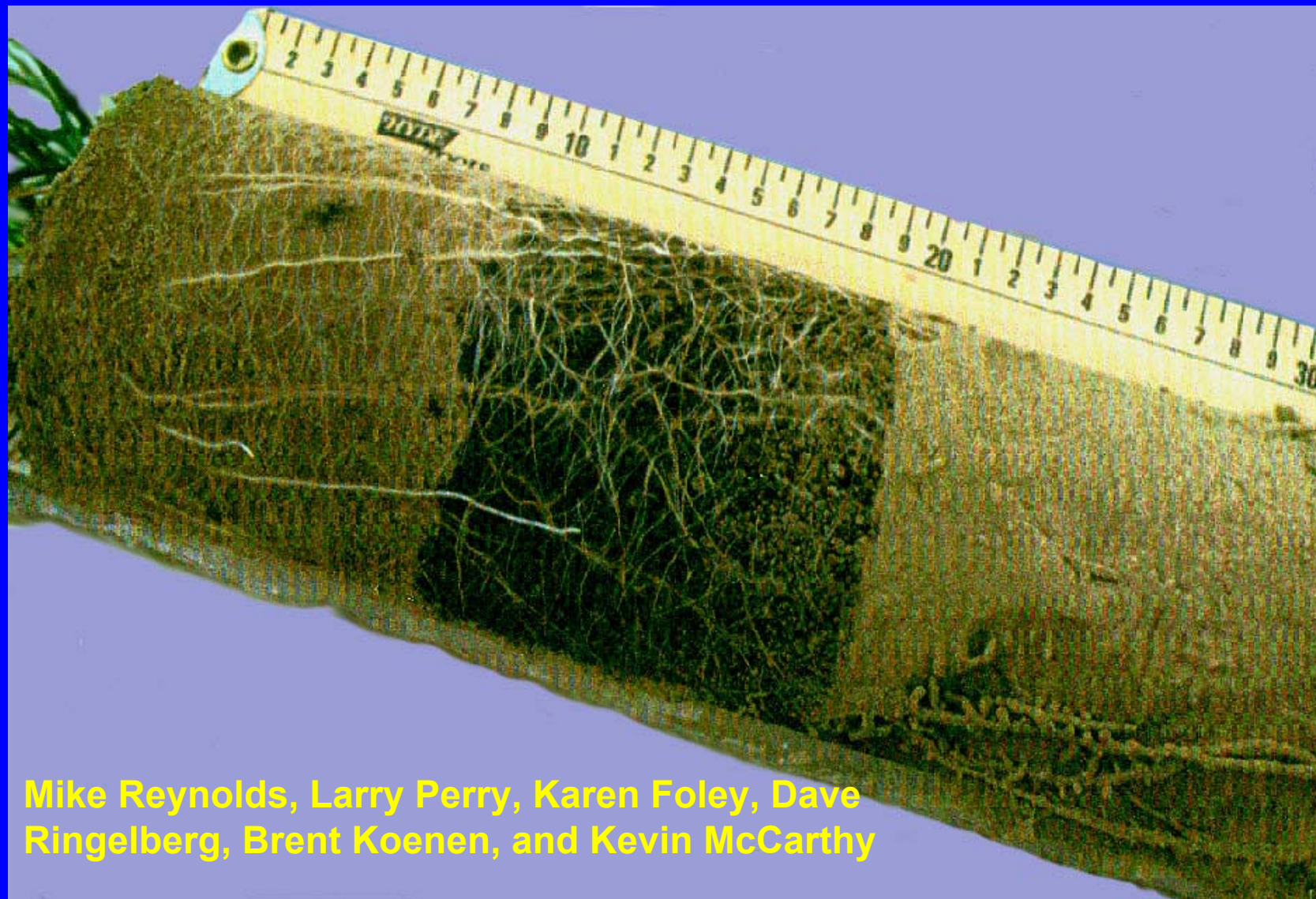
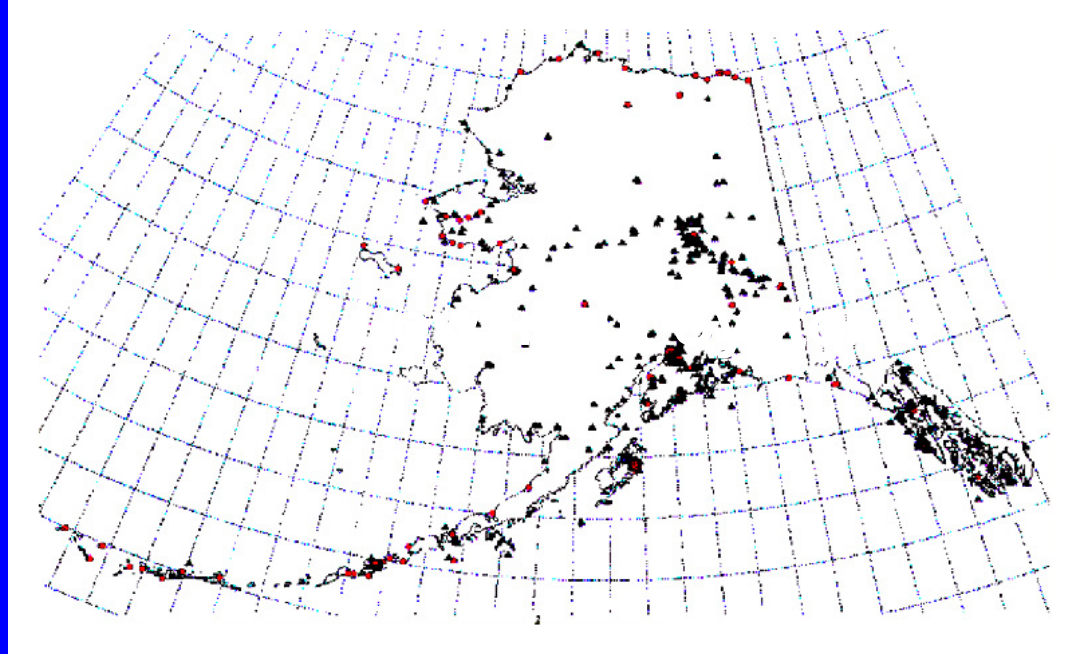


# Rhizosphere-Enhanced Treatment of PAHs at Cold, Remote Locations; Challenges of Application and Monitoring



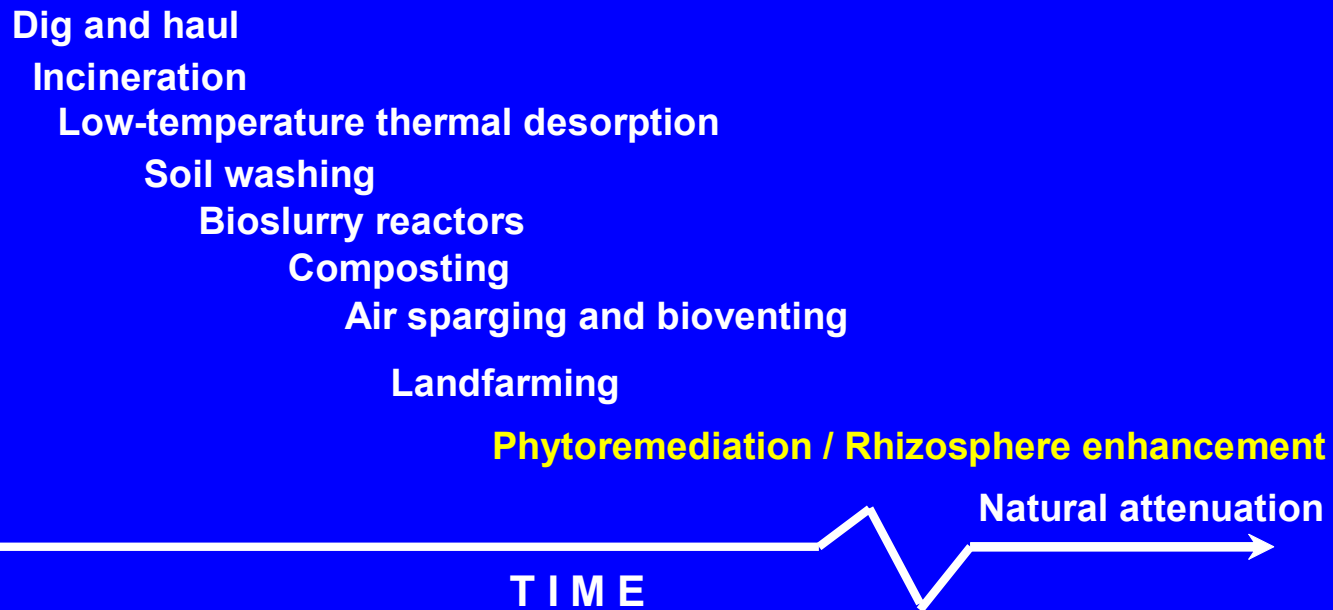
Mike Reynolds, Larry Perry, Karen Foley, Dave Ringelberg, Brent Koenen, and Kevin McCarthy

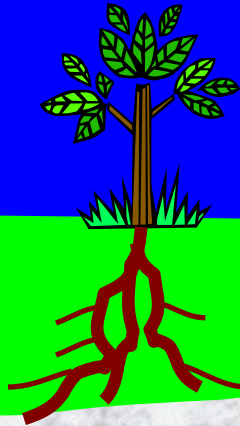
# Federal Cold Region Sites



## High Input Systems

## Low Input Systems





Wide-scale, low-cost, in-situ treatment

- Requires less input
- Requires fewer specifics about a site

Water Soluble Contaminants

~~ Mixing → Natural Attenuation

Relatively constant  
temperature and moisture

Saturated Zone

## Problem

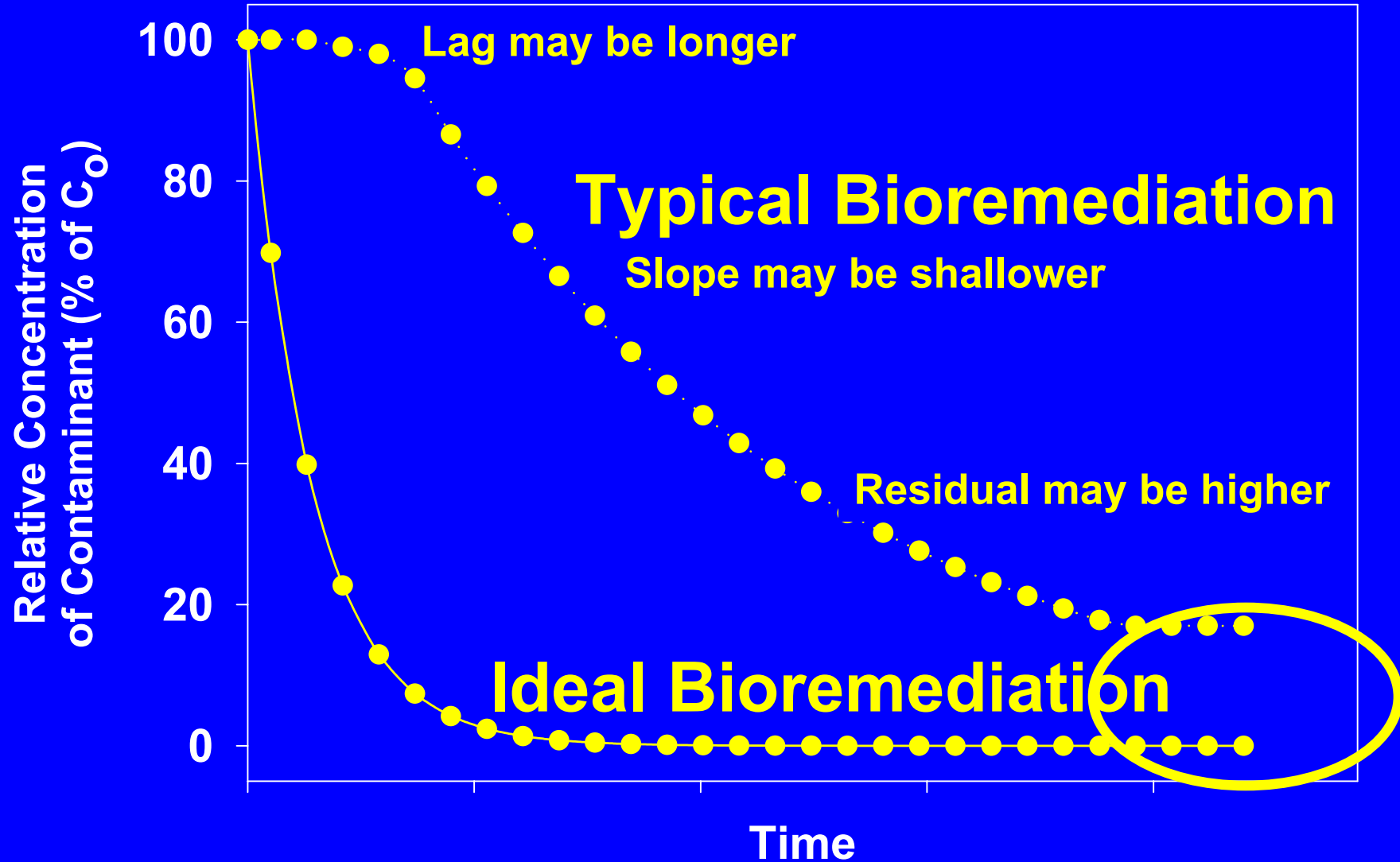
- Remote locations
- Hundreds of sites
- Cleanup is costly
  - Mobilization & demobilization
- Limited alternatives
- Short season
- Relatively slow activity

