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# Response behavior of antibiotic resistance genes and human pathogens to slope gradient and position: an environmental risk analysis in sloping cultivated land

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1 **Title:** Response behavior of antibiotic resistance genes and human pathogens to slope  
2 gradient and position: an environmental risk analysis in sloping cultivated land

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16

17 **Abstract**

18       Soils, especially from farmlands, are key points for the transmission of antibiotic  
19 resistance genes (ARGs) and their hosts from the environment to humans. Sloping  
20 cultivated land is an important agricultural resource, but there is a lack of studies on the  
21 fate and risk of ARGs in sloping land. Moreover, the behavior and drivers of ARGs in  
22 response to slope gradient and position are unclear. In this work metagenomics was  
23 used to investigate the profiles of ARGs, mobile genetic elements (MGEs), and  
24 microbial communities in soils from lands of five slope gradients (5°, 10°, 15°, 20°,  
25 and 25°) with two slope positions (uphill and downhill). The results showed that while  
26 the abundance (except 15°) and diversity (except 20°) of ARGs increased as the slope  
27 gradient increased, the diversity of ARGs with health risk, especially the high-risk ones,  
28 decreased. More abundant and diverse ARGs were more likely to accumulate at  
29 downhill compared to the uphill. Furthermore, 52 bacterial genera and 12 HPB species  
30 were identified as the potential hosts for ARGs with high risk. Moreover, the structural  
31 equation model analysis revealed that the slope gradient and the slope position had both  
32 direct and indirect effects via MGEs on the abundance of ARGs. Further correlation  
33 analysis revealed that the slope gradient had an effect on NO<sub>2</sub><sup>-</sup>N concentration in the  
34 soil. Also, the slope position had an effect on the TP, PN, MBC, and MBN of the soil,  
35 which were also the key factors driving the behavior of ARGs. Overall, this study  
36 provided for the first time comprehensive information on ARGs with health risks and  
37 their pathogenic hosts in sloping farmland and can be of fundamental importance for  
38 controlling antibiotic resistance transmission and be consistent with the "One Health"

39 approach.

40 **Keywords:** Gentle slope farmland; Soil resistome; Human pathogenic bacteria;

41 Metagenomic analysis.

42

43 **Highlights:**

44 ● Metagenomics revealed the health risks posed by ARGs and pathogens in sloping  
45 farmland.

46 ● Gently sloping farmland is a hotspot for antibiotic resistance genes.

47 ● Land with low slope gradients, especially at uphill, are sources of high-risk ARGs  
48 and pathogens.

49 ● The slope's gradient and position affect ARGs' behavior via direct and indirect  
50 ways.

51

## 52 **1 Introduction**

53 Antibiotic resistance, encoded in antibiotic resistance genes (ARGs) is a serious  
54 problem that threatens global public health, and it is expected to cause 10 million deaths  
55 worldwide by 2050 if no control measures are implemented (Zhuang et al., 2021; Singh  
56 et al., 2019; Karkman et al., 2019). Soil is one important reservoir of ARGs that can  
57 also harbor a large number of microorganisms that may act as ARG hosts (Surette et al.,  
58 2017). In addition to this, ARGs and their hosts can be transmitted from the soil to  
59 humans via food crops, thus posing greater threats and challenges to food and health  
60 security (Wang et al., 2022a). Therefore, it is critical to better understand the behavior  
61 and fate of ARGs in agricultural cropland soils.

62 Gently sloping cultivated land, whose slope grading from 6° to 25°, is one of the  
63 main and important agricultural land types for global food production systems (Huang  
64 & Hewings, 2021). It accounts for a substantial 34.76% of the total area of cultivated  
65 land in China (Data bulletin of the third National Land Survey of Ministry of Natural  
66 Resources, PRC, [https://www.mnr.gov.cn/dt/ywbb/202108/t20210826\\_2678340.html](https://www.mnr.gov.cn/dt/ywbb/202108/t20210826_2678340.html)).  
67 Till now studies of ARGs profiles in agricultural soil systems have been primarily  
68 focused on flat agricultural soils ( $\leq 2^\circ$ 、 $2^\circ \sim 6^\circ$ ) based mainly on 16S rRNA sequencing  
69 (Li et al., 2017; Wang et al., 2020b). However, compared to flat soils, gently sloping  
70 agricultural soils have different physicochemical properties (Tosi et al., 2022; Brosens  
71 et al., 2020; Zou et al., 2021) from the flat ones, which can further affect the distribution  
72 of microbial communities and ARGs. This indicates that the behaviors of ARGs' in  
73 these two types of cultivated soils can not be inferred from each other. Hence,

74 discovering the integral profile of ARGs and their behavior mechanism in response to  
75 soil's physiochemical properties and microbial communities in gently sloping  
76 cultivated land is a missing element in literature. This will considerably enhance our  
77 knowledge of occurrence patterns and mechanisms of ARGs in agricultural soils.

78 Slope gradient and slope position are key factors affecting the physicochemical  
79 properties of soil and their correlations with its microbial communities. Previous studies  
80 have demonstrated that these two factors can significantly affect the water content,  
81 organic carbon, nitrogen transfer, and some alkali metal ions in the soil ([Brosens et al.,](#)  
82 [2020](#); [Zou et al., 2021](#); [Hou et al., 2020](#)). It has also been reported that slope gradients  
83 ranging between (0 – 4.31%) showed a negative effect on bacterial and fungal  
84 communities by influencing the soil's pH and ratio of carbon to nitrogen ([Neupane et](#)  
85 [al., 2022](#)). A similar effect was also observed in a forested headwater catchment with  
86 the slope position being able to affect the soil moisture and pH and then shift bacterial  
87 structures ([Shigyo et al., 2022](#)). Along lateritic hillside soils the slope position could  
88 strongly influence the soil's conductivity, pH, clay content, and further bacterial  $\beta$ -  
89 diversity ([O'Brien et al., 2019](#)). At the Shenxiantang tiankeng the different slope  
90 positions along the inverted stone slopes had an impact on microbial abundance instead  
91 of composition, with determining factors the soil's total nitrogen and pH ([Jiang et al.,](#)  
92 [2021](#)). Given that microbial community has been demonstrated as one vital reason for  
93 ARGs' propagation, these findings indicate that the behavior of ARGs could be  
94 significantly affected by slope gradient and position, although very few studies till now  
95 have focused on it.

96 Many previous studies have used metagenomics sequencing to identify multiple  
97 ARGs subtypes in different environments (Liang et al., 2021; Wan et al., 2021), but only  
98 a few genomes actually contain ARGs with high risk to human health (Zhang et al.,  
99 2022b). ARGs with health risks, when carried by human pathogenic bacteria, will tend  
100 to pose a greater threat to human health by making antibiotics invalid and/or less  
101 efficient and therefore increasing infection and mortality. Therefore, identifying and  
102 detecting ARGs highly associated with human activities and health (Li et al., 2020) and  
103 focusing on behavioral changes of risk ARGs in the environment are necessary to assess  
104 the possible health risks caused by ARGs in the environment. Moreover, the risk of  
105 ARGs to the environment depends not only on the autochthonous nature but also on its  
106 potential hosts, especially the pathogenic ones (Li et al., 2023).

107 To reduce these knowledge gaps, metagenomics will be used to investigate the  
108 effects of slope gradient and slope position on the behavior of microbial communities,  
109 ARGs, and MGEs in sloping farmland. Risk ARGs and their potential pathogenic hosts  
110 will be identified by means of existing assessment frameworks. The effects of slope  
111 gradient and slope position on ARGs distribution will be explored. This study provides  
112 important knowledge for assessing the health risk of ARGs in sloping farmland soil.

## 113 **2 Material and Methods**

### 114 **2.1 Sample collection**

115 Sampling was conducted at five sloping red loam cultivating maize located in  
116 Wanzhou District, Chongqing, China (107°55'22"-108°53'25 "E, 30°24'25"-31°14'58  
117 "N). Five sloping lands, with 0° east, were set at 5°, 10°, 15°, 20°, and 25°, marked as  
118 W1, W2, W3, W4, and W5, respectively. W1 was the control group set at flat land. Soils

119 were taken from the top and bottom of the slope and labeled as uphill (U) and downhill  
120 (D). All other parameters and conditions were consistent for all five sites.

121 40 soil samples were collected by repeating sampling four times at 10 sites in the  
122 uphill and downhill of the five slope gradients (W1-W5). One part of each collected  
123 soil sample was stored at 0 – 4 °C for physicochemical analyses, and the other part was  
124 stored at -80 °C for DNA extraction.

## 125 **2.2 Chemical analyses**

126 All soil samples were lyophilized, ground, sieved (<60 mesh), and analyzed for  
127 their chemical properties. Analyses of the soil samples, included organic matter (OC),  
128 microbial carbon (MBC), microbial nitrogen (MBN), total nitrogen (TN), particulate  
129 nitrogen (PN), ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ), nitrite nitrogen  
130 ( $\text{NO}_2^-\text{-N}$ ), soil available potassium (AK), available phosphorus (AP), total phosphorus  
131 (TP), moisture and pH.

132 The soil samples were dried at 105 °C for 24 h to remove moisture, and then their  
133 OC contents were measured by weight loss after burning at 550 °C for 4 h (Hu et al.,  
134 2015; Lu et al., 2019). MBC and MBN were extracted by chloroform fumigation and  
135 determined by carbon and nitrogen analyzer (CN802, VELP, Italy). TN was determined  
136 by the Kjeldahl method, and TP was detected using ammonium molybdate tetrahydrate  
137 spectrophotometry (Liu et al., 2020).  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and  $\text{NO}_2^-\text{-N}$  were determined  
138 by potassium chloride solution extraction spectrophotometry (Bernhard et al., 2018).  
139 The soil samples were mechanically shaken with distilled water for 1 h to obtain the  
140 aqueous soil suspension at a soil/water ratio of 1:10 (w/v) and then soil pH was



141 measured by a pH meter (PHS-25, Leici, China). The specific physical and chemical  
142 properties of soil are shown in Table S1.

### 143 **2.3 DNA extraction and metagenomic sequencing**

144 The FastDNA<sup>®</sup> SPIN Kit for Soil (MP Biomedicals, USA) was used for DNA  
145 extraction. The concentration and purity of the extracted DNA were determined with  
146 TBS-380 and NanoDrop2000, respectively. DNA extract quality was checked on 1%  
147 agarose gel. DNA extract was fragmented to an average size of about 400 bp using  
148 Covaris M220 (Gene Company Limited, China) for paired-end library construction.  
149 Paired-end library was constructed using NEXTFLEX Rapid DNA-Seq (Bioo  
150 Scientific, Austin, TX, USA). Adapters containing the full complement of sequencing  
151 primer hybridization sites were ligated to the blunt end of fragments. Paired-end  
152 sequencing was performed on Illumina Novaseq 6000 (Illumina Inc., San Diego, CA,  
153 USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using NovaSeq  
154 Reagent Kits according to the manufacturer's instructions ([www.illumina.com](http://www.illumina.com)).  
155 Approximately 6 GB of raw data was obtained for each sample and further filtered to  
156 remove adapters and low-quality reads.

