

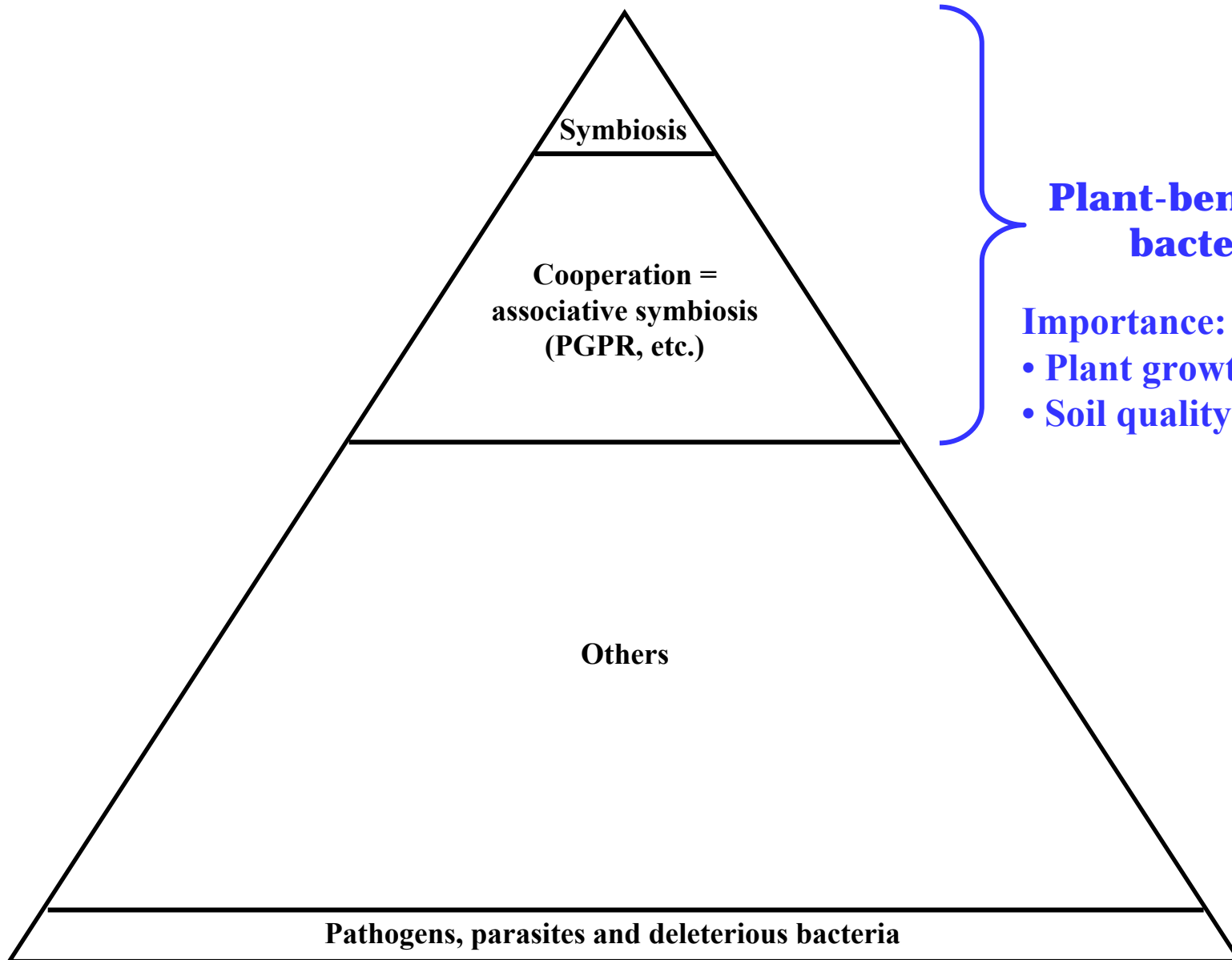


# 16S taxonomic microarray monitoring of plant-associated bacteria

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# Bacteria as they may be seen by the plant