## Limitations

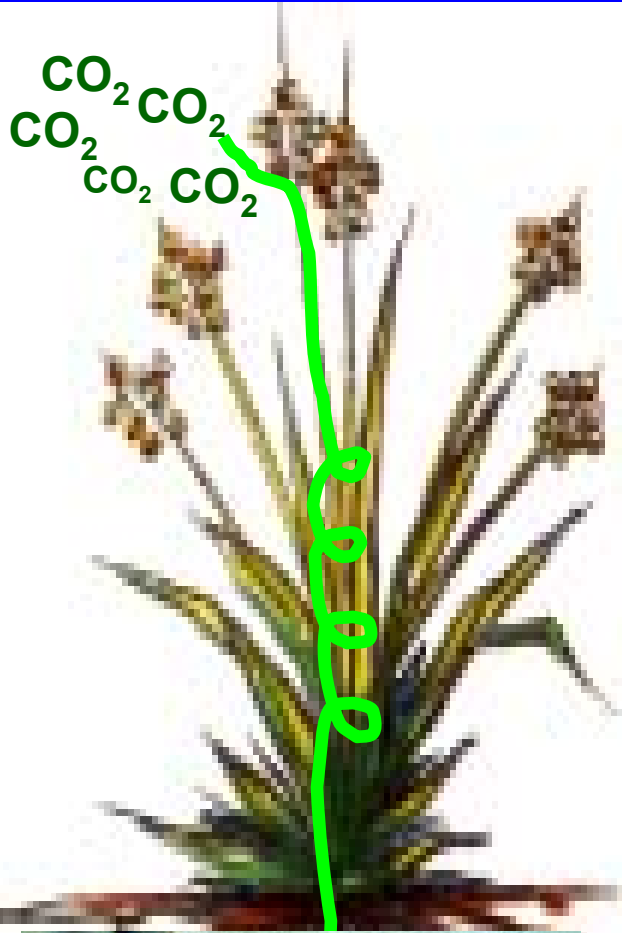
- Temperature
- E-S proximity, MT limitations
- Microorganisms (numbers, diversity, activity, function)
- Nutrient & contaminant availability, bioavailable C
- Aeration

# Impact of Limitations

*Microbial activity in soil is not constant –but starts and stops many times  
f (temperature, moisture, carbon additions, ???)*



# Stimulating Microbial Activity via the Rhizosphere Effect



- Analog enrichment (natural forced molecular evolution)
- Stimulated microbial #s & activity
- Reduced M-T limitations, pseudo-mixing
- Carbon-enriched environment
- Not necessarily plant uptake
- Increased OM - Humification
- ???

# Measuring Contaminant Loss is Difficult

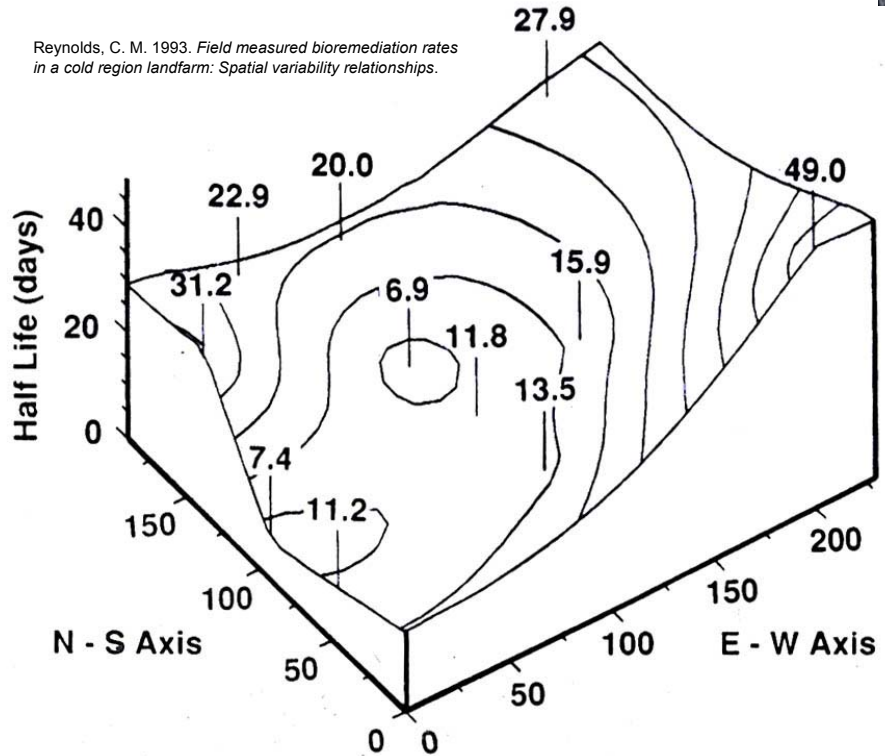
## Landfarm at Fairbanks Airport

- Tilled ~ weekly
- Fertilized and limed
- Irrigated
- Periodically, composite samples taken near 25 nodes
- Calculated half-lives varied ~7-X



Half Life Distribution (days)

Reynolds, C. M. 1993. *Field measured bioremediation rates in a cold region landfarm: Spatial variability relationships.*



**Variability in Contaminant and Product concentrations makes their routine use for monitoring difficult, and this is exacerbated for:**

- Surface soils
- Non “brute-force” treatment methods
- Most situations – whether implementation, regulatory monitoring, or research studies

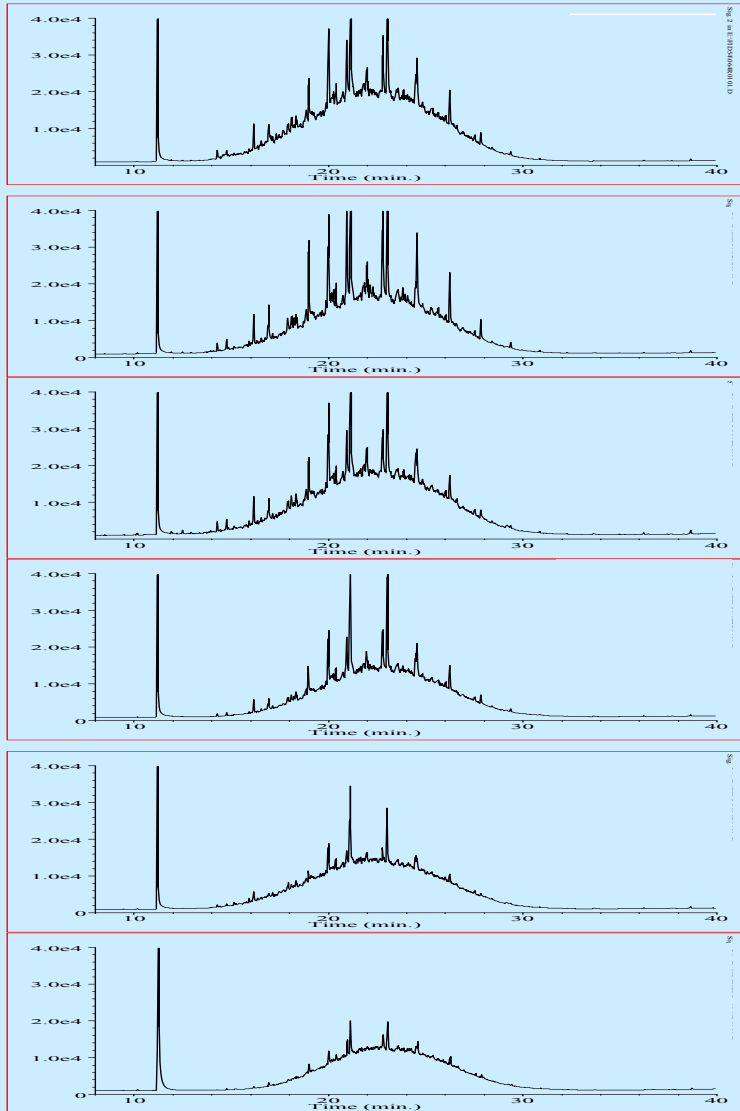
**Replicated  
Grasses, Nutrients, Both, Control  
“Soil sock” approach  
Crude and Diesel**



