Elsevier Editorial System(tm) for Soil Biology and Biochemistry

Manuscript Draft

Manuscript Number:

Title: The influence of wastewater from livestock production and alternate-furrow irrigation upon antibiotic resistance gene abundance in soil planted with pepper (Capsicum annuum L.)

Article Type: Research Paper

Keywords: Livestock wastewater; Alternate-furrow irrigation; Irrigation amount; Antibiotics resistance; Water quality

Corresponding Author: Dr. Zhongyang Li, Ph.D

Corresponding Author's Institution:

First Author: Yuan Liu, Ph.D.

Order of Authors: Yuan Liu, Ph.D.; Erping Cui; Andrew L Neal; Xiaoxian Zhang; Zhongyang Li; Yatao Xiao; Zhenjie Du; Feng Gao; Xiangyang Fan; Chao Hu

Manuscript Region of Origin: CHINA

Suggested Reviewers: Hong Chen PhD
Professor, Department of Environmental Engineering, College of Environmental and Resource S, Zhejiang University
chen_hong@zju.edu.cn
The professor works mainly on antibiotic resistance.

Fangjie Zhao PhD
Professor, College of Resources and Environmental Sciences, Nanjing Agricultural University
fangjie.zhao@njau.edu.cn
The study of the professor are correlated with soil biology and soil chemistry.

Kristen Reyher PhD
Senior Lecturer, School of Veterinary Sciences, University of Bristol
kristen.reyher@bristol.ac.uk
Professor Kristen currently works on antimicrobial resistance.

Vadakattu V.S.R. Gupta PhD
Commonwealth Scientific Industrial Research Organization (CSIRO)
Gupta.Vadakattu@csiro.au
Dr. Gupta Vadakattu investigates on aspects of genetic diversity, functional capability and resilience of soil biota in agricultural soils and functional microbial diversity of aquifer systems

中国农业科学院农田灌溉研究所
Farmland Irrigation Research Institute

Dr. Zhongyang Li
Farmland Irrigation Research Institute
Chinese Academy of Agricultural Sciences
Xinxiang, Henan Province 453002, P.R. China
E-mail: lizhongyang1980@163.com
Mob: +8615090354116

May 29, 2018

Dear Professor Ritz,

Enclosed is an original research article entitled "**The influence of wastewater from livestock production and alternate-furrow irrigation upon antibiotic resistance gene abundance in soil planted with pepper (*Capsicum annuum* L.)**".

Antibiotic resistance genes (ARGs) are classed as contaminants of emerging concern and wastewater derived from livestock production is rich in ARGs. Such wastewater can be reused in agriculture, following appropriate treatments, to alleviate water deficiencies. At the same time, alternate-furrow irrigation has proved to be an effective approach to increasing water-use efficiency that is easy to implement.

Ours is the first study to study the effects of alternate-furrow irrigation on the fate of ARGs introduced by livestock wastewater irrigation in soil-plant system. To reflect reality, we conducted field experiments in which alternate-furrow irrigation at different irrigation rates were compared with conventional furrow irrigation usually adopted by farmers. We conclude that alternate-furrow irrigation has the potential to reduce ARG abundance in rhizosphere soil, but may increase the risk of accumulation of ARGs in plant tissues to some degree.

We believe the paper fits the Aims and Scope of *Soil Biology and Biochemistry*, and will be of interest to readers of your journal, since the study links a water-saving practice to the fate of antibiotic resistance genes in a field study and monitored the ARGs and bacteria communities in the soil. We appreciate your consideration of our manuscript, and look forward to receiving comments from reviewers. If you have any questions, please do not hesitate to contact me.

Sincerely,

Zhongyang Li

Zhongyang Li, corresponding author

Graphical Abstract (for review)

Livestock wastewater (rich in ARGs)

Groundwater

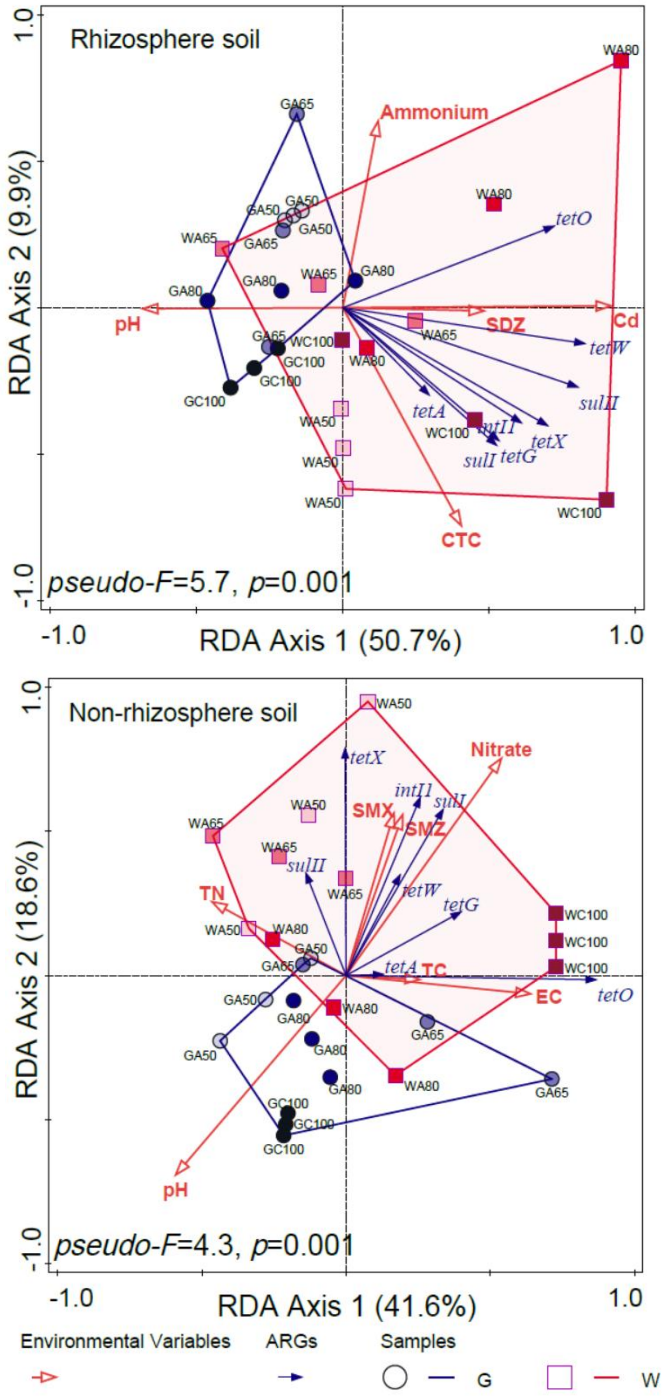
50% irrigation amount

65% irrigation amount

80% irrigation amount

100% irrigation amount

Pepper



***Highlights**

Highlights

- First study of alternate-furrow irrigation effects on ARGs in soil
- Rhizosphere was more sensitive to water source than non-rhizosphere soil
- Cd had greater influence on ARG distribution than antibiotics
- Sulfonomides had a greater influence on ARG distribution than tetracyclines
- Alternate-furrow irrigation reduced ARG abundance in the rhizosphere

The influence of wastewater from livestock production and alternate-furrow irrigation upon antibiotic resistance gene abundance in soil planted with pepper (*Capsicum annuum* L.)

Yuan Liu[a], Erping Cui[a], Andrew L. Neal[b], Xiaoxian Zhang[b], Zhongyang Li[a,*], Yatao Xiao[a], Zhenjie Du[a], Feng Gao[a], Xiangyang Fan[a] and Chao Hu[a]

[a]Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences, Xinxiang 453002, China

[b]Department of Sustainable Agriculture Science, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

*Corresponding author. E-mail address: lizhongyang1980@163.com.

Abstract

One effective approach to treating large amounts of wastewater produced during livestock production is to use it to irrigate crops. However, antibiotic compounds and antibiotic resistance genes (ARGs) associated with livestock wastewater may enter the soil and plants. ARGs are spread readily among microbial populations by mobile genetic elements, and may pose threats to human health. Compared with conventional furrow irrigation (CFI), alternate-furrow irrigation (AFI) can reduce water use and still achieve high yields. These different irrigation methods may influence the fate of ARGs in soil, but few reports have studied the combined effects of AFI and livestock wastewater upon the distribution of ARGs in soil. Here, swine wastewater was used to irrigate cultivated peppers, and compared to relatively ARG-free groundwater. AFI was compared to CFI (100%) at three AFI irrigation rates (80%, 65% and 50% of CFI). The results showed that wastewater irrigation resulted in greater accumulation of antibiotic compounds and ARGs in soil than groundwater irrigation. The effect of wastewater was much more pronounced in the rhizosphere than in the non-rhizosphere soil. Compared with CFI, AFI using wastewater reduced the relative abundance of ARGs in the rhizosphere, but the concentration of antibiotic compounds was largely unaffected; though antibiotic compound concentrations in roots were significantly lower, the abundance of ARGs in roots at 50% and 65% rates and in fruits at 50% rate were significantly increased when using wastewater. The soil bacterial communities did not change significantly between the different irrigation rates, but different behaviours were observed between ARGs and antibiotic compounds at different irrigation rates. Antibiotic compound availability plays an important role in the diffusion of ARGs. In conclusion, AFI with livestock

wastewater can reduce the relative abundance of ARGs in the rhizosphere, but reducing irrigation amount should be employed carefully for the safe agricultural production.

Keywords: Livestock wastewater; Alternate-furrow irrigation; Irrigation amount; Antibiotics resistance; Water quality

1. Introduction

Water used for agricultural production accounts for 50-80% of freshwater consumed globally (Palese et al., 2009). The combined pressures from agricultural production, increasing demand for water from population growth and global climate change has necessitated the practice of recycling wastewater for irrigation to relieve critical water scarcity (Stroosnijder et al., 2012). Concurrently, livestock production is progressing towards larger and more specialized production units, producing greater and more centralized quantities of wastes. For example, in China alone, the production of livestock and poultry manure in 2007 reached 3.9 billion t (Zheng et al., 2015), and the mass of livestock wastewater was about 10 times that of manure. There are potential benefits of using wastewater from livestock production for irrigation due to its richness of nutrients, and this may also be an effective way to reduce pollution resulting from arbitrary discharge.

However, livestock wastewaters are established as reservoirs for both antibiotic compounds and microbial antibiotic resistance genes (ARGs) (Qiao et al., 2018). Development of large-scale concentrated animal feeding operations has increased the extensive use of veterinary antibiotics for infection treatment, disease prevention and growth promotion. Global consumption of defined

daily doses of antibiotics had increased from 2000 to 2015 (Klein et al., 2018). It is estimated that in China alone, 53,800 tons of antibiotics entered the environment in 2013 even after wastewater treatment (Zhang et al., 2015). Residual antibiotic compounds may exert selection pressure on environmental microorganisms, contributing to the spread of resistance genes and antibiotic resistant microorganisms (Pruden et al., 2006). This pressure-driven spread of antibiotic resistance compromises the efficacy of antibiotics in animal and human medicine and is a global public health threat - the United Nations recently warned that antibiotic resistance is a crisis that cannot be ignored and has called for responsible use of antibiotics at the World Antibiotic Awareness Week held in 2017.

During irrigation with livestock wastewater, ARGs are distributed through soil, plants and surface-water runoff (Ghosh and LaPara, 2007; Joy et al., 2013). Sui et al. (2016) studied two farm soils irrigated by swine wastewater with different irrigation periods and found that the total ARGs quantified in soil fell 1.66 log-fold in idle periods over winter compared to periods of irrigation during the summer. Bastida et al. (2017) reported that both water quality and irrigation amount have effects on soil microbial communities of a semi-arid citrus orchard, and Mavrodi et al. (2018) demonstrated that irrigation could influence the overall diversity of the wheat rhizosphere microbiome and the relative abundance of specific operational taxonomic units (OTUs) in a three-year field irrigation experiment by alerting soil water potential and pH. Ma et al. (2018) reported that irrigation water sources affected the accumulation and transport of pharmaceutical and personal care products (PPCPs) in vadose zone soils, but specific ARGs were not included in their study. Antibiotic resistance in soil spreads preferentially along water flow paths (Lüneberg et al., 2018) and ARG dissemination depends on the mobility of individual

antibiotic compounds in soil. Santiago et al. (2016) found that higher soil moisture resulted in higher concentrations of PPCPs, including ofloxacin - a quinolone antibiotic - in recycled wastewater irrigation, suggesting the mobility of PPCPs in soil increased with soil moisture. With the increase in irrigation frequency with reclaimed water, the levels of ARGs increased in soil slurries (Fahrenfeld et al., 2013), and cropping can increase antibiotic mobility due to plant root exudate release and antibiotic sorption to the colloidal fraction of soil (Domínguez et al., 2014; Zou and Zheng, 2013).

In addition to water quality, irrigation period and amount, as well as the irrigation methods of livestock wastewater may also affect the dissemination of ARGs. Since irrigation methods have the potential to influence soil microorganisms, soil antibiotic compound distribution and environmental factors such as soil moisture, soil pH, soil organic matter, soil nutrients, soil heavy metals, these factors may combine to influence the spread and diffusion of ARGs. Few studies, however, have considered the influence of irrigation methods on antibiotic compounds and ARGs in soil-plant systems or association of ARGs with the soil microbiome and other environmental factors.

