## Risks of livestock manure application

#### October 24<sup>th</sup>, Bjorn Berendsen







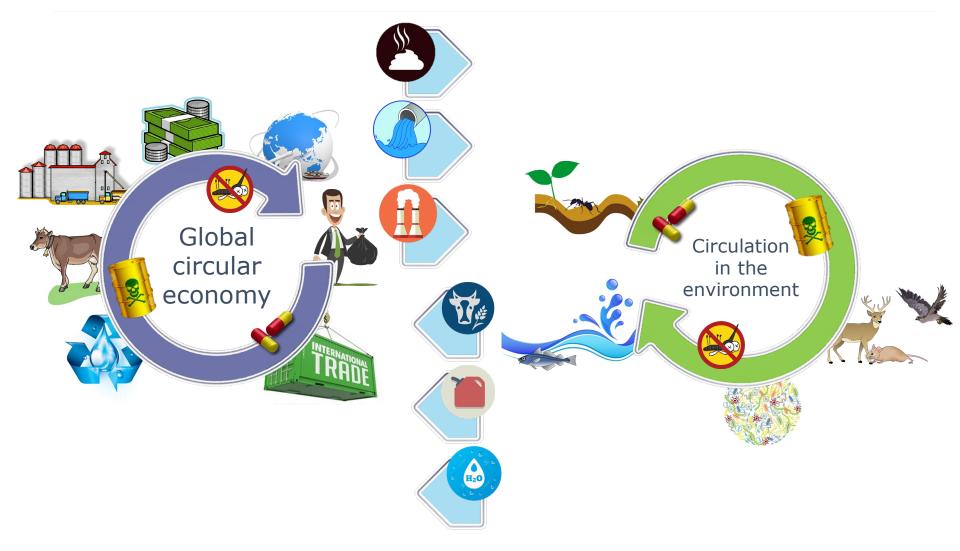
#### Project team

- PSG-WPR: Leo van Overbeek
- ESG-WEnR: Joost Lahr, Jaap van Os, Louise Wipfler
- ASG-WBVR: Dik Mevius, Ales Bossers
- ASG WLR: Paul Hoeksma
- AFSG-Food Micro: Tjakko Abee, Marcel Zwietering
- SSG-WEcR: Nico Bondt, Ron Bergevoet, Tanja de Koeijer
- WFSR: Bjorn Berendsen





## Global One Health approach







## Dissipation routes via manure

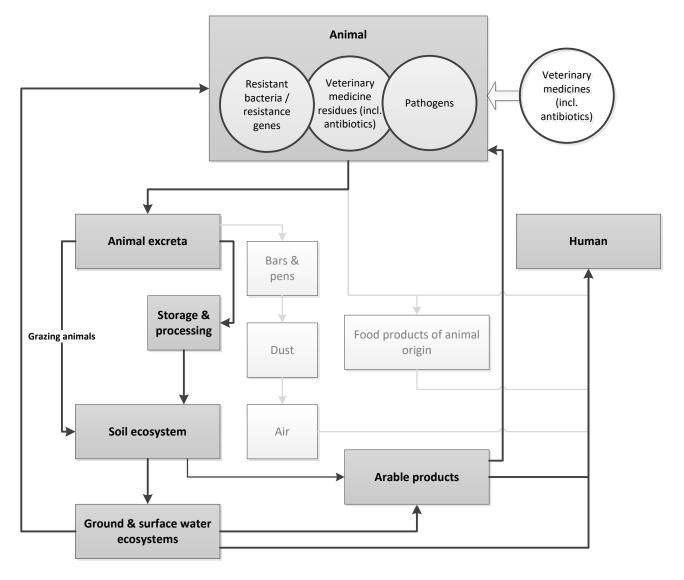


Figure 1. Routes for the spread of manure constituents. The route through animal excreta is highlighted.

