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SHORT COMMUNICATION

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Habitat determines the relationships among bacteria, resistance genes and mobile genetic elements in the soil–plant system

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Abstract

The soil antibiotic resistome is considered to be primarily determined by bacterial community composition. However, the antibiotic resistance of plant microbiota and its association with the soil microbiome in soil–plant systems remain largely unknown. Here, we studied the connections between bacteria and resistance genes (RGs) (mainly antibiotic resistance genes, ARGs) and mobile genetic elements (MGEs) in different cropping systems (rice monoculture, and ryegrass–rice and vetch–rice rotation), growth periods (early, tillering and harvesting stages) and habitats (the soil, rhizoplane and phyllosphere) through high-throughput qPCR and 16S rRNA sequencing. The results showed that habitat was the major factor affecting the distribution of bacteria, RGs and MGEs, whereas the cropping system had less of an effect. The relative abundances of ARGs, multidrug resistance genes, metal resistance genes and integrons were highest in the soil and lowest in the phyllosphere, as was the α-diversity of the soil and plant microbiota. Most importantly, we found that bacteria had the strongest associations with RGs and MGEs in the rhizoplane rather than in the soil and phyllosphere, which might be due to the high network interactions among rhizoplane bacteria. These results suggest that the rhizoplane could be a hotspot for exchange of ARGs in the soil–plant system.

Highlights

- The distributions of bacteria, RGs and MGEs were primarily controlled by habitat.
- The strongest associations were found between rhizoplane bacteria and RGs and MGEs.
- Rhizoplane bacteria had the strongest network associations.

KEYWORDS

antibiotic, resistance genes, habitats, mobile genetic elements, plant microbiota, resistance genes, soil–plant system

1 | INTRODUCTION

An increasing number of studies have shown that the acquisition and dissemination of antibiotic resistance genes (ARGs) in microorganisms, especially pathogenic microbes, can be essential pathways that threaten human health, such as via plant microbiota interfaces (Chen, Cui, Su, Penuelas, & Zhu, 2019; Forsberg et al., 2012; York, 2017; Zhan et al., 2018). In soil–plant systems, the intensive application of organic fertilizers, such as animal manures, exacerbates this threat (Cheng, Chen, Su, & Yan, 2013; Fahrenfeld et al., 2014). Organic fertilizers are rich in nutrients and organic matter and are applied to enhance soil fertility and crop growth; however, they also contain high levels of antibiotics, antibiotic-resistant bacteria (ARB), ARGs and mobile genetic elements (MGEs), such as transposons, integrons and plasmids (Chen et al., 2017; Cheng et al., 2013; Joy et al., 2013). These MGEs can facilitate the horizontal gene transfer (HGT) of ARGs from manure-borne ARB to soil bacteria (Chen et al., 2019; van Elsas, Turner, & Bailey, 2003). Therefore, due to the selective effect of antibiotics in manure and the spread of ARB and ARGs, manure-fertilized soils are considered to be reservoirs of antibiotic resistance.

Several studies have reported that a higher abundance of ARGs is found in the plant microbiota (e.g., lettuce, tomato and carrot) in manure-fertilized soil (Marti et al., 2013; Rahube et al., 2014; Zhu et al., 2017). Furthermore, by analysing the distribution of ARGs and MGEs in struvite, soil, the rhizosphere and the phyllosphere, Chen et al. (2017) indicated that bacteria could act as vectors for ARG transfer from struvite to the rhizosphere and even to the phyllosphere. This result suggests that the spread of some ARB may be an important way to increase the content of ARGs (Xie, Shen, & Zhao, 2018). However, despite studies showing that bacterial community composition determines soil ARG content (Forsberg et al., 2014; Hu et al., 2018), its relation to plant microbiota resistance in soil–plant systems remains unclear, especially among different cropping systems.

In this study, based on high-throughput qPCR and bacterial 16S rRNA sequencing, we detected 58 resistance genes (RGs) and 21 MGEs in various cropping systems (rice monoculture, and ryegrass–rice and vetch–rice rotation), growth periods (early, tillering and harvesting stages) and habitats (soil, the rhizoplane and the phyllosphere). High availability of nutrients and carbon sources around the roots of plants is beneficial to the growth and reproduction of bacteria (Eisenhauer et al., 2017; Lindow & Brandl, 2003), which is expected to increase HGT. Rhizoplane bacteria would have stronger interactions than soil and phyllosphere bacteria. Therefore, we hypothesize that the linkage between bacterial community composition and the microbial resistome is the strongest in the rhizoplane, which would lead to the rhizoplane being a hotspot for ARG exchange in soil–plant systems.

2 | MATERIALS AND METHODS

Here is a brief description of the materials and methods. The detailed process can be found in the Supporting Information.

