Uncovering the prevalence and drivers of antibiotic resistance genes in soils across different land-use types

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Abstract

The emergence and spread of antibiotic resistance genes (ARGs) in soil due to animal excreta and organic waste is a major threat to human health and ecosystems, and global efforts are required to tackle the issue. However, there is limited knowledge of the variation in ARG prevalence and diversity resulting from different land-use patterns and underlying driving factors in soils. This study aimed to comprehensively characterize the profile of ARGs and mobile genetic elements and their drivers in soil samples collected from 11 provinces across China, representing three different land-use types, using high-throughput quantitative polymerase chain reaction and 16S rRNA amplicon sequencing. Our results showed that agricultural soil had the highest abundance and diversity of ARGs, followed by tea plantation and forest land. A total of 124 unique ARGs were detected in all samples, with shared subtypes among different land-use patterns indicating a common origin or high transmission frequency. Moreover, significant differences in ARG distribution were observed among different geographical regions, with the greatest enrichment of ARGs found in southern China. Biotic and abiotic factors, including soil properties, climatic factors, and bacterial diversity, were identified as the primary drivers associated with ARG abundance, explaining 71.8% of total ARG variation. The findings of our study demonstrate that different land-use patterns are associated with variations in ARG abundance in soil, with agricultural practices posing the greatest risk to human health and ecosystems regarding ARGs. Our identification of biotic and abiotic drivers of ARG abundance provides valuable insights into strategies for mitigating the spread of these genes. This study emphasizes the need for coordinated and integrated approaches to address the global antimicrobial resistance crisis.

Keywords: antibiotic resistance genes; large-scale field survey; land-use patterns; shared ARGs; environmental factors

1 Introduction

Worldwide, the prevalence of antibiotic resistance genes (ARGs) in microbial communities is considered a public health challenge, which is associated with the increased use of antibiotics in humans and animals and has generated extensive concern (Kuppusamy et al., 2018; Martinez, 2009; Sarmah et al., 2006; Wang et al., 2022). Soil has been well-documented as a rich reservoir of ARGs, and China has witnessed the most serious symptoms of this urgent challenge (Lyu et al., 2020; Qiao et al., 2018). In recent years, a few studies focused on ARGs in cropland and pristine forest soils, revealing large-scale spatial patterns in China (Du et al., 2020; Song et al., 2021). The prevalence of ARGs with different fertilization and cropping regimes has also been previously reported (Wang et al., 2018). Clearly, further research is warranted to better understand the ARG profiles for different land-use types at a national scale.

The distribution profile of ARGs in soil ecosystems with different land-use types located in the same geographical region remains poorly characterized. The enrichment of ARGs in soils under different land-use patterns may be different owing to the degree of anthropogenic impact, which underscores the imperative for complementary studies. Forest soils contain less ARG contamination primarily due to minimal impact from anthropogenic activities (Song et al., 2021; Xiang et al., 2020). Several reports indicate that high diversity of ARGs has been documented in forest soils, emphasizing forest environments are also likely hidden reservoirs of ARGs (Hu et al., 2018; Radu et al., 2021). A regional case in the Amazon rainforest confirmed that deforestation and subsequent shifts to other land-use regimes enriched the diversity and abundance of the ARGs (Lemos et al., 2021). Further evidence suggests that ARG abundance is significantly higher in cropland than in forest land (Zhu et al., 2022). In stark contrast to forest environments that have relatively limited human impact, common agricultural practices include the introduction of livestock manure, sewage sludge, and biosolids to tea plantations and agricultural soil that are assumed to promote the enrichment of ARGs (Cerqueira et al., 2020; Heuer et al., 2011; Urra et al., 2019). Furthermore, this phenomenon may have been exacerbated by agricultural practices that replaced synthetic fertilizers with manure in recent years (Ji et al., 2020; Udikovic-Kolic et al., 2014; Zhang et al., 2020). In particular, agricultural soils that are more exposed to long-term application of manure have significantly altered patterns of antibiotic-resistant bacteria with proliferation and increased persistence of ARGs in soils (Liu et al., 2022; Peng et al., 2017). Also, as noted previously, ARG pollution can be transferred to the surrounding environment through runoff and aerosols in a long-term and persistent manner, which enhances the dissemination of ARGs among different land-use types (Bai et al., 2022; Cáliz et al., 2022; Zhang et al., 2021).

Several studies have confirmed that soil properties and bacterial community diversity are the dominant drivers in shaping ARG diversity and abundance (Wei et al., 2022; Wu et al., 2023; Yang et al., 2019). For example, soil physicochemical variables, such as soil organic carbon (SOC) and total nitrogen (TN) content, are strongly associated with the distribution and prevalence of ARGs (Guo et al., 2020; Wang et al., 2020). Clay affects the distribution of ARGs by shaping bacterial communities (Awasthi et al., 2019; Wang et al., 2020). pH can alter the hydrophobicity of antibiotics in soils by affecting electrostatic repulsion between the negatively charged soil surface and anionic forms of antibiotics and further reduce the abundance of ARGs by removing antibiotics (Conde-Cid et al., 2020; Li et al., 2020). Heavy metals as widespread and persistent contaminants in soil may trigger the co-selection of ARGs and contribute to their maintenance and propagation (Baker-Austin et al., 2006; Seiler and Berendonk, 2012). A nationwide survey of the geographic distribution of major ARGs in agricultural soils revealed a strong correlation between sulfonamide resistance genes and mean annual temperature (MAT) and mean annual precipitation (MAP) (Zhou et al., 2017). Warming had a positive effect on the amount and proportion of natural ARGs in forest soils, and climatic seasonality is associated with the dissemination of ARGs and was found to be a critical driver of their global occurrence (Delgado-Baquerizo et al., 2022; Z. Li et al., 2022). Soil properties and the bacteria associated with them act jointly to determine ARG profiles (Chen et al., 2016; Xie et al., 2018; Zhou et al., 2020). The primary driver of ARG diversity and geographic distribution in natural wetlands is the bacterial community structure (Yang et al., 2019). Furthermore, bacterial abundance and diversity are the key drivers affecting the evolution of ARGs in aerobic composting (Chen et al., 2022). However, previous large-scale investigations of the prevalence and drivers of ARGs have not considered geographic differences in regional climate factors.

