

# Antibiotic resistance genes in agricultural soils in Germany

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## Introduction and aim of the study

Antibiotic resistance genes (ARGs) in agricultural soils represent an important environmental reservoir with potential relevance for human and animal health. Agricultural management practices, particularly the application of organic fertilisers, may introduce antibiotics and resistant bacteria into soils and promote their persistence and dissemination.

The aim of this study was to assess, at a national scale,

- the occurrence and relative abundance of selected ARGs in agricultural soils across Germany,
- to explore potential co-occurrence patterns of ARGs with pharmaceutical residues, and
- the influence of fertilisation practices on ARG distribution.

## Sampling

- 51 agricultural sites across Germany (50 arable land, 1 grassland)
  - Sampling period: 2022–2024
  - Soil layers: 0–5 cm and 5–20 cm
- 3 reference soils from the German Environmental Specimen Bank (minimal anthropogenic influence)
- Information on fertilisation practices compiled where available

## Gene panel

- A targeted set of ARGs conferring resistance to key antibiotic classes was analysed including macrolides (*ermA*, *ermB*), tetracyclines (*tetA*), aminoglycosides (*aacC4*, *aadA1*), trimethoprim (*dfrA1*),  $\beta$ -lactams (*blaCTX-M1*), quinolones (*qnrS*) and colistin (*mcr-3*).
- The class 1 integrase gene (*intl1*) was included to assess the potential role of mobile genetic elements in ARG dissemination.

## Analytical approach

- Quantification of genes by qPCR, normalised to 16S rRNA
- Gene presence/absence and relative abundance assessed

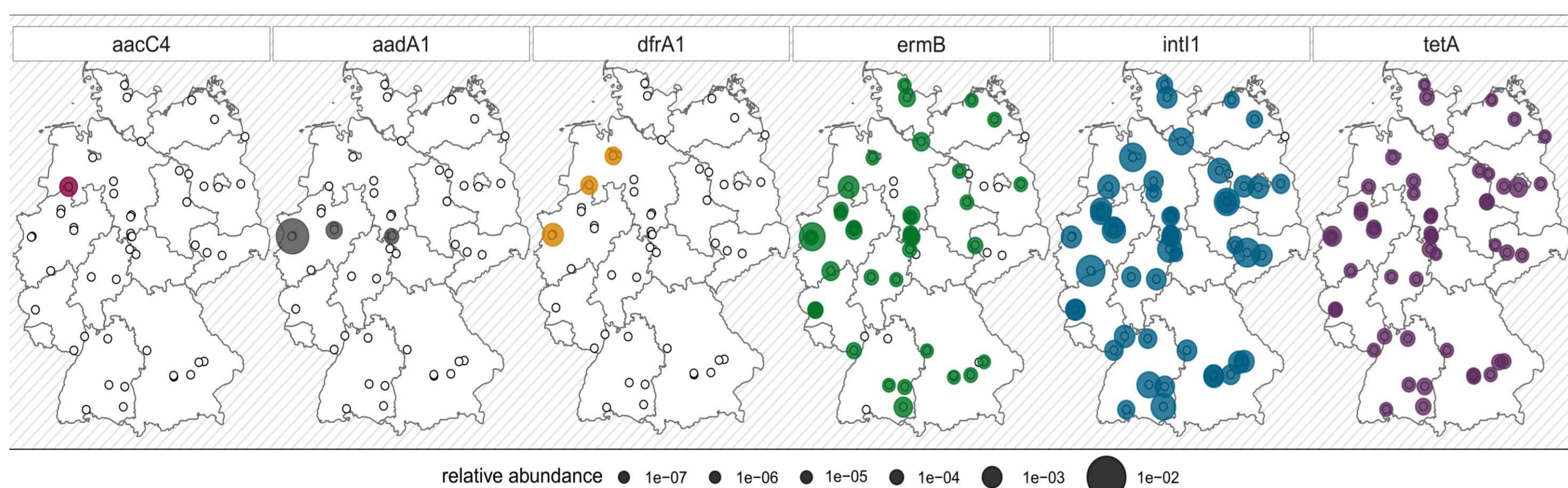


Figure 1: Spatial distribution of relative ARG/*intl1* abundance across Germany

## Occurrence of ARGs and *intl1* (Fig. 1, 3)

- *intl1*, *tetA*, and *ermB* were the most frequently detected genes and were detected even in reference soils, indicating background presence
- *aacC4*, *aadA1* and *dfrA1* were only sporadically found
- *blaCTX-M1*; *qnrS*; *mcr-3*, and *ermA* were not detected

## Spatial patterns across Germany (Fig. 1)

- *intl1*, *tetA*, and *ermB* are widely distributed across Germany
- The rare ARGs are mainly clustered in northwestern regions that overlap with areas of high veterinary antibiotic sales. This suggests a link between livestock density, manure application and ARG distribution.

