

# Short and long term microbiological effects of wastewater irrigation

D. Minz, T. Oved, A. Shaviv, T. Farkash, E. Jurkevitch,  
R. Halabi and L. Kautsky

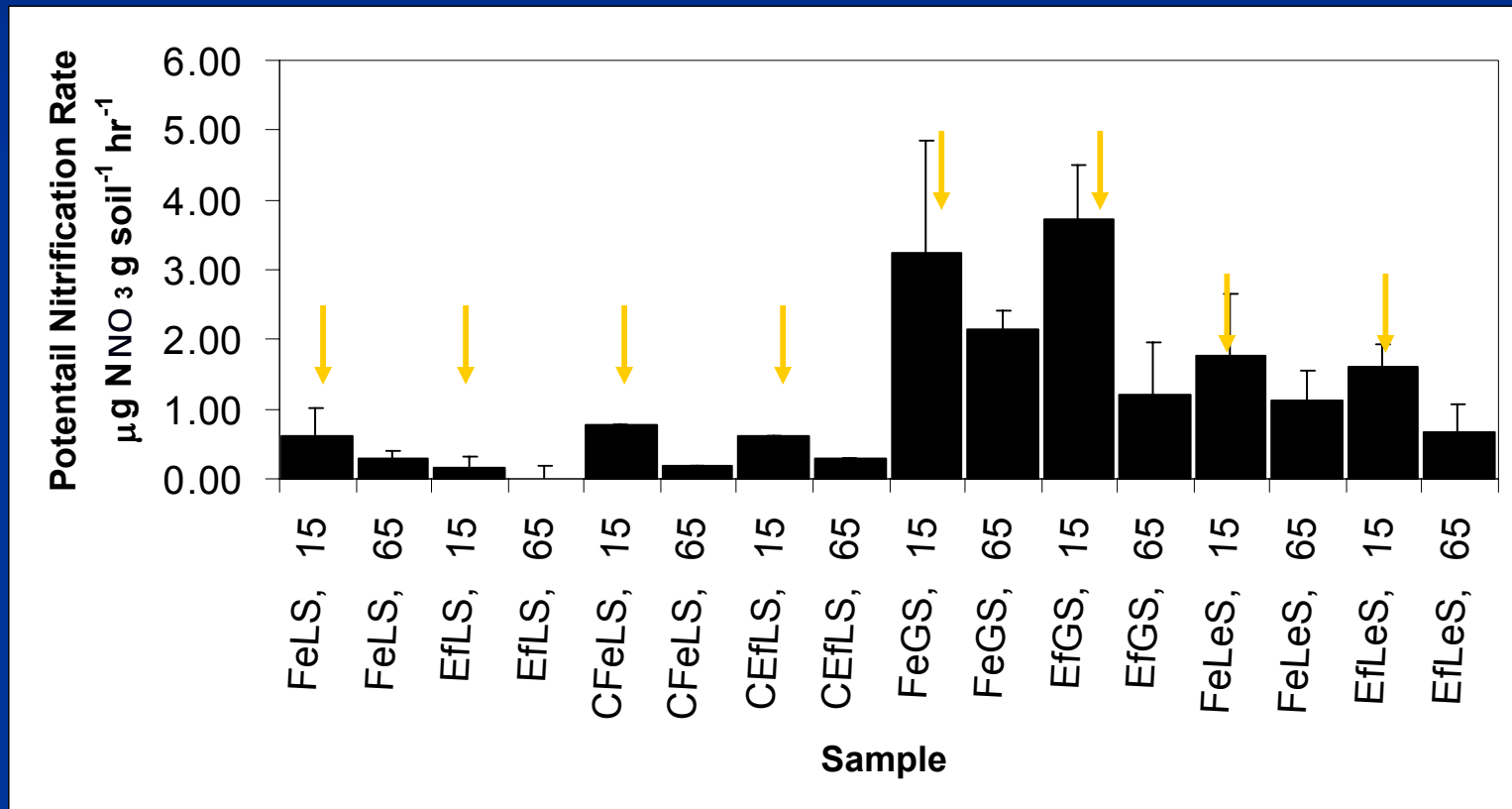


# Topics covered:

- Effect of waste-water irrigation on microbial activities and ammonia oxidizing bacteria in soils (Oved, Kautsky and Shaviv)
- Effect of wastewater irrigation on nitrogen fixation in legumes (Farkash and Jurkevitch)
- Survival of enteric bacteria in soil (Halabi and Kautsky)

# Effect of waste-water irrigation on microbial activities in soils

# Effect of waste-water irrigation on nitrification potential of soils



Slight decrease in nitrification potential in soils irrigated with waste

# To study nitrifying bacteria in soil

Nitrifiers are **hard to cultivate**,  
many different ones are **similar in  
shape**



PURE FANTASY.

**SMIRNOFF**

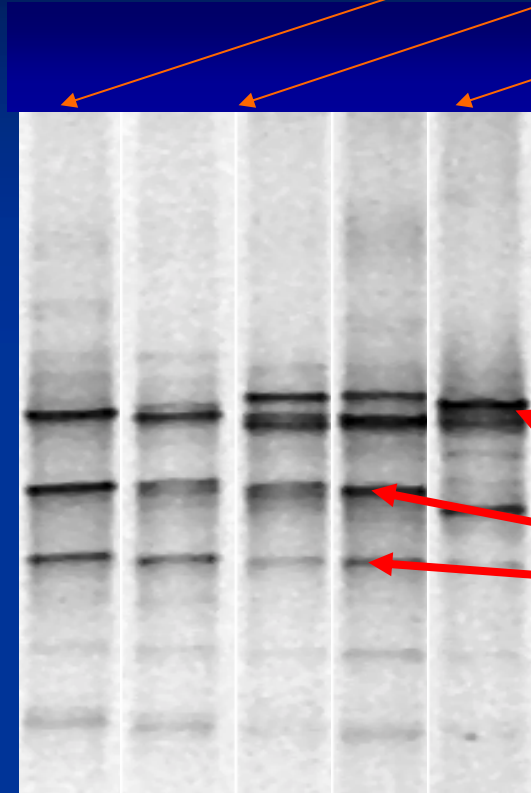
# To study nitrifying bacteria in soil

Nitrifiers are hard to cultivate,  
many different ones are similar in  
shape

combine molecular techniques  
that are not cultivation-  
dependent

# Molecular fingerprinting of microbial community (DGGE)

Each lane represents one sample/experiment



Each band represents different sequence/bacteria

Different location is due to sequence differences

# Ammonia monooxygenase (*amoA*)

Targets ONLY ammonia  
oxidizing and ALL ammonia  
oxidizing bacteria

1<sup>st</sup> sampling

2<sup>nd</sup> sampling

3<sup>rd</sup> sampling

Sample:

FeLS

EiLS

CFeLS

CEiLS

FeLS

EiLS

CFeLS

CEiLS

FeLS

FeLS

EiLS

EiLS

CFeLS

CFeLS

CEiLS

CEiLS

Depth:

15

15

15

15

15

15

15

15

15

65

15

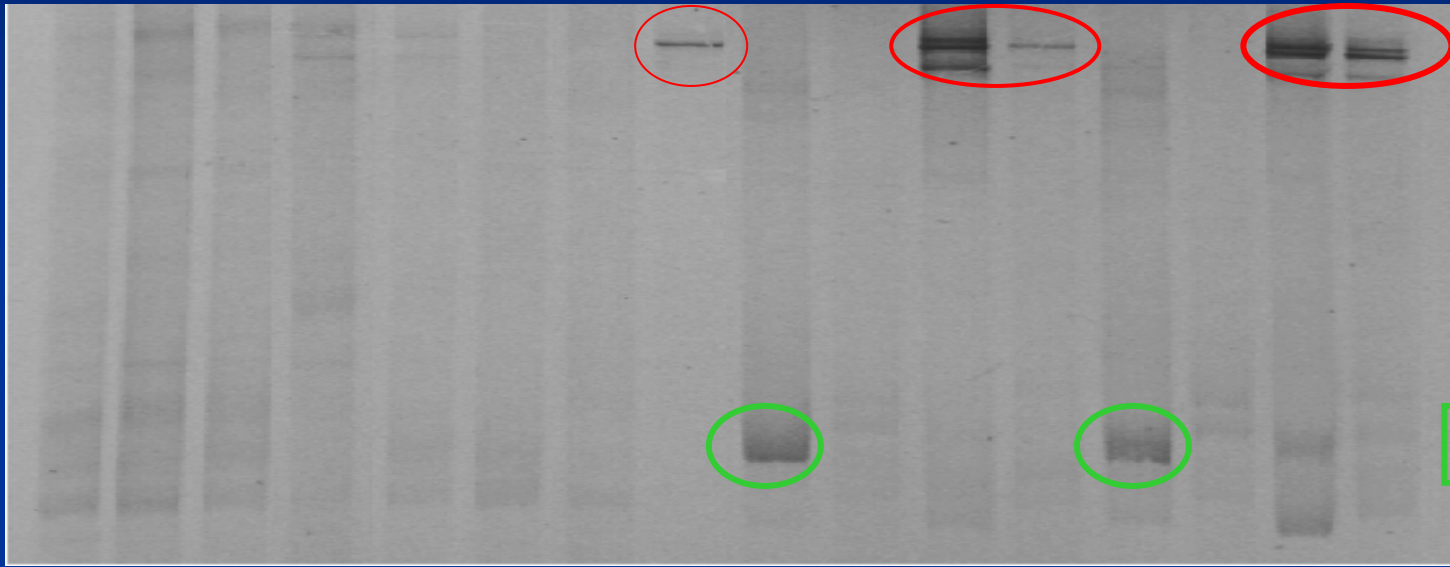
65

15

65

15

65

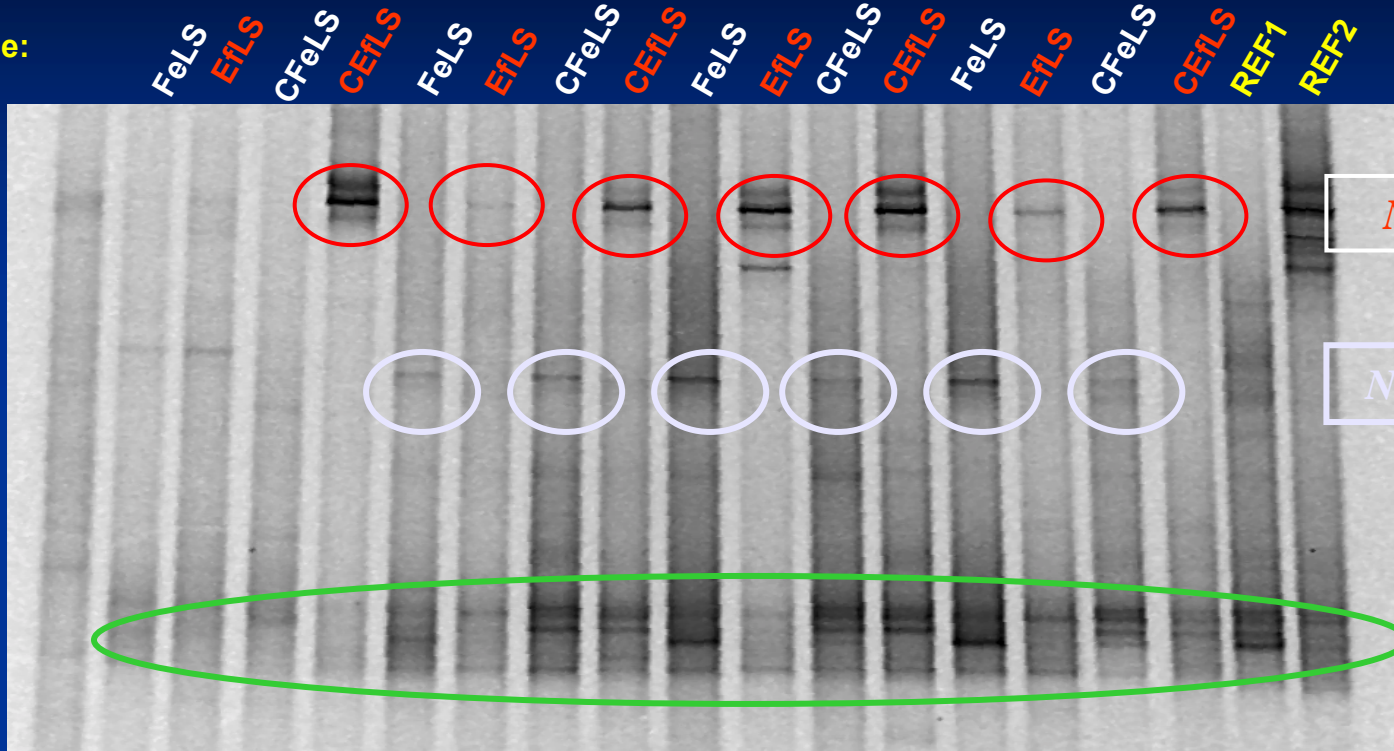


*Nitrosomonas*

*Nitrospira*

4<sup>th</sup> sampling 5<sup>th</sup> sampling 6<sup>th</sup> sampling 7<sup>th</sup> sampling

