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Suppressing *Ralstonia solanacearum* and Bacterial Antibiotic Resistance Genes in Tomato Rhizosphere Soil through Companion Planting with Basil or Cilantro

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Abstract: The effects of companion planting on soil antibiotic resistance genes (ARGs) and associated microbial composition have remained largely unclear until now. In this study, we assessed the changes in the soil microbiome and ARGs frequencies for tomato growing soils that were companion planted with basil (TB) or cilantro (TC) using a metagenome approach. The abundance of the phytopathogen *Ralstonia solanacearum* was significantly lower in the TC or TB treatments compared to the tomato monoculture soils (TT). A significant enrichment of *Pseudomonas* and *Aquabacterium* and a depletion of *Nocardioides* and *Streptomyces* were observed in the TC treatment. Interestingly, both TC and TB companion planting reduced the absolute abundance and the number of subtypes of ARGs. The TC soil showed the lowest numbers of unique ARG subtypes, especially the ARGs resistant to vancomycin and rifamycin, as well as those associated with multidrug resistance. Furthermore, network analysis further revealed that *Nocardioides* and *Streptomyces* were potential hosts of ARGs, whereas *Flavobacterium* negatively correlated with *mdtG*, suggesting a suppressive effect in reducing ARGs. Together, our results suggest that the companion planting of tomatoes with basil or cilantro can reduce the risk of ARG accumulation, making it a feasible farming management tool to promote soil and plant health in sustainable agriculture.

Keywords: bacterial wilt disease; companion planting; rhizosphere soil; antibiotic resistance genes; microbial community

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1. Introduction

Antibiotic resistance is widely recognized as one of the most serious challenges facing public health worldwide in this century, leading to a high risk to food security and human health [1]. It has been estimated that deaths caused by antibiotic resistance worldwide are nearly 0.7 million per year and are estimated to be 10 million per year by 2050 [2]. It has been shown that antibiotic resistance can evolve via the horizontal acquisition of antibiotic resistance genes (ARGs), a phenomenon which seriously exacerbates the threat of antibiotic resistance in agriculture [3]. Hence, ARGs have been attracting a great deal of attention in recent decades. Given that ARGs are ubiquitous in a range of environments, an increasing proportion of current studies have focused on the distribution and transmission of ARGs in various matrices, such as soil [4], water [5], and compost [6]. Considering that the agroecosystem is closely associated with human health, it is extremely crucial to understand the presence, behavior, and transmission of ARGs in farm soil to help design patterns that eliminate the associated health risks.

Whereas the agroecosystem plays a vital role in providing humans with abundant high-quality food products, agricultural ecosystems have recently been recognized as an ideal environment for the acquisition and dissemination of ARGs [7]. The presence of ARGs in the microbial communities of agricultural soil is usually caused by the application of antibiotics to livestock both to treat disease and as growth stimulants, and the subsequent disposal of treated effluents, manure, and wastewater onto agricultural lands [8,9]. In addition, recent studies have shown that other agricultural practices, including the application of herbicides [10], fungicides [11], and fumigants [12], could strongly affect soil ARGs and antibiotic resistance. Moreover, the agronomic factors associated with a considerable re-shaping of the soil resistome, i.e., the frequency of antibiotic resistance in soil bacteria, have been identified as intercropping [13] and crop rotation [14]. Despite the increasing recognition of the rapid proliferation of ARGs in agricultural soils [15], there have been few systematic studies assessing the relationships between companion planting and soil ARGs.

Modern industrial arable farming, based on crop monocultures, is recognized as an unsustainable form of agricultural production, encouraging issues such as increased disease pressure and reduced levels of specific nutrients in the soil, subsequently resulting in lower crop yields [16,17]. The occurrence of soil-borne phytopathogens in agriculture results in significant changes in the composition of the soil microbiome [18]. Bacterial communities have been reported to have received ARGs mainly through horizontal gene transfer (HGT), which can be mediated by mobile genetic elements (MGEs), with the composition of the soil microbial community being associated with the bacterial resistome [19]. However, the complex dynamics of the soil microbiome and its associated impacts on the soil resistome in the presence of a soil-borne disease outbreak have not yet been fully realized.

Bacterial wilt, caused by *Ralstonia solanacearum*, is one of the most important soil-borne bacterial diseases of crops. Taking the tomato family as an example, tomato wilt resulted in a 26% reduction in fresh tomato fruit production, rising to 91% in the presence of a severe bacterial tomato wilt disease [20], which therefore represents the main constraint on tomato production [21]. Moreover, *R. solanacearum* has recently been recognized as being host to a range of ARGs, including *amA*, *bacA*, and *ermB* [22]. Therefore, there is an urgent need to investigate novel strategies for controlling bacterial tomato wilt and eliminating ARGs from agricultural soils, without causing harm to the environment.

Companion planting is widely known for its advantages in improving crop land use efficiency and reducing pests and diseases in crop production [23]. In recent years, there has been a growing interest in suppressing crop disease or pest attack through the use of companion crops [24]. For example, some annual crops, especially spices and medicinal plants, can reduce the occurrence of pests if grown as intercrops in or around the main crop because of their pungent aromatic odor in the field [25]. Aromatic medicinal plants can also significantly increase the soil's organic nitrogen content, available nitrogen, and water content [26]. Basil (*Ocimum basilicum* L.) and cilantro (*Coriandrum sativum* L.) are spices and medicinal plants commonly grown in the Middle-Lower Yangtze Region of China and can be considered as potential companion plants to tomatoes due to their similar light and water needs [27,28]. However, the effects on the soil microbiome, soil-borne phytopathogen survival, and the associated resistome of companion planting tomatoes with basil or cilantro need investigation.