2.1 | Experimental design, sampling and DNA extraction

Pig manure samples (before composting) were collected from a pig farm in Changzhou, Jiangsu, China (31.44°N, 119.45°E). This pig farm has a modern piggery and an area of approximately 60,000 m^2, and it has been operating for more than 20 years. Antibiotics are commonly used as a routine additive to pig feed (forbidden on 1 July 2020) to promote animal growth and reduce disease. Soil samples without antibiotics were collected within 15 cm of the surface soil from an agricultural field that has long been used for growing rice. The soil is classified as Ge-Eutric Gleysols based on the World Reference Base (WRB) (IUSS, 2015). Total carbon, total nitrogen, ammonium nitrogen, nitrate nitrogen, carbonates and pH in the soil were: 10.17 g kg^{-1}, 0.95 g kg^{-1}, 64.12 mg kg^{-1}, 10.87 mg kg^{-1}, 55 g kg^{-1} and 7.65, respectively. The soil texture is clay 9.9%, silt 62.2% and sand 27.9%. The soil and pig manure were thoroughly mixed after pretreatment and then filled into 27 plastic pots (20 × 30 cm, diameter × height), each containing 4 kg of the mixture (see Supporting Information for details). The mixed mass ratio of soil to pig manure (13.81 g N kg^{-1} pig $manure^{-1}$) was 81:1 based on the standard N fertilizer application (375 kg ha^{-1}) ratio of rice in China. Because organic fertilization is commonly used to improve soil fertility and organic matter content, especially for the acidic and infertile soils in southern China, and it can rapidly increase agricultural soil fertility, our experimental design was based on the treatment of adding pig manure as organic fertilizer. From April to November 2017, a pot experiment was set up with three different cropping systems in pig manure-applied soils to see how resistance was transmitted in the soil-cropping system in the process of improving soil fertility.

The systems were as follows: rice monoculture, ryegrass (*Lolium perenne*, 130–160 seedlings planted per plot)–rice rotation and vetch (*Vicia sativa*, 40–60 seedlings planted per plot)–rice rotation. All 27 pots (with

and without plants) were placed in a glassed-in greenhouse. During the experiment, there was plenty of light and no wind, the average light duration was 9 h and the average temperature was 26.5°C. The water used in this study was pure water (the residual chlorine had been removed by 72 h of sunlight exposure). Microbial samples were collected from the soil, rhizoplane and phyllosphere using destructive sampling after 1.5 months of green manure seedling growth (see Supporting Information). There were no samples from the rhizoplane or phyllosphere in the rice monoculture system during the green manure planting period. Therefore, only 21 samples were collected in the early stages. Of these 21 samples, three were collected from soils without green manure applied and nine were collected from the soil, rhizoplane and phyllosphere with ryegrass treatments. Similarly, another nine samples were collected from the vetch treatment. Green manure was ploughed into the soil immediately after sampling, and rice was planted (four rice plants per pot) in soils without green manure, with ryegrass, and with vetch treatments 1 month after green manure decomposition. Subsequently, microbial samples of soil, the rhizoplane and the phyllosphere were collected at the tillering and harvesting stages of rice. In this study, in three soil–plant systems (rice monoculture, and ryegrass–rice and vetch–rice rotation), we obtained a total of 75 samples (21 early samples + 27 tillering samples + 27 harvest samples). All samples were stored at −20°C for DNA extraction. Additionally, we measured 10 typical antibiotics based on previous antibiotic detection methods (Liang et al., 2017) in collected soils, pig manure and pig manure-applied soils. Specifically, detailed assay procedures and concentrations for antibiotic residues can be found in the Supporting Materials and Methods.

2.2 | PCR amplification and 16S RNA sequencing analysis

For bacterial community composition, the 16S rRNA gene was amplified in triplicate PCRs using the 515F and 907R primers (Tamaki et al., 2011). Detailed amplification procedures can be found in the Supporting Materials and Methods. After the raw reads were quality filtered and merged, the remaining unique reads were chimera checked compared with the gold.fa database (http://drive5.com/uchime/gold.fa), and clustered into operational taxonomic units (OTUs) by QIIME2 with a 97% similarity cutoff. The taxonomic assignment of OTUs was performed by the Ribosomal Database Project classifier with a minimal 70% confidence score (Wang, Garrity, Tiedje, & Cole, 2007). Taxonomic assignment was performed using the SILVA database (Quast et al., 2012).

2.3 | High-throughput fluorescence quantitative PCR (HT-qPCR) of RGs and MGEs

HT-qPCR analysis was performed on an Applied Biosystems ViiA™ 7 Real-Time PCR System (Wcgene Biotechnology, Shanghai). Specific PCR amplification processes can be found in the Supporting Information. Eighty validated primer sets targeted four major classes of ARGs (27 tetracycline RGs, four sulphonamide RGs, three quinolone RGs and five macrolide RGs), eight metal resistance genes (MRGs), 11 multidrug resistance (MDR) genes, three major classes of MGEs (three integrase genes, six plasmids and 12 transposase genes) and a 16S rRNA gene (Table S2) (Wang et al., 2016; Zhu et al., 2013). In the following sections, RGs were used to represent ARGs, MRGs and MDR genes. According to Wang et al. (Wang, Qiao, Chen, Su, & Zhu, 2015), we used a threshold period (Ct) of 31 as the detection limit. The abundances of RGs and MGEs were calculated using the ΔCt method (Equation 1, 2) (Karkman et al., 2016):

$$\Delta Ct = Ct_{(RGs/MGEs)} - Ct_{(16S\ rRNA)} \quad (1)$$

$$F = 2^{-\Delta Ct} \quad (2)$$

where $Ct_{(RGs/MGEs)}$ and $Ct_{(16S\ rRNA)}$ represent the threshold cycles of the RG/MGE and 16S rRNA genes, respectively, and F is the relative abundance of the final calculated RGs or MGEs.

