

***Identification of antimicrobial resistant  
bacteria and investigation of horizontal  
gene transfer in agricultural soil***

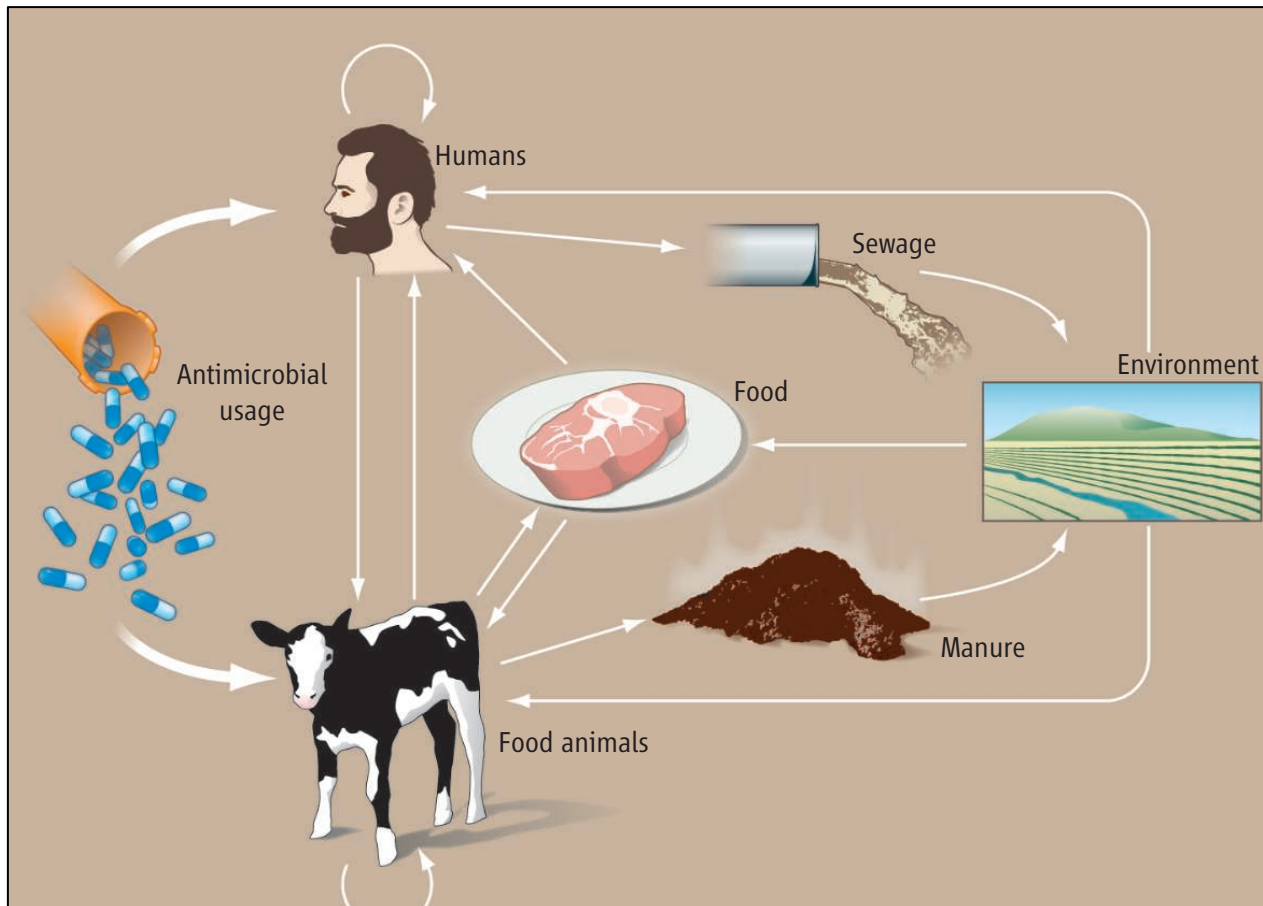
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4th – 7th March 2019

2nd Global NAMRIP Symposium

Kampala, Uganda

# Sources of antimicrobial resistance



- livestock-derived routes
- human-derived routes

# The principle

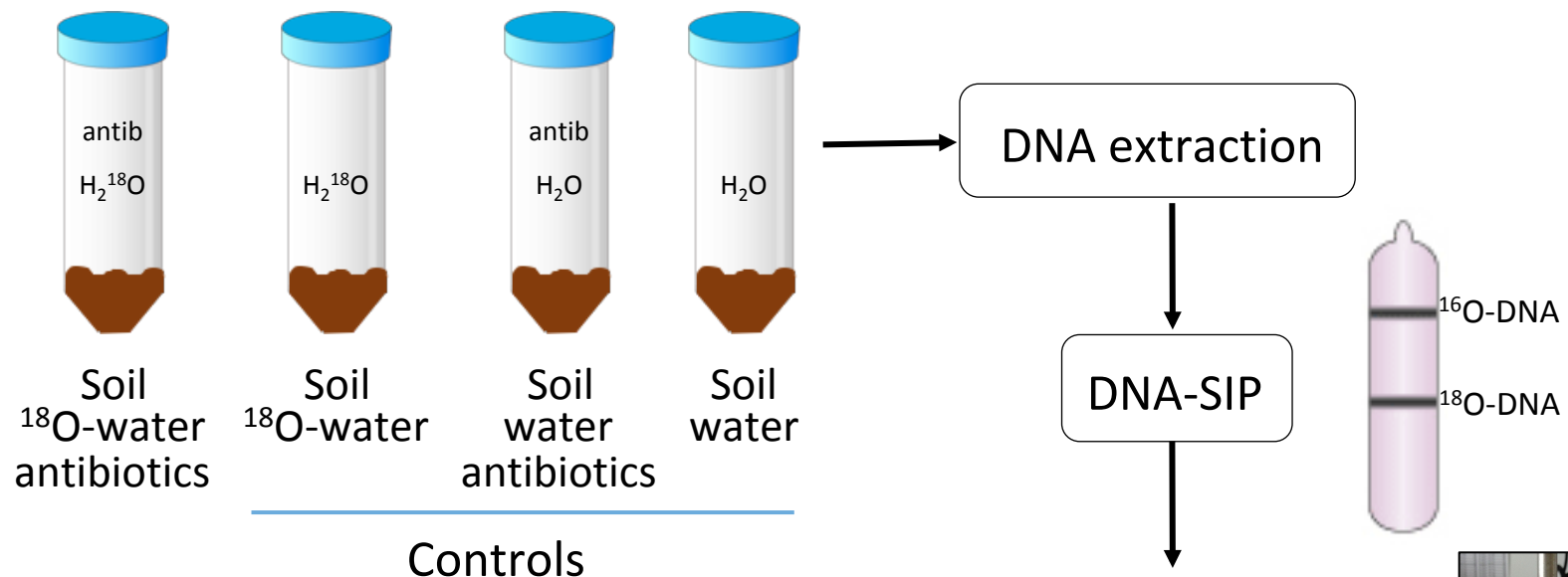
- Addition of an antibiotic to soil will kill or inhibit growth of bacteria sensitive to that antibiotic.
- Bacteria with resistance to the antibiotic can continue to grow.
- Therefore, in the presence of antibiotic and  $^{18}\text{O}$ -water, only resistant bacteria will become labelled

