# Effects of Trophic Level and Land Use on the Variation of Animal Antibiotic Resistome in the Soil Food Web

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**ABSTRACT:** In recent years, it has been increasingly recognized that soil animals are hidden reservoirs of antibiotic resistance genes (ARGs) and play a vital role in spreading ARGs in soil ecosystems. However, little is known about the variation of ARGs among different animals in the soil food web and effects of trophic levels and land uses on them. We characterized the antibiotic resistomes of 495 soil animal samples collected from six regions across China, including two different land uses. A total of 265 ARGs were detected in all animal samples, and relative abundances of ARGs in animals were significantly higher than in soils. In addition, significant differences in ARGs were observed among different animal groups. Twelve common ARGs were identified among all animal groups, accounting for 17.4% of total ARGs abundance. A positive and significant correlation was found between $\delta^{15}N$ values (trophic level) and total ARGs abundance in animals. The relative abundance of ARGs in the soil food web from arable land was higher than forest land. Changes in soil antibiotics may indirectly affect animal resistome by altering soil ARGs. This study suggests that the risk of ARGs spreading through the food web is greater in arable than in forest ecosystems.

**KEYWORDS:** antibiotic resistance genes, soil food chain, One Health, shared ARGs, dispersal, large-scale field survey, different ecosystems, environmental factors
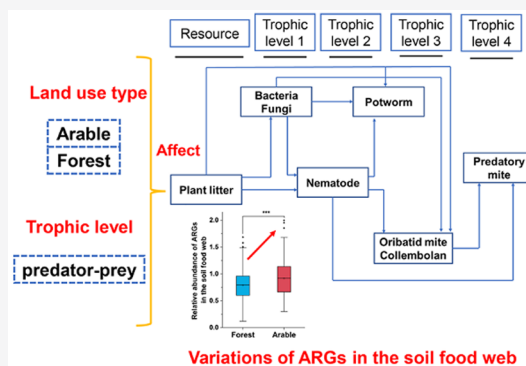
## 1. INTRODUCTION

Antibiotic resistance is ubiquitous in environmental bacteria.[1] However, in recent years, due to the overuse of antibiotics in humans and livestocks, the number and abundance of antibiotic resistance genes (ARGs) in the environment has increased rapidly.[2−6] The emergence of ARGs in the environment has become one of the greatest threats to human health. Many studies have confirmed that soil ecosystems are important reservoirs of ARGs due to manure and sludge.[2,5,7] The dispersal of ARGs in soil ecosystems can harm human health.[7] For example, many studies have shown that ARGs in the soil can be transferred to vegetables.[8−10] Currently, many vegetables are consumed raw, and the ARGs in these vegetables can be transmitted to human through the food chain.[11] Therefore, it is important to understand and manage the dynamics of ARGs in soil ecosystems.

Soil animals are an important component of soil ecosystems, performing many important ecological functions.[12] Soil animals have a high species diversity and are distributed in almost all soils.[13,14] Since soil animals tend to ingest organic matter,[15,16] the application of organic fertilizers is expected to enrich ARGs in the soil animal microbiome.[17,18] Further, diverse antibacterial substances and antibiotic gene clusters have been detected in the intestinal tract of soil animals,[19−21] suggesting that soil animals may possess abundant intrinsic

resistance. Thus, the ARGs of soil animals may play an important role as a resistome in soil ecosystems. In recent years, several studies have focused on the response of soil animal resistomes to environmental changes.[18,22,23] These studies have indicated that diverse and abundant ARGs can be detected in the microbiome of soil animals and that environmental changes significantly affect the resistome of soil animals. Furthermore, some soil animals are ideal food for chickens, birds, and fish.[24−26] Therefore, changes in the ARGs harbored in soil animals may affect the abundance of ARGs in chickens, birds and fish through the food chain, affecting human health. However, most of the studies on ARGs in soil animals have been conducted on single sites or single species, and no systematic studies have been conducted on a large scale and multiple species.

The study of the transfer of pollutants in the food chain is significant in ecological risk assessment.[27] In soil ecosystems,

animals form a complex food web through predator–prey relationships.[28] Many contaminants (e.g., heavy metals[29] and nanoparticles[30]) have been found to transmit through the soil food chain and affect the health of organisms at high trophic levels. In terms of ARGs, microcosm experiments have shown that ARGs from pig manure can be transmitted through the food chain into the microbiomes of high-trophic animals.[31] However, little is known about the dispersal of ARGs in the soil food web from the field, which will affect the risk assessment of ARGs transmission in the soil food chain and in the environment in general. This is because microcosm experiments simplify the microbiome and resistome of soil animals, thus failing to reflect the risk of actual transmission of ARGs[31] under field conditions. Several studies have confirmed that bacterial communities and ARGs are significantly correlated in various environments.[3,32,33] In addition, a recent study on the microbiome of soil food webs demonstrated that the diversity of animal bacterial community increases with increasing trophic levels.[34] Therefore, we hypothesized that the variability of animal ARGs in soil food webs is related to their trophic levels.

With the increase in human activity, land use patterns are also changing.[35] This change in land use significantly impacts soil properties and vegetation.[35–39] Soil properties and vegetation, in general, have important effects on the microbiome and resistome of animals. In our study on ARGs in the collembolan microbiome, we collected collembolan samples from three land uses and found different distribution patterns of collembolan ARGs in parks, parkways and arable lands.[23] A recent study has shown that the conversion of forests to arable land increases the presence of zoonotic pathogens in ecosystems.[37] The conversion of forests to arable land is the most common land use change in wake of increasing food supply to feed more people.[37] However, there is a lack of understanding on the dynamics of ARGs in the soil food web between forests and arable lands. Because arable land is more exposed to chemical stresses (e.g., heavy metals and antibiotics) than forests,[36,37] we hypothesized that the abundance of ARGs in the soil food web from arable land is higher than in forest land.