#### Risks of livestock manure application

#### Goals:

- Selection of the most relevant antibiotics and pathogens
- Optimisation of the analytical methods for antibiotic analysis
- Study the fate of antibiotics and pathogens
- Model manure distribution and antibiotic load
- Study longitudinal effect of antibiotics in manure on resistance
- Study circulation of pathogens and antibiotics in the ecosystem





#### Risks of livestock manure application

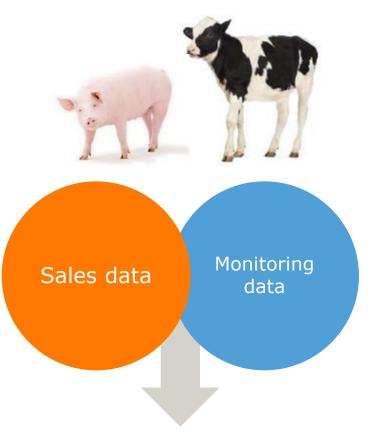
#### Goals:

- Selection of the most relevant antibiotics and pathogens
- Optimisation of the analytical methods for antibiotic analysis
- Study the fate of antibiotics and pathogens
- Model manure distribution and antibiotic load
- Study longitudinal effect of antibiotics in manure on resistance
- Study circulation of pathogens and antibiotics in the ecosystem





#### Selection of most relevant antibiotics



10 prioritized antibiotics







## Selection of most relevant pathogens

- Expert meeting:
  - Wageningen UR
  - GD
  - RIVM
  - enkele
  - Ministeries
  - Provincies
  - Waterschappen







#### Pathogens and antibiotics in the chain

#### Goals:

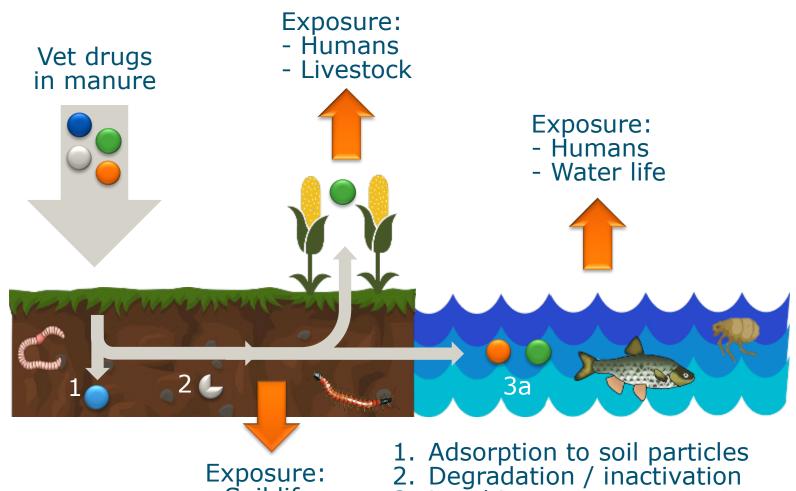
- Selection of the most relevant antibiotics and pathogens
- Optimisation of the analytical methods for antibiotic analysis
- Study the fate of antibiotics and pathogens
- Model manure distribution and antibiotic load
- Study longitudinal effect of antibiotics in manure on resistance
- Study circulation of pathogens and antibiotics in the ecosystem