2.4 | Statistical analysis

Network inference (CoNet) was used to construct a network of interactions between bacteria and between bacteria and RGs and MGEs in Cytoscape (version 3.8.0). Detailed information can be found in the Supporting Materials and Methods. The Shannon-Weiner index and richness index were calculated to estimate the bacterial α diversity by using the vegan package (Dixon, 2003) in R (version 4.0.2) (https://www.r-project.org/). Significant differences in the relative abundances of RGs and MGEs, bacterial α diversity, and the relative abundances of different bacteria among the soil, rhizoplane and phyllosphere were evaluated by ANOVA with post hoc tests of Tukey's HSD in

R. Mantel path analysis (Hartmana, Richardsona, Vilgalysb, & Brulandc, 2008) was employed to assess the association of intra- and inter-habitat bacteria with RGs and MGEs.

3 | RESULTS AND DISCUSSION

3.1 | Distribution of RGs, MGEs and bacterial communities in soil–plant systems

Principal component analysis (PCA) and three nonparametric multivariate analyses were employed to determine the effects of cropping systems, habitats and growth periods on RGs, MGEs and bacterial communities. Our results showed that regardless of RGs, MGEs or bacteria, their distributions were mainly affected by habitat and then by growth period, whereas different cropping systems had no significant influence on them (Figure S2, Table S3). This result may be because both the RGs and the MGEs were predominantly controlled by their host bacteria (Forsberg et al., 2014; Hu et al., 2018). Compared with growth periods and short-term planting of green manure (Table S4), there were significant differences among habitats, including resources (e.g., moisture content and nutrient availability) and physicochemical properties (e.g., pH) (van Elsas et al., 2003; Vandenkoornhuyse, Quaiser, Duhamel, Van Amandine, et al., 2015). Hence, bacteria were mainly affected by habitats because resources and physicochemical properties are the main abiotic factors affecting bacterial community composition (Leff et al., 2015; Liu et al., 2020). Based on these results, we focused on the differences in RGs, MGEs and bacteria and their relationships in separate plant compartments.

We found that RGs (such as ARGs, MRGs and MDRs) and MGEs (such as integrons) had the highest relative abundance in the soil, followed by that in the rhizoplane, and the lowest abundance was in the phyllosphere except for plasmids and transposons (Figure 1a and b). Moreover, bacterial community diversity showed a similar pattern, with the highest α-diversity in the soil and the