To address these goals, we investigated the composition of ARGs in 495 soil animal samples collected from six regions across China. They included five abundant and functionally important animal groups (nematodes, collembolans, potworms, oribatid mites, and predatory mites) in the soil food web[28,40] and two different land uses. First, we used high-throughput quantitative PCR to characterize the relative abundance, number of detections, and community structure of ARGs in the soil food web. Second, we identified core ARGs and the co-occurrence network of ARGs for each soil animal group, providing insights into the intrinsic ARGs in the food web and potential interactions between ARGs. Third, we identified the ARGs shared among soil animal groups, to delineate which ARGs are more likely to spread through the food web. Fourth, we used a linear mixed-effects model to investigate the effects of trophic level and land use on the variability of animal ARGs in the soil food web. Finally, several statistical methods were used to decipher the driving factors shaping the dynamics of ARGs in soil food webs. To our knowledge, this study represents the largest data set of ARGs obtained from the soil food web, which may be useful in understanding the dispersal of ARGs in soil ecosystems and the general environment.

## 2. MATERIALS AND METHODS

**2.1. Sampling Sites and Sample Collection.** We selected six representative sites (Shenyang, SY; Kaifeng, KF; Ningbo, NB; Changsha, CS; Xiamen, XM; Kunming, KM) in China (range of latitude, 24.9°−41.7° N and range of longitude, 102.95°−123.72° E), covering four climatic zones. At each sampling site, soil and animal samples were collected from two neighboring ecosystems with different land uses (forest, F and arable land, A). The arable lands have been historically converted from forests. We collected five replicate soil and animal samples from the soil surface (0−6 cm) of each location. For soil animal samples, enough soil was collected in the field and brought to the laboratory for animal separation. More details on the collection of soil and animal samples can be found in a previous literature.[34]

**2.2. Isolation of Soil and Animal DNA.** This study focused on five abundant and functionally important animal groups in the soil food web, including nematodes, collembolans, potworms, oribatid mites, and predatory mites. The improved Berlese dry extraction and modified Baermann wet funnel methods were used to separate xerocoles (collembolans, oribatid mites, and predatory mites) and mesocoles (nematodes and potworms) from soil samples, respectively. To ensure the representativeness of the sample, we selected the dominant species of each soil animal group from each sampling site to extract their DNAs. Soil animal species were identified by morphological characteristics and DNA barcoding. The numbers of soil animal samples varied per collected site due to the difference of soil animal diversity at each sampling site. In total, we obtained 60 nematode samples, 210 collembolan samples, 50 potworm samples, 90 oribatid mite samples, and 85 predatory mite samples, which covered dominant and functionally important animal species in the soil food web. We used 0.5% sodium hypochlorite to sterilize soil animal surfaces. A microelectric tissue homogenizer was used to homogenize animal tissues before DNA extracting. The DNeasy Blood and Tissue Kits (QIAGEN, Dusseldorf, Germany) and FastDNA Spin Kit for Soil (MP Biomedical, California, USA) were selected for animal and soil DNA isolation, respectively, according to the manufacturer's instructions. Further details are provided in our previous study.[34]