Sample:



*Nitrosomonas I*

*Nitrosomonas II*

*Nitrospira*

# Loess

Sample:

5<sup>th</sup> sampling

6<sup>th</sup> sampling

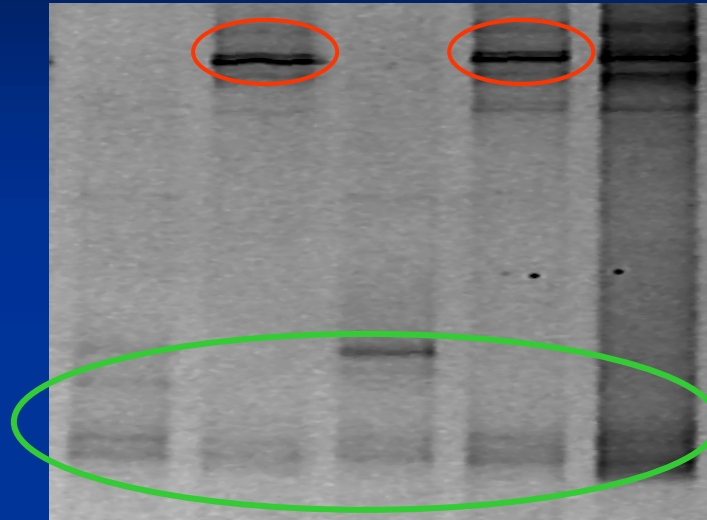
FeLeS

EtLeS

FeLeS

EtLeS

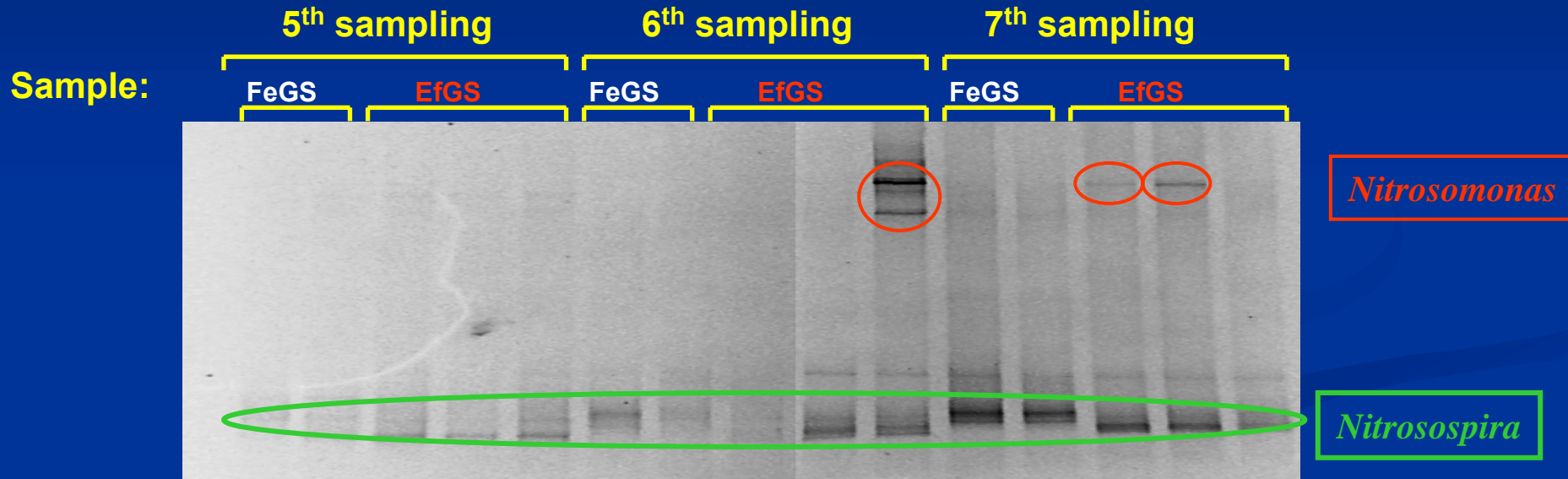
REF2



*Nitrosomonas*