In this study, we aimed to assess the effects of companion planting tomatoes with basil or cilantro on suppressing the occurrence of tomato bacterial wilt disease, manipulating the soil microbiome, and concomitantly decreasing the abundance and diversity of ARGs in the soil. With this goal in mind, we conducted a greenhouse experiment to investigate the effects of companion planting tomatoes with basil or cilantro on the abundance of *R. solanacearum* and ARGs in soil, using quantitative real-time PCR and metagenomic sequencing methods. The results of this study could provide a basis for reducing the risks of both soil-borne disease outbreaks and ARG transmission.

2. Materials and Methods

2.1. Experimental Design

This study was conducted in the greenhouse facility of Nanjing Agricultural University, Jiangsu Province, China, from September to November 2021. The field experiment was designed in a completely randomized manner, with five replicates for each treatment: tomato monoculture control (TT), tomato intercropped with cilantro (TC), and tomato intercropped with basil treatment (TB). Each replicate contained 1.2 kg of planting matrix, which consisted of a sterilized vermiculite substrate mixed with soil at a ratio of 5:3 (v/v) and then loaded into a plastic turnover box (435 mm length × 335 mm width × 140 mm height). The soil used was collected from the vegetable greenhouse facility of Nanjing Vegetable Science Research Institute, Jiangsu Province, a coastal province to the north of Shanghai, from a greenhouse which had been used to grow tomato plants continuously for 13 seasons. Before planting, tomato seeds were surface sterilized by soaking in a 50% (v/v) aqueous solution of commercial bleach (3.5% sodium hypochlorite, NaClO) for 10 min and then rinsing three times with sterile distilled water. Then, the seeds were sown in seed trays and placed in an incubator to germinate. Basil and cilantro seeds were sown directly into seed trays. Then, tomato, basil, and cilantro seedlings, each selected for uniform sizes, were transplanted into boxes. The plant density values reported by Girma et al. [29] were used, who found that the companion planting of tomatoes and basil at the ratio of 1:1 provided the best yield advantage over growing tomato as a monoculture. Specifically, there were three tomato plants and three basil or cilantro plants in each box for TB or TC companion planting treatments, respectively, whereas six tomato plants per box were used for the TT monoculture control.

2.2. Rhizosphere Soil Collection

Before the companion planting experiment began, 50 g of the original soil was collected from each box, prior to transplanting the seedlings, for use as a negative control. The tomato roots were collected after 45 days of growth and were pooled together. The soil which was not tightly bound to the roots was removed by vigorously shaking. Subsequently, the remaining ("rhizosphere") soil was obtained through the following steps. In the first step, the harvested tomato roots were placed into a 250 mL tissue culture bottle filled with 150 mL of sterile water, shaken at 170 rpm for 30 min at room temperature, and then centrifuged for 5 min at 4000× g. The supernatant was discarded, and the pellet was collected as rhizosphere soil, which was stored at −80 °C.

2.3. DNA Extraction, Construction of Sequencing Libraries, and Metagenomic Sequencing

Soil (about 5 g) DNA was extracted using the Qiagen DNeasy PowerMax soil kit (Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were determined using a Qubit 3.0 fluorometer and a NanoDrop One spectrophotometer, which are both produced by Thermo Fisher Scientific (Waltham, MA, USA). Metagenomic sequencing libraries were prepared according to the manufacturer's instructions, using NEB Next® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, Rowley, MA, USA). The library quality was evaluated using the Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and the Qsep400 High-Throughput Nucleic Acid Protein Analysis system. The libraries were sequenced using the Illumina NovaSeq 6000 system platform (Illumina, San Diego, CA, USA), which generates about 150 bp paired-end reads.

2.4. Raw Sequencing Data Processing and Taxonomy Profiling

The clean data were obtained from raw metagenomic sequencing reads using Trimmomatic v. 0.36 [30]. The scaffolds were then de novo assembled by running MEGAHIT v1.0.6 [31] and were used to generate scaftigs. Further, the open reading frames (ORFs) were predicted for scaftigs longer than 500 bp using MetaGeneMark v. 3.38 [32]. After removing redundancy using CD-HIT v. 4.7 [33], the initial unique gene catalog was clustered

at 95% identity with 90% coverage. Subsequently, the clean data from each sample were mapped to a non-redundant gene catalog and BLAST with the NCBI database to obtain the taxonomy information, following previous methods [34,35].

2.5. Antibiotic Resistance Genes (ARG) Prediction

The ARGs-OAP 2.0 [36] were used to identify and classify ARG-like reads as the previously described pipeline. In brief, metagenomic reads were examined against the integrated, structured ARG database [37] and the Structured Antibiotic Resistance Gene (SARG) database, which was constructed by integrating the Antibiotic Resistance Gene Database (ARDB) and the Comprehensive Antibiotic Resistance Database (CARD) [38–41]. Subsequently, ARG-like sequences were annotated to generate the ARG profiles for different soil samples. Furthermore, the metagenomic binning method was used to acquire the draft bacterial genome using MetaBAT2. After checking for completeness and contamination, the binned contigs were annotated for taxonomy information according to GTDBTk. In the final step, the CARD database was used to explore the antimicrobial resistance (AMR) information via Resistance Gene Identifier (RGI) software (version 5) [38].