**2.3. High-Throughput Quantitative PCR (HT-qPCR) of Antibiotic Resistance Genes (ARGs).** A spiking test with a dilution series of the template DNA from animals and soils was performed by adding standard amounts of ARGs to determine the effects of inhibitors before HT-qPCR. Our results revealed that the impact of inhibitors was negligible in the present study. The SmartChip Real-time PCR (Warfergen) was used to determine the composition of ARGs in the soil food web. This study used 321 primer sets targeting 320 ARGs and one 16S rRNA gene conferring resistance to major antibiotics classes. Information on these primers can be found in the Supporting Information. PCR conditions and system were as in our previous study,[41] and each sample was amplified three times. For each HT-qPCR, a nontemplated negative control and a positive control containing plasmid DNA were performed. The plasmid DNA carrying known amounts of ARGs was used as a quantification calibrator to monitor assay variation over time. Three positive replicates were considered to detect one resistance gene. We discarded the result with amplifications efficiency beyond the range (90%−110%). At
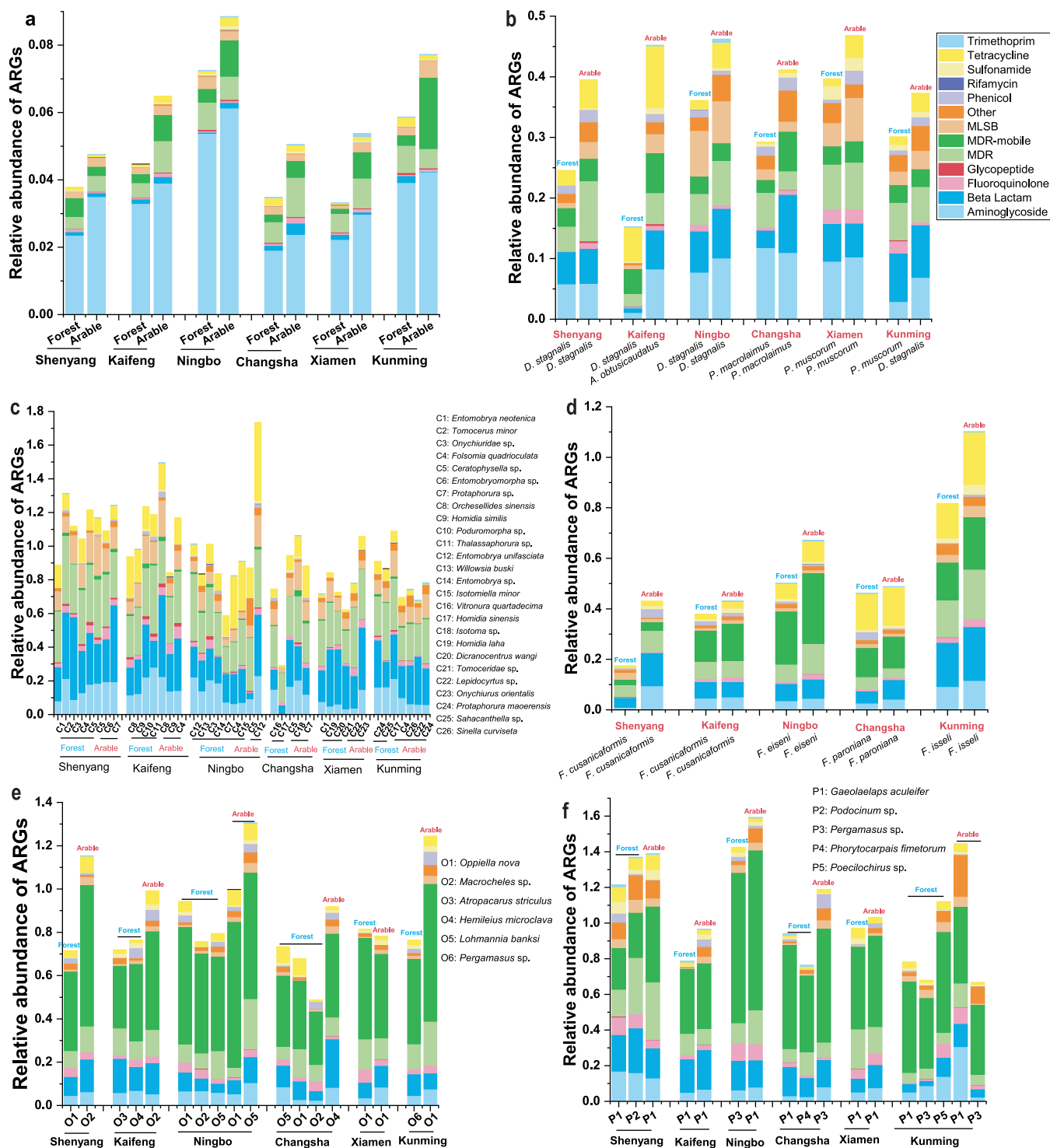
**Figure 1.** Relative abundance of ARGs in the soil food web: (a) soil; (b) nematode; (c) collembolan; (d) potworm; (e) Oribatid mite; and (f) predatory mite.

the same time, a series of diluted plasmid was also selected as positive control in our study, including a minimum concentration of one copy per well. At this minimum concentration, we found that the average Ct of ARGs was about 31. Therefore, in the present study, the detection limit of ARGs was set at a threshold cycle (Ct) of 31. In our results, none of the negative controls (Ct > 31) detected the target resistance gene. The relative abundance of ARGs (defined as

the number of copies of ARGs per 16S rRNA gene) was calculated on the basis of our previous studies.[33,41]

**2.4. Analysis of Nitrogen Stable Isotope and Bacterial Community.** In our study, the $\delta^{15}N$ value of soil animal body tissue was employed to represent the trophic level of soil animal in the food web. The $^{15}N$ isotope signature of soil animal sample was determined by using a Delta V Advantage isotope ratio mass spectrometer (Thermo Finnigan). We selected urea as an internal reference for quality control, and

the precision of measurement was <0.10‰. We amplified the V4 region of 16S rRNA gene of obtained DNA by using the 515*F*/806*R* primer set to analyze bacterial communities of samples. The Illumina MiSeq platform was employed to perform high-throughput sequencing, and the obtained bacterial sequences were analyzed via Quantitative Insights Into Microbial Ecology, following the online instruction. The operational taxonomic units (OTUs) were clustered at the 97% sequence similarity, and we used the SILVA (v138) SSU reference database to identify the taxonomy of OTUs. More details could be found in our previous study.[34]

**2.5. Analysis of Soil Physical and Chemical Properties.** A laser-scattering particle analyzer (Beckman Coulter, Inc.) was used to determine the clay content in dried soils (0.5 g). We used a pH meter to measure the pH of the soil samples at a soil−water ratio of 1:2.5. The soil total nitrogen and carbon (0.2 g) were measured using a CNS elemental analyzer (Elementar). The soil total organic carbon and nitrogen were detected using a TOC/TON analyzer (Shimadzu). An inductively coupled plasma-mass spectrometer (Thermo Fisher Scientific) was used to determine the heavy metal concentration in the soil samples, following the methods of previous studies.[42,43] To extract antibiotics from soil, 5% acetic-ACN and 0.1 M EDTA-McIlvaine buffer (1:1, v/v, pH = 4) was used. We used liquid chromatography coupled with tandem mass spectrometry (LC−MS/MS) to determine the concentration of antibiotics according to a published study.[44] Soil physical and chemical properties are summarized in Table S1 and Figure S1. There were clear differences in clay content, pH, and soil carbon and nitrogen concentrations among the sampling sites ($P < 0.05$; Table S1). Except for in Shenyang and Ningbo, heavy metal concentrations in arable land were generally higher than in forests at the other sampling sites (Table S1). In Kaifeng, Ningbo, Xiamen, and Kunming, antibiotic concentration in arable soil was significantly higher than in forest soil ($P < 0.05$; Figure S1).

