Science of the Total Environment 742 (2020) 140409

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Enhanced removal of ciprofloxacin and reduction of antibiotic resistance genes by earthworm *Metaphire vulgaris* in soil

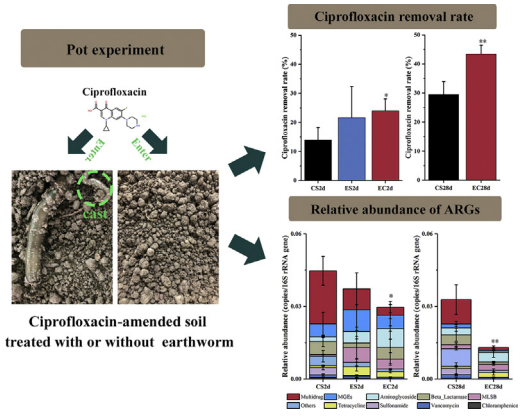
Qiang Pu [a,b], Hong-Tao Wang [a,b], Ting Pan [a,b], Hu Li [a], Jian-Qiang Su [a,*]

[a] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen 361021, China
[b] University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China

HIGHLIGHTS

- Ciprofloxacin removal is significantly enhanced by earthworm activities.
- Earthworm gut is a hotspot for ciprofloxacin removal.
- ARG abundance significantly decreased in earthworm cast compared to soil.
- Earthworm can be a cost-effective option for antibiotic removal and ARG reduction.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 April 2020
Received in revised form 12 June 2020
Accepted 19 June 2020
Available online 20 June 2020

Editor: Fang Wang

Keywords:
Antibiotic contamination
Antibiotic resistome
Antibiotic degrading bacteria
Earthworm cast

ABSTRACT

Antibiotic residues could promote the dissemination of antibiotic resistance genes (ARGs) in the environments, and biodegradation represent a major route for antibiotic removal. Previous studies have showed that earthworm could enhance the degradation of certain organic contaminants, however, its effectiveness in ciprofloxacin removal and ARG reduction in soil remains unclear. In the present study, high-performance liquid chromatography, 16S rRNA gene sequencing and high-throughput quantitative PCR were employed to explore the effects of earthworm addition on ciprofloxacin removal and ARG abundance in ciprofloxacin-amended soil. Ciprofloxacin removal was significantly higher in earthworm cast as compare to control soil, and ARG abundance in earthworm cast was significantly lower than that of control soil. Procrustes analysis together with Mantel test showed that the ARG profiles were strongly associated with bacterial communities, indicating that the lower abundance of ARGs in cast samples could be attributed to changes in bacterial community compositions by earthworm activity. *Flavobacterium* and *Turicibacter* were enriched in cast samples, which were negatively correlated with ciprofloxacin concentration ($p < 0.05$), implying their potential roles in ciprofloxacin removal. These results suggested that earthworm gut is a hotspot for ciprofloxacin removal, and could be an option for mitigation of antibiotic pollution in soil.

1. Introduction

Antibiotics are commonly used for preventing infectious diseases and promoting the growth of livestock (Jechalke et al., 2014). However,

* Corresponding author.
E-mail address: jqsu@iue.ac.cn (J.-Q. Su).

https://doi.org/10.1016/j.scitotenv.2020.140409
0048-9697/

some of the antibiotics are poorly absorbed and excreted to the environments via feces in the form of parent compounds or antibiotically active metabolites (Jechalke et al., 2014; Sarmah et al., 2006). Direct use of manure fertilizers or manure-derived compost would introduce a large amount of antibiotics into soil and consequently various antibiotics have been frequently detected in soil (Xie et al., 2012; Zhang et al., 2016). Antibiotics in soil pose potential risk to organisms in ecosystem. For example, enrofloxacin inhibited the growth, reproductive rate, and respiratory rates of earthworm (Li et al., 2015), and sulfamethoxazole inhibited rice growth (Liu et al., 2009). Antibiotics significantly affected soil microbial activity and diversity (Kong et al., 2006). In addition, antibiotics would enrich antibiotic-resistant bacteria and resistance genes in the environments due to selective pressure exerted by antibiotic pollution (Bush et al., 2011; Xiong et al., 2015).

Ciprofloxacin is one of the broad-spectrum fluoroquinolones against most of gram-negative bacteria by targeting bacterial type II topoisomerases, gyrase and topoisomerase IV and causing irreversible damage to bacterial DNA (Aldred et al., 2014). Ciprofloxacin has been widely used to treat infections in both human therapies and animal industries, for example, more than 7,500,000 prescriptions containing ciprofloxacin were documented for therapies in the United States (CDC, 2013), and 4882 tons of ciprofloxacin were consumed for livestock in 2013 in China (Zhang et al., 2015). Irrational use of ciprofloxacin has resulted in the prevalence of ciprofloxacin resistance among clinical bacterial isolates (Nuesch-Inderbinen et al., 2015). Ciprofloxacin is only partially metabolized and excreted via urine and feces as the parent compound or metabolites with antibacterial activity, resulting in the enrichment of ciprofloxacin in soil, which may impact soil microorganisms and fauna (Pico and Andreu, 2007; Xie et al., 2016).

Biodegradation is one of the major routes for removal of antibiotics in both wastewater treatment systems (Mueller et al., 2013; Prado et al., 2009; Yang et al., 2011) and natural environments (Ouyang et al., 2019). Ciprofloxacin could be degraded by isolates (Pan et al., 2018; Prieto et al., 2011) or bacterial consortium in a co-metabolic manner (Feng et al., 2019). However, ciprofloxacin is generally recalcitrant to transformation and biodegradation, and was frequently detected in soil (Y.W. Li et al., 2011; Pico and Andreu, 2007). Ciprofloxacin had a high K_d (adsorption coefficient) value (150–61,000), suggesting that ciprofloxacin had strong adsorption potential in soil and sediment, and could be accumulated in soil (Tolls, 2001). Therefore, an efficient and safe way to enhance the degradation of ciprofloxacin is essential.

Earthworms play an important role in soil nutrient cycling via changing soil structures and regulating soil bacterial community structures and activities (Gomez-Brandon et al., 2011; Hoang et al., 2016; Huang and Xia, 2018). Previous studies have reported that earthworm aided in the removal of the organic contaminants, including polycyclic aromatic hydrocarbons (PAHs) (Contreras-Ramos et al., 2008), pesticides (Hao et al., 2018) and antibiotics. Cao et al. (2018) found that activity of earthworm increased the abundance of *Anaerolineae*, *Flavobacteriaceae*, and *Pseudomonas* in soil amended with oxytetracycline, thus promoting the degradation of oxytetracycline. Mougin et al. (2013) used [2-^{14}C]-ciprofloxacin to investigate the effect of earthworm on ciprofloxacin degradation in soil, and they found that the mineralization of ciprofloxacin in the soil with earthworm was higher than that of non-earthworm control. These studies demonstrated that changes of microbial communities by earthworm activity could have significant effect on ciprofloxacin degradation (Liao et al., 2016), however, we knew little about the role of earthworm gut microbes in ciprofloxacin removal. Due to the feeding process of earthworm, soil will pass through earthworm gut. We hypothesized that earthworm activity would enhance ciprofloxacin removal which could occur in earthworm gut. We further hypothesized that the removal of ciprofloxacin would result in lower abundance of ARGs. To address these hypotheses, a microcosm experiment was setup to investigate the effects of earthworm on ciprofloxacin removal. The change of bacterial community structures and ARGs was analyzed by 16S rRNA gene sequencing and high-throughput quantitative PCR to evaluate their correlation with ARGs and to explore potential ciprofloxacin-degrading bacteria.