*Nitrospira*

# קרקע גרומוסול

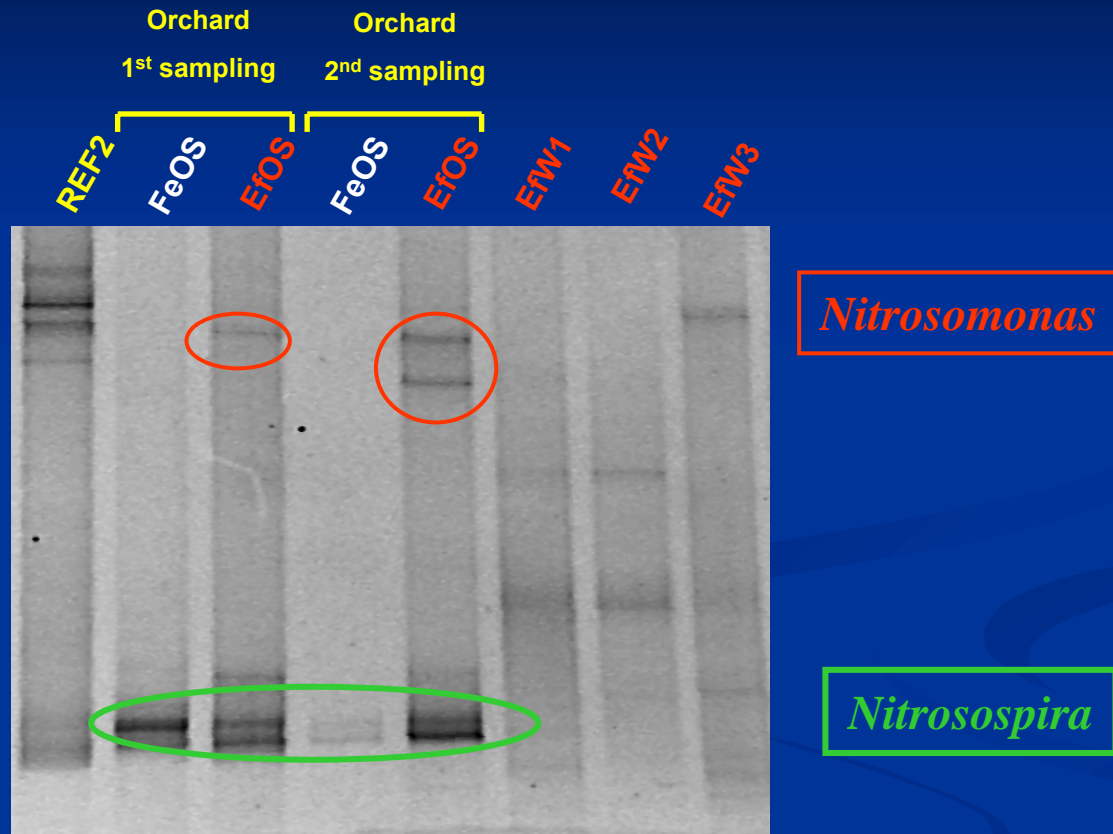


# Comparison of different lysimeters, different soils, different depths

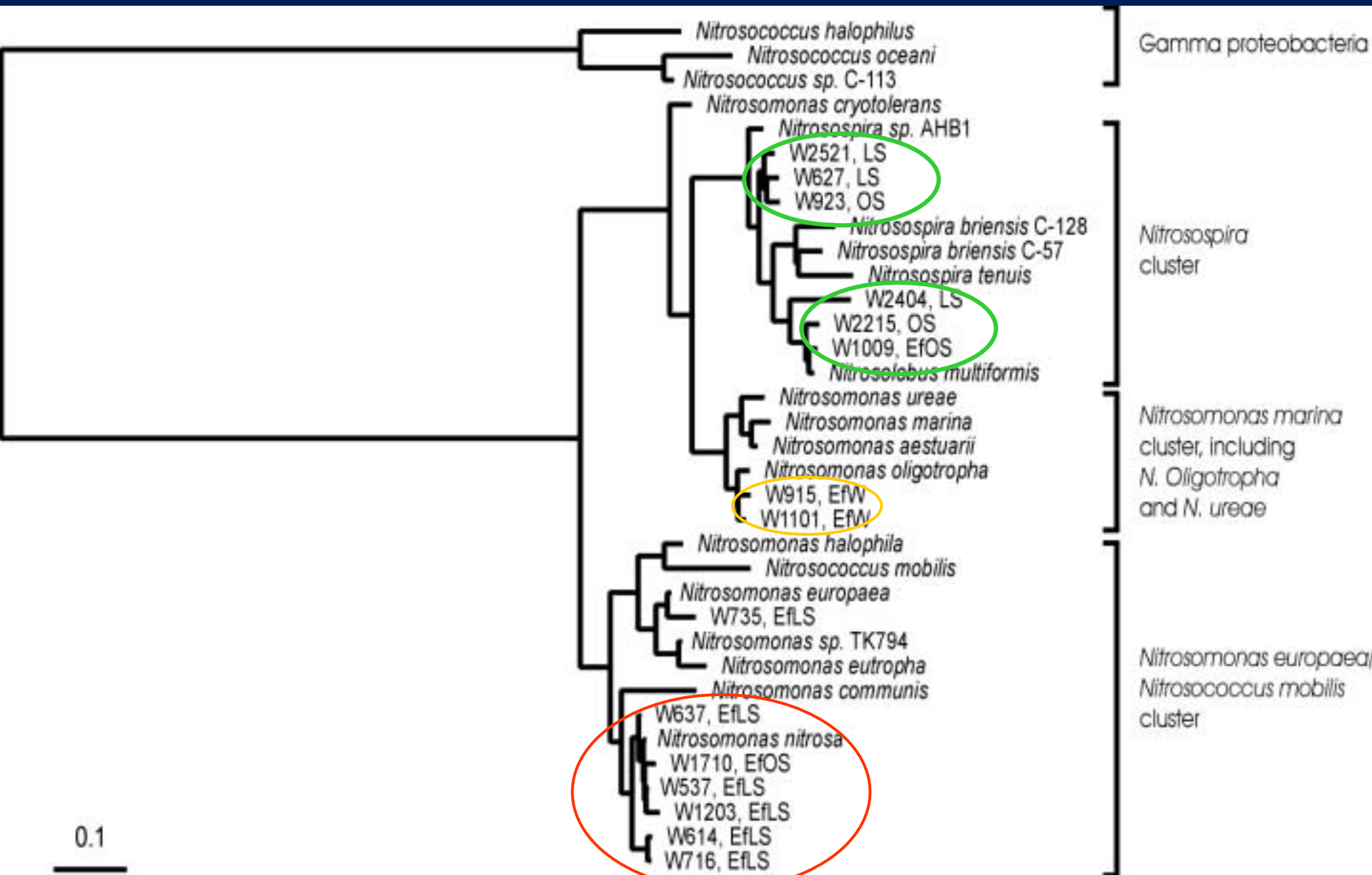
- Same experimental system
- Same student
- Same Dror
- Same audience...