**2.6. Statistical Analysis.** The relative abundance and number of ARGs in soil and animals are presented as mean values. Variance analysis was used to compare the significance of differences in abundance and number among different sampling sites or animal groups in IBM SPSS version 22. In this study, the significance level of the differences was set at $P < 0.05$. A linear mixed-effects model was used to evaluate the significance of trophic level and land use effects on the variability of ARGs in the soil food web. The model included sites as random effects and likelihood ratio test, which was conducted using the lme4 package in R.[45] The vegan 2.5−6 package of R[46] was selected to perform principal coordinates analysis (PCoA) on the basis of Bray−Curtis distance, PERMANOVA (Adonis), Procrustes analysis, and Mantel test. The PERMANOVA was used to determine the effects of sample type, host species, site, land use, and trophic level on ARG variation in the soil food web. Procrustes analysis and Mantel test were employed to determine the relationship between bacterial communities and ARG profiles in the soil food web. Bubble plots were used to present the composition of the core ARGs for each soil animal group. Core ARGs were defined as the average relative abundance of ARGs greater than 0.003. The ARGs were detected in at least 50% of samples. To visualize the correlation between environmental factors and the abundance of ARGs, we used the pheatmap package in R. Climatic data (annual precipitation and annual mean temperature) were extracted from WorldClim in R. Venn was created

online (http://bioinformatics.psb.ugent.be/webtools/Venn/). Spearman correlations among ARGs were determined using the psych package in R, and the co-occurrence network of ARGs was visualized using Gephi 0.9.2. To create bubble plots and PCoA plots, the ggplot2 package in R was selected. Histograms and box plots were drawn using OriginPro (2021). Although data on soil animal bacterial communities and $\delta^{15}N$ values have been reported in our previous study,[34] here, only data were used for correlation analysis with ARGs.

## 3. RESULTS

**3.1. Antibiotic Resistome Characterization in Soil Food Webs.** Of the 320 ARGs assayed, a total of 265 were detected in all soil animal samples with an average relative abundance of 0.85 (Figure S2). Aminoglycoside (8.5%), β-lactam (12.8%), MDR (13.4%), and MDR-mobile (22.4%) were the four dominant ARGs in the soil animal resistome (Figure S2a). The highest relative abundance of β-lactam resistance genes (0.24) was detected in the collembolan samples compared to other soil animals ($P < 0.05$). The relative abundance and number of ARGs detected varied significantly among different soil animal groups ($P < 0.05$; Figure S2). The relative abundance of ARGs in the collembolan, oribatid mite, and predatory mite samples was significantly higher than that in the nematode and potworm samples ($P < 0.05$). Compared to other soil animals ($P < 0.05$), predatory mites had the highest relative abundance of ARGs (1.08) and nematodes had the highest number of ARGs detected (34). The resistance mechanisms of ARGs detected in soil animal samples were similar to those in soil, with antibiotic deactivation (0.46) being the dominant resistance mechanism in all samples (Figure S3).