### 157 **2.4 ARGs and MGEs annotation and ARGs risk assessment**

158 ARGs were annotated against the Comprehensive Antibiotic Resistance Database  
159 (CARD, Version 3.0.9, <https://card.mcmaster.ca/home>) using Diamond  
160 (<http://www.diamondsearch.org/index.php>, v 0.8.35) through the amino acid sequences  
161 of non-redundant gene sets (e-value  $\leq 10^{-5}$ , identity >60 %). The NCBI RefSeq database  
162 (<https://www.ncbi.nlm.nih.gov/refseq>) and the ISfinder database (

163 is.biotoul.fr/) were used to identify plasmids and insertion sequences. The amino acid  
164 sequence aligned by BLAST (e-value  $\leq 10^{-5}$ ) was at least 90 bp, and the sequence with  
165 identity > 90 % were identified as plasmid and ISs.

166 The abundance of ARGs and MGEs was expressed in “ppm” (part per million, one  
167 ARGs-/MGEs- like sequence per million sequences) (Yang et al., 2013) and it was  
168 calculated according to the following formula (Zhao et al., 2018):

$$169 \text{ ppm}_i = \frac{R_i * 10^6}{\sum_1^n (R_j)}$$

170  $R_i$  represents the abundance in a sample (the number of reads compared with Gene<sub>i</sub> in  
171 the sample).  $\sum_1^n (R_j)$  is the sum of the corresponding reads of all genes in the sample.

172 The health risk of annotated ARGs was evaluated based on the database provided  
173 by Zhang et al. (2022b), which includes four ranked risks for 2561 ARG subtypes  
174 considering their human accessibility, mobility, pathogenicity, and clinical availability.  
175 Thereinto, the ARGs at risk were classified into 4 levels, with Q1 ranked as the highest  
176 risk, followed by Q2, Q3, and Q4.

## 177 **2.5. Microbial characterization and human pathogenic bacteria identification**

178 Microbial composition was characterized by NR database using BLASTP via  
179 Diamond software (<http://ab.inf.uni-tuebingen.de/software/diamond/>) with the amino  
180 acid sequences of non-redundant gene sets (e-value  $\leq 10^{-5}$ , identity >60 %). The  
181 prioritized HPB were identified according to A-to-Z database from the National  
182 Infection Prevention and Control Manual (<https://www.hartmann-science-center.com/en/hygiene-knowledge/pathogens-a-z>) (Zhang et al. 2022b).

## 184 **2.6 Statistical analyses**

185 Averages, standard deviations, and correlations for all data were calculated using  
186 IBM SPSS Statistics 26.0 (IBM Corporation, Armonk, NY, USA). Analysis of  
187 similarities (ANOSIM) was conducted to assess the significance of the differences  
188 ( $p < 0.05$ ) between samples for the assayed variables. Shared and unique ARGs harbored  
189 by different sloping habitats were visualized by the bipartite network analyses using the  
190 Yifan Hu layout in Gephi. A significant correlation between two variables (i.e. ARG  
191 and physiochemical parameter) with Spearman's correlation coefficient ( $\rho > 0.8$  and  $p$ -  
192 value  $< 0.01$ ), was visualized Gephi 0.9.2 software using the Fruchterman Reingold  
193 layout (Bastian et al., 2009).

## 194 **2.7 Data availability**

195 Sequence data associated with this study have been deposited in the National  
196 Center for Biotechnology Information (NCBI) with BioProject accession number  
197 PRJNA930649.

198

## 199 **3 Results and Discussion**

### 200 **3.1 Profiles of ARGs**

201 A total of 139 ARG subtypes (20 types) were detected in the soils from the gently  
202 sloping cultivated land system. The most diverse ARGs were those resistant to  
203 multidrug (63 subtypes), followed by tetracycline (12), glycopeptide (8),  
204 fluoroquinolone (8), and aminoglycoside (8). It was also observed that ARGs for  
205 multidrug (abundance of 19574 ppm, relative abundance of 71.91%) and  
206 aminocoumarin (2597 ppm, 9.54%) dominated in all samples (Fig. 1a). Of these ARGs,  
207 *rpoB2* (relative abundance of 29.97%, resistant to multidrug), *rpoB* mutant (10.10%,

208 multidrug), *parY* mutant (5.55%, aminocoumarin), *MuxB* (5.28%, multidrug), and *novA*  
209 (3.99%, aminocoumarin) were predominant subtypes frequently detected (Fig. 1a). The  
210 dominance of *rpoB2* was also reported in other metagenomics studies involving various  
211 environmental media, such as mangrove sediments, lakes, and urban sewage (Imchen  
212 & Kumavath, 2021; Ren & Luo, 2022; Yang et al., 2022b). Further results revealed that  
213 a total of 24 resistance genes were ubiquitous in all slope gradients with a 100%  
214 detection rate and a relative abundance of >1%. These 24 core ARGs included  
215 multidrug (14), aminocoumarin (2), glycopeptide (2), rifamycin (2), MLS (1),  
216 mupirocin (1), peptide (1), and tetracycline (1) resistance genes (Table S2). The  
217 classification of resistance mechanisms showed that antibiotic efflux was the dominant  
218 resistance mechanism with the relative abundance of 41.8%, followed by antibiotic  
219 target alteration and replacement (40.1%), and antibiotic target alteration (12.8%) (Fig.  
220 S1). This might be attributed to the fact that antibiotic efflux pumps are the main  
221 mechanisms of multidrug resistance genes (Li and Nikaido, 2009), since it was  
222 observed that a substantial 84% of the 63 ARG subtypes for multidrug could develop  
223 resistance via antibiotic efflux in this study.

224 [Figure 1]

225 The total abundance of ARGs in soils coming from the five sloped lands (5°, 10°,  
226 15°, 20°, and 25°) were 5323, 5538, 5095, 5562, and 5702 ppm, respectively, with ARG  
227 subtypes of 102, 103, 107, 107, and 114 in turn (Fig. 1b). The total abundance of ARGs  
228 tended to increase with increasing the slope gradient, except for the 15°. To be more  
229 specific, the total abundance of ARGs at the highest slope of 25° (site W5) increased

230 by 7% (379 ppm) compared to the lowest slope of 5° (site W1). Moreover, the diversity  
231 of ARGs also tended to increase with the slope gradient (except for the 20°, which had  
232 the same diversity as at 15°). For example, 12 more ARGs subtypes were observed at  
233 the highest slope 25° (W5) than at the lowest slope 5° (W1). Considering that the  
234 cultivated lands with slopes of 6 – 25° and 2 – 6° are defined as gently (W2-W5 in this  
235 study) and flat (W1), respectively (Huang & Hewings, 2021), a comparison of ARGs'  
236 profiles in the soils of these two slope grading lands have been conducted. Therefore,  
237 the average abundance of ARGs in W2 (10°), W3 (15°), W4 (20°), and W5 (25°) was  
238 regarded as the abundance of ARGs in soil of gently sloping cultivated land (Table S3).  
239 Results showed that ARGs in soils from lands with gently slopes were more diverse  
240 (135 subtypes) and abundant (5474 ppm) than those in soils of flat sloping cultivated  
241 land (102 subtypes, 5323 ppm). To be more specific, 37 ARGs subtypes were detected  
242 only in gently sloping cultivated lands, and 94 ARGs subtypes were more abundant in  
243 gently sloping land than in flat sloping land (Table S2). In addition, the abundance of  
244 ARGs in either gently or flat sloping land was much higher than those reported  
245 previously, which might be attributed to (a) the different research approaches (i.e. qPCR  
246 or metagenomics) (He et al., 2023; Chi et al., 2022), and (b) the soil properties of the  
247 sloping land, such as the soil's organic carbon, available phosphorus, pH, salinity, and  
248 content of carbonates (Blaschke et al., 2000; Simansky et al., 2019). It has been  
249 previously reported that the soil properties can significantly affect the distribution of  
250 ARGs (Wang et al., 2020a) or can have a limiting effect on the propagation of ARGs  
251 (Zhang et al., 2020b).

252 The response of 139 ARG subtypes to the slope position was also investigated.  
253 126 ARG subtypes were detected in the uphill with total abundance of 13478 ppm,  
254 while 130 subtypes with abundance of 13743 ppm were obtained in the downhill soil  
255 (Fig. 1c). Moreover, higher abundance of ARGs was observed at uphill (2688 ppm)  
256 than downhill (2634 ppm) in flat sloping land, and the opposite phenomenon (2697  
257 ppm at uphill and 2777 ppm at downhill) was obtained in gently sloping land. These  
258 suggested that the soil at downhill in gently sloping farmland was more likely to be a  
259 hotspot for ARGs accumulation compared to a flat sloping land. This may be attributed  
260 to the transfer of soil substrates from the uphill to the downhill or from the surface to  
261 the deep soil caused by rainfall erosion and gravity effects (Simansky et al., 2019;  
262 Neverman et al., 2023). Given that soil substrates (e.g. soil aggregates) can be an  
263 important storage unit of ARGs (Xu et al., 2023), unbalancing the presence of soil  
264 aggregates and changing their size can have an impact on the ARG profile in the soil  
265 (Cheng et al., 2022).

266 The shared and unique ARG subtypes of the samples from the five slope gradients  
267 at the two positions were also characterized by bipartite association network (Fig. 1d).  
268 It was found that the number of shared ARG subtypes in the uphill soils was 56 (13  
269 types) and that in downhill soil was 54 (12 types), with multidrug, aminocoumarin, and  
270 glycopeptide being the dominant resistance genes, accounting for more than 83% in all  
271 shared ARGs. The number and abundance of unique ARG subtypes were relatively  
272 small and low, only 3 (in WU4)- 6 (in WU2 and WU3, respectively) subtypes were  
273 identified as the unique ARGs in sites of five slope gradients with two positions, and

274 only *vatF* against MLS was >5 ppm. This is in contrast to the large number of unique  
275 ARGs found in northeastern black soils (Wang et al., 2022b). The reasons for this  
276 disparity may stem from the differences in (a) the span of the sampling area (Tang et  
277 al., 2021), (b) the physicochemical properties due to soil type (Wang et al., 2020b;  
278 Zhang et al., 2020b), and (c) in the annotation method of the ARGs assay (Lu et al.,  
279 2020).

