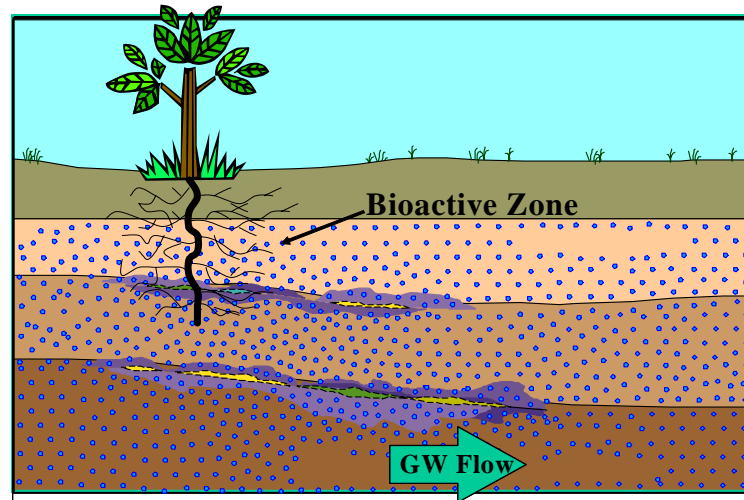


# Phytoremediation of TCE at Two Superfund Sites



## Assessing Methanotroph Activity in the Rhizosphere

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# Personnel Related to This Project

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- **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](http://www.superfund.ufl.edu)

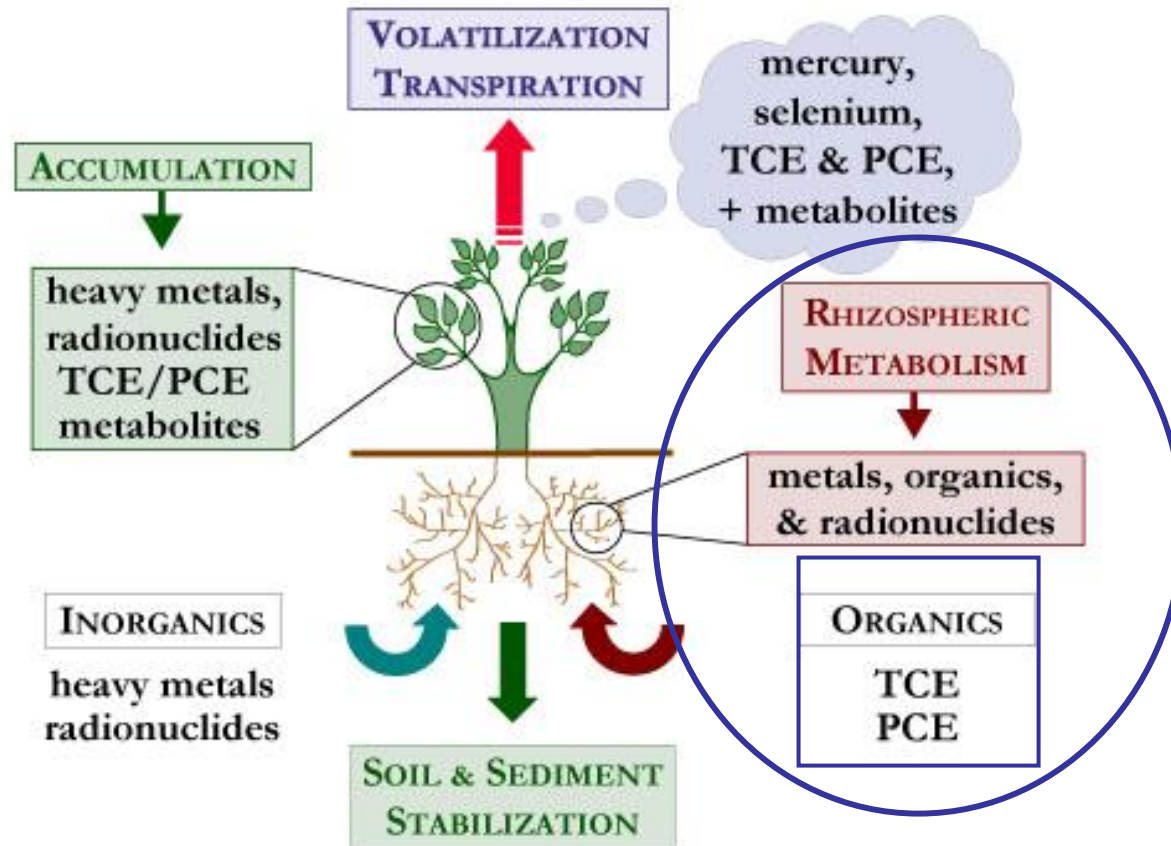
# Overview

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



# Phytoremediation of Contaminants



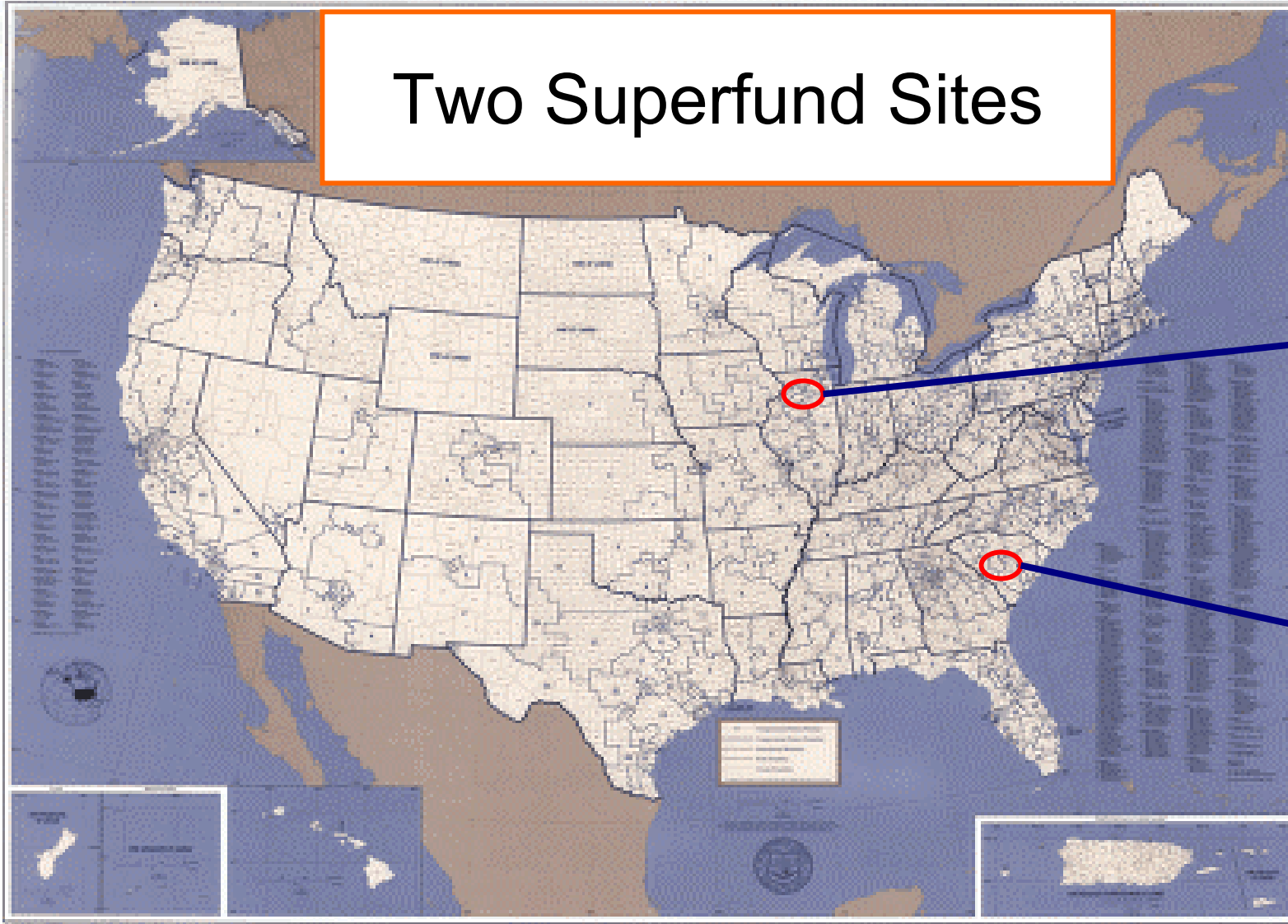
Source:

  
The University of Georgia  
Savannah River Ecology Laboratory



— Focus of this study

# Two Superfund Sites



**LaSalle,  
Illinois  
(LaSalle  
Electric  
Utilities)**

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

# 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.

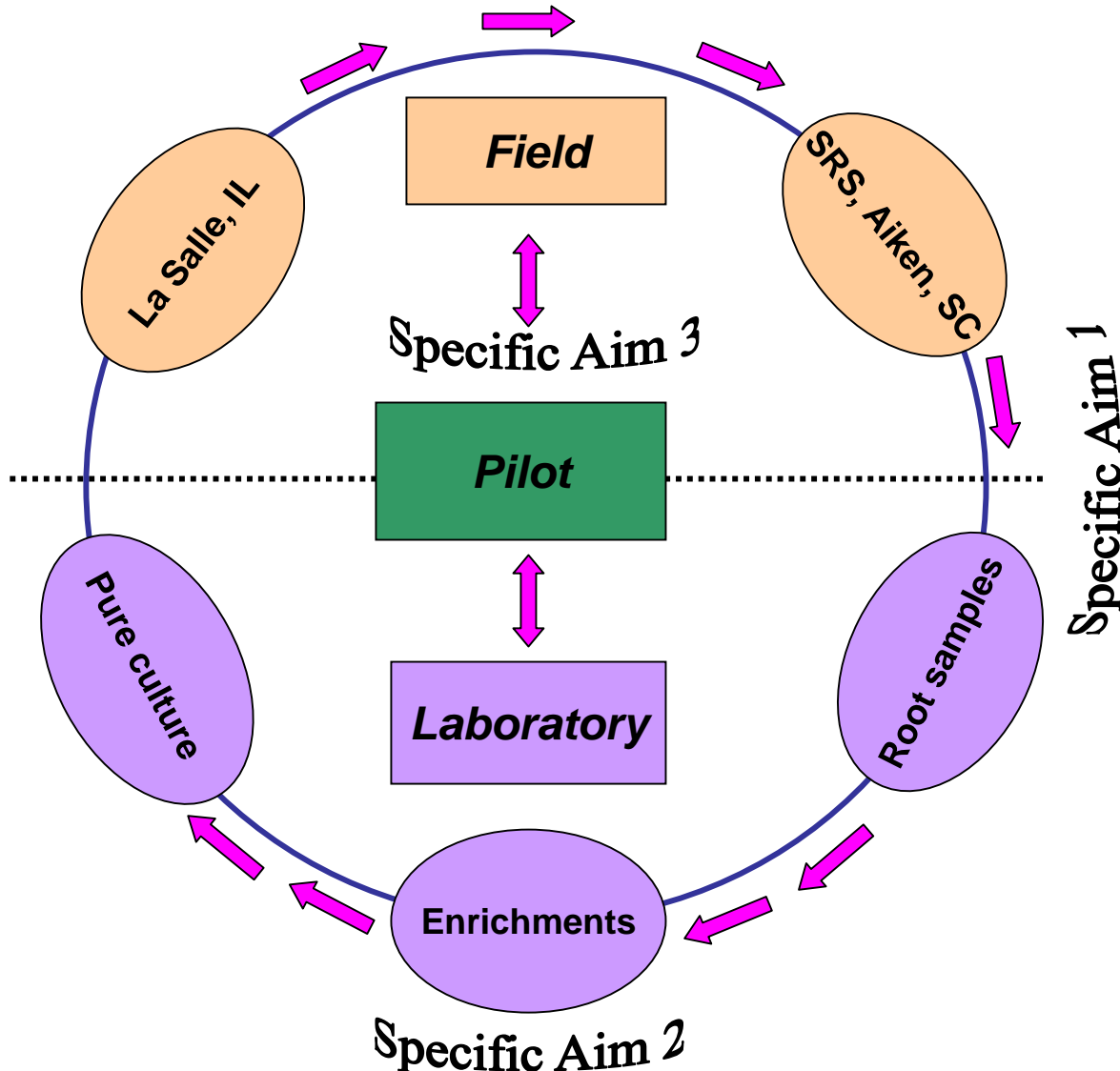


# Broad Goals

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- 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



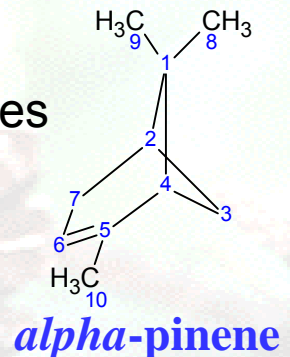
## **Specific Aims:**

1. Microbial Community and Activity Analysis of Rhizosphere Samples
2. Laboratory Enrichments and Activity Measurements
3. Bench-scale Studies