2.6. Determination of the Abundance of R. solanacearum

The abundance of *R. solanacearum* was determined with fluorescence real-time quantitative PCR (qPCR) [42,43]. The primer pair was flicF/flicR (5′-GAACGCCAACGGTGCGAACT-3′/5′-GGCGGCCTTCAGGGAGGTC-3′), which yields about 400 bp PCR products. Amplification conditions were 10 μL of (2×) SYBR®Premix Ex *Taq* (Thermo Fisher Scientific, Waltham, MA, USA), 0.2 μM (10 mM L^{-1}) upstream primer, and 0.2 μL (10 mM L^{-1}) downstream primer, 0.4 μL of (50×) ROX Reference Dye (Thermo Fisher Scientific, Waltham, MA, USA), 2 μL of template DNA (about 50 ng), and 6.8 μL of sterile water. The PCR amplification program included pre-denaturation at 95 °C for 30 s, pre-denaturation at 95 °C for 5 s, extension at 60 °C for 34 s, and 40 cycles. The process was performed using the Applied Biosystems™ 7500 Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The amplification of each of the samples was repeated independently three times, with sterile ultrapure water as a negative control. Finally, the result for each sample was represented as log (copies/g dry soil) number of copies formed per g of dry soil.

2.7. Statistical Analysis

The indices among different soil samples were compared by the ANOVA and LSD methods in SPSS 25.0 (SPSS, Chicago, IL, USA). Principal coordinate analysis (PCoA), conducted by the Bray–Curtis method was used to compare the differences among frequencies of microbial taxa and ARGs in the R 'vegan' package [44]. Then, the permutational multivariate analysis of variance (PERMANOVA) was conducted to assess the effects of companion planting on the composition of the soil microbial community and ARGs. Further, the relationships between the composition of soil bacteria and ARGs were examined based on redundancy analysis (RDA). To explore the potential ecological clusters of soil taxa strongly correlated to ARG subtypes, co-occurrence networks were finally built using the Gephi software package (version 0.10.1. https://gephi.org/, accessed on 16 December 2023) with the default parameters [45].

3. Results

3.1. R. solanacearum Abundance

Compared with the control situation of a tomato monocrop (TT), tomatoes planted in companion with basil (TB) or cilantro (TC) significantly reduced the abundance of *R. solanacearum* in the tomato rhizosphere ($p < 0.05$, Figure 1). Specifically, the TB treatment showed a 3.64% reduction in pathogens in comparison with the TT control. Meanwhile, the TC treatment displayed the lowest pathogen abundance, with an 8.46% reduction compared to the TT control.

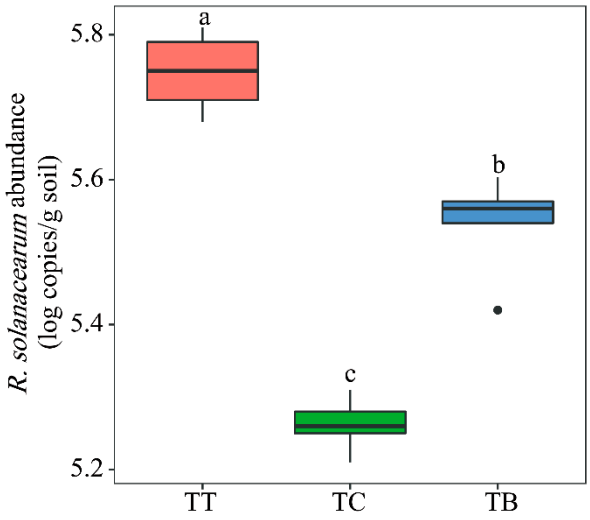


Figure 1. Abundance of *Ralstonia solanacearum* in different treatments. Different letters represent significant differences ($p < 0.05$) based on ANOVA and LSD tests. TT, tomato monocropping control; TC, tomatoes intercropped with cilantro; TB, tomatoes intercropped with basil.

3.2. Soil Microbial Composition

The metagenomic sequencing generated approximately 10.1–13.5 Gb reads for each sample. Then, about 8.4–11.8 Gb high-quality reads were retained for each sample after quality control, which yielded 42,061,797 contigs on average (a mean length of 864 bp for N50, and 544 bp for N90) (Table S1). For the soil samples, 411,392 open reading frames (ORFs) on average were obtained, with a mean size of 912 bp per sample (Table S2).

As shown in Table S3, bacteria, occupying 30.48% of the total sequences, were identified as the dominant microbial domain. The principal coordinate analysis (PCoA) showed that the composition of the bacterial community was significantly (PERMANOVA, $p < 0.001$) different among all treatments (Figure 2A). Among the bacterial domains, Proteobacteria were found to be the most abundant, followed by Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Verrucomicrobia, Planctomycetes, Firmicutes, and Cyanobacteria, which made up the top ten most abundant phyla and the majority of the ORFs (Figure 2B). Compared to the original soil prior to planting, the crop type in the planted soil significantly altered the relative abundance of the top 10 phyla (Figure S1). More specifically, the relative abundances of Proteobacteria and Verrucomicrobia were significantly higher, while those of Actinobacteria, Acidobacteria, Chloroflexi, and Firmicutes were significantly lower in the TC treatment compared to the TT control and the TB treatment ($p < 0.05$, Figure S1).

