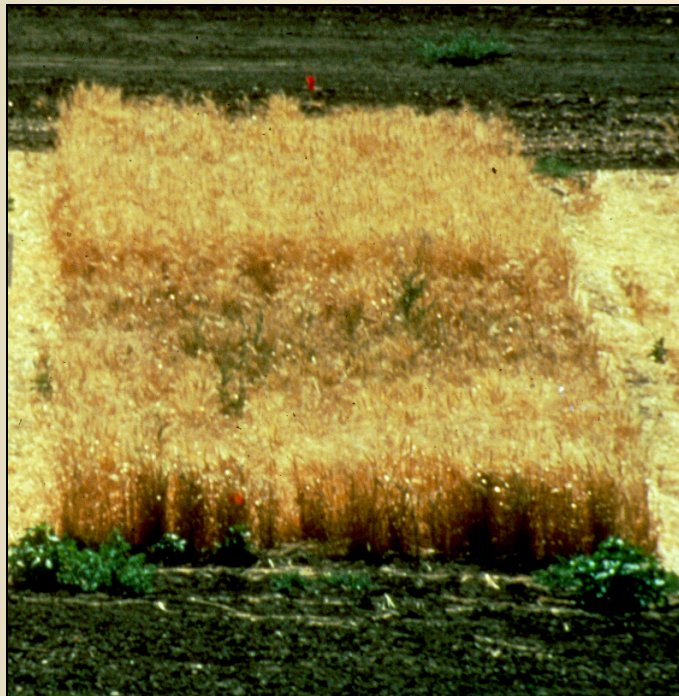


# Characterization of Soil Biology



# Why--What is the question or problem you want to address?

## Specific disease problem or microbial complex?



**Damping-off of wheat  
by *Pythium* resulting in  
diminished stand**



**Apple replant disease  
incited by a consortium  
of microorganisms**

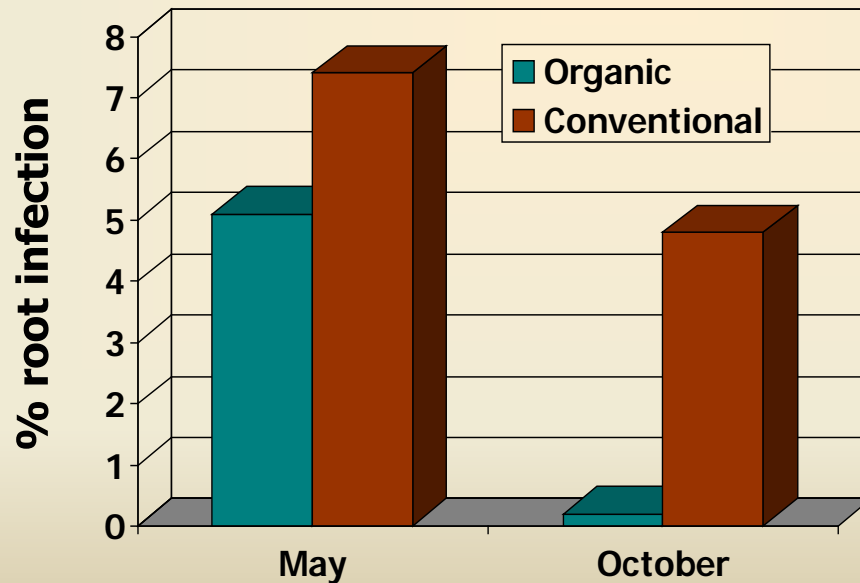
# When?

Soil microbial populations vary throughout the year and the appropriate time to sample will vary in a similar fashion.

For example:

*Pythium* spp.---spring

*Pratylenchus* spp.--fall





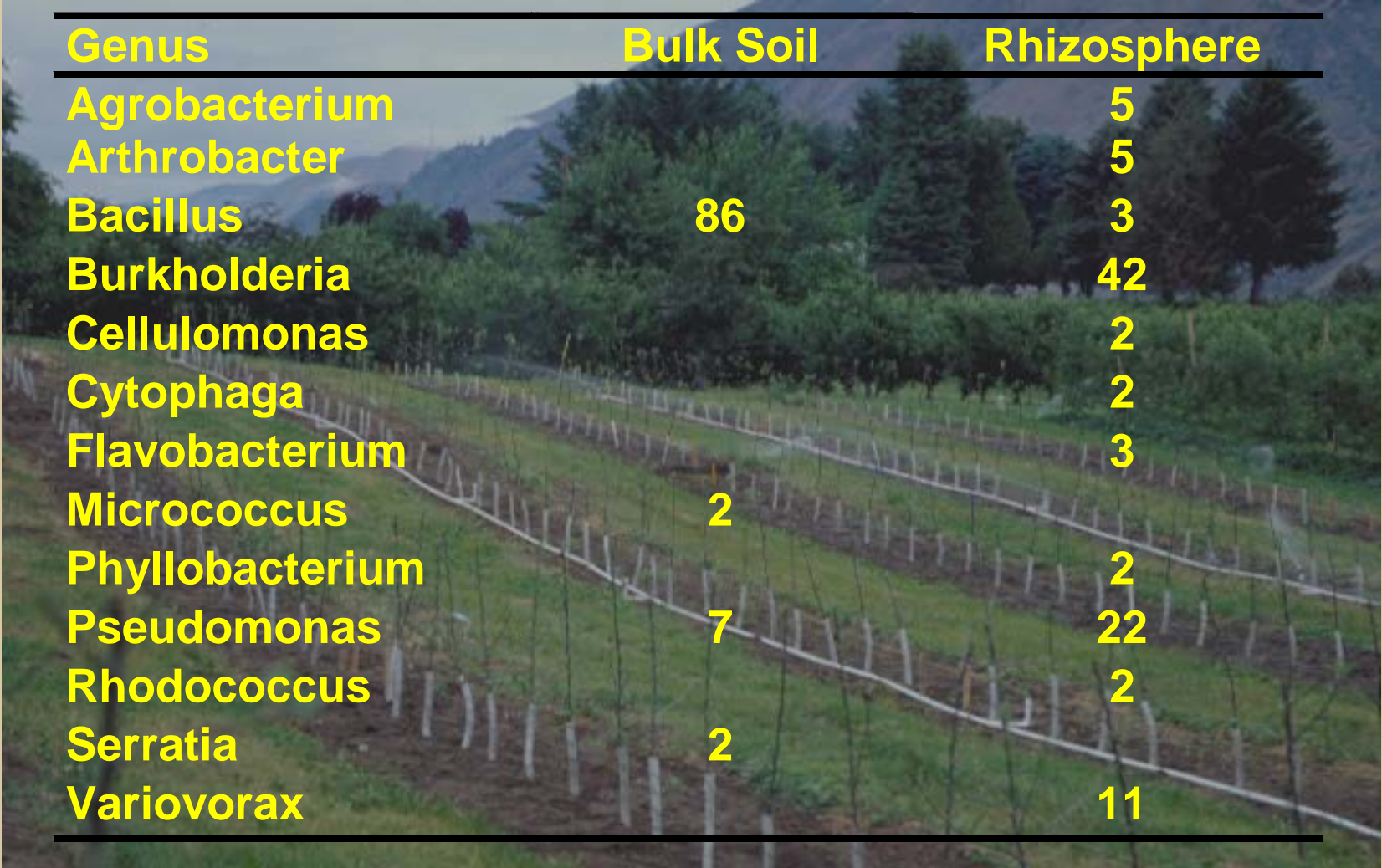
**Where?--**

**Plant row vs. aisle**



**Rhizosphere vs. Bulk soil**

# Relative Composition (%) of the Bacterial Community Recovered from 1-yr-old Apple Orchard



Genus	Bulk Soil	Rhizosphere
Agrobacterium		5
Arthrobacter		5
Bacillus	86	3
Burkholderia		42
Cellulomonas		2
Cytophaga		2
Flavobacterium		3
Micrococcus	2	
Phyllobacterium		2
Pseudomonas	7	22
Rhodococcus		2
Serratia	2	
Variovorax		11