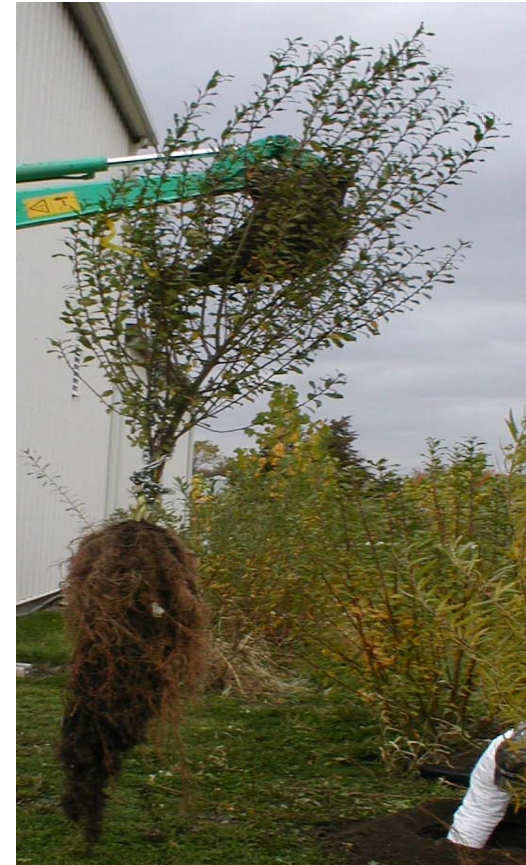
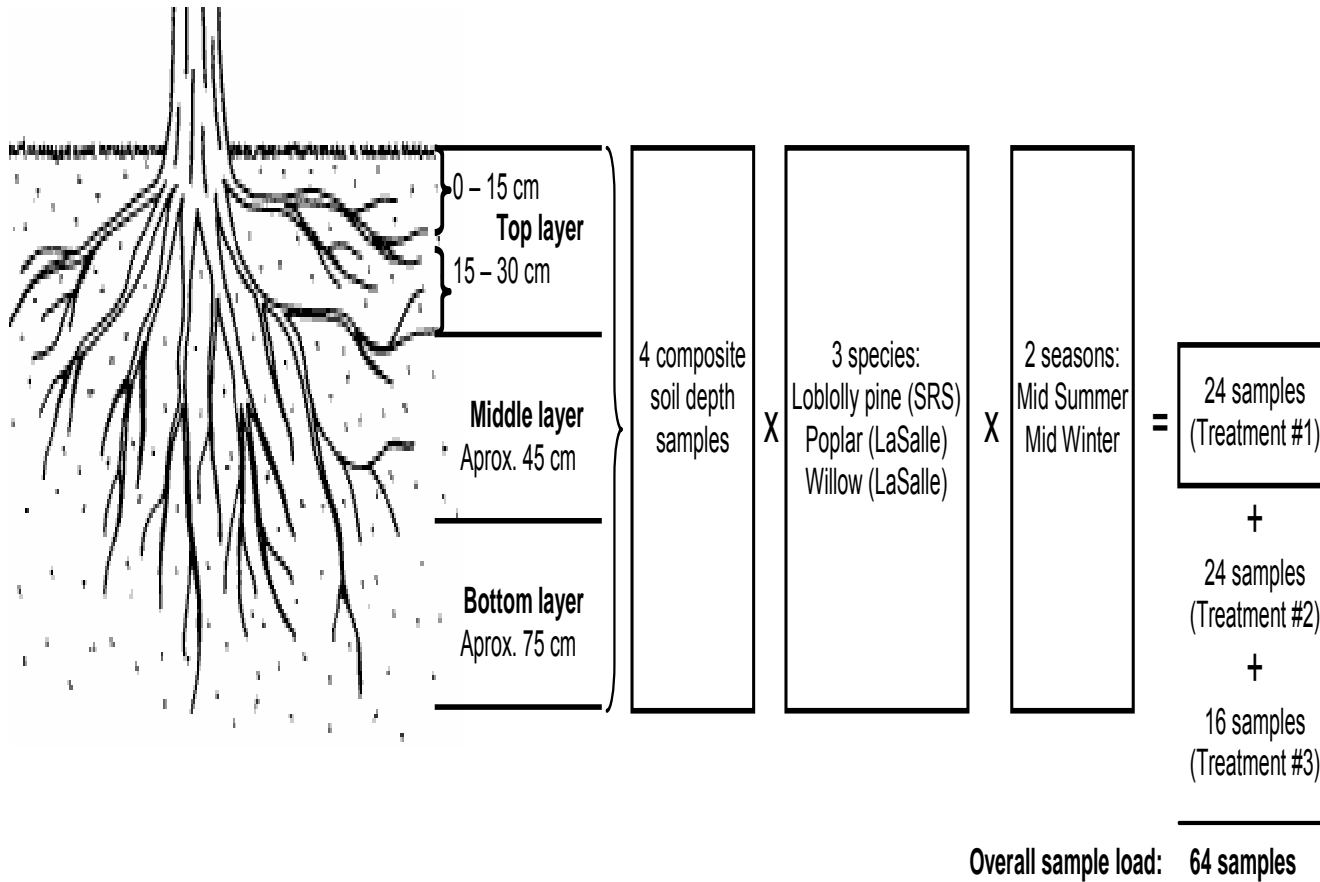


# Questions Addressed Here

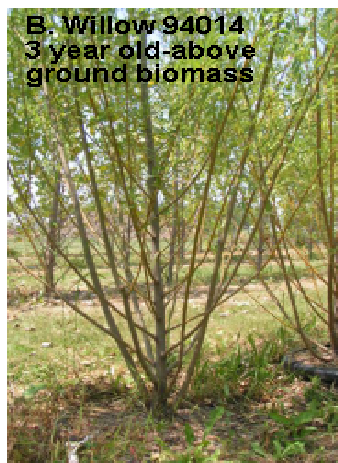
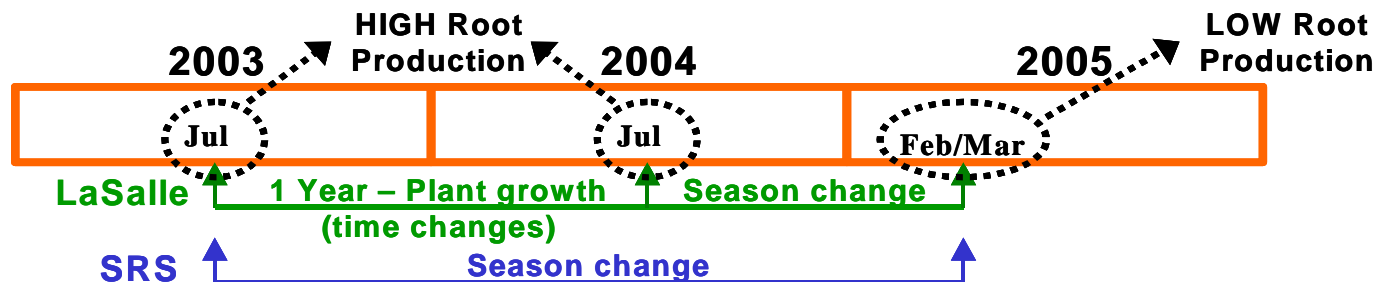
- **Specific Aim 1: Rhizosphere Samples**
  - How do heterotrophic and methanotrophic counts and activity differ
    - By plant type?
    - In roots and soil?
    - With nonvegetated controls?
    - With different moisture levels?
- **Specific Aim 2: Enrichments and Pure Cultures**
  - What effect do terpenes have on TCE biodegradation processes involving methanotrophs?
  - What are the dominant methanotroph species?



# Sampling Protocol



# Sampling Schedule



**July 2003**

**July 2004**

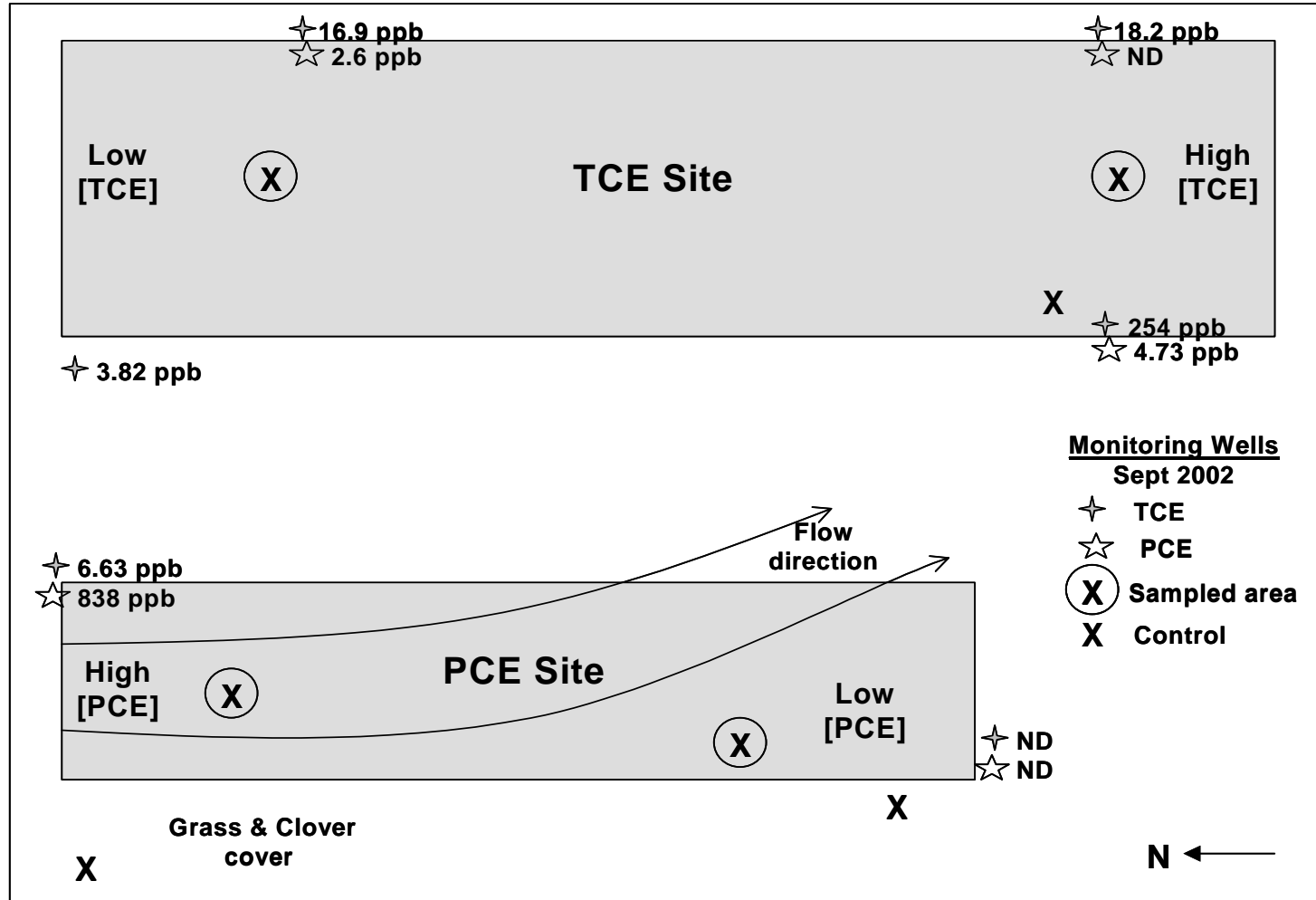
Next sampling at both sites:  
February/March 2005