2. Materials and methods

2.1. Microcosm experiment setup

Soil was collected from a vegetable field in Ningbo, Zhejiang province, China (29°45′N, 121°54′E). The soil was air-dried and passed through a 2 mm mesh sieve before microcosm experiment. The concentration of ciprofloxacin in soil was 11.25 ng/g, and other properties were described in supplementary information Table S1. Ciprofloxacin concentration was measured using high performance liquid chromatography (L-2000, Hitachi). Ciprofloxacin hydrochloride was purchased from Sigma-Aldrich (China), and a stock solution of 1000 mg/L ciprofloxacin was prepared with Milli-Q water. The earthworm genus

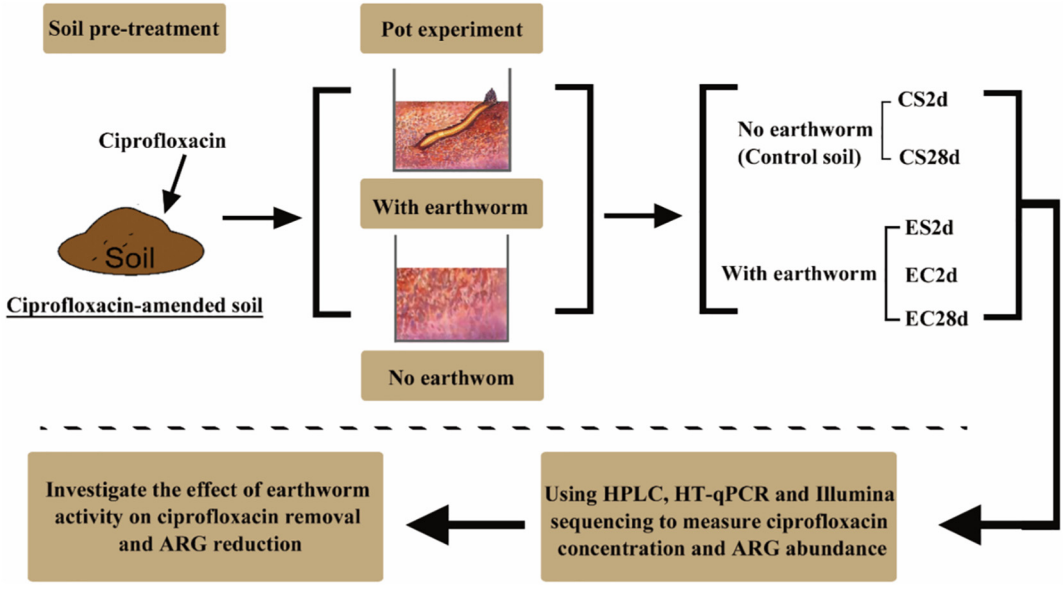


Fig. 1. The schematic diagram of the microcosm experiments.

Metaphire is widely distributed in soil ecosystem, living in the topsoil and feeding on the organic matters including organic pollutants (Drake and Horn, 2007; Sun et al., 2013). In the present study, *Metaphire vulgaris* (110–140 mm in length and 4–6 mm in width) was purchased from a commercial company in Ningbo, China and were acclimated for 14 days in collected soil at 25 °C prior to experiments.

Microcosm experiments (Fig. 1) were setup in polyethylene plastic containers (20 × 15 × 18 cm) covered by polyethylene lids with small holes, each containing 2 kg of dried soil. Ciprofloxacin was added to soil with a final concentration of 10 mg/kg of dry soil. According to US Environmental Protection Agency and the organization for Economic Cooperation and development (OECD) (No. 222) protocols (Cogliano et al., 1998; OECD, 2003), ten earthworms were rinsed with sterile water to remove soil particles and added into each pot, ciprofloxacin-amended soil without earthworm was used as control. All pots were incubated for 28 days according to condition reported by Wang et al. (2019). Each treatment was performed with five replicates. Sterile water was supplied to each pot every two days to maintain 30% of soil moisture. Undisturbed soil with earthworm (ES), control soil (CS) and earthworm cast (EC) samples were collected at day 2 and day 28, respectively, and were stored in −20 °C for further analysis. At the same time, all of earthworms were collected at day 28 and were sacrificed with absolute ethanol according to the protocol of Organization for Economic Cooperation and Development (OECD) (2003).

2.2. Measurement of ciprofloxacin concentration

Ciprofloxacin was extracted according to the study of Hong et al. (2018) with minor modifications. Briefly, soil and cast samples (2 g) were placed into a glass tube containing 25 mL EDTA-McIlvaine buffer (Huang et al., 2013), shaken at 25 °C and 150 rpm for 30 min and pelleted by centrifugation at 25 °C and 1500 ×*g* for 10 min. The supernatant was transferred to a new tube. The pellet was re-extracted twice with 20 and 15 mL EDTA-McIlvaine buffer, respectively. The supernatants from three extraction were pooled and diluted to 500 mL ultrapure water in a brown glass bottle. The resulting aqueous phase was concentrated and purified in solid-phase extraction (SPE) column via HLB (6 cc, waters, USA). The column was then eluted with 12 mL methanol, air dried and dissolved in 1 mL 50% methanol (v/v).

Ciprofloxacin was analyzed using High-performance liquid chromatography (HPLC, Hitachi L-2000, Japan) with an extend-C18 column (250 mm × 4.6 mm) to separate the analytes. The mobile phase and detection condition was adopted from the study of Pan et al. (2018).

Quality control was conducted independently in control soil at a concentration of 10 mg/kg of dry soil with the same extraction and detection procedure. The result showed that the recovery rate was 98%, which indicated that the procedure was suitable for the determination of ciprofloxacin.

The ciprofloxacin removal rate was calculated referring to the concentration of ciprofloxacin in different samples.

$$\text{Ciprofloxacin removal rate} = (C_{d0} - C_d)/C_{d0} \times 100\% \quad (1)$$

where C_{d0} is the ciprofloxacin concentration in soil collected at day 0, C_d is the ciprofloxacin concentration in soil or cast collected at day 2 and day 28.

2.3. DNA extraction, 16S rRNA gene sequencing and analysis

Earthworm gut was collected according to the study of Wang et al. (2019). DNA from soil and earthworm gut were extracted from 0.5 g solid using FastDNA Spin Kit (MP Biomedical, USA) following the manufacturer's instructions. The DNA quantity and quality were measured using spectrophotometer (NanoDrop Technology, Wilmington, USA) and gel electrophoresis with a 1.0% agarose gel.

The V4 region of 16S rRNA gene was amplified with the primer 515F: GTGCCAGCMGCCGCGGTAA and reverse primer 806R: GGAC TACHVGGGTWTCTAAT, which was tagged with unique barcode for each sample. PCR amplification was conducted according to the study of Ding et al. (2019). The amplicons were purified, pooled and sequenced on an Illumina platform (Microanaly, Shanghai, China). Data were analyzed by Quantitative Insights Into Microbial Ecology (QIIME, version1.9) (Caporaso et al., 2010). The sequences were clustered into operational taxonomic units (OTUs) using UCLUST algorithm with a phylotype defined at 97% similarity, and the OTUs were classified using RDP classifier (Edgar, 2010). Shannon index, observed species, PD whole tree and Chao1 were used to evaluate alpha diversity for each sample at equal sequencing depth. All sequences from this study were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number PRJNA600100.

