

Antibiotics and antibiotic resistance genes in agricultural soils: A systematic analysis

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Critical Reviews in Environmental Science and Technology

DOI: 10.1080/10643389.2022.2094693

Published: 03/04/2023

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA): Wu, J., Wang, J., Li, Z., Guo, S., Li, K., Xu, P., Ok, Y. S., Jones, D. L., & Zou, J. (2023). Antibiotics and antibiotic resistance genes in agricultural soils: A systematic analysis. *Critical* Reviews in Environmental Science and Technology, 53(7), 847-864. https://doi.org/10.1080/10643389.2022.2094693

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1	Type of paper: Research Article	
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- 2 Number of figures: 9
- 3 Number of text pages: 29

4 A systematic analysis of antibiotics and antibiotic resistance genes in

5 global agricultural soils

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25 Abstract

Antibiotics and antibiotic resistance genes (ARGs) reach the agricultural soils through 26 fertilization in the form of livestock manure are the main environmental pollutants at 27 28 present. This study discussed the sources and distribution characteristics of antibiotics and ARGs in global agricultural soils, revealed the main driving factors affecting 29 antibiotic concentration and ARGs abundance, analyzed the environmental risk, and 30 31 environmental impact of typical antibiotics, and summarized the common elimination 32 methods in current research. We retrieved the papers related to antibiotics and ARGs, and extracted 2093 observations from 135 papers. The results showed that cattle manure, 33 chicken manure, swine manure, and sewage sludge were the primary pollution sources 34 of antibiotics and ARGs in agricultural soils, and sulfonamides, tetracycline, 35 fluoroquinolones, and their corresponding ARGs were the main pollution types. The 36 37 distribution of the antibiotic concentration and the ARGs abundance was affected by land-use types and soil physicochemical parameters. The mobile genetic elements 38 (MGEs) play a vital role in promoting the dissemination of ARGs, and sull genes 39 strongly correlate with *intI*1. The occurrence, fate, and ecotoxicity of antibiotics and 40 ARGs in agricultural ecosystems have become the most pressing environmental issues. 41 Our results provide a systematic analysis of the environmental behavior of antibiotics 42 43 and ARGs in agricultural soils.

Keywords: veterinary antibiotic; antibiotic resistance genes; agricultural soil;
environmental factors; ecological risk; horizontal transfer

- 46 Synopsis statement: A systematical description for the distribution of antibiotics and
- 47 antibiotic resistance genes in agricultural soils worldwide.

48 Graphical Abstract



50 1 Introduction

Antibiotics are widely used in many countries worldwide to treat human diseases and protect the health of animals in agricultural fields ^{1,2}. Antibiotics cannot be fully absorbed in animals, and large amounts of antibiotics and their degradation products were introduced into agricultural ecosystems in the form of fertilization and irrigation through antibiotic-contaminated manures and sewage sludge ^{3–5}. More importantly, accounting for selective pressure on the environmental resistome, antibiotics are considered to be the driver of increasing antibiotic resistance genes (ARGs)⁶.

The increasing use of fertilizers in agriculture poses a rapidly increasing threat of 58 antibiotic and ARGs contamination ⁵. Distribution of antibiotic and ARGs abundance 59 varied greatly under different land-use patterns in soils. It has been documented that the 60 concentration of antibiotics differs significantly, typically in the µg antibiotic per kg 61 soil⁷. ARGs can spread from animal farms to surrounding agricultural soils, posing a 62 potential high risk to environmental ecology⁸. Remarkably, antibiotics and ARGs 63 contamination status in vegetable, paddy, and upland soil vary greatly. Continuously 64 manure fertilization significantly increased the ARGs abundances in typical greenhouse 65 vegetable production bases, and antibiotic types and vegetable species affected the 66 distribution of ARGs in soil 9,10. The occurrence and abundance of most ARGs 67 increased significantly in wheat soils irrigated with wastewater ¹¹. Organic fertilizer 68 partially replaced mineral fertilizer significantly increased the relative abundance of *sul* 69 genes in north China under wheat-maize rotation ¹². Long-term application of organic 70 fertilizer usually increases antibiotic concentration and ARGs abundance in paddy soil, 71 72 and paddy soil showed higher ARG accumulation than dryland soil, corresponding to higher microbial biomass ^{13,14}. 73

Land-use of livestock and poultry manure is an essential agricultural measure. Intensive animal husbandry is the main cause of the increasing environmental contamination of antibiotics and ARGs in soil ¹⁵. Antibiotic-resistant bacteria (ARB) reproduction was induced in soils modified by cow manure ¹⁶. The long-term

application of chicken manure significantly increased the abundance and diversity of 78 ARGs and various classes of antibiotics in soil ¹⁷. The application of untreated swine 79 80 manure resulted in antibiotic and ARGs pollution, and changed the composition of soil microbial community ^{18,19}. Application of antibiotic-contaminated biogas slurry and 81 residue in the field increased the occurrence of ARB and ARGs ²⁰. Irrigated with tap 82 water spiked with different concentrations of antibiotics significantly altered microbial 83 populations in pot experiments ²¹. Therefore, the pollution of antibiotics and ARGs 84 caused by manure application is worth pondering. 85