# Chilworth experimental site

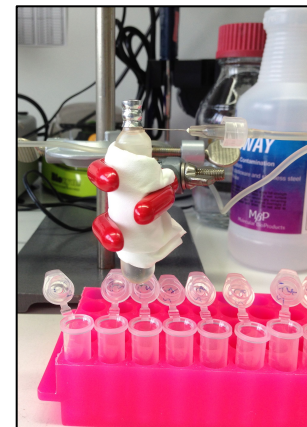
Sandy/loam soil



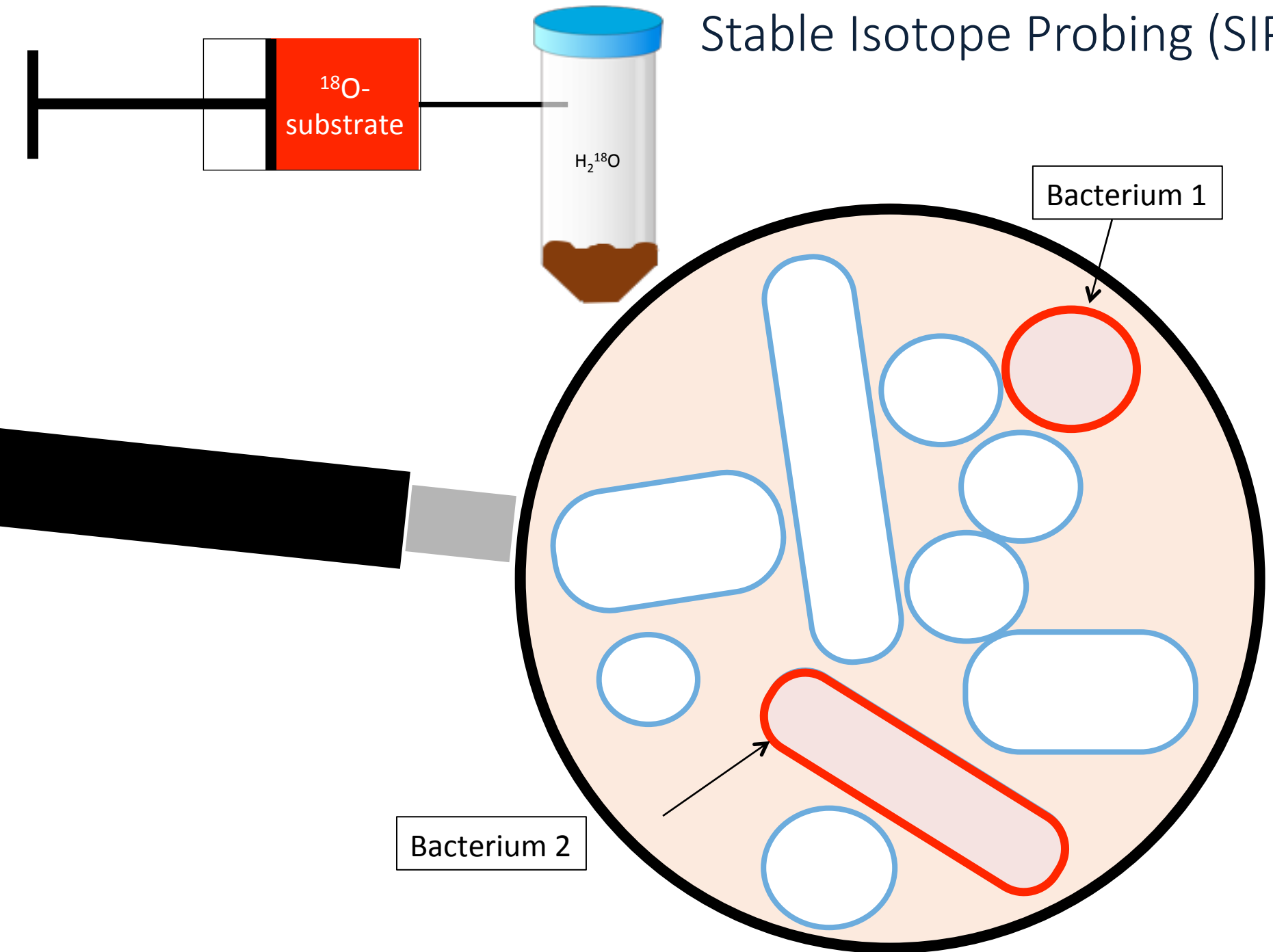
# Which bacteria constitute the soil resistome in soil?