The heatmap plotting the relative abundances of the top 20 most abundant genera shows a clear alternation among the original, TT, TC, and TB soils (Figure 2C). Specifically, *Pseudomonas*, *Nocardioides*, *Sphingomonas*, *Lysobacter*, *Aquabacterium*, and *Streptomyces* were identified as the dominant genera (displaying more than 1% relative abundance in at least one treatment) (Table S4). Furthermore, *Pseudomonas* and *Aquabacterium* were significantly enriched in the TC soil compared to the TT and TB soils ($p < 0.05$, Table S4).

The volcano map shows significant differences in genus abundance of the rhizosphere bacterial communities across the soil samples from the different treatments (Figure 3). Compared to the TT control, 33 microbial genera were significantly enriched in the TC treatment, with *Asfivirus*, *Alishewanella*, *Albitalea*, *Acidovorax*, *Bingvirus*, *Chitinibacter*, *Populus*, *Nevskia*, *Rhizobacter*, and *Schlegelella* identified as the top 10 enriched genera. In the TB treatment, 75 microbial genera were significantly enriched compared to TT, with *Achromobacter*, *Brevibacterium*, *Dermatobacter*, *Deinococcus*, *Gilsonvirus*, *Luckybarnesvirus*, *Oscillochloris*, *Trichoderma*, and *Wilnyevirus* identified as the top 10 enriched microbial genera.

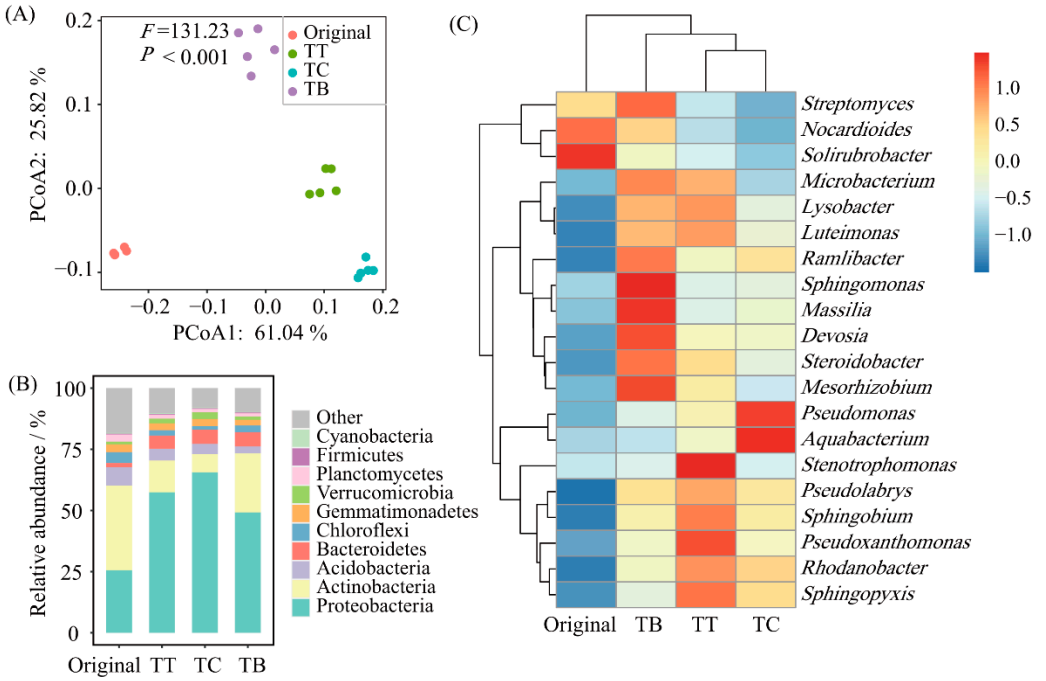


Figure 2. Principal coordinate analysis (PCoA) plots, depicting the differences in soil microbial composition in the rhizosphere (**A**). Stacked bar chart showing the relative abundance (%) of the main identified phyla (**B**). The heatmap displaying the differences in the relative abundance of the top 20 most abundant microbial genera (**C**).

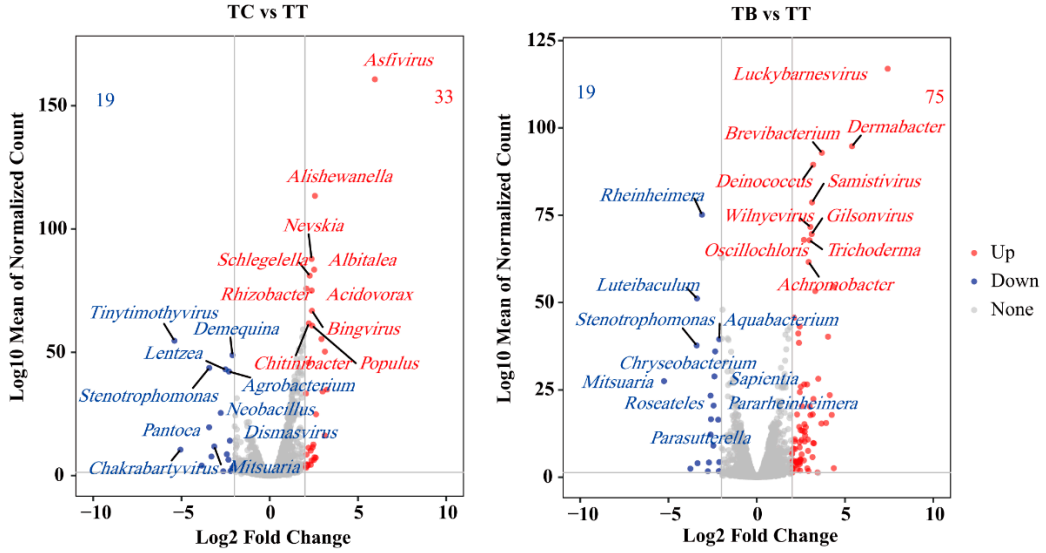


Figure 3. Volcano maps of significant changes in genus abundance among the different treatments.