#### The fate of antibiotics

- Soil life



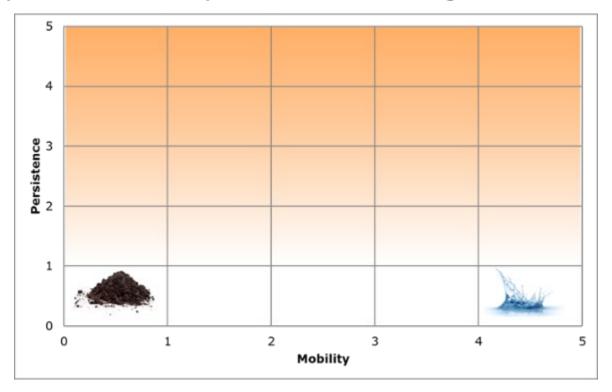
3. Leaching

a. To surface water

b. Uptake by crops

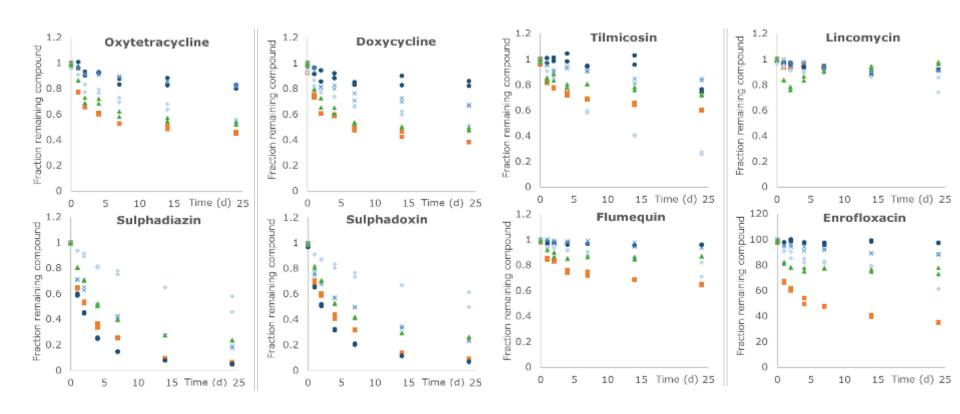
#### The fate of antibiotics

- Persistence = 'the continued or prolonged existence'
- Mobility = 'the ability to move among reservoirs'





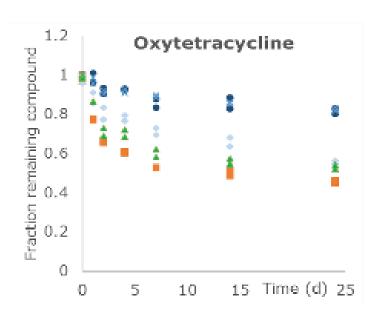


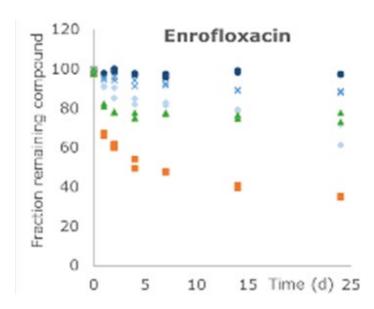






 Diphasic degradation observed for especially tetracyclines and quinolones. Binding to organic particles might contribute to their persistence.









| Compound group | Very persistent DT90 > 1 y | Persistent  DT90 > 6 m | Moderately persistent DT90 > 3m | Slightly persistent DT90 > 1 m | Non-<br>persistent<br>DT90 ≤ m |
|----------------|----------------------------|------------------------|---------------------------------|--------------------------------|--------------------------------|
| Tetracyclines  |                            | X                      |                                 |                                |                                |
| Sulphonamides  |                            |                        | Х                               | ×                              | X                              |
| Quinolones     | Х                          | х                      |                                 |                                |                                |
| Macrolides     |                            | х                      | Х                               |                                |                                |
| Lincosamides   | Х                          |                        |                                 |                                |                                |
| Pleuromutilins | X                          |                        | х                               |                                |                                |



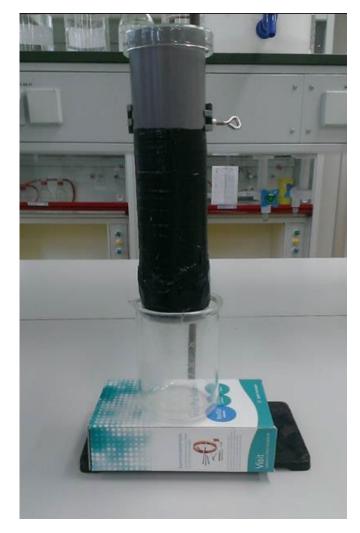


- Persistence in manure depends mainly on animal species.
- The tested antibiotics, with exception of the sulphonamides are moderately to very persistent.
- Even after 9 months storage, some antibiotics can persist in manure and can be transferred to agricultural soils.
- Based on the persistence and frequency of use, currently, environmental exposure to oxytetracycline, doxycycline, flumequine, tilmicosin and lincomycin seems most likely.