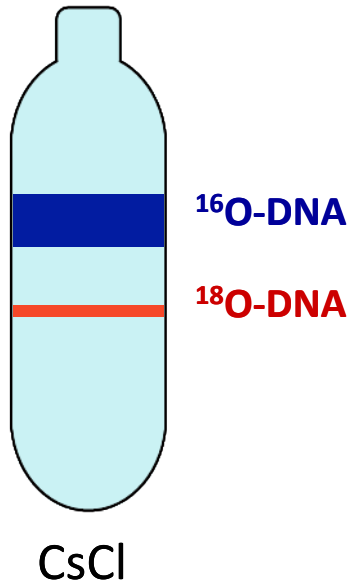
- Antibiotics: mem, ctx, cip, tmp: 100  $\mu\text{g}/\text{ml}$  (each)
- Soil (1 g) in 1.5 ml final volume (water or  $\text{O}^{18}$ -water)
- Antibiotic addition: every 2 days
- Incubate 200 rpm, dark, room temperature
- Sampling time: 2 (only treatment) & 4 days (treatment and controls)
- Triplicates for all incubations



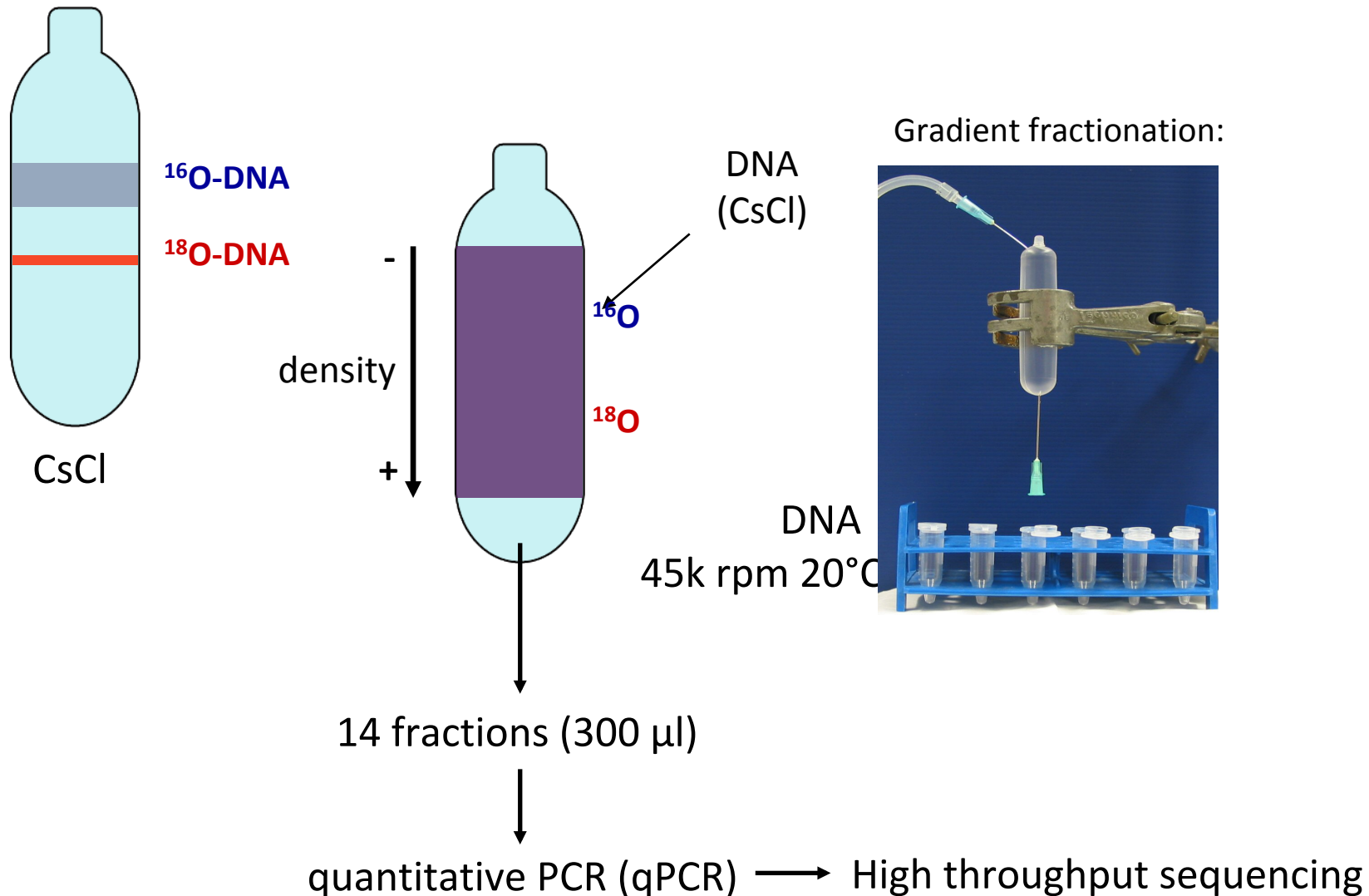
# Stable Isotope Probing (SIP)