Soil properties, including pH, soil organic carbon (SOC), total nitrogen (TN), soil 86 87 texture, and heavy metals, are the key factors determining the change and distribution of antibiotics and ARGs. The texture of soil with manure application determines the 88 persistence of target ARGs, thus influencing the fate of antimicrobial resistance in the 89 soil ²². The adsorption potential of sulfonamides increases with decreasing pH because 90 91 of the abundance of positively charged species capable of electrostatic binding to soil mineral surfaces under acidic conditions ²³. Reducing the manure pH promotes 92 93 degradation of sulfonamides and inhibits *sul* gene-level transfer, which may be a simple and effective management measure to reduce antibiotic resistance ²⁴. Pot experiments 94 comparing antibiotic resistance in three typical soils found that soil with the highest 95 SOC was least affected by swine slurry amendment ²⁵. In general, SOC strongly 96 influenced the retention of sulfonamides; the higher the SOC content, the higher the 97 adsorption capacity, and the lower the desorption capacity due to competition for 98 adsorption sites ^{26,27}. Clay improver greatly influenced the change of original ARB in 99

100 chicken manure compost, and the diversity and relative abundance of ARB in 4% clay was greater than that in high dose clay ²⁸. The soil TN was positively correlated with 101 sul1 in the agricultural soils of the Yangtze River Delta, China²⁹. The soluble organic 102 nitrogen was a critical explanatory variable for the model to determine the swine 103 manure-water distribution coefficients of typical veterinary antibiotics ³⁰. Metal 104 contamination plays an essential selective role in spreading antibiotic resistance 105 through the co-selection mechanisms ^{31,32}. However, many studies fail to adequately 106 address the role of soil properties as a factor affecting the distribution of antibiotics and 107 ARGs in agricultural soils. 108

109 Horizontal gene transfer of ARGs is considered a significant contributor to the spread of antibiotic resistance ^{33,34}. The class I integron-integrase gene (*intI*1) is a good 110 proxy for ARGs pollution ³⁵. There is an excellent positive co-occurrence pattern of 111 ARGs and mobile genetic elements (MGEs) in manure-amended greenhouse soils ³⁶. 112 113 The abundance of *intI*1 increased significantly in the soil exposed to pig manure, and was positively correlated with ARGs in a long-time field experiment ³⁷. Another study 114 115 revealed sub-inhibitory heavy metals widely present in various environments could promote resistance by promoting the horizontal transfer of ARGs ³⁸. This means that 116 the analysis of MGEs contamination status is imperative to better understand ARGs 117 118 dissemination in the soil.

Until now, numerous reports demonstrate the sources, spatial patterns, driving
factors, and environmental implications of antibiotics and ARGs in global estuarine and

coastal environments ^{39,40}, no comprehensive study of antibiotics and ARGs in 121 agricultural soils has been conducted. In this study, we collated the occurrence, 122 123 distribution, and risk of antibiotics and ARGs in agricultural soil. The objectives were to (1) analyze the pollution characteristics of antibiotics and the occurrence of ARGs in 124 agricultural soil under different land-use modes, and determine the potential ecological 125 risk of typical antibiotics in soil; (2) analyze the relationship between soil properties 126 and antibiotic concentration and ARGs abundance, and reveal the key factors affecting 127 its pollution characteristics; (3) discuss the coexistence mode of ARGs and antibiotics, 128 129 and the horizontal transfer of ARGs in the soil, and summarize the common elimination methods and effects in current researches. 130

131 2 Methodology

132 **2.1 Data collection**

To survey antibiotics and ARGs in agricultural soil, we performed a systematic search 133 using the ISI Web of Science (Thomson Reuters, New York, NY, USA), Google Scholar 134 (Google, Mountain View, CA, USA), and China National Knowledge Infrastructure 135 (CNKI, Beijing, China). Our search terms included ("soil*" OR "agricultural soil*") 136 AND ("antibiotic*" OR "antibiotic resistance gene*" OR "ARG*" OR "resistome*"). 137 Studies included in this study had to meet the following criteria: (a) both laboratory 138 field and incubation studies were selected; (b) only studies that had an adequate number 139 of replicates; (c) the means, standard errors, and replication of the variables could be 140 extracted directly from the text, tables, or digitized graphs. The following information 141

was documented for each study: the type of experiment, site, planting type,
contaminated type, soil physiochemical parameter, type of antibiotic and ARGs, the
concentration of antibiotics, and abundance of ARGs. A total of 2039 observations were
taken from 135 papers (Text S1). The distribution location of observations is shown in
Fig. 1. China accounted for about 85.87% of observations, and other countries,
including Israel, Brazil, Korea, Pakistan, Spain, and the USA accounted for 14.13%.