2.4. High-throughput qPCR of antibiotic resistance genes

A total of 295 primer sets were used to analyze resistance genes (Chen et al., 2018b) by Wafergen SmartChip Real-Time PCR System (WaferGen Biosystems, USA). The PCR program was initiated at 95 °C for 10 min, followed by 40 thermal cycles of 30 s at 95 °C, 30 s at 60 °C, and the melting process was automatically generated by Wafergen software. The qPCR results were analyzed by SmartChip

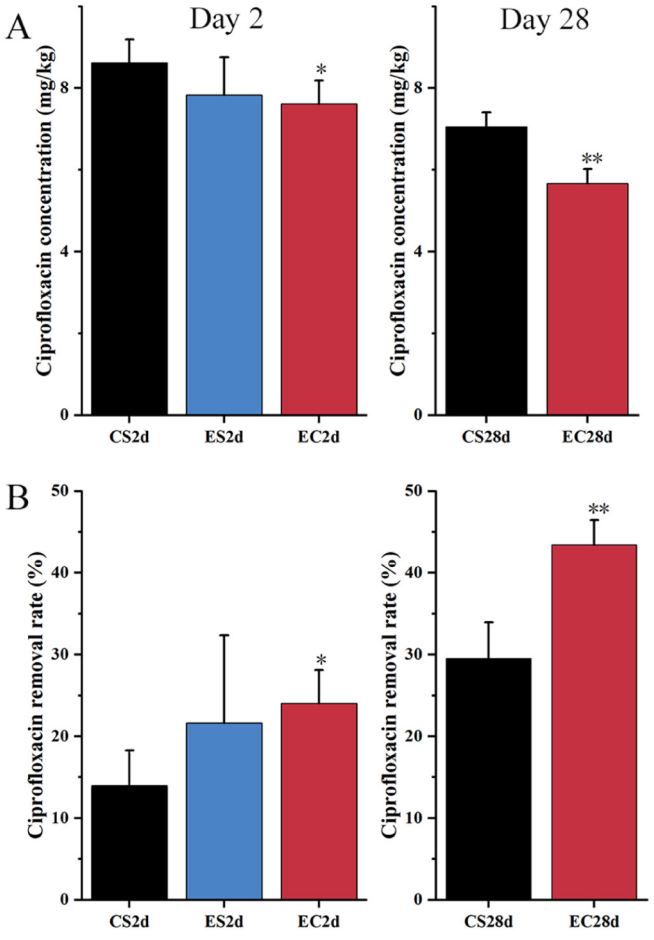


Fig. 2. Ciprofloxacin concentration (A) and removal rate (B) in different samples from microcosm experiments. CS2d, control soil collected at day 2; CS28d, control soil collected at day 28; ES2d, undistributed soil with earthworm collected at day 2; EC2d, earthworm cast collected at day 2; EC28d, earthworm cast collected at day 28. Significance of cast and control samples were tested using *t*-test: $^{*}p < 0.05$ and $^{**}p < 0.01$. Error bar represent the standard deviations of five repetitions.

qPCR software. The relative abundance of ARGs were calculated according to the study of Chen et al. (2018a).

2.5. Statistical analysis

Averages and standard deviations were conducted using Excel 2016 (Microsoft office 2016, Microsoft, USA). Adonis test, Procrustes analysis, and mantel test were performed in R (version 3.4.3) with "vegan" (Oksanen et al., 2014). *t*-Test was conducted using SPSS 20 (SPSS Inc., Chicago, USA). All statistical tests were considered significant at $p < 0.05$. The histogram and box diagrams were generated using Origin 2018 (OriginLab, USA). Principal co-ordinates analysis (PCoA) was used to evaluate the ARG profiles and bacterial communities among different treatments using Canoco version 5.0. Heatmap was used to show relative abundance of ARGs using R (version 3.4.3) with "pheatmap" (Team, 2005). Manhattan plot was used to show the fold change of relative abundance of genera in cast samples compared to control samples.

3. Results and discussion

3.1. Effect of earthworm activity on the removal of ciprofloxacin in soil

In the present study, all earthworms survived and significant difference was not observed in the body weight of earthworm in soil amended with 10 mg/kg ciprofloxacin between day 2 and day 28 samples (Table S2). All soil in pots have been "digested" by earthworm after 28 days of incubation, therefore, soil samples taken from day 28 were considered cast samples and labeled as EC28d. The concentration of ciprofloxacin in control soil was 8.62 and 7.06 mg/kg for CS2d and CS28d, respectively (Fig. 2A), corresponding to ciprofloxacin removal rates of 14% and 30% for CS2d and CS28d, respectively (Fig. 2B). In addition, the concentrations of ciprofloxacin were 7.62 and 5.67 mg/kg for EC2d and EC28d, respectively (Fig. 2A) and the ciprofloxacin removal rates were 24% and 43% for CE2d and CE28d, respectively (Fig. 2B). Further analysis of ciprofloxacin removal in undistributed soil with earthworm showed that ciprofloxacin removal of ES2d was similar with that of CS2d (Fig. 2B). These results indicated that earthworm activity significantly (*t*-test, $p < 0.05$) enhanced the ciprofloxacin removal in cast as compare to control samples, which was consistent with previous reports on earthworm activity enhanced the removal of organic pollutants (Contreras-Ramos et al., 2006; Contreras-Ramos et al., 2008; Lin et al., 2016), for example, enhanced removal of organic pollutants in soil with earthworm have been demonstrated for atrazine (Lin et al., 2018) and pentachlorophenol (Lin et al., 2016). The change of soil microbial communities and the absorption state of organic contaminant to soil induced by earthworm activity were proposed as major mechanisms responsible for the promoted degradation of contaminants in soil (Lin et al., 2016; Lin et al., 2018; Phuong-Thi et al., 2012). In this