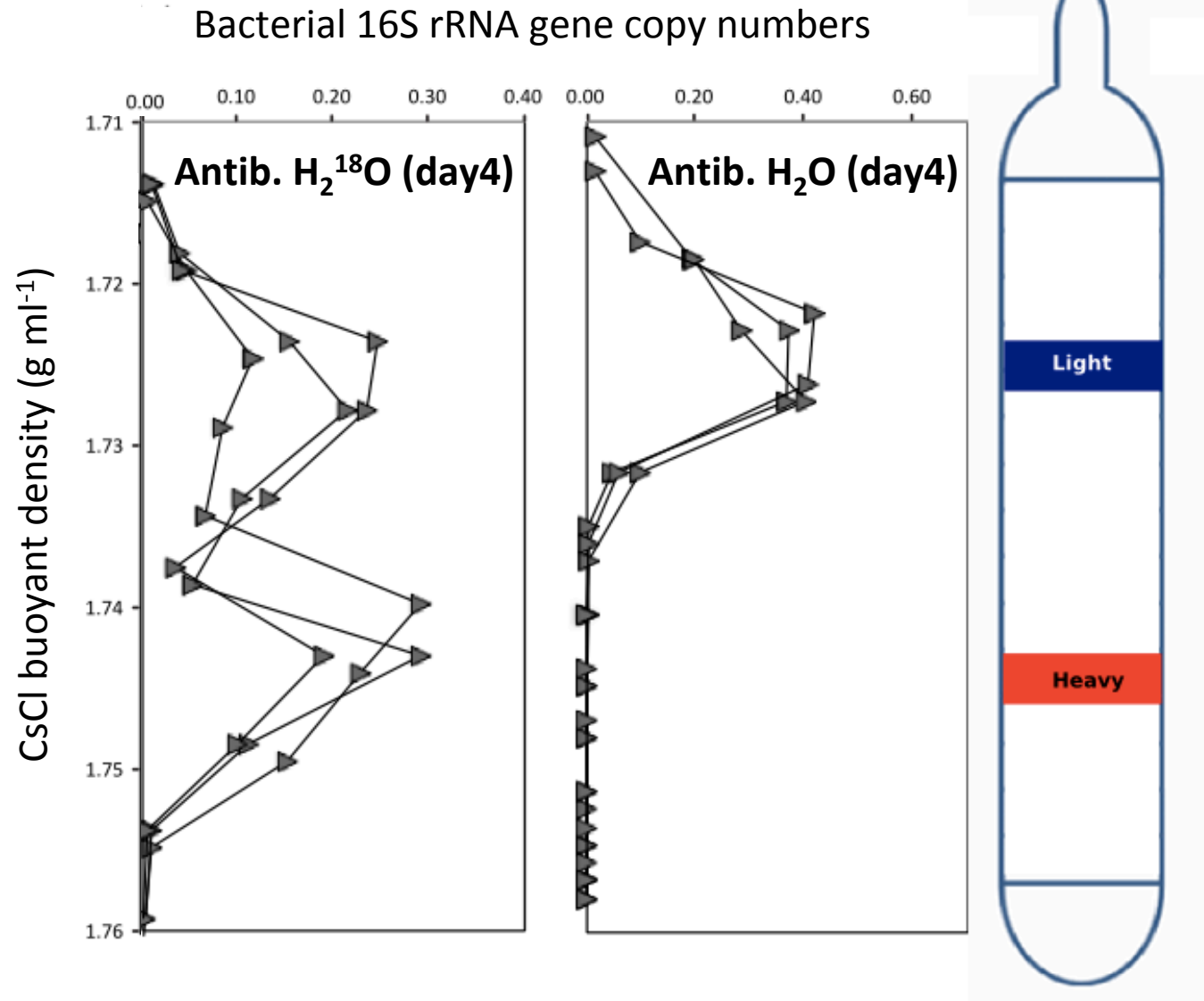
# Stable Isotope Probing (SIP) of nucleic acids

