The impact of the use of organic fertilisers in ruminants farms and the selection and spread of AMR

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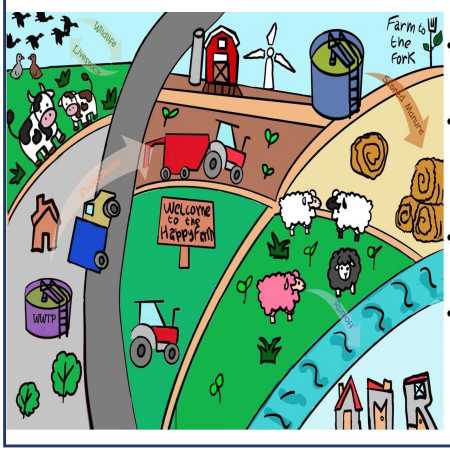
1. INTRODUCTION

- Antimicrobial Resistance (**AMR)** is ranked one of the top ten global public health threats facing humanity by WHO.
- Antimicrobials used in human and vet medicine end up in the environment and may impact the agricultural and environmental ecosystem.
- The spread of antimicrobial resistance genes (ARG) into soils, may limit the use of organic fertilizers in grasslands.
- Pasture soils treated with organic fertilisers are at higher risk of exposure to antimicrobial drug residue and AMR genes (ARGs), and may result in higher prevalence and diversity of ARGs and resistant bacteria in soil and in the gut flora of animals that graze on it.

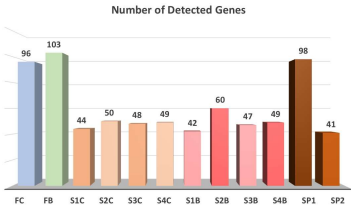
2. METHODS

- **Pilot study**: **Sewage-sludge** experimental model.
- The model comprises a "sludge pellets-treated" plot and a control plot.
- Soil samples (n= 25 and animal faeces n= 50) were collected before and after sludge pellets application.
- DNA extraction. DNeasy PowerSoil Kit + MP FAST beads.



- **Current project**: Farm full picture.
- Fertilisation practices comparison (**manure**, slurry and sludge pellets).
- **Soils**, **waterways**, **animals** and **septic tanks**.
- **ARGs** vs **resistant bacteria** vs **antimicrobial** and **heavy metals** residues analysis.

3. RESULTS



- A total of 125 ARGs and MGEs qPCR were identified in one or more samples

Fig.1- Distribution of positive ARG and MGE assays across different samples. FC- Faecal Control; FB- Faecal sludge pellets; S1C-S4C- Soil Control; S1B- Soil sludge pellets (pre-application- Spring); S2B- Soil sludge pellets (post-application-Spring); S3B- Soil sludge pellets (pre-application-Autumn); S4B- Soil sludge pellets (post application-Autumn); SP1-SP2- Sludge pellets (Spring-Autumn).

- **Sludge pellets** "Sp2" (Autumn), showed a higher relative abundance, namely MGE.

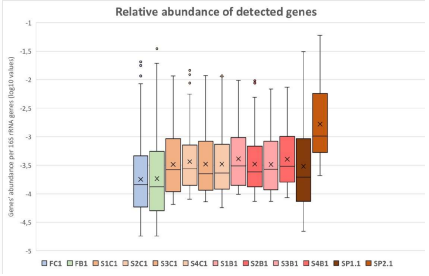
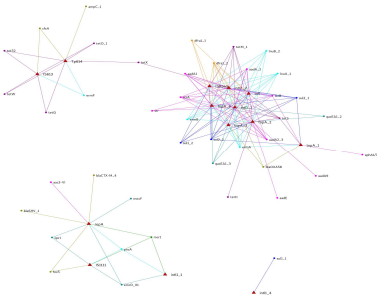


Fig.2- Genes relative abundance per 16S rRNA in the DNA samples (log10 values). FC1- Faecal Control; FB1- Faecal sludge pellets; S1-S4C1- Soil Control; S1B1- Soil sludge pellets (pre-application- Spring); S2B1- Soil sludge pellets (post-application-Spring); S3B1- Soil sludge pellets (pre-application-Autumn); S4B1- Soil sludge pellets (post application-Autumn); SP1.1-SP2.1- Sludge pellets (Spring-Autumn).



- The network analysis showed that ARGs detected in soil and faecal samples co-occurred with integrons, insertions sequences, transposases and plasmid related genes.

Fig. 3- Network Analysis of ARG and MGE Co-occurrences: Spearman's correlation of p > 0.8 with p-value < 0.05.

4. CONCLUSIONS

- No significant difference between sludge pellets-treated pasture and non-treated pasture samples.
- Sludge pellets presented a high number of ARGs and MGEs, which may be a concern for long-term application.

5. FUTURE WORK

- Impact of different strategies for grassland fertilisation on AMR selection

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