148

Fig. 1 The distribution of data collected from different countries. The point sizes
represent the number of replications, and different colors denote antibiotics and ARGs.

151 **2.2 Ecological risk assessment of antibiotics**

A risk quotient (RQ) was used to evaluate the potential risk and calculated as follows
 ^{41,42}:

154
$$RQ = \frac{MEC}{PNEC_{soil}}$$
(1)

155
$$PNEC_{soil} = PNEC_{water} \times K_d$$
 (2)

156
$$PNEC_{water} = EC_{50}/AF$$
 (3)

where MEC is the actual measured concentration in the environment ($\mu g k g^{-1}$), PNEC_{soil} 157 is the predicted no-effect concentration in soil (µg kg⁻¹), and PNEC_{water} is the predicted 158 no-effect concentration in water, and which represents the maximum drug 159 concentration that will not have an adverse effect on the microorganisms or the 160 ecosystem in the environment under the existing cognition ($\mu g L^{-1}$). K_d is the soil-water 161 162 partition coefficient (L kg⁻¹). EC₅₀ is the half-maximum effect concentration (mg L^{-1}). EC₅₀ is all obtained from the literature. AF is the evaluation factor, which is the 163 recommended value of the European Water Framework Directive (1,000). The 164 ecological risk parameters of antibiotics are summarized in Table S1. 165

166 RQ was used to assess the ecological risk of the target antibiotic. The RQ values 167 were mainly divided into four levels: $RQ \ge 1$ high risk; $0.1 \le RQ < 1$ medium risk; 0.01 168 $\le RQ < 0.1$ low risk; $RQ \le 0.01$ no risk ⁴³.

169 2.3 Statistical analysis

The mean concentrations of antibiotics and ARGs abundance were either provided in the publications or calculated using the original data. Concentrations that were "not detected" or "below detection limit" were entered as zero values. To minimize the differences in background bacterial abundances, the absolute number of all detected genes was further normalized to that of the ambient 16S rRNA gene. We used linear mixed-effects models to test the fixed effects of planting type, contaminated type, type of antibiotic, and ARGs on the concentration of antibiotics and the ARGs abundance,
respectively. Study ID was included as a random effect. The "nlme" package was used
to conduct linear mixed-effects models in R version 4.0.3 (R Core Team, 2020).
Multiple comparisons were made using the Tukey HSD test. The network analysis was
visualized on the Gephi platform (version 0.9.2).

181 3 Results

182 **3.1 Antibiotics in agricultural soils**

183 **3.1.1 Distribution characteristics of antibiotics in agricultural soils**

184 The concentrations of antibiotics in soil samples are shown in Fig. 2. In this survey, beta-lactam, sulfonamides, tetracyclines, and fluoroquinolones classes were the 185 dominant antibiotics with a concentration of 364.16, 241.17, 176.98, and 112.02 µg kg⁻ 186 ¹, respectively, much higher than others (Fig. 2A). Simultaneously, tetracycline, 187 chlortetracycline, oxytetracycline, sulfamethoxazole, norfloxacin, and ciprofloxacin 188 have higher concentrations, and their concentrations differed in several orders of 189 magnitude (Fig. S1). Applications of different manure could make a difference in the 190 contamination status of antibiotics. Soil applied with cattle, chicken, and swine manure 191 contains higher concentrations of antibiotics, followed by sewage sludge and mix 192 manure (P < 0.05; Fig. 2B). Concentrations of antibiotics in vegetable and paddy soil 193 194 were significantly higher than other planting types (P < 0.01; Fig. 2C).



Fig. 2 Concentrations of antibiotics in global agricultural soils. Violin plots show the concentration of antibiotics (A) for each type of antibiotics, (B) in the soil with different manure applied, and (C) from different land-use types. Different lowercase letters in each panel indicate significant differences between groups. The solid line inside the box represents the median.

200 **3.1.2** Correlation between antibiotics and soil physicochemical parameters

201	The correlations between the major environmental variables and the concentration of
202	antibiotics were analyzed for all studied data. A significant positive correlation was
203	observed between antibiotics and SOC ($P < 0.05$) and several heavy metals (e.g., Pb,
204	Hg, and Na) ($P < 0.01$; Fig. 3), respectively. In contrast above factors, the concentration
205	of antibiotics indicated negative correlations with clay, TN, Cd, and As ($P < 0.01$).



fluoroquinolones antibiotic showed a significant positive correlation with pH (P < 0.05), while showed a significant negative correlation with TN (P < 0.05). Moreover, the strongly negative relationship between macrolides and clay, and several heavy metals (e.g., Cu, Zn, and Pb), the significant correlation between aminoglycosides, amphenicols, and environmental factors existed in the soils in this study.