3.3. Abundance and Composition of ARGs

In total, 23 ARGs, consisting of 1329 subtypes, were detected across all soil samples (Figure 4). Genes encoding multidrug resistance and resistance to aminoglycoside, bacitracin, beta-lactam, fosmidomycin, macrolides-lincosamides-streptogramins (MLS), quinolone, rifamycin, tetracycline, or vancomycin were found. Among these, beta-lactam resistance genes, comprising 902 subtypes, were identified as the most frequent ARGs. However, the beta-lactam resistance genes only accounted for 4.5% of the total ARGs (Figure 4A). Furthermore, multidrug resistance genes, consisting of 77 subtypes, were recognized as the most abundant resistance genes across all treatments, accounting for up to 40.86% of the total ARGs (Figure 4B).

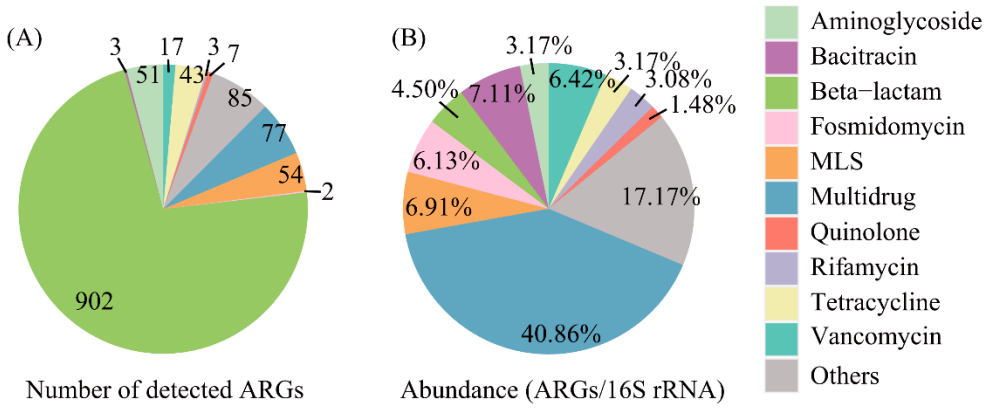


Figure 4. Number (**A**) and normalized abundance (**B**) of ARGs across various treatments, identified using the metagenomics method.

The PCoA results demonstrated that the composition of ARGs was significantly (p = 0.011) different across the various cropping treatments (Figure 5A). Among the 1329 ARG subtypes detected, 408 were general to all samples, while 35 unique subtypes were detected in the original soil, compared to 47 in TT, 28 in TC, and 45 in TB (Figure 5B). The ARG absolute abundance was lowest in the TC soil, followed by the TB and TT treatment soils. Specifically, the absolute abundance of ARGs detected in the original, TT, TC, and TB soils was 794, 1378, 1038, and 1123 ppm, respectively (Figure 5C), with multidrug, bacitracin, MLS, vancomycin, fosmidomycin, beta-lactam, aminoglycoside, tetracycline, rifamycin, and quinolone resistance genes being the ten most abundant ARGs among the original, TT, TC, and TB treatments (Figure 5D).