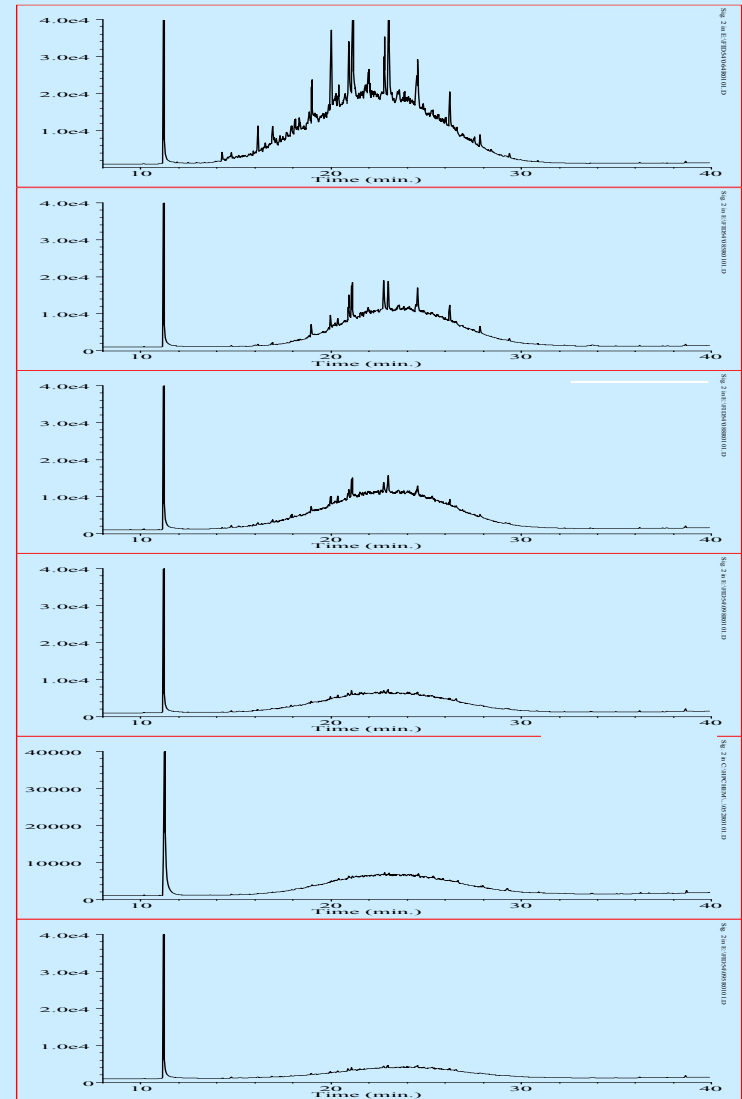


# The rhizosphere-effect really does work ... Fairbanks data

## Control



## Vegetated



$t_i$

~700 days

$t_f$

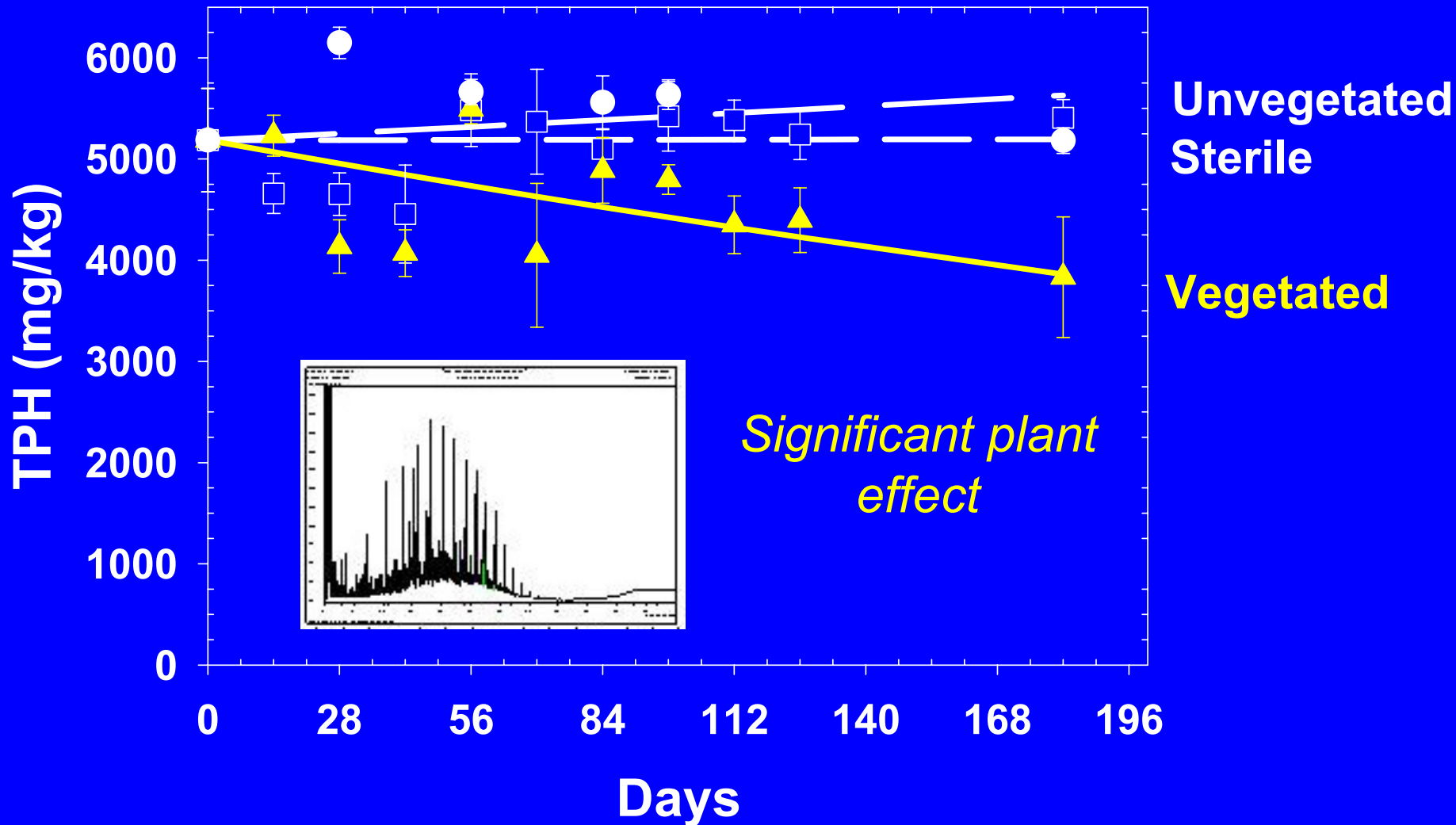
Lab study

Alaska soil

Winter rye

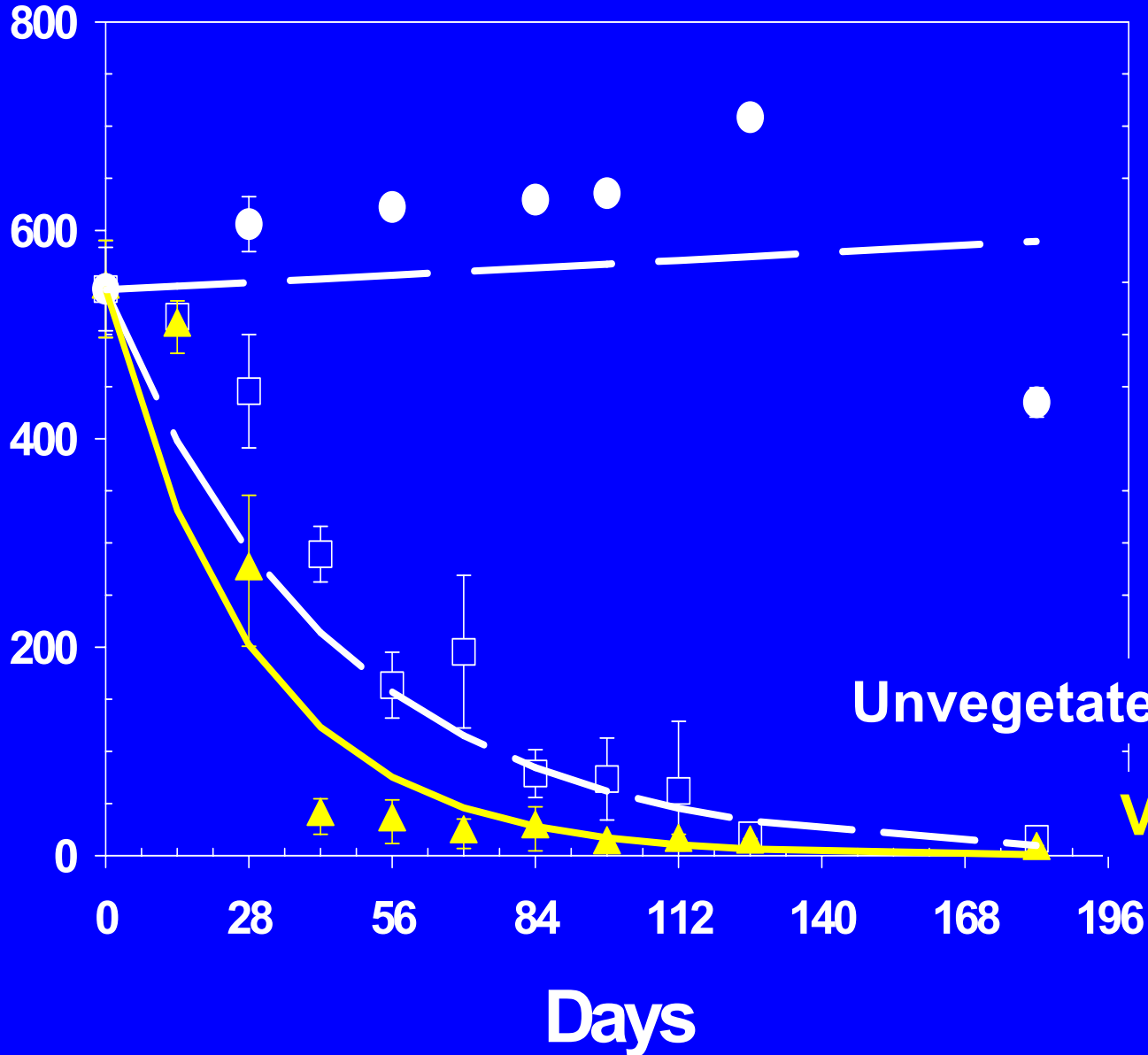
No nutrients added

# TPH Changes



# Hexadecane

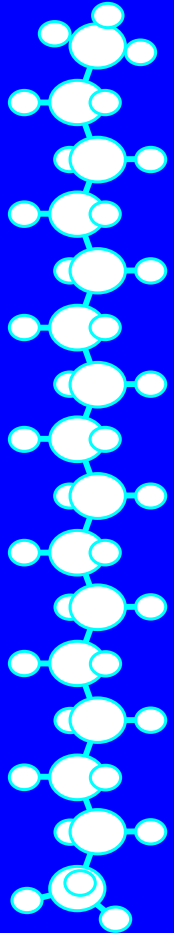
*Easy to degrade,  
Little difference*



**Sterile**

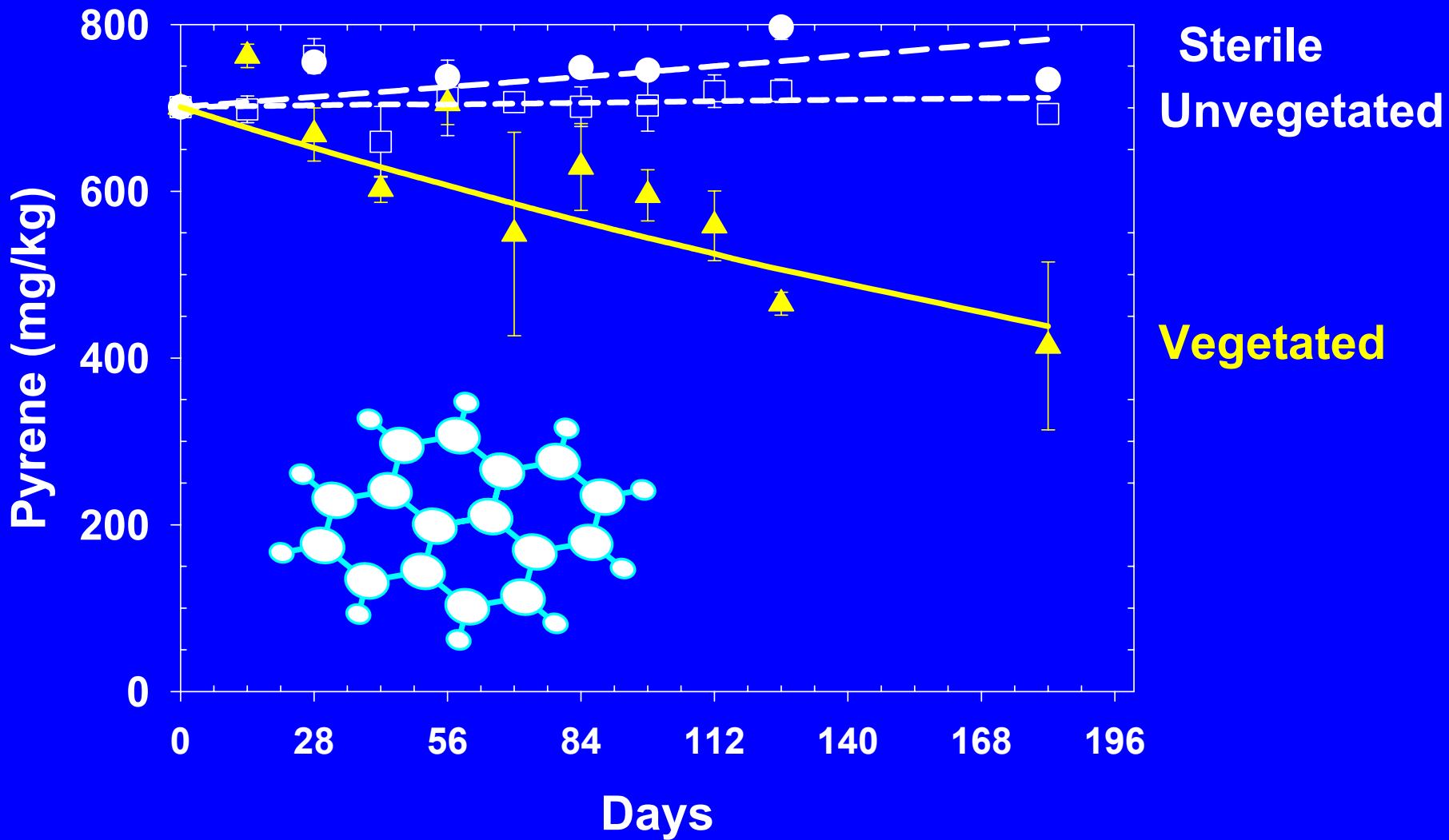
**Unvegetated**

**Vegetated**



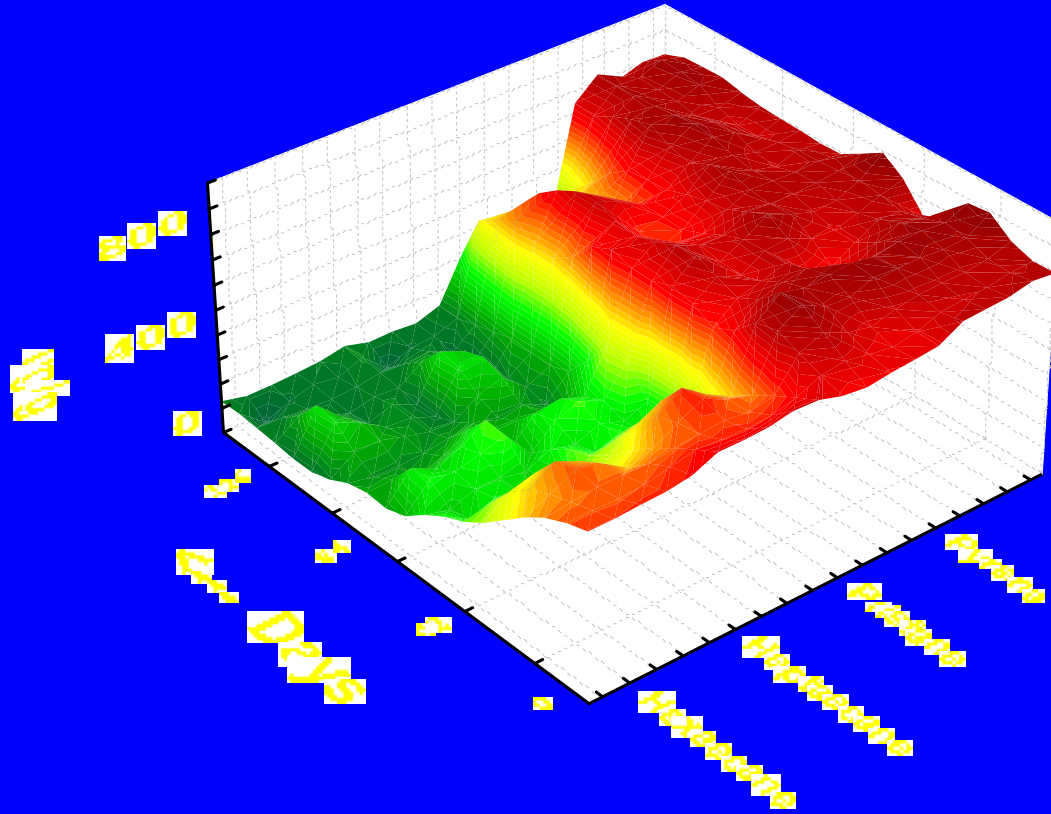
# Pyrene

*Recalcitrant, big  
difference*

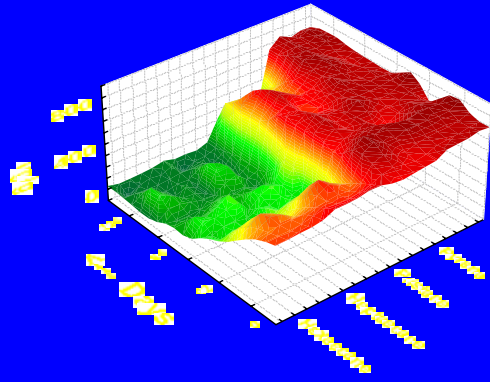


# Selected Compounds

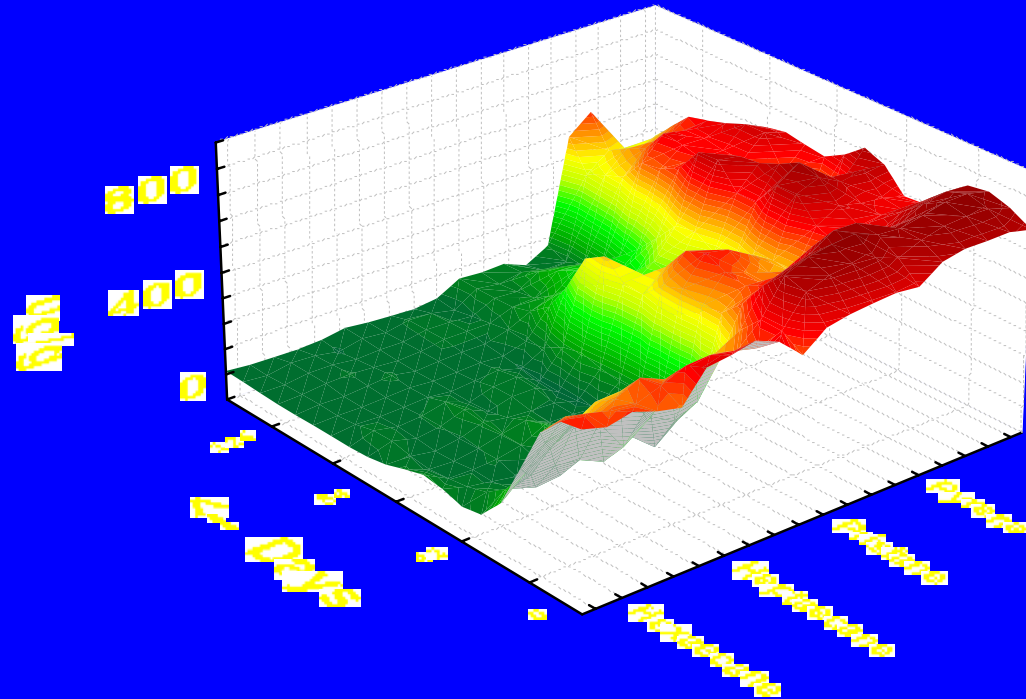
## Non-vegetated



**Selected Compounds  
Non-vegetated**

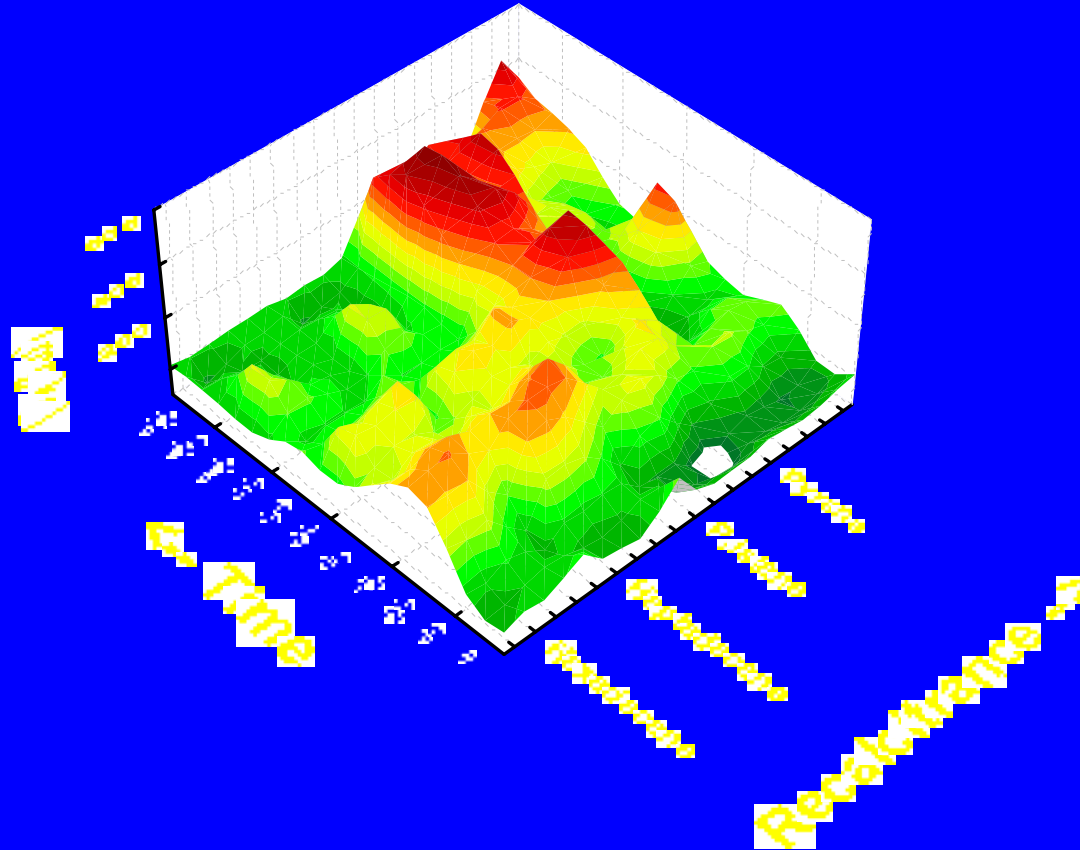


**Selected Compounds  
Vegetated**