Many irrigation methods are being used in agriculture, especially in arid or semi-arid regions with the goal of increasing water use efficiency (WUE). Conventional furrow irrigation (CFI) is one of the most common methods, but it has poor WUE. Alternate-furrow irrigation (AFI) has been developed as a more efficient practice than CFI, which is also easy to implement (Graterol et al., 1993; S. Kang et al., 2000a; Kang et al., 2000b). AFI moistens alternate halves of the soil and root zone every irrigation aimed to promote the synthesis of abscisic acid (ABA) by roots in the dry half to reduce stomatal conductance and hence transpiration, and has been replacing CFI in

most semiarid regions as the dominant irrigation method. In this study, we studied the effects of adopting either CFI or AFI on the spread of antibiotic compounds and ARGs in an irrigated pepper cultivation field experiment, using swine wastewater or groundwater (as a control). We hypothesized that irrigation methods, water quality and irrigation amount all influence the abundance of ARGs in soil. Our study aimed to investigate the effect of livestock wastewater irrigation with AFI on the diffusion of ARG in soil and plants and find the association between environmental factors and the ARGs diffusion, which could facilitate our thorough understanding of the environmental risk of ARGs during the livestock wastewater irrigation and provide some reference for the safe irrigation using livestock wastewater in agriculture production.

2. Materials and Methods

2.1. Soil

The experiment was carried out in a vinyl tunnel at the Agriculture Water and Soil Environmental Field Science Research Station, Chinese Academy of Agricultural Science at Xinxiang (Henan Province, 35°15′44″N, 113°55′6″E). The vinyl tunnel acted only to intercept rainwater and had no temperature, light, CO_2 or moisture control. The field soil is classified as a fluvo-aquic soil (Chinese Soil System). The chemical properties of the top soil (0-20 cm) were as follows: pH 8.5, electrical conductivity 87.7 mS m^{-1}, organic matter (OM) 9.0 g kg^{-1}, total N 0.7 g kg^{-1}, nitrogen as nitrate 136 mg kg^{-1}, nitrogen as ammonium 7.9 mg kg^{-1}， available K 252 mg kg^{-1}, available P 33.2 mg kg^{-1}， total Cu 25.7 mg kg^{-1}, total Zn 72.4 mg kg^{-1}, total Pb 22.0 mg kg^{-1}, total Cd 0.60 mg kg^{-1}, available Cu 1.5 mg kg^{-1}, available Zn 1.8 mg kg^{-1}, available Pb 1.9 mg kg^{-1},

available Cd 0.20 mg kg^{-1}.

2.2. Water

Groundwater and swine wastewater were used in our study. The groundwater was pumped to the field through the plastic pipes with a flow meter from a depth of 4.5 m beneath the ground level at the experimental site. Swine wastewater was sampled from a fermentation tank in a hoggery near the research station. The pig farm has an annual stock of about 3,000 pigs, annually producing approximately 40,000 t of wastewater. Water properties were presented in Table 1.

2.3. Plant cultivation

Pepper (*Capsicum annuum* L., Fulong F1) was cultivated as a model crop, as it is a vegetable eaten regularly and is typically cultivated in vinyl tunnels. A mixture of perlite and vermiculite (1:1 weight) was used as the seedling culture, which was then transferred into a seedling-nursing disk (4 × 8 cavaties, 5.3 cm in top diameter, 2.7 cm in bottom diameter, 5.8 cm in height, and a small hole at the bottom). Pepper seeds were sown into the prepared cultures on April 14, 2017 and provided with Hoagland and Amon nutrient solutions (708 mg L^{-1} $Ca(NO_3)_2 \cdot 4\ H_2O$, 1011 mg L^{-1} KNO_3, 230 mg L^{-1} $NH_4H_2PO_4$, 493 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 40 mg L^{-1} NaFe-EDTA, 2.86 mg L^{-1} H_3BO_3, 2.13 mg L^{-1} $MnSO_4 \cdot 4\ H_2O$, 0.22 mg L^{-1} $ZnSO_4 \cdot 7\ H_2O$, 0.08 mg L^{-1} $CuSO_4 \cdot 5\ H_2O$, 0.02 mg L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$). After one month, healthy and uniform-sized seedlings were selected and transplanted to the field plots. Rows were spaced 50 cm apart and plants were spaced 50 cm apart along each row. There were 3 ridges of pepper plants and 4 furrows in each plot. Furrow depth was 30 cm. To ensure survival of the transplanted seedlings, each plot was watered

147 with 400 L (250 m^3 ha^{-1}) of ground water via CFI immediately after the transplantation. This full
148 irrigation amount was chosen based upon local farmers' experience.
149
150 *2.4. Field experiment*
151 Before transplanting, the soil was supplied with base fertilizers consisting of 180 kg
152 $CO(NH_2)_2$ ha^{-1}, 450 kg $Ca(H_2PO_4)_2 \cdot H_2O$ ha^{-1}, and 240 kg KCl ha^{-1}. A top dressing of 90 kg
153 $CO(NH_2)_2$ ha^{-1} was applied on July 21, August 12 and September 3 respectively, so that the total
154 amount of applied $CO(NH_2)_2$ was 450 kg ha^{-1}. Each plot was 2 × 8 m, and there were 50-cm
155 intervals between every two plots to avoid the interaction of water between adjacent plots. 400 L
156 of groundwater via CFI was irrigated to each plot every 7 days until June 19, then groundwater or
157 swine wastewater at different irrigation amounts were employed until August 23. All treatments
158 are as follows:
159 1. GC100 (CFI with 100% of 400L groundwater about every 10 days)
160 2. GA50 (AFI with groundwater by using 50% of GC100)
161 3. GA65 (AFI with groundwater by using 65% of GC100)
162 4. GA80 (AFI with groundwater by using 80% of GC100)
163 5. WC100 (CFI with wastewater of GC100 about every 10 days)
164 6. WA50 (AFI with ground water by using 50% of WC100)
165 7. WA65 (AFI with ground water by using 65% of WC100)
166 8. WA80 (AFI with ground water by using 65% of WC100)
167 All treatments were irrigated approximately at the same time on the same day, and three
168 replicates were set for every treatment. Comparison of CFI at 100% rate and AFI at 50% rate was

used to establish the effects of AFI according to Kang et al. (2000b), and comparison of 50%, 65% and 80% rates was to establish the optimal irrigation amount of AFI that was effective in reducing AGRs dispersion without compromising pepper yield. A bare plot with base fertilizer application but without cultivation and irrigation (BK) was also set in order to monitor any changes in ARG abundance in the soil not caused by cropping and irrigation.

To ensure a good yield, all plots were irrigated with 400 L of groundwater via CFI every 7 days between August 23 and harvest. Pepper plants were harvested on October 9 and divided into roots, stems, leaves, and fruits. And at the same time, the topsoil (0-20 cm) of each treatment was sampled; soil shaken off the roots at harvest were considered as non-rhizosphere soil (NRS), soil adhering to the roots was brushed off and collected as rhizosphere soil (RS). Soil collected from 5 random plants was combined for each plot. In BK, three replicate soil samples were collected. Plants were washed thoroughly with sterile saline solution (8.5 g L^{-1} NaCl) to remove adhering particles and surface microbes. Sub-samples of the soil and plant samples were stored at -80 ºC, the rest was air- or oven-dried before determination of various chemical parameters.

2.5. Measurement of soil chemical properties

Soil pH was measured according to the national environmental protection protocol NY/T 1377-2007 (Jin et al., 2018) using a PHS-3C pH meter (Shanghai Leici, China). Soil electrical conductivity (EC) was determined according to the standard HJ802-2016 (National environmental protection standards of the PRC, 2016) by conductivity meter DDS-307 (Shanghai Leici, China). Soil organic matter (OM) was analyzed by NY/T 1121.6-2006 (Jin et al., 2018). Soil total N (TN) was analyzed by NY/T 1121.24-2012 (Jin et al., 2018) with a Kjeldahl Analyzer KDN-08A

(Shanghai Hongji, China). Soil nitrate nitrogen and ammonium nitrogen were extracted according to the protocols LY/T 1228-2015 (Zuo et al., 2018) and NY/T 1848-2010 (Standards of agricultural industry of the PRC, 2010), and then were determined by UV-5500(PC) UV/VIS spectrophotometer (Shanghai Yuanxi, China). Soil available potassium was extracted by ammonium acetate, and determined by ICP-OES iCAP7400 (ThermoFisher, USA). Soil available phosphorous was determined according to NY/T 1121.7-2014 (Wang et al., 2018) with UV-5500(PC) UV/VIS spectrophotometer. Soil total Cu, Zn, Pb and Cd was extracted by HNO_3-HF-$HClO_4$, and analyzed with ICP-MS iCAP Qc (ThermoFisher, USA). Soil available Cu, Zn, Pb, Cd was determined according to HJ 804-2016 (Jiang and Zhou, 2018), and measured with ICP-OES iCAP7400.

2.6. Antibiotic Compound Analysis

Six antibiotic compounds typically used in livestock production (Tang et al., 2015) were determined in our study according to the procedure of Cheng et al. (2016), with some minor modifications. The compounds were Tetracycline (TC), Chlortetracycline (CTC), Oxytetracycline (OTC), Sulfadiazine (SDZ), Sulfamethoxazole (SMX) and Sulfamerazine (SMZ).

Water samples (10 mL) were filtered through 0.45 μm glass fiber filters and 0.80 g L^{-1} Na_2EDTA was added to the samples and allowed it to react for 1 h. Then, 0.1 M HCl or NaOH was used to adjust the pH of samples to 5.0. Oasis HLB plates (60 mg, Waters, USA) were successively activated with 2.0 mL methanol, 2.0 mL ultra-pure water and 1.0 mL ultra-pure water (pH 5.0 ± 0.2). Samples were passed through the plates at a rate of 0.4 mL min^{-1}. The plates were rinsed with 2 mL ultra-pure water and dried under nitrogen gas for 30 min. Once dried, the plates

were eluted with 2 mL of a mixture of methanol : acetonitrile (1:1, v/v). The eluates were dried under gentle nitrogen gas at 40℃ and later diluted to a volume of 100 μL with methanol : water (1:1, v/v). Finally, the treated samples were analyzed by Ultra-high Performance Liquid Chromatography tandem Mass Spectrometry (UPLC-MS/MS).

Freeze-dried solid samples (75 mg) were extracted in 3 mL of a mixed solution of 1.5 mL methanol and 1.5 mL Na_2EDTA-McIlvaine with ultrasonication (50 kHz) for 10 min, then centrifuged at 3,000 rpm for 20 min. The procedure repeated three times and the supernatants collected after each step were pooled. 1 mL of each supernatant mixture was diluted to 10 mL with ultra-pure water. The same process was followed with the liquid samples.

The final extracts were analyzed by a UPLC-MS/MS system equipped with an Agilent 1290 Infinity UHPLC and an Agilent 6470 Triple Quadruple MS/MS (Agilent Technologies, USA). All target antibiotics were separated on an XSelect HSS T3 Column (2.5 μm, 2.1 × 100 mm, Waters Co., Massachusetts, USA) and identified and quantified by MS/MS in multi-reaction monitoring (MRM) mode. MS/MS analysis was performed in the positive electrospray ionization (ESI) mode. The specific instrument conditions of the six compounds are summarized in Table S1.

2.7. DNA extraction

FastDNA SPIN Kits (MP Biomedicals, CA) were used to extract total DNA from soil, plant and water samples. Plant tissue was ground in liquid nitrogen before extraction. To determine the concentration and quality of the extracted DNA, spectrophotometric analysis (NanoDrop ND-2000c, Thermo Fisher Scientific, Waltham, MA) and 1.5% agarose gel electrophoresis were used.

235

236 *2.8. MiSeq pyrosequencing*

237 PCR amplification of the bacterial 16S rRNA gene V3–V4 variable region was performed
238 using the forward primer 5'-ACTCCTACGGGAGGCAGCAG-3' (338F) and the reverse primer
239 5'-GGACTACHVGGGTWTCTAAT-3' (806R) (Xu et al., 2016). The reaction mixture and
240 thermal profile of the PCR amplifications were according to Huang et al. (2016). After the PCR
241 products were purified, they were adjusted to equal quantities, and paired-end 2×300 base pair (bp)
242 sequencing was performed on an Illumina MiSeq sequencing platform by Shanghai Personal
243 Biotechnology Co., Ltd. (Shanghai, China).

244 Sequences were examined for quality using the default arguments in the split_libraries python
245 script with the exception of increasing primer mismatch from 0 to 2, and then assigned to each
246 sample based on unique 10-bp barcodes. After removing barcode and primer sequences, the
247 sequences were clustered into operational taxonomic units (OTUs) at a level of 97% sequence
248 similarity and annotated using BLAST searches against the Greengenes (Release 13.8,
249 http://greengenes.secondgenome.com/, bacteria) database using the Quantitative Insights into
250 Microbial Ecology (QIIME) software package version 1.8.0 (Caporaso et al., 2010).

251

252 *2.9. Relative Quantification of ARGs and* intIl

253 A total of seven ARGs (*tetA*, *tetG*, *tetO*, *tetW*, *tetX*, *sulI* and *sulII*), the class 1 integron
254 integrase, *intI1*, and the 16S rRNA gene were amplified and quantified using quantitative
255 polymerase chain reaction (qPCR) using a SYBR Green approach at Shanghai Personal
256 Biotechnology Co., Ltd (Shanghai, China). All qPCR reactions were repeated three times. The

primer description can be found in Table S2 of the Supporting Information. All qPCR reactions were performed using CFX-96 touch real-time PCR detection system (Bio-Rad, USA). Cycle conditions were: 95 °C for 5 minutes, followed by 45 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. A threshold cycle (C_t) of 36 was used as the detection limit (Malvick and Impullitti, 2007). Generally, the technical triplicates were tested during separate testing occasions (plate and day of testing) as a method of quality control. The $2^{-\Delta\Delta Ct}$ method of comparison (Livak and Schmittgen, 2001; Zhu et al., 2013) was used to compare relative abundance between samples:

$$\Delta C_t = C_{t,(\text{ARG or } intI1)} - C_{t,(16S)}$$

$$\Delta\Delta C_t = \Delta C_{t,(\text{Target})} - \Delta C_{t,(\text{Ref})}$$

where C_t is the threshold cycle, ARG is one of the antibiotic resistance gene assays, *intI1* is the *intI1* gene assay, 16S is the 16S rRNA gene assay, Target is the experimental sample, and Ref is the reference sample. The reference sample used for comparison depended on the purpose of the analysis. When comparing differences of ARG abundance between groundwater and wastewater, the groundwater was selected as the reference sample. When the purpose was to reveal the changes of ARG abundance in different soils among all treatments, the original soil before cultivation and fertilization was selected as the reference sample for all the soil samples. When the purpose was to reveal the changes of ARG abundance in different plant tissues among all treatments, the root of GC100 was selected as the reference sample.