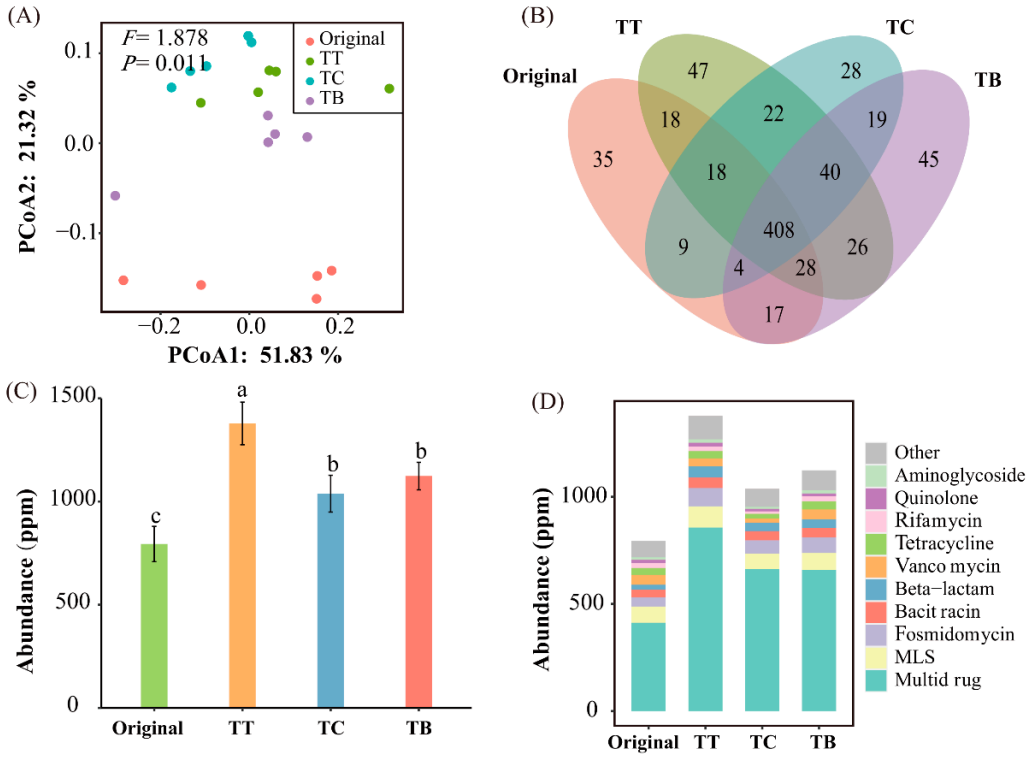


Figure 5. PCoA plots displaying the composition of ARG subtypes among all treatments (**A**). Venn diagram showing the common and unique ARGs for all treatments (**B**). Stack bar chart depicting the absolute abundance (ARG/ppm) of ARG subtypes for each treatment (**C**). Boxplot illustrating the absolute abundance of major ARG subtypes for each treatment (**D**). Different letters represent significant differences ($p < 0.05$).

As shown by Figure 6, the multidrug resistance genes were significantly depleted ($p < 0.05$) in the intercropped TB and TC soils compared to the monocrop TT soil. In addition to the genes encoding resistance to tetracycline, the genes for rifamycin are also significantly enriched in the TB soil compared to the TT soil. In contrast, the genes encoding resistance to rifamycin, together with those encoding resistance to vancomycin are significantly lower in the TC soil.

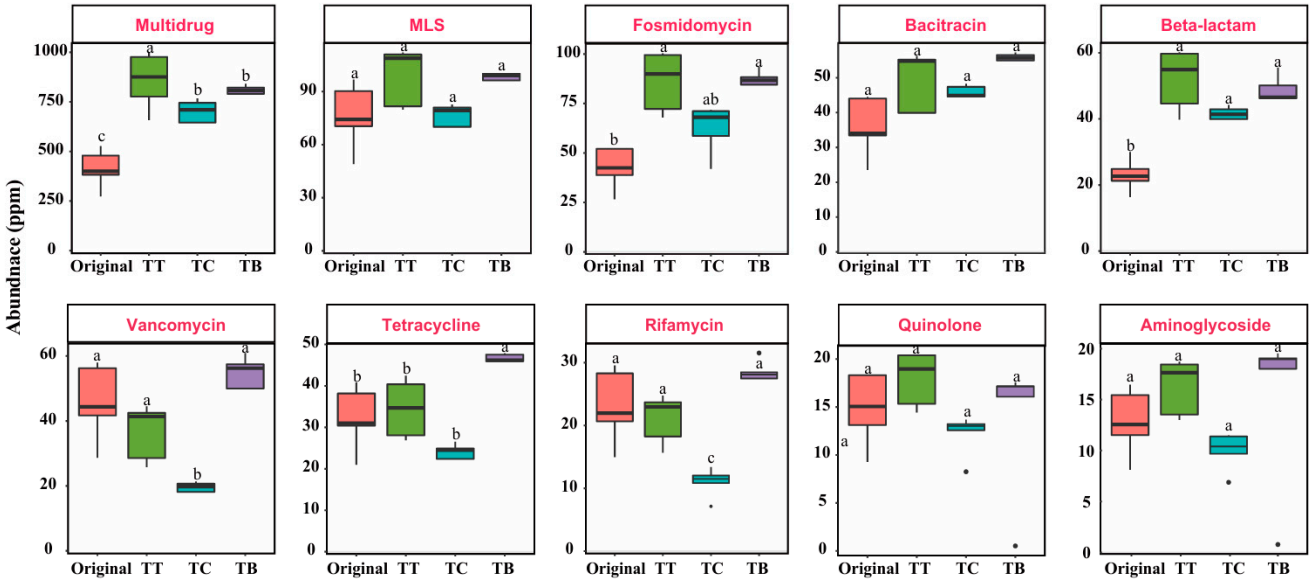


Figure 6. Boxplot depicting the abundance of major ARG types for all treatments. Different letters represent significant differences ($p < 0.05$).

3.4. Relationships between Soil Bacterial Community Composition and ARGs

Networks based on ARG subtypes and microbial genera (relative abundance > 0.01%) were constructed to identify the potential hosts and antagonists of ARG subtypes present in soil (Figure 7). The final built network in the TC soil contained 142 edges and 67 nodes, containing 36 ARGs and 31 microbial genera (Table S5). Among these, 11 genera known as Actinobacteria and 17 genera affiliated to Proteobacteria were regarded as the major potential microbial hosts. Meanwhile, the final constructed network in the TB soil consisted of 82 edges and 57 nodes, including 24 ARGs and 33 genera (Table S6). Within the 33 genera, seven genera classified as Actinobacteria and 24 genera identified as Proteobacteria were deemed the major potential microbial hosts.

Specifically, in the TC soil, the relative abundance of *Streptomyces* was significantly and positively correlated with the frequency of MLS resistance genes (*mgtA*, *oleD*, and *erm*(41)), the multidrug resistance gene (*mdtG*), and the tetracycline resistance gene (*tetV*), whereas the relative abundance of *Sphingosinicella* was significantly and negatively correlated with the frequency of the multidrug resistance gene (*mexT*), the tetracycline resistance gene (*tetR*), and the polymyxin resistance genes (*icr-Mo* and *mcr-2.1*). Furthermore, *Flavobacterium* was negatively correlated with mdtG, encoding a subtype of multidrug resistance. In the TB soil, the relative abundance of *Streptomyces* was significantly and positively correlated with the frequency of the multidrug resistance gene *oprM*. In addition, *Nocardioides* was found to be significantly and positively linked to the MLS resistance genes (*mgtA*, *oleD*, and *erm*(41)) and the tetracycline resistance gene (*tetV*) in the TC soil, while it was found to be significantly and positively correlated to the aminoglycoside resistance gene (*aac(2′)-Ic*) in the TB soil.