215

216 Fig. 3 Spearman correlations between antibiotics and environmental factors in soil.

217 Pairwise comparisons are shown, with a color gradient denoting spearman's correlation 218 coefficient. *** P < 0.001, ** P < 0.01, and * P < 0.05. Cu, copper; Zn, zinc; Pb, lead; Cd, 219 cadmium; Ni, nickel; Cr, chromium; As, arsenic; Hg, mercury; Na, sodium; Ca, calcium; Mg, 220 magnesium.

221 **3.1.3 Potential ecological risk of antibiotics**

To better elucidate the ecological risk of antibiotics in agricultural soils, the RQ values of concentrations of sulfonamides, tetracyclines, and fluoroquinolones classes in soil were analyzed (Fig. 4). In this study, the RQ of the three antibiotic classes detected were all less than one. The RQ of norfloxacin is significantly higher than the other antibiotics of all target compounds and may present a relatively higher ecological risk, followed by the sulfadiazine. Contrarily, the RQ values of other antibiotics examined here were below 0.1 poses a low or no environmental risk. We can speculate that the soil was seriously polluted with antibiotics, leading to ARGs contamination and potential ecological risks.



Fig. 4 Risk quotient values of various antibiotics in agricultural soil. The RQ values could be divided into four levels according to the European Commission: $RQ \ge 1$ high risk; $0.1 \le RQ$ </br/>
4 represents the values of 0.01 and 0.01, respectively. RQ values greater than one is not shown in

the figure for clearer display (6% of total observations).

237 **3.2 ARGs in agricultural soils**

238 **3.2.1** The abundance of ARGs in agricultural soils

The abundance of ARGs varied greatly across soils. ARGs conferred resistance to 239 multidrug, sulfonamide, and tetracycline classes were the most abundant ARG subtypes 240 in samples, while other ARGs were found at much lower levels (Fig. 5A). Additionally, 241 the abundance of the *sul*1 and *sul*2 genes was higher than that of other *sul* genes, the 242 tetM, tetW, tetO, and tetX were the most abundant across tet genes, and intI1 was the 243 most abundant integron genes (Fig. S2). The abundance of ARGs was found at much 244 245 higher levels in soil applied with cattle, chicken, swine manure, and mixed manure (P < 0.05; Fig. 5B). It is noteworthy that a similar distribution pattern was observed for 246 ARGs among planting types compared with antibiotics (Fig. 5C). 247



Fig. 5 The abundance of ARGs in global agricultural soils. The abundance of ARGs in soil
(A) for each type of AGRs, (B) in the soil with different manure applied, and (C) from different
land-use types. Different lowercase letters in each panel indicate significant differences
between groups. The solid line inside the box represents the median.

3.2.2 Correlation between ARGs and soil physicochemical parameters

254

255	correlated with the clay content generally but negatively correlated with the SOC, TN,
256	and metallic elements (e.g., Cd, Ni, Cr, As, and Hg) ($P < 0.01$; Fig. 6).
257	Specifically, sulfonamide and tetracycline classes exhibited significant negative
258	correlations with pH ($P < 0.05$). Almost no correlations were found between most heavy
259	metals and sulfonamide ARGs except for Na, Ca, and Mg ($P < 0.05$). Cu, Zn, and Pb
260	are correlated positively with tetracycline ARGs ($P < 0.01$). However, we found an
261	opposite trend between other various heavy metals and tetracycline ARGs. Moreover,
262	none of the ARGs detected above in samples showed a significant correlation with SOC,
263	TN, and clay.

Correlation analysis showed that the abundance of target ARGs in soil was positively

Unlike the above two ARGs, the analysis revealed that beta-lactam and multidrug 264 ARGs were significantly and positively correlated with pH. A stronger correlation was 265 found observed between beta-lactam and pH (P < 0.01) than between multidrug and pH 266 (P < 0.05). Our results did not show statistically significant correlations between the 267 beta-lactam and multidrug ARGs with SOC and clay. Beta-lactam ARGs showed a 268 significantly negative correlation with TN but a significantly positive correlation with 269 270 various heavy metals (P < 0.01). Furthermore, the MGEs were positively correlated with Ni (P < 0.05), while other soil physicochemical parameters were less intensely or 271 even not associated. 272





Fig. 6 Spearman correlations between abundance of ARGs and environmental factors in

soil. Pairwise comparisons are shown, with a color gradient denoting spearman's correlation coefficient. *** P < 0.001, ** P < 0.01, and * P < 0.05. Cu, copper; Zn, zinc; Pb, lead; Cd, cadmium; Ni, nickel; Cr, chromium; As, arsenic; Hg, mercury; Na, sodium; Ca, calcium; Mg, magnesium.