The number of ARGs detected in soil animals was significantly lower than that in soils. However, the relative abundance of ARGs was considerably higher in soil animal samples ($P < 0.05$; Figure 1 and Figure S4). The relative abundance and numbers of ARGs in soil animals were significantly different ($P < 0.05$) among different sites. There were also significant differences in the abundance and number of ARGs among different animal species at the same site ($P < 0.05$). MDR-mobile ARGs were abundantly present in all oribatid mite (0.44) and predatory mite (0.49) species at different sites (Figure 1e,f). Aminoglycoside was the dominant ARG in all soil samples at different sites (Figure 1a and Figure S4a). The PCoA of ARGs showed that animal and soil samples were separated and clustered by sample type (Figure S5a). Furthermore, PERMANOVA showed that, in the soil animal resistome, sample type and animal species could explain 64.1% and 78.1% of variations, respectively ($P < 0.05$; Table S2). Along the PCo1 axis (explaining 42.7% of variations), the ARG distribution of collembolan samples was distinctly different from the other soil animal samples. We further found that each sampling site presented similar trends (Figure S6). Animal samples were also clustered on the basis of sampling sites in each soil animal group (Figure S5). The sampling site significantly affected variations in resistome of each soil animal group ($P < 0.05$; Figure S7), explaining 53.4% for nematodes, 23.3% for collembolans, 60.8% for potworms, 25.1% for oribatid mites, and 48.2% for predatory mites. The effect of sampling sites on nematodes and potworms was greater than that on collembolans, oribatid mites, and predatory mites. At each sampling site, the distance in ARG distribution between
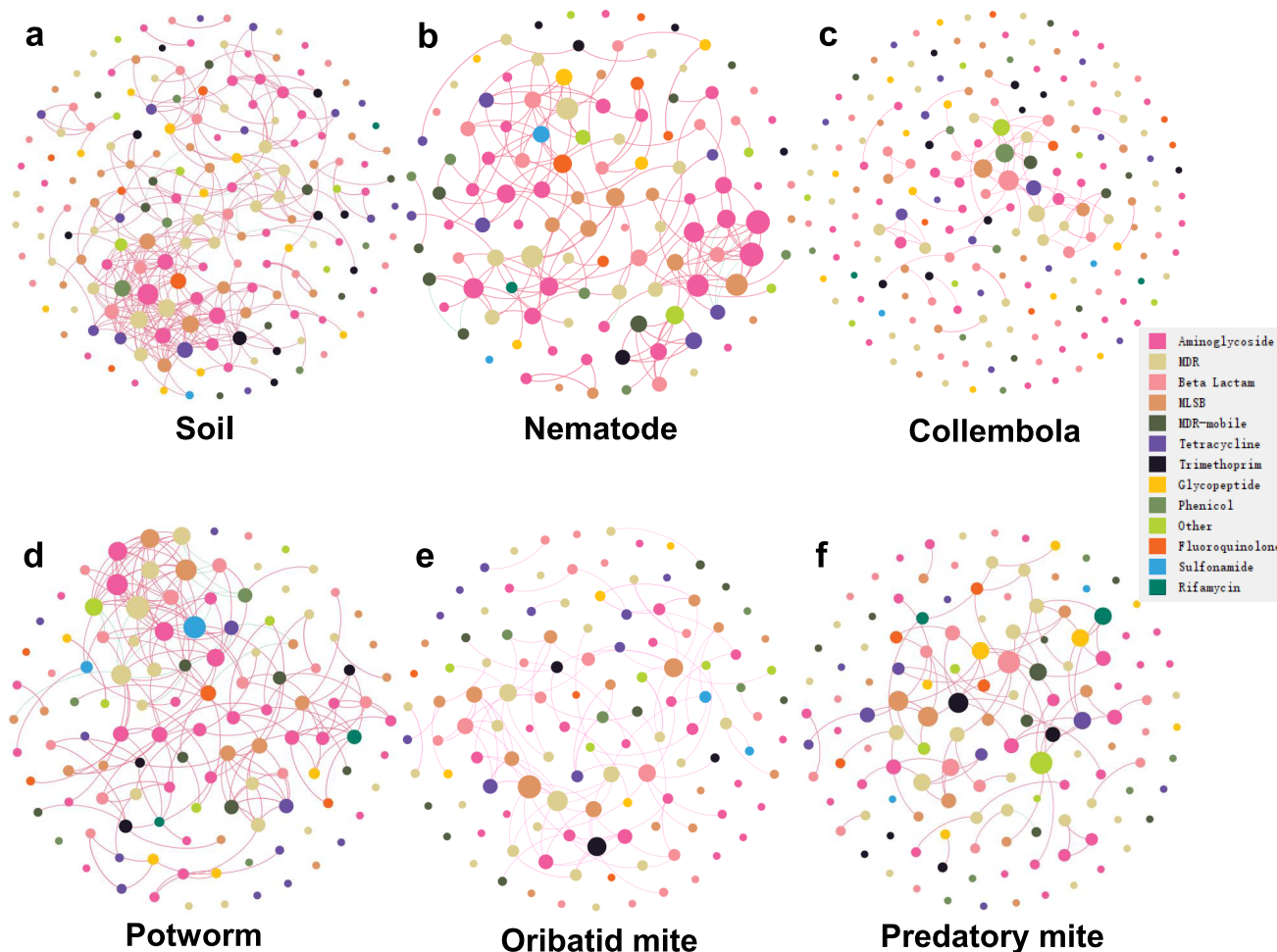
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**Figure 2.** Relationships between ARGs in the soil food web. Co-occurrence networks of (a) soil and (b−f) different animals ARGs. The size of the circle indicated the importance of the antibiotic resistance gene (the more edges that are significantly correlated, the bigger the circle size) in networks.
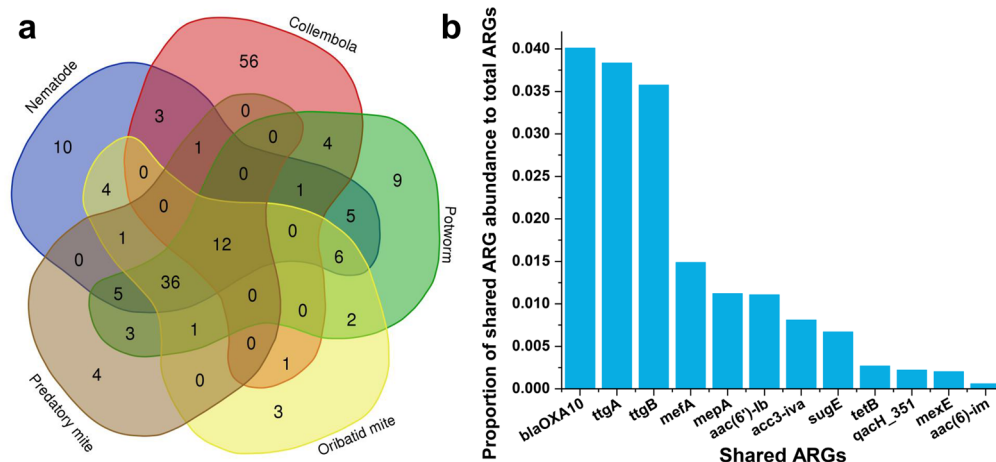


**Figure 3.** Shared ARGs in the soil food web. (a) Venn revealing the number of shared ARGs between different soil animals. (b) Proportion of abundance of ARG shared by all soil animals to total ARGs.

oribatid mite and predatory mite samples was obviously less than that between other soil animal groups (Figure S6).