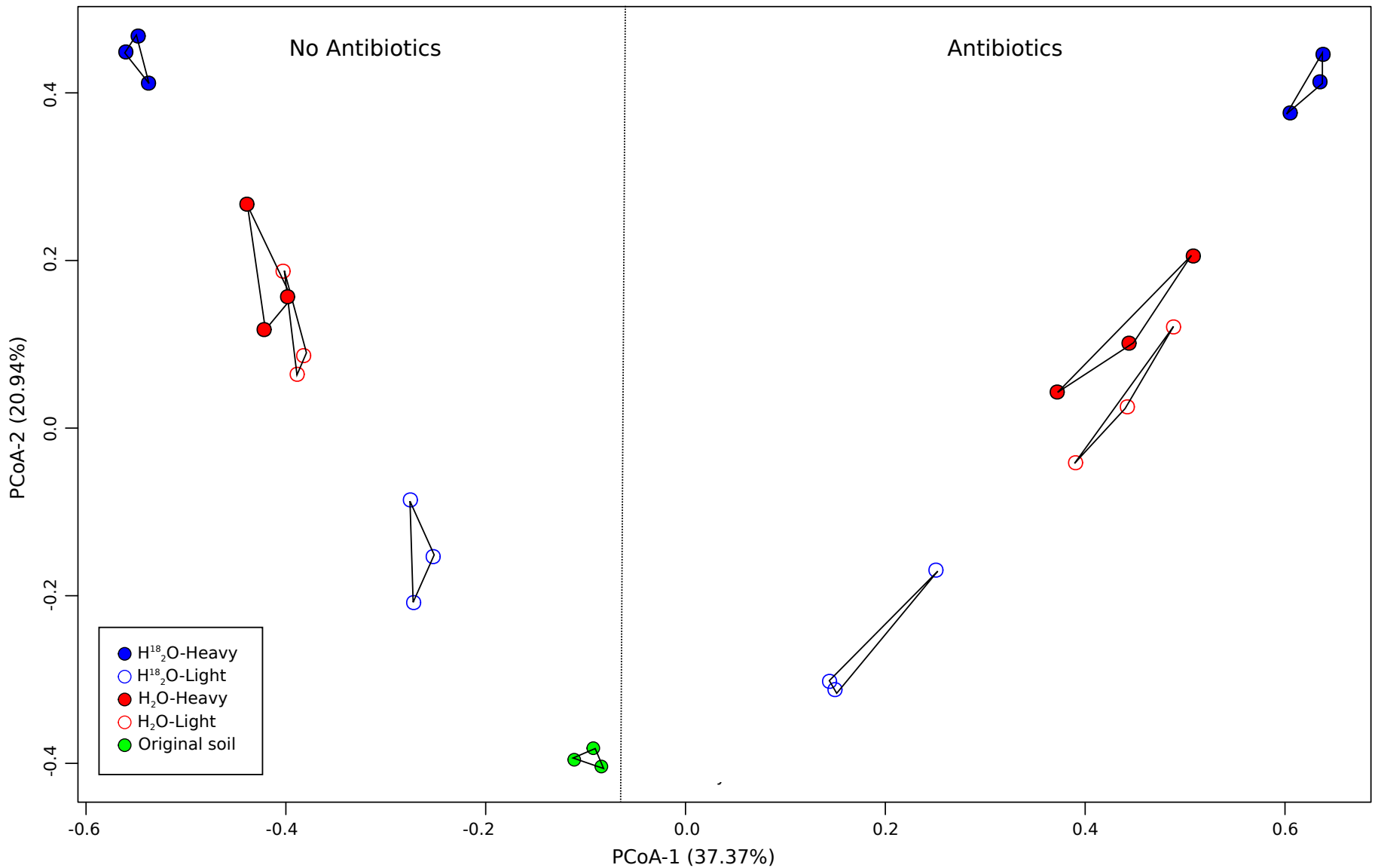


# Stable Isotope Probing (SIP) of nucleic acids



# Abundance of bacterial 16S rRNA gene in CsCl density gradients after $\text{H}_2^{18}\text{O}$ incubation

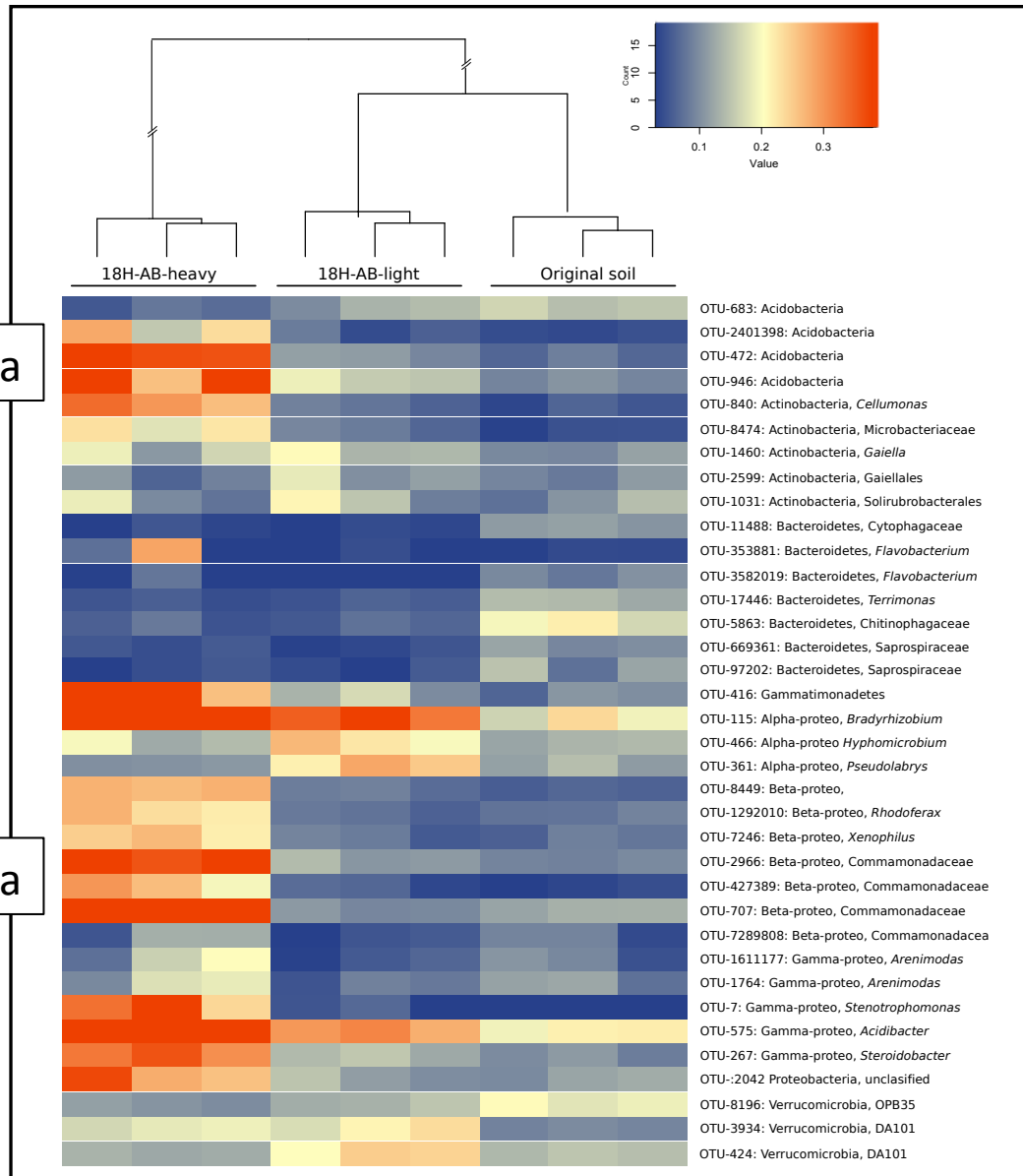




Day 2

Acidobacteria

Proteobacteria

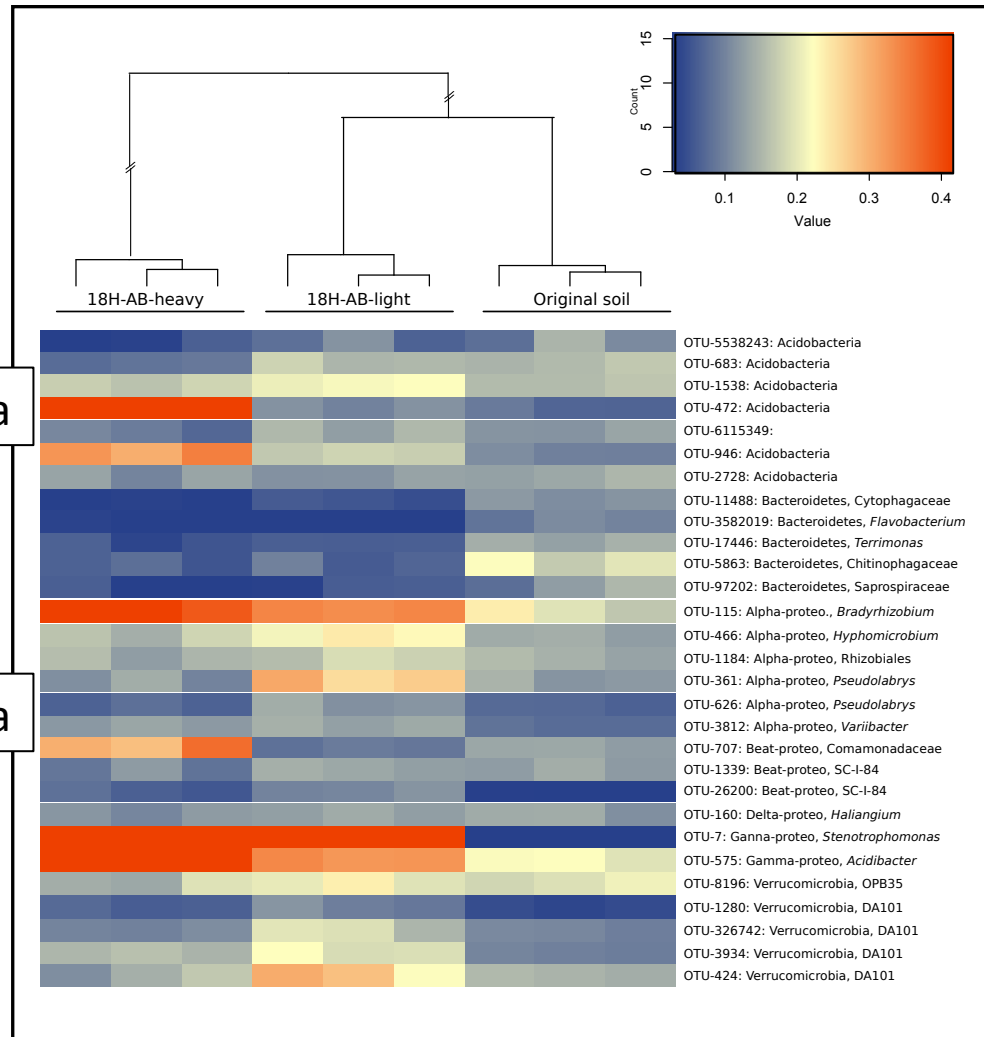


- Heatmap showing the relative abundance of selected OTUs  
- OTUs with the highest contribution to PCA ordination were selected

Day 4

Acidobacteria

Proteobacteria



- Heatmap showing the relative abundance of selected OTUs
- OTUs with the highest contribution to PCA ordination were selected