2.10. Statistical analysis

Statistical comparison of antibiotic resistance gene abundance and environmental parameters were performed with the software package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL,

279 USA). Mean differences between treatments were assessed by analysis of variance (ANOVA).
280 *Post-hoc* pairwise comparisons of the treatment-means were performed using Duncan's multiple
281 range test. Differences were considered significant at $p < 0.05$. Correlation tests were performed
282 using Pearson's correlation coefficient.

283 MicrobiomeAnalyst (Dhariwal et al., 2017) was used for the analysis of OTU data. A
284 minimum mean abundance of 14 across all treatments was used as a cut-off, together with a low
285 variance filter to remove those OTUs associated with the lowest 10% of the coefficients of
286 variance, determined using the inter-quantile range. Abundance data was scaled using Cumulative
287 Sum Scaling (CSS) (Weiss et al., 2017). Principal coordinate analysis (PCoA) of soil bacterial
288 communities at the OTU-level was used, based on weighted UniFrac phylogenetic distance
289 (Lozupone et al., 2011). We also employed hierarchical bi-clustering of OTUs associated with the
290 different treatments employing Ward's minimum variance method to identify clusters. To test for
291 significant OTU divergence between different soils, we used permutation multivariate analysis of
292 variance (PERMANOVA) based again on OTU weighted UniFrac distance. Where significant
293 divergence between communities was detected, we also tested for homogeneity of multivariate
294 dispersion between groups using PERMDISP (Anderson and Walsh, 2013). Where no significant
295 difference in the multivariate dispersion was observed, we assumed the significant effects
296 observed in PERMANOVA were ascribed to treatment.

297 Differences between relative antibiotic resistance and integron integrase gene abundances
298 were assessed using Principal Coordinate Analysis (PCoA) based upon Gower distances
299 (Kuczynski et al., 2010) in PAST 3.20. Two-factor PERMANOVA with 9,999 Monte Carlo
300 permutations was conducted in PAST to evaluate the divergence of ARGs between different

treatments also using Gower distances. Where significant treatment effects were identified, Redundancy Analysis (RDA) was used to assess the relationships between ARG abundance and various environmental factors in CANOCO 5 (ter Braak, 1988). For each RDA model, interactive-forward-selection of environmental variables was used to identify the predictors of ARG abundance. Before analysis, all environmental variables were transformed to *z*-scores. Statistical significance of each RDA model was assessed based upon 999 Monte Carlo permutations.

3. Results

3.1. Concentrations of antibiotic compounds and relative abundance of ARGs and intI1 *in irrigation waters.*

We measured the concentration of six antibiotic compounds, and the abundance of seven ARGs and *intI1* in wastewater and groundwater used to irrigate the plots. Concentrations of TC, CTC, OTC, SMX, SMZ and SDZ in groundwater were 7.11, 9.00, 15.65, 5.03, 6.11 and 3.06 ng L^{-1}, respectively, and in wastewater were 354.23, 311.35, 5471.26, 4.94, 4.56 and 9.16 ng L^{-1}, respectively. Tetracycline concentrations in wastewater were significantly higher than tetracyclines in groundwater and sulfonamides in wastewater. Relative to their abundances in groundwater, *tetA*, *tetG*, *tetO*, *tetW*, *tetX*, *sulI*, *sulII* and *intI1* genes in wastewater were 9.2-, 176.1-, 30.8-, 483.3-, 3.5-, 88.0-, 1206.4- and 6.8-fold more abundant, respectively.

3.2. The chemical properties of soil following irrigation

323 Soil chemical properties, including pH, electrical conductivity, organic matter, total nitrogen,
324 nitrate-nitrogen, ammonium-nitrogen and bioavailable heavy metals, are presented in Figs. S1 and
325 S2. The pH and bioavailable zinc (Zn) were higher in groundwater-irrigated soils, while the
326 content of organic matter, total nitrogen, nitrate nitrogen and bioavailable cadmium (Cd) were
327 higher in wastewater-irrigated soils. Electrical conductivity (EC), organic matter (OM) and
328 nitrate-nitrogen were higher in non-rhizosphere soil than in rhizosphere soil, while
329 ammonium-nitrogen had the opposite trend.
330
331 *3.3. Concentrations of antibiotic compounds in soil*
332 *3.3.1. Water quality effects and AFI irrigation rate effects*
333 Concentrations of sulfonamides in soil were much lower than that of tetracyclines (Fig. 1),
334 following the pattern observed for the irrigation waters. Wastewater irrigation resulted in
335 accumulation of more antibiotics in soil than groundwater irrigation. With increasing irrigation
336 rates under AFI, there was no significant increase in antibiotics concentrations in rhizosphere soil
337 regardless of the water sources. The same was true for non-rhizosphere soil.
338 *3.3.2. Alternate-furrow irrigation effects*
339 Under wastewater irrigation, there were no significant differences in concentrations of the
340 antibiotic compounds between CFI and AFI at 50% irrigation rates in either rhizosphere or
341 non-rhizosphere soils. Comparison of AFI and CFI under groundwater irrigation indicated that
342 tetracycline concentrations were significantly reduced in rhizosphere soil by AFI, but
343 sulfonamides were significantly increased, suggesting that AFI did not have a consistent effect

upon the different classes of antibiotics in soil.

3.3.3. Other effects

There were no significant differences of sulfonamide concentrations between the original soil and wastewater-irrigated soils, and no significant differences between rhizosphere and non-rhizosphere soils. Irrigation with groundwater did not increase the concentration of antibiotics significantly in soil, except in rhizosphere soil under CFI. For tetracyclines, wastewater irrigation resulted in a significant increase in their concentrations in rhizosphere soils under all treatments compared to the original, unirrigated soil. Under CFI, the concentrations of tetracycline compounds were significantly higher in the rhizosphere than in the non-rhizosphere soils with either water source, while under AFI, this occurred only with wastewater and at the rate of 50 and 65%. When the irrigation rate under AFI increased to 80%, however, the difference in concentrations of the antibiotic compounds between the rhizosphere and non-rhizosphere soils disappeared. The concentrations of sulfonamides in the soil of the bare plot were lower than the original soil, while tetracyclines remained unchanged.

3.4. Bacterial community composition

Overall, 2,626 OTUs were identified in a total of 3,914,770 amplicon sequences (average sequences per sample 72,495; range 94,029-40,558). Dominant phyla in the soils were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Gemmatimonadetes, Firmicutes and Chloroflexi, which together accounted for over 93% of all OTUs (Fig. 2). The relative abundance of Actinobacteria and Firmicutes was higher in wastewater-irrigated than groundwater-irrigated

365 soils, but not significantly. Compared with the original soil, Actinobacteria, Gemmatimonadetes,
366 Bacteroidetes and Chloroflexi increased in the soil of the bare plot.

367 PCoA revealed separation of bacterial communities between rhizosphere and non-rhizosphere
368 soils. Rhizosphere-associated OTUs showed reduced variability compared to
369 non-rhizosphere-associated OTUs (Fig. 3). This separation was also evident from cluster analysis
370 (Fig. S3). Two-factor PERMANOVA (Table 2) indicated a significant divergence in soil OTUs,
371 dependent upon irrigation water source but not on irrigation rate.

372

373 *3.5. Relative abundance of ARGs and* intI1 *in soil*

374 *3.5.1. Correlation between relative abundance of* intI1 *and ARGs*

375 All seven ARGs were positively correlated with *intI1* (Table 3), suggesting that *intI1* may play
376 an important role in the mobility of ARGs. The association between *sulI* and *intI1* genes was the
377 strongest of all the genes studied in both rhizosphere (r = 0.97, p < 0.001) and non-rhizosphere
378 soil (r = 0.68, p < 0.001), and consistent with the trend of relative abundance among all
379 treatments.

380 *3.5.2. Water quality effects in rhizosphere and non-rhizosphere soils.*

381 Relative to groundwater, the abundance of ARGs and *intI1* in rhizosphere and non-rhizosphere
382 soils were increased significantly by wastewater irrigation (Fig. 4, Table 2). Furthermore,
383 abundance of ARGs and *intI1* between the different soils showed separation in PCoA ordination
384 (Fig. 5). In the rhizosphere soils, groundwater- and wastewater-irrigation treatments were
385 separated on the first PCoA axis (associated with 67% of the variation in gene abundance). No

such separation of soils based upon the abundance of ARGs was evident in the non-rhizosphere soils, where groundwater-irrigated and wastewater-irrigated soils were separated on the second PCoA axis which accounted for only 15% of the variation in gene abundance. There was also a clear separation of wastewater irrigation rates along the first axis. Similar overall patterns were evident in RDA, which identified strong and significant associations between ARGs and *intI1* and the increased concentrations of antibiotic compounds in the wastewater-irrigated soils which was not evident in groundwater-irrigated soils (Fig. 6). For rhizosphere soils, RDA separated the effects of irrigation water source on the first axis, representing 51% of the variability accounted for by the model. Bioavailable Cd (accounting for 23.8% of variability, *pseudo-F* = 9.2; $p = 0.001$), pH (12.1% of variability, *pseudo-F* = 3.4; $p = 0.039$) and the concentration of the sulfonamide compound SDZ (12.1% of variability, *pseudo-F* = 3.0; $p = 0.042$) were strongly associated with this separation of wastewater- and groundwater-irrigated soils. All ARG and integron integrase genes showed some level of association with increased Cd and SDZ concentrations. None was associated with the increased pH, evident in groundwater-irrigated soils. Ammonium-nitrogen (6.9% of variability, *pseudo-F* = 2.9; $p = 0.05$) and the tetracycline compound CTC (6.3% of variability, *pseudo-F* = 2.9; $p = 0.04$) were more closely associated with the second axis, suggesting a reduced association with the influence of irrigation water source and less influence upon the abundance of ARGs.

Such a strong separation between irrigation water sources was not evident in non-rhizosphere soils, where ground- and wastewater were separated on the second axis, which accounted for only 19% of the variability. In these non-rhizosphere soils, EC (10.6% of variability, *pseudo-F* = 3.5; $p = 0.02$) and total nitrogen (12.0% of variability, *pseudo-F* = 3.0; $p = 0.041$) exerted the greatest

influence on the primary axis (accounting for 42% of variability) while the tetracycline compound TC (10.4% of variability, *pseudo-F* = 3.1; $p = 0.047$) a lesser influence. This separation is driven largely by the application of wastewater at the 100% rate. The genes *tetO* and *tetA* are associated with this axis, the latter only weakly. All other genes are associated with the increased concentrations of the sulfonamide compounds SMX (10.0% of variability, *pseudo-F* = 2.7; $p = 0.049$) and SMZ (8.3% of variability, *pseudo-F* = 3.1; $p = 0.038$), and nitrate-nitrogen (6.9% of variability, *pseudo-F* = 2.8; $p = 0.037$).

3.5.3. The effects of Alternate-furrow irrigation and varying irrigation amounts

Wastewater irrigation resulted in increased abundance of ARGs and *intI1* in rhizosphere soil, especially at higher irrigation rates. The effects of AFI and varying irrigation amounts were evident in PCoA (Fig. 5) and two-factor PERMANOVA identified a significant effect of irrigation rate (Table 2). Different irrigation rate effects were also evident in RDA, with a few exceptions especially in wastewater irrigated plots (Fig. 6).

The abundance of ARGs and *intI1* in rhizosphere soil was significantly reduced by adopting AFI compared to CFI, suggesting that the abundance of ARGs in the pepper rhizosphere can be reduced by AFI. Such significant differences were not observed for groundwater irrigation, possibly because the abundance of ARGs was already low at the full irrigation rate. When irrigated with wastewater, the 80% rate was associated with the highest relative abundance of ARGs and *intI1* in rhizosphere soil; but there was no significant difference in abundance of the ARGs and *intI1* between the lower rates (50 and 65%). Again, there was no effect of reducing irrigation rates upon the abundance of ARGs when using groundwater irrigation.

3.6. The Relative abundance of ARGs and intI1 *in plant tissues*

3.6.1. Water quality effects

The general irrigation with wastewater resulted in an increase in ARGs abundance within different plant tissues compared to the groundwater irrigation, especially in the roots (Fig. 7). For *intI1*, the significant gene abundance increase due to wastewater irrigation was only found in the stems at 80% rate and in the fruits at 100 and 50% rates.

3.6.2. Alternate-furrow irrigation (AFI) effects

Under groundwater irrigation, AFI did not influence the abundance of genes in plant tissues notably, relative to CFI. When using wastewater to irrigate the plots, AFI at half the irrigation rate significantly decreased ARG abundance in stems, but significantly increased ARG abundance in roots, leaves and fruits as well as *intI1* abundance in roots, stems and fruits compared to CFI. Hence, AFI effects upon ARG abundance in plant tissues were inconsistent with the effects observed on soil ARG abundance.

