1   # Antibiotic resistance genes (ARGs) in agricultural soils from

2   # the Yangtze River Delta, China

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Abstract

As an important reservoir of intrinsic antimicrobial resistance, soil is subjected to increasing anthropogenic activities that creates sustained selection pressure for the prevalence of antibiotic resistance genes (ARGs), thus constituting an important environmental dissemination pathway to human exposure. This study investigated the levels and spatial distributions of three classes of ARGs in relation to a range of co-occurring chemical mixtures and soil properties at a regional scale of the Yangtze River Delta (YRD), China. The selected eight ARGs were all detected in 241 agricultural soil samples with relative abundances ranging from 1.01×10^{-7} to 2.31×10^{-1} normalized to the 16S rRNA gene. The *sul*II and *tet*G were the dominant ARGs with a mean relative abundance of 6.67×10^{-3} and 5.25×10^{-3}, respectively. The ARGs were mainly present in agricultural soils alongside Taihu Lake and Shanghai municipality, the most agriculturally and economically vibrant area of the YRD region. Antibiotics, rather than other co-occurring pollutants and soil properties, remain to be the dominant correlate to the ARGs, suggesting their co-introduction into the soils via irrigation and manure application or the sustained selection pressure of antibiotics from these sources for the proliferation of ARGs in the soils. While the current dataset provided useful information to assess the ARGs pollution for mitigation, future studies are warranted to reveal the complete picture on the potential transfer of antimicrobial resistance from soil to agricultural produces to human consumption and associated health implications.

41   soils

42

1. Introduction

The advent of antibiotics in the 20th century revolutionized modern medicine and livestock industry. The sustained selection pressure of antibiotics on bacteria due to their misuse and overuse across the globe, however, has led to the increased prevalence of antibiotic resistance genes (ARGs) in a wide variety of clinical pathogens and commensal bacteria and hence the emergence of antibiotic-resistant pathogens and "superbugs". Outside the clinical and livestock sectors, the environment, as the remaining pillar of the "One Health" loop, represents a critical reservoir and transmission pathway of antimicrobial resistance (Berendonk et al., 2015). Since the first recognition of antibiotic resistance genes (ARGs) as emerging environmental contaminants (Pruden et al., 2006), ARGs pollution has aroused considerable concerns for both the ecological environment and human health.

Soil has the largest and most diverse microflora, and soil microbiota is one of the origins of antibiotic resistance genes (D'Costa et al., 2011; Forsberg et al., 2012). However, the increasing use and misuse of antimicrobials in humans and animals in recent decades has contributed to a rise in both the diversity and prevalence of antimicrobial resistance in soils, particularly in areas affected by human and animal wastes, such as organic manures and reclaimed wastewater. Consequently, the agricultural soil could possibly become an important reservoir of ARGs, which may pose potential health risks to humans (Sun et al., 2017). ARGs in agricultural soil could

63 be transferred to the human body via crop (Zhu et al., 2017) and air particulate matter

64 exposure pathways (McEachran et al., 2015; Xie et al., 2018). The sulfonamide (*sul*)

65 genes found in agricultural soils of China ranged from 10^{-6} to 10^{-2} gene copies/16S

66 rRNA gene copies (Zhou et al., 2017), whereas the tetracycline (*tet*) genes in soils

67 across the world ranged from 10^{-6} to 10^{-2} gene copies/16S rRNA gene copies (Ji et al.,

68 2012; Wang et al., 2015). Given that the *sul* and *tet* resistance genes were often detected

69 in manure or manure-amended soils (Qiao et al., 2018), application of manure fertilizer

70 and wastewater irrigation could be the main anthropogenic sources of ARGs for

71 agricultural soil environment (Wu et al., 2010; Cheng et al., 2016; Zhou et al., 2017).