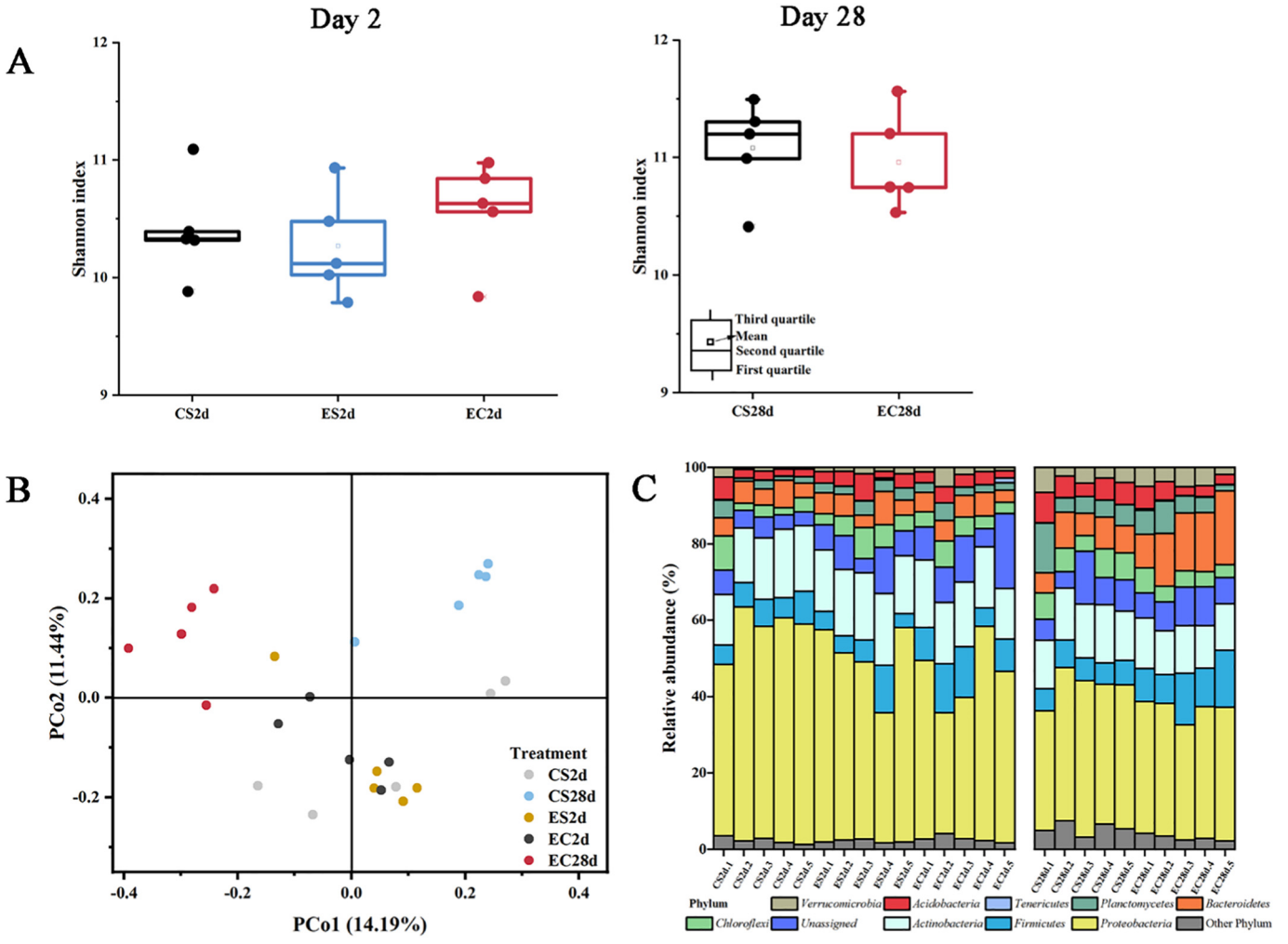


Fig. 3. Shannon index showing the estimated bacterial diversity in different samples (A). PCoA based on Bray-Curtis distance showing the overall distribution pattern of bacterial communities in different samples (B). Phylum level diversity of different samples (C). Low abundance of the phylum is categorized into "Other phylum". CS2d, control soil collected at day 2; CS28d, control soil collected at day 28; ES2d, undistributed soil with earthworm collected at day 2; EC2d, earthworm cast collected at day 2; EC28d, earthworm cast collected at day 28. Each column is labeled with the sample name with numbers representing replicates.

study, significant higher removal of ciprofloxacin was observed in earthworm cast samples than that in control soil (Fig. 2B). Earthworm gut harbors abundant microorganisms (Singh et al., 2015) and earthworm cast was characterized by higher levels of nutrients and microbial biomass and activities than bulk soil (Don et al., 2008; Monard et al., 2008; Sizmur et al., 2011), therefore, we speculated that enhanced ciprofloxacin removal by earthworm could occur in earthworm gut.

3.2. Earthworm activity changed soil bacterial community composition and structure

We then compared the bacterial community structures between earthworm cast and soil. The diversity of bacterial community was similar between earthworm cast and control soil (Fig. 3A), which was confirmed by the Chao 1 index, PD-whole tree and observed species (Fig. S1). While, PCoA based on Bray-Curtis distance and hierarchical clustering analysis showed that EC28d was separated with control samples (Fig. 3B and S2), suggesting a significant difference in bacterial community structures between earthworm cast and soil. Previous studies have showed that the change in bacterial communities after earthworm activity could enhance the removal of antibiotic (Bernard et al., 2012; Hery et al., 2008; Mummey et al., 2006). Therefore, the shift of bacterial communities in cast samples could be an important factor contributing to the higher ciprofloxacin removal in cast (Liao et al., 2016).

Earthworm activity significantly changed the microbial community compositions with increased relative abundance of *Bacteroidetes* and decreased *Proteobacteria*, *Firmicutes* and *Actinobacteria* in cast samples as compare to control samples (Fig. 3C). *Proteobacteria*, *Firmicutes* and *Actinobacteria* were the most abundant phyla in all samples, accounting for 71%, 69% and 78% of the total reads in ES2d, EC2d and CS2d, respectively, and 57% and 57% for CS28d and EC28d, respectively (Fig. 3C). These results were consistent with the reports that *Proteobacteria*, *Actinobacteria* and *Firmicutes* were dominant microbes in antibiotic-amended soil (Cleary et al., 2016; Leclercq et al., 2016; D. Li et al., 2011), and they were the major predicted source phyla of ARGs (Forsberg et al., 2014; Sun et al., 2019). Further analysis of bacterial community at genus level found that *Burkholderia* was dominant in ES2d (11.4%), EC2d (7.1%) and CS2d (9.9%), respectively, while the predominant genera was *Pseudomonas* (8.5%) in CS28d, and *Flavobacterium* (11%) in EC28d (Fig. S3).

Comparison of bacterial compositions between cast and control soil samples identified significant (t-test, $p < 0.05$) difference in the relative