3.6.3. Irrigation amount effects under AFI

When using wastewater for irrigation, the abundance of ARGs and *intI1* in plant roots did not decrease in response to decreased irrigation rates. The abundance of ARGs in roots reached its maximal values at the 65% irrigation rate while the abundance of *intI1* in roots reached its maximum under the 50% irrigation rate. For groundwater irrigation, no significant differences in ARG abundance were observed in roots in response to different irrigation rates. Regardless of the water source, there was no consistent increase in ARG abundance in stem and leaf materials due to increasing irrigation amount. The abundance of ARGs in fruits was the lowest in all plant

451 materials tested in this study. For fruits, under AFI wastewater irrigation, the abundance of ARGs
452 and *intI1* at the 50% irrigation rate was significantly higher than that at 65 and 80% rates.
453
454 *3.7. The concentration of antibiotic compounds in plant tissues*
455 Antibiotic compounds in all plant tissues were above the detection limit. Similar to soil,
456 concentrations of sulfonamide compounds in plant tissues were much lower than tetracycline
457 compounds (Fig. 8). Wastewater irrigation resulted in a greater accumulation of antibiotic
458 compounds in tissues compared to groundwater irrigation with a few exceptions. Compared to CFI
459 with wastewater, antibiotic compound concentrations in roots were significantly lower in AFI at
460 the 50% irrigation rate. Under groundwater irrigation, tetracycline concentrations in roots, and
461 sulfonamide concentrations in stems and fruits of AFI with 50% irrigation amount were
462 significantly lower than that of conventional furrow irrigation. AFI had an apparently negative
463 effect upon accumulation of antibiotic compounds in plant tissues.
464 Under wastewater irrigation, there was no significant difference in concentration of antibiotic
465 compounds in roots, stems or leaves among the three AFI irrigation amounts; but antibiotic
466 compound concentrations in fruits with 65% irrigation amount were significantly increased. Under
467 groundwater irrigation, the tetracyclines in roots and leaves of 80% irrigation amount were
468 significantly lower than that of 50% irrigation amount, the sulfonamides in stems of 50%
469 irrigation amount and in fruits of 80% irrigation amount were both significantly higher than that of
470 other two irrigation amounts. In all treatments, the concentration of the sulfonamides in plant roots
471 were higher than that in the associated soil.
472

4. Discussion

The source of water used to irrigate the pepper plots in this study had significant effects on the soil bacterial communities, the concentrations of antibiotic compounds and the abundance of ARG and integron integrase genes in rhizosphere soil and plants. Adopting alternate-furrow irrigation reduced the abundance of ARGs in soil, consistent with our hypothesis. Under groundwater irrigation, different irrigation rates had no significant effect upon the abundance of ARGs in soil. However, wastewater irrigation amount effects were much more notable. Nevertheless, irrigation rate under alternate-furrow irrigation had no consistent effects upon the abundance of ARGs in plants and the concentrations of antibiotic compounds in soil and plants.

4.1. Correlation between relative abundance of intI1 *and relative abundance of ARGs*

In this study, we regularly observed closely associated behaviour of the *sulI* and *intI1* genes. This was evident in both correlation analyses (Table 3) and RDA (Fig. 6). Previous studies have demonstrated that the integron integrase, *intI1*, is an important marker for vectors associated with the propagation of antibiotic resistance (Gillings et al., 2008). *intI1* typically is associated with *sul1* at the 3'-conserved region, capturing gene cassettes that confer additional and combined resistance to hosts (Heuer and Smalla, 2007): this may explain the strong association in these soils. Such associations have been observed in other studies. In a long-term experimental study by Peng et al. (2017), significant associations between ARGs and *intI1* were observed, especially, as here, between *sulI* and *intI1*. Du et al. (2014) also found that *tetX*, *intI1*, and *sulI* exhibited similar trends with *tetG* in five wastewater treatment plants. In the work of Lin et al. (2016), the *intI1* abundance correlated significantly with *sulI*, *sulII*, *tetA*, *tetG* and *tetW* abundance, demonstrating

494 a close association between *intI1* and these ARGs. Wang et al. (2014) also found that there is a
495 strong correlation between the abundance of *intI1* and the abundance of *sulI*, *sulII* and *tetG* in
496 reclaimed water.

497 *4.2. Correlation between antibiotics and relative abundance of ARGs*

498 Following the general trend of antibiotic compound distribution in irrigation water, in soil the
499 concentration of sulfonamides was much lower than tetracyclines. Soil particles show a greater
500 adsorption of tetracyclines than sulfonamides (Hamscher et al., 2005). As a result, sulfonamides
501 are highly mobile, and can leach readily from soil. Tetracyclines, however, are less mobile and can
502 accumulate in soil. When the antibiotics and ARGs associated with wastewater were incorporated
503 into soil via irrigation, it is expected that ARG abundance would initially increase before attaining
504 a plateau, and then decrease due to degradation of antibiotic compounds (Heuer et al., 2008) and
505 leaching. All ARGs studied here were positively correlated with concentrations of these two
506 classes of antibiotic compound in the rhizosphere, while in the non-rhizosphere soil the positive
507 correlations existed between antibiotic compound concentrations and sulfonamides, especially
508 SMX and SMZ. In non-rhizosphere soils, there was no consistent trend (Table 3). In RDA, it was
509 evident that ARG abundance was more associated with the more bioavailable sulfonamides than
510 tetracyclines in both rhizosphere and non-rhizosphere soils, demonstrating that antibiotic
511 bioavailability, especially of the less concentrated but more mobile sulfonamides, played an
512 important role in the spreading of ARGs.

513 *4.3. Water quality effects on ARGs*

514 Higher ARG abundance and antibiotic compound concentrations were found after wastewater

515 irrigation in this study, consistent with previous reports (Ji et al., 2012; Negreanu et al., 2012).
516 This phenomenon probably arose due to higher ARG abundance and antibiotic compound
517 concentrations in wastewater. RDA provided evidence that wastewater-irrigated soil was
518 associated with more antibiotic compounds, bioavailable Cd, nitrogenous compounds and had a
519 higher EC, whereas groundwater-irrigated soils were associated with increased pH. The
520 abundance and distribution of ARGs was variable in soils under wastewater irrigation, particularly
521 in response to the varied rates of irrigation. This was not the case for groundwater-irrigated soils
522 where the different rates of irrigation had little effect upon the abundance and distribution of genes,
523 probably because of the relatively low abundance of genes in the groundwater. We also observed
524 significantly different bacterial communities in soil, dependent upon the source of irrigation water
525 and soil compartment, but the rate of irrigation had no obvious effects upon OTU abundance and
526 diversity (Fig. 3). The combination of the different antibiotics, ARGs, heavy metals, and nutrients
527 in soil as a result of water quality and the responsive soil bacteria difference resulted in the
528 significant difference of ARG abundance between irrigations with these two types of water.

529 *4.4. Effect of AFI on the relative abundance of ARGs*

530 AFI achieved a high fruit yield with only 50% of the amount of water used in CFI (Fig. S4).
531 At the same time, AFI reduced the abundance of ARGs, but not the concentration of antibiotic
532 compounds, in the pepper rhizosphere. Despite similar antibiotic compound concentrations in the
533 rhizosphere soils between CFI and AFI with wastewater, the abundance of ARGs was consistently
534 lower under AFI, reflecting that AFI reduced mobility or bioavailability of antibiotic compounds
535 for the spread of ARGs. Though AFI moistens only half of the soil in each irrigation, the
536 difference in water matrix potential between soils in the dry and watered furrows can drive the

537 water to flow from the irrigated half to the dry half across the root zone, increasing water use
538 efficiency as a result (Graterol et al., 1993; Kang et al., 2000b). Root uptake actively moves
539 distant water into the rhizosphere, but this was reduced under AFI due to the decrease in
540 transpiration. Furthermore, because most antibiotics were tetracyclines which are adsorptive to
541 soil, root-induced water flow could only bring limited mobile antibiotics into the rhizosphere.
542 Combination of these physical processes rendered the antibiotics in the rhizosphere under AFI
543 lower than that under CFI. It has been reported that AFI can maintain high bacterial biomass, even
544 in severe water deficit irrigation (Wang et al., 2008), consistent with our findings. AFI had no
545 significant effect on soil pH, EC, nitrogen or heavy metals (Figs. S1 and S2). Thus, the reduced
546 effects on the abundance of ARGs by AFI compared to CFI mainly depended on the decreased
547 bioavailability of Cd and antibiotics.

548 Biologically, AFI - akin to partial drought stress - promotes synthesis of abscisic acid (ABA)
549 by the roots in the dry parts (Kang et al., 2000b) and this hormone has been found capable to
550 modulating plant-pathogen interactions apart from regulating leaf-stomatal conductance (Fan et al.,
551 2009). ABA might play an important role in the increase of ARG abundance in root endophytes,
552 which may explain the higher abundance of ARGs in the roots under wastewater irrigation in this
553 paper. Currently, there are no studies delineating the mechanisms by which ABA influences ARG
554 diffusion and identifying the association of ABA and ARG diffusion in soil and plants should
555 therefore be a focus of future studies.

556 *4.5. Effect of AFI irrigation amount on the relative abundance of ARGs*

557 When the AFI irrigation amount of wastewater was increased from 50 to 80%, ARGs
558 increased in abundance significantly in the pepper rhizosphere. Increasing irrigation amount did

559 not have significant effect upon the bacterial communities in our study, possibly as a result of the
560 period of CFI with groundwater immediately before the harvest. Because the ARG abundance in
561 groundwater was relatively low in comparison to wastewater, no significant effects of irrigation
562 rate was observed in groundwater treatments. When using wastewater to irrigate soil, available Cd
563 was associated with the irrigation rates in rhizosphere soil, and increased EC was associated with
564 the irrigation rates in non-rhizosphere soil (Fig. 6).

565 In wastewater-irrigated rhizosphere soil, antibiotic compound behaviour at the 80% irrigation
566 rate was markedly different from that at the 50 and 65% rates. At the 80% rate, the water supply
567 rate exceeded soil infiltration rate and surface water runoff occurred subsequently. As a result,
568 there were no significant difference in soil water content beneath the dry and the wetted furrows
569 (Wang et al., 2008). This could have alerted some microbial and plant physiological processes
570 unique to root under water stress as under the 50 and 65% rates, such as root exudations and
571 soil-plant-microbe interaction. As such, similar antibiotics contents in rhizosphere and
572 non-rhizosphere soil were found. In 80% irrigation treatments, due to the lower antibiotics in
573 rhizosphere soil, the concentration of antibiotics and ARG abundance in plant roots were lower
574 too. Unlike the water quality effects, the irrigation rate effects were mainly associated with the
575 ARGs, heavy metals, and nutrients, total concentration of antibiotics in addition to the
576 bioavailability of antibiotics.

577 *4.6. Rhizosphere effect on the relative abundance of ARGs*

578 At high wastewater irrigation rates (100% and 80%), all ARGs were more abundant in
579 rhizosphere soil, due either to a wider spread of genes through the bacterial community, or to a
580 greater abundance of ARG-harbouring cells in the rhizosphere soil. At low irrigation rates (50%

and 65%), there was no significant difference in ARG abundance. ARGs behaved differently and responded to different environmental factors in the different soil compartments (Figs. 5 and 6). The rhizosphere soil was more responsive to water quality than non-rhizosphere soil. In rhizosphere soil, the behavior of the ARGs were influenced by available Cd and SDZ. ARG abundance was also influenced by CTC to a lesser degree, possibly because of the increased bioavailability resulting from the higher organic matter in rhizosphere (Hung et al., 2009). In a similar fashion, in non-rhizosphere soils genes were influenced by the more mobile SMX and SMZ compounds than the less mobile TC. Antibiotic bioavailability and mobility in soil proved to be very important for the diffusion of ARGs.

Our recent results from rhizobox experiments filled with the same soil had shown that repeating irrigation with wastewater rendered the ARG abundance in rhizosphere higher than that in non-rhizosphere soil at day 30 and day 60 (Cui et al., 2018), and it was likely that the ARG abundance in rhizosphere soil was also higher in this study before CFI with groundwater was restored. There was a gap of almost 60 days between the last wastewater irrigation and harvest, and therefore, the ARGs attenuated during this period and the differences of their abundances between rhizosphere and non-rhizosphere soil was likely to change temporally. At high irrigation rates, the soil could maintain a high water content, and weakly adsorbed sulfonamide compounds were relatively easy to move to the rhizosphere soil driven by root-induced water movement. Thus, increasing irrigation rates delivered more antibiotic compounds to the soil, some of which moved to the rhizosphere even after wastewater irrigation ceased. Since root does not actively take up antibiotics, the antibiotics build up in the rhizosphere, promoting ARGs production compared to the non-rhizosphere soil. At low irrigation rate, the availability of antibiotic compound is low and

603 the soil is more aerobic. Such conditions might make microbes capable of secreting enzymes to
604 degrade antibiotic compounds less competitive. This, along with ARGs attenuation, could reduce
605 the ARGs abundance in rhizosphere.

606 **5. Conclusions**

607 Our research studied differences in ARG distribution in soil and plant tissues following
608 conventional furrow irrigation (CFI) and alternate-furrow irrigation (AFI), comparing
609 groundwater and wastewater sources at different irrigation rates. ARG abundance in the
610 rhizosphere was more sensitive to wastewater-irrigation than in the non-rhizosphere soil.
611 Compared with CFI, AFI reduced ARG abundance in the rhizosphere, but could risk the
612 occurrence of ARGs in plant tissues. Water quality had a manifest effect on the spread of ARGs:
613 the genes were more responsive to wastewater irrigation than to groundwater irrigation. Under
614 AFI with wastewater, decreasing the irrigation amount could reduce the ARG abundance in the
615 rhizosphere, but not the ARG accumulation in plant tissues. Antibiotic compound bioavailability
616 was of great significance in dispersion of ARGs. Further research is required to achieve water
617 savings without a risk to public health arising from the wider dissemination of antibiotic resistance
618 in microorganisms under livestock wastewater irrigation.