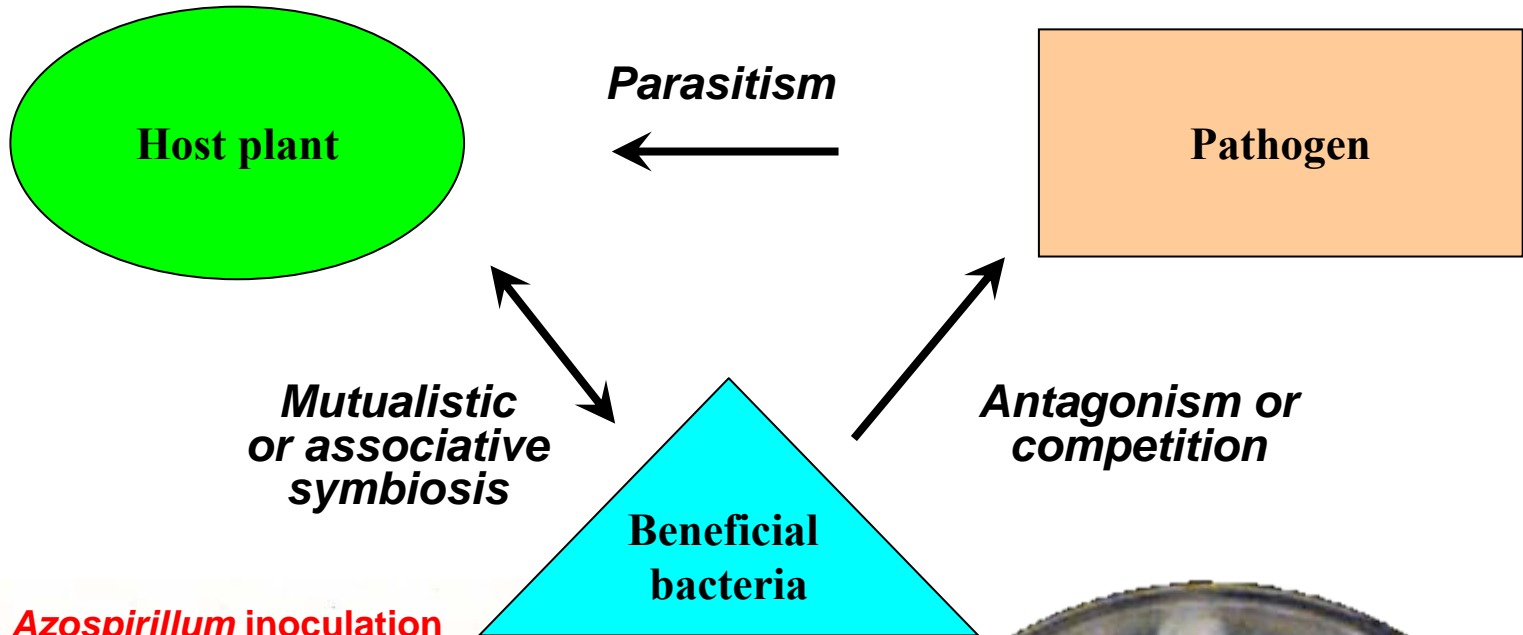


**Plant-beneficial  
bacteria**

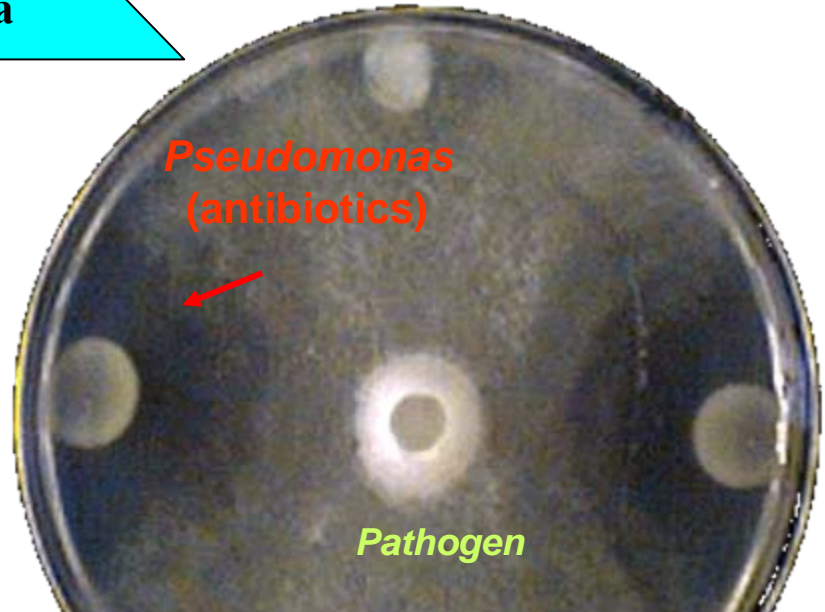
**Importance:**

- Plant growth and health
- Soil quality

# Plant-beneficial rhizosphere bacteria



*Azospirillum* inoculation



# Monitoring of plant-beneficial rhizosphere bacteria: a few major issues

## Species of plant-beneficial bacteria not easy to identify:

- ▶ Plant-beneficial bacteria probably exist among non-cultured taxa.
- ▶ Certain plant-beneficial bacteria are related to human pathogens.

## Relation between plant-beneficial traits and taxonomy:

- ▶ Good relation: associative nitrogen fixation in *Azospirillum* spp., etc.
- ▶ Bad relation: symbiotic nitrogen fixation in *Rhizobium* spp., etc.

## Knowledge of plant-beneficial traits:

- ▶ Incomplete: type III secretion in phloroglucinol-producing *Pseudomonas fluorescens*.
- ▶ Horizontal gene transfer can modify plant-beneficial properties (*acdS* and *hrcN* in *P. fluorescens*, etc.).

