

# Antibiotic resistance genes in manure-amended paddy soils across eastern China: Occurrence and influencing factors

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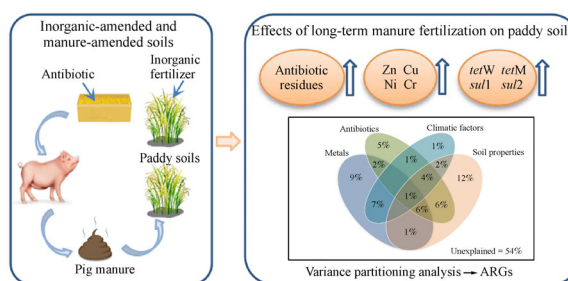
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## HIGHLIGHTS

- Manure fertilization resulted in antibiotic residues and increased metal contents.
- The *tet* and *sul* genes were significantly enhanced with manure fertilization.
- Soil physicochemical properties contributed to 12% of the variations in ARGs.
- Soil metals and antibiotics co-select for ARGs.

## GRAPHIC ABSTRACT



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## ABSTRACT

Pig manure, rich in antibiotics and metals, is widely applied in paddy fields as a soil conditioner, triggering the proliferation of antibiotic resistance genes (ARGs) in soil. However, comprehensive studies on the effects of manure fertilization on the abundance of ARGs and their influencing factors are still insufficient. Here, pig manure and manure-amended and inorganic-amended soils were collected from 11 rice-cropping regions in eastern China, and the accumulation of antibiotics, metals, and ARGs was assessed simultaneously. The results showed that manure fertilization led to antibiotic residues and increased the metal content (i.e., Zn, Cu, Ni, and Cr). Tetracycline and sulfonamide resistance genes (*tetM*, *tetO*, *sul1*, and *sul2*) were also significantly enhanced with manure fertilization. According to variance partitioning analysis, the most important factors that individually influenced ARGs were soil physicochemical properties, accounting for 12% of the variation. Significant correlations between soil nutrients and ARGs indicated that manure application enhanced the growth of resistant microorganisms by supplying more nutrients. Metals and antibiotics contributed 9% and 5% to the variations in ARGs, respectively. Their co-occurrence also increased the enrichment of ARGs, as their interactions accounted for 2% of the variation in ARGs. Interestingly, Cu was significantly related to most ARGs in the soil ( $r = 0.26-0.52$ ,  $p < 0.05$ ). Sulfapyridine was significantly related to *sul2*, and tetracycline resistance genes were positively related to doxycycline. This study highlighted the risks of antibiotic and ARG accumulation with manure fertilization and shed light on the essential influencing factors of ARGs in paddy soils.

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## 1 Introduction

To promote animal growth and prevent disease, antibiotics are added to animal feed, and most antibiotics can not be

absorbed and metabolized by animals (Rahman et al., 2018). In fact, international health organizations disapprove the use of antibiotics for animal growth promotion, and several countries, such as the European Union (EU), have recognized this and taken steps to reduce the use of antibiotics animal production (Heuer et al., 2011a). However, antibiotics are still widely used in the livestock industry in some Asian countries to increase production

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