#### The fate of antibiotics - mobility

- Soil column experiment
  - Packed with wetted soil
  - 300 µg of the antibiotics applied
  - 50 ml water daily
  - Isolate eluate daily
  - Isolate 2 cm soil layers after 15 days

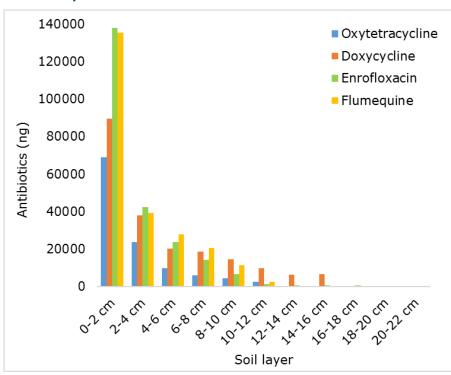




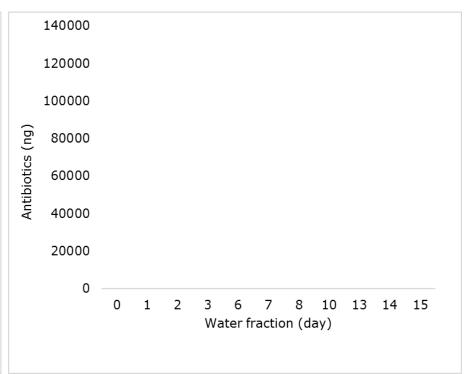


## The fate of antibiotics - mobility

#### Soil layers



#### Water fractions

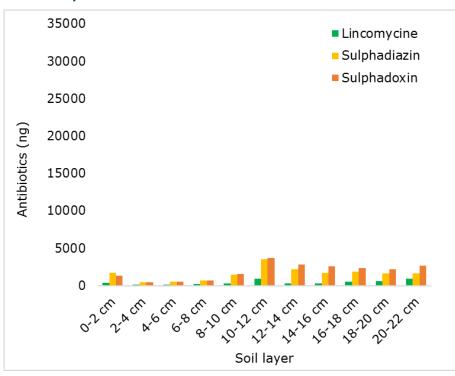