Therefore, our knowledge of plant-beneficial bacteria needs to be broadened

# **Disease-suppressive soils, where plant-beneficial rhizosphere bacteria are at work**

**They are soils in which the pathogen:**

- ▶ **Does not establish or persist,**
- ▶ **establishes but causes little or no damage,**
- ▶ **or establishes but causes significant disease only the first few years.**

**Two types:**

- ▶ **Induced suppressive soils: crop monoculture important**
- ▶ **Long-standing/natural suppressive soils : monoculture not required**

**Comparison of disease-suppressive and disease-conducive soil:**

- ▶ **Useful to monitor the functioning of plant-beneficial bacteria**
- ▶ **Will help define biodiversity criteria for soil quality and impact studies**

# Taxonomic microarray targeting 16S rRNA gene *rrs*

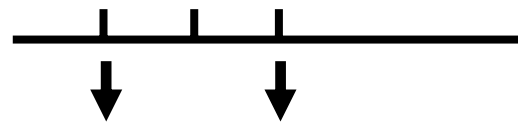
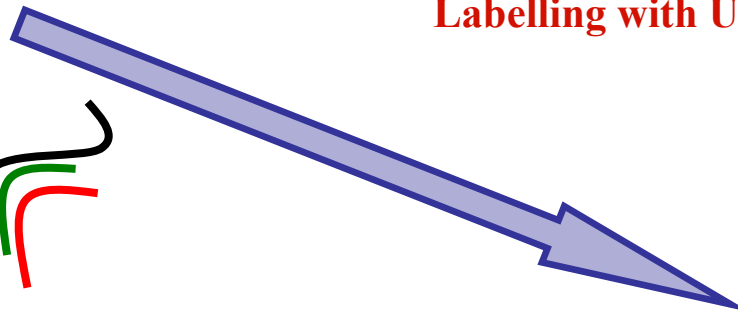
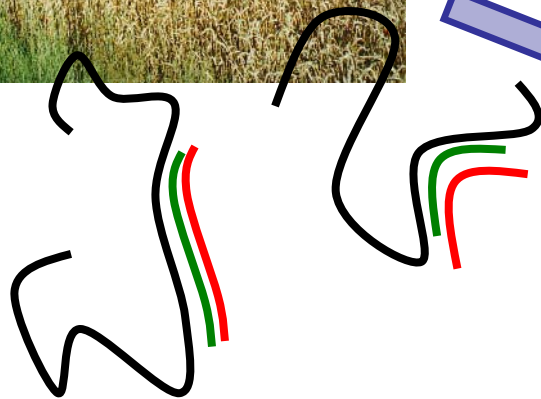