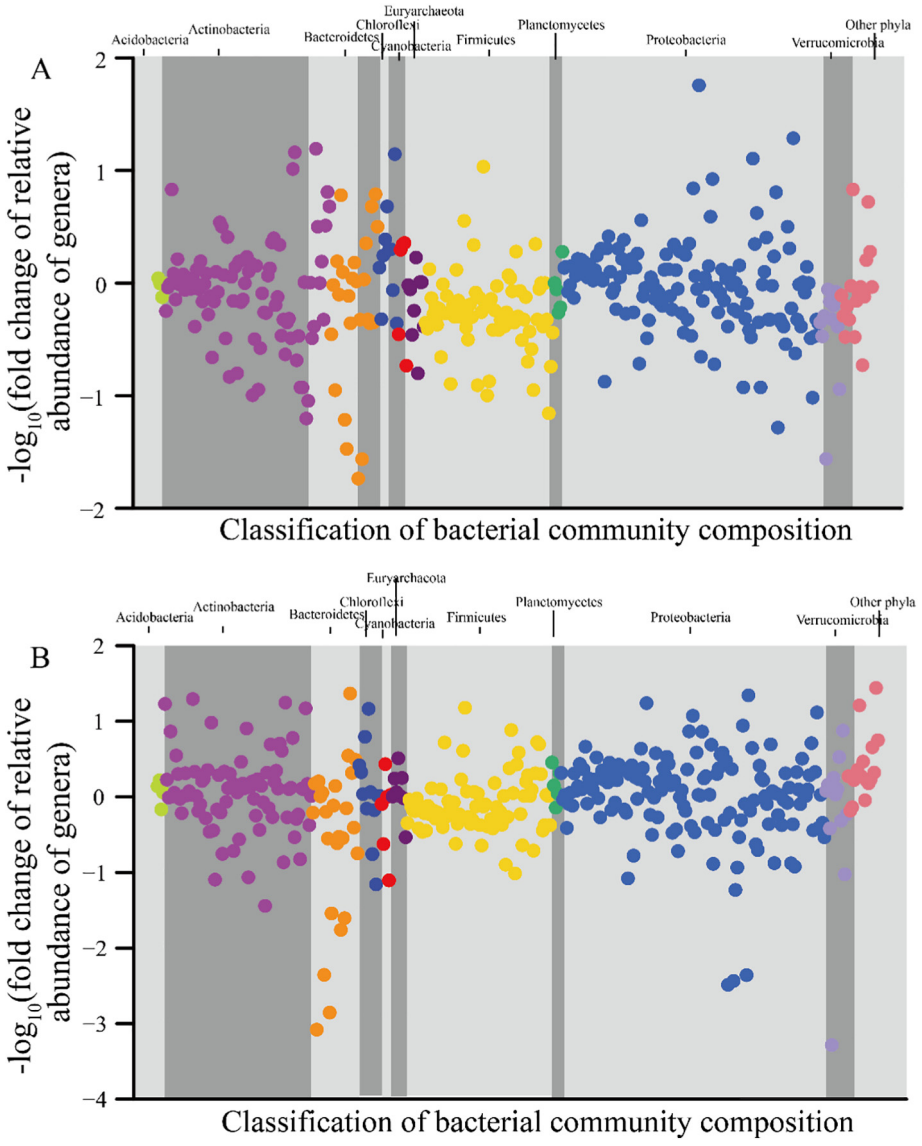


Fig. 4. Manhattan plots showing the fold changes of relative abundance of genera in comparison of EC2d and CS2d (A) and comparison of EC28d and CS28d (B). The color of each dot represents the taxonomic affiliation of genus level.

Table 1
Pearson correlation coefficient of the relative abundance of *Flavobacterium*, *Turicibacter*, ciprofloxacin concentration and ARG abundance after the earthworm activity.

	Flavobacterium	*Turicibacter*	Ciprofloxacin	ARGs
Flavobacterium	1	0.61**	−0.633**	−0.625**
Turicibacter		1	−0.558**	−0.681**
Ciprofloxacin			1	0.583**
ARGs				1

Asterisks represent the level of significance: *, $p < 0.05$; **, $p < 0.01$.

abundance of 30 genera in comparison between EC2d and CS2d, and 54 genera in comparison between EC28d and CS28d, respectively (Fig. 4, Table S3 and S4). In addition, we found that enrichment of microorganisms in EC28d was higher than that in EC2d (Fig. 4). Further analysis of enriched genera in EC2d and EC28d found that these enriched genera belonged to a wide range of bacterial phyla, where the dominant phyla were *Firmicutes* and *Actinobacteria* for EC2d, whereas *Firmicutes* and *Proteobacteria* for EC28d (Table S3 and S4). *Flavobacterium*, *Turicibacter*, *Bacillus*, and *Skermanella*, were detected in both EC2d and EC28d samples, which accounted for 3.6% and 11.5% of total bacterial community compositions in EC2d and EC28d, respectively. Correlation analysis showed that the relative abundance of *Flavobacterium* and *Turicibacter* were significantly negatively correlated with ciprofloxacin concentration (Table 1), implying they were potential ciprofloxacin degrading bacteria. Previous studies have observed that these two genera had the capacity to remove high concentration of organic contaminant (Gonzalez and Hu, 1991; Ju and Zhang, 2014; Ros et al., 2017; Saber and Crawford, 1985; Somara and Siddavattam, 1995). However, the removal of ciprofloxacin by these two genera was not reported, further studies are needed to depict their roles in ciprofloxacin degradation.

3.3. Antibiotic resistome and associated bacteria in soil

The detected number of ARGs showed no significant difference in cast samples compared with control samples (Fig. S4), and the major mechanisms of resistance were antibiotic deactivation, efflux pumps and cellular protection in both cast and control samples (Fig. S5 and S6). Genes conferring resistance to multidrug, beta_lactams and aminoglycosides were the dominant genes detected in all treatments (Fig. S7). PCoA based on Bray-Curtis distance showed that the ARG profiles in cast samples were divergent but formed a distinct cluster separated from control samples along PCo1 (explained 40.79% of the total variance) (Fig. S8). In addition, 54 ARGs were detected in earthworm gut after incubation in ciprofloxacin-amended soil, which was slightly higher than