Given the above, the factors affecting ARG distribution are complex, and this study was aimed to investigate the variations in prevalence of ARGs and potential driving mechanisms involved in the propagation of ARGs in three land-use regimes (forest land, tea plantation, and agricultural land) at a national scale. By combining high-throughput quantitative polymerase chain reaction (HT-qPCR)-based SmartChip systems and Illumina sequencing of bacterial 16S rRNA genes, the specific objectives of this study were as follows: (1) to compare the occurrence of the abundance and diversity of ARGs among different land-use patterns; (2) to reveal the controlling biotic and abiotic factors driving ARG profiles; and (3) to discuss practical measures to control ARG pollution under specific land-use patterns. The outcomes will provide insights into feasible strategies regarding ARG reduction in soils.

2 Materials and Methods

2.1 Sampling sites and sample collection

The representative sites were selected from 11 provinces (AH, Anhui; FJ, Fujian; GZ, Guizhou; HEN, Henan; HUN, Hunan; JS, Jiangsu; JX, Jiangxi; SC, Sichuan; SX, Shanxi; YN, Yunnan; ZJ, Zhejiang) in China (range of latitude, 25.0°-33.5°N, and range of longitude, 101.6°-120.3°E), covering multiple climate zones. Each sampling point selected had to cover three different land use patterns (forest land, tea plantation, and agricultural land) and without livestock farms and farmers raising livestock in a scattered manner nearby. At each sampling site, the upper 20 cm of soil was collected from three neighboring ecosystems. Three individual samples were combined randomly selected within the target areas into large composite samples to ensure the representativeness of samples (three samples per land-use pattern for the same sampling site and 1.5 kg of soil per sample).

According to our survey, the addition of exogenous fertilizers with minimal human interference did not affect forest land. Each tea plantation had been in operation for at least 10 years. The agricultural land had been annually cropped with traditional crops (e.g., rice, wheat, and vegetable). Tea plantations and agricultural land were managed according to conventional agricultural practices, which usually received manure application (e.g., swine manure, cattle manure, and chicken manure). Collected soil samples were transported to the laboratory within three days. A total of 33 soil samples were sieved through a 2 mm mesh and thoroughly homogenized. A portion of each soil sample was air-dried for chemical property measurements, and another portion was stored at -80°C until DNA extraction. Detailed information on the sampling sites is listed in Supporting Information (SI) Table S1.

2.2 Measurements of soil properties

Soil physicochemical properties, including soil pH, SOC, TN, NH_4^+, NO_3^-, and soil texture (clay, sand, and silt) were measured using conventional methods as described previously (Cheng et al., 2016; Wang et al., 2020; Zhu et al., 2022). Soil pH was measured by a pH probe (PHS-3C, Shanghai, China) at a soil-to-water ratio of 1:2.5 (*w*/*v*). Soil total C and N were measured with a Multi N/C 3100 analyzer

(AnalytikJena, Jena, Germany). Skalar San Plus segmented flow analyzer (Skalar Analytical, Breda, Netherlands) was used to measure the soil mineral N (NH_4^+ and NO_3^-). Heavy metal (Cu, Zn, Pb, Cd, Ni, Cr, As, and Hg) concentrations were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Fisher Scientific, USA) after acid digestion ($HClO_4$, HNO_3, and HF) according to EPA Method 3050B. The annual average temperature and precipitation were extracted from the WorldClim database (www.worldclim.org/). Detailed information on the soil physicochemical properties is presented in SI Table S1.

2.3 DNA extraction and Illumina sequencing

Soil DNA was extracted from 0.25 g of fresh soil by the MoBio PowerSoil™ DNA Isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA). The concentration and quality of DNA were measured with a Nanodrop ND-2000 spectrophotometer (Thermo Scientific, USA).

To analyze the composition of the bacterial community, the V4 hypervariable region of the 16S rRNA gene was amplified using universal primers 520F (5’-AYTGGGYDTAAAGNG-3’) and 802R (5’-TACNVGGGTATCTAATCC-3’). After the libraries passed quality control, the results of the Smear analysis were examined using a Fragment Analyzer. The mixed libraries were purified by gel cutting using the QIA quick gel recovery kit (Qiagen, Venio, The Netherlands). The sequencing was performed using an Illumina MiSeq PE300 (Illumina Inc., San Diego, CA, USA). The data were analyzed by the QIIME pipeline (version 1.9.1) (Caporaso et al., 2010; Kemp and Aller, 2004). Sequences were clustered into operational taxonomic units (OTUs) by the UCLUST clustering method to classify at a 97% similarity level (Edgar, 2013), and used the SILVA (v132) SSU reference database to define the taxonomy of OTUs. The observed species, Chao1 index, and Shannon index were used to evaluate alpha diversity for each sample (Schloss et al., 2009). The Illumina raw sequence reads were submitted to the

National Center for Biotechnology Information Sequence Read Archive database (accession number: PRJNA914204).

2.4 High-throughput quantitative PCR of ARGs

HT-qPCR was performed by the SmartChip Real-time PCR system (Wafergen Inc., CA, USA) to determine the abundance and composition of ARGs and mobile genetic elements (MGEs) in soil samples. The system is capable of processing 5184 nano-well reactions in parallel at one time. A total of 144 primer sets were selected (Table S2), targeting 137 ARGs conferring resistance to major antibiotics subtypes, 6 MGEs, and 16S rRNA gene (Su et al., 2015; Wolters et al., 2018; Zhu et al., 2013). Amplification conditions were: 95 °C for 10 min, followed by 40 cycles of 30 s at 95 °C and 30 s at 60 °C. The melting curve was automatically generated by Wafergen software. Wells with multiple melting peaks or amplification efficiencies above the acceptable range (90-110%) were dropped. To ensure reproducibility, a threshold cycle (CT) of 31 was used as the detection limit in all three technical replicates. Only samples with two or more detections in three replicates and meeting a deviation of <20% were regarded as positive. The abundance of ARGs and MGEs was normalized using 16S rRNA genes from the Wafergen platform to minimize the error caused by the different abundance of background bacteria and variation in DNA extraction and analysis efficiency. The CT measured by the WaferGen qPCR was used to calculate the copy number of ARGs via Copy Number = $10^{(31-CT)/(10/3)}$ (Chen et al., 2017; Zhu et al., 2018). The absolute abundance (copies/g) of ARGs and MGEs was calculated by multiplying the relative abundance by the absolute number of gene copies of 16S rRNA in the same sample (Fu et al., 2021).