**Lyzimeter artifact?**

# Orchard Loam



# Phylogenetic analysis of AOB from irrigation experiments



# Conclusions- wastewater irrigation

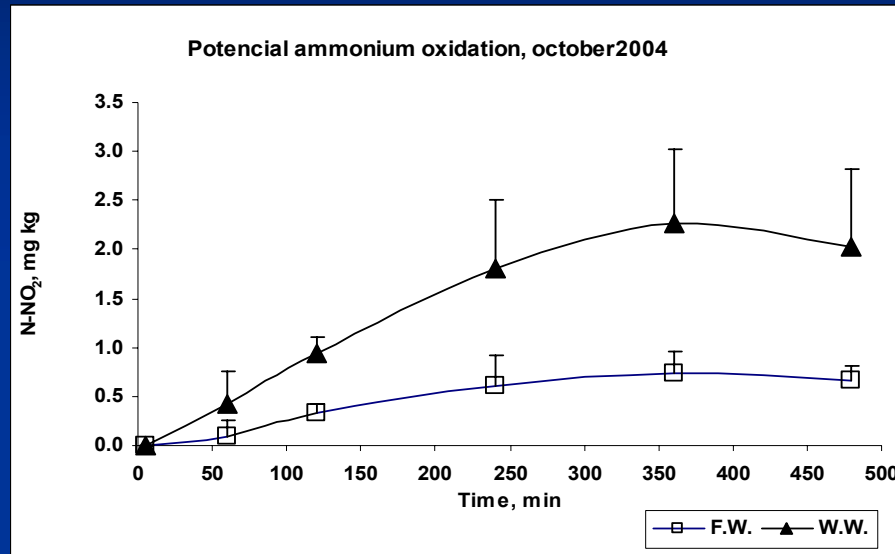
- Major shift in ammonia oxidizing community from soil *Nitrosospira* to *Nitrosomonas* in waste-water irrigated soils
- Shift levels are dependent on soil type

# Questions:

- What caused the shift? (?)
- Are the *Nitrosomonas* originated from wastewater? (not necessarily)
- How common in nature/agricultural soils?  
(very)
- How does the change affect nitrification?  
(?)
- Is the shift reversible? (?)