***We can subtract Vegetated from Non-Vegetated to visual rhizosphere effect***

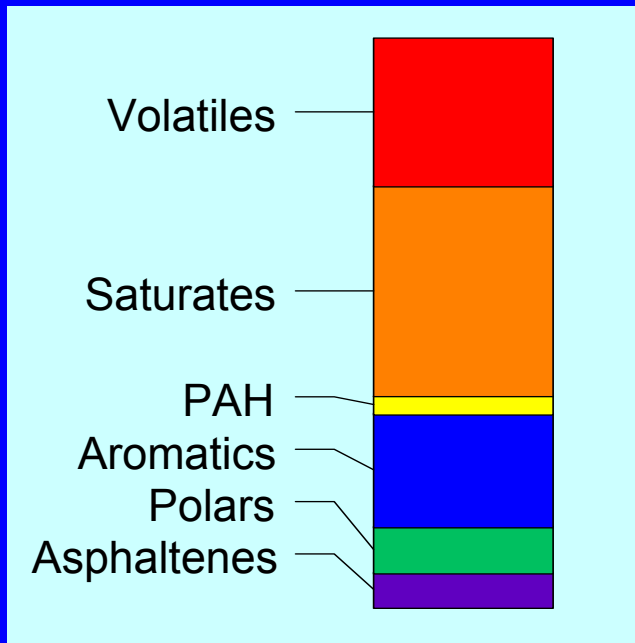
**Benefit due to Rhizosphere Effect**  
**[ Non-veg ] - [ Vegetated ]**  
**is  $f$  (Compound and Time)**



***Rhizosphere effect has Time and Compound Components***

*Biomarkers...*

# Petroleum - a Complex Mix of hydrocarbons



*Generalized Composition of Crude Oil*

## Compositional Variability

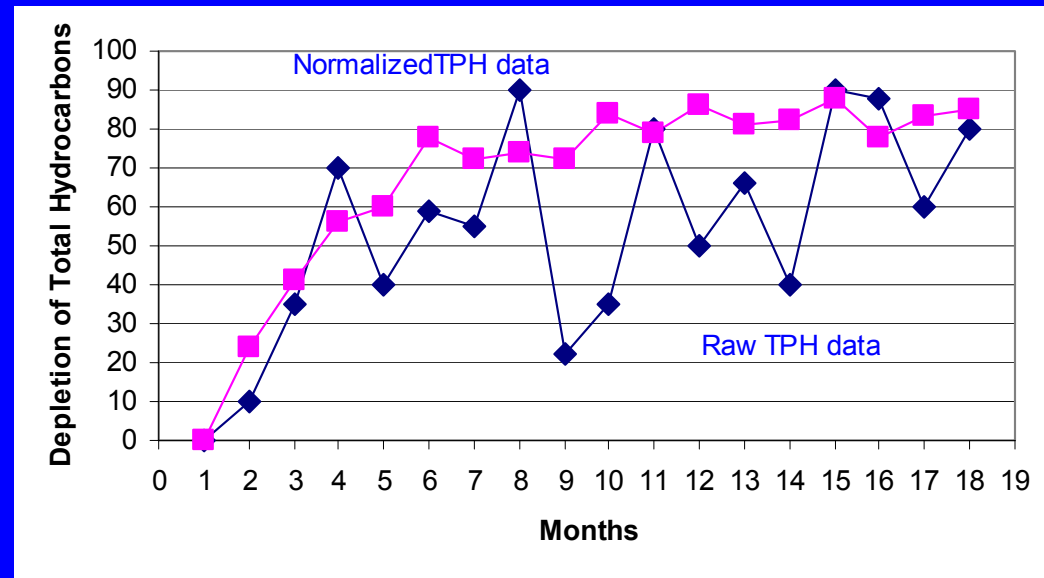
- May consist of 100s to 1000s of hydrocarbons
- Natural or remediation-enhanced weathering of hydrocarbons in soil substantially alters its composition
- Chromatographic methods yield most accurate measures of extractable hydrocarbons
- Inherent variability in TPH can be minimized by normalization to a recalcitrant marker compound