# What?--

**General Community**

**Based on what parameters**

**Plant Pathogens**

**Bacteria, fungi, nematodes**

**Plant Beneficials**

**Mycorrhizae**

**Rhizobium**

**PGPR**

**Other Functional Groups**



# Characterization of Soil Biology: Molecular Methods

## Analysis of Fatty Acids

GC-FAME

PLFA

## DNA-Based Methods

Sequence Analysis-16S rRNA gene

PCR

DGGE

RFLP

ETC

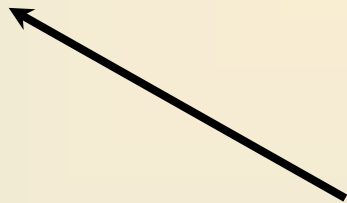
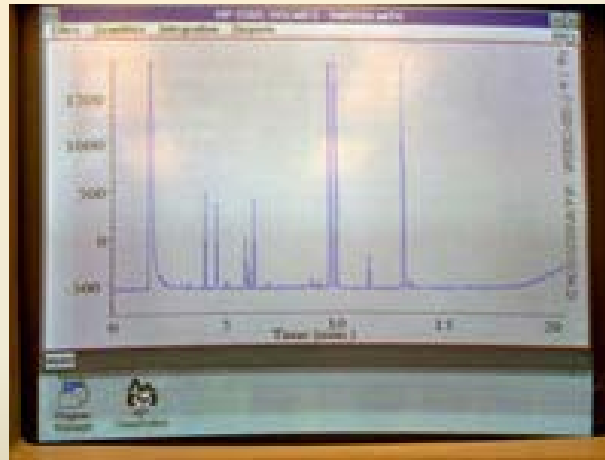
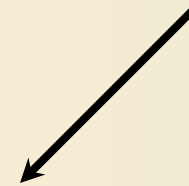


# Analysis of Fatty Acids

Extraction from soil  
or micro-organism



Speciation based on Comparison  
to library of profiles



Examine for  
Indicator Fatty Acids





# DNA-Based Methods

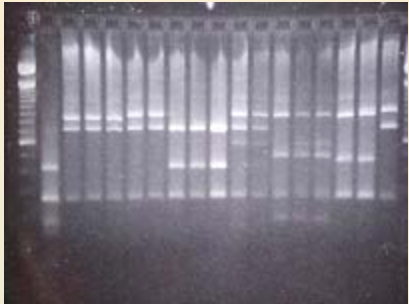


**Amplification of rDNA  
(or other target)**

**Extract DNA from  
Environmental Sample**



**Sequence Analysis**



**RFLP Analysis**



**DGGE**

## Advantages:



vs.



- Methods are culture independent
  - Estimated that  $<1\%$  of soil microbial community can be cultured using traditional methods
  - Many culturable bacteria are often present in soil in a viable but non-culturable state
- Shifts in community composition are more easily detected.

## **Advantages:**

**-Identification does not require specialized training in mycology, bacteriology, nematology, etc., etc.**

## **Disadvantages:**

- Identifications are only as good as the library**
- Many non-culturable organisms are unknown both in ID as well as function**
- Require specialized equipment**
- PLFA methods have not consistently detected shifts even with dramatic changes in soil management over extended (years) periods.**

## Practical Application:

- ◆ Research Capacity
- ◆ Delimiting and Detecting Biological Markers
- ◆ Screening for the Presence of Known Pathogens
- ◆ Determining Mycorrhizal Colonization
- ◆ Monitoring Changes in Composition of Microflora
- ◆ ETC





# 16S rDNA Genotype Composition of Fluorescent *Pseudomonas* spp. Populations from Apple Grown in Wheat Cultivated WVC-A Soils

Genotype	Con.	'Eltan'	'Hill81'	'Madsen'	'Penawawa'	'Lewjain'
1	19	18	19	19	14	10
2	0	0	0	1	5	1
5	0	1	0	0	0	0
9	2	0	2	0	5	9
10	2	2	3	0	0	0
11	1	0	0	0	0	1
13	1	1	0	1	2	1
14	1	0	0	0	0	0
15	0	0	0	2	0	0
17	1	1	0	0	0	2
18	0	0	1	1	1	1
20	0	1	0	0	0	0
21	0	1	0	0	0	0
23	0	0	1	0	0	1
27	0	0	0	0	0	0
29	0	2	4	0	1	1
30	0	0	0	0	0	2
33	0	1	0	0	0	1
34	0	2	0	2	0	0
% inhibitory strains	13%	10%	10%	15%	40%	43%