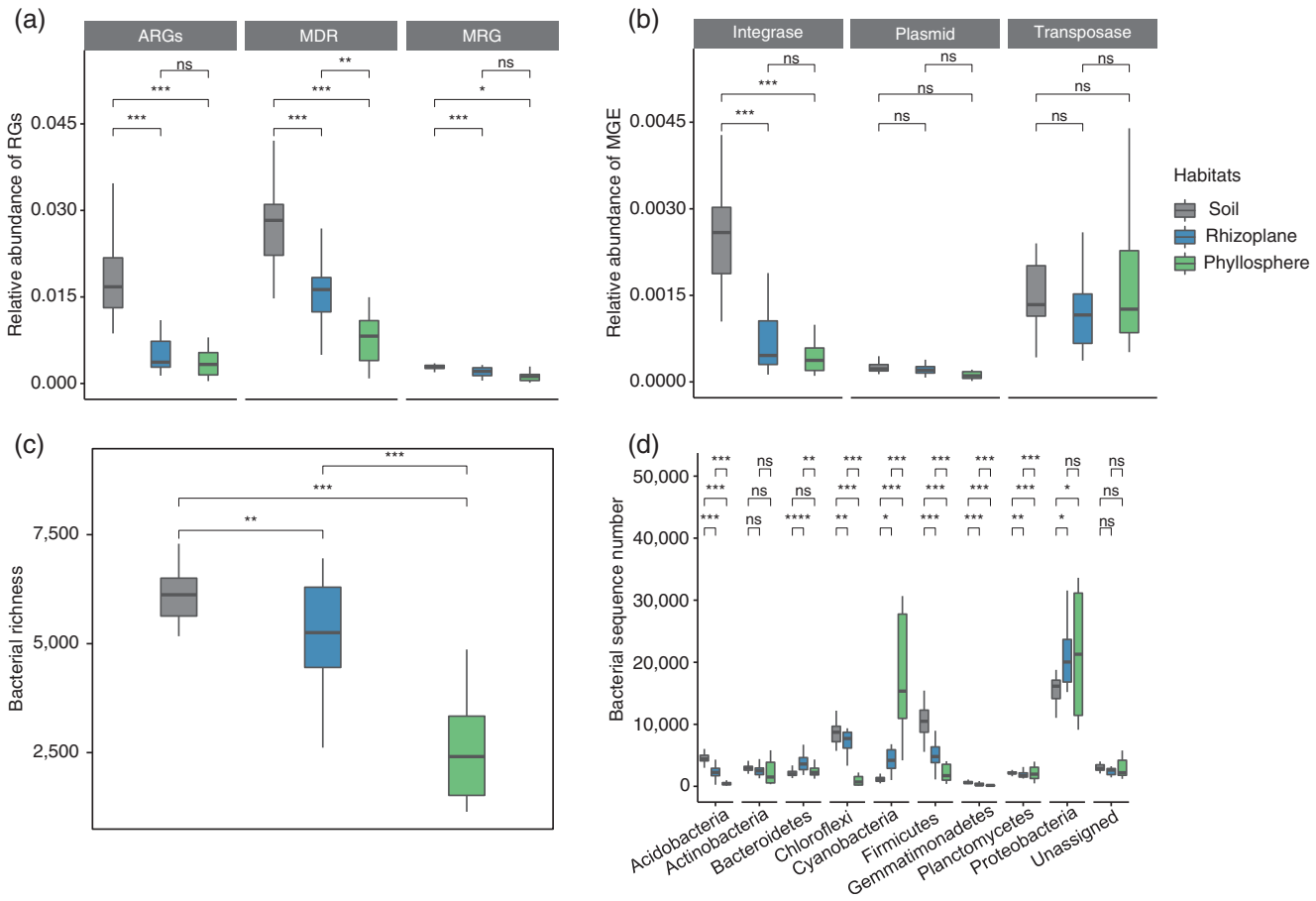

FIGURE 1 Effects of habitats on resistance genes (RGs), mobile genetic elements (MGEs) and bacteria. Relative abundance of different RGs (a) and MGEs (b) in the soil, rhizoplane and phyllosphere. (c) Bacterial richness in the soil, rhizoplane and phyllosphere. (d) Relative abundance of dominant bacteria in different habitats. Significance test: "Ns" $p > 0.05$, "*" $p < 0.05$, "**" $p < 0.01$, "***" $p < 0.001$

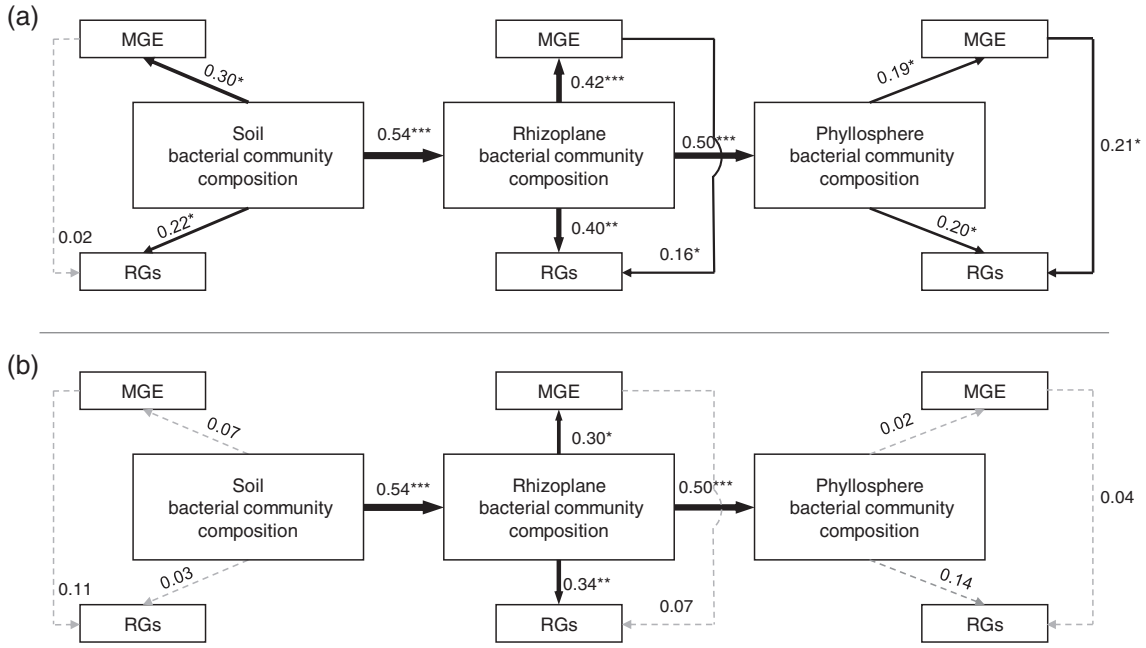

FIGURE 2 Relationships between bacteria and shared (a) resistance genes (RGs) and mobile genetic elements (MGEs), and unique (b) RGs and MGEs in different habitats. Solid black lines represent a significant correlation ($p < 0.05$), whereas dashed grey lines represent an insignificant correlation. The width of the line reflects the strength of the correlation