279 **3.2.3** Correlations between ARGs and *intI*1

280	Based on the correlation analysis between the most prevalent mobile genetic elements
281	(<i>intI</i> 1) and ARGs (Fig. 7), there were strong positive correlations between <i>intI</i> 1 and the
282	abundance of sulfonamide ARGs (sul1) in soils ($P < 0.01$) and weaker correlations with
283	the rest of ARGs. This might indicate that sull is generally located on the more readily
284	disseminated class I integron and a lower frequency of horizontal gene transfer caused
285	by <i>intI</i> 1 for other types of ARGs. This is not surprising given that these correlations do
286	not entirely reflect variations in ARGs dissemination based on existing data.





Fig. 7 Spearman correlations between the abundance of *intI*1 and ARGs in soil. Pairwise comparisons are shown, with a color gradient denoting spearman's correlation coefficient. *** P < 0.001, ** P < 0.01, and * P < 0.05.

291 4 Discussion

292 **4.1 Effects of land-use on the distribution of antibiotics and ARGs**

The distribution characteristics of antibiotics and ARGs were significantly affected by land-use patterns. Our finding provides evidence that both the concentration of antibiotics and the abundance of ARGs were higher in the paddy fields than that in uplands, which may be attributed to differences in the degradation of antibiotics and the migration capacity of ARGs in soil under different planting types. The rate of transformation and degradation of antibiotics in the soil largely depends on many

abiotic factors, and anaerobic conditions significantly prolonged the half-life of 299 antibiotics ⁴⁴. Paddy soil generally showed a higher accumulation of ARGs due to 300 higher microbial biomass that host populations of ARGs under anaerobic conditions ¹⁴. 301 Increasing the amount of manure in a paddy-upland rotation system resulted in a sharp 302 303 increase in soil ARGs; however, the high moisture content may block the path of contact from the manure-improved soil to the phyllosphere, thereby affecting the dissemination 304 of ARGs ^{45,46}. Therefore, it should be fully considered that multiple soil factors may 305 drive the distribution of antibiotics and ARGs to confirm the different fates of them in 306 307 the soil introduced by manure under aerobic and anaerobic conditions.

308 Prior studies that have noted the behavior of antibiotics and ARGs proved to be governed by the combination of soil and plants under the real planting conditions ⁴⁷. 309 Further analysis showed that vegetable species had a significant effect on the content 310 311 of antibiotics and ARGs in the soil, and the soil planting pakchoi accumulates more than other vegetables (Fig. 8). Antibiotics and ARGs absorbed by plants are further 312 harmful to human health as they travel up the food chain. Therefore, it is may represent 313 314 higher health risks than other vegetables when consumed pakchoi by humans. Plant root exudates indirectly affect the fate of antibiotics by changing soil properties during 315 vegetable growth ¹⁰. Antibiotics accumulate in plants through passive absorption by 316 water ⁴⁸, which is the main reason for dissipation and removal of antibiotics effects 317 differed in the soil of different vegetable growing types. The growth of plant roots alters 318 319 the soil microbial composition, and the selectivity of plants to environmental bacteria may be an important factor leading to the difference of distribution of ARGs ^{49,50}. 320

Additionally, different types of antibiotics are absorbed and accumulated in different parts of plants ^{51,52}. Although this aspect is not involved in this study, the environmental behavior of complex types of antibiotics in agricultural soils warrants more exhaustive investigation.



Fig. 8 Concentrations of antibiotics and abundance of ARGs in vegetable soils. Violin plots
show the concentration of antibiotics (A) and abundance of ARGs (B) for each type of vegetable.
Different lowercase letters in each panel indicate significant differences between groups. The
solid line inside the box represents the median.

4.2 Effects of fertilization on the distribution of antibiotics and ARGs

The contamination of antibiotics and ARGs in the soil is related to fertilization methods and local farming practices. The amount of antibiotics taken by farm animals varies by livestock species and region, and the abundance of ARGs is related to the amount of antibiotics used by these animal species ⁵³. Previous studies found significant differences in the abundance of ARGs in different types of farm soils, with the highest

concentrations in pig farm soils ⁵⁴. This also accords with our observations above that 336 swine manures generally had higher levels of ARGs, followed by soils applied cattle 337 338 and chicken manure, and the ARGs contamination caused by the mixed-use of manure should not be ignored. Pork production is the main pillar industry of animal husbandry, 339 340 and antibiotics are widely used in pig breeding due to the disease types and incidence rate of pigs being relatively high compared with other animals ⁵⁵. Metagenomic 341 sequencing indicated that the long-term application of swine manure could increase 342 microbial diversity and reshape ARGs in agricultural soils ⁵⁶. Cattle manure is widely 343 used as fertilizer because it is an excellent source of nutrients, they exhibit more 344 abundant ARGs than poultry manure, and the application of fresh cattle manure may 345 lead to a greater transmission potential of antimicrobial resistance genes in the soil ⁵⁷. 346 347 The current study found that a lower bacterial diversity in poultry litter might be linked to the higher total solids ⁵⁸, which in turn affects the abundance of ARGs in the soil. 348 Taken together, it is essential to reasonably control the abuse of antibiotics in livestock 349 350 and poultry breeding.