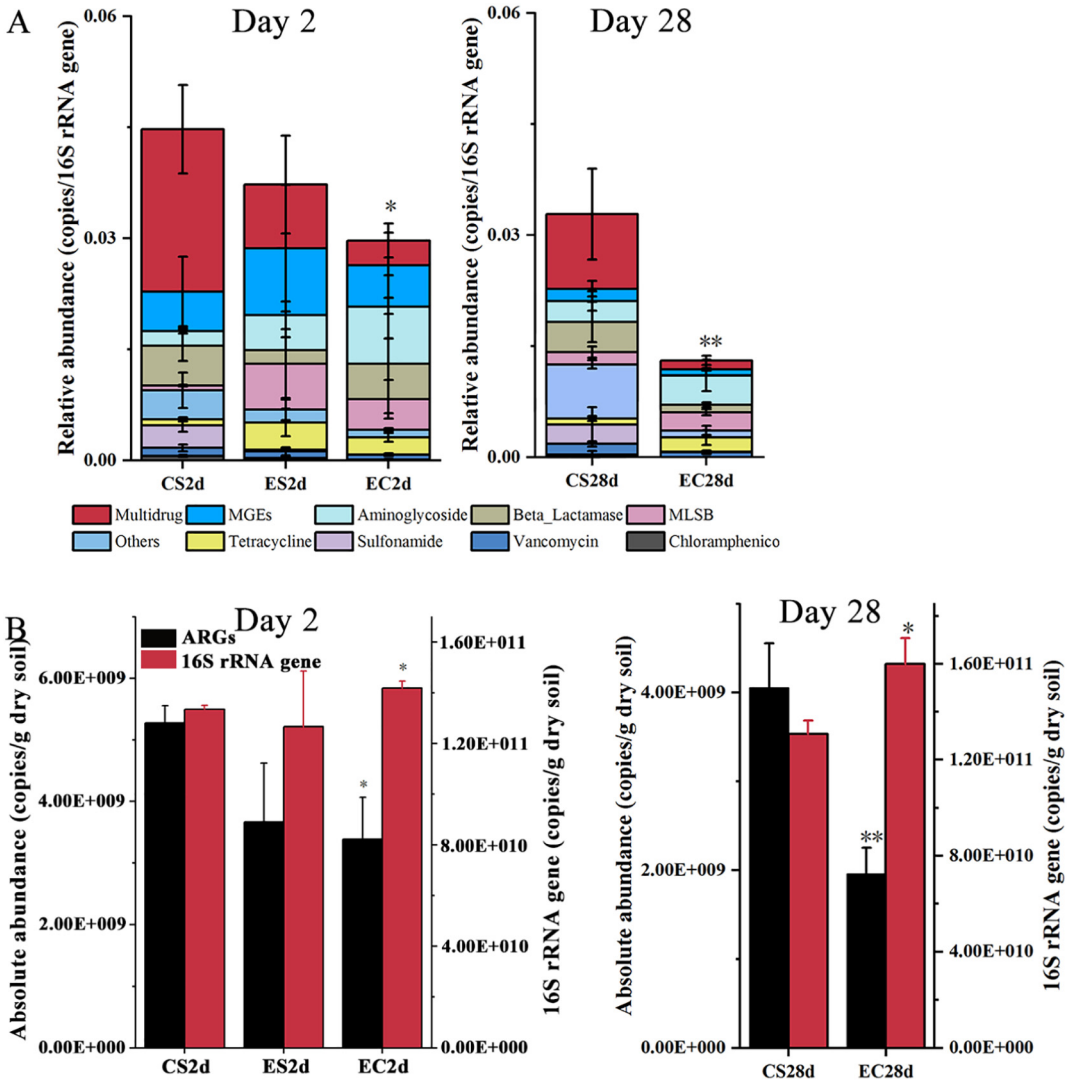


Fig. 5. The relative abundance (copies/16S rRNA gene) (A) and absolute abundance (B) of ARGs in different samples from microcosm experiments. ARGs were classified according to the antibiotics to which they conferred resistance. CS2d, control soil collected at day 2; CS28d, control soil collected at day 28; ES2d, undistributed soil with earthworm collected at day 2; EC2d, earthworm cast collected at day 2; EC28d, earthworm cast collected at day 28. Significance of cast and control samples were tested using t-test: *$p < 0.05$ and **$p < 0.01$.

that in earthworm gut at the beginning of incubation (49 ARGs) (Fig. S9). The absolute abundance of 16S rRNA gene was significantly higher in cast samples as compare to control samples (Fig. 5). Nevertheless, the relative abundance and absolute abundance of ARGs were significantly decreased in EC2d as compare to CS2d, and a similar result was observed in the sample of EC28d compared with CS28d (Fig. 5A and B), indicating that earthworm activities could decrease ARG abundance in cast samples. Since antibiotic is one of the major selecting pressure for enrichment of ARGs (Berendonk et al., 2015; Chen et al., 2013; Karkman et al., 2018; Li et al., 2017), the lower level of ARG abundance in cast could be partially attributed to the lower concentration of ciprofloxacin in cast.

Procrustes analysis indicated that ARG profiles and bacterial community structures were significantly correlated in both cast and control samples, which was confirmed by Mantel test (Fig. S10). These results were consistent with previous studies that ARG profiles were strongly correlated with bacterial communities (Di Cesare et al., 2016; Jia et al., 2015; Jia et al., 2017; Zhou et al., 2020; Zhu et al., 2020), suggesting that the lower abundance of ARGs in earthworm cast could also be attributed to the changes of bacterial communities in cast samples.

4. Conclusion

This study found that earthworm could promote ciprofloxacin removal and decrease ARG abundance in cast, indicating that earthworm gut was the hotpot for ciprofloxacin removal in ciprofloxacin-amended soil. The lower ciprofloxacin concentration and shift of bacterial community structures contributed to the lower abundance of ARGs in cast. In addition, two potential ciprofloxacin-degrading bacteria were identified in this study. These results highlight the potential roles of earthworm in antibiotic removal and further mitigating the development of antibiotic resistance in antibiotic polluted soil.

CRediT authorship contribution statement

Qiang Pu: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Hong-Tao Wang:** Methodology, Investigation. **Ting Pan:** Methodology, Investigation. **Hu Li:** Conceptualization, Methodology, Investigation. **Jian-Qiang Su:** Conceptualization, Validation, Formal analysis, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was financially supported by the National Key Research and Development Program of China-International collaborative project from Chinese Ministry of Science and Technology (2017YFE0107300), National Natural Science Foundation of China (31722004, U1805244), K. C. Wong Education Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140409.

References

Aldred, K.J., Kerns, R.J., Osheroff, N., 2014. Mechanism of quinolone action and resistance. Biochemistry 53, 1565–1574.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Buergmann, H., Sorum, H., Norstrom, M., Pons, M.-N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., Luis Martinez, J., 2015. Tackling antibiotic resistance: the environmental framework. Nat. Rev. Microbiol. 13, 310–317.

Bernard, L., Chapuis-Lardy, L., Razafimbelo, T., Razafindrakoto, M., Pablo, A.-L., Legname, E., Poulain, J., Bruels, T., O'Donohue, M., Brauman, A., Chotte, J.-L., Blanchart, E., 2012. Endogeic earthworms shape bacterial functional communities and affect organic matter mineralization in a tropical soil. ISME J 6, 213–222.

Bush, K., Courvalin, P., Dantas, G., Davies, J., Eisenstein, B., Huovinen, P., Jacoby, G.A., Kishony, R., Kreiswirth, B.N., Kutter, E., Lerner, S.A., Levy, S., Lewis, K., Lomovskaya, O., Miller, J.H., Mobashery, S., Piddock, L.J.V., Projan, S., Thomas, C.M., Tomasz, A., Tulkens, P.M., Walsh, T.R., Watson, J.D., Witkowski, J., Witte, W., Wright, G., Yeh, P., Zgurskaya, H.I., 2011. Tackling antibiotic resistance. Nat. Rev. Microbiol. 9, 894–896.

Cao, J., Wang, C., Dou, Z., Liu, M., Ji, D., 2018. Hyphospheric impacts of earthworms and arbuscular mycorrhizal fungus on soil bacterial community to promote oxytetracycline degradation. J. Hazard. Mater. 341, 346–354.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, 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., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

CDC, 2013. Antibiotic resistance threats in the United States. Available from. http://www.cdc.gov/drugresistance/threat-report-2013/.

Chen, B., Liang, X., Huang, X., Zhang, T., Li, X., 2013. Differentiating anthropogenic impacts on ARGs in the Pearl River Estuary by using suitable gene indicators. Water Res. 47, 2811–2820.

Chen, Q.L., An, X.L., Zheng, B.X., Ma, Y.B., Su, J.Q., 2018a. Long-term organic fertilization increased antibiotic resistome in phyllosphere of maize. Sci. Total Environ. 645, 1230–1237.

Chen, Q.L., Fan, X.T., Zhu, D., An, X.L., Su, J.Q., Cui, L., 2018b. Effect of biochar amendment on the alleviation of antibiotic resistance in soil and phyllosphere of Brassica chinensis L. Soil Biol. Biochem. 119, 74–82.

Cleary, D.W., Bishop, A.H., Zhang, L., Topp, E., Wellington, E.M.H., Gaze, W.H., 2016. Long-term antibiotic exposure in soil is associated with changes in microbial community structure and prevalence of class 1 integrons. FEMS Microbiol. Ecol. 92.

Cogliano, V.J., Koppikar, A.M., Canis, J.M., Gibson, S.C., Gift, J.S., Messing, A.W., Ricci, G.R., Tuffly, B.L., 1998. Methodolgy for Evaluating Potential Carcinogenicity in Support of Reportable Quantity Adjustments Pursuant to CERCLA Section 102; EPA/600/8-89/53 (NTIS PB93230027). U.S. Environmental Protection Agency, Washington, D.C.

Contreras-Ramos, S.M., Alvarez-Bernal, D., Dendooven, L., 2006. Eisenia fetida increased removal of polycyclic aromatic hydrocarbons from soil. Environ. Pollut. 141, 396–401.