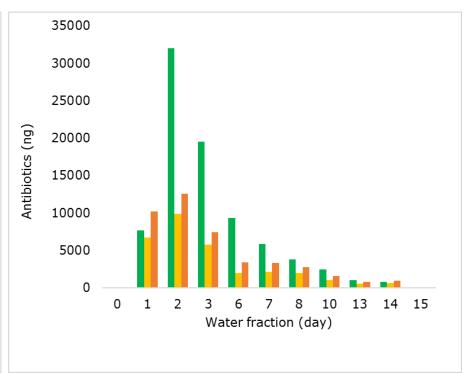


## The fate of antibiotics - mobility

#### Soil layers



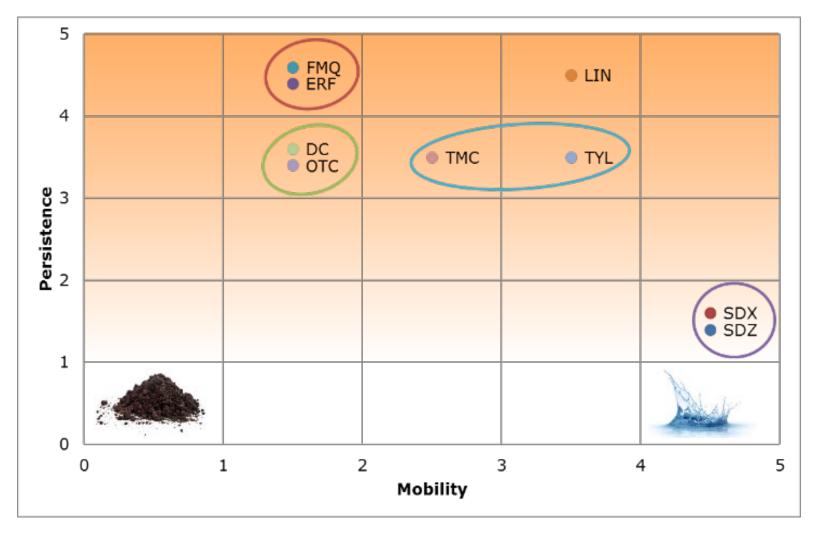
#### Water fractions







#### The fate of antibiotics

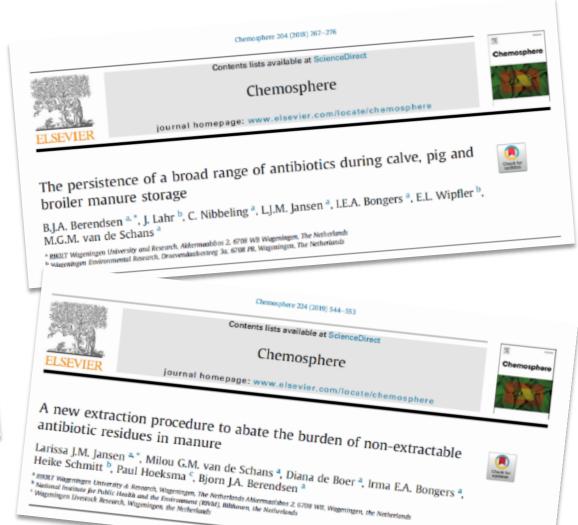






# The fate of antibiotics









#### Pathogens and antibiotics in the chain

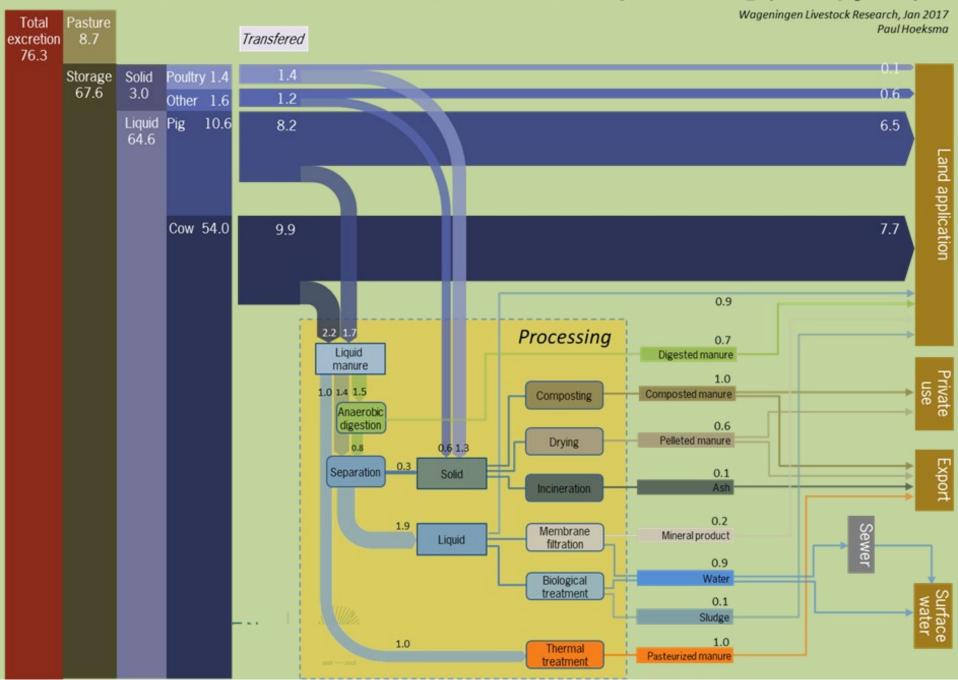
#### Goals:

- Selection of the most relevant antibiotics and pathogens
- Optimisation of the analytical methods for antibiotic analysis
- Study the fate of antibiotics and pathogens
- Model manure distribution and antibiotic load
- Study longitudinal effect of antibiotics in manure on resistance
- Study circulation of pathogens and antibiotics in the ecosystem





#### Livestock manure NL - excretion, transfer and processing (Mton/year)



#### Pathogens and antibiotics in the chain

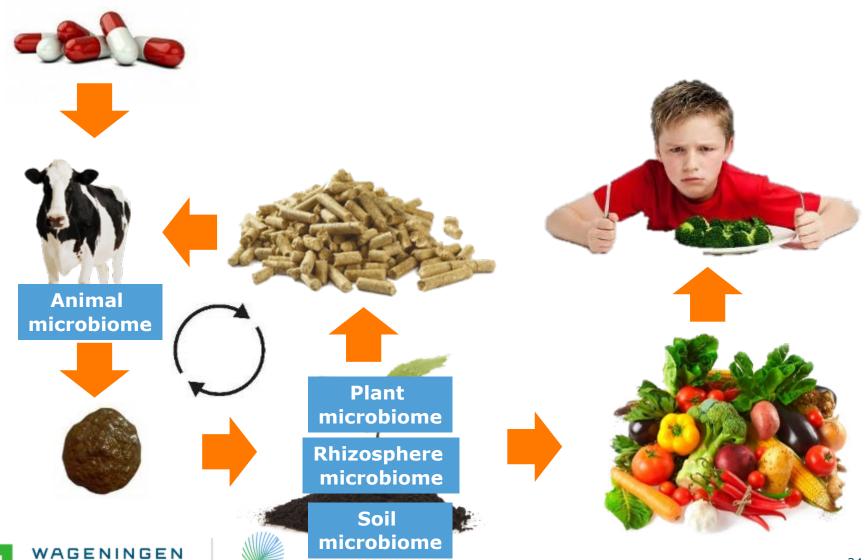
#### Goals:

- Selection of the most relevant antibiotics and pathogens
- Optimisation of the analytical methods for antibiotic analysis
- Study the fate of antibiotics and pathogens
- Model manure distribution and antibiotic load
- Study longitudinal effect of antibiotics in manure on resistance
- Study circulation of pathogens and antibiotics in the ecosystem