lowest α-diversity in the phyllosphere (Figure 1c and Figure S3). It can be expected that soil has the highest resource heterogeneity and that the phyllosphere has relatively homogeneous resources and high light exposure (Lindow & Brandl, 2003; van Elsas et al., 2003). However, for different bacterial taxa, their relative abundance was not always consistent with the pattern (Figure 1d). For example, we found that the relative abundances of Proteobacteria and Cyanobacteria were the highest in the phyllosphere and the lowest in the soil. Consistent with previous studies (Rastogi et al., 2012), our results showed that Proteobacteria were the dominant bacteria among the phyllospheric bacteria. However, inconsistent with some studies on the plant phyllospheric microbiome (e.g., *Arabidopsis thaliana*, *Oryza sativa* and *Lactuca sativa*) (Delmotte et al., 2009; Knief et al., 2012; Rastogi et al., 2012), we found that Cyanobacteria also accounted for a large proportion of the rice phyllospheric bacterial community. Some studies have shown that many Cyanobacteria members have strong multihabitat colonization abilities, including colonization in the rhizoplane and phyllosphere (Furnkranz et al., 2008; Nilsson, Bhattacharya, & Bergman, 2002). This result means that some bacteria with multihabitat adaptability (e.g., Cyanobacteria) can be actively and/or passively transformed from the soil to the root surface and even to plant leaves. This process may increase the associations of bacterial communities in soil–plant systems, thus affecting the relationship between antibiotic-resistant groups and RGs and MGEs.

3.2 | Relationships among bacteria, RGs and MGEs in soil–plant systems

It is generally believed that RGs can be transferred horizontally between different bacterial cells through MGEs (such as integrons, plasmids and transposons) (Chen et al., 2019; van Elsas et al., 2003). Therefore, the abundance of RGs may be positively correlated with that of MGEs. In this study, based on linear fitting analysis, our results confirmed that there was a significant positive correlation between RGs and MGEs ($p < 0.001$, Figure S4a). However, we found that only 18% (R^2 in Figure S4a) of variations in RGs could be explained by MGE changes. This result may be because the correlation between RGs and MGEs can be influenced by the type of RGs and the type of MGEs. For example, the results showed that ARGs had a higher correlation with integrase genes in the same habitat than that of other types of RGs and MGEs (Figure S4b). In addition to the HGT pathway, the growth and propagation of some ARB carrying single or multidrug-resistant genes may be an essential way to increase the abundance of RGs (Xie et al., 2018), thus weakening the correlation between RGs and MGEs within a given habitat because these ARB may be able to spread among different plant compartments (Chen et al., 2019). Additionally, we found that the correlation strength between RGs and MGEs was affected not only by gene types but also by habitats (Figure S4b). Specifically, the strongest correlation between RGs and MGEs occurred in the rhizoplane,