Extraction of DNA from the rhizosphere

PCR amplification of *rrs*

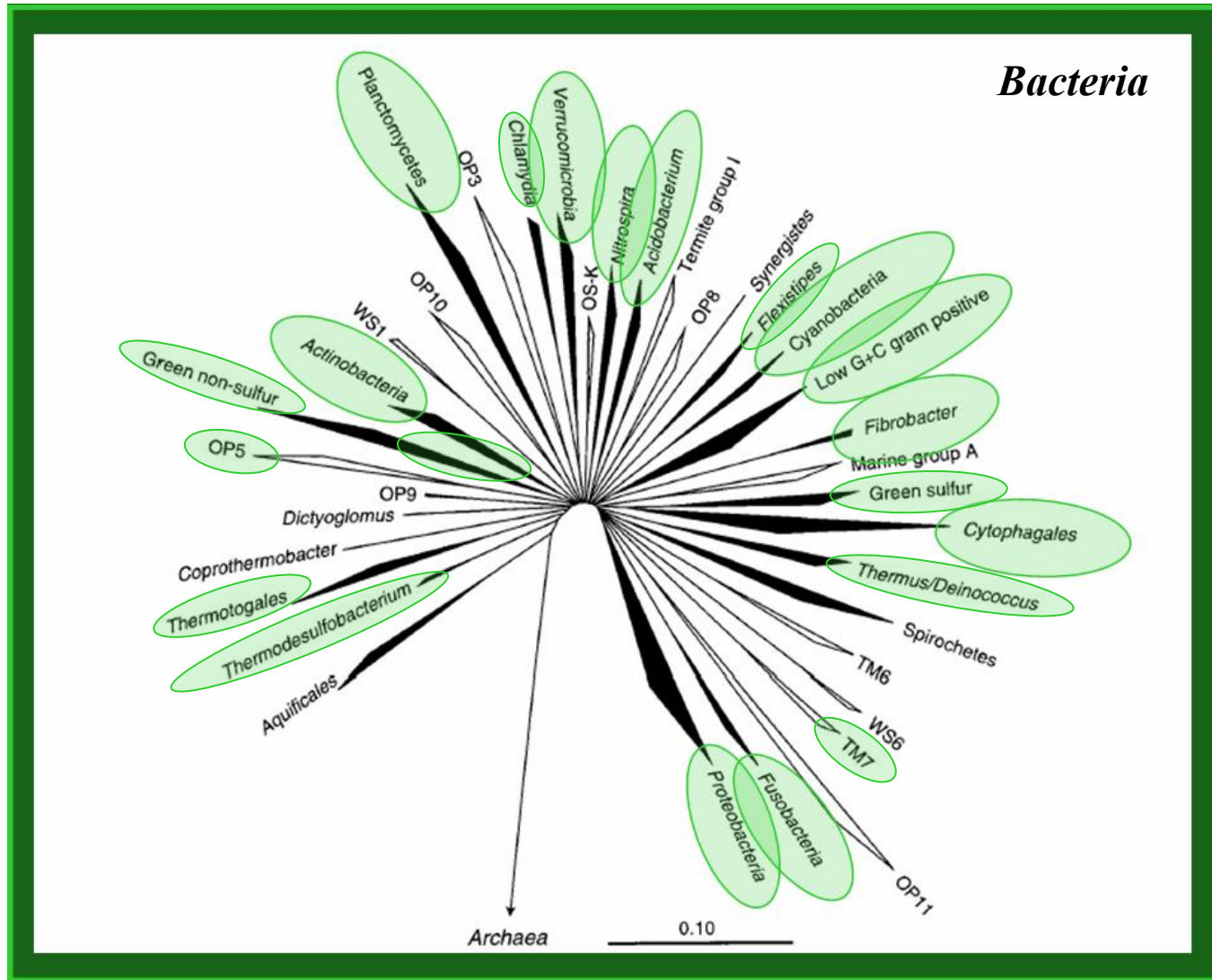
Labelling with UTP-Cy3 by transcription *in vitro*

Hybridisation



*Taxonomic information*

# Probe set



575 then 1400 individual probes (replicated 4 times per slide).  
Target bacteria at different taxonomic levels (phyla to genomic species).