### 280 **3.2 Identification of risk genes**

281 A total of 72 subtypes (8745 ppm) with health risk were identified from all 139  
282 ARGs, based on the current risk assessment framework. These included 38 subtypes  
283 (relative abundance of 59.1%) in Q1, 12 subtypes (18.0%) in Q2, 9 subtypes (6.1%) in  
284 Q3, and 13 subtypes (16.7%) in Q4, respectively (Fig. 2a), with dominant ARGs against  
285 multidrug (6395 ppm, 73.1%), followed by glycopeptide (614 ppm, 7.0%), peptide (504  
286 ppm, 5.8%), MLS (426 ppm, 4.9%), and rifamycin (425 ppm, 4.9%). The abundances  
287 of risky ARGs were 1585 (W1), 1882 (W2), 1715 (W3), 1779 (W4), and 1783 (W5)  
288 ppm, with subtype number of 56, 61, 61, 60, and 59, respectively (Fig. 2b, c). It can be  
289 seen that the abundance and diversity of risk ARGs did not change regularly with slope  
290 gradient, but when it comes to slope grades, the lowest overall health risk of ARGs was  
291 obtained in flat sloping cultivated land (W1), while gently sloping farmland posed  
292 higher risk, especially in W2. In addition, the abundance of risk ARGs was higher in  
293 the uphill soils (4404 ppm, 66 subtypes) than in the downhill ones (4340 ppm, 69  
294 subtypes) (Fig. 2b, c). These phenomena were in contrast with the distribution of all  
295 ARGs that showed that the most abundant and diverse ARGs (5702ppm, 114 subtypes)

296 occurred in the highest slope gradient (W5) (Fig. 1b), and they tended to accumulate in  
297 the downhill soils (Fig. 1c). Therefore, more attention should be paid regarding ARGs  
298 with health risk in soils especially at uphill in gently sloping cultivated land with low  
299 slope gradients (e.g. 10° in this study).

300 [Figure 2]

301

302 Furthermore, ARGs with the highest health risk (Q1) were emphasized. Among the 38  
303 ARGs in Q1, the multidrug resistance genes (4886 ppm, 31 subtypes) were the most  
304 predominant, followed by peptide (2143 ppm, 2), aminoglycoside (279 ppm, 2),  
305 fluoroquinolone (225 ppm, 2), and MLS (137 ppm, 1) (Table S4). The abundance of  
306 ARGs in Q1 was also the highest in W2 (10°) with 1139 ppm, but decreased to 971  
307 ppm in W3 (15°), and gradually accumulated to 1066 ppm as slope gradients increased.  
308 Besides, a relatively low abundance (979 ppm) was obtained in W1 (Fig. 2b). This  
309 change was consistent with the distribution of risk ARGs (Q1-Q4), and this result was  
310 mainly caused by multidrug resistance genes, such as *MexF*, *oqxB*, *ceoB*, *mexQ*, and  
311 *AxyY*, that had the highest abundance in W2 and showed the same trend of change with  
312 the risk ARGs. In addition, as far as the slope position is concerned, the highest  
313 abundance and diversity of high-risk ARGs was observed in the downhill soils (2607  
314 ppm, 38 subtypes) compared with those at uphill (2563 ppm, 36 subtypes) (Fig. 2b, c).  
315 To be more specific, the higher abundance of high-risk ARGs (Q1) in the downhill soils  
316 was mainly contributed by 21 subtypes, consisting of *mexQ* and *adeF* increased the  
317 most at downhill, up to 48 ppm and 23 ppm, respectively (Table S4).



318 Notably, the shared high-risk ARGs accounted for >98% of the total abundance of  
319 high-risk ARGs, and 16 risk genes including 3 ARGs in Q1 (*arlR*, *Mex*, *baeR*), 4 ARGs  
320 in Q2 (*oleB*, *qacH*, *QepA2*, *mdtA*), 4 ARGs in Q3, and 5 ARGs in Q4 were found only  
321 in gently sloping cultivated soils (not present in the site W1) (Fig. 2a). Moreover, 2  
322 multidrug resistance genes, *smeE* (Q1) and *qacH* (Q2) were found to be strongly  
323 correlated with changes of the slope gradient, and their abundance increased with slope  
324 gradient increasing (Fig. 2a). These results all indicate that risk ARGs are more  
325 prevalent in gently sloping cultivated soils than in flat sloping cultivated land, and that  
326 the risk rank, abundance, and diversity of ARGs are closely related to the slope gradient.  
327 Meanwhile, it is worth highlighting that the multidrug resistance genes in this study  
328 showed high abundance, high diversity and high risk. Multidrug resistance (MDR)  
329 genes are one of the most common as well as widespread resistance gene classes in the  
330 environment (Liu et al., 2023), and the high prevalence of MDR genes in soil has also  
331 raised concerns in recent years (Qian et al., 2021; Yi et al., 2022). However, treating  
332 MDR infections can be difficult because these genes are resistant to multiple antibiotics  
333 (Zhang et al., 2020a), and therefore the risk of MDR genes, which emerged in this study,  
334 also needs to be taken into account.

### 335 **3.3 Distribution of MGEs**

336 Metagenomics analysis also revealed changes in the abundance as well as the  
337 diversity of two important MGEs, namely plasmids and insertion sequences (ISs), and  
338 therefore further evaluation of the horizontal transfer potential of ARGs in different  
339 slopes was carried out. 2027 types of plasmids with the total abundance of 14454 ppm

340 were identified in sloped soils, and the abundances of the top 14 named plasmids under  
341 each slope gradient are shown in [Fig. 3a](#). Both high abundance and large diversity of  
342 plasmids were observed in the soils from lands with the highest slope gradients (W5,  
343 25°, 3380 ppm, 1230 types), while the lowest abundance and diversity was observed in  
344 the 5° sloping soils (site W1) with 2423 ppm and 926 types. Moreover, the abundance  
345 and diversity of plasmids in gently sloping cultivated soils (3008 ppm, 1959 types) were  
346 also much higher than those in flat sloping cultivated soils (2423 ppm, 926 types) ([Fig.](#)  
347 [3c, e](#)). These indicated that ARGs in gently sloping cultivated soils, especially in the  
348 high slope gradient, were more likely to undergo intercellular plasmid-mediated  
349 horizontal gene transfer and propagation. Similar propagation of ARGs through  
350 plasmids in the soil environment has also been widely reported ([Jiang et al., 2022](#); [Meng](#)  
351 [et al., 2022](#); [Zhang et al., 2022a](#)), and bacterial communities ([Bennett et al., 2008](#);  
352 [Ellabaan et al., 2021](#)) and soil nutrients ([Lu et al., 2020](#)) were also found to affect the  
353 migration and propagation of ARGs by plasmid-mediated. This might be the main  
354 reason for the differences observed in the abundance and diversity of plasmid in soils  
355 with different slope gradients.

356 ISs are small mobile genetic elements that rely on transposase genes (Tnp), and  
357 they are inserted directly into the ends of the region encoding the resistance gene to  
358 enable gene transfer to make bacteria acquire multiple resistance ([Partridge et al., 2018](#);  
359 [Siguier et al., 2014](#)). A total 478 types of ISs belonging to 24 families were identified  
360 in soils from lands with five slopes, with IS3, IS5, IS21, IS256, and IS30 being the top  
361 five most abundant families ([Fig. 3b](#)) and accounting for more than 52% of the total ISs

362 abundance (5168 ppm). It could be seen that the variation of ISs' abundance and  
363 diversity were irregular and not significantly affected by the slope gradient and position  
364 (Fig. 3d, f). However, a relatively high abundance and diversity were generally  
365 observed in soils from high sloping farmlands (20°), thereby suggesting that a stronger  
366 potential for horizontal transfer of ARGs was more likely to appear in high slope  
367 gradient soils.

368 [Figure 3]

369 Furthermore, the correlation analysis of MGEs (including all ISs families and top  
370 150 abundant plasmids) with high-risk ARGs (Q1 level) showed that a total of 15 MGEs  
371 (including 7 ISs and 8 plasmids) were identified with 14 high-risk ARGs with  
372 significant positive correlations ( $p > 0.8$ ,  $p < 0.01$ ) (Fig. 3g). It should be also noted that  
373 18 high-risk ARGs encoded resistances for multidrug (11), aminoglycoside (1),  
374 fluoroquinolone (1), and peptide (1). This indicates that many multidrug class high-risk  
375 ARGs were associated with MGEs, corroborating the conclusion that multidrug  
376 resistance arises mainly through the accumulation of resistance genes on MGEs by  
377 horizontal gene transfer (Kim et al., 2021). Also, the ARG-MGE co-occurrence  
378 associations implied the potential mobility of ARGs (Zhao et al., 2021b), thus  
379 indicating that the migration and spread of these multidrug high-risk genes in sloping  
380 farmland soil should be paid more attention.

### 381 **3.4 The composition of microbial community in sloping cultivated soils**

382 A total of 190 microbial phyla were detected in the soils from lands at the different  
383 five slope gradients, including 16646 bacterial, 274 archaeal, and 467 fungal species.

384 For bacteria, 8 phyla (relative abundance > 1%) dominated and contributed by 96.7%  
385 of the total bacterial abundance (Fig. S2). Meanwhile, 19 classified genera which  
386 consisted of *Nocardioides* (relative abundance of 11.85%), *Sphingomonas* (7.43%), and  
387 *Pseudarthrobacter* (6.75%), were recognized as the predominant genera (Fig. S3). As  
388 far as the slope gradient is concerned, excluding the W1 site (5°), it was found that the  
389 bacterial abundance tended to increase with the gradient. To be more specific, the  
390 bacterial abundance in sites W2-W4 (10°-25°) were 688911, 715915, 857646, and  
391 860438 ppm, respectively (Fig. 4a). At the same time, the diversity of bacteria did not  
392 vary regularly with the slope gradient, with the lowest diversity in W1 (5°, 1904) and  
393 the highest diversity in W4 (20°, 1969) (Fig. 4b). Moreover, the total bacterial  
394 abundance of 2130896 ppm in soils at downhill positions was much higher than the  
395 uphill ones with 1779594 ppm (Fig. 4e), and these differences were mainly due to  
396 *Nocardioides*, *Sphingomonas*, and *Phycococcus* (Fig. S3). In addition, Wilcoxon rank-  
397 sum test on bacterial genera revealed significant differences ( $p < 0.05$ ) in the abundance  
398 of 15 bacterial genera between soils of uphill and downhill, hereinto, 5 genera showed  
399 higher abundance in the uphill soils and other 10 genera dominated at downhill (Fig.  
400 4c). Furthermore, LEfSe analysis (LDA values > 2.5) identified that 3 genera, namely  
401 *Phycococcus*, *Massilia*, and *Geodermatophilus*, which could be considered as downhill  
402 **biomarkers** and had significant effects on the abundance differences at bacterial genus  
403 level between slope positions (Fig. 4c). As reported, *Phycococcus* was considered as an  
404 important genus of bacteria in Cd-stressed soils in a study which explored the effects  
405 of heavy metal addition in soil microbial communities (Wang et al., 2020c). *Massilia*

406 has been screened and identified in mining soils (Feng et al., 2016), farmland soils (Lee  
407 et al., 2017), and sludge (Rodríguez-Díaz et al., 2014) contaminated with heavy metals,  
408 and was found to be an important resistant microorganism in heavy metal-stressed  
409 environments and showed significant resistance to cadmium contamination (Zhou et al.,  
410 2021). The accumulation of these metal-resistant bacteria in the downhill soil indicates  
411 that in addition to the risk of ARGs, there may also be a potential risk of heavy metal  
412 pollution in the downhill soil, and that antibiotic-metal co-resistance carried by  
413 microorganisms needs to be emphasized.