Contreras-Ramos, S.M., Alvarez-Bernal, D., Dendooven, L., 2008. Removal of polycyclic aromatic hydrocarbons from soil amended with biosolid or vermicompost in the presence of earthworms (Eisenia fetida). Soil Biol. Biochem. 40, 1954–1959.

Di Cesare, A., Fontaneto, D., Doppelbauer, J., Corno, G., 2016. Fitness and recovery of bacterial communities and antibiotic resistance genes in urban wastewaters exposed to classical disinfection treatments. Environ. Sci. Technol. 50, 10153–10161.

Ding, J., Zhu, D., Li, H., Ding, K., Chen, Q.-L., Lassen, S.B., Ke, X., O'Connor, P., Zhu, Y.-G., 2019. The gut microbiota of soil organisms show species-specific responses to liming. Sci. Total Environ. 659, 715–723.

Don, A., Steinberg, B., Schoening, I., Pritsch, K., Joschko, M., Gleixner, G., Schulze, E.D., 2008. Organic carbon sequestration in earthworm burrows. Soil Biol. Biochem. 40, 1803–1812.

Drake, H.L., Horn, M.A., 2007. As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. Annu. Rev. Microbiol. 61, 169–189.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.

Feng, N.X., Yu, J., Xiang, L., Yu, L.Y., Zhao, H.M., Mo, C.H., Li, Y.W., Cai, Q.Y., Wong, M.H., Li, Q.X., 2019. Co-metabolic degradation of the antibiotic ciprofloxacin by the enriched bacterial consortium XG and its bacterial community composition. Sci. Total Environ. 665, 41–51.

Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., Dantas, G., 2014. Bacterial phylogeny structures soil resistomes across habitats. Nature 509, 612.

Gomez-Brandon, M., Aira, M., Lores, M., Dominguez, J., 2011. Epigeic earthworms exert a bottleneck effect on microbial communities through gut associated processes. PLoS One 6.

Gonzalez, J.F., Hu, W.S., 1991. Effect of glutamate on the degradation of pentachlorophenol by flavobacterium sp. Appl. Microbiol. Biotechnol. 35, 100–104.

Hao, Y.Q., Zhao, L.X., Sun, Y., Li, X.J., Weng, L.P., Xu, H.J., Li, Y.T., 2018. Enhancement effect of earthworm (Eisenia fetida) on acetochlor biodegradation in soil and possible mechanisms. Environ. Pollut. 242, 728–737.

Hery, M., Singer, A.C., Kumaresan, D., Bodrossy, L., Stralis-Pavese, N., Prosser, J.I., Thompson, I.P., Murrell, J.C., 2008. Effect of earthworms on the community structure of active methanotrophic bacteria in a landfill cover soil. ISME J 2, 92–104.

Hoang, D.T.T., Razavi, B.S., Kuzyakov, Y., Blagodatskaya, E., 2016. Earthworm burrows: kinetics and spatial distribution of enzymes of C-, N- and P- cycles. Soil Biol. Biochem. 99, 94–103.

Hong, B., Lin, Q.Y., Yu, S., Chen, Y.S., Chen, Y.M., Chiang, P.C., 2018. Urbanization gradient of selected pharmaceuticals in surface water at a watershed scale. Sci. Total Environ. 634, 448–458.

Huang, K., Xia, H., 2018. Role of earthworms' mucus in vermicomposting system: biodegradation tests based on humification and microbial activity. Sci. Total Environ. 610, 703–708.

Huang, Y.J., Cheng, M.M., Li, W.H., Wu, L.H., Chen, Y.S., Luo, Y.M., Christie, P., Zhang, H.B., 2013. Simultaneous extraction of four classes of antibiotics in soil, manure and