*Biomarkers...*

# Benefits to Normalizing TPH to Hopane

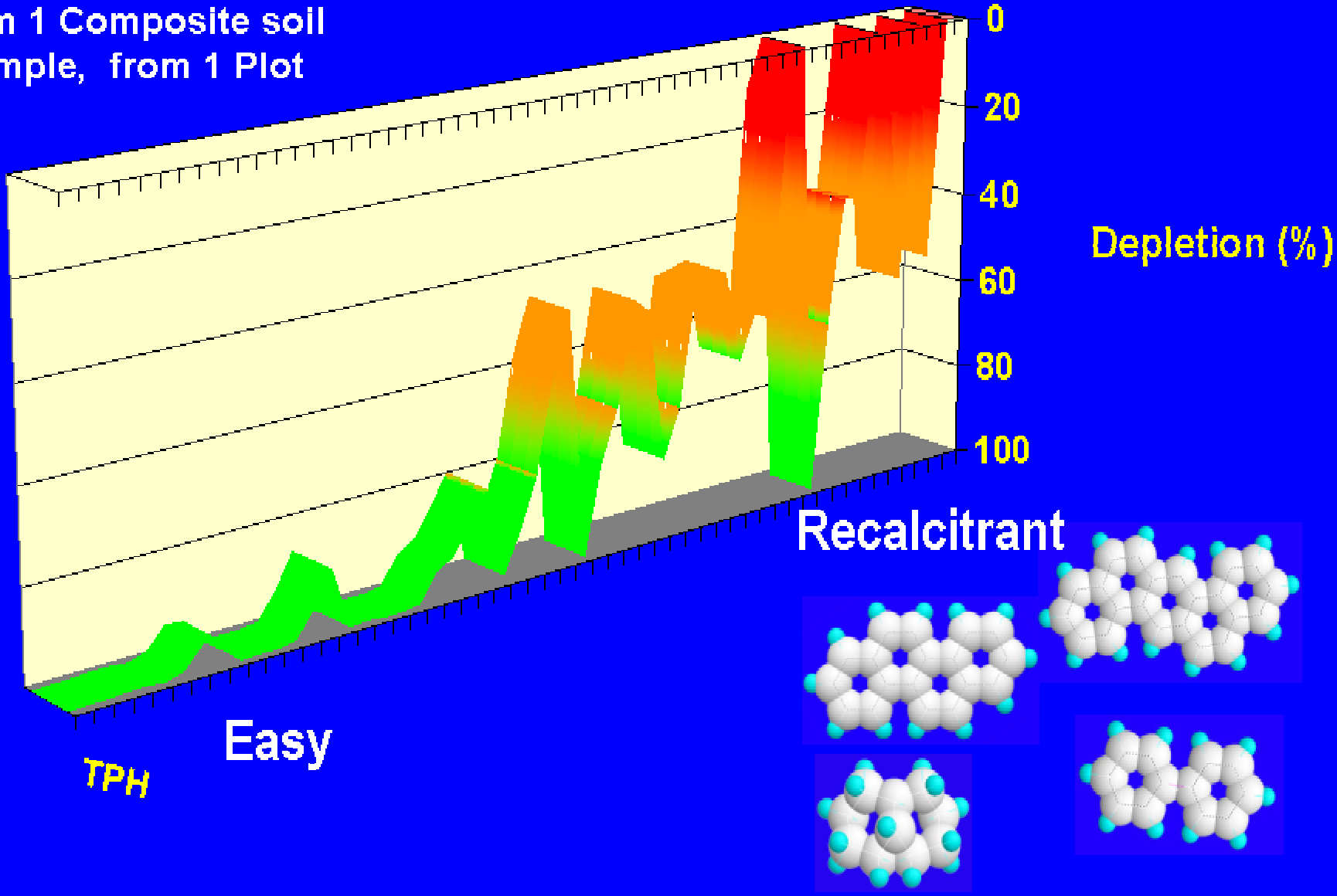
- Precision (concentration data) can be increased when data are normalized to internal marker such as hopane
  - Raw TPH soil data has inherently high variability
  - Concentration data can become more variable as petroleum weathers
- Normalizes TPH concentration data



**Assumes that contaminant at a site has uniform composition at t=0**

# In addition to TPH, we can normalize other compounds w.r.t a biomarker

From 1 Composite soil Sample, from 1 Plot





## Two Field Demo Sites in Korea

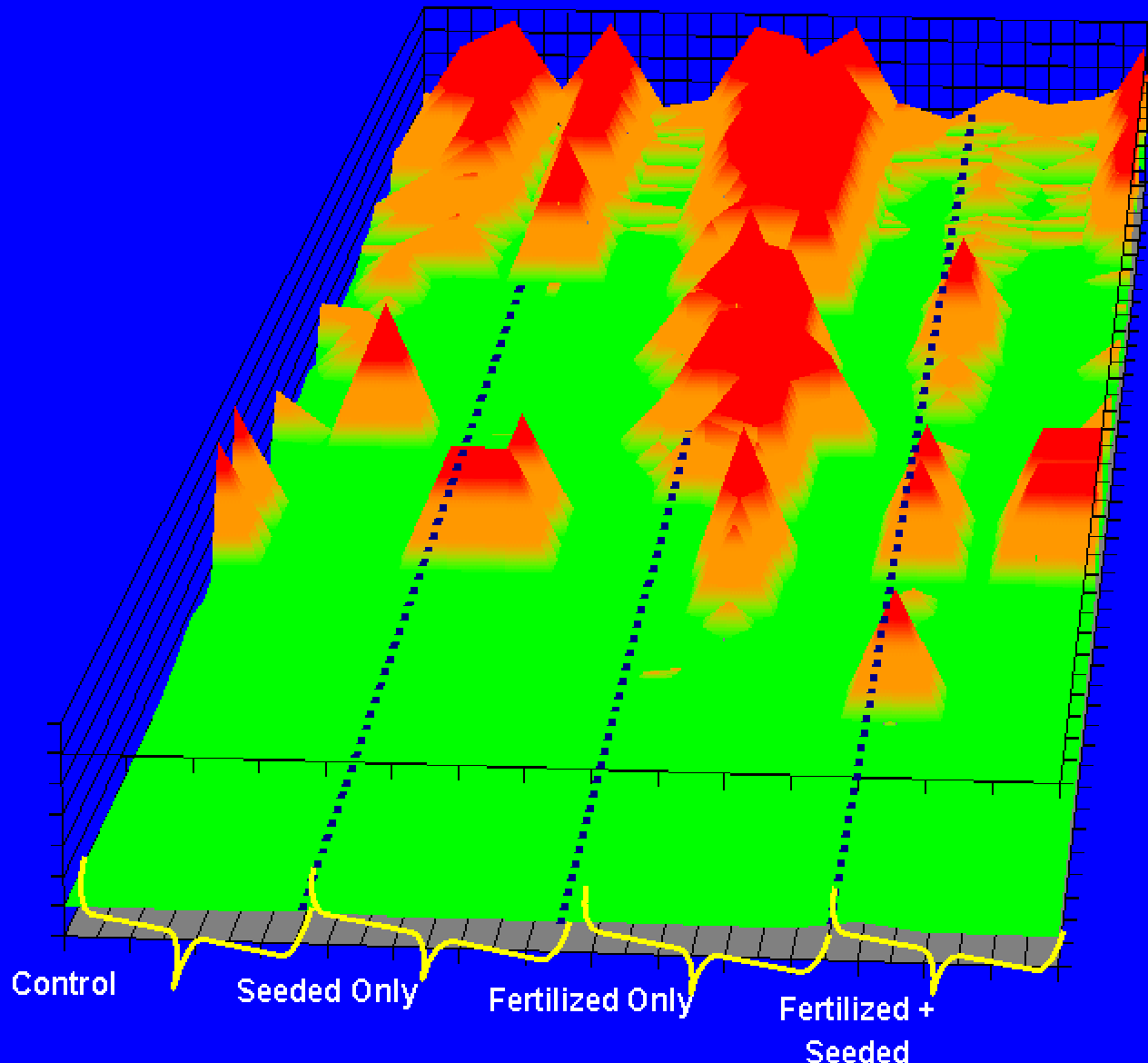
Not cold sites, but in practice similar problems to cold regions

**Factorial**  
**Ryegrass (Yes / No)**  
**Nutrients (Yes / No)**  
**4 reps**  
**Composite samples**



# Korea – Site 1

16 graphs (1 per soil sample) of previous graphs "stacked" together



Recalcitrant

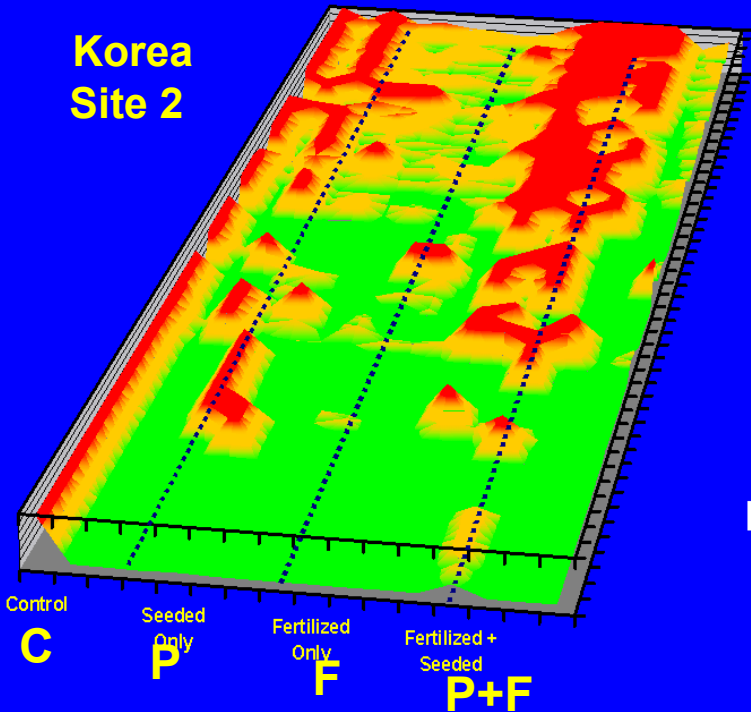
Easy

Depletion data from 4 reps of 4 trts

# Monitoring Rhizosphere-enhanced remediation...

- Petroleum
  - 1000s of compounds
  - biodegrade at different rates
  - by different *enzymes*
- We can exploit differential rates
  - confirm degradation
  - compare treatments
- And it may also give us better insight

Korea Site 2

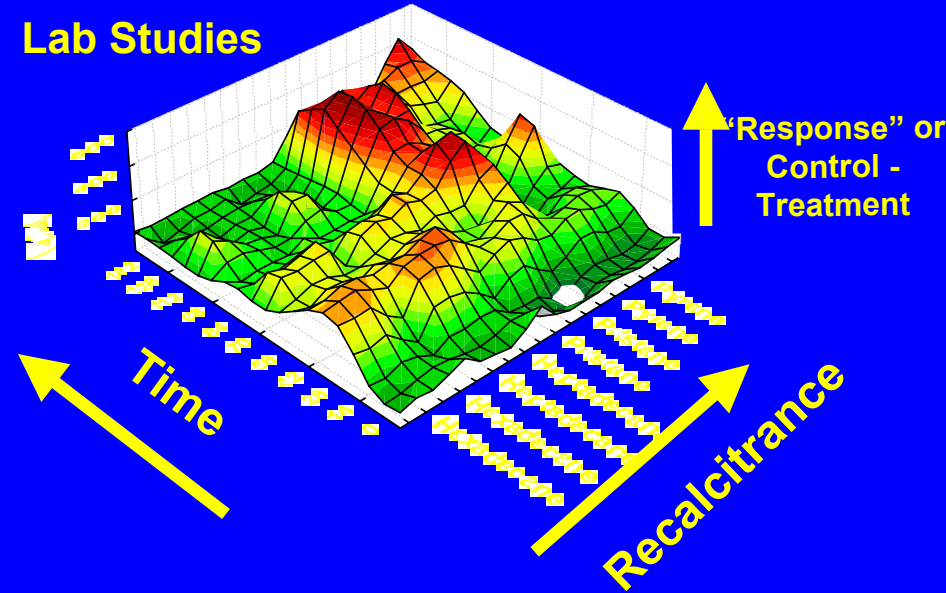


Recalcitrant

Easy

## Benefit due to Rhizosphere Effect is $f$ (Compound and Time)

Lab Studies



★ Data also suggest that for effective monitoring, you must know:

- How to sample
- What to look for
- When to look for it...
  - and *when* is  $f$  (microbial processes), not our calendars
  - ... and microbial processes are  $f$  (conditions)...{H<sub>2</sub>O, Temp., Carbon additions}