414 No significant difference ( $p>0.05$ ) was obtained in the composition and abundance  
415 of archaea and Nitrososphaera was always found as the most abundant archaeal phylum,  
416 followed by Candidatus\_Nitrosocosmicus, Candidatus\_Nitrosotalea, and  
417 Methanosarcina (Fig. S4). Also, a total of 5 fungal phyla were detected in all sites,  
418 containing 92 identified genera (Fig. S5). The abundance of fungi (1905 ppm) was  
419 substantially lower than that of archaea (43049 ppm), but the fungal diversity (92  
420 genera) was higher than archaea's (58 genera) (Fig. 4a, b). Based on the Shannon index  
421 and Simpson index, alpha diversity of fungal communities at the phyla level had  
422 significant structural differences ( $p<0.01$ ) between uphill and downhill soils regardless  
423 of the slope gradients (Fig. S6). In addition, it is noteworthy that the abundance of fungi  
424 in the W1 site (995 ppm) was much higher than those of the other sites (156 ppm, 167  
425 ppm, 143 ppm, and 445 ppm in W2, W3, W4, and W5, respectively) (Fig. 4a). This may  
426 be due to the fact that land with slopes would induce soil erosion, which causes land  
427 degradation and has a significant negative impact on the soil fungal community (Hao

428 et al., 2022; Du et al., 2021), and thus decreasing fungal abundance and diversity in  
429 gently sloping farmlands. [Figure 4]

430 Notably, a total of 56 human pathogenic bacteria (HPB) species in 38 genera were  
431 identified in all studied sites, including 5 superbugs (*Acinetobacter baumannii*,  
432 *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and  
433 *Enterobacter cloacae*). The most abundant HPB were *Bacillus cereus* (4066 ppm,  
434 relative abundance of 29.2%), followed by *Pseudomonas spp.* (2683 ppm, 19.3%) and  
435 *Acinetobacter baumannii* (678 ppm, 4.9% ) (Fig. 4d). Regarding the slope position,  
436 HPB abundance did not differ significantly in the uphill (6932 ppm) and downhill (6986  
437 ppm) soils, while their diversity was greater at uphill (56 species) than downhill (51  
438 species). As far as the slope gradients were concerned, the highest total abundance of  
439 HPB (2905 ppm) was detected in the soils in W1, with the lowest slope gradient (5°),  
440 while the lowest abundance (2693 ppm) occurred in soils with slope gradient of 15°  
441 (Fig. 4a, b). Besides, in gently sloping farmland (W2-W5), the diversity of HPB  
442 increased when the slope gradient was increased (Fig. 4b). Nevertheless, *Acinetobacter*  
443 *Baumannii* (superbug) was reduced by 376 ppm from 10° (W2) to 25° (W5), and the  
444 highest total abundance of 5 detected superbugs was also observed at W2 (10°). These  
445 suggested that HPB, especially superbugs that are dangerous to human health, may be  
446 more likely to accumulate in soils with relatively low slope gradients. Further  
447 investigation based on correlation analysis showed that AK in sloping soils was the key  
448 factor affecting HPB, especially *Acinetobacter baumannii*, *Staphylococcus aureus*, and  
449 *Salmonella spp.* ( $\rho>0.8$ ,  $p<0.01$ ) (Fig. S7). Similarly, the positive correlation of AK

450 with potentially pathogenic genera was found in other soils (Liu et al., 2022), therefore,  
451 the high AK content detected in the soil of the site W1 (Table S1) could be the reason  
452 for the enrichment of HPB in the soil with low slope gradient.

### 453 **3.5 Potential hosts and potential pathogen hosts of risk ARGs**

454 The network analysis between all ARGs with high risk (Q1, 38 subtypes) and  
455 bacterial communities (top 150 abundant genera) ( $\rho > 0.8$ ,  $p < 0.01$ ) was carried out. The  
456 co-occurrence network was composed of 71 nodes and 61 edges, including 52 genera  
457 and 19 ARGs in Q1 (Fig. 5a). There was a strong positive correlation between 41 genera  
458 and 19 high-risk ARGs. In particular, bacteria in genera *Aromatoleum*,  
459 *Paraburkholderia*, *Lysobacter*, *Brevundimonas*, and *Erythrobacter* correlated with  
460 more than 2 high-risk ARGs, and 6 high-risk ARGs of *adeJ*, *efrA*, *macB*, *mtrA*, *oqxB*,  
461 and *ugd*, were potentially carried by at least 3 bacterial genera.

462 Alarmingly, potential pathogenic hosts for high-risk ARGs (Q1) were also  
463 revealed. The correlation network analysis showed that a total of 13 HPB species were  
464 significantly correlated with 12 ARGs in Q1 (Fig. 5b), among which *Enterococcus*  
465 *faecalis* was significantly positively correlated with 2 high-risk ARGs (*MexD* and  
466 *bacA*). As reported, *Enterococcus faecalis* was the major pathogens causing  
467 community- and healthcare-associated infections, with an ability to acquire resistance  
468 to multiple antimicrobials (Aung et al., 2023; Mchugh et al., 2022). The greater  
469 abundance of *Enterococcus faecalis* in W1 (7.6 ppm), compared to gently sloping lands  
470 (2.0 ppm, 2.4 ppm, 3.2 ppm, and 2.7 ppm in W2, W3, W4, and W5, respectively)  
471 regardless of slope position, suggested a twin-risk of pathogenicity and antibiotic

472 resistance caused by *Enterococcus faecalis* in flat sloping cultivated land. Moreover,  
473 *MexW* (Q1), which resists a variety of antibiotics, was significantly positively  
474 associated with 3 HPB species. Similar results were reported previously and particular  
475 *MexW* was recognized as the gene resistance to metals and biocides (Liu et al., 2018;  
476 Yang et al., 2022a). Thereby, the high abundance of *MexW* in W2-W5 (73.4 ppm, 70.0  
477 ppm, 83.0 ppm, and 58.8) (Table S4) indicated that high-risk ARGs in gently sloping  
478 farmland may have a complex risk of co-resistance (antibiotic-metal-pesticide co-  
479 resistance) and pathogenicity.

### 480 **3.6 Determining the direct and indirect effects on the behavior of ARGs and** 481 **revealing their behavior mechanism**

482 SEM was constructed to explore the mechanism of slope gradient and slope  
483 position on the behavior of all ARGs and ARGs with health risk. As illustrated in Fig.  
484 6a, both the slope gradient and position can directly and significantly affect positively  
485 the behavior of ARGs. Moreover the slope position can also indirectly affect the ARGs'  
486 behavior by influencing MGEs. Moreover, the soil properties directly affected ARGs'  
487 behavior and possessed indirect effect via influencing bacterial and fungal community  
488 structures. Notably, the insignificant effects of the slope gradient and slope position on  
489 soil properties were observed in SEM, and these were not consistent with those reported  
490 in other studies (Zou et al., 2021; Neupane et al., 2022; Shigyo et al., 2022). The reasons  
491 for this might be that the soil properties in SEM were for the overall physicochemical  
492 properties, while when it comes to specific correlation analysis, the significant  
493 relationship between the slope gradient and soil NO<sub>2</sub><sup>-</sup>-N, as well as slope position and



494 TP, PN, MBC, and MBN, respectively, were obtained (Fig. S7). These suggested that  
495 the slope gradient and position may influence the profile of soil ARGs by affecting the  
496 specific soil properties.

497 Furthermore, bacteria as the main potential hosts of ARGs had a significant direct  
498 effect on ARGs and they could also indirectly influence ARGs by affecting MGEs  
499 (Fig. 6a). In the affecting pathway of soil properties-bacteria-ARGs, a positive  
500 effect followed by a negative one was observed and this could be attributed to the  
501 properties of soil. To be more specific, resources in the soil that are available for  
502 microbes (dominated by bacteria) would positively correlate with microbial  
503 abundance, which was negatively related with microbial competition, that needed  
504 antibiotics production, thereby inducing a shift of antibiotics resistance (Chen et  
505 al., 2022). Furthermore, all effects between MGEs and ARGs were direct, which  
506 was also widely demonstrated previously (Zhao et al., 2021a). Comparing the total  
507 effect of each factor on ARGs, the abundance of ARGs was mostly influenced  
508 negatively by bacterial community and positively by MGEs. Apart from the slope  
509 gradients and MGEs, the total effects of other factors on the profile of ARGs were  
510 all negative, and this might be due to the negative offsetting from indirect effects  
511 (Fig. 6c).

512 [Figure 6]

513 For ARGs with health risk, the slope gradient was found to have a significant  
514 positive relationship with risk ARGs, while the impact of the slope position was not  
515 significant on the factors studied in SEM (Fig. 6b). Regarding the total effects, risky

516 ARGs were mainly influenced by MGEs and HPB, both positively and directly (Fig.  
517 6d). This indicates that the increase of MGEs and HPB in sloping cultivated soil will  
518 lead to an increase in the abundance of risky ARGs, which is due to the fact that risk  
519 ARGs always appear on the same segment of genes as MGEs (Zhang et al., 2022a), and  
520 HPB is often observed as potential hosts for risky ARGs (Li et al., 2023).