**3.2. Core ARGs and Co-Occurrence Analysis of ARGs.**
Core ARGs for each soil animal group (average relative abundance of ARGs > 0.003; ARGs were detected in at least 50% of the samples) were found to be 31 ARGs for nematode,

32 ARGs for collembolans, 20 ARGs for potworms, 22 ARGs for oribatid mites, and 22 ARGs for predatory mites (Figures S8−S12). For each soil animal group, the sampling site had a significant effect on the core resistome of the soil animal ($P < 0.05$; PERMANOVA). Abundant $czcA$ resistance genes were detected in all samples of oribatid (0.43) and predatory (0.46)

**Table 1. Effects of Trophic Level and Land Use on Variations of ARGs in the Soil Food Web[a]**

| | trophic level ($\delta$ N15 value) | | | land use (forest and arable) | | |
|---|---|---|---|---|---|---|
| | estimate | P | $R^2$ | estimate (forest) | P | $R^2$ |
| aminoglycoside | −0.0013 | 0.122 | 0.0046 | **−0.0274** | **<0.001** | **0.0313** |
| $\beta$-lactam | 0.0008 | 0.581 | 0.0006 | **−0.0452** | **<0.001** | **0.0376** |
| fluoroquinolone | **0.0009** | **0.004** | **0.0165** | −0.0038 | 0.0522 | 0.0046 |
| glycopeptide | −0.0002 | 0.060 | 0.0065 | −0.0001 | 0.792 | <0.001 |
| MDR | −0.0002 | 0.873 | <0.001 | **−0.0294** | **<0.001** | **0.0164** |
| MDR-mobile | **0.0131** | **<0.001** | **0.0364** | **−0.0547** | **<0.001** | **0.0121** |
| MLSB | −0.0007 | 0.295 | 0.0021 | **−0.0224** | **<0.001** | **0.0411** |
| other | **0.0018** | **<0.001** | **0.0294** | **−0.0246** | **<0.001** | **0.0988** |
| phenicol | 0.0002 | 0.246 | 0.0025 | **−0.0072** | **<0.001** | **0.0442** |
| rifamycin | −0.0001 | 0.052 | 0.0076 | −0.0002 | 0.339 | 0.0019 |
| sulfonamide | **0.0005** | **0.005** | **0.0161** | **−0.0026** | **0.010** | **0.0105** |
| tetracycline | −0.0002 | 0.802 | <0.001 | **−0.0127** | **0.012** | **0.0062** |
| total abundance | **0.0146** | **<0.001** | **0.0214** | **−0.2385** | **<0.001** | **0.0800** |

[a]The linear mixed effects model was employed to evaluate the significance of the effect, including site as a random effect with the likelihood ratio test.
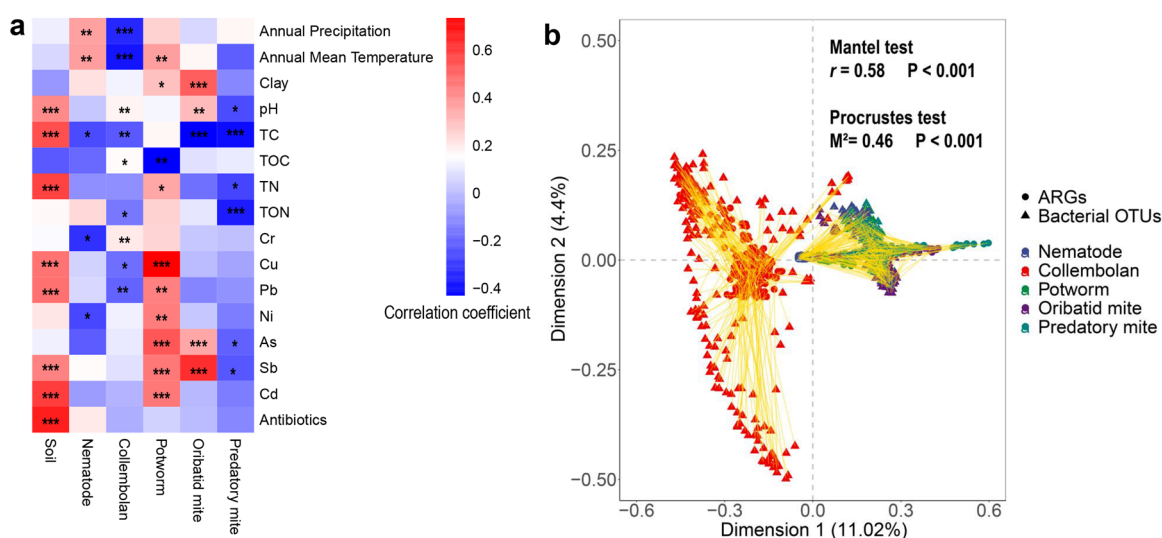


**Figure 4.** Environmental factors and bacterial variation explain antibiotic resistome variation. (a) Heatmap revealing Pearson's correlation between environmental factors and relative abundance of ARGs. "*" indicated $P < 0.05$; "**" indicated $P < 0.01$; "*" indicated $P < 0.001$. (b) Procrustes analysis depicting the correlation between bacterial OTUs and ARGs in the soil food web on the basis of Bray−Curtis distances (9999 permutations).

mites (Figures S11 and S12). The *fexA* (0.15) was the dominant resistance gene in all collembolan samples (Figure S9). Network analysis showed that the co-occurrence patterns of ARGs were different among different sample types (Figure 2 and Table S3). More edges were identified in soil samples (307) than in animal samples (69−194). Nematodes and potworms had more edges and higher average degree and density than collembolans, oribatids mites, and predatory mites. The collembolans had the highest average clustering coefficient (0.674) in the co-occurrence network compared to the other samples.