# Conclusions

- Acidobacteria are highly abundant after two days of incubation with antibiotics
- We also observed the presence of *Stenotrophomonas* after four days of incubation with antibiotics

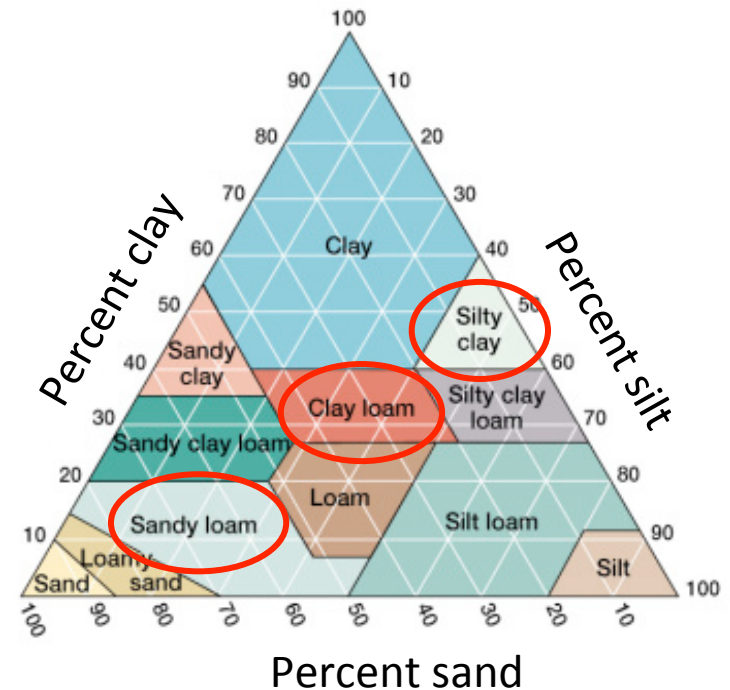
# Conclusions

- Acidobacteria are highly abundant after two days of incubation with antibiotics
- We also observed the presence of *Stenotrophomonas* after four days of incubation with antibiotics
- The results indicate that both non-pathogenic soil-bacteria as well as potential clinical pathogens are present in this agricultural soil, but it is still unclear if horizontal gene transfer between these groups can occur.