## Benefits of Sampling Protocol:

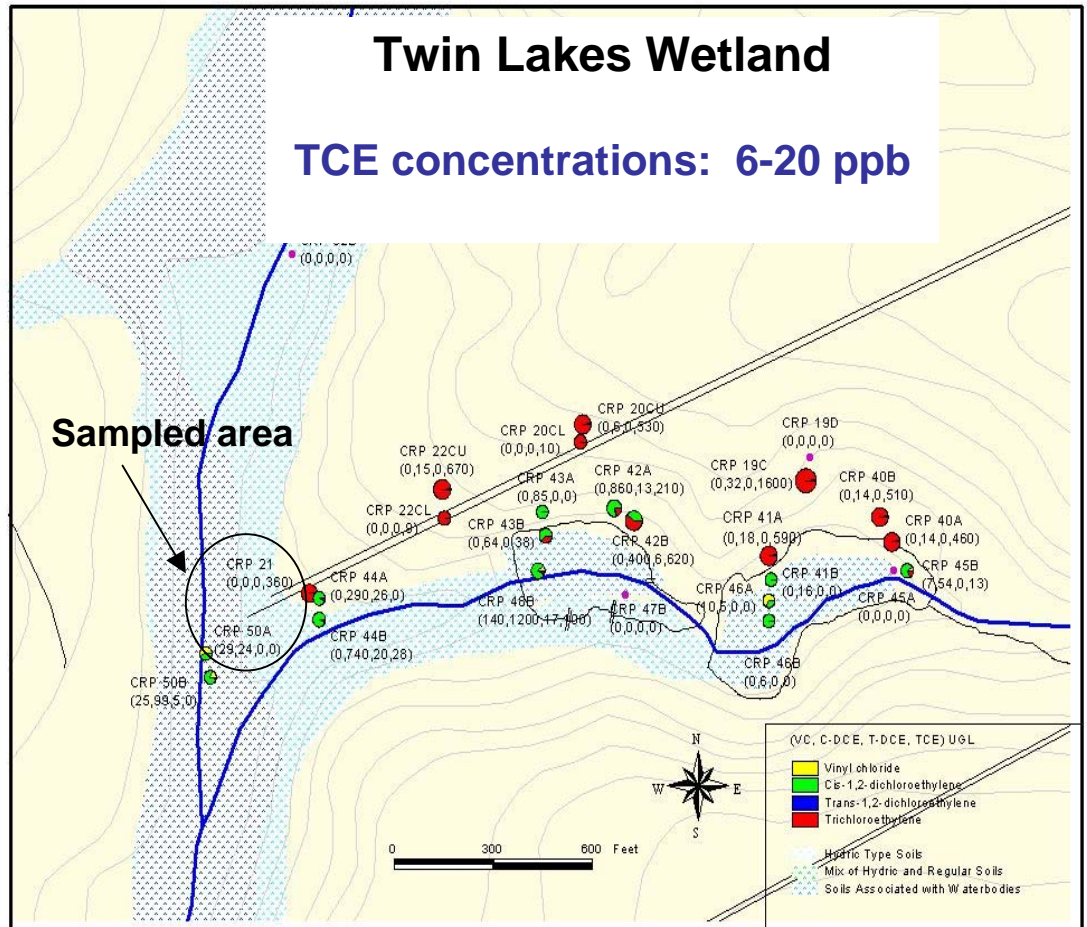
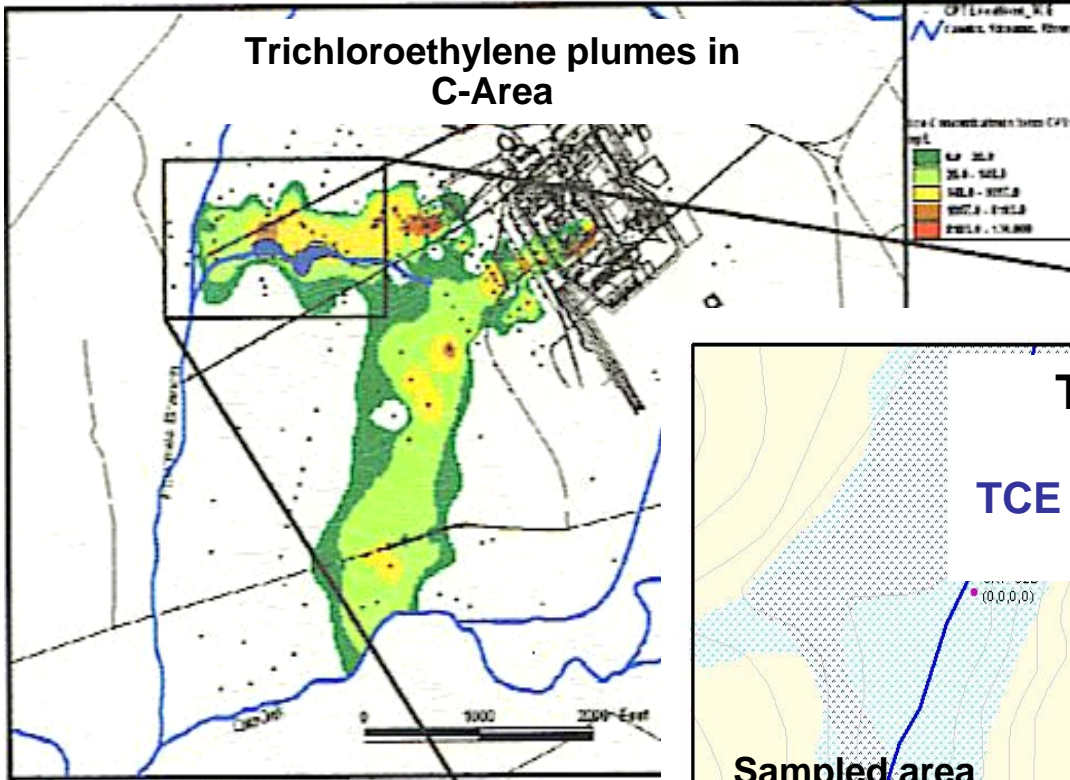
Allows for determining effects of *stage of plant growth, season, and plant maintenance techniques* on populations and activities