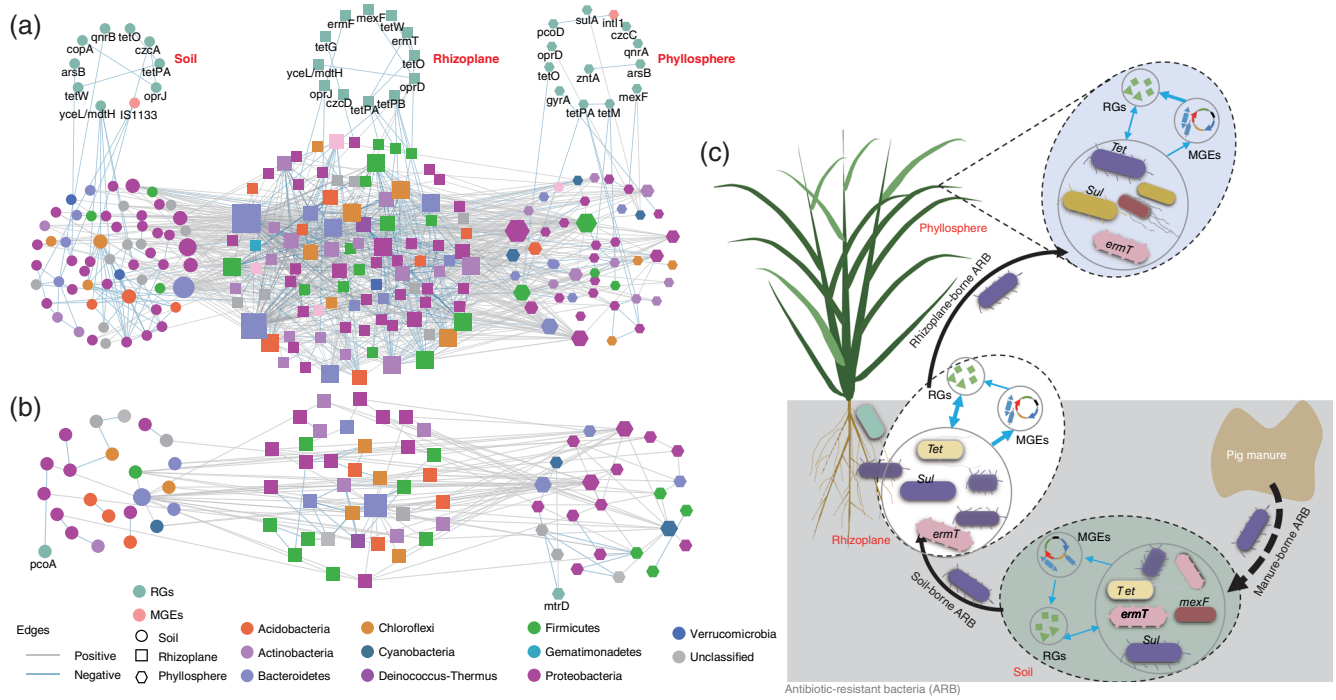

FIGURE 3 Network interactions between bacteria and shared (a) resistance genes (RGs) and shared mobile genetic elements (MGEs) and unique (b) RGs and unique MGEs. The node size represents the number of degrees that the node contains. The azure and grey lines represent the positive and negative correlations among nodes, respectively. (c) Conceptual diagram of the connections among bacteria, RGs and MGEs in different habitats. The width of the line reflects the strength of the connection. With the application of organic fertilizers, antibiotic-resistant bacteria (ARB) from the manure could spread into the soil and influence the community composition of soil bacteria and the relationship among bacteria, RGs and MGEs. ARB were expected to strengthen the connections between rhizoplane bacteria and RGs and MGEs during plant growth. This process is related to water flow, plant recruitment, higher bacterial community biomass and bacterial interactions. As the plant grows, ARBs would passively or actively colonize in the phyllosphere and influence the community composition of phyllosphere bacteria, increasing the resistome

followed by that in the phyllosphere and that in the soil. This result means that the rhizoplane may be a key site for HGT (van Elsas et al., 2003).

In this study, we found that almost all 58 RGs (including eight MRGs) and 21 MGEs were detected simultaneously in the soil, rhizoplane and phyllosphere (Table S2). This result suggested that there may be a close correlation between RGs and MGEs in the soil, rhizoplane and phyllosphere (Chen et al., 2017). In addition, considering the possibility of an intrinsic resistome within a habitat, we classified the RGs and MGEs into "shared" and "unique" categories, respectively. Shared RGs and MGEs indicate that the same RG or MGE is significantly correlated with the same bacteria at the phylum level simultaneously in two or more habitats (the soil, rhizoplane and phyllosphere). For example, *intl1* was classified as a shared gene because it was significantly correlated with Proteobacteria in both the soil and the rhizoplane (Figure S5a). Accordingly, RGs and MGEs are defined as "unique" when a significant correlation occurred only in one habitat. For example, *tetT* was classified as a unique gene because it was significantly correlated with Actinobacteria only in the soil (-Figure S5b). We found that the number and total abundance of shared RGs were significantly higher than those of unique RGs regardless of habitat (Figure S5 and Table S5). Moreover, our results showed that although the relative abundance of shared genes was not significantly different among different habitats (Figure S6), the correlation between shared genes and bacterial community composition was stronger in the rhizoplane than in the soil and phyllosphere (Table S5). A previous study showed that ARGs could be transferred from struvite to the plant surface through bacteria as a spreading vector (Chen et al., 2017). Therefore, the high connection between shared RGs and bacteria means that sharing RGs may have a high risk of dissemination in soil–plant systems.

We further used Mantel path analysis to assess the sequential relationship among soil, rhizoplane and phyllospheric bacteria, and to determine whether they would affect the relationship between RGs and MGEs. The results showed there was a significant correlation between adjacent-habitat bacterial community

compositions (Figure 2, $p < 0.001$). Additionally, we found that changes in bacterial community composition can not only directly affect the abundance of RGs and MGEs within the native habitat but also indirectly affect their abundance by changing the community composition of the adjacent-habitat bacteria (Figure 2). Moreover, compared with the soil and phyllosphere, the correlations between genes and bacterial communities were the strongest in the rhizoplane (Figure 2 and Table S5). This result may be because plant mucilage and root exudates produced during plant growth can provide abundant organic carbon for bacteria in a short time in the rhizoplane (Dennis, Miller, & Hirsch, 2010; Eisenhauer et al., 2017). This process can increase the relative abundance of ARB and directly enhance the rhizoplane resistome (Forsberg et al., 2014). Meanwhile, high nutrient availability helps to improve microbial activity. The increase in microbial activity may contribute to HGT (van Elsas et al., 2003), which is expected to strengthen the relationship between bacteria and RGs and MGEs. Therefore, bacteria had the highest connections with RGs and MGEs in the rhizoplane compared with the phyllosphere and soil (Figure 3c).

3.3 | Changes in the interactions among bacteria, RGs and MGEs in different habitats

Previous studies have shown that the bacterial community composition may be the determinant of soil ARG content (Forsberg et al., 2014). In this study, we found that the effects of bacteria on RGs and MGEs varied from habitat to habitat (Figures 2 and 3c). In addition to the abiotic factors such as resource availability and physicochemical properties, this may also be due to the difference in interactions among microbes in different habitats (Huang et al., 2019; Lindow & Brandl, 2003). It has been shown that enhanced interspecific interactions may enhance the frequency of HGT in microorganisms by increasing bacterial conjugation (Braga, Dourado, & Araújo, 2016; Tecon, Ebrahimi, Kleyer, Erev Levi, & Or, 2018). This process will promote the connections of bacteria with RGs and MGEs. In our study, based on the analysis of the bacterial cooccurrence network, we found that there was a strong network interaction among rhizoplane microbes (Figure 3a and b). Specifically, the number of edges, clustering coefficients, network density and average network degree of the bacterial network in the rhizoplane were significantly higher than those of the soil and phyllosphere, regardless of the shared or unique RGs and MGEs (Table S6). Therefore, there was a strong relationship between the rhizoplane bacteria and RGs and MGEs (Figure 3c). In contrast, there was no significant difference in relationships between bacterial communities and RGs and MGEs in the soil and phyllosphere, along with less difference in bacterial interactions (Figure 3).