## Horizontal gene transfer (HGT) in soil

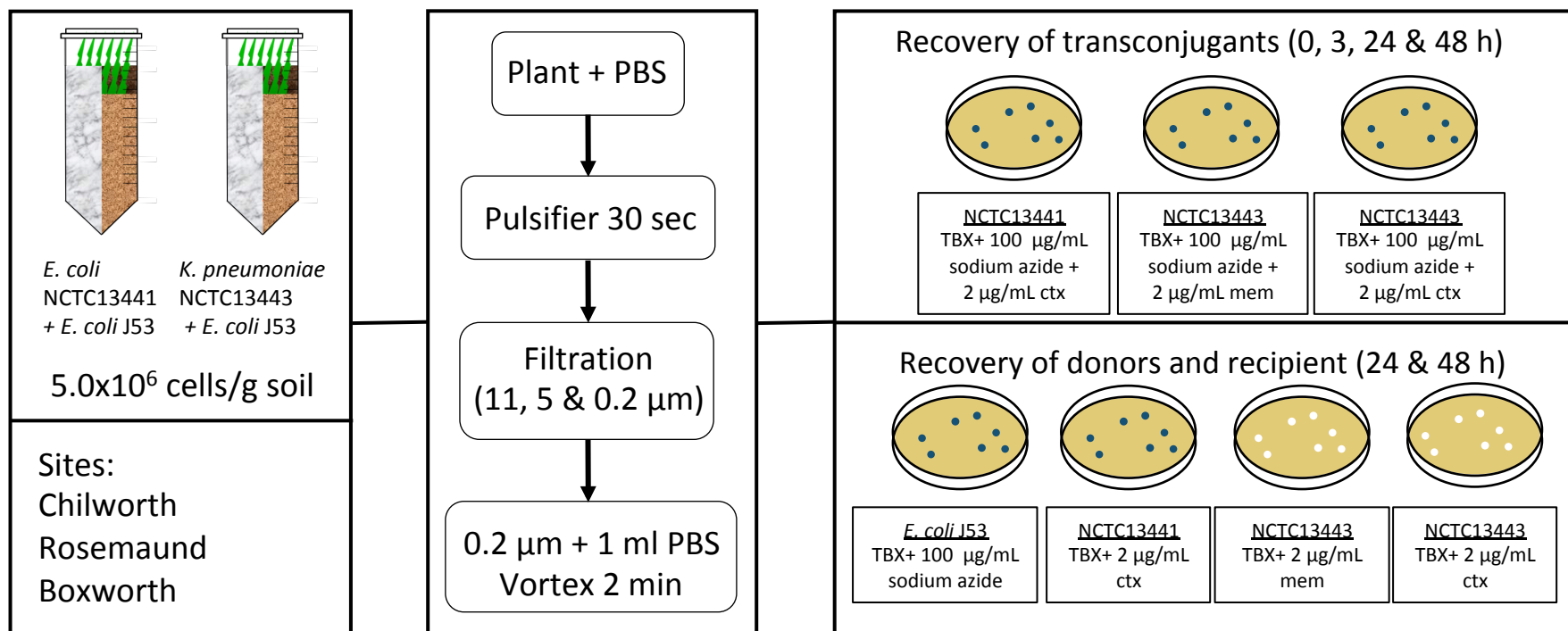
**Can HGT between two defined bacterial strains occur in a soil environment?**

# Soil sampling



Chilworth (sandy loam)  
Boxworth (Clay loam)  
Rosemaund (Silty clay)

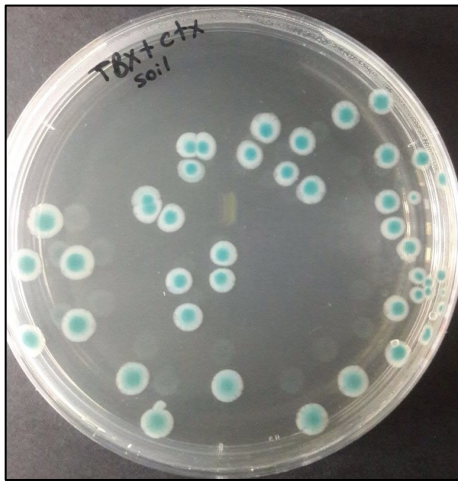
# Methods



- Sodium azide-resistant *E. coli* J53 (J53 Ar<sup>R</sup>) RECIPIENT
- *Klebsiella pneumoniae* NCTC13443 (encodes bla<sub>NMD-1</sub>) DONOR
- *Escherichia coli* NCTC13441 (encodes bla<sub>CTX-M-15</sub>) DONOR
- Destructive samples: 0, 3, 24 and 48 h, incubation at room temp.
- Triplicates for all incubations
- Total samples: 72 plants
- Some transconjugants kept in glycerol 20%

References:  
 Scott et al., 2006  
 Wolffs et al., 2006  
 Warnes et al., 2012  
 Highmore et al., 2017

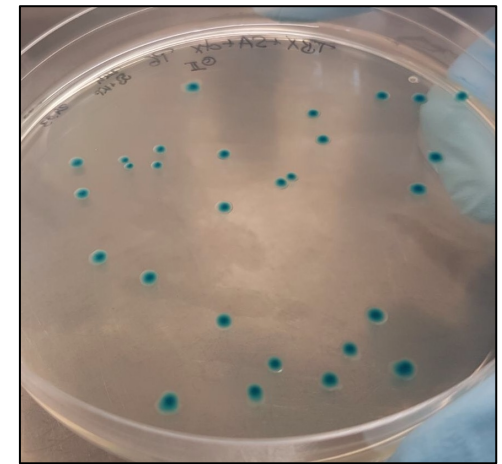
# Recovery from the soil after 24 h incubation (examples)



*Escherichia coli*  
NCTC13441



*Klebsiella pneumoniae*  
NCTC13443

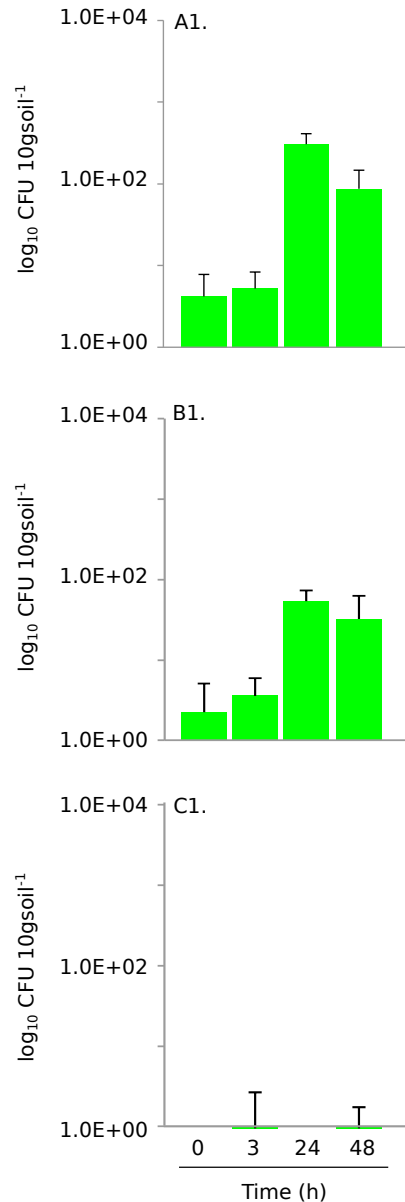


*Escherichia coli* J53 SA  
resistant carrying the  
plasmid pEK499 with gene  
 $\text{bla}_{\text{CTX-M-15}}$  (ctx resistance)