72   Numerous studies showed that the ARGs diversity and abundance in agricultural

73 soils were influenced by multiple factors, such as antibiotics, heavy metals, and Class

74 I integron (*int*I1). It was reported that there was a significant and positive correlation

75 between ARGs and antibiotics in soil environment (Huang et al., 2013; Zhu et al., 2013;

76 Cheng et al., 2016). The overuse of antibiotics exerts selective pressure on

77 microorganisms and ARGs, which exacerbates the contamination of ARGs (Witte,

78 2000; Luo et al., 2010; Xiong et al., 2015). Furthermore, ARGs pollution in soil

79 environment is on a great scale, with some levels surging by > 15-fold since the 1970s

80 (Knapp et al., 2010). The co-selection of heavy metal and antibiotics on ARGs can also

81 increase the ARGs pollution in soils (Zhang et al., 2015a, b; Lin et al., 2016). As one

82 of the mobile genetic elements (MGEs), the *int*I1 exists both in Gram-positive and

83 Gram-negative bacteria (Gillings et al., 2015) and contributed to the transfer of ARGs

84 in soils. Therefore, the environmental behavior of ARGs is highly related to the soil

85 types, climatic environment, and land use. Through a regional field study, the key

86 factors influencing the fate of ARGs in soil could be revealed and evaluated.

87 The Yangtze River Delta (YRD) is the most populated and economically prosperous

88 region in China. The study area of the YRD covers Shanghai municipality, northern

89 Zhejiang and southern Jiangsu provinces. The population of the YRD region has

90 exceeded 110 million, resulting in large agricultural growth in the region. Our previous

91 studies indicated that polychlorinated biphenyls (PCBs) (Sun et al., 2016a),

92 organochlorine pesticides (OCPs), phthalate esters (PAEs), polybrominated diphenyl

93 ethers (PBDEs) (Sun et al., 2016b), and antibiotics (Sun et al., 2017) in agricultural

94 soils were widely detected. The combined organic pollution may pose ecological risks

95 and have an effect on the profiles of ARGs in agricultural soils. Therefore, it is strictly

96 necessary to investigate the occurrence and spatial distribution of ARGs, and synthesize

97 the data on co-occurring chemical mixtures and soil properties to evaluate the

98 influencing factors on the fate of ARGs to effectively control the ARGs pollution in the

99 YRD region.

100 The study aims 1) to reveal the occurrence and distribution of ARGs in agricultural

101 soils of the YRD region; and 2) to evaluate the major influencing factors on the fate of

102 ARGs in soils. The findings provided a better understanding of ARGs contamination in

103 highly human-impacted regions in the world.

2. Materials and methods

2.1. Sample collection

106 A total of 241 surface agricultural soil samples (0–15 cm) were collected from the

107 Yangtze River Delta in the east of China in June 2014. The sampling region covered a

108 total area of 45,800 square kilometers in eastern Zhejiang, Shanghai, and southern

109 Jiangsu (Figure 1). Detailed information on the sampling sites was presented in our

110 previous study (Sun et al., 2016b). Five subsamples were drawn with a bamboo scoop

111 at each site and mixed into a single sample. The distance of each subsample core was

112 10-20 m and the sampling site had an area of 400 m^2. The collected soil samples were

113 then sent to the laboratory and stored in brown containers at ultra-low temperature of

114 −80 °C for subsequent analysis.

115 ***2.2. DNA extraction of soil samples***

116 The extraction of DNA was conducted with a DNeasy PowerSoil Kit (QIAGEN,

117 German) from three replicates of 0.25 g each soil following the manufacturer's

118 instructions. The concentration of extracted DNA was measured by the Nanodrop

119 (Thermo Fisher, Germany). The quality of DNA was determined by running 1.5%

120 agarose gel electrophoresis. The extracted DNA was kept at −20 °C before analysis.

121 The target genes including eight ARGs, *int*I1 and 16S rRNA were purified by

122 polymerase chain reaction (PCR) assay. The duplicate PCR reactions were carried for

123 each gene; while ultrapure water was used as a control to ensure the accuracy of the

124 PCR results. PCR reaction consists of 2.0 µL 10 × PCR buffer (Mg^{2+} free), 0.8 µL

125 $MgCl_2$ (25 mM), 2 µL dNTPs (10 mM), 1 µL template DNA (75 ng), 1 µL of each

126 primer (10 µM), 0.3 µL Taq DNA polymerase (5 Unit) and 16.9 µL double-distilled

127 H_2O. The temperature program of PCR was followed: 5 min at 95 °C for initial

128 denaturation, 40 cycles of 15 s at 95 °C, 30 s at the annealing temperature, 30 s at 72 °C

129 for the final step of extension. The annealing temperature and primers of target genes

130 are illustrated in Table S1 of Supporting Information.