#### 521 **4 Conclusions**

522 The risk of antibiotic resistance was studied in terms of ARGs with health risk,  
523 their potential pathogenic hosts, and MGEs in soils from sloping cultivated land, which  
524 is a global important agricultural land type. The soils from sloping cultivated land were  
525 recognized as a hotspot for ARGs, which tended to enrich in gently sloping farmland  
526 with high slopes and downhill position. For risky ARGs and HPB, more attention  
527 should be paid on multidrug ARGs and five superbugs in soils at low slope gradient and  
528 uphill, respectively. The slope gradient and slope position were found to affect the  
529 composition of ARGs through direct effects, and the slope position can also indirectly  
530 affect the ARGs' behavior by influencing MGEs. Moreover, ARGs were mainly  
531 affected by bacterial community and MGEs, and risky ARGs were greatly influenced  
532 by HPB and MGEs. Overall, this study contributes to better understanding of the risk  
533 differences in ARGs and HPB and highlights the importance of monitoring antibiotic  
534 resistance in gently sloping farmland, especially at downhill position, in order to be  
535 consistent with the One Health approach.

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## 540 **References**

- 541 Bastian M, Heymann S, Jacomy M. Gephi: an open source software for exploring and  
542 manipulating networks. ICWSM. 2009.
- 543 Bennett, P.M., 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of  
544 antibiotic resistance genes in bacteria. *Br. J. Pharmacol.* 153 (S1), S347–S357.
- 545 Bernhard, N., Moskwa, L.M., Schmidt, K., Oeser, R.A., Aburto, F., Bader, M.Y.,  
546 Baumann, K., von Blanckenburg, F., Boy, J., van den Brink, L., Brucker, E.,  
547 Budel, B., Canessa, R., Dippold, M.A., Ehlers, T.A., Fuentes, J.P., Godoy, R.,  
548 Jung, P., Karsten, U., Koster, M., Kuzyakov, Y., Leinweber, P., Neidhardt, H.,  
549 Matus, F., Mueller, C.W., Oelmann, Y., Oses, R., Osses, P., Paulino, L., Samolov,  
550 E., Schaller, M., Schmid, M., Spielvogel, S., Spohn, M., Stock, S., Stroncik, N.,  
551 Tielborger, K., Ubernickel, K., Scholten, T., Seguel, O., Wagner, D., Kuhn, P.  
552 2018. Pedogenic and microbial interrelations to regional climate and local  
553 topography: New insights from a climate gradient (arid to humid) along the  
554 Coastal Cordillera of Chile. *Catena*, 170, 335-355.
- 555 Blaschke, P.M., Trustrum, N.A., Hicks, D.L. 2000. Impacts of mass movement  
556 erosion on land productivity: a review. *Progress in Physical Geography-Earth  
557 and Environment*, 24(1), 21-52.
- 558 Brosens, L., Campforts, B., Robinet, J., et al. Slope Gradient Controls Soil Thickness  
559 and Chemical Weathering in Subtropical Brazil: Understanding Rates and  
560 Timescales of Regional Soilscape Evolution Through a Combination of Field  
561 Data and Modeling. *Journal of Geophysical Research-Earth Surface*, 2020,  
562 125(6).
- 563 Chen, Q.-L., Hu, H.-W., Yan, Z.-Z., Zhu, Y.-G., He, J.-Z., Delgado-Baquerizo, M.  
564 2022. Cross-biome antibiotic resistance decays after millions of years of soil  
565 development. *Isme Journal*, 16(7), 1864-1867.
- 566 Cheng, J.-H., Tang, X.-Y., Cui, J.-F. 2022. Distinct aggregate stratification of  
567 antibiotic resistome in farmland soil with long-term manure application. *Science  
568 of the Total Environment*, 833.
- 569 Chi, S., Xu, W., Han, Y. 2022. ARGs distribution and high-risk ARGs identification  
570 based on continuous application of manure in purple soil. *Science of the Total  
571 Environment*, 853.
- 572 Du, L., Guo, S., Gao, X., Li, W., Li, X., Hou, F., Wang, R. 2021. Divergent responses  
573 of soil fungal communities to soil erosion and deposition as evidenced in topsoil  
574 and subsoil. *Science of the Total Environment*, 755.
- 575 Ellabaan, M.M.H., Munck, C., Porse, A., Imamovic, L., Sommer, M.O.A. 2021.  
576 Forecasting the dissemination of antibiotic resistance genes across bacterial  
577 genomes. *Nature Communications*, 12(1).

578 G.D. Feng, S.Z. Yang, H.P. Li, et al. *Massilia putida* sp. nov., a dimethyl disulfide-  
579 producing bacterium isolated from wolfram mine tailing *Int. J. Syst. Evol.*  
580 *Microbiol.*, 66 (1) (2016), pp. 50-55, 10.1099/ijsem.0.000670

581 Hao, W., Xia, B., Xu, M. 2022. Erosion-deposition positively reconstruct the bacterial  
582 community and negatively weaken the fungal community. *Catena*, **217**.

583 H. Lee, D.U. Kim, S. Park, et al. *Massilia chloroacetimidivorans* sp. nov., a  
584 chloroacetamide herbicide-degrading bacterium isolated from soil *Ant.*  
585 *Leeuwenhoek Int. J. Gen. Mole. Microbiol.*, 110 (6) (2017), pp. 751-758,

586 He, L.-X., He, L.-Y., Gao, F.-Z., Zhang, M., Chen, J., Jia, W.-L., Ye, P., Jia, Y.-W.,  
587 Hong, B., Liu, S.-S., Liu, Y.-S., Zhao, J.-L., Ying, G.-G. 2023. Mariculture  
588 affects antibiotic resistome and microbiome in the coastal environment. *Journal*  
589 *of Hazardous Materials*, 452.

590 Hou, G., Delang, C.O., Lu, X. 2020. Afforestation changes soil organic carbon stocks  
591 on sloping land: The role of previous land cover and tree type. *Ecological*  
592 *Engineering*, 152.

593 Hu, W., Zheng, G., Fang, D., Cui, C., Liang, J., Zhou, L., 2015. Bioleached sludge  
594 composting drastically reducing ammonia volatilization as well as decreasing  
595 bulking agent dosage and improving compost quality: a case study. *Waste*  
596 *Manag.* 44, 55–62.

597 Huang, Y., Hewings, G. 2021. More Reliable Land Price Index: Is There a Slope  
598 Effect? *Land*, 10(3).

599 Imchen, M., Kumavath, R. 2021. Metagenomic insights into the antibiotic resistome  
600 of mangrove sediments and their association to socioeconomic status.  
601 *Environmental Pollution*, 268.

602 Jiang, C., Feng, J., Zhu, S.F., et al. Characteristics of the Soil Microbial Communities  
603 in Different Slope Positions along an Inverted Stone Slope in a Degraded Karst  
604 Tiankeng. *Biology Basel*, 2021, 10(6).

605 Jiang, Q., Lu, W., Zhang, L., Jin, Y., Wang, Y., Chen, J., Ye, Z., Xiao, M. 2022.  
606 Promotion mechanism of self-transmissible degradative plasmid transfer in  
607 maize rhizosphere and its application in naphthalene degradation in soil. *Journal*  
608 *of Environmental Sciences*, 115, 240-252.

609 Karkman, A., Parnanen, K., Larsson, D.G.J. 2019. Fecal pollution can explain  
610 antibiotic resistance gene abundances in anthropogenically impacted  
611 environments. *Nature Communications*, 10.

612 Li, L.G., Huang, Q., Yin, X., Zhang, T., 2020. Source tracking of antibiotic resistance  
613 genes in the environment - challenges, progress, and prospects. *Water Res.* 185,  
614 116127.

615 Li J, Xin Z, Zhang Y, et al. Long-term manure application increased the levels of  
616 antibiotics and antibiotic resistance genes in a greenhouse soil[J]. *Applied Soil*  
617 *Ecology*, 2017,121:193-200.

618 Li, W., Pang, L., Chatzisyneon, E., Yang, P. 2023. Effects of micron-scale zero valent  
619 iron on behaviors of antibiotic resistance genes and pathogens in thermophilic  
620 anaerobic digestion of waste activated sludge. *Bioresource Technology*, 376.

621 Liang, H., Wang, F., Mu, R., Huang, J., Zhao, R., Li, X., Yu, K., Li, B. 2021.  
622 Metagenomics analysis revealing the occurrence of antibiotic resistome in salt  
623 lakes. *Science of the Total Environment*, 790.

624 Liu, C., Chen, Y., Li, X., Zhang, Y., Ye, J., Huang, H., Zhu, C., 2020. Temporal effects  
625 of repeated application of biogas slurry on soil antibiotic resistance genes and  
626 their potential bacterial hosts. *Environ. Pollut.* [https://doi.org/10.1016/j.en](https://doi.org/10.1016/j.envpol.2019.113652)  
627 [vpol.2019.113652](https://doi.org/10.1016/j.envpol.2019.113652).

628 Liu, H., Li, S., Qiang, R., Lu, E., Li, C., Zhang, J., Gao, Q. 2022. Response of Soil  
629 Microbial Community Structure to Phosphate Fertilizer Reduction and  
630 Combinations of Microbial Fertilizer. *Frontiers in Environmental Science*, 10.

631 Liu, X., Yang, S., Wang, Y., Zhao, H.-P., Song, L. 2018. Metagenomic analysis of  
632 antibiotic resistance genes (ARGs) during refuse decomposition. *Science of the*  
633 *Total Environment*, 634, 1231-1237.

634 Liu, Z., Zhao, Y., Zhang, B., Wang, J., Zhu, L., Hu, B. 2023. Deterministic Effect of  
635 pH on Shaping Soil Resistome Revealed by Metagenomic Analysis.  
636 *Environmental Science & Technology*.

637 Lu, W., Wang, M., Wu, J., et al. Spread of chloramphenicol and tetracycline resistance  
638 genes by plasmid mobilization in agricultural soil. *Environmental Pollution*,  
639 2020, 260.

640 Lu, Y., Zheng, G., Zhou, W., Wang, J., Zhou, L., 2019. Bioleaching conditioning  
641 increased the bioavailability of polycyclic aromatic hydrocarbons to promote  
642 their removal during co-composting of industrial and municipal sewage sludges.  
643 *Sci. Total Environ.* 665, 1073–1082.

644 M. Rodríguez-Díaz, F. Cerrone, M. Sánchez-Peinado, et al. *Massilia umbonata* sp.  
645 nov., able to accumulate poly-beta-hydroxybutyrate, isolated from a sewage  
646 sludge compost-soil microcosm *Int. J. Syst. Evol. Microbiol.*, 64 (1) (2014), pp.  
647 131-137, 10.1099/ijs.0.049874-0

648 Mchugh, M.P., Parcell, B.J., Pettigrew, K.A. 2022. Presence of *optrA*-mediated  
649 linezolid resistance in multiple lineages and plasmids of *Enterococcus faecalis*  
650 revealed by long read sequencing. *Microbiology*, (2), 168.

651 Meng, M., Li, Y., Yao, H. 2022. Plasmid-Mediated Transfer of Antibiotic Resistance  
652 Genes in Soil. *Antibiotics-Basel*, 11(4).