Applied sewage sludge increased soil microbial activity, thereby contributing to the risk of the spread of ARGs and MGEs ^{59,60}. Peri-urban agricultural plots were irrigated with treated wastewater and groundwater from rural organic farms suggested the agricultural practices and water quality for irrigation influenced microbial communities and associated resistances ⁶¹. However, in reviewing the literature, the study did not reveal the risk of sludge improvement associated with the development of antibiotic resistance in an agricultural field trial ⁶². Our study demonstrated that antibiotics and ARGs pollution caused by sewage sludge were not significant compared
with other fertilization methods. Hence, it should be inferred carefully due to the
different sludge quality and application practices in various agricultural regions.

361 4.3 Effects of soil environmental factors on the distribution of antibiotics and 362 ARGs

The sorption ability is stronger at a lower pH value and higher clay fraction in the soil 363 ⁶³. Although the effects of pH on the occurrence of soil antibiotics and ARGs have been 364 365 studied extensively, no unified results have been formed. Our results indicated that pH 366 was not strongly correlated with antibiotics and ARGs, and the effects of pH on them were not consistent. Only beta-lactam and tetracyclines and their corresponding ARGs 367 were significantly positively and negatively correlated with pH, respectively. The 368 adsorption mechanism and degradation performance of antibiotics were significantly 369 different in soils with different pH values. Hydrophobic interaction and cation exchange 370 371 might be the primary mechanism of tetracyclines adsorption in acidic and alkaline soil, respectively ⁶⁴. Studies on the effects of pH on the adsorption capacity of 372 373 sulfamethoxazole in pasture soils showed that the adsorption capacity decreased when pH increased ⁶⁵. pH value is an essential parameter of the hydrolysis process of 374 antibiotics; the hydrolysis rate of base catalysis was significantly higher than that of 375 acid catalysis and neutral pH and altered the fate of antibiotics ⁶⁶. Moreover, pH also 376 strongly affected the bacterial diversity and shaped the ARGs profiles, given that 377 bacteria were the dominant carriers of ARGs ⁶⁷. Positive correlations between sorption 378

coefficients and clay content were found for several antibiotics ⁶⁸. Contrary to the results, this study did not detect evidence for the phenomenon, which may relate to the type of antibiotics we analyzed. The findings observed a significant positive correlation between ARGs and clay content in this study mirror those of the previous studies that soil texture was the top edaphic factor that correlated with the abundance of ARGs ⁶⁹.

Nutrient factors in fertilized soil affected the distribution of ARGs, with SOC and 384 TN being contributed significantly to the distribution of ARGs ^{70–72}. In general, soils 385 with higher SOC showed higher adsorption for antibiotics, dissolved organic matter 386 released from farmland straw may reduce the ecological risk of sulfamethoxazole by 387 inhibiting the migration of antibiotic pollutants ⁷³. The removal of SOC by calcination 388 results in a sharp decrease in the adsorption capacity of antibiotics ⁷⁴. This finding 389 supports the result that there was a significant positive correlation between SOC content 390 391 and antibiotics. However, the risk is serious in soils with lower SOC because antibiotics adsorption is limited ⁷⁵. TN was significantly positively correlated with the abundance 392 of target ARGs in wastewater contamination of coastal waters ⁷⁶. Our study suggested 393 394 that TN showed a significant negative correlation with ARGs. This somewhat contradictory result may be due to promoting soil microbial degradation of antibiotics 395 under high TN content ⁷⁷. More research on this topic needs to be undertaken before 396 the association between soil physical and chemical properties, and distribution of 397 antibiotics and ARGs is more clearly understood. 398

399

Large amounts of heavy metals are used in large intensive livestock and poultry

farms worldwide to promote growth and control disease ⁷⁸. As mentioned in the 400 literature review, an obvious co-selection mechanism between heavy metals and ARGs 401 includes co-resistance, cross-resistance, co-regulation, and biofilm phenotypes ³¹. In the 402 first instance, a positive correlation between ARGs and high metal concentrations, some 403 404 antibiotic-resistant bacterial strains also showed tolerance to high concentrations of heavy metals ^{79,80}. In the next place, most ARGs and heavy metal resistance genes are 405 carried on MGEs, which contributes to their spread and reproduction in contaminated 406 soils. Heavy metal resistance genes are thought to play an essential role in the 407 proliferation of ARGs⁸¹. Furthermore, heavy metals can trigger the proliferation of 408 ARGs by increasing the abundance of MGEs or altering the bacterial community 409 structure⁸². 410