4 | CONCLUSION

Based on bacterial 16S rRNA sequencing and the high-throughput quantitative PCR of RGs and MGEs, we found that bacteria had the strongest associations with RGs and MGEs in the rhizoplane rather than in the soil and phyllosphere, although the α-diversity of bacteria and the abundances of RGs and MGEs were not the highest in the rhizoplane. Moreover, there were stronger network interactions among bacteria in the rhizoplane than in the soil and phyllosphere. These results suggest that the rhizoplane could be a hotspot for ARG exchange in the soil–plant system. The findings of our work provide evidence that the cropping-plant system is an important medium for ARG dissemination. Additionally, preventing or disturbing ARG transfer in rhizosphere soils of plants might be valuable for control of the risk of spreading ARGs from contaminated soils to crops and potentially to human beings and animals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ruilin Huang: Formal analysis; investigation; writing-original draft. **Jixian Ding:** Formal analysis; investigation. **Yuwei Guo:** Investigation. **Bo Sun:** Project administration; writing-review & editing. **Yuting Liang:** Conceptualization; project administration; writing-review & editing.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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REFERENCES

Braga, R. M., Dourado, M. N., & Araújo, W. L. (2016). Microbial interactions: Ecology in a molecular perspective. *Brazilian Journal of Microbiology*, *47*, 86–98. https://doi.org/10.1016/j.bjm.2016.10.005

Chen, Q., An, X., Zhu, Y., Su, J., Gillings, M. R., Ye, Z., … Cui, L. (2017). Application of struvite alters the antibiotic resistome in soil, rhizosphere, and phyllosphere. *Environmental Science & Technology*, *51*(14), 8149–8157. https://doi.org/10.1021/acs.est.7b01420

Chen, Q., Cui, H., Su, J., Penuelas, J., & Zhu, Y. (2019). Antibiotic resistomes in plant microbiomes. *Trends in Plant Science*, *24*(6), 530–541. https://doi.org/10.1016/j.tplants.2019.02.010

Cheng, W., Chen, H., Su, C., & Yan, S. (2013). Abundance and persistence of antibiotic resistance genes in livestock farms: A comprehensive investigation in eastern China. *Environment International*, *61*, 1–7. https://doi.org/10.1016/j.envint.2013.08.023

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., … Vorholt, J. A. (2009). Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences*, *106*(38), 16428–16433. https://doi.org/10.1073/pnas.0905240106

Dennis, P. G., Miller, A. J., & Hirsch, P. R. (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology*, *72*(3), 313–327.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M. P., … Mommer, L. (2017). Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Scientific Reports*, *7*, 44641. https://doi.org/10.1038/srep44641

Fahrenfeld, N., Knowlton, K., Krometis, L. A., Hession, W. C., Xia, K., Lipscomb, E., … Pruden, A. (2014). Effect of manure application on abundance of antibiotic resistance genes and their attenuation rates in soil: Field-scale mass balance approach. *Environmental Science & Technology*, *48*(5), 2643–2650. https://doi.org/10.1021/es404988k

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*(7502), 612–616. https://doi.org/10.1038/nature13377

Forsberg, K. J., Reyes, A., Wang, B., Selleck, E. M., Sommer, M. O., … Dantas, G. (2012). The shared antibiotic resistome of soil bacteria and human pathogens. *Science*, *337*(6098), 1107–1111. https://doi.org/10.1126/science.1220761

Furnkranz, M., Wanek, W., Richter, A., Abell, G., Rasche, F., … Sessitsch, A. (2008). Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *The ISME Journal*, *2*(5), 561–570. https://doi.org/10.1038/ismej.2008.14

Hartmana, W. H., Richardsona, C. J., Vilgalysb, R., & Brulandc, G. L. (2008). Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proceedings of the National Academy of Sciences*, *105*(46), 17842–17847. https://doi.org/10.1073/pnas.0808254105

Hu, H., Wang, J., Singh, B. K., Liu, Y., Chen, Y., Zhang, Y., … He, J. (2018). Diversity of herbaceous plants and bacterial communities regulates soil resistome across forest biomes. *Environmental Microbiology*, *20*(9), 3186–3200. https://doi.org/10.1111/1462-2920.14248

Huang, R., McGrath, S. P., Hirsch, P. R., Clark, I. M., Storkey, J., Wu, L., … Liang, Y. (2019). Plant-microbe networks in soil are weakened by century-long use of inorganic fertilizers. *Microbial Biotechnology*, *12*(6), 1464–1475. https://doi.org/10.1111/1751-7915.13487

IUSS. (2015). *World Reference Base (WRB) for soil resources*. Rome, Italy: Food and Agriculture Organization of The United Nations.