*How can we do this?*

# Campion



**Factorial**

**Vegetation (Yes / No)**

**Nutrients (Yes / No)**

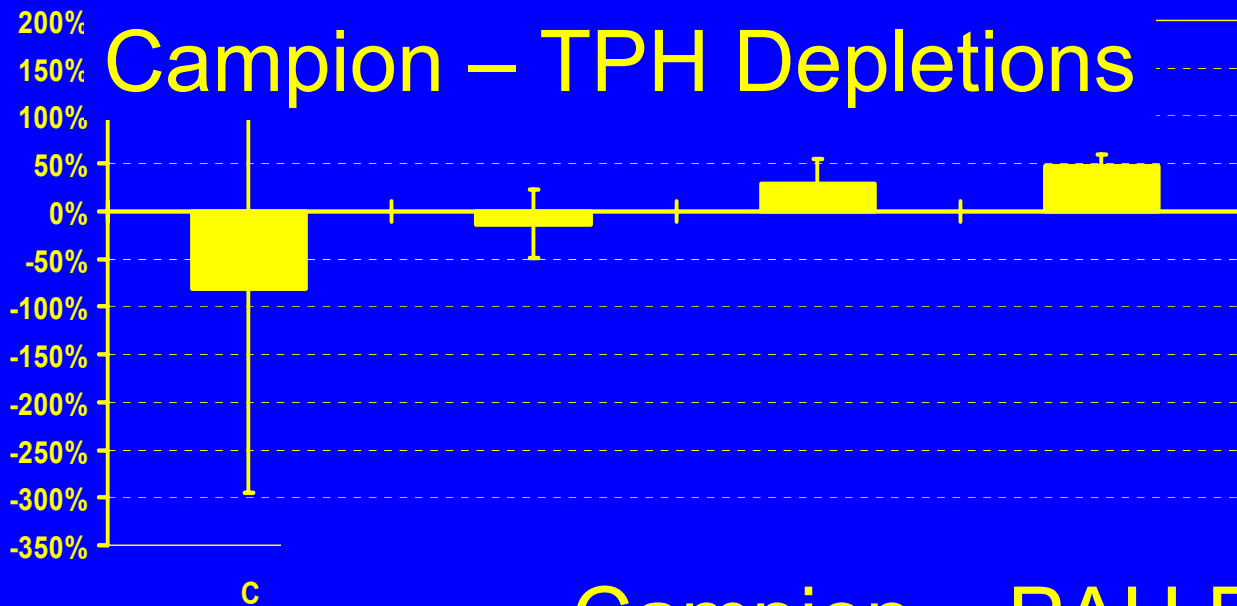
**4 reps**

**Composite and soil sock samples**

# Campion

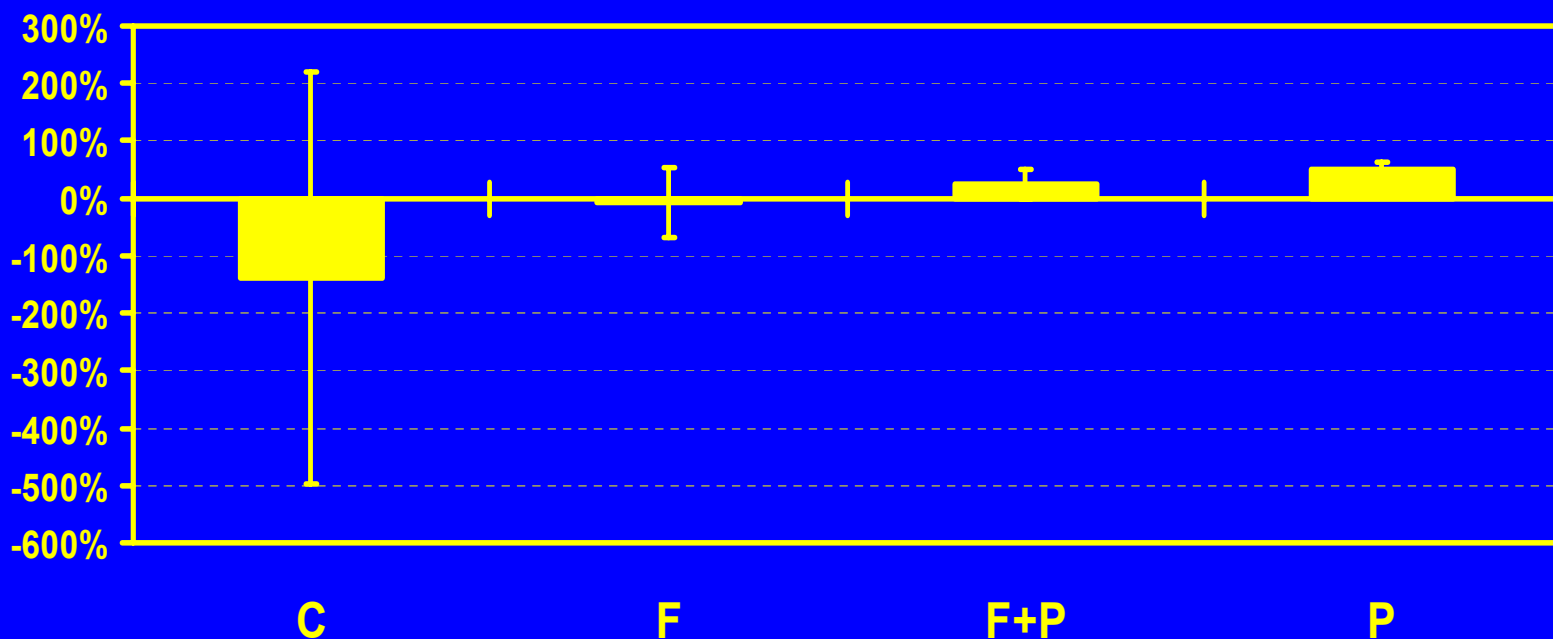


- “Bottom” of bioventing pile
- Previously fertilized and treated



- Weathered at  $t=0$
- “Polishing”
- Most effect is with PAHs

# Campion – PAH Depletions





# Annette Island

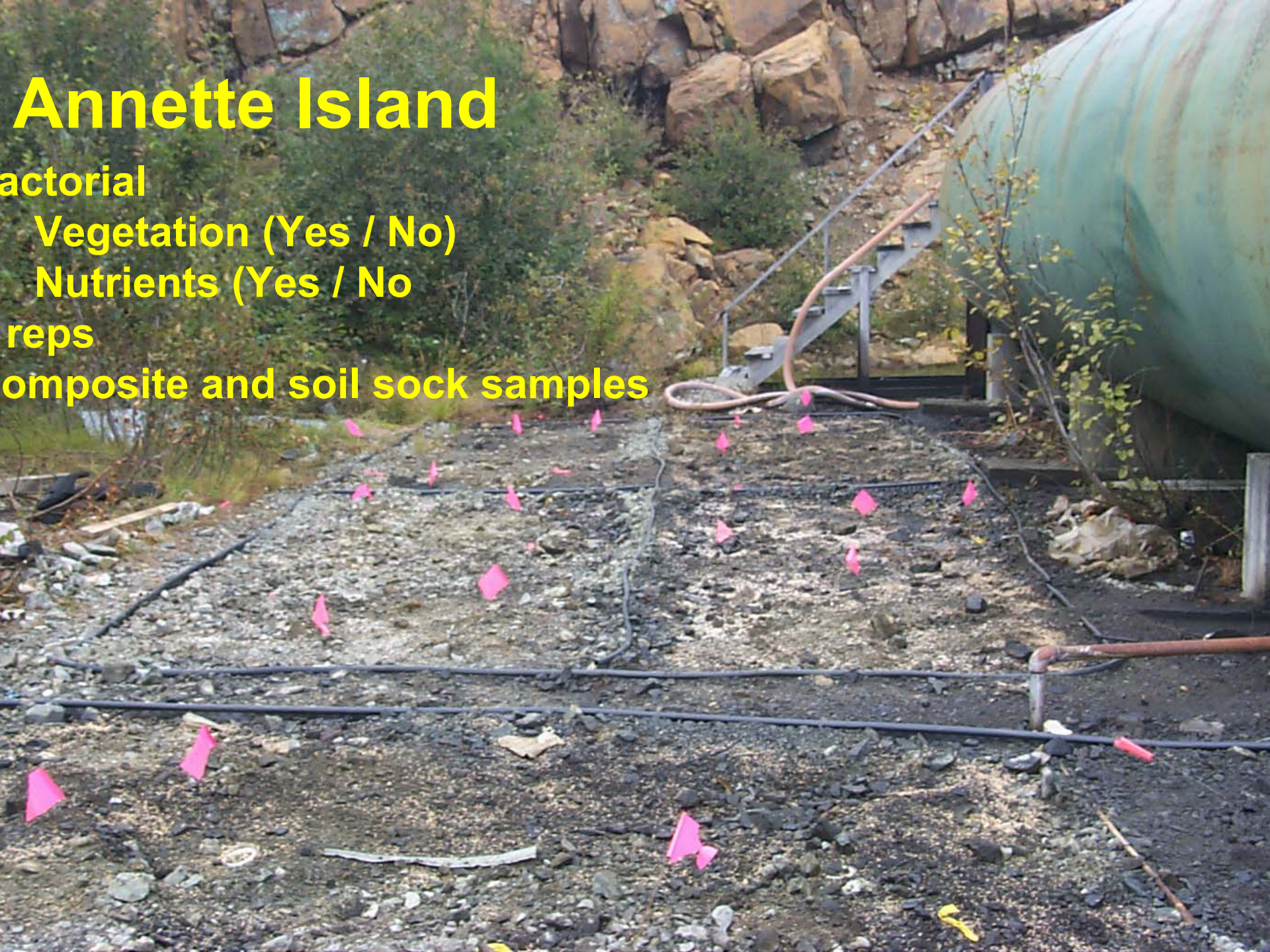
Microbial

Vegetation (Yes / No)

Nutrients (Yes / No)