# Overview of microarray system

## Probes defined in ARB software and checked with Oligo5

- 20-mer oligonucleotides (compromise between specificity and sensitivity)
- T<sub>m</sub>: 65 ± 5°C
- 5' NH<sub>2</sub> group, followed by a C6 spacer preceding the probe sequence

## Target characteristics

- *rrs* (16S rDNA)
- Labeling with Cy3 by *in vitro* transcription (T7-PA + PH)
- Hybridization with 400 ng labeled RNA

**Hybridization temperature** : 50°C (overnight)

## Slides :

- QMT Aldehyde Slides (Quantifoil)
- Home-made spotting

**Image acquisition** : Scanner GenTac LS IV

**Image and data analysis**: GenePix Pro 4.1 and S-plus

**Bioinformatics**: Development of an integrated database (PRABI, Université Lyon 1)

# The case of take-all disease caused by the soil fungus *Gaeumannomyces graminis* var. *tritici* on wheat



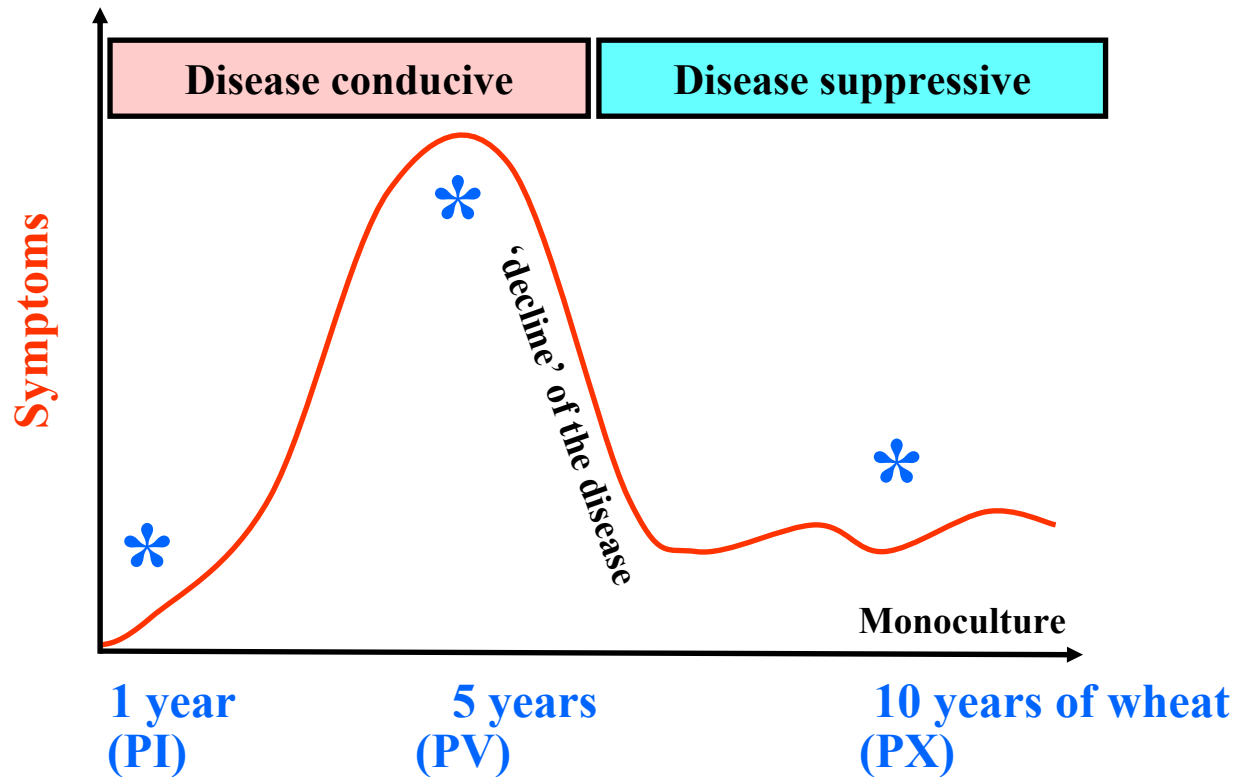
**Disease suppression  
induced by monoculture**



7th year wheat monoculture



# Analysis of the bacterial community of the rhizosphere: who is there?



Luvisol, INRA field site of La Gruche, Brittany (A. Sarniguet)