2.5 Statistical analysis

Statistical analyses were performed in R version 4.0.3 (R Core Team, 2020). The absolute abundance and number of ARGs in soil are presented as mean values. The differences in the abundance of ARGs and MGEs and the relative abundance of the dominant bacteria in soil under different land-use patterns were determined using a one-way analysis of variance (ANOVA) followed by Tukey's test. Non-metric multidimensional scaling (NMDS) analyses based on the Bray-Curtis distance and the permutational multivariate analysis of variance (PERMANOVA, Adonis test) with 999 permutations were employed to determine the dissimilarity in soil microbial diversity based on the OTU in different samples (Legendre and Gallagher, 2001). The "randomForest" package was used for random forest analysis. Correlations between ARGs and bacterial communities were determined using the Mantel test and Procrustes analysis (Delgado-Baquerizo et al., 2018; Peres-Neto and Jackson, 2001). Spearman's correlation matrix between bacterial OTUs and ARGs was calculated using the Hmisc package in R, and the correspondence between bacteria and ARGs was visualized by network analysis in the Gephi platform (version 0.9.2) (Li et al., 2015). Redundancy analysis (RDA) and variance partitioning analysis (VPA) was applied to investigate the relationship between ARGs and the components of the bacterial community, soil physicochemical properties, and climatic factors. Heatmap diagrams were generated to visualize the abundance of ARG classification in different samples with the "ComplexHeatmap" package in R software.

3 Results

3.1 Characterization of the antibiotic resistome in soils

Resistomes were classified into eight dominant ARGs categories (i.e., aminoglycoside, beta-lactamase, chloramphenicol, macrolide-lincosamide-streptogramin B (MLSB), multidrug, sulfonamide, tetracycline, vancomycin, and others) and MGEs, with the distribution of ARGs in each soil sample shown in Fig. 1. A total of 124 unique ARGs and five MGEs were detected across all samples conferring resistance to a wide range of antibiotics. Overall, the absolute abundance of ARGs for beta-lactamase (13.5%), MLSB (16.8%), and multidrug (23.6%) accounted for the majority of constituents in soils, followed by tetracycline (10.6%) and aminoglycoside (13.2%) resistance genes (Fig. S1 and Table S3).

The resistomes varied significantly among different land-use regimes. The abundance of ARGs in agricultural soil was considerably greater than in either tea plantation or forest land ($P < 0.001$; Fig. 1a and Table S3). Specifically, the ARG abundance was significantly increased by 27.9% in soils for tea plantation compared to forest land and 66.1% in agricultural soil compared to the tea plantation, and variations were mainly reflected in aminoglycoside and sulfonamide resistance genes ($P < 0.01$-0.001; Table S3). For collection sites, the survey in 11 provinces of China for major categories of ARGs and MGEs showed that ARG pollution was most severe in southern China, such as Fujian and Yunnan, demonstrating the large discrepancy in the ARG abundance among geographical regions (Fig. 1a).

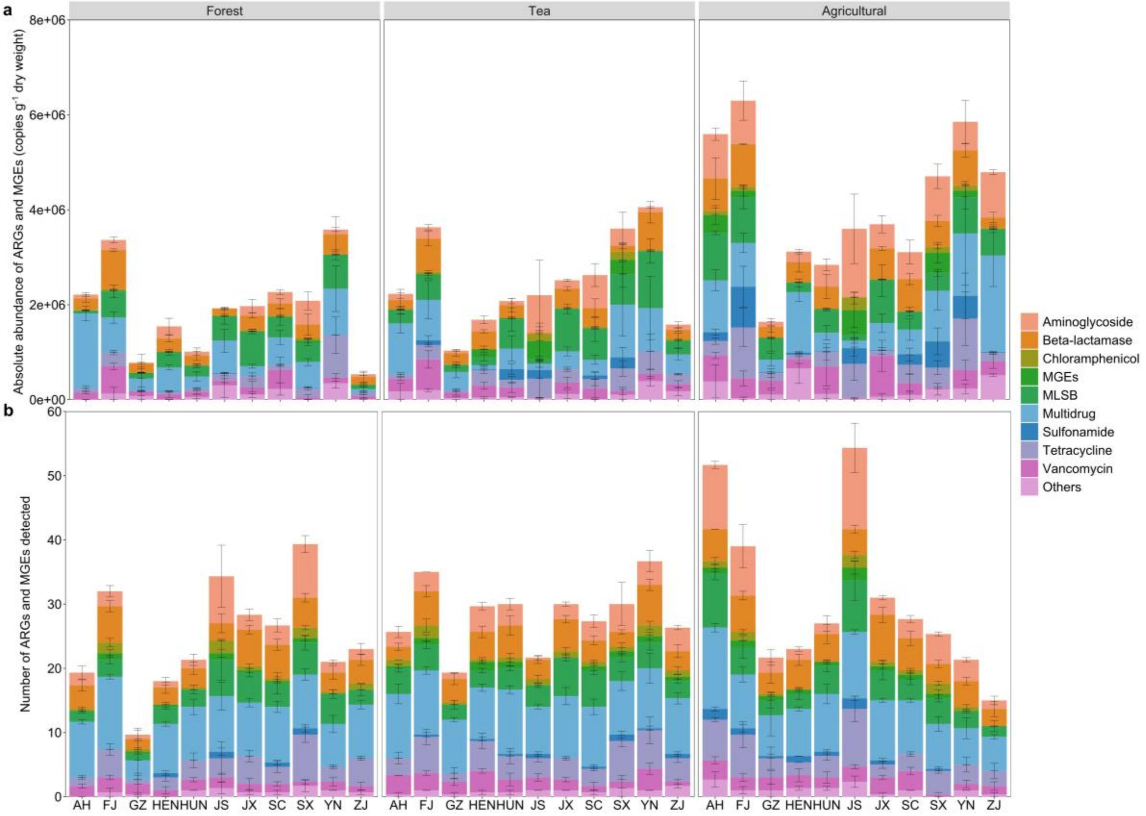

Fig. 1 Absolute abundance (a) and number (b) of ARGs and MGEs detected in soil under different

land-use patterns. AH, Anhui; FJ, Fujian; GZ, Guizhou; HEN, Henan; HUN, Hunan; JS, Jiangsu; JX, Jiangxi; SC, Sichuan; SX, Shanxi; YN, Yunnan; ZJ, Zhejiang.