2.3. Real-time PCR of ARGs, intI1 and 16S rRNA genes

132   This study analyzed eight ARGs including five *tet* genes (*tet*A, *tet*G, *tet*M, *tet*O,

133 *tet*W), two *sul* genes (*sul*I, *sul*II) and one *qnr* gene (*qnr*S), which are the common and

134 typical ARGs in soil environments with three classes. The quantification of selected

135 genes was conducted under the Step One Plus Real-Time PCR Systems (ABI, USA) in

136 three replicates. The primers of real-time PCR were the same as those in the PCR

137 process. The real-time PCR system was carried out in triplicate with a final volume of

138 15 μL, which consisted of 7.5 μL of SYBR Premix Ex Taq™ (TaKaRa), 4.6 μL of

139 double-distilled H_2O, 0.3 mL of 50 × ROX reference dye, 0.3 μL of forward primer (10

140 mM), 0.3 μL of reverse primer (10 mM) and 2 μL of template DNA. We had diluted the

141 DNA template to ensure the DNA concentration was <10 ng/μL for the prevention of

142 amplification inhibition. The temperature program of real-time PCR was performed at

143 95 °C for 30 s, followed by 40 cycles of 5 s at 95 °C, 30 s at the annealing temperature,

144 and 30 s at 72 °C (Table S1). The melt curve stage increased from 60 to 95 °C at the

145 rate of 0.5 °C per read to affirm the specificity. The real-time PCR products were

146 excised, purified and linked to plasmids pMD19-T cloned into Escherichia coli DH5α

147 (Takara) to generate positive controls. According to the BLAST alignment tool

148 (http://www.ncbi.nlm.nih.gov/blast/), the positive clones of the ARGs were used as the

149 calibration standards. The real-time PCR standard curves and amplification efficiency

150 are listed in Table S2.

2.4. Data analysis

Data analysis of ARGs was performed with SPSS Version 20.0, Origin8.0 and

Microsoft Excel 2000. Spearman correlation analysis was applied to assess the

relationships between the abundance of ARGs, *int*I1 and environmental factors. The

spatial distributions of ARG in agricultural soils from YRD region were simulated by

using universal Kriging in ArcGIS 10.2 (ESRI, Redlands, CA, USA). Principal

component analysis (PCA) and redundancy analysis (RDA) were carried out with

Canoco 4.5 to examine the independent contributions of influence factors on ARG

composition. The abundance values of ARGs were expressed on a dry-weight basis and

were log-transformed before the statistical analyses. The data of antibiotics and heavy

metals in agricultural soils of the YRD region came from our previous study (Sun et al.,

2017; Sun et al., 2016a, b).

3. Results and discussion

3.1. Levels of ARGs in soil of the YRD region

The levels of ARGs were calculated through the absolute abundances of ARGs

normalized to that of the 16S rRNA gene in agricultural soils (Figure 2). The relative

abundance of three selected classes of ARGs including tetracycline (*tet*A, *tet*G, *tet*M,

*tet*O and *tet*W), sulfonamide (*sul*I and *sul*II) and quinolone (*qnr*S) resistance genes is

summarized in Table 1. Eight ARGs were all detected in 241 agricultural soils of the

YRD region. The abundances of selected ARGs in agricultural soils ranged from

1.01×10^{-7} to 2.31×10^{-1} copies/16S rRNA copies (Table 1). The *sul* resistance genes were

the most abundant ARGs in agricultural soils which may be caused by the propagation

173 characteristic of *sul*I and *sul*II (Radstrom and Swedberg, 1988). In our previous study,

174 the *sul*, *tet* and *qnr* resistance genes were detected in greenhouse and corresponding

175 open-field soils across China, and the level of *sul* resistance genes were relatively

176 higher than other ARGs in both kinds of agricultural soils (Zeng et al., 2019). In this

177 survey, the maximum abundance of ARGs in the YRD region reached 10^{-1} copies/16S

178 rRNA copies, which means that roughly two bacterial cells contain one ARG copy

179 (assuming four copies of the 16S rRNA gene in one bacterial cell). The mean values of

180 ARGs in agricultural soils of the YRD region were as follows: *sul*II ($6.67×10^{-3}$) > *tet*G

181 ($5.25×10^{-3}$) > *sul*I ($2.92×10^{-3}$) > *tet*M ($6.70×10^{-4}$) > *tet*W ($6.47×10^{-4}$) > *qnr*S ($8.95×10^{-5}$)

182 ^{5}) > *tet*A ($4.07×10^{-5}$) > *tet*O ($2.93×10^{-5}$). The main sources of ARGs in agricultural soils

183 were the application of manure fertilizer and the irrigation of wastewater and reclaimed

184 water, especially in the soil close to feedlots and aquafarm. The ARGs in downstream

185 of the Yangtze River were widely detected (Wang et al., 2019), which is possibly one

186 of the sources of ARGs in agricultural soils of the YRD region.