### 3.3. Shared ARGs among Different Animals in Soil Food Webs.
Twelve shared ARGs, belonging to tetracycline, MLSB, MDR-mobile, MDR, $\beta$-lactam, and aminoglycoside, were identified in all soil animal groups (Figure 3). These 12 shared ARGs accounted for 17.4% of the total abundance of ARGs detected in soil animal samples (Figure 3b). The three dominant genes among the 12 shared ARGs were *blaOXA10* (4.0%), *ttgA* (3.8%), and *ttgB* (3.6%). In addition, there were

36 shared ARGs among nematodes, potworms, oribatid mites, and predatory mites. Fifty-six unique ARGs were found in the collembolan samples, far more than nematodes (10), potworms (9), oribatid mites (3), and predatory mites (4). For the other animals, except for collembolans (30.8%), more than 65% of the ARGs were shared with soils (Figure S13).

### 3.4. Effects of Trophic Level and Land Use on ARG Variations in Soil Food Webs.
The nitrogen stable isotope technique was employed to determine the trophic status of animals in the soil food web, since $\delta^{15}N$ could be enriched in high trophic levels of animals. There was a positive and significant correlation between the total abundance of ARGs and the $\delta^{15}N$ value (trophic level) of soil animals ($P < 0.001$; Table 1). Furthermore, the linear mixed-effects model showed that the relative abundances of fluoroquinolone, MDR-mobile, sulphonamide, and other resistance genes were positively correlated with $\delta^{15}N$ values (trophic level) in soil animals ($P < 0.01$). Compared with other ARG classifications, the variation

of MDR-mobile resistance genes, explained by trophic level, was the highest, reaching 3.64%.

The relative abundance and the number of soil animal ARGs in the arable land were significantly higher than those in the forests by 16.4% and 15.8%, respectively ($P < 0.001$; Figure S14). Similar trends were also found for each soil animal group and sampling site (Figure 1 and Figure S4). In the PCoA, the distributions of sample ARGs of the same soil animal group were clustered by land uses at each sampling site (Figures S5 and S6). A linear mixed-effects model revealed that land use significantly affected the total relative abundance of soil animal ARGs, explaining 8% of the variation ($P < 0.001$; Table 1). Ten of the 13 ARGs classifications were significantly correlated with land use ($P < 0.05$).

### 3.5. Relationships between Environmental Factors and Bacterial Communities and ARG Profiles in Soil Food Webs.
There was a significant positive correlation between mean annual temperature and the total abundance of nematode or potworm ARGs ($P < 0.01$; Figure 4a). Six of the seven heavy metals were significantly correlated with the relative abundance of potworm ARGs ($P < 0.01$). Antibiotics had a significant correlation with the relative abundance of soil ARGs ($P < 0.001$), but the correlation with soil animals was not significant ($P > 0.05$). Procrustes analysis and Mantel test all showed that soil animal ARG profiles were significantly correlated with animal bacterial communities in the soil food web ($P < 0.001$; Figure 4b). Apart from predatory mites, the ARGs of soils were significantly correlated with the ARGs of other animals ($P < 0.05$, Mantel test; Table S4).

## 4. DISCUSSION

The results showed abundant and diverse ARGs in the soil food web, consistent with previous studies on individual species.[23,33] This confirms that animal resistome in the soil food web is an important component of the soil resistome. Furthermore, high levels of $\beta$-lactam resistance genes were detected in the soil collembolan microbiome. This may be due to the possibility that arthropod collembolans may secrete $\beta$-lactams.[47] In this study, the relative abundance of ARGs in xerocoles (collembolans, oribatid mites, and predatory mites) was significantly higher than in mesocoles (nematodes and potworms). There are two possible reasons. One is that xerocoles (collembolans, oribatid mites, and predatory mites) belong to arthropods, and the gut of arthropods generally contains abundant antimicrobial substances.[19,48] The other reason may be due to differences in habitats. Since most antibiotics are insoluble in water and are adsorbed by soil particles,[49] the pressure of antibiotics in soil pore water is relatively low. Mesocoles depend on pore water for their normal life.[50−52]

In this study, the composition of ARGs was significantly different among different sample types or animal species, indicating that sample types and animal species are the two major factors contributing to the variation of ARGs in the soil food web. This suggests that soil animals have a robust selectivity for ARGs. There are three reasons for this suggestion. First, since different soil animal species have different dietary traits,[28,40,53] the ARGs of animals vary with dietray differences.[54] Second, previous studies have shown that bacterial communities are commonly and significantly correlated with ARGs[3,32] and that different animal groups or species had different microbiomes.[34] Therefore, the differences in bacterial communities among animals contribute, at least in

part, to the variation of ARGs in the soil food web. This has been confirmed by Procrustes analysis and Mantel test in our study. Finally, different animals have different enrichment coefficients of pollutants[55−57] and thus different levels of pollution-induced stress. Furthermore, several ARGs (core ARGs) were abundant and prevalent in the soil animal microbiome, suggesting that soil animals may have intrinsic resistance. This may be because the soil animal microbiome provides a unique niche and could secrete some antibacterial substances. Therefore, attention should be paid to the risk of spillage of these ARGs through the soil food web. In the co-occurrence network, the network complexity of mesocoles (nematodes and potworms) was higher than that of xerocoles (collembolans, oribatid mites, and predatory mites), suggesting that the interaction between ARGs is stronger in mesocoles than in xerocoles.