As shown in Fig. 1b, the detected number of ARG subtypes and MGEs ranged from 10 to 54 in each sample. In general, the maximum ARG diversity for each region occurred mostly in agricultural soil, and the largest number of ARGs was detected in agricultural soil in Jiangsu. As expected, the diversity results of ARGs revealed by the Shannon index were generally consistent with a change in the detected number, and the Shannon index of Anhui, Henan, and Zhejiang was significantly different among the three land-use patterns (*P* < 0.05-0.001; Fig. S2). The behavior (including diversity and abundance) of MGEs was similar to that of ARGs among different land-use patterns. Furthermore, a total of 88 ARGs and MGEs were shared among soil samples in different land-use regimes, suggesting that ARG may have a high potential for transferring between soil environments (Fig. S3).

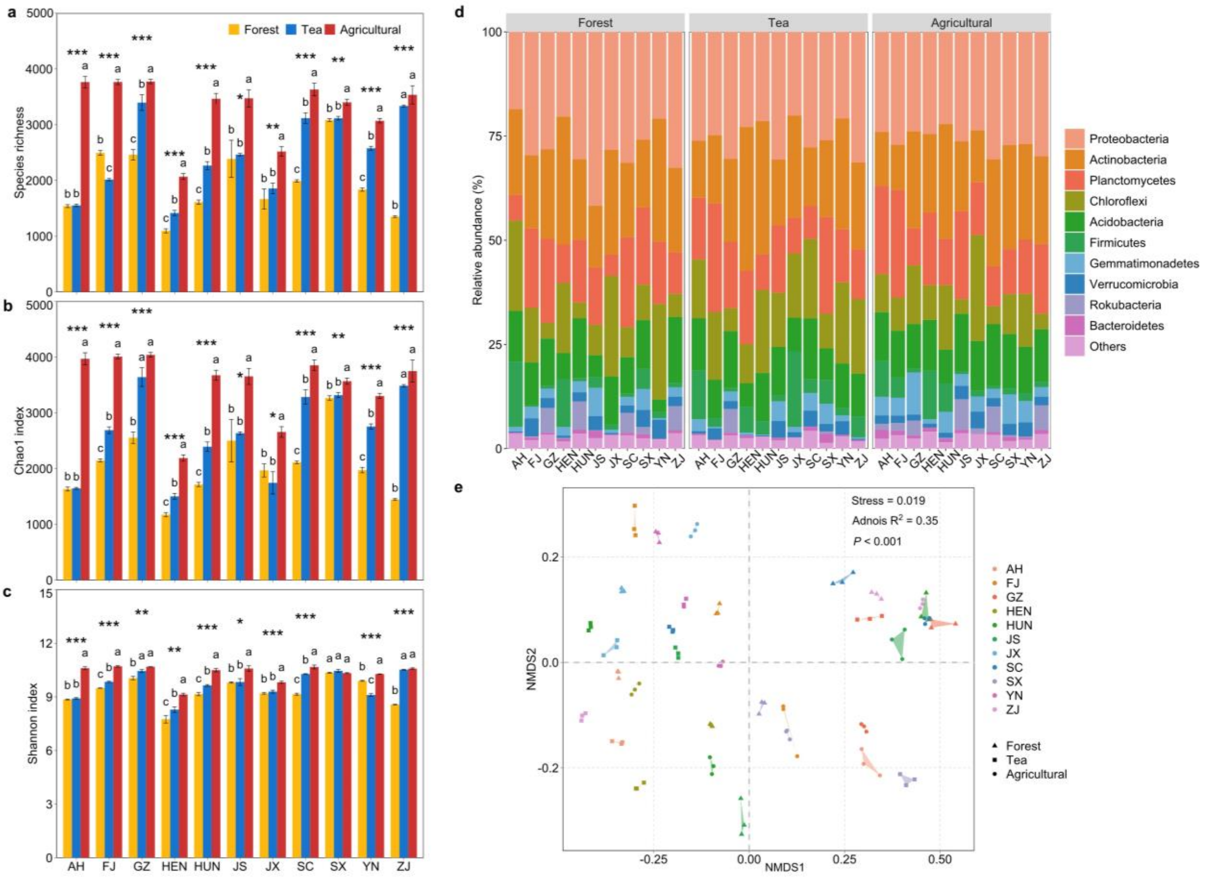

Fig. 2 The alpha diversity (Species richness, Chao1 index, and Shannon index) (a, b, and c), taxonomic profiles of bacterial communities at the phylum level (d), and non-metric multidimensional scaling (NMDS) ordination plot of the Bray-Curtis dissimilarity matrices and the Adonis PERMANOVA analyses of bacterial distribution (e) in soil under different land-use patterns. AH, Anhui; FJ, Fujian; GZ, Guizhou; HEN, Henan; HUN, Hunan; JS, Jiangsu; JX, Jiangxi; SC, Sichuan; SX, Shanxi; YN, Yunnan; ZJ, Zhejiang. Different letters refer to significant differences among different land-use patterns at the $P < 0.05$. The alpha diversity of soil bacteria with significant differences among different land-use patterns in the same region is indicated by asterisks. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

3.2 Characterization of the bacterial community in soils

After assembly and quality filtering, a total of 10,317,764 high-quality sequences were obtained from the soil by amplicon sequencing, ranging from 46,943 to 122,811 per sample, and these sequences were classified into 87,169 OTUs at the 97% similarity cutoff. Rarefaction curves revealed that the depth of bacterial sequencing had reached saturation and was adequate for subsequent community analysis (Fig. S4). The alpha diversity of bacteria, as represented by Species richness, Chao1 index, and Shannon index, showed consistent responses to land-use type, with samples from agricultural soil having significantly higher bacterial diversity, followed by tea plantation and forest land ($P < 0.05$-0.001; Fig. 2a-c).