187   The *sul* resistance genes including *sul*I and *sul*II in agricultural soils ranged from

188 $1.72×10^{-5}$ to $2.81×10^{-1}$ copies/16S rRNA copies, which can encode dihydropteroate

189 synthases (DHPS) for resistance. The widespread and severe contamination of *sul*

190 resistance genes in agricultural soils was related to easy combination of *sul*I and *sul*II

191 with mobile genetic elements (Stoll et al., 2012). The reason was that the *sul*I and *sul*II

192 genes were lightly conferred to class I integrons and small plasmids (Antunes et al.,

193 2005). With reference to many published reports, both genes have been shown to occur

194 with highly similar frequency in the environment (Stoll et al., 2012; Liu et al., 2014;

195 Zhou et at., 2017; Zeng et al., 2019). The high contamination of *sul* resistance genes

196 could also be caused by the extensive use of sulfonamides in human and animals (Kools

197 et al., 2008). Further, the *sul*I and *sul*II genes were detected in human pathogens in

198 agricultural soils (Forsberg et al., 2012; Fang et al., 2015; Li et al., 2017), which can be

199 a threat to human health.

200   The *tet* resistance genes (*tet*A, *tet*G, *tet*M, *tet*O and *tet*W) ranged from 1.01×10^{-7} to

201 8.77×10^{-2} copies/16S rRNA copies in agricultural soils of the YRD region. Tetracycline

202 resistance genes were one of the most widely distributed and heavily contaminated

203 resistance genes in the world. A total of 45 *tet* resistance genes have been identified

204 (Gao et al., 2012). The *tet* genes in this survey have two resistance mechanisms

205 including efflux pumps (*tet*A and *tet*G) and ribosomal protection proteins (*tet*M, *tet*O,

206 and *tet*W) (Zhu et al., 2013). The *tet*G is the dominant *tet* genes in agricultural soils of

207 the YRD region, and *tet*G has high homology with some pathogenic bacteria (Peng et

208 al., 2015), which may pose risks to human health. The *tet*M has the most extensive host

209 range which results in widespread pollution in agricultural soils. With the findings of

210 our previous study, *tet*M and *tet*G were the heavily contaminated genes in agricultural

211 soils of China (Zeng et al., 2019). The *qnr*S gene, which encodes for *qnr* peptide, ranged

212 from 2.17×10^{-7} to 2.20×10^{-3} copies/16S rRNA copies in agricultural soils of the YRD

213 region. In this survey, *int*I1 was detected in agricultural soils, which was an important

214 indicator of ARG mobility and could have significant impact on the fate of ARGs in

215 soils.

216 *3.2. Spatial distribution of ARGs in soils of the YRD region*

217   The spatial distribution of selected ARGs in agricultural soils of the YRD region is

218   presented in Figures 1 and S1. As for *sul* genes, the distinct spatial distribution showed

219   that the abundances of *sul*I and *sul*II genes were comparatively higher in the YRD

220   region around the Taihu Lake, such as the south of Jiangsu and the northwest of

221   Zhejiang. The ARGs levels in soils were easily affected by proximity to the pollution

222   sources. In aquatic environments, higher concentrations of sulfonamides aggravated *sul*

223   genes contamination (Luo et al., 2011) and *sul*-ARGs were the most prevalent ARGs in

224   the water of China (Zhang et al., 2020). Previous report indicated that the abundances

225   of *sul* (*sul*I and *sul*II) and *tet* (*tet*A, *tet*G, and *tet*M) genes in Taihu Lake reached 10^{-2}

226   copies/16S rRNA copies (Yang et al., 2017), which could be the sources of *sul*I and

227   *sul*II genes in agricultural soils with irrigation water of Taihu Lake. The most polluted

228   area for *sul*I is south Jiangsu, and northwest Zhejiang for *sul*II. Meanwhile, the

229   antibiotic residues of these two provinces were relatively higher in the YRD region

230   (Sun et al., 2017) which may be contributed to the ARGs pollution.