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

# LaSalle Site Sampling

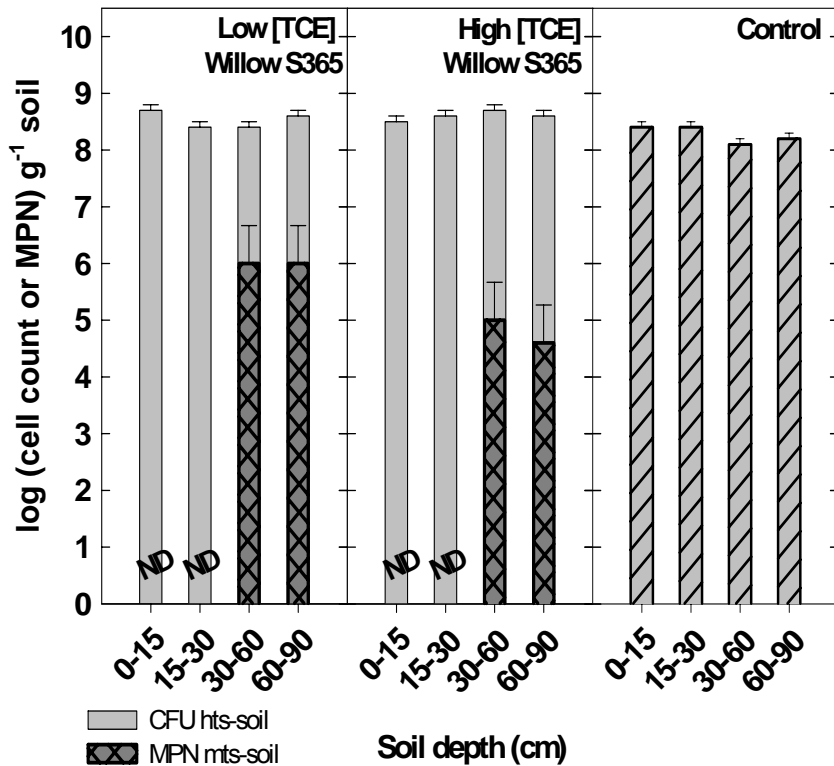


# SRS Sampling

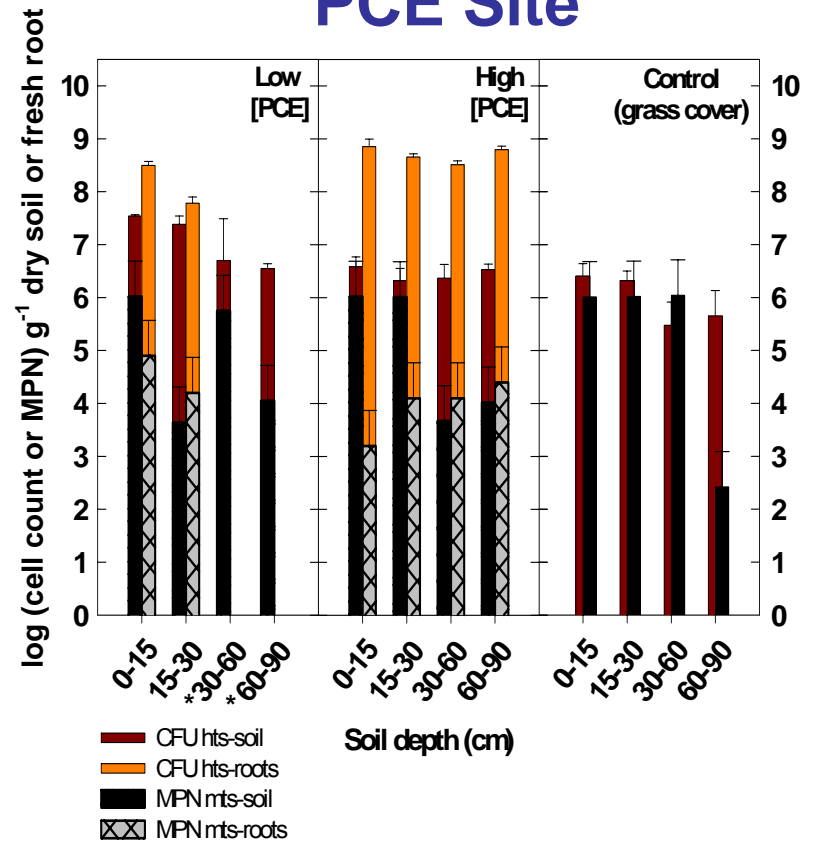


# Microbial Counts in 2003 La Salle Samples

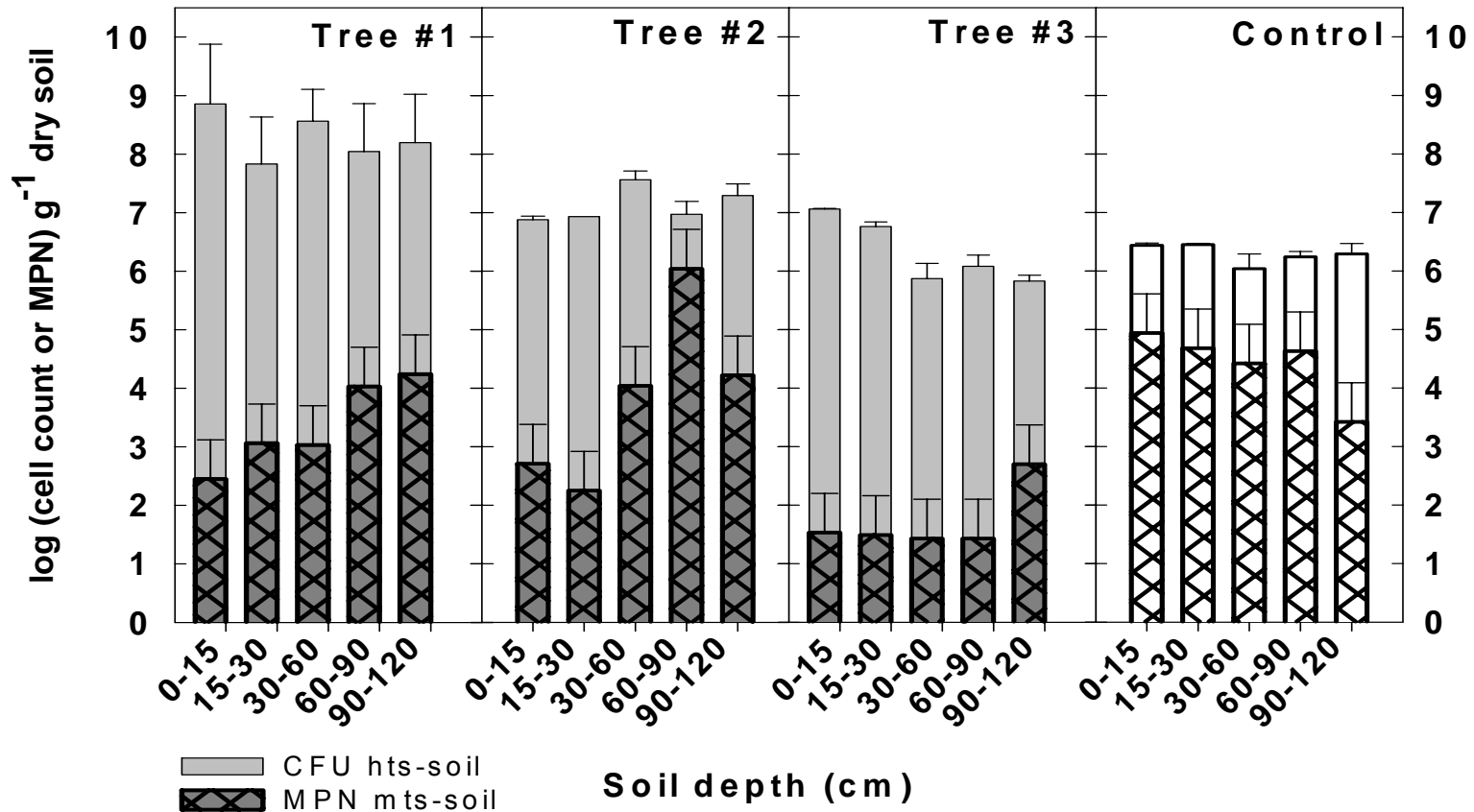
## TCE Site



## PCE Site



# Microbial Counts in 2003 SRS Samples



# Summary of Microbial Counts

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



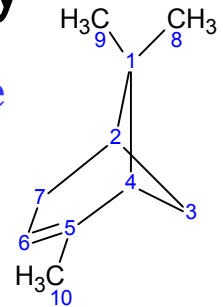