The composition of the bacterial community exhibited distinct changes at the phylum level across land-use patterns. Proteobacteria, Actinobacteria, Planctomycetes, Chloroflexi, Acidobacteria, and Firmicutes were the most dominant phyla in the three land-use regimes, accounting for more than 90% of the whole bacterial community structure (Fig. 2d), and Proteobacteria was the most abundant bacterial phyla in all samples. Differences in the taxonomic composition of bacteria among land-use patterns occurred at the level of Planctomycetes, Acidobacteria, and Gemmatimonadetes, the proportion of which was significantly increased in the agricultural soil (*P* < 0.05; Table S4). NMDS analysis of bacterial profile based on the Bray-Curtis distance revealed a clear separation among different land-use regimes, which was also confirmed by the Adonis test. Further analysis revealed that the bacterial communities also strongly clustered according to geographical location for 11 provinces (*P* < 0.001; Fig. 2e).

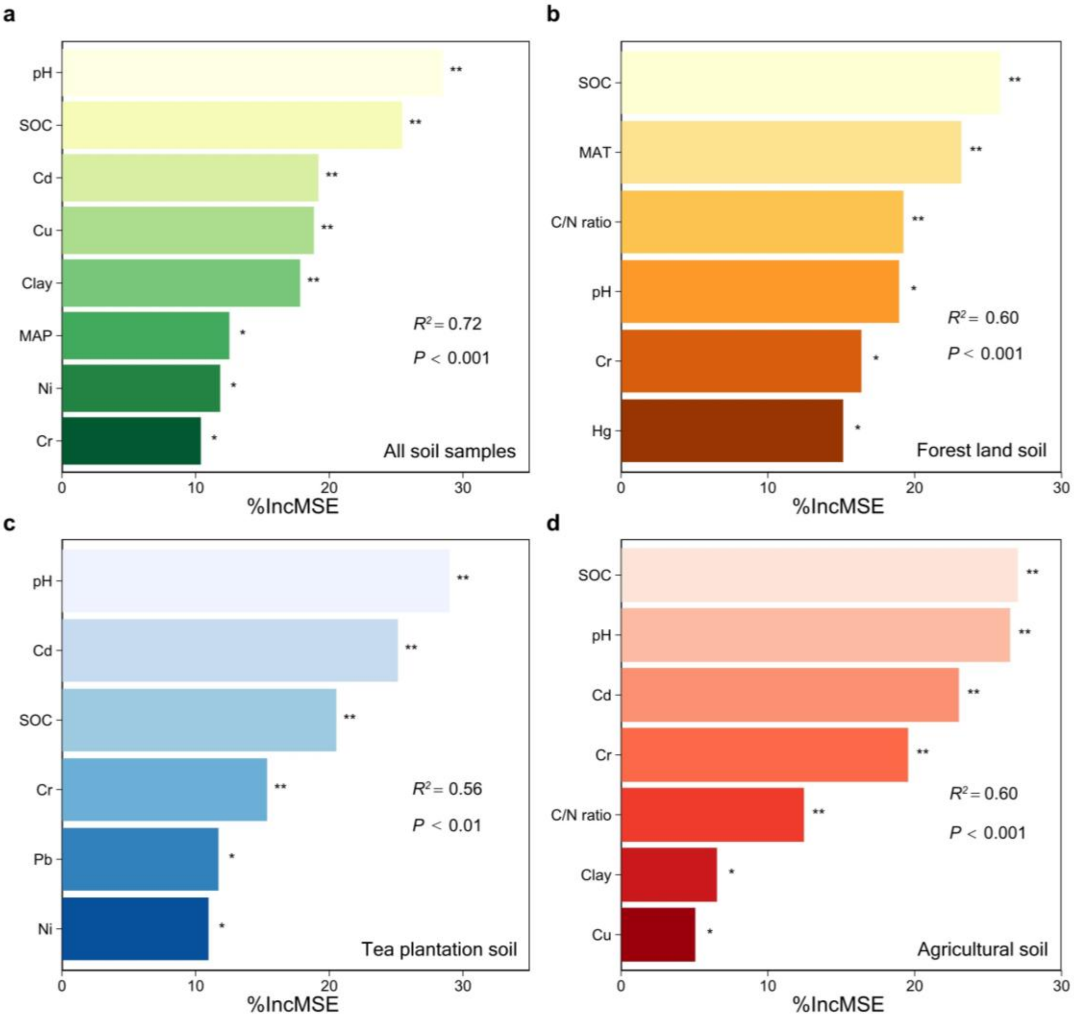

Fig. 3 Main factors affecting the distribution of ARGs characterized by random forest modeling analysis in all soil samples (a), forest land (b), tea plantation (c), and agricultural soils (d), respectively. The figure shows the random forest analysis results with primary predictor importance

(i.e., the percent increase in mean square error [%IncMSE]) of soil properties and climatic factors on ARG profiles from the soil under different land-use patterns. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

3.3 Driving forces for the distribution of ARGs in soils

The random forest model analysis used to identify the key soil properties and climatic factors that influenced ARG abundances in soils (Fig. 3). Overall, in the model of ARG profiles ($R^2 = 0.72$, $P < 0.001$), the significant importance of drivers was in the order of soil pH > SOC > Cd > Cu > clay > MAP > Ni > Cr in all samples (Fig. 3a). Specifically, in the forest land ($R^2 = 0.60$, $P < 0.001$), ARG distribution was greatly affected by SOC, MAT, and C/N ratio (Fig. 3b). Similarly, the relatively vital soil properties in the tea plantation ($R^2 = 0.56$, $P < 0.01$) were soil pH, Cd, and SOC. Comparatively, the effect of soil properties on the profile of ARGs was most significant in the agricultural system ($R^2 = 0.60$, $P < 0.001$), and the relative importance was as follows: SOC > pH > Cd > Cr > C/N ratio > clay > Cu (Fig. 3b-d).