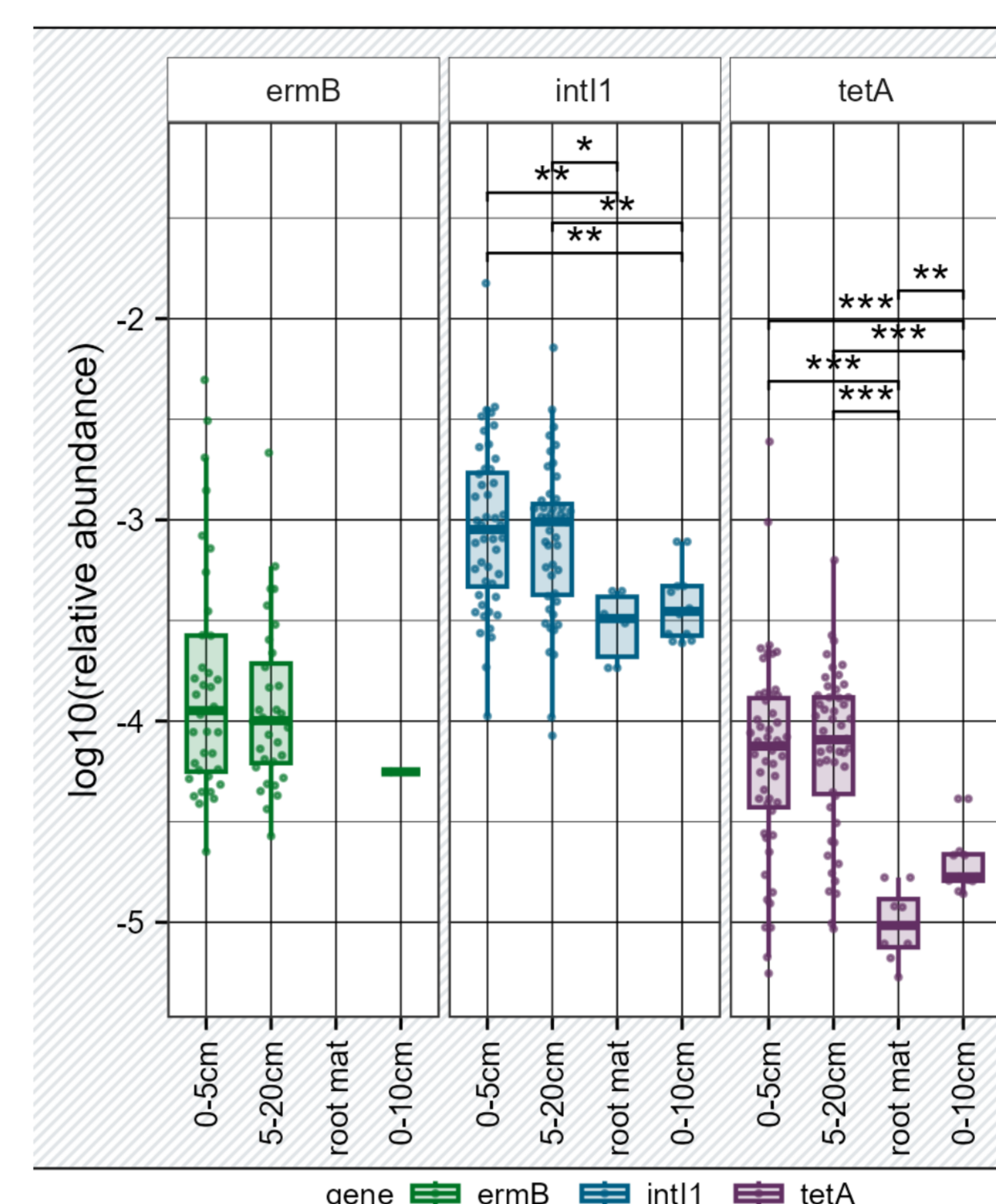


Figure 2: Vertical gene distribution in agricultural (0-5cm; 5-20cm) and reference samples (root mat; 0-10cm)

## Vertical distribution (Fig. 2)

- No significant differences of *ermB*, *intl1* and *tetA* abundances between upper and lower soil layer indicate a relatively uniform vertical distribution in agricultural soils
- Agricultural soils showed significantly higher abundances (*intl1*, *tetA*) than reference soils

## Influence of fertilisation practices (Fig. 3)

- Soils amended with organic fertilizers exhibited significantly higher ARG and *intl1* abundances than reference soils
- Strongest enrichment observed in slurry and slurry/manure-amended soil
- Effects were most pronounced in topsoil, but detectable also in subsoil
- Highlights the relevance of livestock-related inputs for ARG enrichment