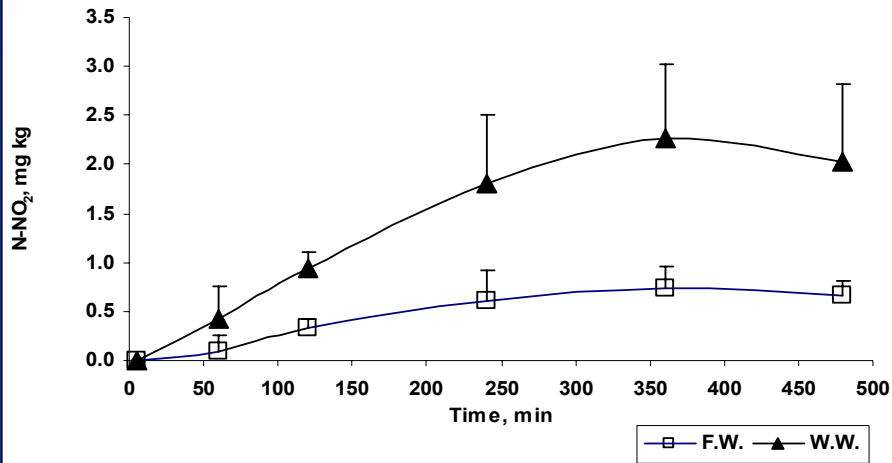
# Microbial activities in soil

# Nitrification potential

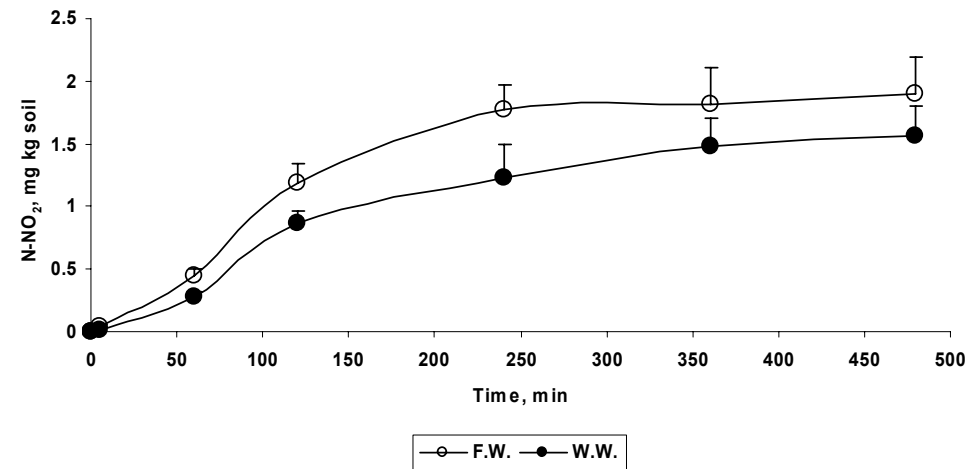


# Nitrification potential

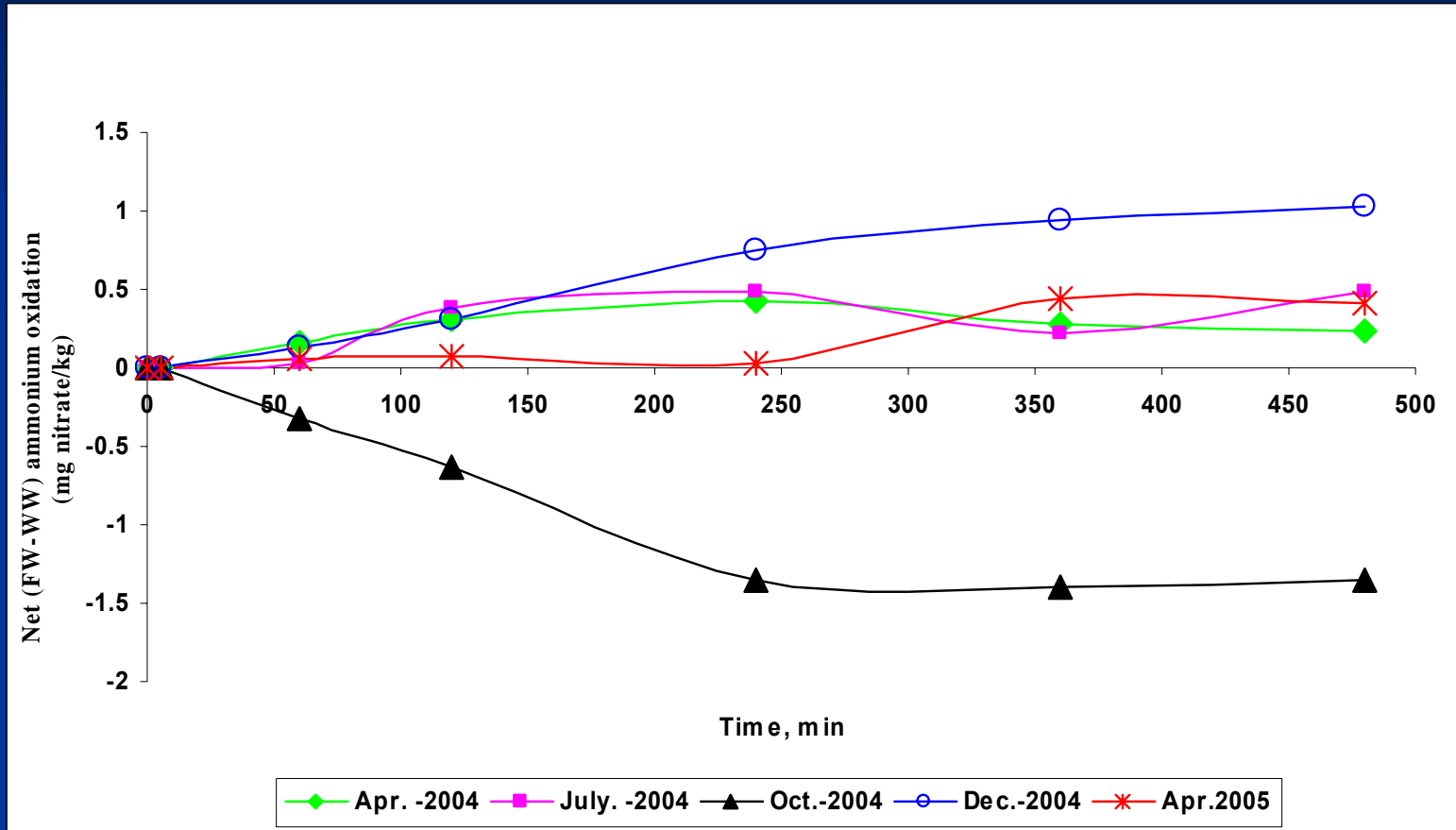
Potencial ammonium oxidation, october2004



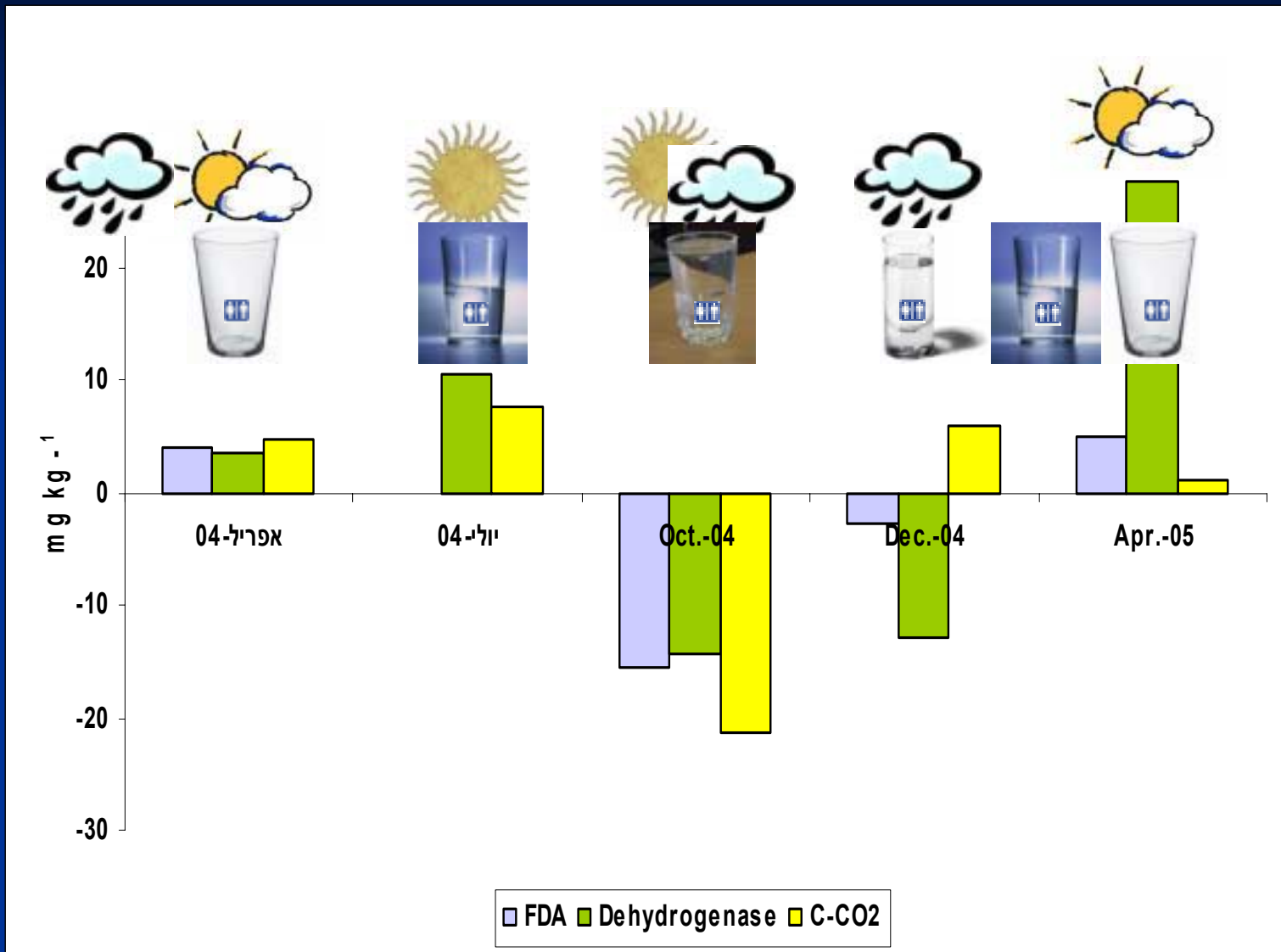
Potencial ammonium oxidation, Beit oren, 2004(5)