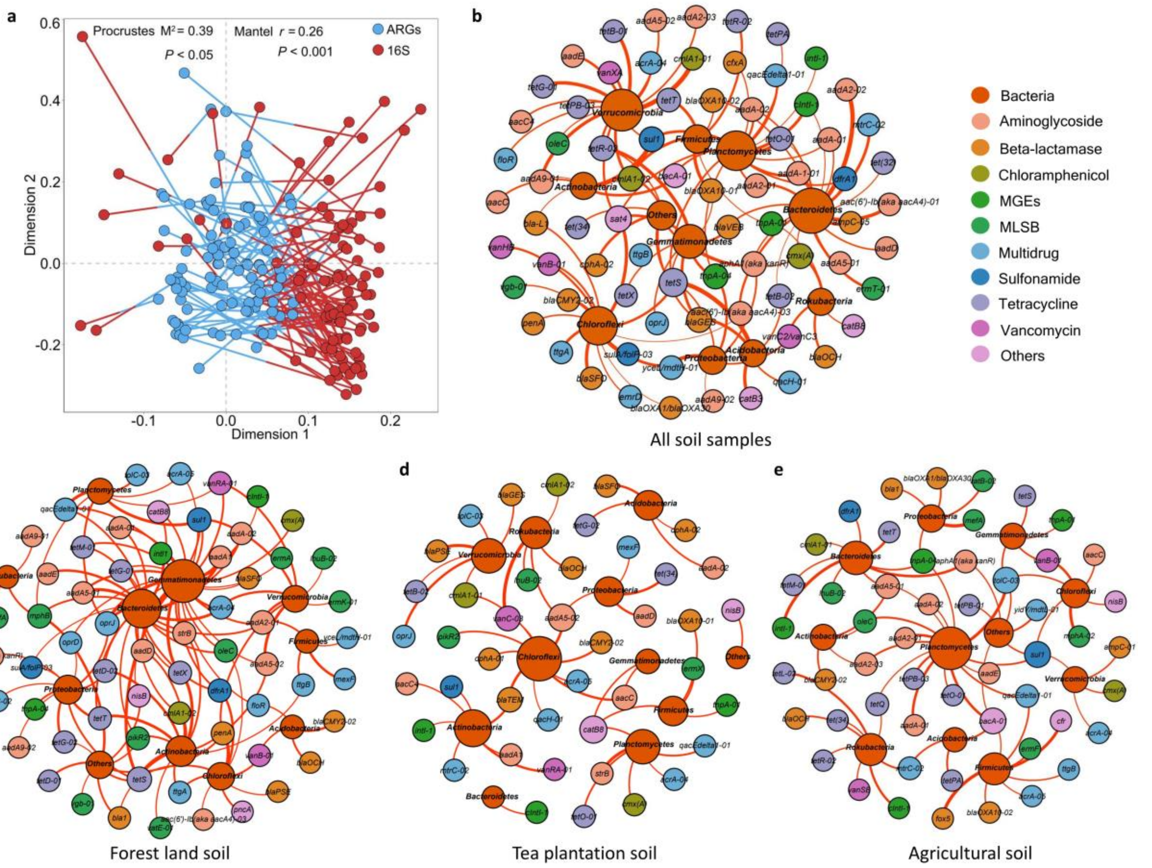

Fig. 4 Association of ARG distribution with the bacterial community (a) and network visualization of ARGs and bacterial community in all soil samples (b), forest land (c), tea plantation (d), and agricultural soils (e), respectively. Procrustes analysis and the Mantel test are used to reveal significant associations between ARG abundance and the bacterial community at the phylum level. Procrustes test between ARG profiles (Bray-Curtis) and bacterial communities using taxonomic dissimilarity metrics (Bray-Curtis), where M^2 represents the sum of the squared deviations (vector residuals) over the first two dimensions (999 permutations). The connection between ARGs and bacterial taxa represents a strong (Spearman's correlation coefficient $r > 0.7$) and significant (P-value < 0.01) correlation. The nodes are colored according to bacterial and ARG types. The node size is proportional to the number of connections, and the edge thickness is proportional to the correlation coefficient.

Using the Procrustes and Mantel tests to explore whether the ARG profiles correlated with the bacterial OTU composition using (Fig. 4a). The results of the Mantel test indicated that ARG profiles were significantly correlated with bacterial composition based on Bray-Curtis distances ($r = 0.26$, $P < 0.001$, permutations = 999). Moreover, the Procrustes analysis showed that most of the ARG data and bacterial 16S rRNA gene OTU data of soil samples exhibited goodness-of-fit based on the Bray-Curtis dissimilarity metrics ($M^2 = 0.39$, $P < 0.005$, permutations = 999).

The co-occurrence patterns among potential host bacteria at the phylum level and individual ARG subtypes were further explored using network analysis covering all main types of ARGs and MGEs, and only strong ($r > 0.7$) and significant ($P < 0.01$) correlations were displayed (Fig. 4b-e). Overall, there were 85 nodes and 115 edges in the network for all samples, and the average degree and modularity index were 2.706 and 0.567, respectively. ARGs and MGEs showed high connectivity with Planctomycetes, Acidobacteria, Gemmatimonadetes, Verrucomicrobi, and Bacteroidetes in soils (Fig. 4b). Comparing specific land-use types, several bacterial phyla, i.e., Gemmatimonadetes and Bacteroidetes, were most significantly associated with multiple ARGs in forest land (Fig. 4c). The ARGs were weakest associated with bacteria in tea plantation soils (Fig. 4d). In contrast, agricultural soil significantly increased the nodes (63) and edges (72) between ARGs and bacteria compared with the tea plantation soils (Fig. 4e). The most abundant bacteria, i.e., Planctomycetes, Firmicutes, and Bacteroidetes, were tightly associated with ARGs, and may be the major carriers of ARGs in agricultural soil. In addition, one ARG may be carried by different host bacteria.