653 Neupane, J., Guo, W., Cao, G., et al. Spatial patterns of soil microbial communities  
654 and implications for precision soil management at the field scale. *Precision*  
655 *Agriculture*, 2022, 23(3), 1008-1026.

656 O'Brien, F. J. M., Almaraz, M., Foster, M. A., et al. Soil Salinity and pH Drive Soil  
657 Bacterial Community Composition and Diversity Along a Lateritic Slope in the  
658 Avon River Critical Zone Observatory, Western Australia. *Frontiers in*  
659 *Microbiology*, 2019, 10.

660 Partridge, Sally R., Kwong, Stephen M., Firth, Neville, Jensen, Slade O..Mobile  
661 Genetic Elements Associated with Antimicrobial Resistance[J].*Clinical*  
662 *microbiology reviews*,2018,31(4):e00088-e00088.

663 Qian, X.; Gunturu, S.; Guo, J.; Chai, B.; Cole, J. R.; Gu, J.; Tiedje, J. M.  
664 Metagenomic analysis reveals the shared and distinct features of the soil

665 resistome across tundra, temperate prairie, and tropical ecosystems. *Microbiome*  
666 2021, 9 (1), 108.

667 Ren, Z., Luo, W. 2022. Metagenomic analysis reveals the diversity and distribution of  
668 antibiotic resistance genes in thermokarst lakes of the Yellow River Source Area.  
669 *Environmental Pollution*, 313.

670 Shigyo, N., Furusawa, H., Yamashita, N., et al. Slope-induced factors shape bacterial  
671 communities in surface soils in a forested headwater catchment. *Catena*, 2022,  
672 214.

673 Siguier, P., Gourbeyre, E., Chandler, M., 2014. Bacterial insertion sequences: their  
674 genomic impact and diversity. *FEMS Microbiol. Rev.* 38 (5), 865–891.

675 Simansky, V., Juriga, M., Mendyk, L. 2019. Slope position and management practices  
676 as factors influencing selected properties of topsoil. *Soil Science Annual*, 70(2),  
677 137-146.

678 Singh, R., Singh, A.P., Kumar, S., Giri, B.S., Kim, K.H. 2019. Antibiotic resistance in  
679 major rivers in the world: A systematic review on occurrence, emergence, and  
680 management strategies. *Journal of Cleaner Production*, 234, 1484-1505.

681 Surette, M.D., Wright, G.D. 2017. Lessons from the Environmental Antibiotic  
682 Resistome. in: *Annual Review of Microbiology*, Vol 71, (Ed.) S. Gottesman, Vol.  
683 71, pp. 309-329.

684 Tang, X., Shen, M., Zhang, Y., Zhu, D., Wang, H., Zhao, Y., Kang, Y. 2021. The  
685 changes in antibiotic resistance genes during 86 years of the soil ripening process  
686 without anthropogenic activities. *Chemosphere*, 266.

687 Tosi, M., Drummelsmith, J., Obregon, D., Chahal, I., Van Eerd, L.L., Dunfield, K.E.  
688 2022. Cover crop-driven shifts in soil microbial communities could modulate  
689 early tomato biomass via plant-soil feedbacks. *Scientific Reports*, 12(1).

690 Wan, S.; Xia, M.; Tao, J.; Pang, Y.; Yu, F.; Wu, J.; Chen, S. Metagenomics Analysis  
691 Reveals the Microbial Communities, Antimicrobial Resistance Gene Diversity  
692 and Potential Pathogen Transmission Risk of Two Different Landfills in China.  
693 *Diversity* 2021, 13, 230.

694 Wang F, Han W, Chen S, et al. Fifteen-Year Application of Manure and Chemical  
695 Fertilizers Differently Impacts Soil ARGs and Microbial Community  
696 Structure[J]. *Frontiers in Microbiology*, 2020a,11:62.

697 Wang, F.H., Sun, R.B., Hu, H.W., Duan, G.L., Meng, L., Qiao, M. 2022a. The overlap  
698 of soil and vegetable microbes drives the transfer of antibiotic resistance genes  
699 from manure-amended soil to vegetables. *Science of the Total Environment*, 828.

700 Wang, J., Zhang, Q., Chu, H., Shi, Y., Wang, Q. 2022b. Distribution and co-  
701 occurrence patterns of antibiotic resistance genes in black soils in Northeast  
702 China. *Journal of Environmental Management*, 319.

703 Wang, L., Wang, J., Wang, J., Zhu, L., Conkle, J.L., Yang, R. 2020. Soil types  
704 influence the characteristic of antibiotic resistance genes in greenhouse soil with  
705 long-term manure application. *Journal of Hazardous Materials*, 392.

706 Wang, X., Dong, G., Liu, X., Zhang, S., Li, C., Lu, X., Xia, T. 2020c. Poly-gamma-  
707 glutamic acid-producing bacteria reduced Cd uptake and effected the rhizosphere  
708 microbial communities of lettuce. *Journal of Hazardous Materials*, 398.

709 Xu, F., Guan, J., Zhou, Y., Song, Z., Shen, Y., Liu, Y., Jia, X., Zhang, B., Guo, P. 2023.  
710 Effects of freeze-thaw dynamics and microplastics on the distribution of  
711 antibiotic resistance genes in soil aggregates. *Chemosphere*, 329, 138678-  
712 138678.

713 Yang, F., Shen, S., Gao, W., Ma, Y., Han, B., Ding, Y., Wang, X., Zhang, K. 2022a.  
714 Deciphering discriminative antibiotic resistance genes and pathogens in  
715 agricultural soil following chemical and organic fertilizer. *Journal of*  
716 *environmental management*, 322, 116110-116110.

717 Yang, Y., Li, B., Ju, F., Zhang, T., 2013. Exploring variation of antibiotic resistance  
718 genes in activated sludge over a four-year period through a metagenomic  
719 approach. *Environ. Sci. Technol.* 47 (18), 10197–10205.

720 Yang, Z., Liu, P., Wei, H., Li, H., Li, J., Qiu, X., Ding, R., Guo, X. 2022b. Alteration  
721 in microbial community and antibiotic resistance genes mediated by  
722 microplastics during wastewater ultraviolet disinfection. *Science of the Total*  
723 *Environment*, 825.

724 Yi, X.; Liang, J.-L.; Su, J.-Q.; Jia, P.; Lu, J.-l.; Zheng, J.; Wang, Z.; Feng, S.-w.; Luo,  
725 Z.-h.; Ai, H.-x.; Liao, B.; Shu, W.-s.; Li, J.-t.; Zhu, Y.-G. Globally distributed  
726 mining-impacted environments are underexplored hotspots of multidrug  
727 resistance genes. *ISME J.* 2022, 16, 2099.

728 Zhang, P., Liu, M., Fu, J., Zhong, C., Zong, G., Cao, G. 2020a. Identification of a  
729 mobilizable, multidrug-resistant genomic island in *Myroides odoratimimus*  
730 isolated from Tibetan pasture. *Science of the Total Environment*, 723.

731 Zhang, S., Wen, J., Wang, Y., Wang, M., Jia, R., Chen, S., Liu, M., Zhu, D., Zhao, X.,  
732 Wu, Y., Yang, Q., Huang, J., Ou, X., Mao, S., Gao, Q., Sun, D., Tian, B., Cheng,  
733 A. 2022a. Dissemination and prevalence of plasmid-mediated high-level  
734 tetracycline resistance gene tet (X4). *Frontiers in Microbiology*, 13.

735 Zhang, Y., Liu, H., Dai, X., Cai, C., Wang, J., Wang, M., Shen, Y., Wang, P. 2020b.  
736 Impact of application of heat-activated persulfate oxidation treated erythromycin  
737 fermentation residue as a soil amendment: Soil chemical properties and antibiotic  
738 resistance. *Science of the Total Environment*, 736.

739 Zhang, Z., Zhang, Q., Wang, T. et al. Assessment of global health risk of antibiotic  
740 resistance genes. *Nat Commun* 13, 1553 (2022b).

741 Zhao, Q., Guo, W.Q., Luo, H.C., Xing, C.M., Wang, H.Z., Liu, B.H., Si, Q.S., Ren,  
742 N.Q. 2021a. Deciphering the transfers of antibiotic resistance genes under  
743 antibiotic exposure conditions: Driven by functional modules and bacterial  
744 community. *Water Research*, 205.

745 Zhao, R., Feng, J., Huang, J., Li, X., Li, B. 2021b. Responses of microbial community  
746 and antibiotic resistance genes to the selection pressures of ampicillin,  
747 cephalexin and chloramphenicol in activated sludge reactors. *Science of the Total*  
748 *Environment*, 755.

749 Zhao, Y., Zhang, X.X., Zhao, Z., Duan, C., Chen, H., Wang, M., Ren, H., Yin, Y., Ye,  
750 L., 2018. Metagenomic analysis revealed the prevalence of antibiotic resistance  
751 genes in the gut and living environment of freshwater shrimp. *J. Hazard. Mater.*  
752 350, 10–18.

753 Zhou, D., Liang, X., Wang, J., Wang, S., Li, X., Ning, Y. 2021. Study on the  
754 regulatory mechanism of the earthworm microbial community in vitro and in  
755 vivo under cadmium stress. *Environmental Pollution*, 279.  
756 Zhuang M, Achmon Y, Cao YP, Liang XM, Chen L, Wang H, Siame BA, Leung KY.  
757 Distribution of antibiotic resistance genes in the environment. *Environ Pollut.*  
758 2021. <https://doi.org/10.1016/j.envpol.2021.117402>.  
759 Zou, X., Zhang, Z., Wu, M., et L. Slope-scale spatial variability of fractal dimension  
760 of soil particle size distribution at multiple depths. *Soil Science Society of*  
761 *America Journal*, 2021, 85(1), 117-131.



762 **Figure caption**

763 **Figure 1.** (a) Overall composition profile of antibiotic resistance genes (ARGs) types  
764 (inner circle) with their subtypes (outer circle). (b) Distribution of the dominant ARG  
765 types and subtypes (relative abundance >1%) in soils from lands of five slope gradients.  
766 Others represent ARG types or subtypes with the relative abundance less than 1%. (c)  
767 The abundance and diversity of ARGs in different slope positions. (d) Bipartite  
768 association networks show unique and shared ARGs in the uphill and downhill with  
769 different slope gradients.

770 **Figure 2.** (a) The abundance of risk ARGs at each site. Total abundance (b) and  
771 diversity (b) of risk ARGs of different risk rank in each group.