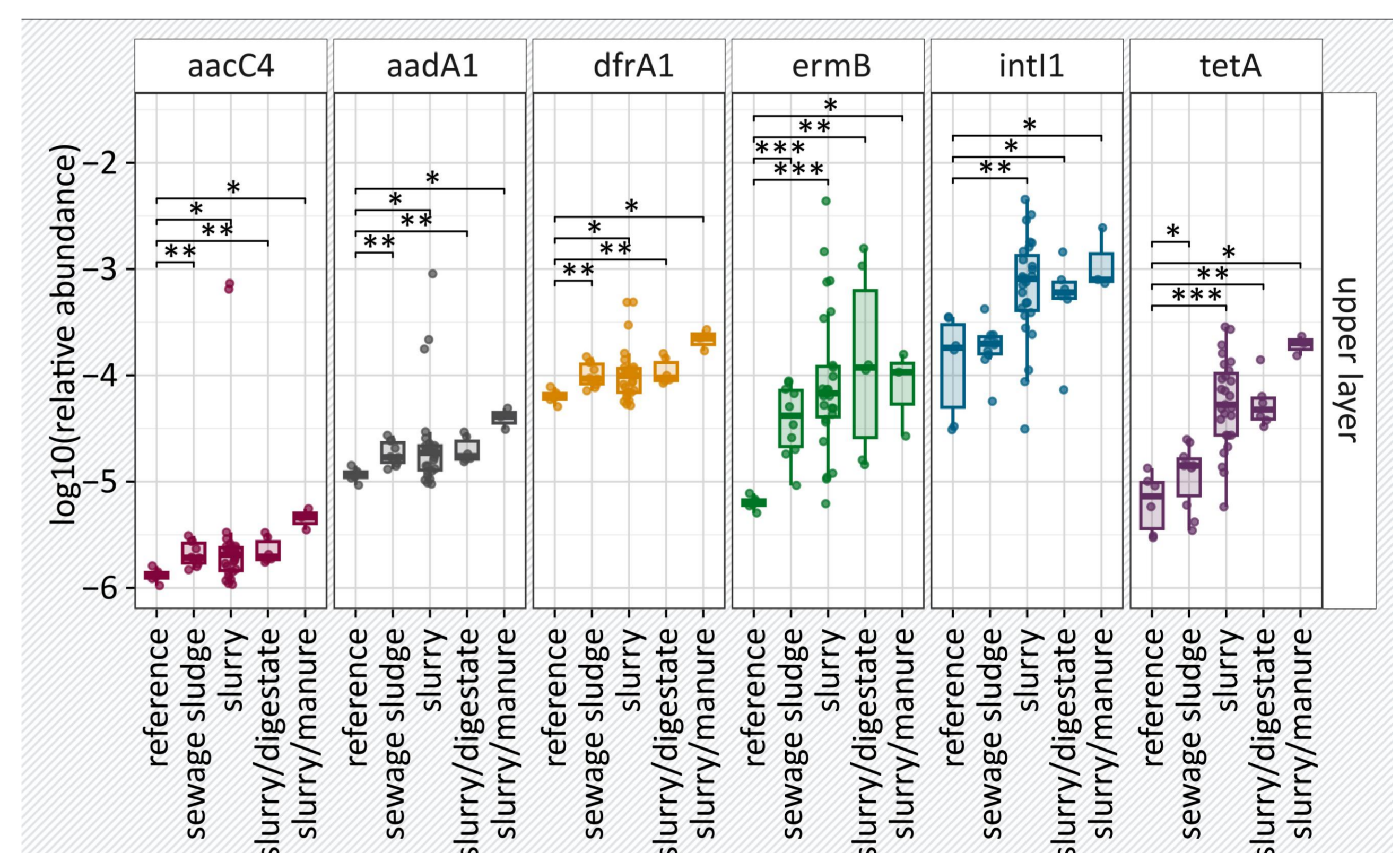


Figure 3: Abundance of ARGs and *intl1* in soils treated with various fertilisation methods compared to reference samples

## ARG co-occurrence and mobile genetic elements (data not shown)

- Moderate correlation ( $R^2=0.6$ ) between *intl1* and *tetA*, indicating a role of horizontal gene transfer
- Weaker association ( $R^2=0.21$ ) between *intl1* and *ermB*, suggesting different mobility patterns
- Supports the use of integrons as indicators of anthropogenic pressure, rather than markers of individual ARGs

## Conclusion:

Despite low detection frequencies of antibiotics, agricultural soils showed higher ARG abundances than reference soils, particularly under organic fertilisation regimes. These findings underline the importance of integrated assessments that jointly consider the presence of chemical residues, agricultural management, and resistance genes when evaluating environmental contributions to human and environmental health risks.

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## Funding:

This project was funded by Federal Ministry for the Environment, Climate Action, Nature Conservation and Nuclear Safety and supervised by the German Environment Agency (FKZ 3720 63 4020)