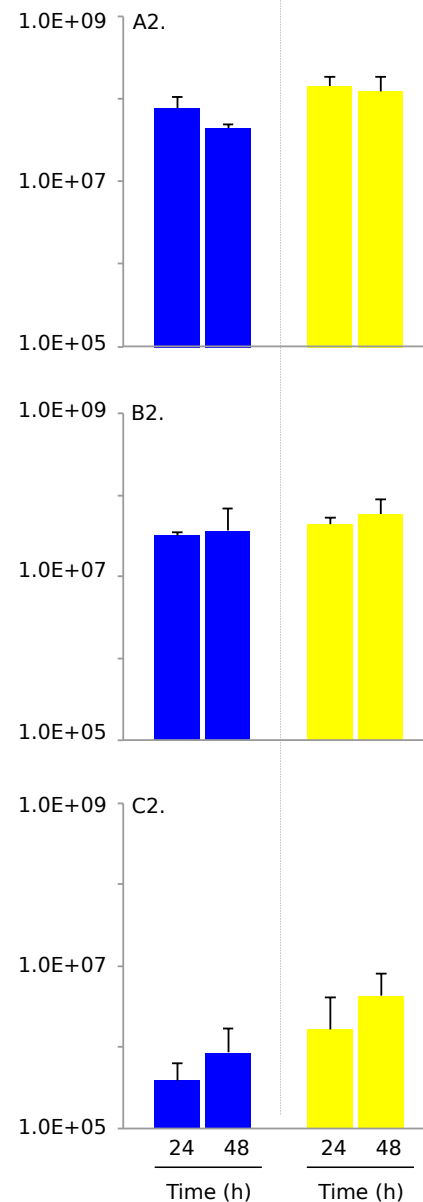
# *Escherichia coli* as donor

Transconjugants  
selected on  
TBX+SA+ctx

Transconjugants  
Recipient: *E. coli* J53  
Donor: *E. coli* NCTC13441



Recovery of recipient and donor  
*E. coli* J53  
TBX+SA  
*E. coli* NCTC13441  
TBX+ctx



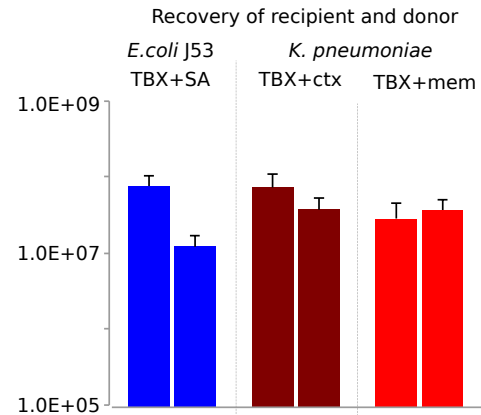
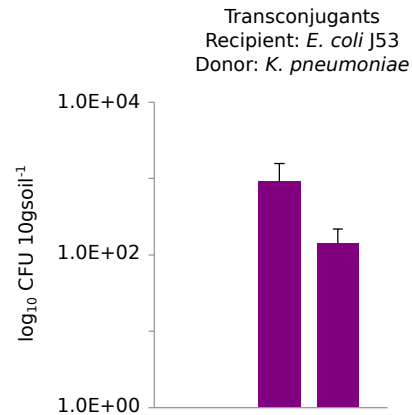
Chilworth

Rosemaund

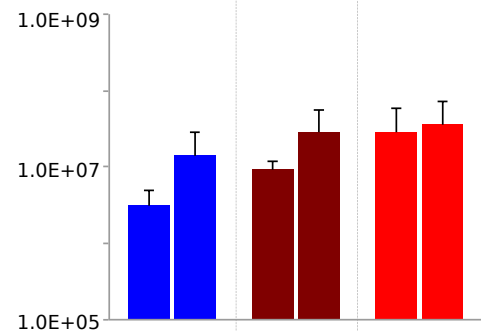
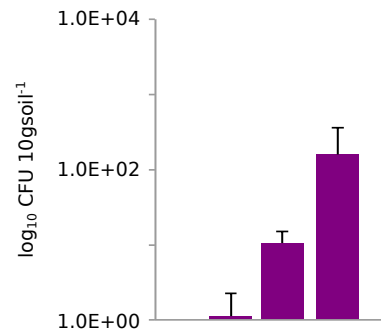
Boxworth

# *Klebsiella pneumoniae* as donor

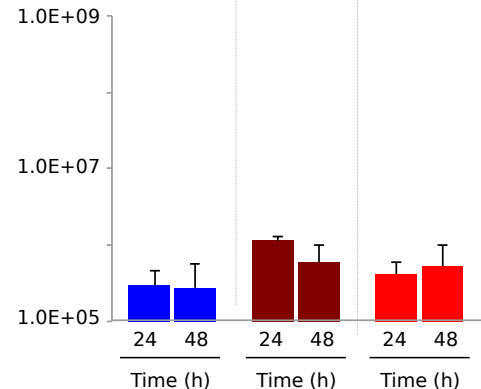
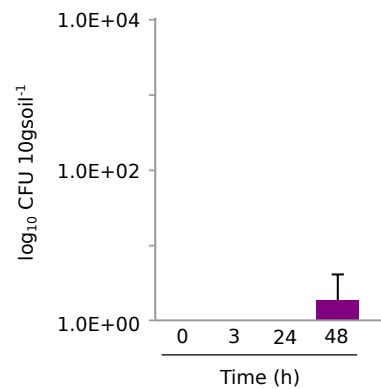
Transconjugants  
selected on  
TBX+SA+ctx



Chilworth



Rosemaund



Boxworth

## Conclusions

- The results indicate that potential pathogens can survive in soil and transfer their plasmid to a recipient after 48 h incubation.
- Transconjugants carrying plasmid conferring resistance through the gene *bla*<sub>CTX-M-15</sub> from both donors (*K. pneumoniae* and *E. coli*) were found.
- No conjugation of the plasmid containing NDM-1 metallo- $\beta$ -lactamase was observed.
- These are early studies confirming that horizontal gene transfer can occur in the soil.

# Acknowledgements

- Prof. Bill Keevil
- Dr. Marc Dumont
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