# Net nitrification potential (FW-WW)



# Net microbial activities (FW - WW)



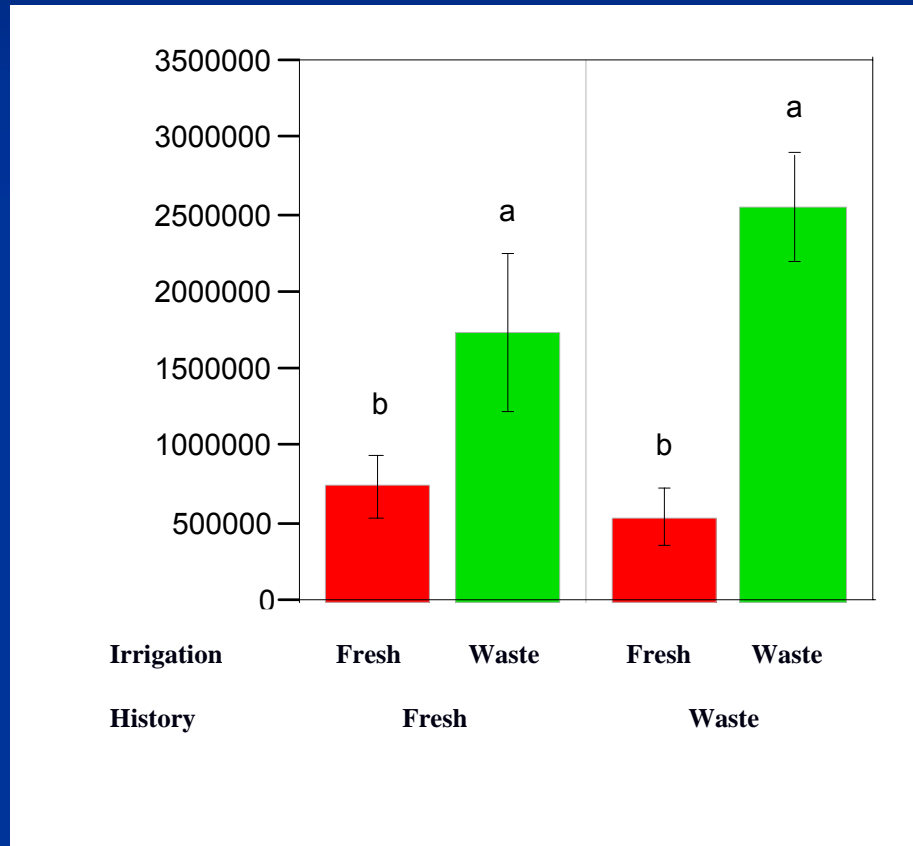
# Conclusions:

- At the **beginning of irrigation** season, in soil previously irrigated by wastewater, microbial activities are lower than in soils previously irrigated by freshwater
- **During irrigation**, situation is shifting to higher activities in soil irrigated with wastewater.

# Effect of wastewater irrigation on nitrogen fixation in legumes



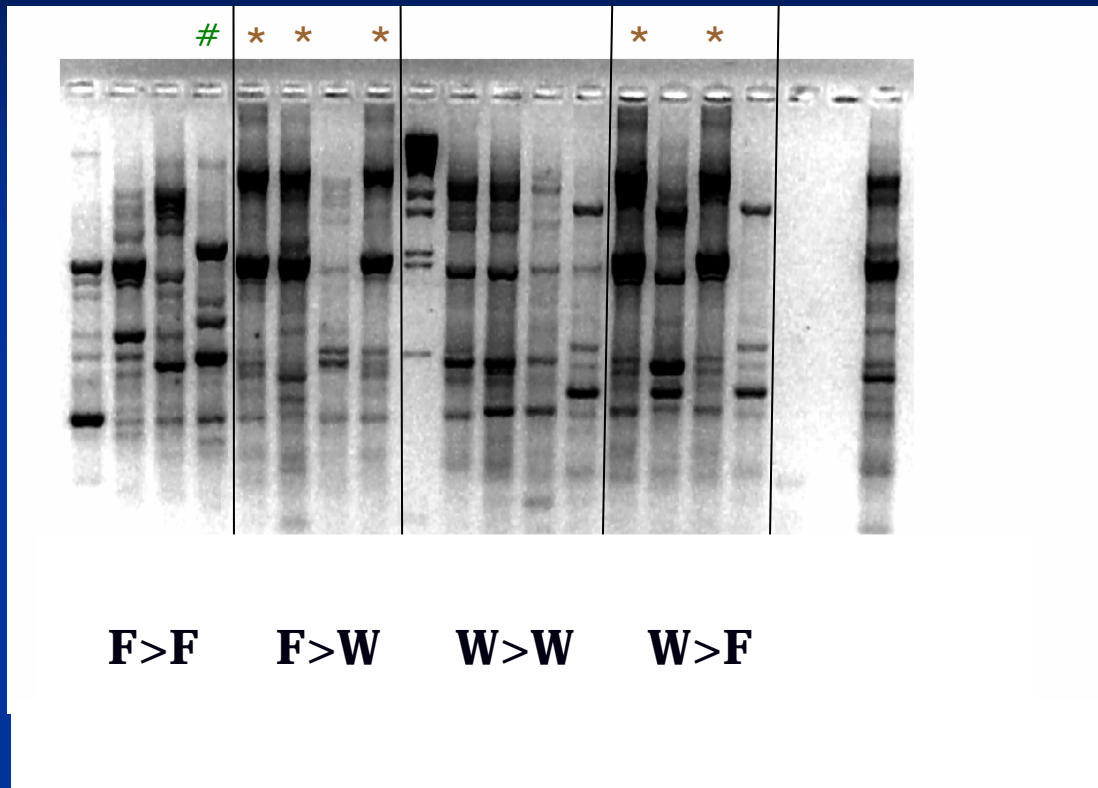
# Nitrogen fixation



# The relevant bacteria in this system

- Always *Mesorhizobium sp*
- ERI C-PCR: Enterobacterial Repetitive Intergenic Consensus (“genomic” method)

# Molecular characterization (ERIC) of *Rhizobium* in nodules



Wastewater-related organism (\*)