# Specific Aim 2

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

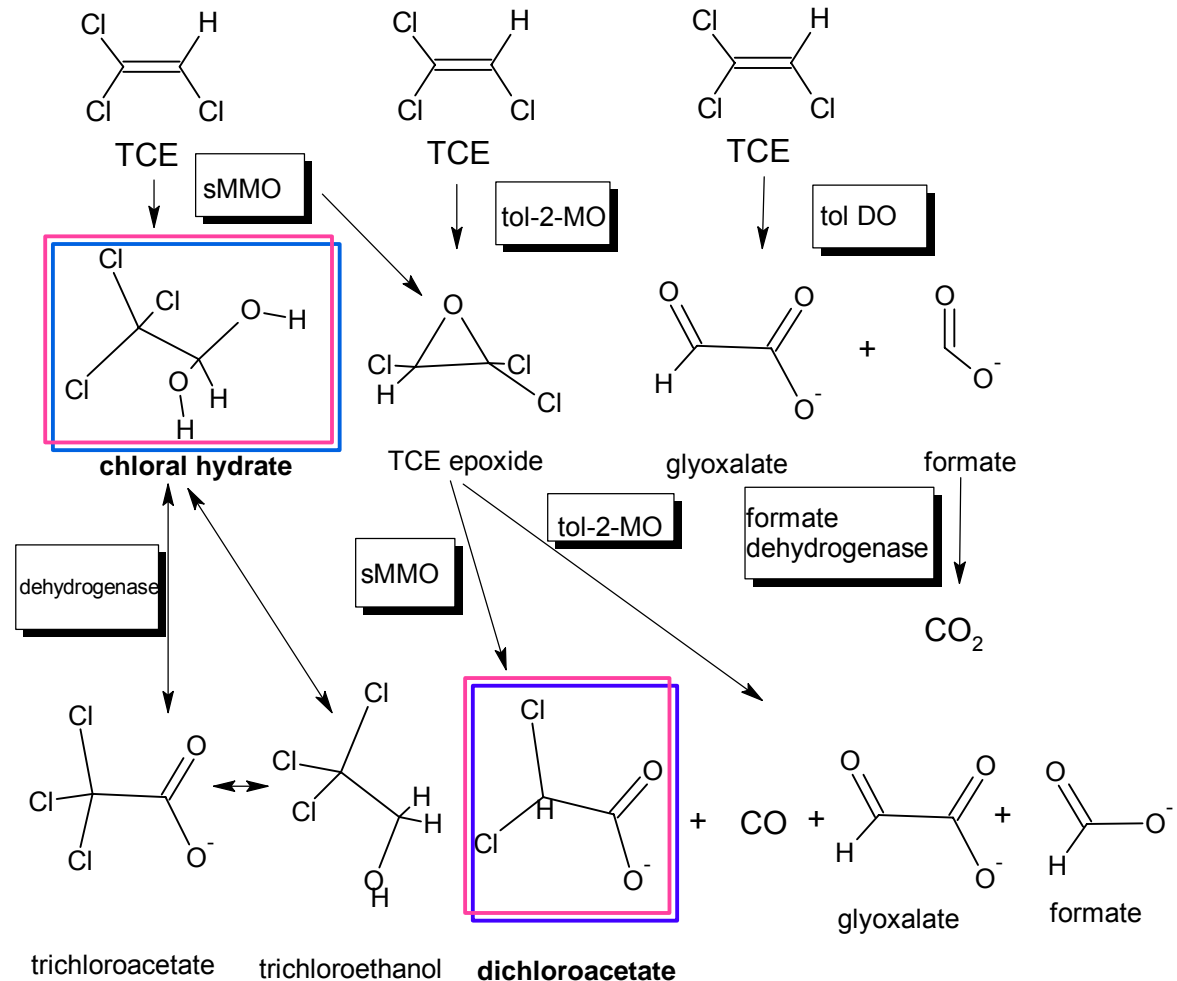
*alpha*-pinene



- 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

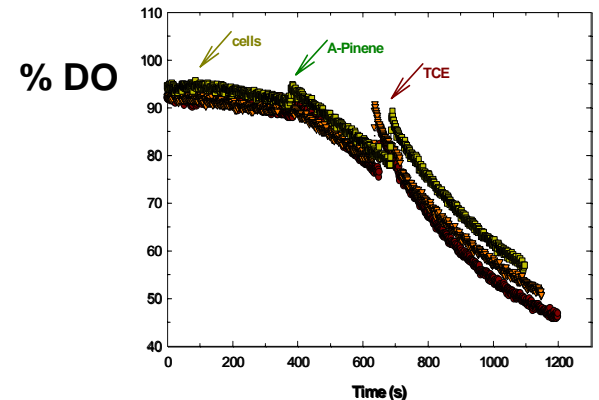
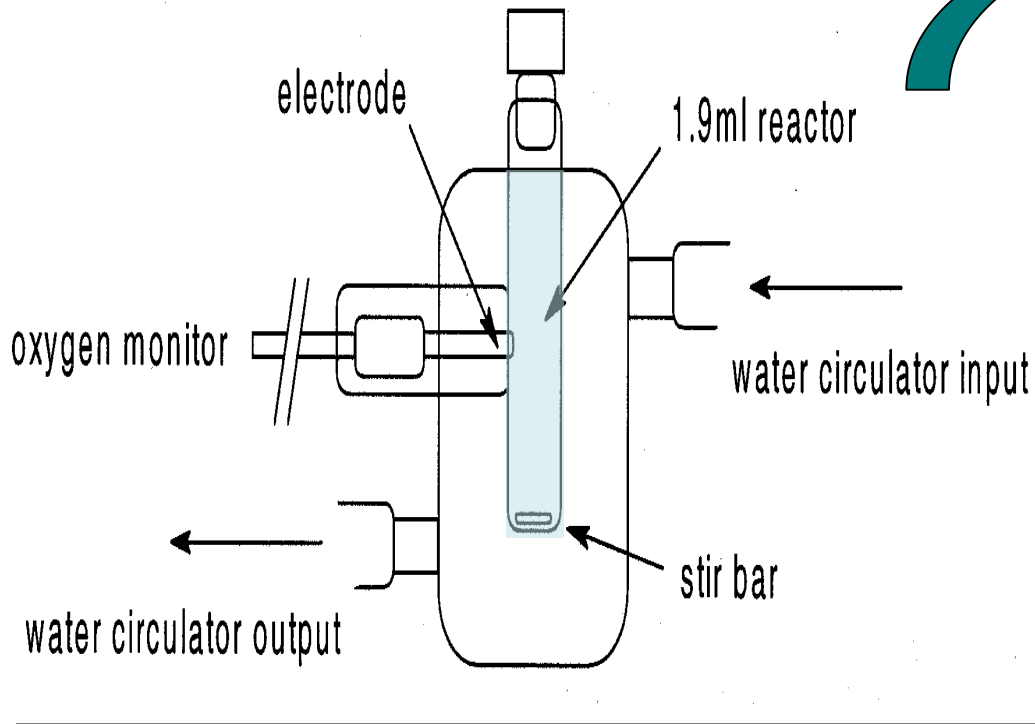
- Most methanotrophs can express particulate methane monooxygenase (pMMO).
- Some methanotrophs can also express soluble methane monooxygenase (sMMO).
- pMMO and sMMO can oxidize TCE.



