

## Introduction

Antimicrobial resistance is severely hampering the treatment of infectious diseases of humans and animals and poses a substantial threat to Public Health and the global economy. Many antibiotic resistance genes inactivating clinically important antimicrobials appear to originate from environmental reservoirs like soil or water compartments. Along the food/feed chain, agricultural soils constitute a source and sink for environmentally-borne and anthropogenically introduced ARGs. Antibiotic resistant bacteria and ARGs from manure generated in animal husbandry interact with endogenously present bacteria and ARGs in agricultural fields and can re-enter the food/feed chain via plant-derived feed contaminated with ARGs.



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## Methodology

Total DNA was isolated from soils collected from maize fields with inorganic and organic fertilization located in the Hydrology Open Air Laboratory (HOAL, Petzenkirchen, Austria), non-agricultural comparator soil from deciduous forest, pristine alpine regions and protected wetlands in a national park, from inner-city park areas and boulevards. Pig manure, compost and faeces were also analysed. ARG relative and absolute abundances were determined per gram soil dry-weight using TaqMan qPCR for the following targets: *sul1*, *ermB*, *vanA*, *tet(W)*, *bla<sub>TEM-1</sub>*, *aph(3')-IIa* and *aph(3')-IIIa*. Bacterial biodiversity was determined by 16S targeted amplicon sequencing.

## Results

- Concentrations of certain ARGs significantly increased 1 day after manure application in exposed agricultural soils and gradually returned to baseline levels present at the onset of the experiment over the crop growing season.
- *vanA* concentrations were not influenced by manure application.
- The forest closely associated to the fields showed more than  $10^5$  copies of *tet(W)*/g soil which corresponded to baseline levels observed in the fields and the tested pristine areas.
- Pristine protected national park wetlands and alpine soil samples showed distinctly lower *sul1* concentrations compared to the background loads observed in manured agricultural fields but similar concentrations with the non-manured field over the whole monitoring period.
- The urban soil shows the highest overall microbial biodiversity, while the organic fertilizer show the lowest biodiversity.

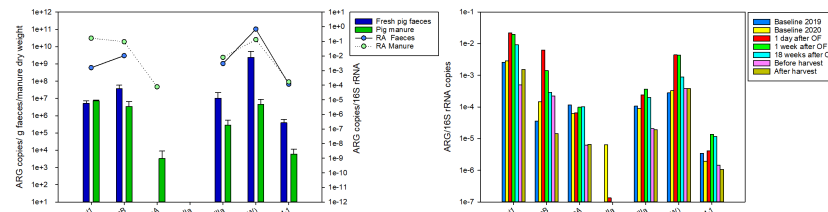


Figure 1. ARGs relative and absolute abundances in fresh pig faeces and manure

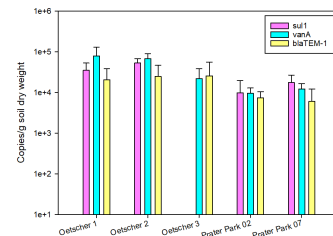


Figure 3. *Sul1*, *vanA* and *bla<sub>TEM-1</sub>* abundance in pristine alpine and inner city soils

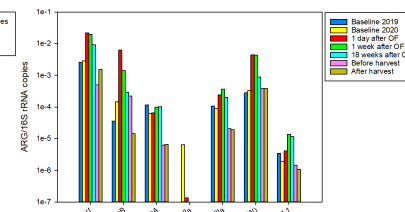


Figure 2. ARGs relative abundance in agricultural manured soil

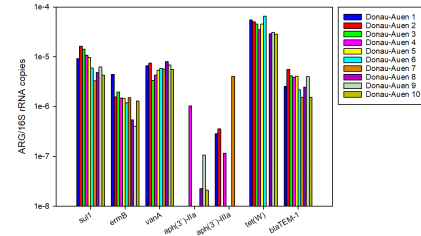


Figure 4. ARGs relative abundance in protected wetlands in a national park soil

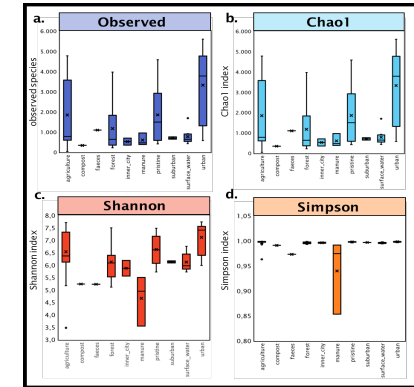


Figure 5. Alpha diversity metrics of different soil types. All boxplots are denoted using the inter-quartile ranges (IQR) with a median as a black line within the boxes. The arithmetic mean is represented as an “x” and single dots depict the outliers. (a) Boxplot of the Observed taxa count in different fields. (b) Boxplot of the species richness estimator Chao1. (c) Boxplot of the species diversity index Shannon. (d) Boxplot of the species diversity index Simpson.

## Conclusions

- Challenge of agricultural fields with ARG-containing manure led to an increase in the abundances of certain soil-borne ARGs.
- ARG dissemination route via freshly introduced manure-derived gut bacteria is not capable to establish exogenous ARGs permanently in exposed soil microbial communities.
- *Tet(W)* and *sul1* are omnipresent in agricultural and “pristine” soils from protected wetlands and alpine regions under low anthropogenic pressure.

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