231   As for *tet* genes, the spatial distribution of five *tet* genes was observed to be higher

232   in Shanghai municipality, such as in the Jiading, Pudong and Baoshan areas. In these

233   areas, the livestock and poultry aquaculture industry are well developed and could be

234   the major sources of ARGs in agricultural soils (Chen et al., 2016; Cheng et al., 2016).

235   Furthermore, the ARGs were detected in coastal areas of China and this can be an

236   important source of ARGs in soil environments (Xu et al., 2019). In soils from the north

237   of Taihu Lake, the *tet* genes also had high abundances which may be heavily irrigated

238   by untreated wastewater with ARGs pollution. The *tet*G was the most contaminated *tet*

239　gene in agricultural soils and mainly distributed in the north Zhejiang, Shanghai, and

240　south Jiangsu. The ARGs can enter the body through crops and have adverse effect on

241　human gut bacteria (Cerqueira et al., 2019). Widespread contamination of soil ARGs

242　poses serious environmental damage and human exposure risks.

243　　The anthropogenic activity could be a dominant source of ARGs in agricultural soils

244　such as land application of manure fertilizer, irrigation of wastewater and reclaimed

245　water (Ji et al., 2012; Mu et al., 2015), which could cause the ARGs pollution. It was

246　reported that the abundance of *tet* and *sul* genes ranged from 10^{-3} to 10^{-1} copies/16S

247　rRNA copies in agricultural soils with manure application of Jiangsu province, China

248　(Zhang et al., 2015c). The ARGs were also detected in agricultural soils with cow

249　manure application of Sardinian (Italy) (Chessa et al., 2016). In addition, the high

250　abundances of ARGs in soils with reclaimed water irrigation were also reported (Wang

251　et al., 2014a; Wang et al., 2014b).

252　*3.3. Correlations between ARGs and chemical pollution and soil properties*

253　　A correlation matrix (Figure 3) was created for the relative abundance of the studied

254　genes (16S rRNA gene, ARGs, intl1), known concentrations of antibiotics and other

255　co-occurring contaminants, and soil physicochemical parameters. The significant

256　correlations were generally concentrated in the antibiotic-*int*l1-ARGs cluster of the

257　correlation matrix. The *sul*II (r = 0.260, *p* < 0.05), *tet*M (r = 0.204, *p* < 0.05), and *tet*W

258　(r = 0.277, *p* < 0.05) were significantly correlated with tetracyclines (TCs) in

259　agricultural soils. The *sul*I (r = 0.376, *p* < 0.05), *sul*II (r = 0.201, *p* < 0.05), *tet*G (r =

260　0.433, *p* < 0.01), *tet*O (r = 0.278, *p* < 0.01), and *qnr*S (r = 0.204, *p* < 0.05) significantly

261   correlated with quinolones (QNs) in agricultural soils. Concerning total antibiotics, *sul*I

262   (r = 0.423, *p* < 0.01), *sul*II (r = 0.302, *p* < 0.01), *tet*G (r = 0334, *p* < 0.01), *tet*M (r =

263   0.267, *p* < 0.05), and *tet*W (r = 0.276, *p* < 0.05) were significantly correlated with total

264   antibiotics. These results highlighted that the co-introduced antibiotics from irrigation

265   and manure application exert sustained pressure for the proliferation of ARGs.

266   Moreover, we observed significant correlations between a number of ARGs and the

267   non-corresponding antibiotics, for example, sulfonamides resistance genes and TCs and

268   QNs, and tetracycline resistance genes and QNs. These demonstrated the presence of

269   cross-resistance between antibiotics and ARGs, which warrant future detailed

270   molecular studies for mechanistic elucidation.

271   The MGEs, such as *int*I1 widely existed in bacteria and facilitated the horizontal

272   transfer of ARGs in soils that could aggravate the contamination of ARGs in soil

273   environments (Ghosh and LaPara, 2007; Luo et al., 2010; Gillings et al., 2015; Ma et

274   al., 2017). In our study, *sul*I (r = 0.137, *p* < 0.05), *sul*II (r = 0.455, *p* < 0.01), *tet*G (r =

275   0.497, *p* < 0.01), and *tet*W (r = 0.364, *p* < 0.01) were significantly correlated with *int*I1

276   genes in agricultural soils of YRD region. These genes (*sul*I, *sul*II, *tet*G, and *tet*W) were

277   widespread and seriously polluted in the YRD region. These results highlighted that

278   mobile genetic elements, such as *int*I1, are critical genetic compartments for the

279   dissemination of ARGs in the soil environment.