# Pure Culture Studies: Oxygen Uptake Analysis to Measure the Effect of $\alpha$ -Pinene on TCE Oxidation

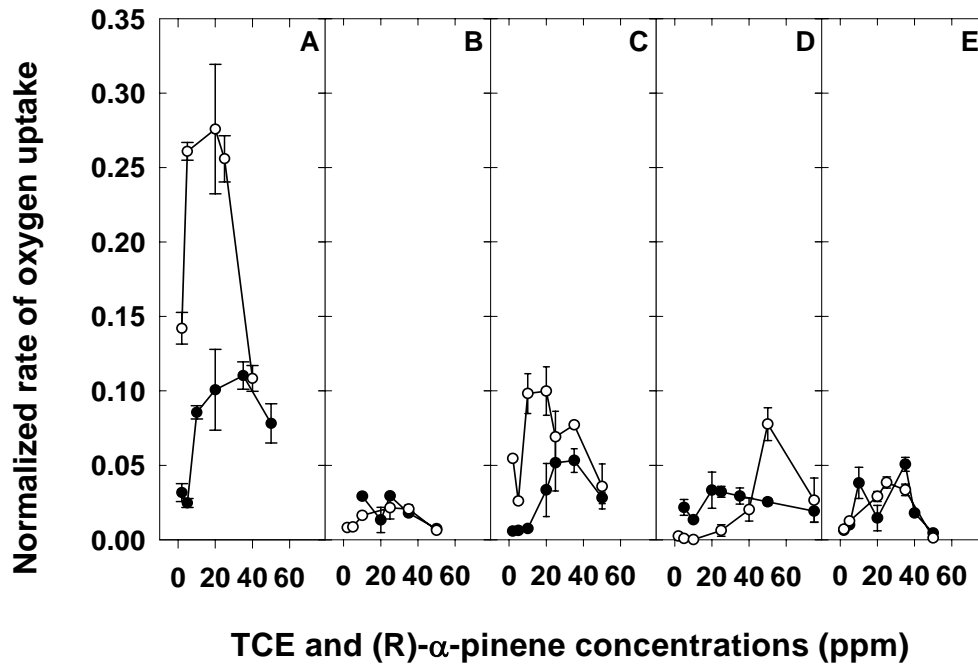
## Methanotrophs +

1. TCE alone
2.  $\alpha$ -Pinene alone
3. TCE +  $\alpha$ -Pinene

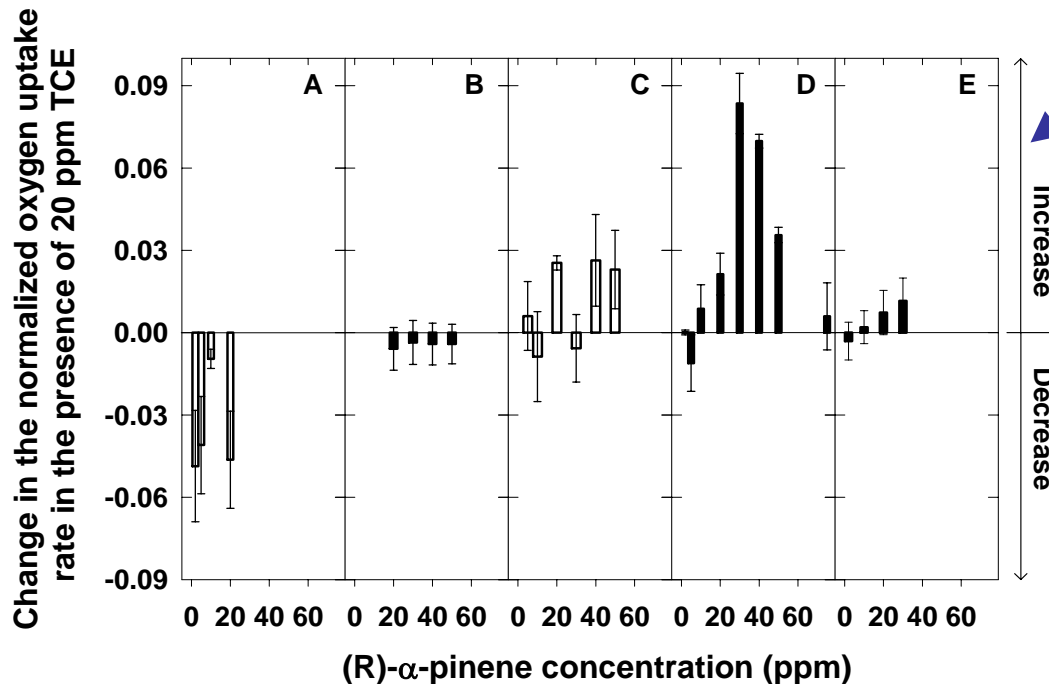


Rates are normalized to methane to produce a dimensionless measure of oxygen uptake.

# 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- $\alpha$ -pinene

# Description of Enriched Mixed Cultures

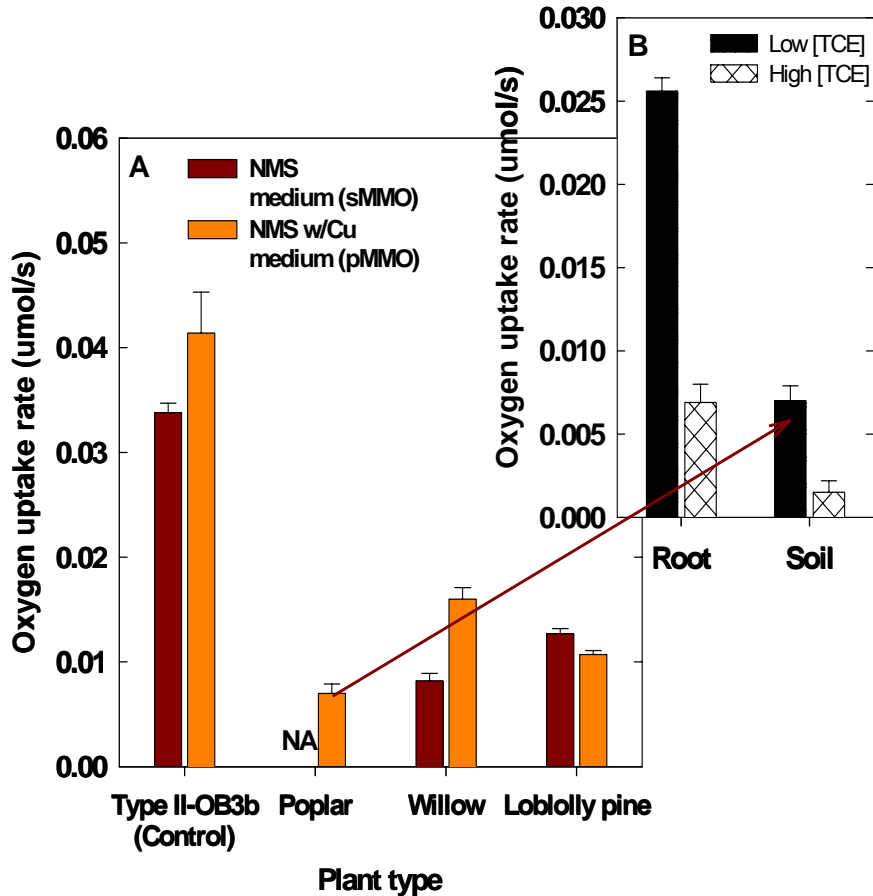
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- Stable mixed methanotrophic-heterotrophic cultures were isolated from the rhizosphere of poplar (LaSalle), willow (LaSalle), and loblolly pine (SRS) trees.
- All mixed cultures can use methane as the only carbon source and exhibit sMMO activity when 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<sub>4</sub>