Similar to our first hypothesis, the total relative abundance of ARGs in animals was significantly and positively correlated with trophic levels in the soil food web. This suggests that ARGs, as biological pollutants, like some chemical pollutants,[55−57] may be transmitted and enriched along the soil food chain. Compared to other resistance gene classifications, we also found that the trophic levels had the highest explanation rates for the variation of MDR-mobile resistance genes in the soil food web. The MDR-mobile resistance genes are generally considered to be mobile resistance genes.[58] Therefore, mobile resistance genes may be more readily transmitted through the soil food chain. Furthermore, we identified 12 resistance genes shared by all animal groups using the Venn diagram, which accounted for 17.4% of the total ARGs abundance. This suggests that abundant and diverse ARGs can be potentially transmitted in the soil food web. The authors previously used a soil model food chain to show that three ARGs from pig manure were transmitted by collembolans to predatory mites at higher trophic levels.[31] These results suggest that ARGs can spread through the food chain in soil ecosystems and that soil animals at higher trophic levels may enrich more ARGs. Given that soil animals with higher trophic levels tend to have a broader range of activities,[59] animals with higher trophic levels might be key players in causing the dispersal of ARGs in soil ecosystems.

The sampling sites significantly influenced the composition and distribution of ARGs in each soil animal group, indicating that environmental factors significantly contribute to the variation of ARGs in each soil animal group. In the examination of environmental factors, mean annual temperature showed a significant positive correlation with the relative abundance of ARGs in nematodes and potworms. This suggested that higher temperatures may increase the ARGs in the microbiome of nematodes and potworms. This may be attributed to mesocoles being more sensitive to temperature changes than xerocoles.[60,61] A similar phenomenon of increased ARG abundance with warming was also observed in a study of plastisphere ARGs.[62] In this study, we found that changes in soil antibiotic concentrations significantly affected the abundance of soil ARGs but not on animals. Meanwhile, soil ARGs were significantly correlated with animal ARGs, except for predatory mites, suggesting that antibiotics indirectly affect the soil animal resistome via modulating the changes in soil ARGs. One possible reason is that the selective pressure of antibiotics causes the production of soil microbial resistance.[63] Because of the adsorptive nature of soils,[49] the bioavailability of antibiotics to soil animals is relatively low.

Therefore, the selective pressure of antibiotics on soil animal ARGs was also weak. The ARGs acquired by soil animals from the soil environment may be facilitated mainly by ingesting resistant microorganisms. Compared with other soil animals, predatory mite has the weakest ability to ingest soil resistant microorganisms,[28] so its regression coefficient with soil ARGs was also the lowest.

The present study supports our second hypothesis by showing that the relative abundance of animal ARGs from arable land is higher than forests in the soil food web. This is mainly because arable land is more susceptible to stress from pollutants (e.g., heavy metals and antibiotics) than forests due to human activities (e.g., application of organic fertilizers and pesticides).[35−37] Our study also confirmed that the concentrations of heavy metals and antibiotics were higher in arable land than in forest land at most sampling sites. Certainly, the difference in vegetation between forest and arable lands is also an important factor. This is because changes in vegetation can affect the food resources of soil animals,[64,65] which in turn can affect changes in their resistome. Previous studies have shown that dietary changes significantly impact animal resistome.[54] Since ARGs are horizontally transmitted to humans and zoonotic pathogens,[66−68] an increase in ARGs due to land use change may pose a higher risk. Recent studies have shown that the conversion of forests to arable land increases the presence of human and zoonotic pathogens in ecosystems.[37]

Through this large-scale field study, we have shown that abundant and diverse ARGs are harbored in the soil animal microbiome. The trophic dynamics of ARGs in soil food web demonstrates that ARGs could be spread through predator−prey interactions in soil ecosystems, and changes in land use could further affect the dispersal of ARGs in the soil food web. It is well-established that soil animals are an ideal food for some terrestrial animals (e.g., chicken and bird). Therefore, in the context of "One Health", ARGs in soil ecosystem and their potential dispersal via food web should be considered in assessing human risks associated with soil contamination of ARGs. Furthermore, our study demonstrates that trophic level of animal has a significant effect on the variation of ARGs in the soil food web. This suggests that we can mitigate the spread of ARGs in the environment by regulating key animal species in the soil food web.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c00710.

> Tables of soil basic properties, comparison of soil fauna resistome composition, ARGs co-occurrence network characteristics, and relationships between soil ARGs and animal ARGs and figures of concentration of antibiotics in soil samples, relative abundance and detected number of ARGs in different soil animal samples, predicted resistance mechanisms conferred by ARGs detected in all samples, number of ARGs detected in the soil food web, the distribution of ARG profiles in soil and animal samples, relative contribution of land use, sampling site, trophic level (N15), and soil faunal species to the variation of each soil animal group resistome, core ARGs of soil animal, shared ARGs between soil and soil animal, and ARGs abundance of soil food web in different land uses (PDF)

> Primer information on ARGs as mentioned in the text (XLSX)

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### Author Contributions

Conceived and designed the experiments: D.Z. and Y.-G.Z. Performed the experiments: D.Z. and J.D. Analyzed the data and prepared the figures: D.Z., J.D., and Y.-F.W. Wrote the paper: D.Z. and Y.-G.Z. All authors read and approved the manuscript.

### Notes

The authors declare no competing financial interest.

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