3 reps

Composite and soil sock samples



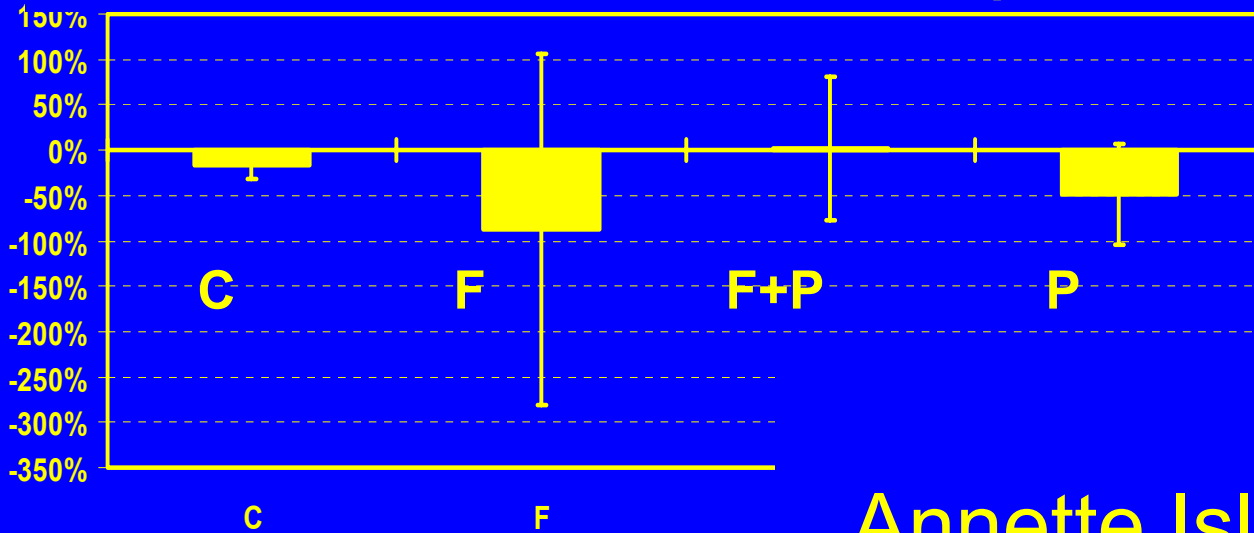
# Annette Island



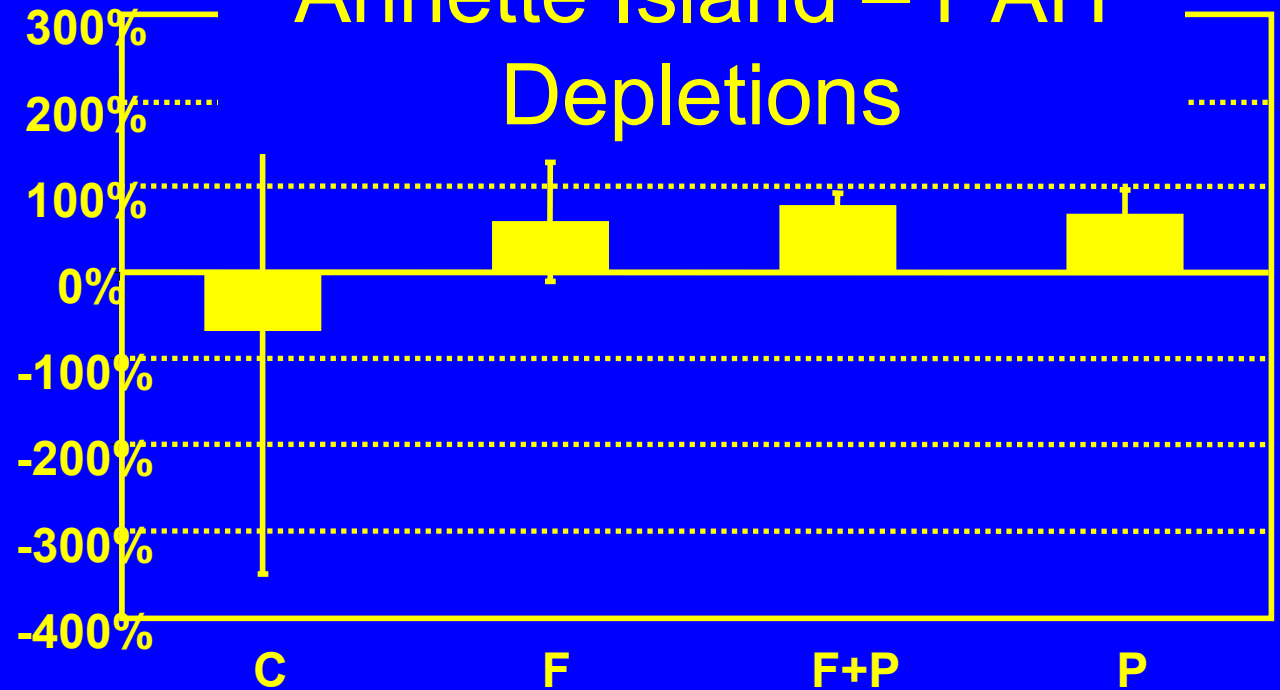
5/16/2000 02:24

# Annette Island – TPH Depletions

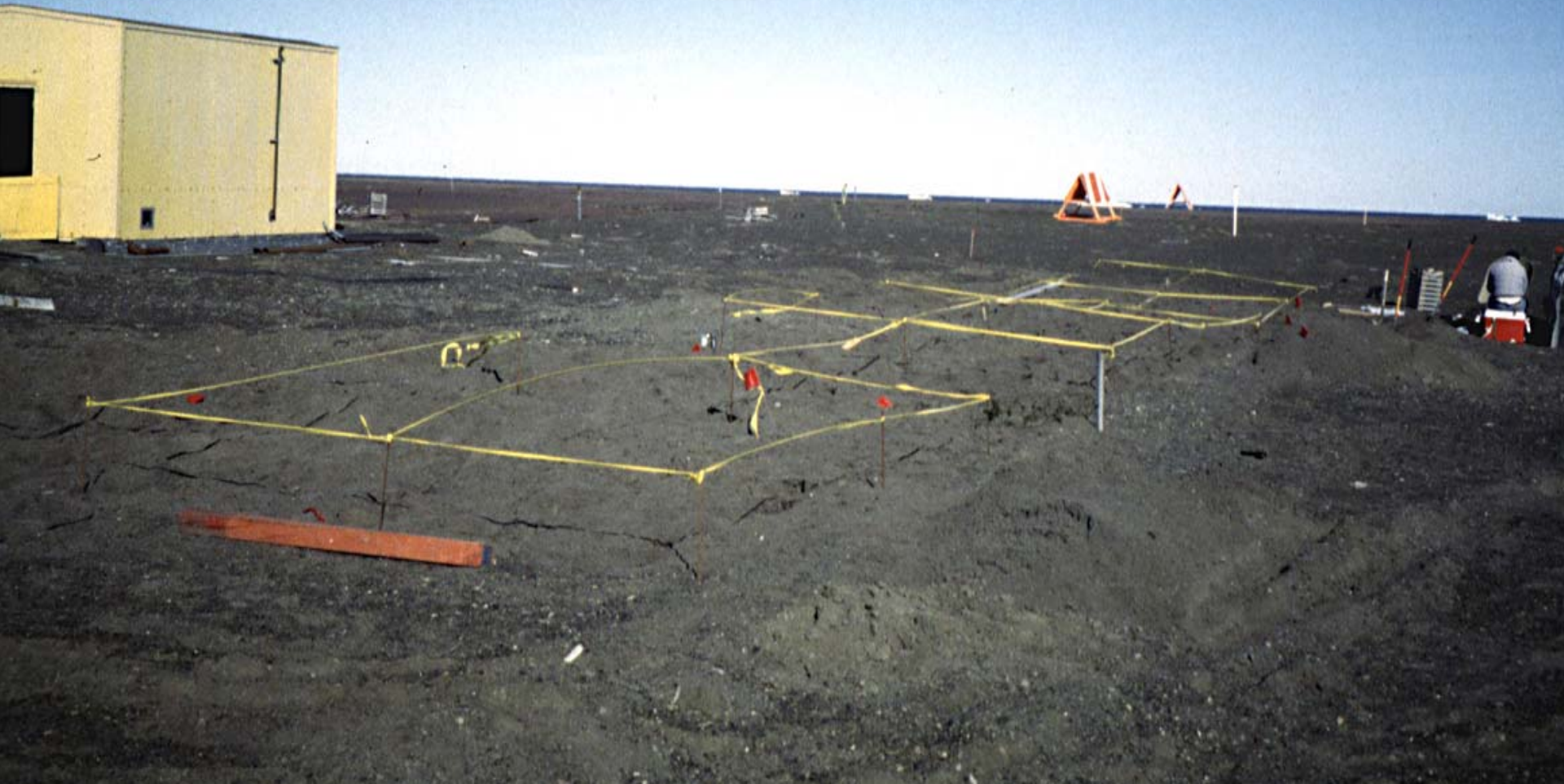
- Non-uniform composition
- Nutrients and (Nutrients and Plants) starting to respond