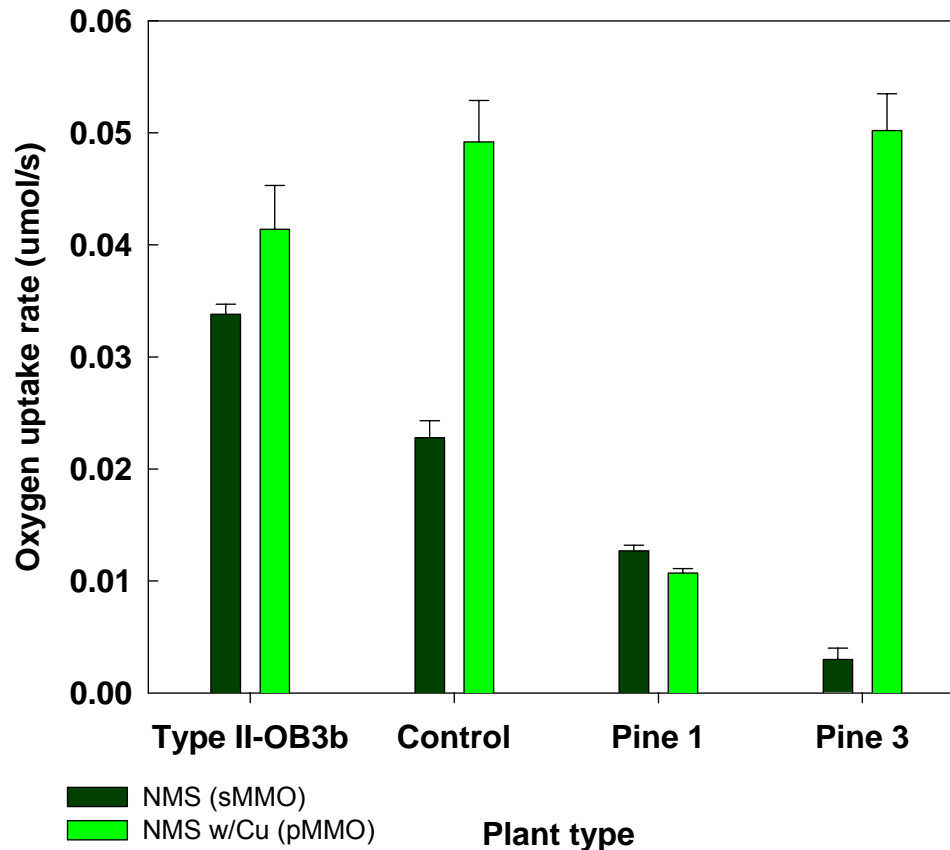
*Cultures isolated from poplar soil and root samples*



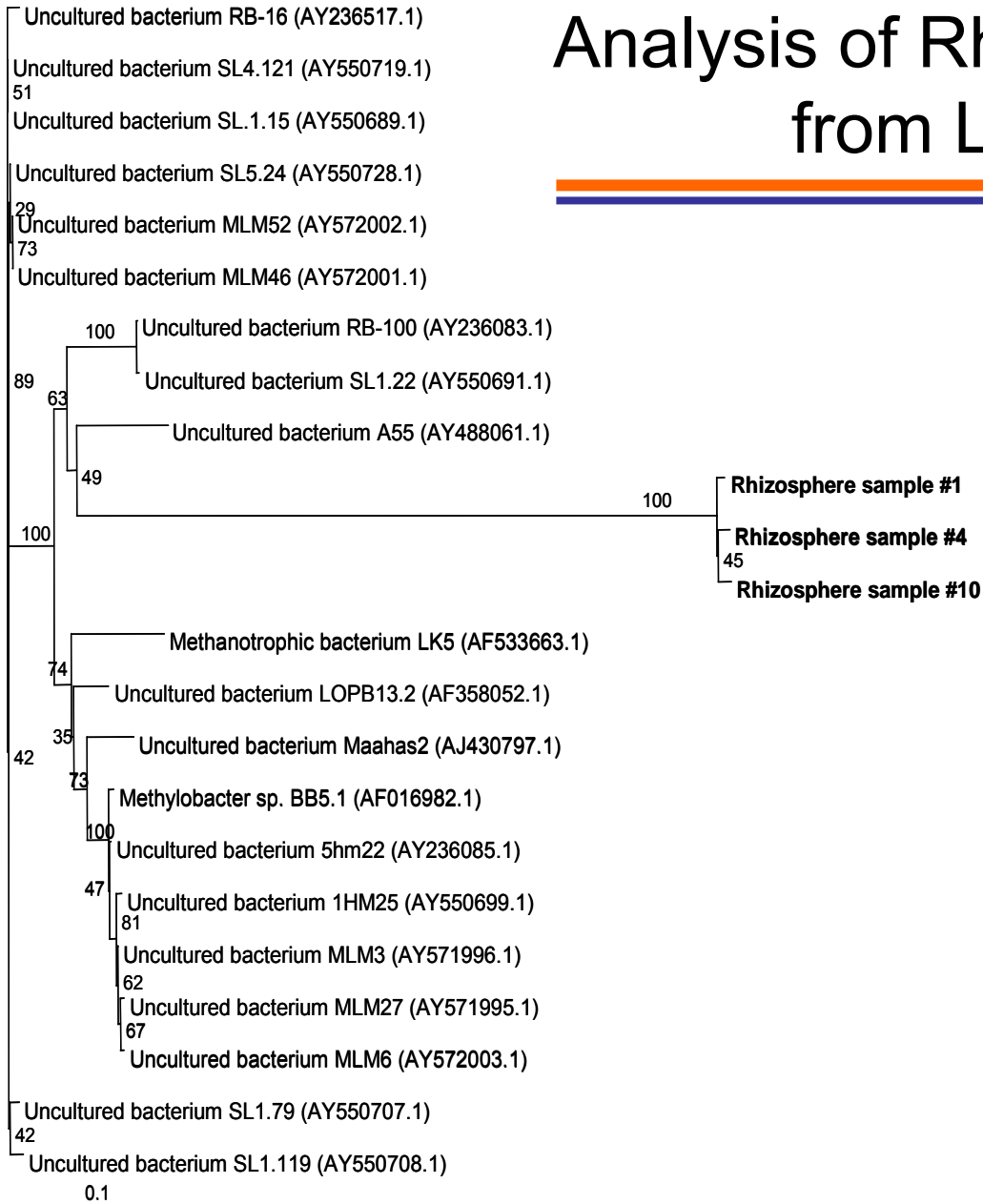
*TCE Zone:  
Cultures isolated from rhizosphere soil samples of all tree types*

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

# Activity of SRS Enrichments in the Presence of CH<sub>4</sub>



# 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



# Conclusions to Date

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- **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

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## – **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 ( $^{14}\text{CH}_4$ ) of samples for methanotrophic activity

## – **Specific Aim 2:**

- Protocol development for analysis for products of *alpha*-pinene and TCE oxidation by pure methanotrophs