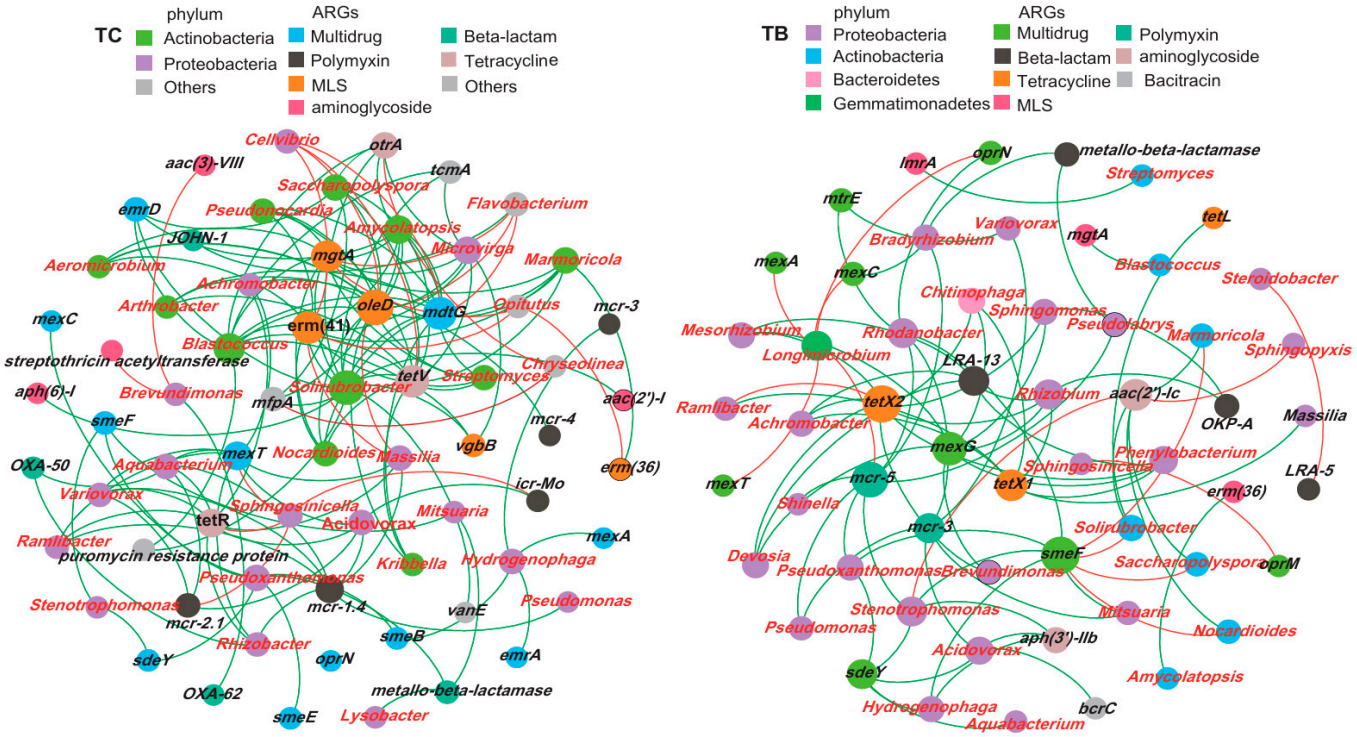


Figure 7. Network analysis showing the correlations between microbial genera and the main ARG subtypes. Connections indicate strong (Spearman's correlation coefficient, $r_S > 0.9$) and significant ($p < 0.01$) correlations. Different colors for nodes mean different types of ARGs and bacterial taxa. Node sizes represent the extent of significant connections. A green edge shows a positive correlation, while a red one means a negative correlation.

4. Discussion

Excessive use of antibiotics may result in higher residues in the soil, promoting the frequency and spread of ARGs in microbial communities and the soil, which may, in turn, threaten environmental safety and human health [46,47]. Microbial communities and antibiotic resistance genes have been studied in different companion planting systems. However, the effects of tomato companion planting with basil or cilantro on the soil microbiome and ARGs in soil are not yet fully understood. The current study demonstrated that companion planting tomatoes with basil or cilantro could reduce the frequencies of both soil-borne *R. solanacearum* and ARGs by altering the composition of the microbial communities.

Companion planting utilizes fewer inputs compared to conventional intensive agriculture and contributes to managing pathogens and pests through the release of antagonistic secondary metabolites from the roots or leaves of the companion crop, thereby reducing pathogens in the other crop [48,49]. In our study, we demonstrated that the companion planting of tomato plants with basil or cilantro could significantly suppress the soil-borne pathogen responsible for tomato bacterial wilt. Similarly, the inhibitory effects of interspecific plant interactions on soil-borne pathogens were described in several previous studies [50–52]. Companion planting basil with tomatoes was found to significantly reduce the survival of *Fusarium* wilt in soil compared to a tomato monoculture [53]. The decrease in disease incidence and severity in intercropped settings may be affected by the following reasons: alternations in the microbial community in the rhizosphere, the activation of host defense, pathogens suppression by root exudates, and signaling compounds produced by the companion plant [24,54,55]. In agreement with a previous study, which demonstrated the biocontrol ability of the soil fungus *Trichoderma harzianum* against *R. solanacearum* [56], in the present study, *Trichoderma* was found to be significantly enriched in the tomato rhizosphere soil intercropped with basil. It has also been reported that basil roots can stimulate bacteria and arbuscular mycorrhizal fungi (AMF), which help prevent tomato disease