619 We measured the properties of soil and crop only at the harvest and did not assess the
620 availability of the antibiotic compounds in the soil. However, the effects of AFI with different
621 irrigation rates on ARG abundance in soil and plants were clearly evident, and the quality of soil
622 and fruits at the harvest is at the center of public concern. We also conjectured from our findings
623 that some microbial and plant physiological processes unique to roots under drought stress might
624 play an important role in ARGs diffusion in the rhizosphere, which needs further research.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (41701265), the Scientific and Technological Project of Henan Province (172102110121), the Central Public-interest Scientific Institution Basal Research Fund (Farmland Irrigation Research Institute, CAAS) (FIRI2016-13 and FIRI2017-14), the National Keypoint Research and Invention Program (2017YFD0801103-2), the National Natural Science Foundation of China (51779260 and 51479201), the Agricultural Science and Technology Innovation Program (ASTIP) of Chinese Academy of Agricultural Sciences and the China Scholarship Council. Work at Rothamsted Research is supported by the United Kingdom Biotechnology and Biological Science Research Council (BBSRC)-funded Soil to Nutrition strategic programme (BBS/E/C/000I0310) and jointly by the Natural Environment Research Council and BBSRC as part of the Achieving Sustainable Agricultural Systems research programme (NE/N018125/1 LTS-M). The authors are grateful to Huaibo Sun and Dr. Congcong Shen for preliminary microbiological data analysis.

Conflicts of interest

The authors declare no conflict of interest.

References

Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecological Monographs 83, 557–574. doi:10.1890/12-2010.1

Bastida, F., Torres, I.F., Romero-Trigueros, C., Baldrian, P., Větrovský, T., Bayona, J.M., Alarcón, J.J., Hernández, T., García, C., Nicolás, E., 2017. Combined effects of reduced irrigation and water quality on the soil microbial community of a citrus orchard under semi-arid conditions. Soil

Biology and Biochemistry 104, 226–237. doi:10.1016/j.soilbio.2016.10.024

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pẽa, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. doi:10.1038/nmeth.f.303

Cheng, W., Li, J., Wu, Y., Xu, L., Su, C., Qian, Y., Zhu, Y.G., Chen, H., 2016. Behavior of antibiotics and antibiotic resistance genes in eco-agricultural system: A case study. Journal of Hazardous Materials 304, 18–25. doi:10.1016/j.jhazmat.2015.10.037

Cui, E.P., Gao, F., Liu, Y., Fan, X.Y., Li, Z.Y., Du, Z.J., Hu, C., Neal, A.L., 2018. Amendment soil with biochar to control antibiotic resistance genes under unconventional water resources irrigation: Proceed with caution. Environmental Pollution 240, 475–484. doi:10.1016/j.envpol.2018.04.143

Dhariwal, A., Chong, J., Habib, S., King, I.L., Agellon, L.B., Xia, J., 2017. MicrobiomeAnalyst: A web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Research 45, W180–W188. doi:10.1093/nar/gkx295

Domínguez, C., Flores, C., Caixach, J., Mita, L., Piña, B., Comas, J., Bayona, J.M., 2014. Evaluation of antibiotic mobility in soil associated with swine-slurry soil amendment under cropping conditions. Environmental Science and Pollution Research 21, 12336–12344. doi:10.1007/s11356-014-3174-3

Du, J., Ren, H., Geng, J., Zhang, Y., Xu, K., Ding, L., 2014. Occurrence and abundance of tetracycline, sulfonamide resistance genes, and class 1 integron in five wastewater treatment plants. Environmental Science and Pollution Research 21, 7276–7284. doi:10.1007/s11356-014-2613-5

Fahrenfeld, N., Ma, Y., O'Brien, M., Pruden, A., 2013. Reclaimed water as a reservoir of antibiotic resistance genes: Distribution system and irrigation implications. Frontiers in Microbiology 4. doi:10.3389/fmicb.2013.00130

Fan, J., Hill, L., Crooks, C., Doerner, P., Lamb, C., 2009. Abscisic Acid Has a Key Role in Modulating Diverse Plant-Pathogen Interactions. PLANT PHYSIOLOGY 150, 1750–1761. doi:10.1104/pp.109.137943

Ghosh, S., LaPara, T.M., 2007. The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME Journal 1, 191–203. doi:10.1038/ismej.2007.31

Gillings, M.R., Krishnan, S., Worden, P.J., Hardwick, S.A., 2008. Recovery of diverse genes for class 1 integron-integrases from environmental DNA samples. FEMS Microbiology Letters 287, 56–62. doi:10.1111/j.1574-6968.2008.01291.x

Graterol, Y.E., Eisenhauer, D.E., Elmore, R.W., 1993. Alternate-furrow irrigation for soybean production. Agricultural Water Management 24, 133–145. doi:10.1016/0378-3774(93)90004-T

688 Hamscher, G., Pawelzick, H.T., Höper, H., Nau, H., 2005. Different behavior of tetracyclines and
689 sulfonamides in sandy soils after repeated fertilization with liquid manure. Environmental
690 Toxicology and Chemistry 24, 861–868. doi:10.1897/04-182R.1

691 Heuer, H., Focks, A., Lamshöft, M., Smalla, K., Matthies, M., Spiteller, M., 2008. Fate of sulfadiazine
692 administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in
693 manure and manured soil. Soil Biology and Biochemistry 40, 1892–1900.
694 doi:10.1016/j.soilbio.2008.03.014

695 Heuer, H., Smalla, K., 2007. Manure and sulfadiazine synergistically increased bacterial antibiotic
696 resistance in soil over at least two months. Environmental Microbiology 9, 657–666.
697 doi:10.1111/j.1462-2920.2006.01185.x

698 Huang, X., Liu, L., Wen, T., Zhang, J., Wang, F., Cai, Z., 2016. Changes in the soil microbial
699 community after reductive soil disinfestation and cucumber seedling cultivation. Applied
700 Microbiology and Biotechnology 100, 5581–5593. doi:10.1007/s00253-016-7362-6

701 Hung, H.-W., Sheng, G.D., Lin, T.-F., Su, Y., Chiou, C.T., 2009. The organic contamination level
702 based on the total soil mass is not a proper index of the soil contamination intensity.
703 Environmental Pollution 157, 2928–2932. doi:https://doi.org/10.1016/j.envpol.2009.07.007

704 Ji, X., Shen, Q., Liu, F., Ma, J., Xu, G., Wang, Y., Wu, M., 2012. Antibiotic resistance gene
705 abundances associated with antibiotics and heavy metals in animal manures and agricultural soils
706 adjacent to feedlots in Shanghai; China. Journal of Hazardous Materials 235–236, 178–185.
707 doi:10.1016/j.jhazmat.2012.07.040

708 Jiang, K., Zhou, K., 2018. Chemical immobilization of lead, cadmium, and arsenic in a
709 smelter-contaminated soil using 2,4,6-trimercaptotriazine, trisodium salt, nonahydrate and ferric
710 sulfate. Journal of Soils and Sediments 18, 1060–1065. doi:10.1007/s11368-017-1822-1

711 Jin, C., Nan, Z., Wang, H., Li, X., Zhou, J., Yao, X., Jin, P., 2018. Effect of Cd stress on the
712 bioavailability of Cd and other mineral nutrition elements in broad bean grown in a loess subsoil
713 amended with municipal sludge compost. Environmental Science and Pollution Research 25,
714 7418–7432. doi:10.1007/s11356-017-0994-y

715 Joy, S.R., Bartelt-Hunt, S.L., Snow, D.D., Gilley, J.E., Woodbury, B.L., Parker, D.B., Marx, D.B., Li,
716 X., 2013. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and
717 runoff following land application of swine manure slurry. Environmental Science and
718 Technology 47, 12081–12088. doi:10.1021/es4026358

719 Kang, S., Liang, Z., Pan, Y., Shi, P., Zhang, J., 2000a. Alternate furrow irrigation for maize production
720 in an arid area. Agricultural Water Management 45, 267–274.
721 doi:10.1016/S0378-3774(00)00072-X

722 Kang, S.Z., Shi, P., Pan, Y.H., Liang, Z.S., Hu, X.T., Zhang, J., 2000b. Soil water distribution,
723 uniformity and water-use efficiency under alternate furrow irrigation in arid areas. Irrigation
724 Science 19, 181–190. doi:10.1007/s002710000019

725 Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H.,
726 Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption

727 between 2000 and 2015. Proceedings of the National Academy of Sciences 201717295.
728 doi:10.1073/pnas.1717295115

729 Kuczynski, J., Liu, Z., Lozupone, C., McDonald, D., Fierer, N., Knight, R., 2010. Microbial
730 community resemblance methods differ in their ability to detect biologically relevant patterns.
731 Nature Methods 7, 813–819. doi:10.1038/nmeth.1499

732 Lin, H., Sun, W., Zhang, Z., Chapman, S.J., Freitag, T.E., Fu, J., Zhang, X., Ma, J., 2016. Effects of
733 manure and mineral fertilization strategies on soil antibiotic resistance gene levels and microbial
734 community in a paddy-upland rotation system. Environmental Pollution 211, 332–337.
735 doi:10.1016/j.envpol.2016.01.007

736 Livak, K.J., Schmittgen, T.D., 2001. Analysis of Relative Gene Expression Data Using Real-Time
737 Quantitative PCR and the 2−ΔΔCT Method. Methods 25, 402–408.
738 doi:https://doi.org/10.1006/meth.2001.1262

739 Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: An effective
740 distance metric for microbial community comparison. ISME Journal.
741 doi:10.1038/ismej.2010.133

742 Lüneberg, K., Prado, B., Broszat, M., Dalkmann, P., Díaz, D., Huebner, J., Amelung, W., López-Vidal,
743 Y., Siemens, J., Grohmann, E., Siebe, C., 2018. Water flow paths are hotspots for the
744 dissemination of antibiotic resistance in soil. Chemosphere 193, 1198–1206.
745 doi:10.1016/j.chemosphere.2017.11.143

746 Ma, L., Liu, Y., Zhang, J., Yang, Q., Li, G., Zhang, D., 2018. Impacts of irrigation water sources and
747 geochemical conditions on vertical distribution of pharmaceutical and personal care products
748 (PPCPs) in the vadose zone soils. Science of the Total Environment 626, 1148–1156.
749 doi:10.1016/j.scitotenv.2018.01.168

750 Malvick, D.K., Impullitti, a E., 2007. Detection and quantification of Phialophora gregata in soybean
751 and soil samples with a quantitative, real-time PCR assay. Plant Disease 91, 736–742.
752 doi:10.1094/PDIS-91-6-0736

753 Mavrodi, D. V., Mavrodi, O. V., Elbourne, L.D.H., Tetu, S., Bonsall, R.F., Parejko, J., Yang, M.,
754 Paulsen, I.T., Weller, D.M., Thomashow, L.S., 2018. Long-Term Irrigation Affects the Dynamics
755 and Activity of the Wheat Rhizosphere Microbiome. Frontiers in Plant Science 9.
756 doi:10.3389/fpls.2018.00345

757 National environmental protection standards of the People's Republic of China, 2016. Soil
758 quality—Determination of conductivity- Electrode method,
759 http://www.doc88.com/p-3025282450539.html

760 Negreanu, Y., Pasternak, Z., Jurkevitch, E., Cytryn, E., 2012. Impact of Treated Wastewater Irrigation
761 on Antibiotic Resistance in Agricultural Soils. Environmental Science & Technology 46,
762 4800–4808. doi:10.1021/es204665b

763 Palese, A.M., Pasquale, V., Celano, G., Figliuolo, G., Masi, S., Xiloyannis, C., 2009. Irrigation of olive
764 groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of
765 soil and fruits. Agriculture, Ecosystems and Environment 129, 43–51.