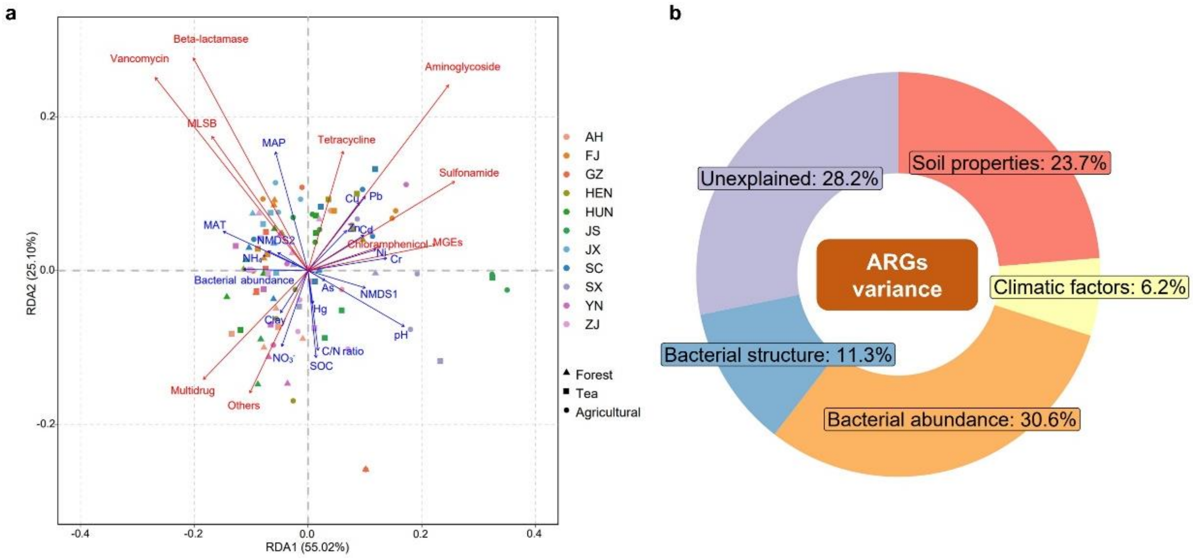

Fig. 5 Redundancy analysis (RDA) (a) and variation partitioning analysis (VPA) (b) depict the contribution of biotic and abiotic factors to the variation of ARGs in soils. AH, Anhui; FJ, Fujian; GZ, Guizhou; HEN, Henan; HUN, Hunan; JS, Jiangsu; JX, Jiangxi; SC, Sichuan; SX, Shanxi; YN, Yunnan; ZJ, Zhejiang. Using soil physicochemical characteristics, climatic factors, bacterial abundance, and bacterial community structure (two axes of NMDS) as explanatory variables. The blue and red arrows represent the explanatory and response variables, respectively, where greater lengths indicate stronger correlations between the pivotal factor and ARG distribution. The angles between arrows reveal the correlations between respective environmental parameters and individual ARGs.

Redundancy analysis (RDA) was used to analyze the effect of soil properties, climatic factors, bacterial abundance, and bacterial structure on the ARG profiles. The results revealed that 80.12% of the total variation could be explained by the first two axes of the RDA (Fig. 5a). Variation partitioning analysis (VPA) indicated that our selected biotic and abiotic factors explained 71.8% of total ARG variation (Fig. 5b). The soil properties contributed 23.7% of total ARG variation and climatic factors (i.e., MAT and MAP) influenced 6.2% of the variance in ARGs. For the biotic factors, bacterial abundance (30.6%) has a larger impact on the distribution of ARGs than bacterial community structure

(11.3%).

4 Discussion

4.1 Occurrence of ARGs in different land-use patterns

Variations in fertilizer application regimes result in different artificial pressure for the proliferation of ARGs among the three land-use patterns. Compared to forest and tea plantation soils, the anthropogenic inputs of manure, biosolids, and sewage sludge to agricultural soils are considered to significantly increase the levels of ARGs as they are reservoirs of ARGs and resistant bacteria (Heuer et al., 2011; Kuppusamy et al., 2018; Udikovic-Kolic et al., 2014). Several studies indicate that agricultural practices are the main source of antibiotics entering the soil, and the concentration of veterinary antibiotics found in cultivated land was generally higher than in forest land, associated with increased ARGs in soils (Zhao et al., 2020b, 2020a). In particular, agricultural areas with continual manure application enhanced the occurrence and number of ARGs in the soil compared to chemical fertilizer (Liu et al., 2022; Peng et al., 2017). Likewise, our results were consistent with the above-mentioned findings that the abundance and diversity of ARGs were sequentially higher in the forest land, tea plantation, and agricultural soils. Nevertheless, for the specific ARG types, the dissimilarity was only in aminoglycoside and sulfonamide, which may be directly related to the type of antibiotics used in the local livestock farming management (Fig. 1 and Table S3). In addition, from the geographical location, the southern region, like Fujian province, has more serious pollution of ARGs, and the trend of higher ARG contamination was also presented in the less economically developed areas like Yunnan province, accounted for by the abuse of manure containing antibiotics (Fig. 1a). Research on large scale patterns of antibiotic resistance in Chinese farmland soils also revealed that ARGs presented distinct geographic regional differences and highlighted the role of anthropogenic activities in shaping ARGs in soils (Du et al., 2020).

Undoubtedly, we cannot ignore the fact that forest soil is a huge potential reservoir of ARGs (Table S3). A nationwide survey of soil resistome from pristine forests across China still detected ARGs conferring to major classes of antibiotics, and a high abundance of ARGs can be found in forest soils (Radu et al., 2021; Song et al., 2021). Geographical distance and environmental variables considerably influence the distribution and abundance of ARGs in forest soils with little human disturbance (Song et al., 2021). More importantly, 68.2% of ARG subtypes co-existed in soil samples with different land-use patterns, indicating a common origin or high transmission frequency (Fig. S3). Spread of ARGs through runoff and airborne between land-use types within the same region is supported by several evidences. Distribution characteristics of ARGs in a representative rivers-reservoir system suggested rainfall-induced seasonal runoff may promote the abundance of ARGs and MGEs (Chen et al., 2019). Long-term atmospheric deposition monitoring found sulfonamide resistance genes and *intI*1 to be long-range and continuously dispersed in free tropospheric aerosols and ARGs from animal feces and feed yards origin accumulated in bioaerosols and were dispersed over long distances (Bai et al., 2022; Cáliz et al., 2022; McEachran et al., 2015; Zhou et al., 2023).