Specifically, there was a significant positive correlation between tetracycline 411 ARGs and Cu, Zn, and Pb, which was confirmed by related studies ^{83,84}. As and Cd as 412 413 common heavy metals pollution types had a relatively high accumulation proportion in the soil, the strong positive correlation between Cd, As, and beta-lactam ARGs in this 414 415 study were found. Cd stress in sludge compost increased the relative abundance of target ARGs in rhizosphere soil, which exacerbated the risk of ARGs transferring from 416 the underground to the aboveground part of plants ⁸⁵. As (III) may coexist with ARGs 417 on the mobile genome as a co-selection driver in paddy soils ⁸⁶. Notably, long-term 418 exposure to far below the minimal inhibitory concentration of certain metals also 419 420 increases bacterial resistance to antibiotics, demonstrating that even low concentrations of heavy metals may pose a considerable risk to the transmission of ARGs⁸⁷. 421

Additionally, the occurrence of co-selection is also related to soil characteristics; Hg-422 driven ARGs were observed in alkaline soils but not in neutral pH soils ⁸⁸. More 423 424 seriously, multiple antibiotics form complexes with metal cations (e.g., Cu and Zn) that impede (or enhance) antibiotic activity 89. In this study, the significant positive 425 correlation between amphenicols, tetracyclines, and heavy metals may be due to the 426 complexes promoting the adsorption of antibiotics in the soil ^{90,91}. Therefore, 427 controlling heavy metal pollution is crucial to reducing the adsorption, transfer, and 428 diffusion of antibiotics and ARGs in agricultural soils. 429

430 4.4 Coexistence of antibiotics, MGEs, and ARGs in agricultural soils

The abundance of ARGs is associated with the overuse and misuse of antibiotics in the 431 soil. To determine the association between antibiotics and ARGs in agricultural soils, 432 we performed network analysis on the antibiotics and ARGs data of the same treatment 433 (Fig. 9). Specifically, tetracyclines are closely related to tetB, tetX, tetM, tetL, tetW and 434 435 tetO, but the correlation between sulfonamides and sul1 and sul2 is weak, although they are the main ARGs. Pot experiment showed that the abundance of sull in 436 sulfamethoxazole-contaminated soil increased significantly ⁹². The emergence of 437 tetracycline and quinolone resistance genes was consistent with the results of antibiotic 438 risk assessments in farm soils fertilized with animal manure ⁹³. It is interesting to note 439 that an antibiotic can be closely associated with the generation of different types of 440 441 ARGs, which may cause more significant risks to the farmland environment and is worth our vigilance. Assessment of the relative contributions of antibiotics to ARGs in 442

a natural farming environment suggests that sulfonamides are significantly associated
with multiple genes and dominate the spread of ARGs ⁹⁴. However, studies have
questioned there was no clear correlation between the presence of targeted antibiotics
and the relative abundance of ARGs in the soil irrigated with treated wastewater ⁹⁵.
Hence, the distribution factors of ARGs in the soil should be considered
comprehensively to assess its environmental impact more accurately.

449 The integrase gene *intl*1 that can be horizontal transferred between bacteria is usually closely associated with ARGs ^{37,96}. The positive correlation between ARGs and 450 integrase genes confirmed that the application of manure fertilizer increased the 451 possibility of ARGs transmission in agricultural soils ^{97–99}. Once ARGs are integrated 452 into successful gene delivery elements, they can persist and spread even without 453 antibiotics². The positive correlation between *intI*1 and *sul*1 in estuarine sediments 454 suggested that *intI*1 may be involved in the occurrence and reproduction of 455 sulfonamides resistance genes ¹⁰⁰, which is consistent with our results (Fig. 7). In 456 addition, the correlation between *intI*1 and beta-lactam ARGs (*bla*_{TEM}, *bla*_{OXA-58}, and 457 458 *bla*_{CTX-M-32}) was also strong, although these results were not statistically significant (Fig. 7). Simultaneously, this study provides evidence that *intI*1 was significantly positively 459 correlated with a variety of ARGs (e.g., tetB, cmlA, and qnrA), which further confirmed 460 the role of *intI*1 in promoting the transmission of ARGs (Fig. 9). 461





Fig. 9 Network visualizes the antibiotics and ARGs in soils. A connection represents a strong Spearman's correlation coefficient $\rho > 0.7$ with statistical significance (P < 0.05). The nodes were colored according to antibiotics and ARGs types. The blue and red lines represent positive and negative correlations, respectively. Node size is proportional to the number of connections, and edge thickness is proportional to the correlation coefficient.