sewage sludge and analysis by liquid chromatography-tandem mass spectrometry with the isotope-labelled internal standard method. Anal. Methods 5, 3721–3731.
Jechalke, S., Heuer, H., Siemens, J., Amelung, W., Smalla, K., 2014. Fate and effects of veterinary antibiotics in soil. Trends Microbiol. 22, 536–545.
Jia, S., Shi, P., Hu, Q., Li, B., Zhang, T., Zhang, X.-X., 2015. Bacterial community shift drives antibiotic resistance promotion during drinking water chlorination. Environ. Sci. Technol. 49, 12271–12279.
Jia, S., Zhang, X.-X., Miao, Y., Zhao, Y., Ye, L., Li, B., Zhang, T., 2017. Fate of antibiotic resistance genes and their associations with bacterial community in livestock breeding wastewater and its receiving river water. Water Res. 124, 259–268.
Ju, F., Zhang, T., 2014. Novel microbial populations in ambient and mesophilic biogas-producing and phenol-degrading consortia unraveled by high-throughput sequencing. Microb. Ecol. 68, 235–246.
Karkman, A., Thi Thuy, D., Walsh, F., Virta, M.P.J., 2018. Antibiotic-resistance genes in waste water. Trends Microbiol. 26, 220–228.
Kong, W.D., Zhu, Y.G., Fu, B.J., Marschner, P., He, J.Z., 2006. The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. Environ. Pollut. 143, 129–137.
Leclercq, S.O., Wang, C., Sui, Z., Wu, H., Zhu, B., Deng, Y., Feng, J., 2016. A multiplayer game: species of Clostridium, Acinetobacter, and Pseudomonas are responsible for the persistence of antibiotic resistance genes in manure-treated soils. Environ. Microbiol. 18, 3494–3508.
Li, D., Qi, R., Yang, M., Zhang, Y., Yu, T., 2011a. Bacterial community characteristics under long-term antibiotic selection pressures. Water Res. 45, 6063–6073.
Li, Y.W., Wu, X.L., Mo, C.H., Tai, Y.P., Huang, X.P., Xiang, L., 2011b. Investigation of sulfonamide, tetracycline, and quinolone antibiotics in vegetable farmland soil in the Pearl River Delta area, southern China. J. Argic. Food Chem. 59, 7268–7276.
Li, Y.S., Hu, Y.X., Ai, X.J., Qiu, J.P., Wang, X.H., 2015. Acute and sub-acute effects of enrofloxacin on the earthworm species Eisenia fetida in an artificial soil substrate. Eur. J. Soil Biol. 66, 19–23.
Li, L.G., Xia, Y., Zhang, T., 2017. Co-occurrence of antibiotic and metal resistance genes revealed in complete genome collection. ISME J 11, 651–662.
Liao, X., Li, B., Zou, R., Dai, Y., Xie, S., Yuan, B., 2016. Biodegradation of antibiotic ciprofloxacin: pathways, influential factors, and bacterial community structure. Environ. Sci. Pollut. R. 23, 7911–7918.
Lin, Z., Bai, J., Zhen, Z., Lao, S., Li, W., Wu, Z., Li, Y., Spiro, B., Zhang, D., 2016. Enhancing pentachlorophenol degradation by vermicomposting associated bioremediation. Ecol. Eng. 87, 288–294.
Lin, Z., Zhen, Z., Ren, L., Yang, J.W., Luo, C.L., Zhong, L.Y., Hu, H.Q., Liang, Y.Q., Li, Y.T., Zhang, D.Y., 2018. Effects of two ecological earthworm species on atrazine degradation performance and bacterial community structure in red soil. Chemosphere 196, 467–475.
Liu, F., Ying, G.G., Tao, R., Jian-Liang, Z., Yang, J.F., Zhao, L.F., 2009. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environ. Pollut. 157, 1636–1642.
Monard, C., Martin-Laurent, F., Vecchiato, C., Francez, A.J., Vandenkoornhuyse, P., Binet, F., 2008. Combined effect of bioaugmentation and bioturbation on atrazine degradation in soil. Soil Biol. Biochem. 40, 2253–2259.
Mougin, C., Cheviron, N., Repincay, C., Hedde, M., Hernandez-Raquet, G., 2013. Earthworms highly increase ciprofloxacin mineralization in soils. Environ. Chem. Lett. 11, 127–133.
Mueller, E., Schuessler, W., Horn, H., Lemmer, H., 2013. Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source. Chemosphere 92, 969–978.
Mummey, D.L., Rillig, M.C., Six, J., 2006. Endogeic earthworms differentially influence bacterial communities associated with different soil aggregate size fractions. Soil Biol. Biochem. 38, 1608–1614.
Nuesch-Inderbinen, M., Abgottspon, H., Saegesser, G., Cernela, N., Stephan, R., 2015. Antimicrobial susceptibility of travel-related Salmonella enterica serovar Typhi isolates detected in Switzerland (2002–2013) and molecular characterization of quinolone resistant isolates. BMC Infect. Dis. 15.
OECD, 2003. Guideline for Testing of Chemicals No 222, Earthworm Reproduction Test (Eisenia fetida/andrei). pp. 1–18 (Paris, 2003).
Oksanen, J., Blanchet, F.G., Kindt, R., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2014. vegan: community ecology package. R package version 2.0-10. http://CRAN.R-project.org/package=vegan.
Ouyang, W.Y., Su, J.Q., Richnow, H.H., Adrian, L., 2019. Identification of dominant sulfamethoxazole-degraders in pig farm-impacted soil by DNA and protein stable isotope probing. Environ. Int. 126, 118–126.
Pan, L.J., Li, J., Li, C.X., Tang, X.D., Yu, G.W., Wang, Y., 2018. Study of ciprofloxacin biodegradation by a Thermus sp isolated from pharmaceutical sludge. J. Hazard. Mater. 343, 59–67.
Phuong-Thi, N., Rumpel, C., Thu-Thuy, D., Jouquet, P., 2012. The effect of earthworms on carbon storage and soil organic matter composition in tropical soil amended with compost and vermicompost. Soil Biol. Biochem. 50, 214–220.
Pico, Y., Andreu, V., 2007. Fluoroquinolones in soil - risks and challenges. Anal. Bioanal. Chem. 387, 1287–1299.
Prado, N., Ochoa, J., Amrane, A., 2009. Biodegradation and biosorption of tetracycline and tylosin antibiotics in activated sludge system. Process Biochem. 44, 1302–1306.
Prieto, A., Moeder, M., Rodil, R., Adrian, L., Marco-Urrea, E., 2011. Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. Bioresour. Technol. 102, 10987–10995.
Ros, M., Oliveira Filho, J.d.S., Perez Murcia, M.D., Angeles Bustamante, M., Moral, R., Dolores Coll, M., Lopez Santisima-Trinidad, A.B., Antonio Pascual, J., 2017. Mesophilic anaerobic digestion of pig slurry and fruit and vegetable waste: dissection of the microbial community structure. J. Clean. Prod. 156, 757–765.
Saber, D.L., Crawford, R.L., 1985. Isolation and characterization of flavobacterium strains that degrade pentachlorophenol. Appl. Environ. Microb. 50, 1512–1518.
Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725–759.
Singh, A., Singh, D.P., Tiwari, R., Kumar, K., Singh, R.V., Singh, S., Prasanna, R., Saxena, A.K., Nain, L., 2015. Taxonomic and functional annotation of gut bacterial communities of Eisenia foetida and Perionyx excavatus. Microbiol. Res. 175, 48–56.
Sizmur, T., Palumbo-Roe, B., Watts, M.J., Hodson, M.E., 2011. Impact of the earthworm Lumbricus terrestris (L.) on As, Cu, Pb and Zn mobility and speciation in contaminated soils. Environ. Pollut. 159, 742–748.
Somara, S., Siddavattam, D., 1995. Plasmid mediated organophosphate pesticide degradation by Flavobacterium balustinum. Biochem. Mol. Biol. Int. 36, 627–631.
Sun, J., Zhao, Q., Jiang, J., Qiu, J., 2013. New Amynthas species (Oligochaeta: Megascolecidae) from south and central Hainan Island, China and estimates of evolutionary divergence among some corticis-group species. J. Nat. Hist. 47, 1143–1160.
Sun, W., Gu, J., Wang, X., Qian, X., Peng, H., 2019. Solid-state anaerobic digestion facilitates the removal of antibiotic resistance genes and mobile genetic elements from cattle manure. Bioresour. Technol. 274, 287–295.
Team, R.D.C., 2005. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria www.R-project.org.
Tolls, J., 2001. Sorption of veterinary pharmaceuticals in soils: a review. Environ. Sci. Technol. 35, 3397–3406.
Wang, H.T., Zhu, D., Li, G., Zheng, F., Ding, J., O'Connor, P.J., Zhu, Y.G., Xue, X.M., 2019. Effects of arsenic on gut microbiota and its biotransformation genes in earthworm Metaphire sieboldi. Environ. Sci. Technol. 53, 3841–3849.
Xie, Y.F., Li, X.W., Wang, J.F., Christakos, G., Hu, M.G., An, L.H., Li, F.S., 2012. Spatial estimation of antibiotic residues in surface soils in a typical intensive vegetable cultivation area in China. Sci. Total Environ. 430, 126–131.
Xie, W.Y., Yang, X.P., Li, Q., Wu, L.H., Shen, Q.R., Zhao, F.J., 2016. Changes in antibiotic concentrations and antibiotic resistome during commercial composting of animal manures. Environ. Pollut. 219, 182–190.
Xiong, W.G., Sun, Y.X., Ding, X.Y., Wang, M.Z., Zeng, Z.L., 2015. Selective pressure of antibiotics on ARGs and bacterial communities in manure-polluted freshwater-sediment microcosms. Front. Microbiol. 6.
Yang, S.F., Lin, C.F., Lin, A.Y.C., Hong, P.K.A., 2011. Sorption and biodegradation of sulfonamide antibiotics by activated sludge: experimental assessment using batch data obtained under aerobic conditions. Water Res. 45, 3389–3397.
Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ. Sci. Technol. 49, 6772–6782.
Zhang, H.B., Zhou, Y., Huang, Y.J., Wu, L.H., Liu, X.H., Luo, Y.M., 2016. Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures. Chemosphere 152, 229–237.
Zhou, S.-y.-d., Zhu, D., Giles, M., Daniell, T., Neilson, R., Yang, X.-r., 2020. Does reduced usage of antibiotics in livestock production mitigate the spread of antibiotic resistance in soil, earthworm guts, and the phyllosphere? Environ. Int. 136.
Zhu, D., Ding, J., Yin, Y., Ke, X., O'Connor, P., Zhu, Y.-G., 2020. Effects of earthworms on the microbiomes and antibiotic resistomes of detritus fauna and phyllospheres. Environ. Sci. Technol. 54, 6000–6008.