280   The result of laboratory experiments proved that non-antibiotic chemicals may co-

281   select ARGs via co-resistance and cross-resistance (Berg et al., 2010; Ye et al., 2017;

282   Zhang et al., 2015a, b). Heavy metals (zinc, copper and mercury) with critical levels in

283 the environment would induce co-selection of ARGs in bacteria (Imran et al., 2019).

284 Most of the studied chemicals (e.g., PCBs, PBDEs, OCPs, PAEs, and Cu), however, did

285 not correlate with *int*l1 or ARGs, except for an occasional statistical significance for Zn

286 and *int*l1. The findings of this analysis reflected that the role of these co-occurring

287 chemicals may not be vital as expected in co-selecting ARGs. The field relevance of

288 the co-selection by sub-inhibitory concentrations of non-antibiotic agents observed in

289 the laboratory experiments need to be fully examined.

290 Most of the soil physicochemical parameters tested appeared not to be associated

291 with the bacterial abundance and tested MGEs and ARGs, except for a weak correlation

292 between pH and the bacterial abundance indicated by 16S rRNA gene. That being said,

293 the influence of soil properties on the bacterial assembly warrant future studies.

294

4. Conclusions

296 This study established a baseline of the occurrence, abundance and distribution of

297 sulfonamide, tetracycline and quinolone resistance genes in agricultural soils on a

298 regional scale of the YRD, and provided basic data of ARGs contamination in China.

299 The hotspots of agriculture soil ARGs were concentrated in the area alongside Taihu

300 Lake and Shanghai municipality, the most agriculturally and economically vibrant of

301 the YRD region. Among the co-occurring chemical contaminants and soil

302 physicochemical conditions, antibiotics remain to be the dominant factor for the ARGs,

303 suggesting their co-introduction into the soils via irrigation and manure application or

304 the sustained selection pressure of antibiotics from these sources for the proliferation

of ARGs in the soils. Future studies are warranted to understand the potential transfer of antimicrobial resistance from soil to crop produces to human consumption and associated health implications. This study provides useful information on the levels and regional characteristics of ARGs pollution in China, which can play its part in prevention and regulation of ARGs in the world.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

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521   **Figure and Tables:**

522   **Figure 1.** Spatial distributions of selected ARGs in agricultural soils of the YRD region.

523   (A) *sul*I, (B) *sul*II, (C) *tet*G, (D) *qnr*S.

524

525   **Figure 2.** The concentrations of selected ARGs in agricultural soils of the YRD region.

526

527   **Figure 3.** Heatmap showing the spearman correlation between the relative abundance

528   of selected genes and concentrations of antibiotic and non-antibiotic chemicals, soil

529   physicochemical parameters and population density (*$p<0.05$; **$p<0.01$; ***$p<0.001$).
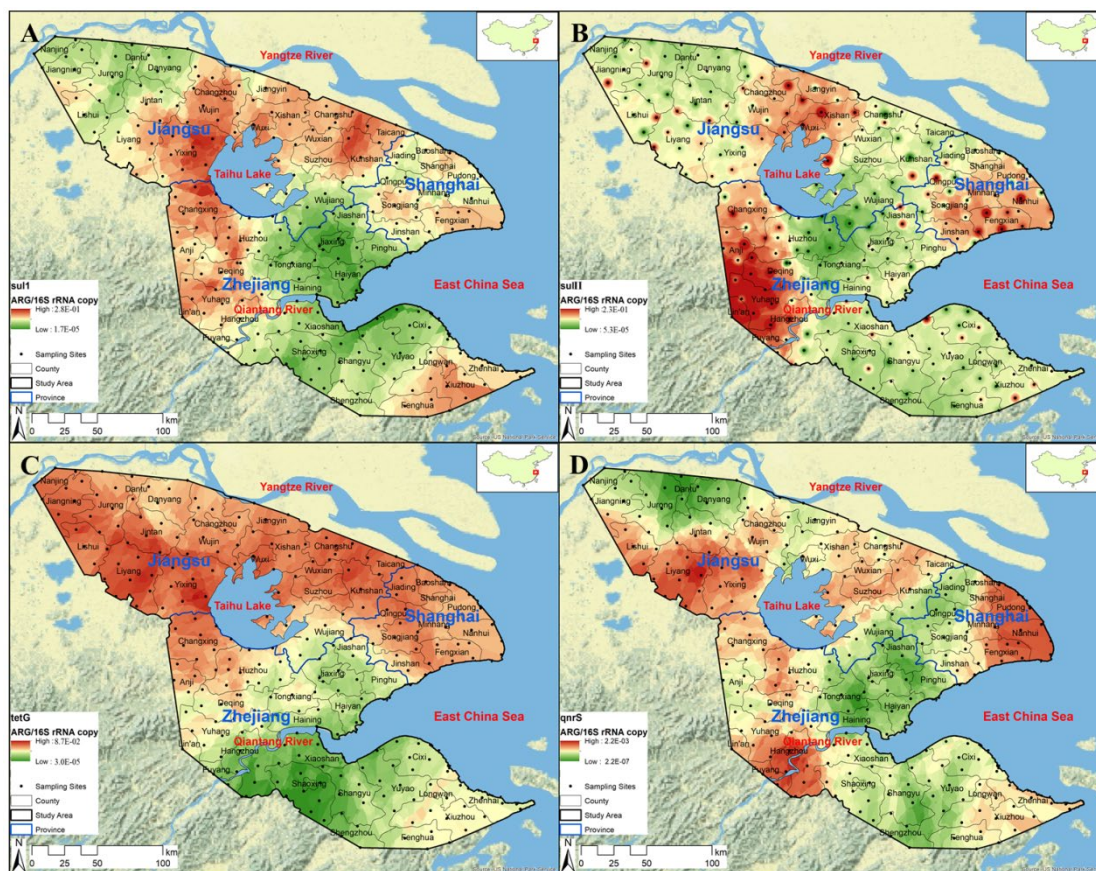
530