outbreaks and increase tomato yield [57]. Companion planting tomatoes with cilantro can increase tomato yield and reduce pests compared to tomato monoculture [28,58]. Therefore, the higher fruit fresh weight and lower *R. solanacearum* abundance in the intercropped tomato soil reported in previous studies may be caused by the ability of the plant–plant interaction to regulate root exudates and/or recruit beneficial microorganisms to suppress the pathogen [59–62]. All these results together support a previous study that suggested [63] the soil microbiome plays crucial roles in keeping bacterial *R. solanacearum* at bay.

Increased crop diversity leads to changes in the soil microbial composition, particularly increasing the abundance of plant growth-promoting microorganisms, e.g., members of the phylum Actinobacteria [64]. Companion planting alters the soil microbial community composition [65], especially rhizosphere microorganisms [66]. Some microorganisms in the rhizosphere of tea trees, when intercropped with peas, showed marked differences, especially the phyla of Acidobacteria and Proteobacteria [67]. Companion planting pear trees with basil and summer savory increased the species richness of both bacterial and fungal communities [68]. This increase may be caused by the effect of root exudates on soil microbial communities [69–71]. In the present study, we found that companion planting tomatoes with either basil or cilantro altered the soil microbial communities, possibly as a result of the root exudates and volatile organic compounds emanating from these aromatic plants. It is widely known that various factors, including plant species, root growth, root exudates, and soil properties, could lead to differences in the soil ARG group by manipulating the microbial community and the antibiotic response of community members [72]. Some studies have shown that, compared to unplanted soil, crop monocultures increase the abundance of ARGs in the soil, whereas, compared to monocultures, intercropping reduces the abundance of ARGs in the soil [13]. These are findings similar to those from our current research.

In agreement with a previous study showing a potential link between phytopathogen incidence and the spread of ARGs [73], our study found that a decrease in the density of *R. solanacearum* in the rhizosphere displayed a strong positive correlation with the reduction in the abundance of ARGs. In addition, we observed that companion planting tomatoes with different herb crops resulted in changes in the composition of soil microbial communities, supporting the findings from a recent study, which suggested that the regulation of the soil microbiome could both improve soil disease suppression and reduce the number of soil ARGs [74]. Similar to a previous study, where Actinomycetes and Proteobacteria were potential microbial hosts [75], our study revealed that Proteobacteria and Actinobacteria were the two most important phyla affecting the composition of ARGs. By using a network approach to analyzing potential bacterial hosts of ARGs at the genus level, we found that *Streptomyces* was associated with the tetracycline resistance gene (*tetV*), the multidrug resistance gene (*mdtG*), and the MLS resistance genes (*mgtA*, *oleD*, *erm*(41)), indicating that *Streptomyces* may be the host of multiple antibiotic resistance genes; a previous study reported that *Streptomyces* may be the main microbial host of ARGs in soil [75], which suggests that the decrease in ARG abundance observed in the current study in the rhizosphere soil of tomatoes intercropped with cilantro may be caused by a decrease in *Streptomyces* abundance resulting from companion planting. Furthermore, it is well accepted that one ARG subtype may be carried by a number of different microorganisms [76,77]. Our study confirmed this phenomenon with, for example, the tetracycline resistance gene (*tetV*) being associated with *Nocardioides*, *Streptomyces*, *Kribbella*, *Marmoricola*, *Blastococcus*, *Arthrobacter*, *Saccharopolyspora*, *Amycolatopsis*, *Pseudonocardia*, and *Microvirga*. Our results suggest that the succession of microbial community composition, occurring in response to companion planting, plays a major role in the manipulation of soil ARGs.

5. Conclusions

Our results show that companion planting tomatoes with basil or (especially) cilantro contributed to reducing *R. solanacearum* density and total ARGs abundance and diversity, in terms of ARG subtypes. Companion planting tomatoes with cilantro lowered the fre-

quency of genes conferring resistance to multidrugs, vancomycin, and rifamycin, whereas companion planting with basil mainly decreased the frequency of genes for multidrug resistance. Companion planting further altered the network patterns of ARGs with their potential hosts. Therefore, companion planting with cilantro or basil provides a promising way to alleviate the contamination of agricultural soils with both soil-borne *R. solanacearum* and ARGs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14061129/s1, Figure S1: Histogram showing the relative abundance of top abundant phyla in soils from different treatments. Different letters above the histogram indicates a significant difference at $p < 0.05$ level; Table S1: Overview of sequences mergeability and number of annotations made for shotgun-metagenome datasets representing each soil sample; Table S2: Overview of genes catalog assembly to open reading frames (ORFs); Table S3: Relative abundance (%) of identified kingdom in different soil samples; Table S4: Relative abundances (%) of top 50 microbial genera in different samples; Table S5: Spearman correlations between bacterial community and ARGs in TC treatment; Table S6: Spearman correlations between bacterial community and ARGs in TB treatment.

Author Contributions: H.L., Z.S., R.L. and Q.S. designed the study; T.L., S.L., M.G. and Y.O. performed all experiments; T.L., S.L., Y.O. and Z.S. wrote the majority of the manuscript; X.D., H.L., Z.S. and R.L. provided comments and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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