772 **Figure 3.** The composition of top 14 plasmids (a) and top 10 ISs (b) in five slope  
773 gradients. Total abundance of plasmids (c) and ISs (d) in soils from each group.  
774 Diversity of plasmids (e) and ISs (f) in soils from each group. (g) Heat map of the  
775 correlation between MGEs (including all ISs families and top 150 abundant plasmids)  
776 and high-risk ARGs (Q1 level). Significant differences: \* means  $p < 0.05$ , \*\* means  $p$   
777  $< 0.01$ .

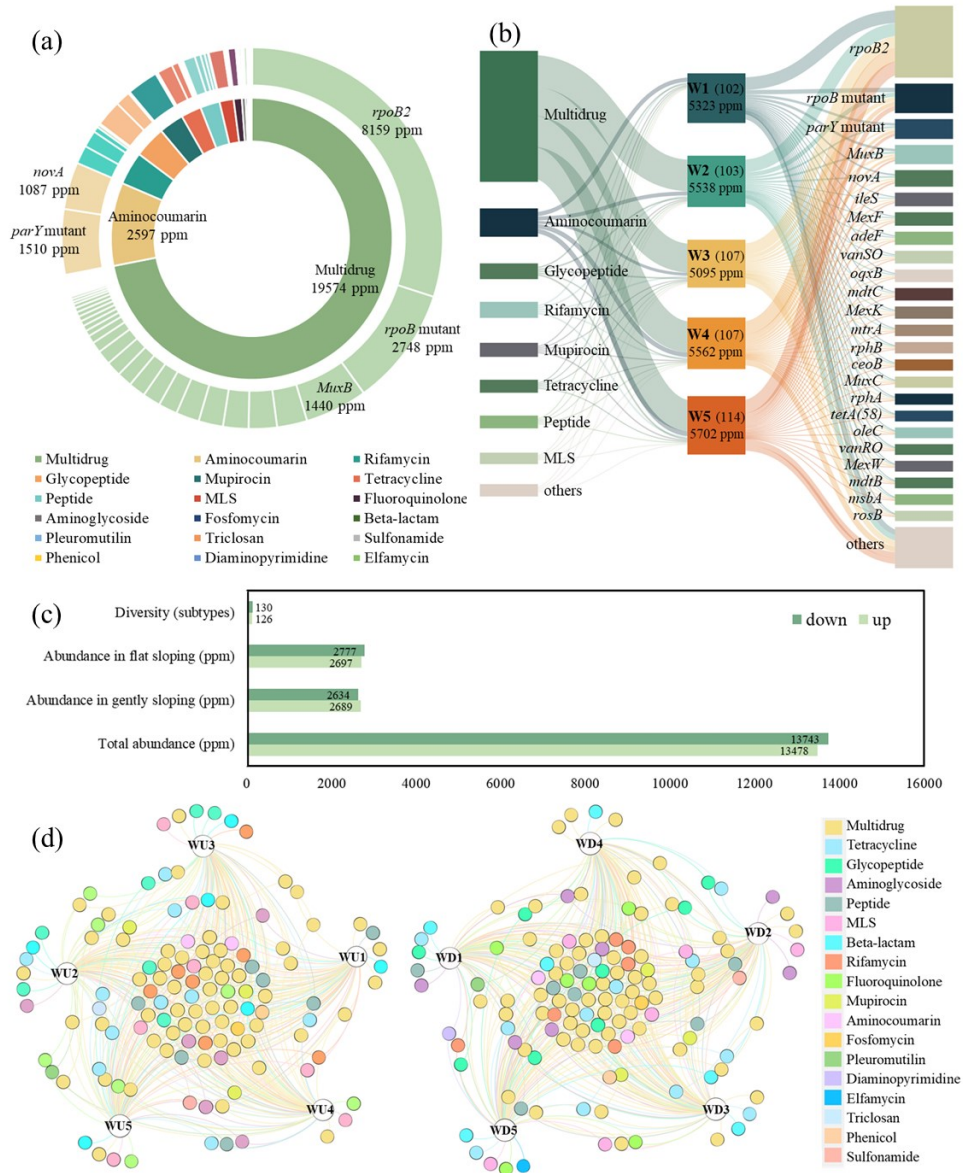
778 **Figure 4.** The abundance (a) and diversity (b) of bacterial, archaea, fungi, and HPB  
779 community at five slope gradients. (c) Differences in the abundance of bacterial  
780 communities in the uphill and downhill, exhibiting genera as the dominant genera in  
781 the uphill or downhill. Significant differences: \* means  $p < 0.05$ . (d) Abundance bubble  
782 plots of the top 20 HPB at different slope gradients and positions. (e) The abundance  
783 and diversity of bacterial, archaea, fungi, and HPB community at two slope positions.

784 **Figure 5.** (a) Network analysis of the correlation between ARG subtypes at high risk  
785 and predominant genera (top 150 in abundance). (b) The correlation ( $R > 0.8$ ,  $p < 0.01$ )  
786 among high risk ARGs subtypes, human pathogenic bacteria and MGEs.

787 **Figure 6.** (a) SEM showing the relationships among slope gradient, slope position, soil  
788 property, bacterial composition, fungal composition, archaeal composition, metabolic  
789 pathways, MGEs, and ARGs in the sloping cultivated soil. (b) SEM showing the  
790 relationships among slope gradient, slope position, soil property, HPB, MGEs, and risk  
791 ARGs in the sloping cultivated soil. Numbers adjacent to the arrows are path  
792 coefficients, the red and blue lines indicate positive and negative effects, respectively.  
793 The significance levels are represented by \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), and \*\*\* ( $P$   
794  $< 0.001$ ). (c) The direct and indirect effects of slope gradient, slope position, soil  
795 property, bacterial composition, fungal composition, archaeal composition, metabolic  
796 pathways, and MGEs on ARGs from SEM. (d) The direct and indirect effects of slope  
797 gradient, slope position, soil property, HPB, and MGEs on risk ARGs from SEM.