## Pathogens and antibiotics in the chain



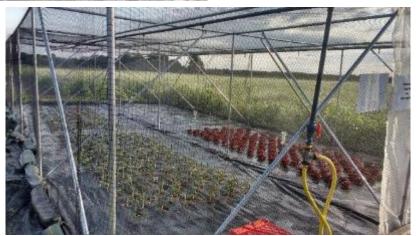








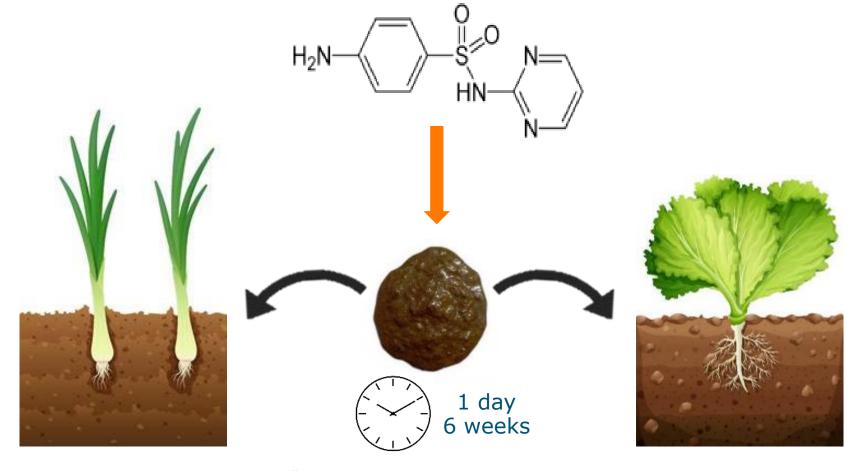






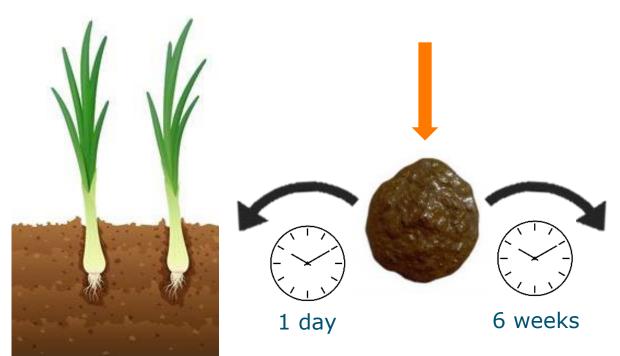


Sufadiazine (SDZ); mobile antibiotic











SDZ in manured soil:

 $Planting = 366 \mu g/kg$ 

 $Harvest = 82.2 \mu g/kg$ 



 $Planting = 273 \mu g/kg$ 

 $Harvest = 66.6 \mu g/kg$ 





|             | Manure treatment |           |           |  |  |  |  |
|-------------|------------------|-----------|-----------|--|--|--|--|
|             | Untreated -6 wee |           | -1 day    |  |  |  |  |
|             | SDZ (ppb)        | SDZ (ppb) | SDZ (ppb) |  |  |  |  |
| Rhizosphere | ND               | 0.47      | 0.47      |  |  |  |  |
| Root        | ND               | ND        | ND        |  |  |  |  |
| Shoot       | ND               | 0.07      | 0.08      |  |  |  |  |





|             | Manure treatment |               |           |               |           |               |  |  |
|-------------|------------------|---------------|-----------|---------------|-----------|---------------|--|--|
|             | Untreated        |               | -6 weeks  |               | -1 day    |               |  |  |
|             | SDZ (ppb)        | LogCFU/g soil | SDZ (ppb) | LogCFU/g soil | SDZ (ppb) | LogCFU/g soil |  |  |
| Rhizosphere | ND               | 5.79          | 0.47      | 6.58*         | 0.47      | 6.57*         |  |  |
| Root        | ND               | 4.63          | ND        | 6.04*         | ND        | 5.69*         |  |  |
| Shoot       | ND               | 4.10          | 0.07      | 3.78          | 0.08      | 3.65          |  |  |

\* CFU values are significantly different (P<0.001) from nonfortified manure





- Sulfadiazine was taken up by lettuce and leek plants in low quantities.
- Higher SDZ-resistant bacterial numbers were found in the rhizosphere and roots of lettuce and leek

• *E. coli* was transmitted from manure to lettuce and leek plants and could persist in the leek rhizosphere during winter time.





# Take home message







### Take home message

- The risks of pathogens in manure for humans, animals and the environment is currently considerd to be low.
- Antibiotic residues occur in many reservoirs and we can now predict where the risk of occurrence of individual compounds is the highest.
- Antibiotic treatments cause alterations in the manure microbiome which is still observed in soil, rhizosphere and root after manure application.
- Interdisciplinary research involving different science groups yields new perspectives and high impact results.





## Thank you!

WPR: Leo van Overbeek

WEnR: Joost Lahr, Louise Wipfler,

Jaap van Os

WLR: Paul Hoeksma

WBVR: Dik Mevius, Alex Bossers

WECR: Nico Bondt, Tanja de Koeijer

WFSR: Milou van de Schans, Mariel

Pikkemaat