766 doi:10.1016/j.agee.2008.07.003

767 Peng, S., Feng, Y., Wang, Y., Guo, X., Chu, H., Lin, X., 2017. Prevalence of antibiotic resistance
768 genes in soils after continually applied with different manure for 30 years. Journal of Hazardous
769 Materials 340, 16–25. doi:10.1016/j.jhazmat.2017.06.059

770 Pruden, A., Pei, R., Storteboom, H., Carlson, K.H., 2006. Antibiotic Resistance Genes as Emerging
771 Contaminants: Studies in Northern Colorado †. Environmental Science & Technology 40,
772 7445–7450. doi:10.1021/es060413l

773 Qiao, M., Ying, G.G., Singer, A.C., Zhu, Y.G., 2018. Review of antibiotic resistance in China and its
774 environment. Environment International. doi:10.1016/j.envint.2017.10.016

775 Santiago, S., Roll, D.M., Ray, C., Williams, C., Moravcik, P., Knopf, A., 2016. Effects of soil moisture
776 depletion on vegetable crop uptake of pharmaceuticals and personal care products (PPCPs).
777 Environmental Science and Pollution Research 23, 20257–20268.
778 doi:10.1007/s11356-016-7194-z

779 Standards of agricultural industry of the People's Republic of China, 2010. Method for determination of
780 ammonium nitrogen, available phosphorus and rapidly-available potassium in neutrality or
781 calcareous soil Universal extract-colorimetric method,
782 http://www.doc88.com/p-7816449638897.html

783 Stroosnijder, L., Moore, D., Alharbi, A., Argaman, E., Biazin, B., van den Elsen, E., 2012. Improving
784 water use efficiency in drylands. Current Opinion in Environmental Sustainability.
785 doi:10.1016/j.cosust.2012.08.011

786 Sui, Q., Zhang, J., Chen, M., Tong, J., Wang, R., Wei, Y., 2016. Distribution of antibiotic resistance
787 genes (ARGs) in anaerobic digestion and land application of swine wastewater. Environmental
788 Pollution 213, 751–759. doi:10.1016/j.envpol.2016.03.038

789 Tang, X., Lou, C., Wang, S., Lu, Y., Liu, M., Hashmi, M.Z., Liang, X., Li, Z., Liao, Y., Qin, W., Fan,
790 F., Xu, J., Brookes, P.C., 2015. Effects of long-term manure applications on the occurrence of
791 antibiotics and antibiotic resistance genes (ARGs) in paddy soils: Evidence from four field
792 experiments in south of China. Soil Biology and Biochemistry 90, 179–187.
793 doi:10.1016/j.soilbio.2015.07.027

794 ter Braak, C.J.F., 1988. CANOCO-an extension of DECORANA to analyze species-environment
795 relationships. Vegetatio 75, 159–160. doi:10.1007/BF00045629

796 Wang, F.H., Qiao, M., Lv, Z.E., Guo, G.X., Jia, Y., Su, Y.H., Zhu, Y.G., 2014. Impact of reclaimed
797 water irrigation on antibiotic resistance in public parks, Beijing, China. Environmental Pollution
798 184, 247–253. doi:10.1016/j.envpol.2013.08.038

799 Wang, J., Kang, S., Li, F., Zhang, F., Li, Z., Zhang, J., 2008. Effects of alternate partial root-zone
800 irrigation on soil microorganism and maize growth. Plant and Soil 302, 45–52.
801 doi:10.1007/s11104-007-9453-8

802 Wang, Y., Zhang, T. an, Lyu, G., Guo, F., Zhang, W., Zhang, Y., 2018. Recovery of alkali and alumina
803 from bauxite residue (red mud) and complete reuse of the treated residue. Journal of Cleaner

804 Production 188, 456–465. doi:10.1016/j.jclepro.2018.04.009

805 Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J.R.,
806 Vázquez-Baeza, Y., Birmingham, A., Hyde, E.R., Knight, R., 2017. Normalization and microbial
807 differential abundance strategies depend upon data characteristics. Microbiome 5.
808 doi:10.1186/s40168-017-0237-y

809 Xu, N., Tan, G., Wang, H., Gai, X., 2016. Effect of biochar additions to soil on nitrogen leaching,
810 microbial biomass and bacterial community structure. European Journal of Soil Biology 74, 1–8.
811 doi:10.1016/j.ejsobi.2016.02.004

812 Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L., 2015. Comprehensive evaluation of
813 antibiotics emission and fate in the river basins of China: Source analysis, multimedia modeling,
814 and linkage to bacterial resistance. Environmental Science and Technology 49, 6772–6782.
815 doi:10.1021/acs.est.5b00729

816 Zheng, Z., Liu, J., Yuan, X., Wang, X., Zhu, W., Yang, F., Cui, Z., 2015. Effect of dairy manure to
817 switchgrass co-digestion ratio on methane production and the bacterial community in batch
818 anaerobic digestion. Applied Energy 151, 249–257. doi:10.1016/j.apenergy.2015.04.078

819 Zhu, Y.-G., Johnson, T. a, Su, J.-Q., Qiao, M., Guo, G.-X., Stedtfeld, R.D., Hashsham, S. a, Tiedje,
820 J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms.
821 Proceedings of the National Academy of Sciences of the United States of America 110, 3435–40.
822 doi:10.1073/pnas.1222743110

823 Zou, Y., Zheng, W., 2013. Modeling manure colloid-facilitated transport of the weakly hydrophobic
824 antibiotic florfenicol in saturated soil columns. Environmental Science and Technology 47,
825 5185–5192. doi:10.1021/es400624w

826 Zuo, L., Yang, R., Zhen, Z., Liu, J., Huang, L., Yang, M., 2018. A 5-year field study showed no
827 apparent effect of the Bt transgenic 741 poplar on the arthropod community and soil bacterial
828 diversity. Scientific Reports 8. doi:10.1038/s41598-018-20322-3

829

830

831 Figure captions

832 Fig. 1. The concentration of antibiotic compounds in irrigated and unirrigated soils.

833 Fig. 2. The relative abundance of soil bacterial phyla in irrigated and unirrigated soils.

834 Fig. 3. Principal Coordinate Analysis of soil bacteria communities at the OTU-level based on
835 weighted UniFrac distance metrics.

836 Fig. 4. The abundance of antibiotic resistance genes and *intI1* in soil relative to the original soil
837 before fertilization and cultivation.

838 Fig. 5. Principal Coordinate Analysis of antibiotic resistance and class I integron integrase genes
839 using Gower distance metrics in rhizosphere (A) and non-rhizosphere soil (B).

840 Fig. 6. Redundancy Analysis presenting the association of antibiotic resistance and class I integron
841 integrase genes with environmental factors in rhizosphere (A) and non-rhizosphere soil (B).

842 Fig. 7. The relative abundance of antibiotic resistance and class I integron integrase genes in plant
843 materials.

844 Fig. 8. The concentration of antibiotic compounds in plant materials.

845

Tables

Table 1. Properties of groundwater and wastewater.

	pH	EC	COD[a]	TDS[b]	N	P	Ca	Mg	Fe	Zn	Mn
	-	μS/cm	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	μg/L
Groundwater	8.07	1985	104	2251	0.550	-	55.5	122	1.07	0.021	178
Wastewater	8.40	2588	330	1681	325.6	16.6	47.6	38.6	0.88	0.366	120

	Pb	Cd	Cu	Cr	As	Hg	NO_3^-	PO_4^{3-}	SO_4^{2-}	K^+	Na^+
	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Groundwater	0.654	0.050	2.45	13.3	9.85	0.065	-	-	844	2.95	514
Wastewater	1.729	0.107	73.16	30.0	2.10	0.178	2.70	4.94	319	212.3	257

Note: a, chemical oxygen demand. b, total dissolved solids; the content of N, P, Ca, Mg, Fe, Zn, Mn, Cu, Pb, Cd, Cr, As, Hg refers to the total content.

Table 2. Two-way Permutational multivariate analysis of variance of bacterial community and ARGs in rhizosphere soil and non-rhizosphere soil.

Soil compartment	Source of variation	Bacterial community		ARGs	
		F	*P*	F	*P*
Rhizosphere soil	Water source	13.31	<0.001	30.27	<0.001
	Irrigation amount	1.07	0.38	9.69	<0.001
	Interaction	1.01	0.41	7.16	<0.001
Non-rhizosphere soil	Water source	2.39	0.02	11.23	<0.001
	Irrigation amount	1.07	0.35	5.87	<0.001
	Interaction	1.05	0.38	5.99	<0.001

Table 3. Pearson correlation coefficients between soil ARGs and *intI1* abundance as well as between soil antibiotics concentrations and coefficients between soil ARGs and *intI1* abundance. * refers to correlation is significant at the 0.05 level (2-tailed). TC refers to Tetracycline, CTC refers to Chlortetracycline, OTC refers to Oxytetracycline, SDZ refers to Sulfadiazine, SMX refers to Sulfamethoxazole, and SMZ refers to Sulfamerazine.

		tetA	*tetG*	*tetO*	*tetW*	*tetX*	*sulI*	*sulII*	*intI1*
Rhizosphere soil	SMX	0.062	0.320	0.341	0.353	0.315	0.397	0.443*	0.346
	SMZ	0.189	0.443*	0.338	0.346	0.330	0.576**	0.458*	0.489*
	SDZ	0.068	0.401	0.347	0.312	0.290	0.516**	0.421*	0.447*
	TC	0.197	0.250	0.045	0.034	0.119	0.210	0.176	0.179
	OTC	0.248	0.439*	0.012	0.289	0.380	0.491*	0.439*	0.430*
	CTC	0.379	0.545**	0.085	0.439*	0.573**	0.604**	0.524**	0.531**
	intI1	0.563**	0.946**	0.497*	0.682**	0.702**	0.971**	0.754**	
Non-rhizosphere soil	SMX	0.055	0.071	0.145	0.175	0.421*	0.351	0.620**	0.288
	SMZ	0.000	0.040	0.174	0.199	0.408*	0.392	0.715**	0.270
	SDZ	0.057	0.166	0.104	0.084	0.303	0.271	0.455*	0.194
	TC	0.028	-0.139	0.236	0.034	-0.033	-0.082	0.557**	-0.151
	OTC	0.000	-0.082	0.146	-0.032	-0.138	-0.180	0.469*	-0.205
	CTC	0.045	0.143	0.438*	-0.110	-0.127	-0.086	0.110	-0.205
	intI1	0.318	0.566**	0.127	0.356	0.638**	0.681**	0.210	

Figure

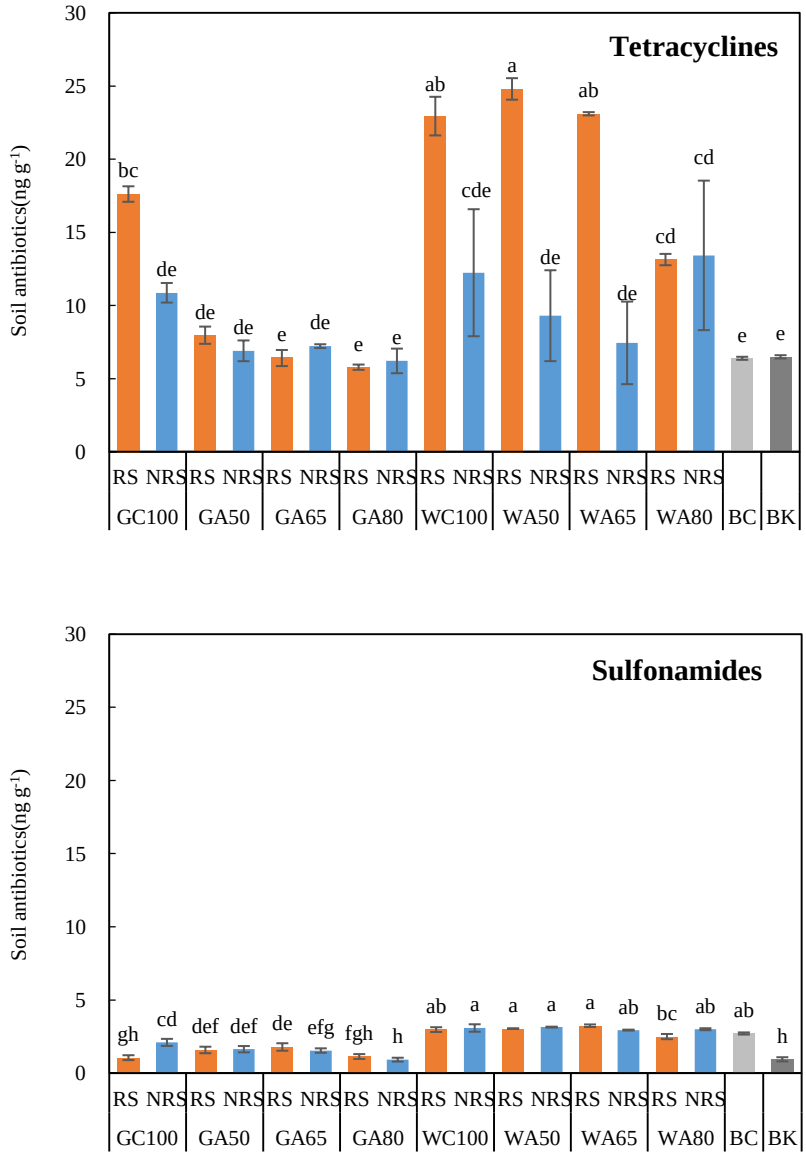


Fig. 1. The concentration of antibiotic compounds in irrigated and unirrigated soils. The concentration of tetracyclines is the sum of the concentrations of tetracycline, chlortetracycline and oxytetracycline (Table S3). The concentration of sulfonamides is the sum of the concentrations of sulfadiazine, sulfamethoxazole and sulfamerazine (Table S3). RS refers to rhizosphere soil, NRS refers to non-rhizosphere soil, G refers to groundwater, W refers to livestock wastewater, C refers to conventional furrow irrigation, A refers to alternate-furrow irrigation. 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively. BC refers to the original soil before fertilization and cultivation, BK refers to bare plot soil with base fertilizer only but no cultivation and no irrigation. The data are expressed as the mean concentration ± standard deviation. Different lower case letters above the columns represent significant difference between treatments at $p < 0.05$ determined from Duncan's *post hoc* pairwise comparisons.