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Figure 1

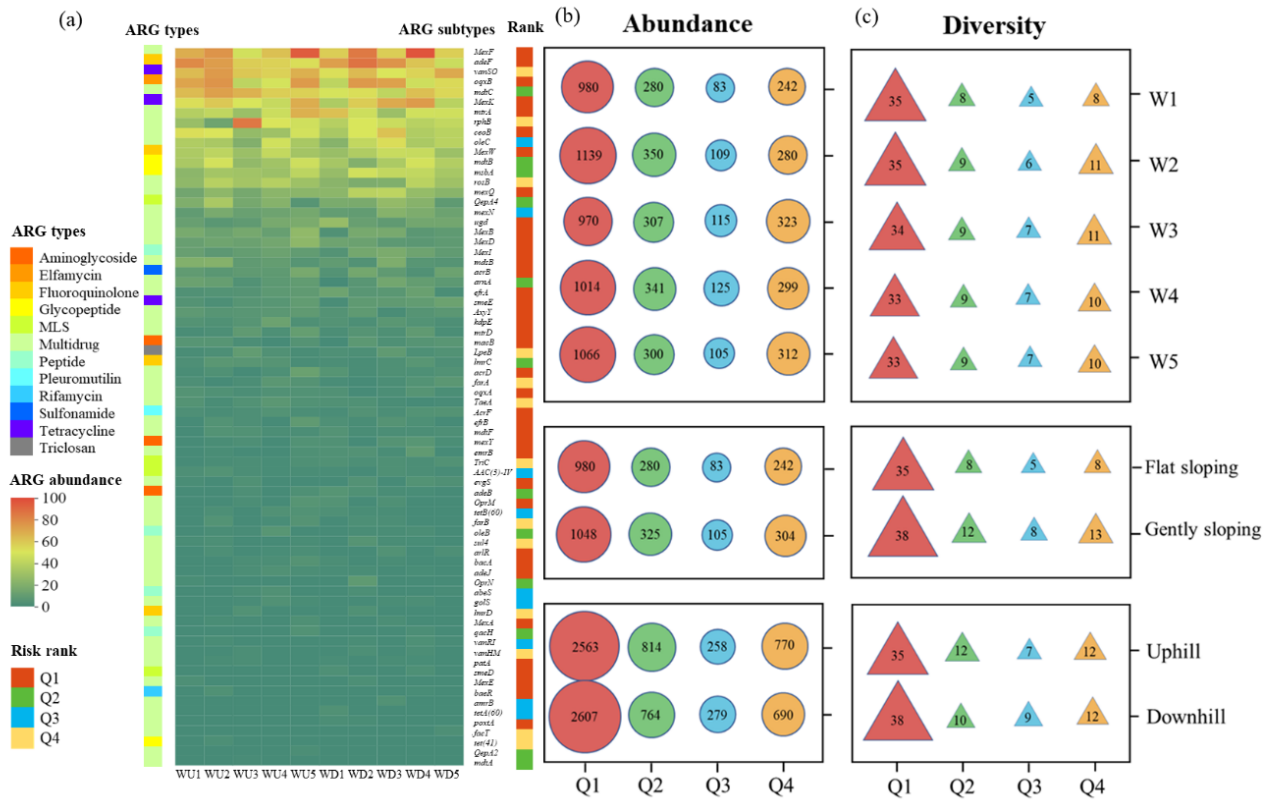


Figure 2

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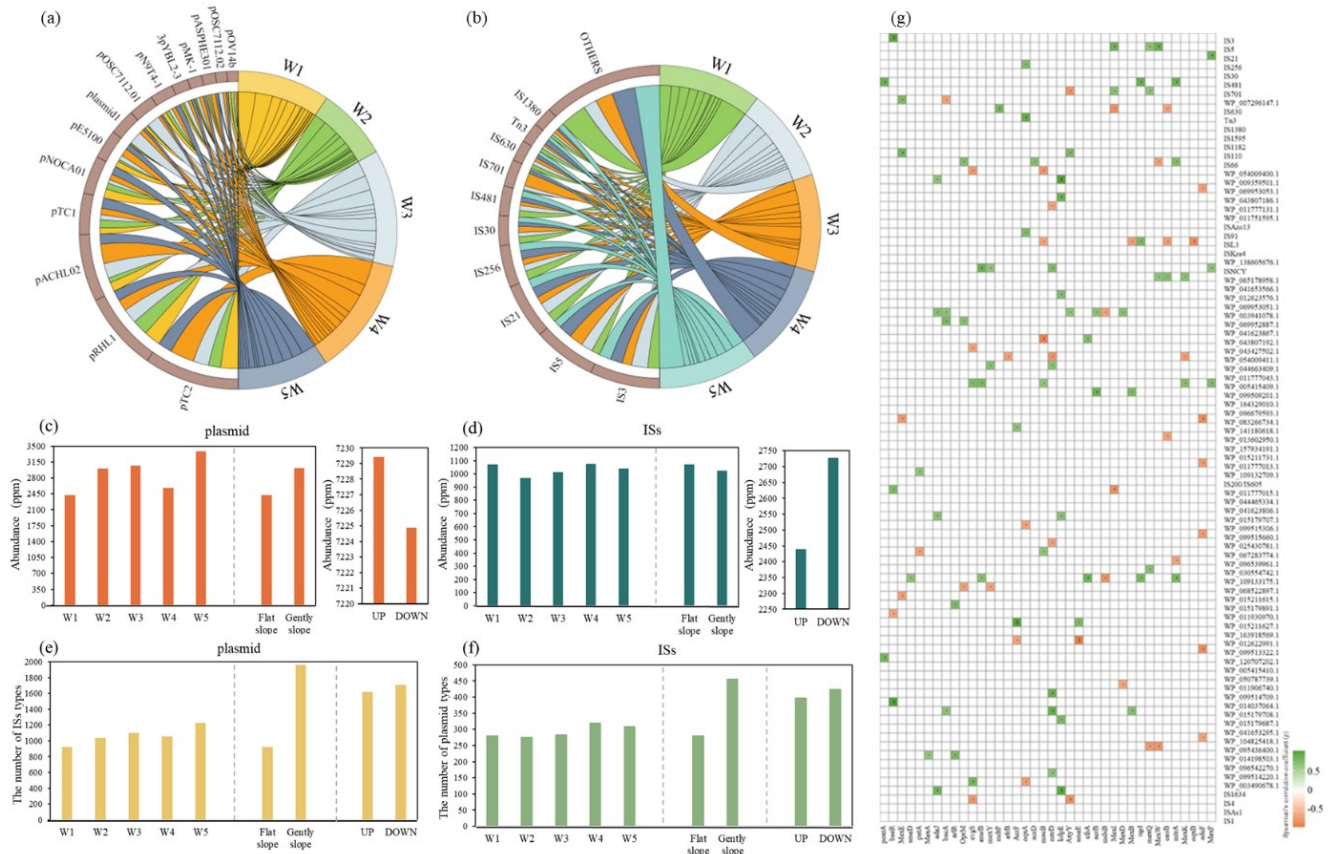
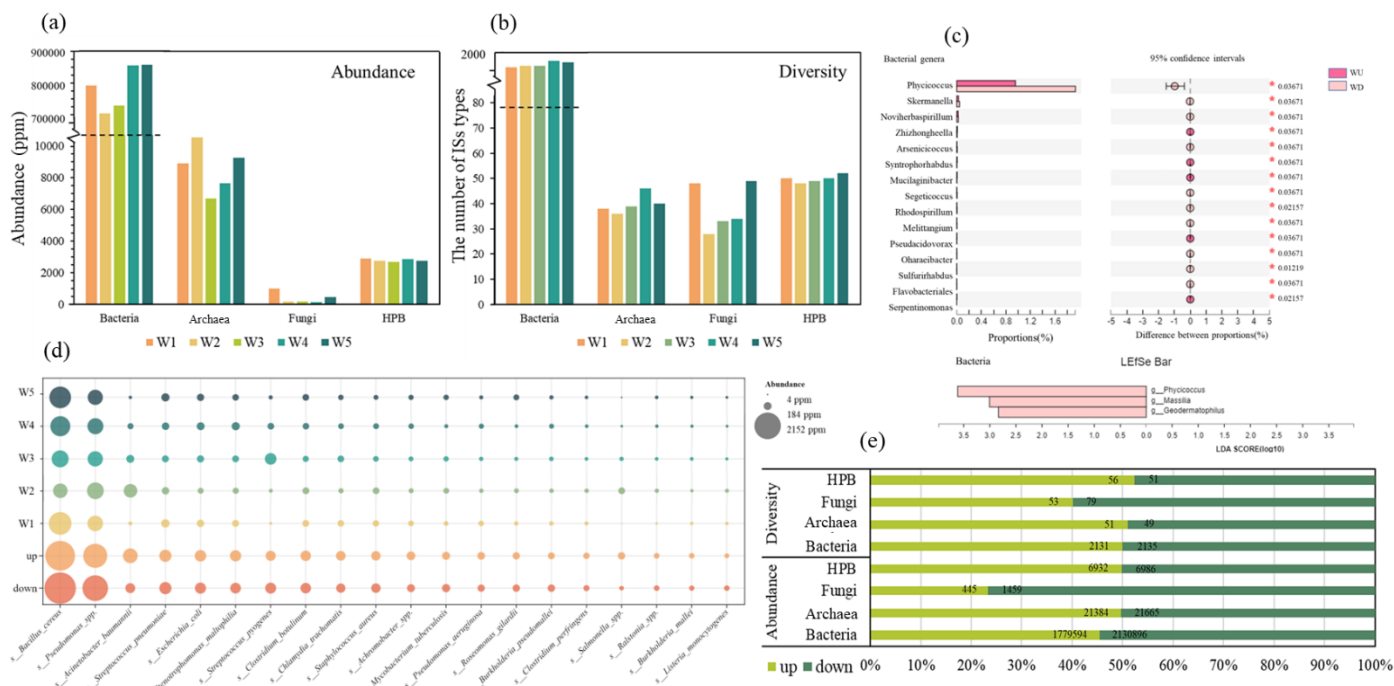


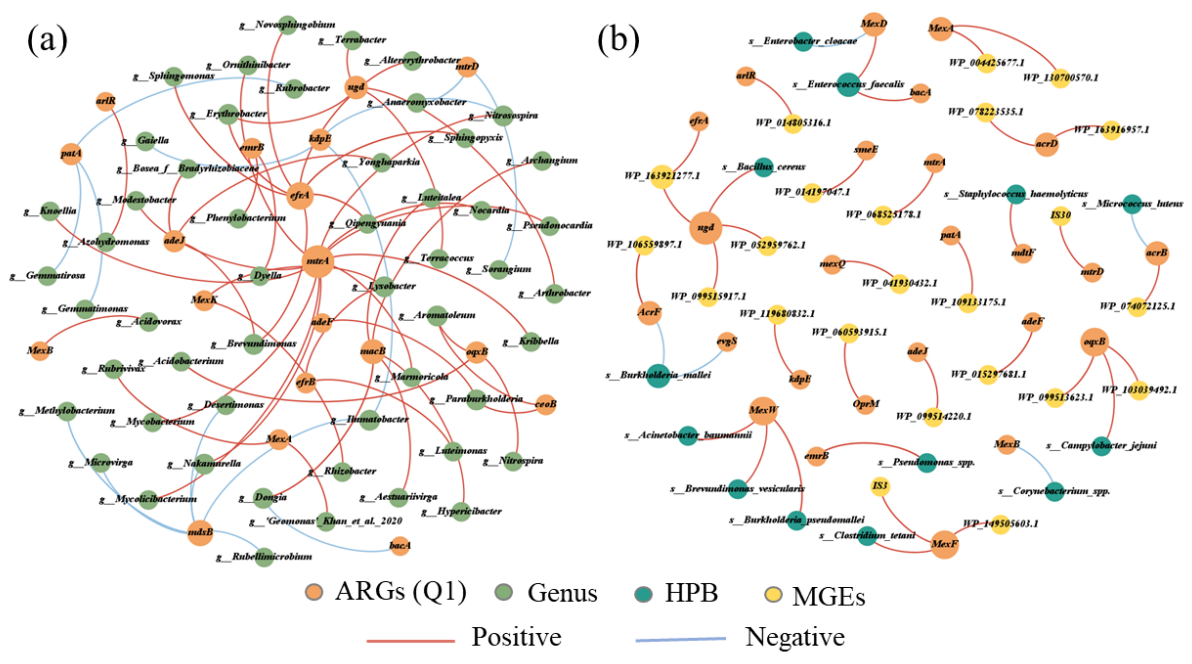
Figure 3



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Figure 4

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Figure 5

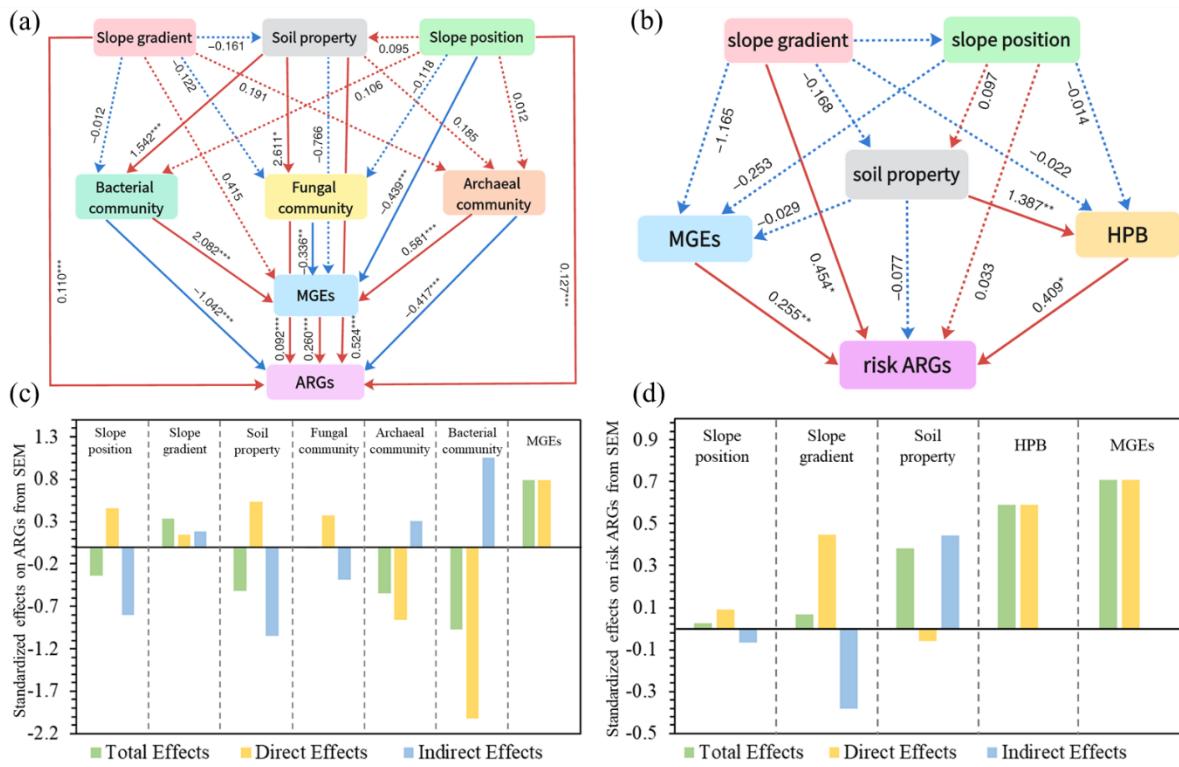


Figure 6