531   **Table 1**

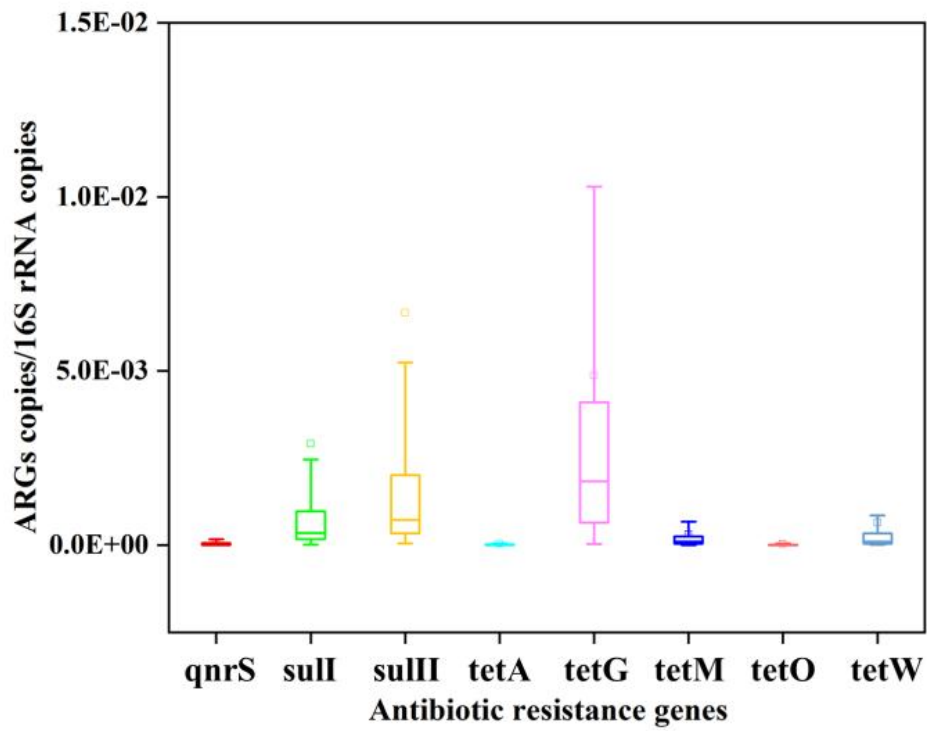532   The abundances of ARGs in agricultural soils of the YRD region.