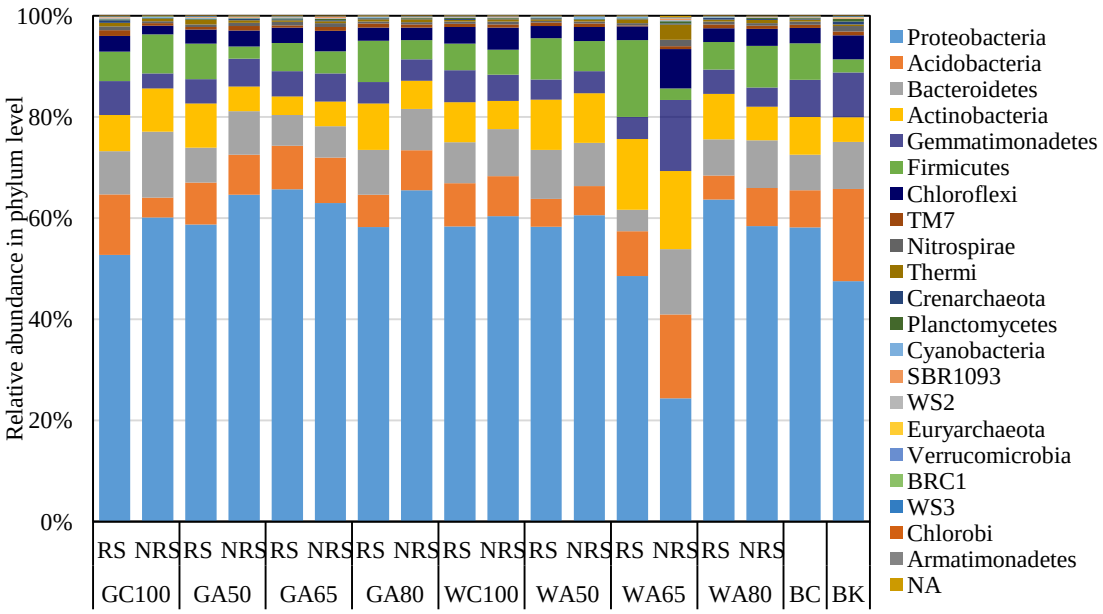
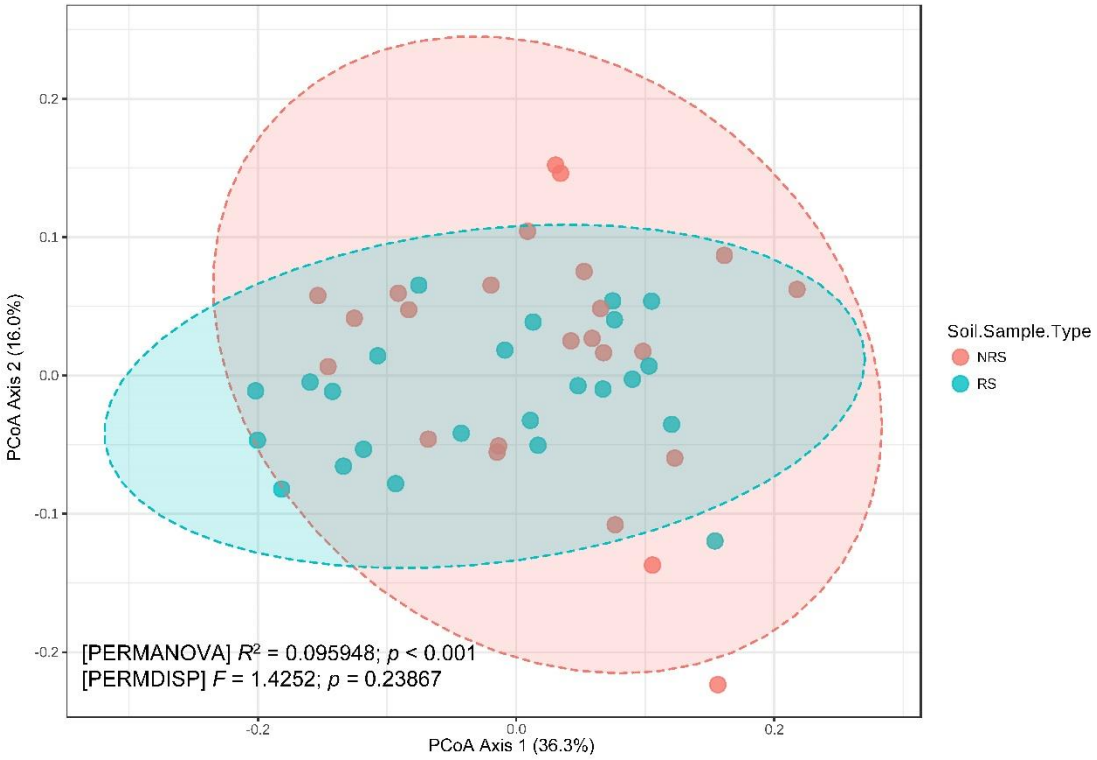
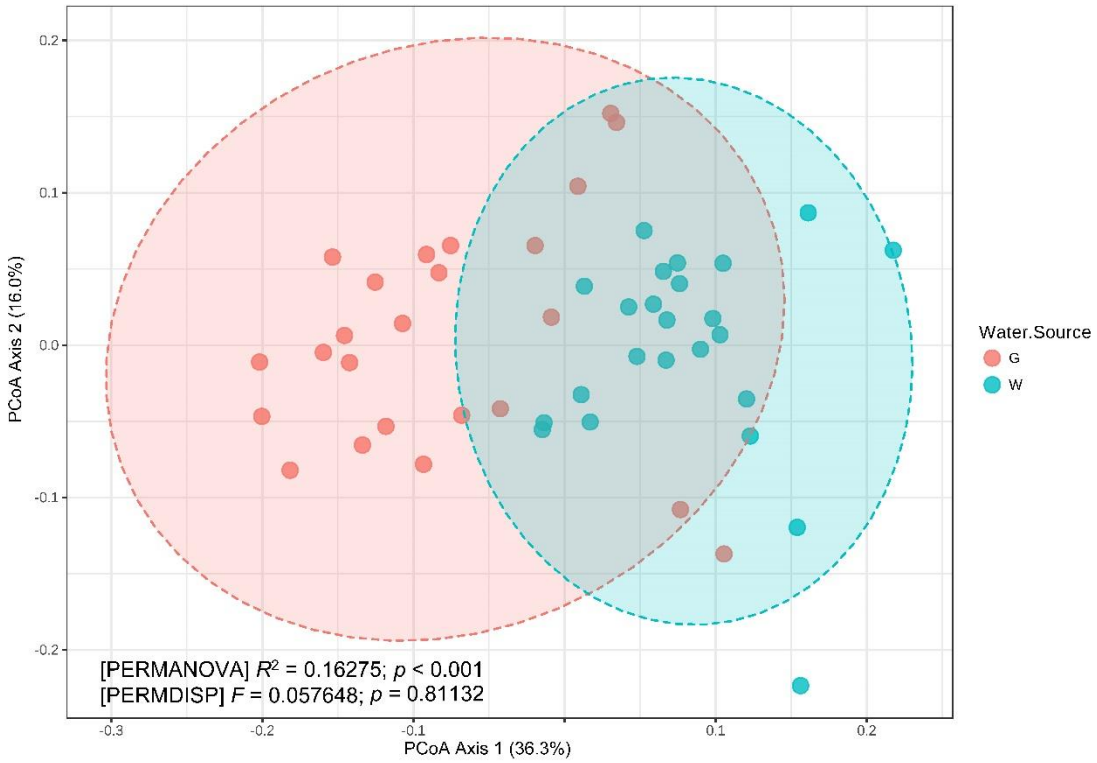


Fig. 2. The relative abundance of soil bacterial phyla in irrigated and unirrigated soils. RS refers to rhizosphere soil, NRS refers to non-rhizosphere soil, G refers to groundwater, W refers to livestock wastewater, C refers to conventional furrow irrigation, A refers to alternate-furrow irrigation. 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively. BC refers to the original soil before fertilization and cultivation, BK refers to bare plot soil with base fertilizer only but no cultivation and no irrigation.


PCoA Axis 2 (16.0%)
PCoA Axis 1 (36.3%)
[PERMANOVA] R^2 = 0.095948; p < 0.001
[PERMDISP] F = 1.4252; p = 0.23867
Soil.Sample.Type
NRS
RS


PCoA Axis 2 (16.0%)
PCoA Axis 1 (36.3%)
[PERMANOVA] R^2 = 0.16275; p < 0.001
[PERMDISP] F = 0.057648; p = 0.81132
Water.Source
G
W

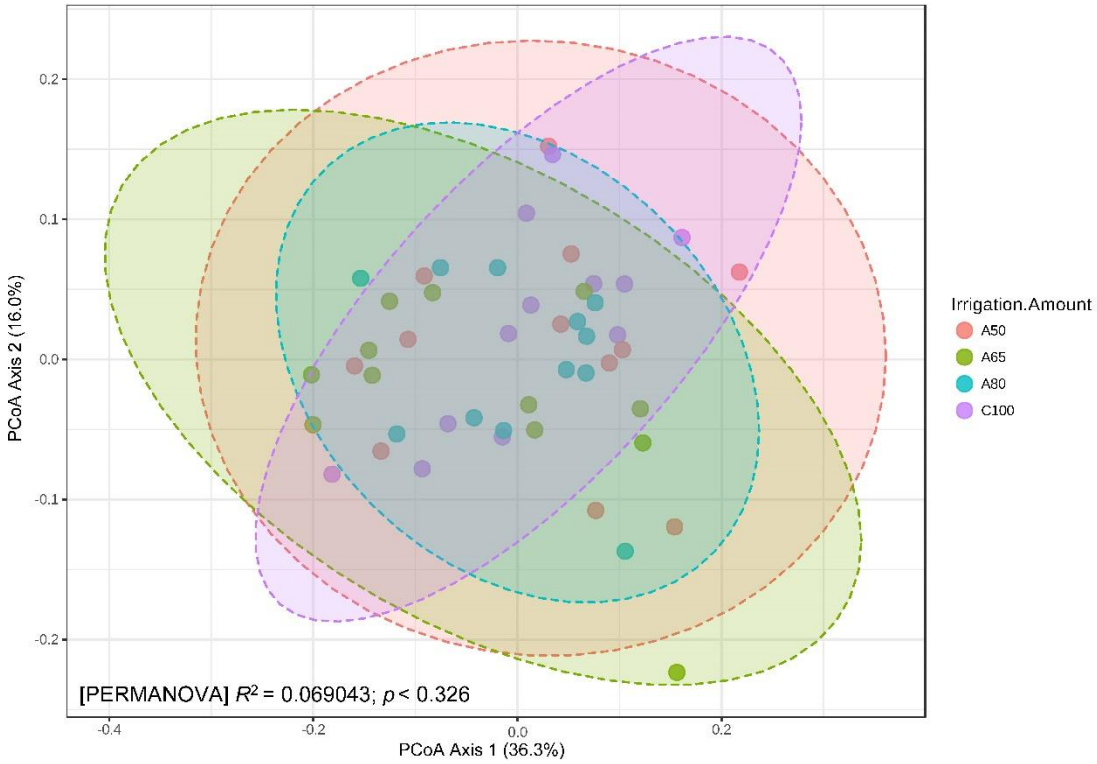


Fig. 3. Principal Coordinate Analysis of soil bacteria communities at the OTU-level based on weighted UniFrac distance metrics. RS refers to rhizosphere soil, NRS refers to non-rhizosphere soil, G refers to groundwater, W refers to livestock wastewater, C refers to conventional furrow irrigation, A refers to alternate-furrow irrigation. 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively.

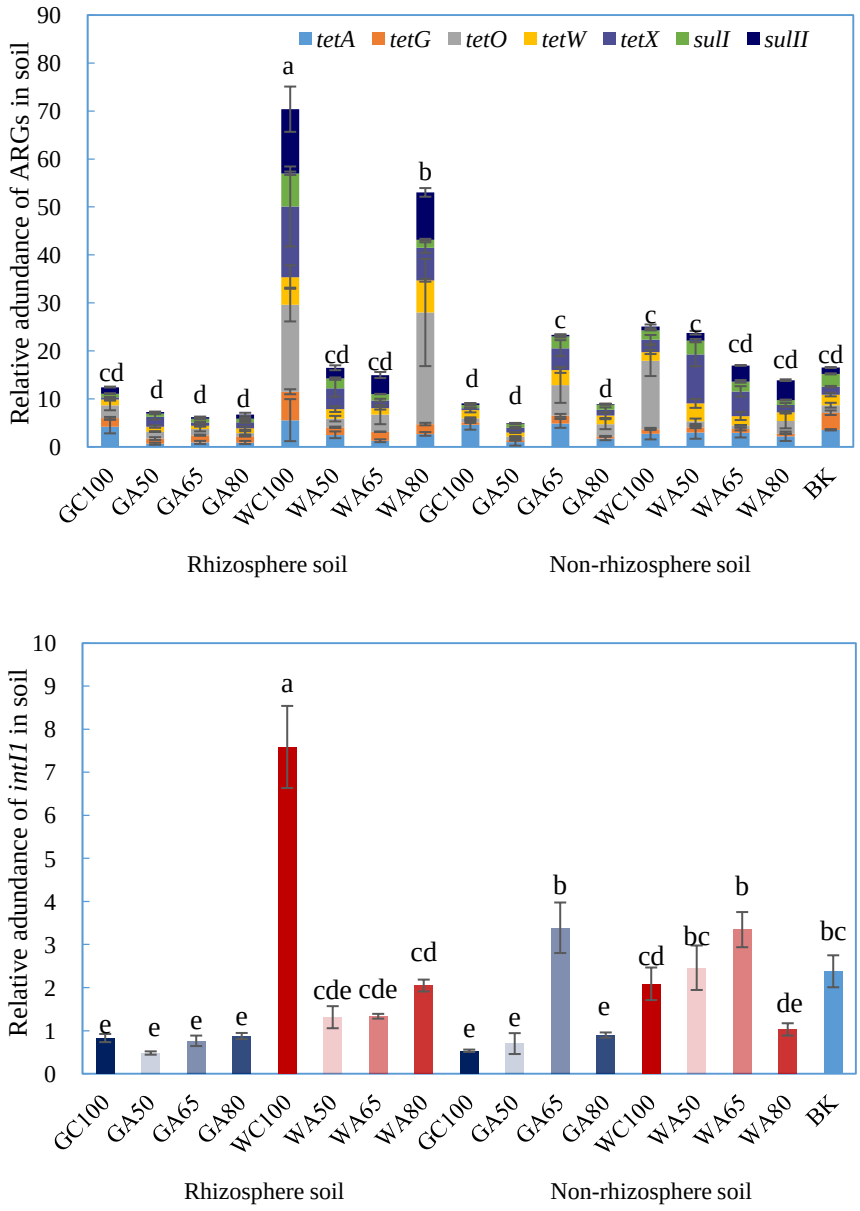


Fig. 4. The abundance of antibiotic resistance genes and *intI1* in soil relative to the original soil before fertilization and cultivation. G refers to groundwater, W refers to livestock wastewater, C refers to conventional furrow irrigation, A refers to alternate-furrow irrigation. 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively. BK refers to bare plot soil with base fertilizer only but no cultivation and no irrigation. The data are expressed as the mean ± standard deviation. Different lower case letters above the columns represent significant difference between treatments at $p < 0.05$.