Freshwater-related organism (#)

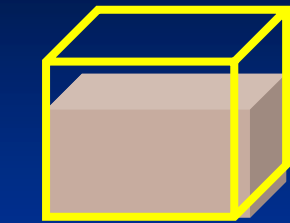
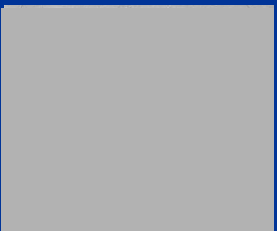
Freshwater >freshwater	Freshwater >wastewater	Wastewater >freshwater	Wastewater >wastewater	
<b>17%</b>	<b>23%</b>	<b>23%</b>	<b>47%</b>	Wastewater -related organism
<b>29%</b>	<b>6%</b>	<b>6%</b>	<b>0%</b>	Freshwater -related organism

# Conclusions:

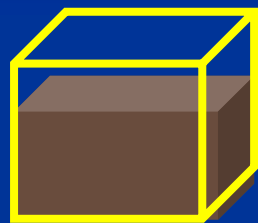
- Wastewater irrigation affects community of Rhizobia in nodules
- Plants irrigated with wastewater fix more nitrogen

# Survival of enteric bacteria in soil

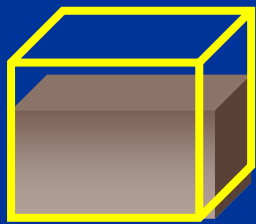
GFP labeled  
*coliforms*



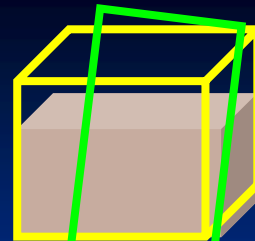
Control soil



Sewage + sludge



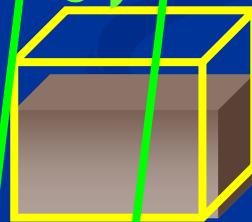
Sewage + compost



Control soil



Sewage + sludge



Sewage + compost

Sterilized

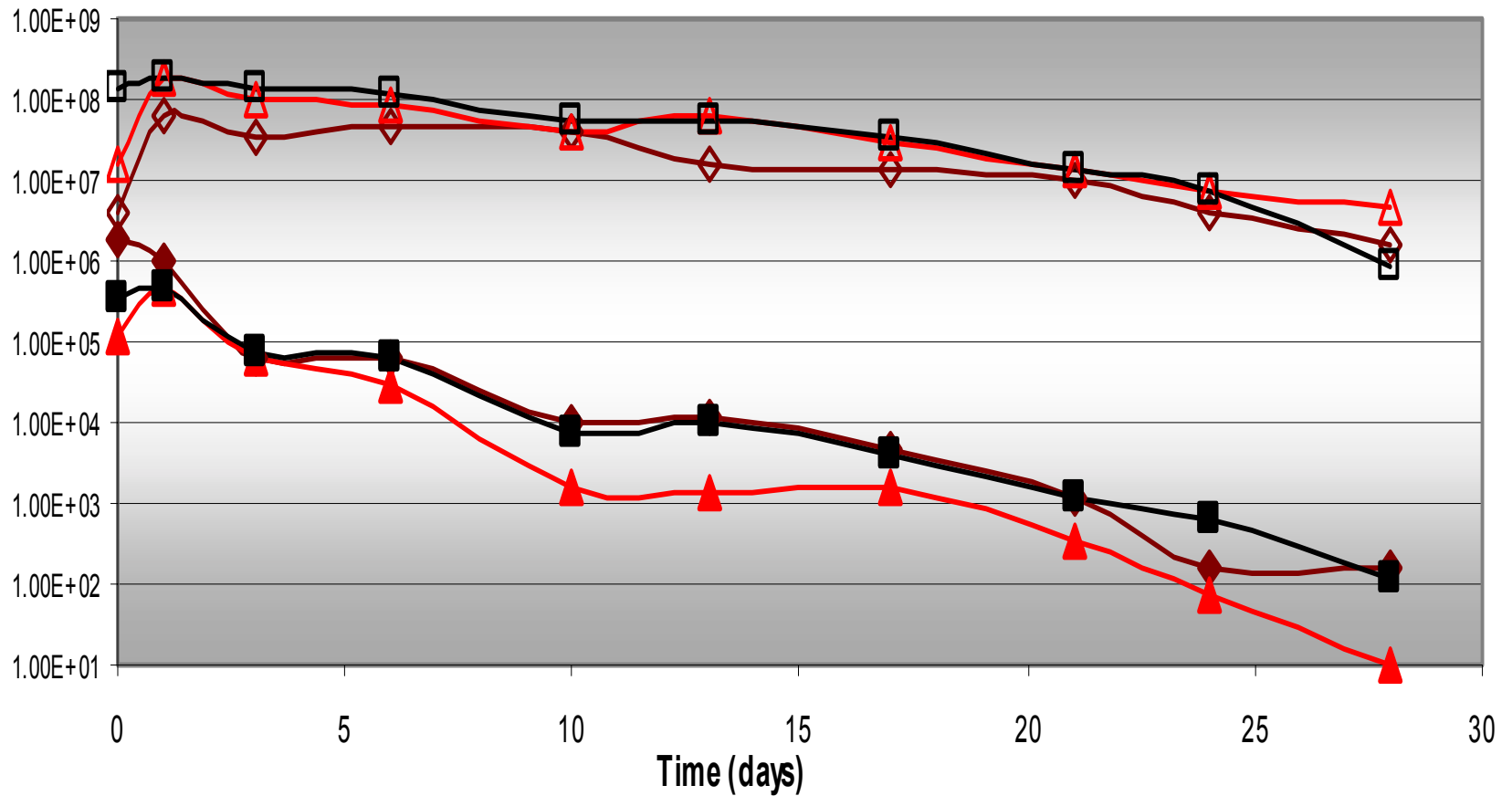


Viable  
counts



DNA

# Results



# Conclutions

- A reduction in enteric bacteria was observed in fresh but not in sterile soils, suggesting reduction may be a biological process.
- This process is enhanced in sludge-amended, wastewater-irrigated soils suggesting existence of more effective or larger number of competing/predating organisms in the sludge amended soils.