# Annette Island – PAH Depletions



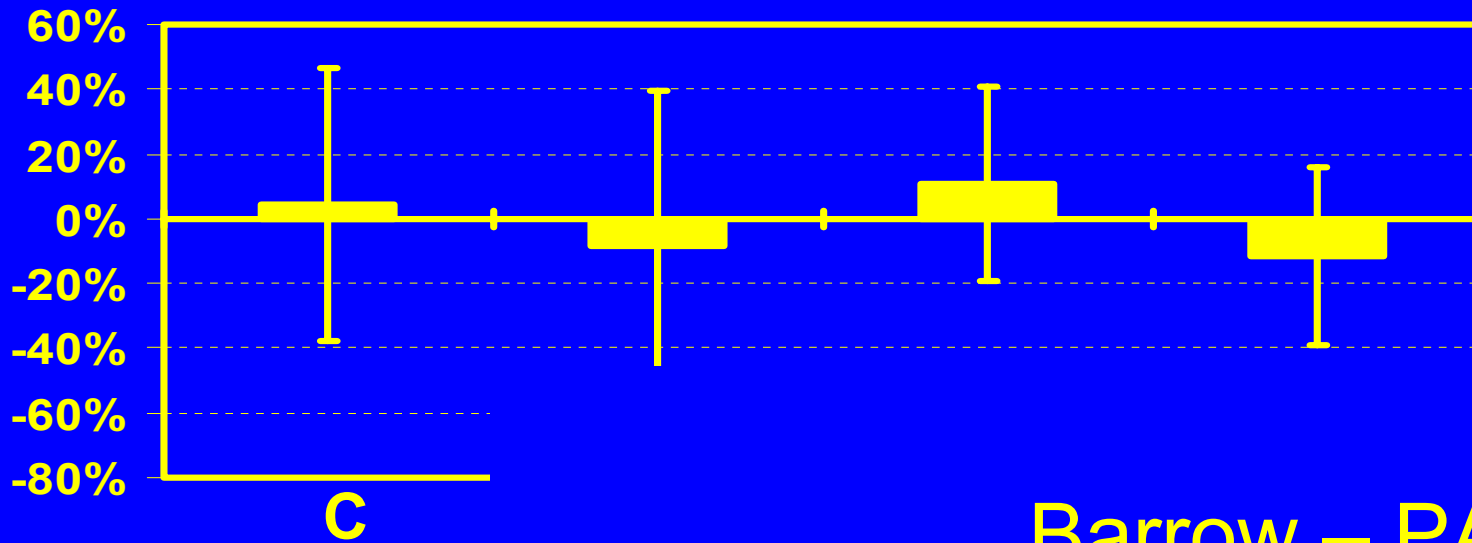
# Barrow



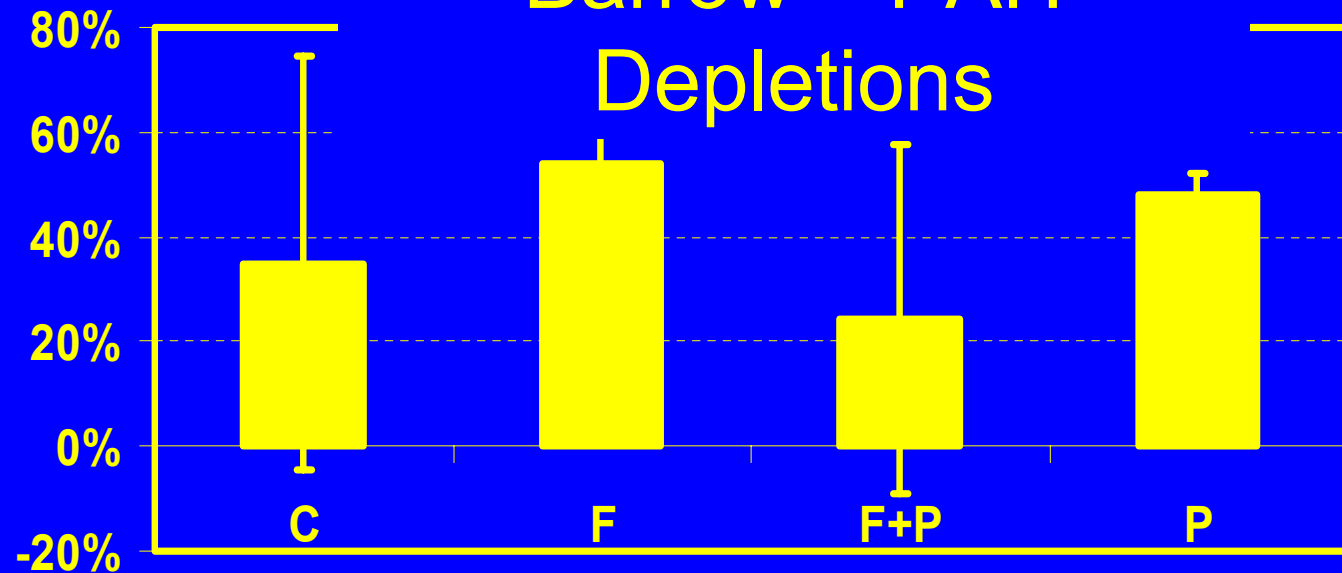
# Barrow

9/11/2000 10:4

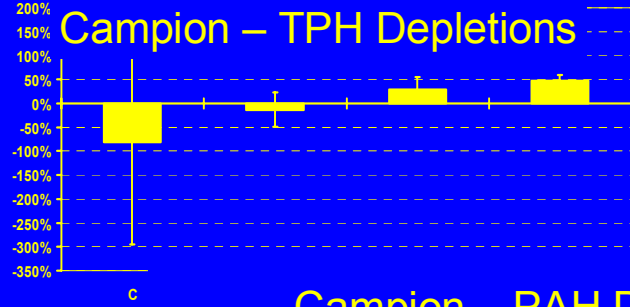
# Barrow - TPH Depletions



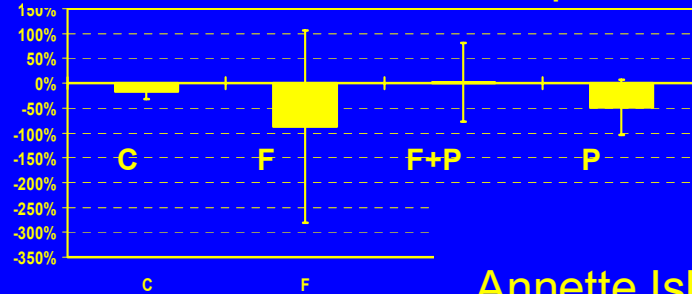
# Barrow - PAH Depletions



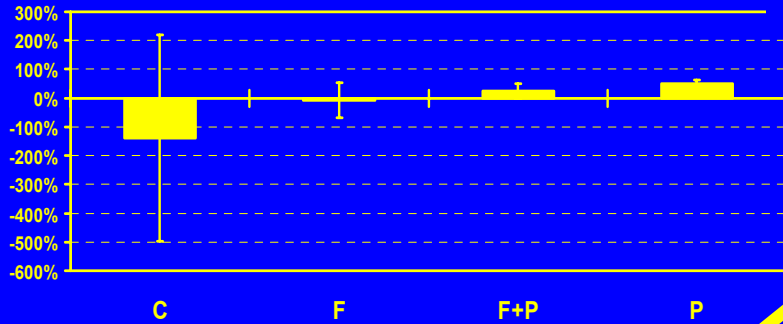
### Campion – TPH Depletions



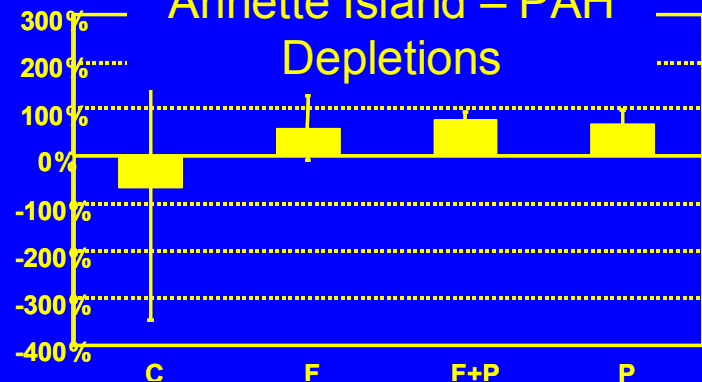
### Annette Island – TPH Depletions



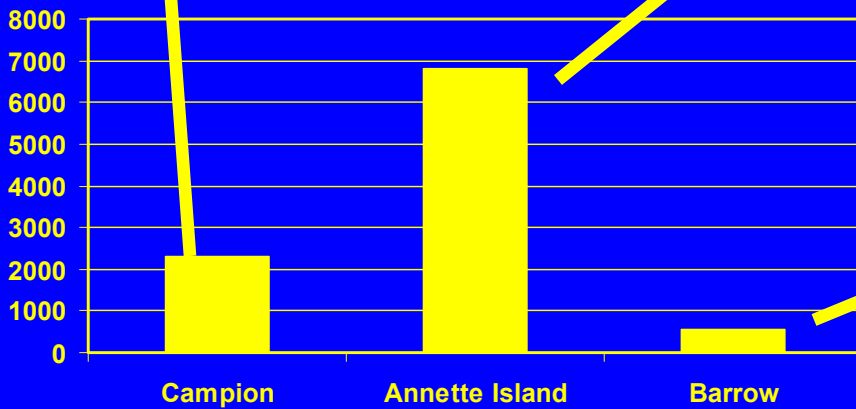
### Campion – PAH Depletions



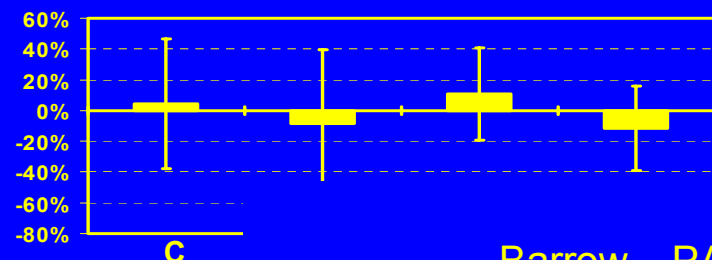
### Annette Island – PAH Depletions



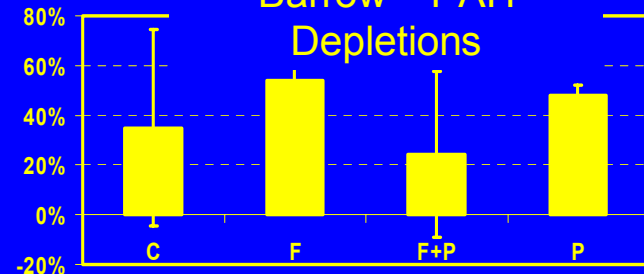
### Non-Freezing Degree Days



### Barrow - TPH Depletions

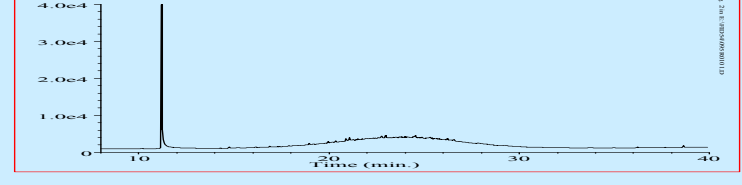
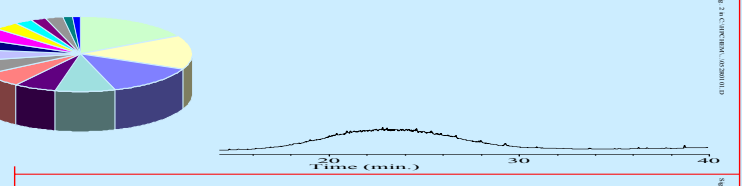
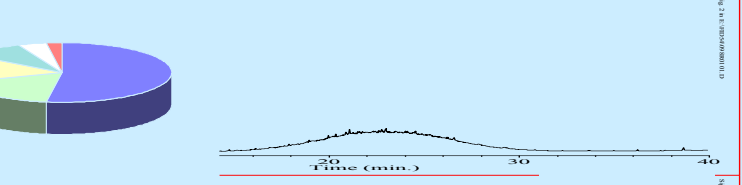
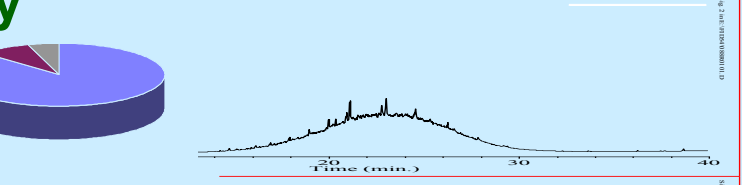
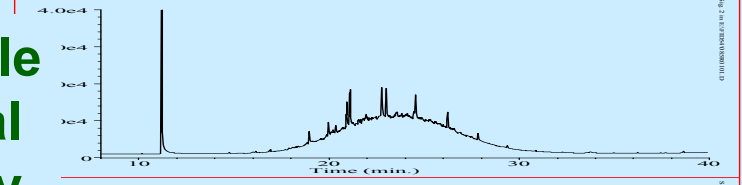
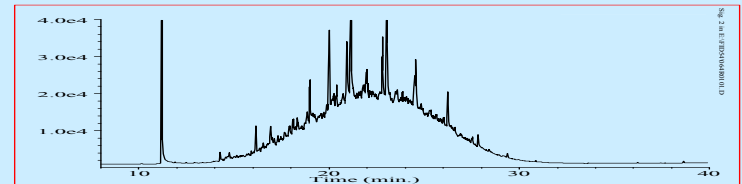
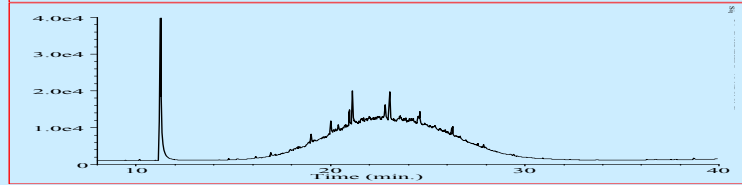
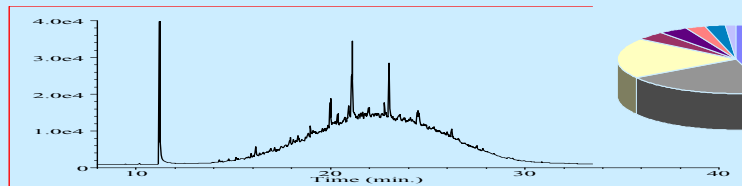
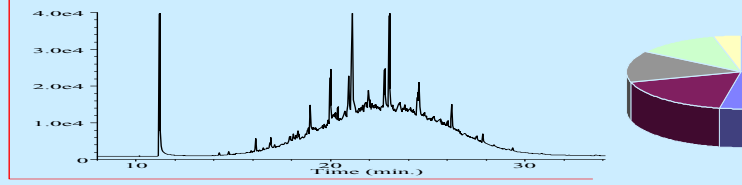
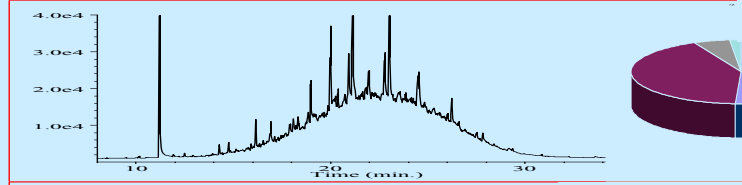
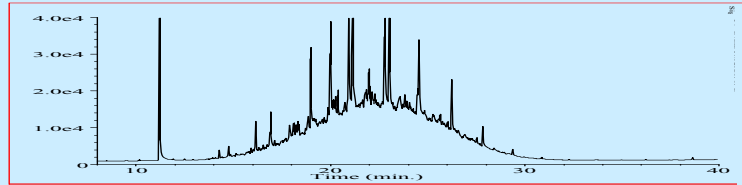
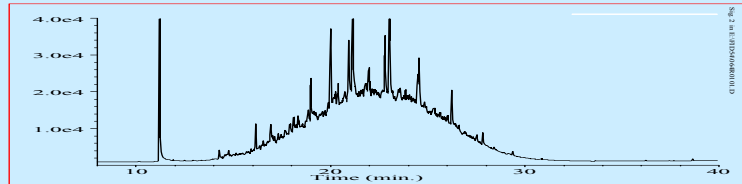


### Barrow – PAH Depletions

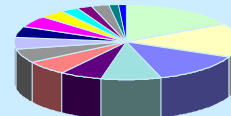
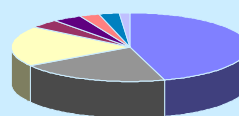
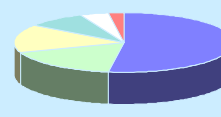
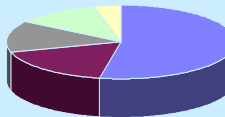
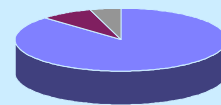


# Rhizosphere effect improves degradation...and Contaminant decreases are related to microbial shifts

**Control** **Vegetated**



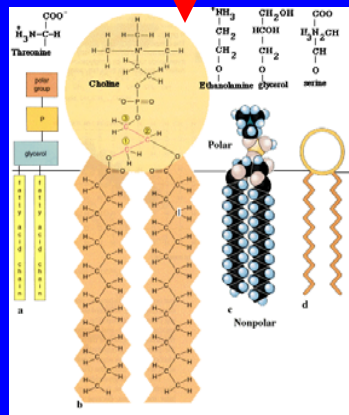
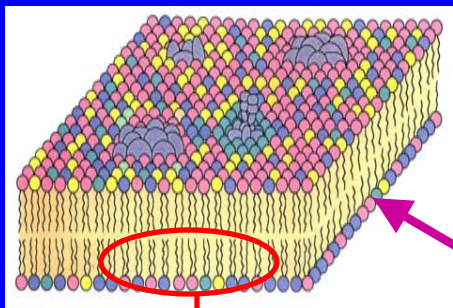
$t_i$   
|  
**Culturable  
Bacterial  
Diversity**



$t_f$



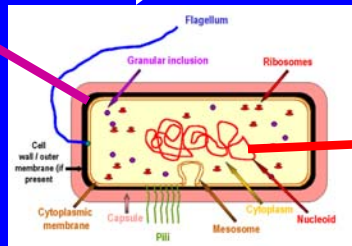
# How can we characterize microorganisms in soil???



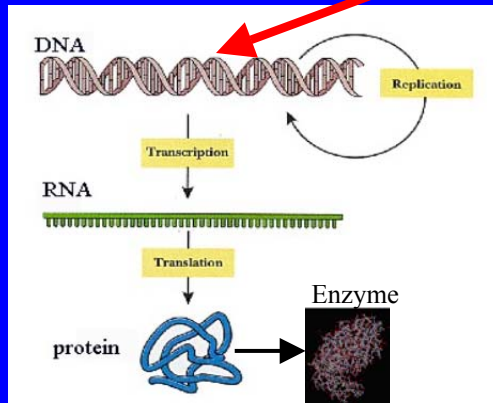
**Phenotype**

**PLFA (Phospholipid fatty acids)**

- Biomass
- Community Composition
- Physiological Status



**Genotype**



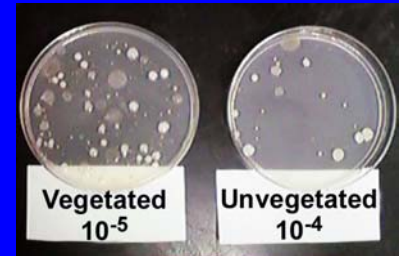
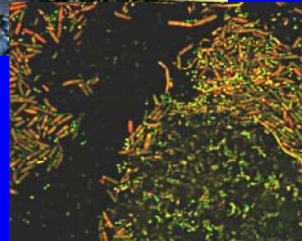
**PCR (Polymerase Chain Reaction)**

**t-RFLP (Terminal Restriction Fragment Length Polymorphism)**

**TGGE and DGGE (temperature/density gradient gel electrophoresis)**

**RT-PCR (real-time or quantitative PCR)**

- Almost an expression of activity - Use DNA fragments for in situ estimate of activity
- Soon – use RNA fragments for in situ estimate of gene expression → enzyme synthesis



**Culturable**

**Selective Media & FAME**

- CFUs/g
- Identification of culturable organisms

# Summary

- It's a system
- We can adjust fertilizer and select plant species
- Water and temperature more difficult to alter

## Monitoring ...??

- “Standard” methods may not be appropriate
- Multiple lines of evidence
  - More “selective” chemical techniques
  - Possibly microbial techniques?
- The best response variable for monitoring may change with “status” of the system
- Phyto
  - Generally  $\cong$  fertilizer initially
  - Phyto  $>$  fertilizer as recalcitrance  $\uparrow$ 
    - Fits well with concept of root-released carbon