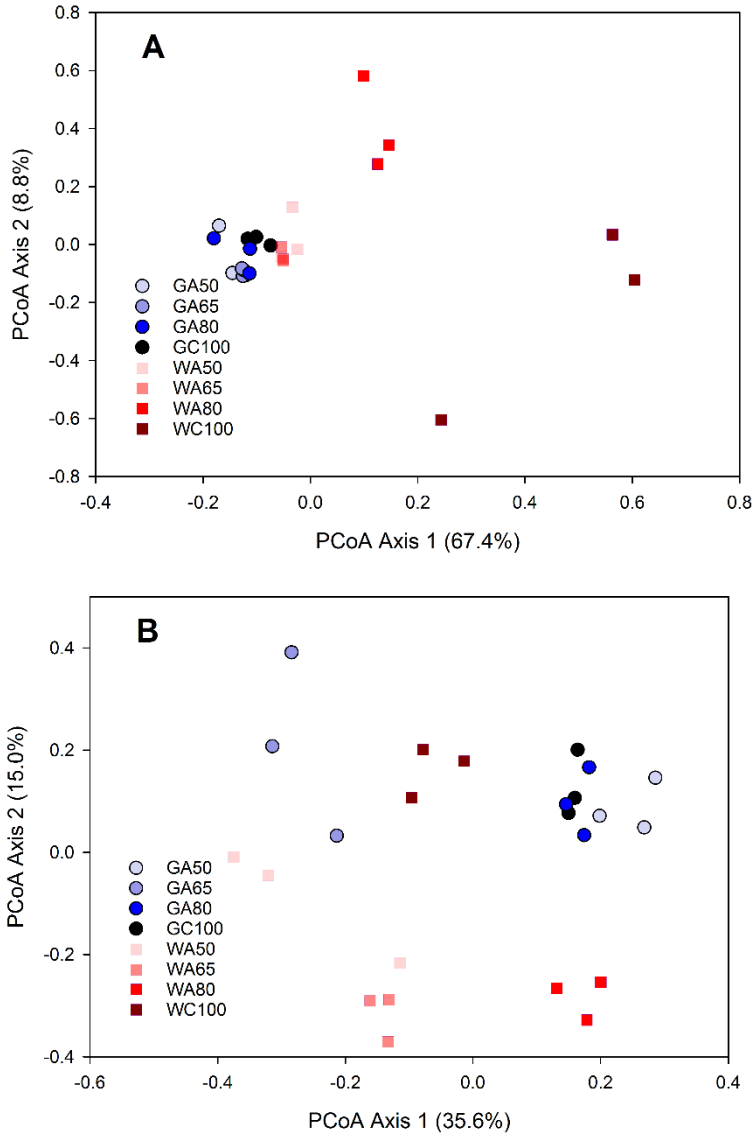


Fig. 5. Principal Coordinate Analysis of antibiotic resistance and class I integron integrase genes using Gower distance metrics in rhizosphere (A) and non-rhizosphere soil (B). G refers to groundwater, W refers to livestock wastewater, C refers to conventional furrow irrigation, A refers to alternate-furrow irrigation. 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively.

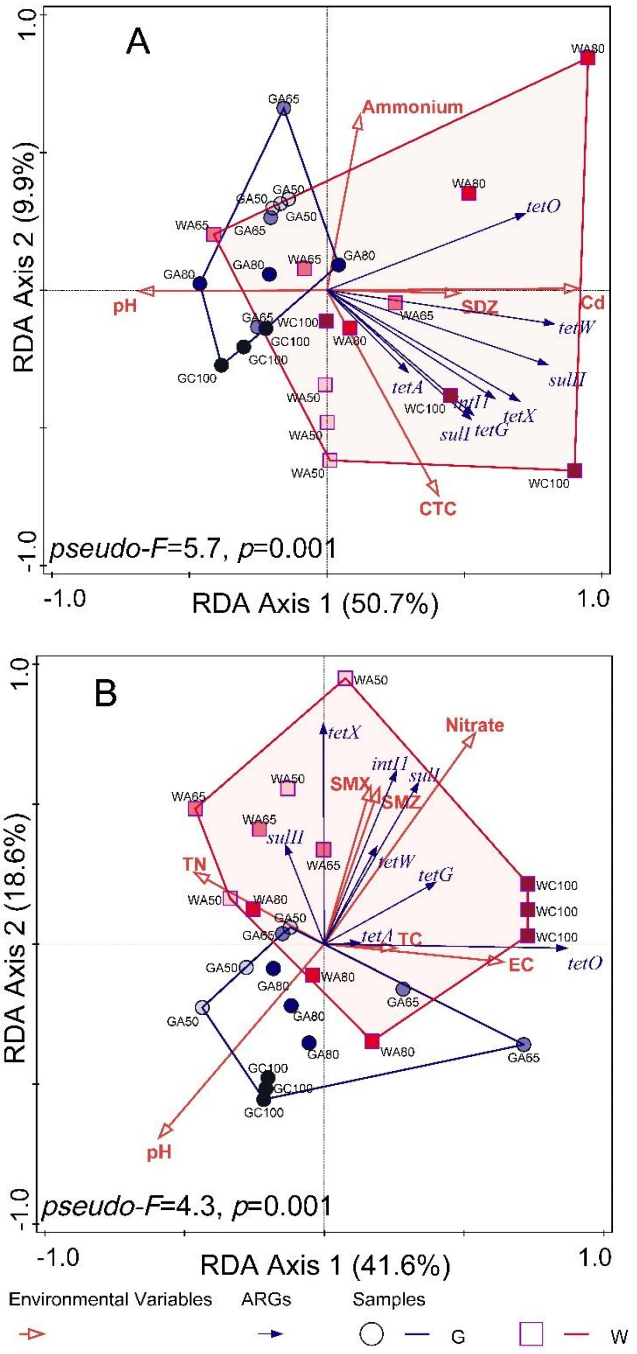


Fig. 6. Redundancy Analysis presenting the association of antibiotic resistance and class I integron integrase genes with environmental factors in rhizosphere (A) and non-rhizosphere soil (B). Environmental variables were selected using Forward Selection. G refers to groundwater, W refers to livestock wastewater. C refers to conventional furrow irrigation, A refers to alternate-furrow irrigation. 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively. TC refers to Tetracycline, CTC refers to Chlortetracycline, SDZ refers to Sulfadiazine, SMX refers to Sulfamethoxazole, and SMZ refers to Sulfamerazine, TN to total nitrogen, EC to electrical conductivity, and Cd to available cadmium.

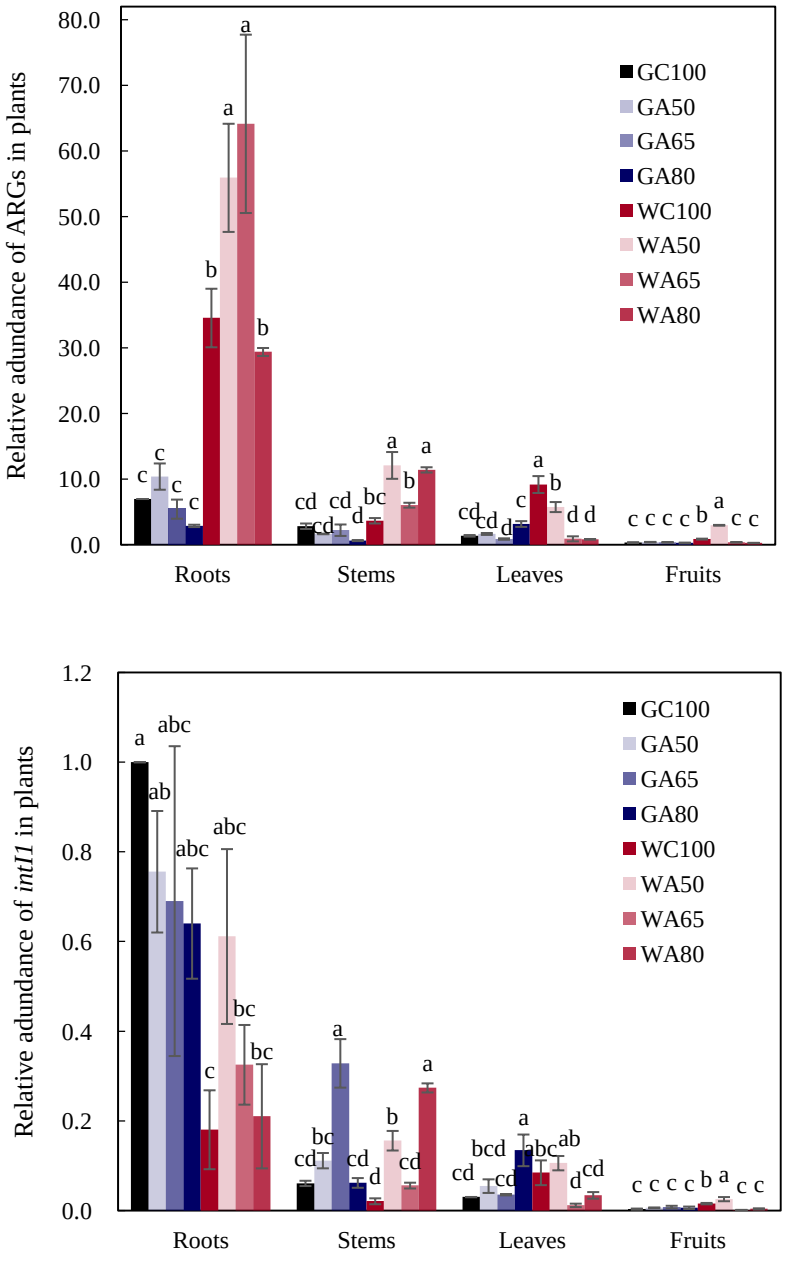


Fig. 7. The relative abundance of antibiotic resistance and class I integron integrase genes in plant materials. The relative abundance of antibiotic resistance genes is the sum of the relative abundance of *tetA*, *tetG*, *tetO*, *tetW*, *tetX*, *sulI* and *sulII* (Table S4). 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively. The data are expressed as the mean ± standard deviation. Different lower case letters above the columns represent significant difference between treatments at $p < 0.05$.

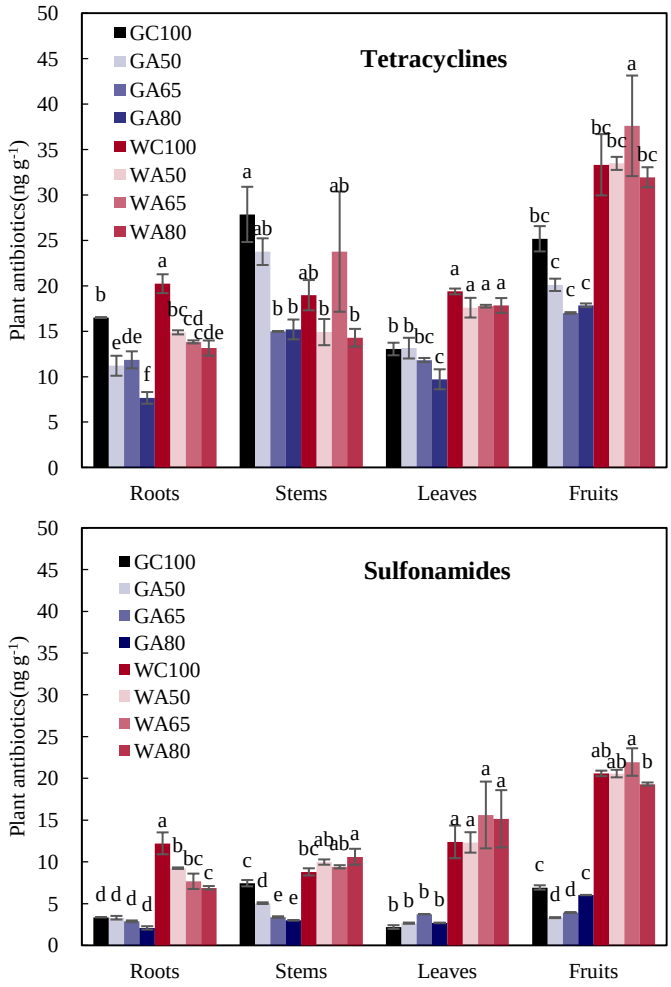


Fig. 8. The concentration of antibiotic compounds in plant materials. The concentration of tetracyclines is the sum of the concentrations of tetracycline, chlortetracycline and oxytetracycline (Table S5). The concentration of sulfonamides is the sum of the concentrations of sulfadiazine, sulfamethoxazole and sulfamerazine (Table S5). G refers to groundwater, W refers to livestock wastewater, C refers to conventional furrow irrigation, A refers to alternate-furrow irrigation. 100, 50, 65 and 80 refer to 100%, 50%, 65% and 80% of full irrigation amount per plot, respectively. The data are expressed as the mean ± standard deviation. Different lower case letters above the columns represent significant difference between treatments at $p < 0.05$.

Supplementary Material for online publication only

Click here to download Supplementary Material for online publication only: Supplemental data.docx