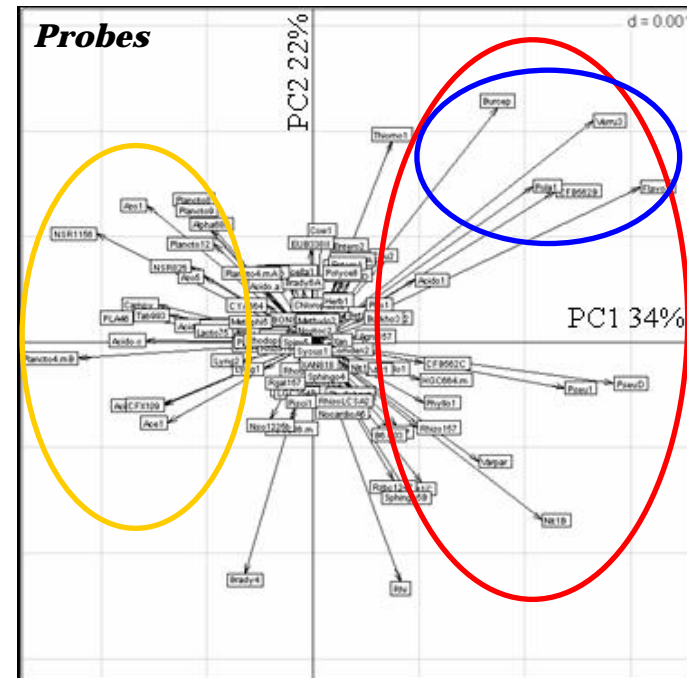
# PCA of microarray data (575 probes)

## PV (disease)

*γ-Proteobacteria (Pseudomonas),  
α-Proteobacteria (Sphingomonadaceae,  
Rhizobiaceae, Phyllobacteriaceae),  
β-Proteobacteria (Burkholderia cepacia,  
Nitrosospira, Variovorax, Acidovorax,  
Thiomonas, Polaromonas), Bacteroidetes,  
Flavobacteria (Flavobacterium),  
Verrucomicrobia, Actinobacteria.*

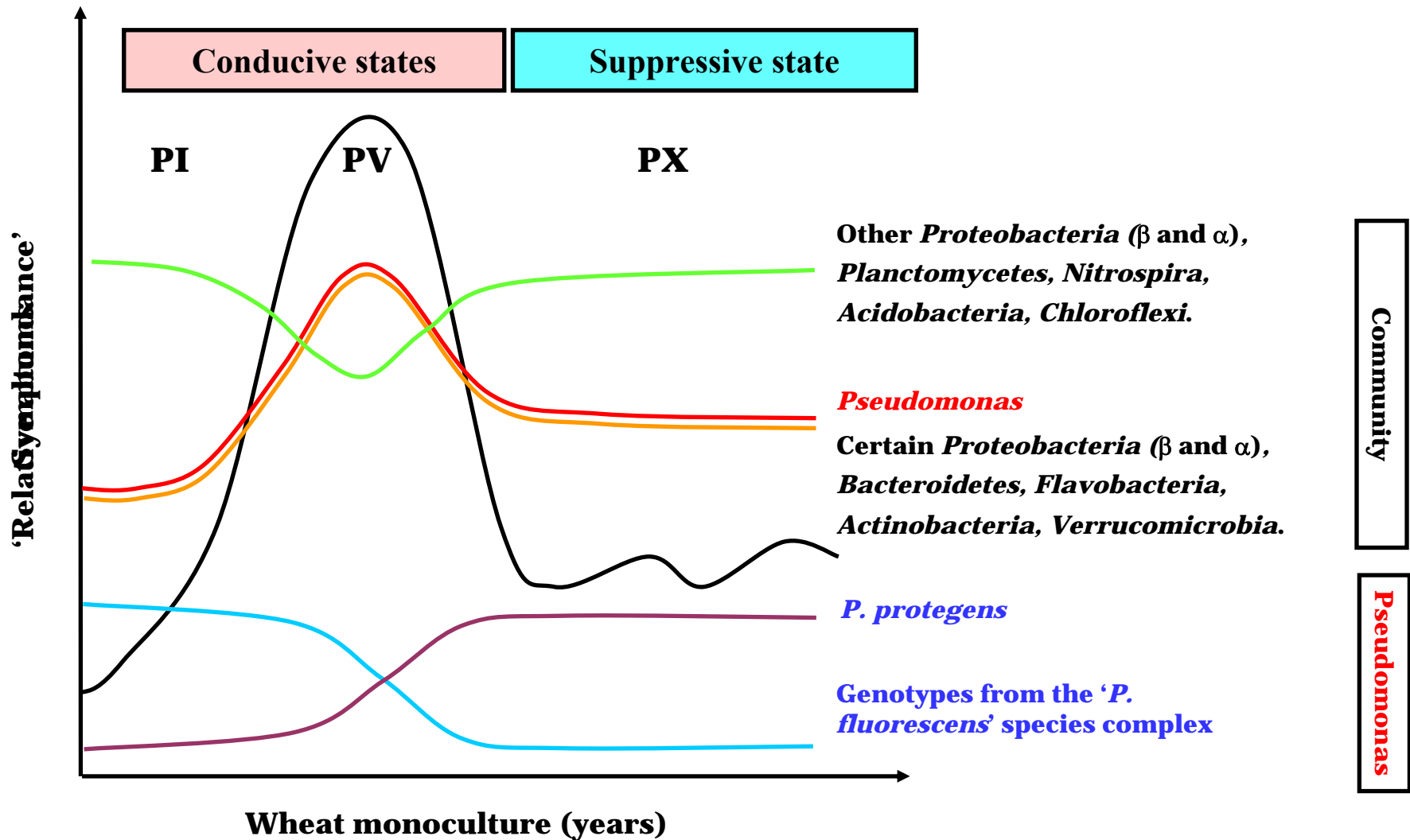
## PX (suppressiveness)