4.2 Biotic and abiotic factors influencing the distribution of ARGs

As we expected, the soil properties and climatic factors that influence the persistence and spread of ARGs and antibiotic residues vary in relative importance under different land-use practices (Cycoń et al., 2019; Wu et al., 2023). Overall, ARG distribution was strongly influenced by pH, SOC, and multiple heavy metals (Cu, Zn, Pb, Cd, Ni, Cr, As, and Hg) (Fig. 3a). A study targeting the large-scale profile of antibiotic resistome in forest soils found significant relationships between temperature and spatial variation in ARGs (Song et al., 2021). Our study confirmed that forest soil ARGs were mainly associated with SOC, MAT, and C/N ratios, and further supported this explanation (Fig. 3b). This may promote the degradation of antibiotics due to the high organic carbon and nitrogen content induced by plant litter as well as the fact that forest vegetation regulates temperature differences (Zhang et al., 2023). Shared bacteria and resistome in the soil-plant systems suggested the possibility of transfer for ARGs through the internal plant tissue (Zhang et al., 2019). Resistomes were altered by plant identity (Zheng et al., 2022), and forest vegetation may have a better capacity to absorb ARGs compared to tea and agricultural crops. pH is the primary abiotic factor affecting the variation of ARG abundance in tea plantation soils (Fig. 3c). Increasing the pH was associated with a decrease in target ARGs from manure slurries (Li et al., 2020). SOC and pH were the top two soil attributes affecting ARG abundance in agricultural soils (Fig. 3d), which showed a significant negative correlation with ARGs (Wang et al., 2020; Wu et al., 2023). Heavy metals, such as Cd and Cu (Figs. 3 and 5b), introduced into the soil with long-term fertilization driving the risk of antibiotic resistance co-selection is a concern (Baker-Austin et al., 2006). The use of zinc as an agricultural feed additive has driven the development of antibiotic resistance in metal-exposed bacteria due to the selective effect of metals on genetic elements that carry both metal and antibiotic resistance genes (Dickinson et al., 2019; Poole, 2017). Co-occurrence of ARGs and heavy metals are characterized much more frequently in human pathogens than in bacteria that are less frequently associated with humans (Li et al., 2017). Furthermore, it was found that warming and increased precipitation have positive impacts on specific ARGs (e.g., beta-lactamase, vancomycin, and MLSB) but have negative impacts on other categories (Fig. 5a), and climate seasonality was also determined as a key driver of their global profile (Delgado-Baquerizo et al., 2022; Z. Li et al., 2022). On the whole, it can be observed that pH and SOC have a negative effect on the majority of ARG types, while several heavy metals and MAP have a positive effect in the present study.

Bacterial community composition (i.e., abundance and structure) is also a primary driver shaping ARG diversity and evolution (Chen et al., 2022; Yang et al., 2019), with a greater influence than abiotic factors (Fig. 5b). By network analysis, Planctomycetes, Acidobacteria, and Gemmatimonadetes were considered as the major potential hosts of ARGs, and the variation in ARG abundance may be related to their carrying aminoglycoside and sulfonamide in soils (Fig. 4b and Tables S3-4). The high connectivity of ARGs with several bacterial phyla (i.e., Gemmatimonadetes and Bacteroidetes) in forest systems reflected the capacity of forest soils as potential reservoirs of ARGs (Fig. 4c). In contrast, the weakest correlation between ARG and host bacteria was in tea plantation soils, and ARGs were mainly associated with Chloroflexi (Fig. 4d). Previous studies have also found enrichment of soil resistome was strongly influenced by bacterial taxa in manure-amended soils, including Gemmatimonadetes and Chloroflexi (Zhang et al., 2019). Although Proteobacteria*,* as the most enriched bacterial phylum, was considered the major potential host of ARGs in agricultural soils (T. Li et al., 2022), Planctomycetes, Firmicutes, and Bacteroidetes may be the main carriers of ARGs in this study (Fig. 4e). In addition, the positive correlation of MGEs with sulfonamide and aminoglycoside further indicates their role in carrying ARGs (Gaze et al., 2011; Peng et al., 2017).

4.3 Mitigation options for ARG contamination from different land-use patterns

To date, ARGs are an increasing global crisis in human health (Zhang et al., 2022), and it is crucial to propose strategies to mitigate ARGs for specific soil environments. Antibiotic-resistant bacteria and ARGs can persist and transmit to vegetables with the application of manure (Pu et al., 2019). Hence, developing cost-effective antibiotic abatement methods are fundamental approaches to mitigate ARG contamination from the source. Currently, aerobic composting and aerobic and anaerobic digestion of manure and sewage sludge are effective methods to reduce ARGs in soils (Wang et al., 2022). For forest systems, the absorption and accumulation of ARGs by plants should be fully utilized. It has been reported that plants with an enrichment effect on organic pollutants in combination with biochar showed stronger attenuation of typical antibiotics and ARGs in soils (Liang et al., 2017). Increasing vegetation coverage near the tea plantation and agricultural land can be considered a sustainable ecological approach. Agricultural field and tea plantation soils are more likely to be contaminated by anthropogenic sources such as cultivation activities compared to forest systems (Xiang et al., 2020). pH, as the most influential factor affecting ARGs in tea plantation soils (Fig. 3c), implied that reasonable fertilization measures to mitigate the process of soil acidification in tea plantations might contribute to the alleviation of ARG contamination. A systematic global analysis of ARGs found that the abundance of ARGs was negatively correlated with SOC and TN in agricultural soil (Wu et al., 2023), which was consistent with the observations in this study (Fig. 5a). This implied that maintaining high soil nutrients may outcompete introduced ARG bearing bacteria. Meanwhile, soil quality should be selected carefully when growing crops. For example, higher clay and heavy metals may significantly enhance the abundance of antibiotic-resistant bacteria (Awasthi et al., 2019; Seiler and Berendonk, 2012). Moreover, as ARGs are normally carried by host bacteria, it could be promising to explore efficient biological control agents to maintain soil nutrients for crop growth while reducing the reproduction of bacteria carrying ARGs. We also acknowledge that there are some limitations in this study, such as soil samples were collected from three land-use patterns in several provinces in this study, subsequent studies could enhance the representativeness of the samples by collecting multiple samples from a particular location. In addition, the environmental risk of ARGs closely associated with human pathogens in each land-use pattern was not clarified and the dynamic pattern of ARGs over time was not consistently tracked.

5 Conclusion

Through the large-scale field investigation of the presence of ARGs in different land-use practices across 11 provinces across China, this study has provided compelling field evidence that the abundance of ARGs increased by 66.1% in agricultural soils compared to tea plantations and by 27.9% in tea plantation compared to forest land soils. Notably, the shared subtypes suggest that ARGs may have a common origin or high transmission frequency between different soil environments. Although there was no further evidence to determine the transfer pathway of ARGs in our study, the findings still can extend our sights for regulating ARGs. The forest soil also showed a high abundance and detected number of ARGs despite the forest environments being rarely affected by human activities. Intrinsically, variance in ARGs induced by changes in soil properties (i.e., pH, SOC, Cd, Cu, and clay), climatic factors (i.e., MAT and MAP), and bacterial community (i.e., abundance and structure) are the main drivers shaping ARGs profiles in different land-use patterns. Finally, these findings are essential for evaluating the potential public risks posed by ARGs in soil systems, and we highlight the necessity to target ARG reduction by adopting efficient practices under different land-use regimes, especially in soil types and properties of importance.

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