468 **4.5 Environmental implications and mitigation strategies of antibiotics and ARGs**

The antibiotics and ARGs burden have serious implications for human health and the agricultural environment. In most cases, agricultural management adopts the repeated use of manure-containing antibiotics, whereas little is known about the long-term effects of this measure on natural microbial communities in the soil, which plays a crucial role in ecological processes such as the nitrogen cycle in the soil ^{3,101,102}. A more sophisticated analysis of the fate and effects of antibiotics accounting for spatiotemporal variability of microorganisms in agricultural soils is necessary. In

addition, The obvious consequence of antibiotic release is the emergence of resistant 476 bacteria with the increasing use of antibiotics in the natural environment, and the high 477 mortality caused by multidrug-resistant bacterial infections is of particular concern ^{103–} 478 ¹⁰⁵. The presence of antibiotics in agricultural soils can adversely affect plant growth 479 480 and productivity, their absorption, and accumulation in crops, and ultimately affect human health through the food chain ^{106–108}. Antibiotic compounds were ubiquitous in 481 soil samples from organic vegetable farms. The norfloxacin is a typical quinolone 482 antibiotic which could pose a high risk in vegetable soils ^{109,110}. This study further 483 confirms the ecological risk of norfloxacin. Sulfanilamide and tetracycline are most 484 common in farms, wastewater treatment plants, and soil, and the highest concentrations 485 were above 10 mg kg⁻¹ in agricultural soils from multiple feedlots ^{111,112}. These findings 486 487 seem to be consistent with our research, and this may be due to unique structure of them affecting the degradation and migration processes in the soil. Hence, it is necessary to 488 understand the fate and transmission mechanisms of antibiotics and ARGs and develop 489 490 effective technologies to minimize the risk of antibiotic resistance.

In general, anaerobic and aerobic digestions of compost, manure, or sludge are reasonable disposal measures to reduce antibiotic resistance in agricultural soils at source ¹¹³. The removal effect of ARGs, ARB, and transposon genes was pronounced after aerobic composting ¹¹⁴. Anaerobic digestion can reduce the relative abundance of macrolide-lincosamide-streptogramin and tetracycline resistance genes in biogas slurry ²⁰. Adsorbent materials such as biochar used as a soil amendment can effectively remove antibiotic residues in feces and reduce the ecological risk of their release into the environment ^{115–117}. In addition, constructed wetlands are considered an economical and efficient ecological restoration technology are emerging in recent years. Their performance in removing antibiotics is superior to many traditional wastewater treatment technologies under certain conditions ¹¹⁸. With the increasing pollution of antibiotics and ARGs in agricultural soils, it is necessary to grasp the mechanisms of their adsorption, migration, and degradation. It is imperative to understand the expression and inhibition of ARGs for the study of their removal mechanisms.

Regulated management in the treatment of antibiotics and ARGs prior to exposure 505 to the soil is lacking. The Ministry of Agriculture and Rural Affairs of the People's 506 507 Republic of China formulated the National Action Plan for the Reduction of Veterinary Antimicrobial Use (2021-2025). This plan aims to effectively improve the ability and 508 level of the safe, standardized, and scientific use of veterinary antimicrobials in 509 510 livestock and poultry breeding and establish a sound and strict implementation of a management system for the safe use of veterinary drugs. China faces the most severe 511 challenge of any country in dealing with antimicrobial resistance due to the large 512 513 production and usage of antibiotics. In fact, the long-term effects of antibiotics and ARGs on natural microbial communities and agricultural ecosystems are still largely 514 unknown. The transmission of antibiotic-resistant bacterial might occur on a worldwide 515 scale ¹¹⁹. The widespread nature of antibiotics and ARGs in agricultural soils is well 516 documented in countries. Nevertheless, limited researches from low-income countries 517 hinder a comprehensive understanding of antibiotics and ARGs, and this knowledge 518 519 gap limits our ability to assess the risk of antibiotic resistance outbreaks in agricultural

520 ecosystems ³⁹. As such, antibiotics and ARGs should be treated as a global health issue
521 that all countries strive to solve.

522 **5 Conclusions**

In summary, this study systematically described the distribution of antibiotics and 523 ARGs in agricultural soil. Cattle manure, chicken manure, swine manure, and sewage 524 sludge are the primary sources of antibiotic pollution in agricultural soil. For ARGs, the 525 pollution caused by the application of mixed manure cannot be underestimated. 526 527 Sulfanilamide, tetracyclines, fluoroquinolones, and corresponding ARGs were the main pollution types, and the abundance of multidrug resistance was also high in agricultural 528 soil. Land-use types and environmental variables regulated the distributions of 529 antibiotics and ARGs. There were significant differences in antibiotic concentration and 530 ARGs abundance under different planting types and soil physicochemical parameters 531 (e.g., pH, SOC, clay content, TN, and heavy metals). It is worth emphasizing that the 532 533 int/1 plays a vital role in promoting the dissemination of ARGs. In addition, it is necessary to find appropriate conditions and scientific management methods for the 534 535 removal of antibiotics and ARGs to play a greater efficiency. Hence, a more sophisticated analysis of the occurrence and fate of different antibiotic and ARGs 536 classes to combat this type of increasingly serious pollution. 537

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957 Supplementary Materials Captions

- 958 Figure S1 Concentrations of different types of antibiotics in global agricultural soils.
- 959 **Figure S2** Abundances of different types of ARGs in global agricultural soils.
- 960 Text S1 Studies included in this analysis exploring the antibiotics and ARGs in global
- 961 agricultural soils.
- 962 **Table S1** Antibiotics ecological risk parameters.