*α-Proteobacteria (Azospirillum),  
Planctomycetes, Nitrospira,  
Acidobacteria, Chloroflexi,  
Firmicutes (Thermoanaerobacter).*



Distinctions between PV (disease) and PX (disease suppressiveness)

# Overview of bacterial dynamics in relation with decline of take-all disease of wheat



# Soil suppressiveness to black root rot of tobacco (*Thielaviopsis basicola*)

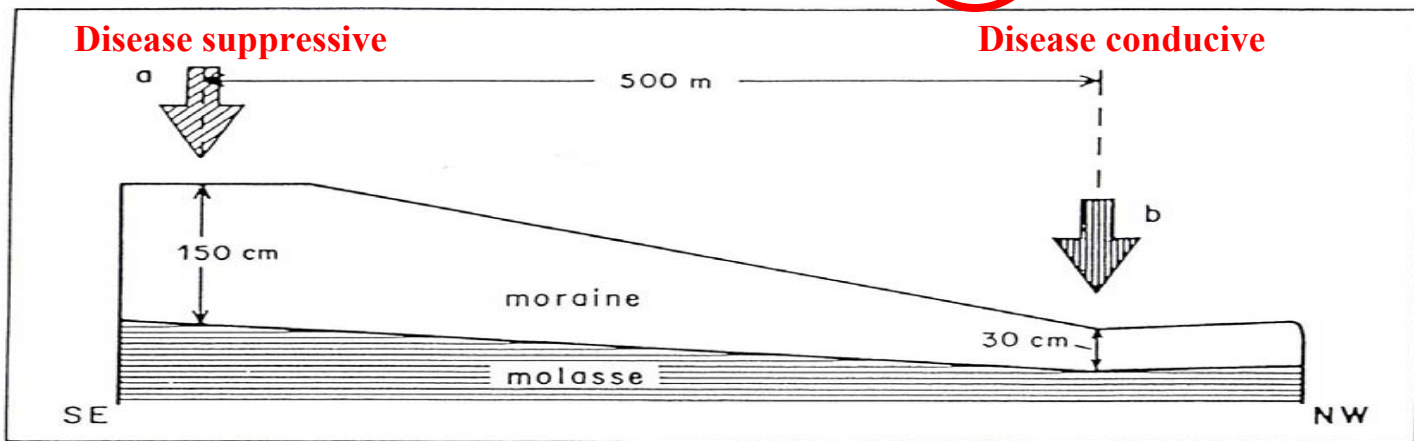


Fig. 3. Longitudinal soil profile: a = suppressive, b = not suppressive.

**Morens (Switzerland): brunisols**

# Conclusion

## In this study:

- ▶ Identification of taxa (other than *Pseudomonas*) that perhaps contribute to disease suppressiveness of soils.
- ▶ Certain taxa prevalent in different types of suppressive soils (*Pseudomonas*, ...) or conducive soils (Actinobacteria, ...).

## 16S microarray approach is useful for diversity studies in Microbial Ecology:

- ▶ Direct identification of microbial taxa present in the community (rather than molecular fingerprints).
- ▶ Possibility to assess the co-occurrence of taxa.
- ▶ Its potential is largely compatible with the scale of bacterial diversity.

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