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Figure 1. Spatial distributions of selected ARGs in agricultural soils of the YRD region.
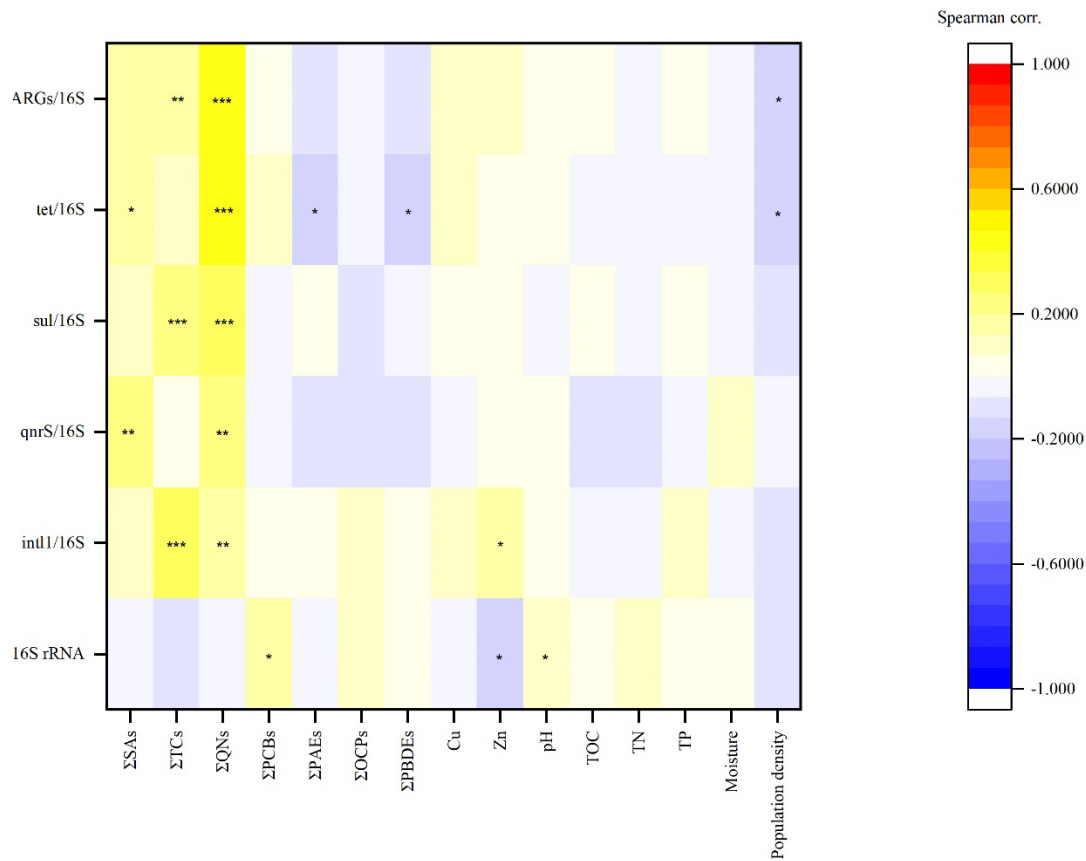
(A) *sul*I, (B) *sul*II, (C) *tet*G, (D) *qnr*S.

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541 **Figure 2.** The concentrations of selected ARGs in agricultural soils of the YRD region.

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Figure 3. Heatmap showing the spearman correlation between the relative abundance of selected genes and concentrations of antibiotic and non-antibiotic chemicals, soil physicochemical parameters and population density (*p<0.05; **p<0.01; ***p<0.001).

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Table 1

The abundances of ARGs in agricultural soils of the YRD region.

ARGs	Mean	Median	Min	Max
*sul*I	2.92×10^{-3}	3.51×10^{-4}	1.72×10^{-5}	2.81×10^{-1}
*sul*II	6.67×10^{-3}	7.21×10^{-4}	5.25×10^{-5}	2.31×10^{-1}
*tet*A	4.07×10^{-5}	1.15×10^{-5}	1.01×10^{-6}	2.03×10^{-3}
*tet*G	5.25×10^{-3}	1.84×10^{-3}	3.01×10^{-5}	8.77×10^{-2}
*tet*M	6.70×10^{-4}	1.02×10^{-4}	3.03×10^{-6}	9.56×10^{-3}
*tet*O	2.93×10^{-5}	6.49×10^{-6}	1.01×10^{-7}	9.22×10^{-4}
*tet*W	6.47×10^{-4}	1.01×10^{-4}	1.01×10^{-6}	2.35×10^{-2}
*qnr*S	8.95×10^{-5}	2.11×10^{-5}	2.17×10^{-7}	2.20×10^{-3}
*int*I1	1.10×10^{-4}	3.42×10^{-5}	5.48×10^{-7}	1.56×10^{-3}

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