Joy, S. R., Bartelt-Hunt, S. L., Snow, D. D., Gilley, J. E., Woodbury, B. L., Parker, D. B., … Li, X. (2013). Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. *Environmental Science & Technology*, *47*(21), 12081–12088. https://doi.org/10.1021/es4026358

Karkman, A., Johnson, T. A., Lyra, C., Stedtfeld, R. D., Tamminen, M., Tiedje, J. M., … Virta, M. (2016). High-throughput quantification of antibiotic resistance genes from an urban wastewater treatment plant. *FEMS Microbiology Ecology*, *92*(3), fiw014. https://doi.org/10.1093/femsec/fiw014

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., … Vorholt, J. A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *The ISME Journal*, *6*(7), 1378–1390. https://doi.org/10.1038/ismej.2011.192

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., … Fierer, N. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences*, *112*(35), 10967–10972. https://doi.org/10.1073/pnas.1508382112

Liang, Y., Pei, M., Wang, D., Cao, S., Xiao, X., … Sun, B. (2017). Improvement of soil ecosystem multifunctionality by dissipating manure-induced antibiotics and resistance genes. *Environmental Science & Technology*, *51*(9), 4988–4998. https://doi.org/10.1021/acs.est.7b00693

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, *69*(4), 1875–1883. https://doi.org/10.1128/AEM.69.4.1875

Liu, S., Wang, H., Tian, P., Yao, X., Sun, H., Wang, Q., … Delgado-Baquerizo, M. (2020). Decoupled diversity patterns in bacteria and fungi across continental forest ecosystems. *Soil Biology and Biochemistry*, *144*, 107763.

Marti, R., Scott, A., Tien, Y., Murray, R., Sabourin, L., Zhang, Y., … Topp, E. (2013). Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. *Applied and Environmental Microbiology*, *79*(18), 5701–5709. https://doi.org/10.1128/AEM.01682-13

Nilsson, M., Bhattacharya, J., & Bergman, A. N. R. A. (2002). Colonization of roots of rice (*Oryza sativa*) by symbiotic Nostoc strains. *New Phytologist*, *156*(3), 517–525.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., … Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based

tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Rahube, T. O., Marti, R., Scott, A., Tien, Y., Murray, R., Sabourin, L., … Topp, E. (2014). Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. *Applied and Environmental Microbiology*, *80*(22), 6898–6907. https://doi.org/10.1128/AEM.02389-14

Rastogi, G., Sbodio, A., Tech, J. J., Suslow, T. V., Coaker, G. L., … Leveau, J. H. J. (2012). Leaf microbiota in an agroecosystem: Spatiotemporal variation in bacterial community composition on field-grown lettuce. *The ISME Journal*, *6*(10), 1812–1822. https://doi.org/10.1038/ismej.2012.32

Tamaki, H., Wright, C. L., Li, X., Lin, Q., Hwang, C., Wang, S., … Liu, W. T. (2011). Analysis of 16S rRNA amplicon sequencing options on the Roche/454 next-generation titanium sequencing platform. *PLoS One*, *6*(9), e25263. https://doi.org/10.1371/journal.pone.0025263

Tecon, R., Ebrahimi, A., Kleyer, H., Erev Levi, S., & Or, D. (2018). Cell-to-cell bacterial interactions promoted by drier conditions on soil surfaces. *Proceedings of the National Academy of Sciences*, *115*(39), 9791–9796. https://doi.org/10.1073/pnas.1808274115

van Elsas, J. D., Turner, S., & Bailey, M. J. (2003). Horizontal gene transfer in the phytosphere. *New Phytologist*, *157*, 525–537.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Van Amandine, L., … Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist*, *206*, 1196–1206.

Wang, F., Qiao, M., Chen, Z., Su, J., & Zhu, Y. (2015). Antibiotic resistance genes in manure-amended soil and vegetables at harvest. *Journal of Hazardous Materials*, *299*, 215–221. https://doi.org/10.1016/j.jhazmat.2015.05.028

Wang, F., Stedtfeld, R. D., Kim, O., Chai, B., Yang, L., Stedtfeld, T. M., … Sul, W. J. (2016). Influence of soil characteristics and proximity to Antarctic research stations on abundance of antibiotic resistance genes in soils. *Environmental Science & Technology*, *50*(23), 12621–12629. https://doi.org/10.1021/acs.est.6b02863

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Xie, W. Y., Shen, Q., & Zhao, F. J. (2018). Antibiotics and antibiotic resistance from animal manures to soil: A review. *European Journal of Soil Science*, *69*(1), 181–195. https://doi.org/10.1111/ejss.12494

York, A. (2017). Historical influences on antibiotic resistance. *Nature Reviews Microbiology*, *15*(10), 577. https://doi.org/10.1038/nrmicro.2017.111

Zhan, J., Liang, Y., Liu, D., Ma, X., Li, P., Liu, C., … Zhou, Z. (2018). Antibiotics may increase triazine herbicide exposure risk via disturbing gut microbiota. *Microbiome*, *6*(1), 224. https://doi.org/10.1186/s40168-018-0602-5

Zhu, Y., Johnson, T. A., Su, J., Qiao, M., Guo, G., Stedtfeld, R. D., … Tiedje, J. M. (2013). Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proceedings of the National Academy of Sciences*, *110*(9), 3435–3440. https://doi.org/10.1073/pnas.1222743110

Zhu, Y., Zhao, Y., Li, B., Huang, C., Zhang, S., Yu, S., … Su, J. (2017). Continental-scale pollution of estuaries with antibiotic resistance genes. *Nature Microbiology*, *2*(4), 16270. https://doi.org/10.1038/nmicrobiol.2016.270

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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