Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Soil-derived bacterial root commensals induce systemic alterations of defence-related secondary metabolism and response in tomato whole plants for favoring resistance against Fusarium wilt

Zhaoxia Jin

jinzx2018@163.com

Dalian Polytechnic University

Xuan Liu
Binyan Li
Xinyuan Chen
Yanyan Wang
Fang Yu
Ping Kou
Long Chen
Yangyang Guo

Research Article

Keywords: Rhizosphere soil microbiota, Metabolic reprogramming, Systemic resistance, Phenylpropanoid pathway

Posted Date: November 13th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7838786/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at Plant and Soil on October 15th, 2026. See the published version at https://doi.org/10.1007/s11104-026-09138-7.

Abstract

Aims

Root-associated beneficial microbes orchestrate systemic defense priming in plants, yet the underlying metabolism-mediated plant-microbe interplay remains poorly understood.

Methods

Here, it was demonstrated that synthetic bacterial consortia (*Bacillus velezensis* YY13, *B. subtilis* JN1, and *Pseudomonas chlororaphis* JN72) colonizing tomato roots confer resistance against Fusarium wilt. Untargeted metabolomics and transcriptional reprogramming analysis were employed. The impact of root-exuded flavonoids on the rhizosphere soil microbiome was also investigated.

Results

Rhizosphere soil colonization by the Syncoms reduced disease severity by 33.49% and enhanced biomass accumulation. Untargeted metabolomics revealed systemic alterations in phenylpropanoid derivatives, with roots showing elevated feruloyltyramine glycosides (2.1-fold) and leaves accumulating quercetin-O-rutinoside (1.8-fold), while redirecting carbon flux from lignin precursors to antifungal metabolites. Transcriptional reprogramming exhibited spatiotemporal specificity, with the early upregulation of PAL/4CL/CHS in roots preceding F3H activation in leaves, thereby synchronizing JA/ET-mediated PR gene induction. Crucially, root-exuded flavonoids (naringenin, quercetin) reshaped rhizosphere soil microbiomes, enriching Actinobacteria (27%) and recruiting plant-protective genera (*Bacillus*, *Pseudomonas*). This restructured microbiota amplified defense priming through JA/ET-PR positive feedback loops.

Conclusions

Our findings unveil a tripartite defense mechanism where microbial consortia 1) reprogram phenylpropanoid channeling to prioritize defensive metabolites over structural polymers, 2) elicit tissue-specific immune transcription, and 3) sustain resistance via flavonoid-mediated microbiome recruitment. This phyto-microbial loop paradigm advances the design of synthetic communities for microbiome-assisted crop protection.

1. Introduction

An array of pathogenic microbes consistently poses challenges to plants. The tomato, a crucial economic crop worldwide, is widely cherished by consumers for its rich nutritional benefits, including vitamins and lycopene. However, its cultivation faces numerous obstacles, such as fluctuations in soil

microbial populations, which can lead to severe plant diseases and substantial losses in yield. Among the most damaging of these are soil-dwelling plant pathogens, particularly the wilt fungi *Fusarium oxysporum*, which represent a significant threat to agricultural productivity(Jin et al., 2024). At present, effective treatments to counter infections caused by fungal wilt pathogens are lacking, primarily due to their resilience in the soil, broad host range, and diverse strategies for infection(Gómez-Lama Cabanás C and Mercado-Blanco J., 2025). Consequently, managing such diseases remains a complex challenge. Traditionally, the reliance on chemical pesticides has been the primary strategy, but this approach introduces several issues, including diminished soil biodiversity, the development of pathogen resistance, and the persistence of pesticide residues in the environment. In light of this, farmers are increasingly being encouraged to reduce their dependence on chemical solutions and adopt more environmentally friendly cultivation practices. Biological solutions have gained considerable attention worldwide, owing to their environmental advantages, ease of application, and potential to offer long-lasting and effective protection(Gómez-Lama Cabanás C and Mercado-Blanco J., 2025).

Healthy plants host a vast diversity of commensal microbiota in the rhizosphere soil, including bacteria and fungi, which would greatly benefit plant growth and health(Teixeira et al.,2019; Zhou et al.,2023). For instance, when sugar beet and tomato plants encounter soil-borne pathogens such as *Rhizoctonia solani* or *Ralstonia solanacearum*, they enlist the help of native *Flavobacterium* rhizobacteria to combat these wilt-inducing threats(Russ et al.,2024; Wei et al.,2019; Yin et al.,2022). Numerous mutualistic microbes, primarily from the rhizospheric genera *Bacillus*, *Pseudomonas*, *Trichoderma*, and *Streptomyces*, have been shown to provide substantial protection to host plants and hold promise for controlling soil-borne diseases(Wei et al.,2019; Trivedi et al.,2017). Consequently, when developing antagonists or probiotic biocontrol agents, it is crucial to consider these plant microbial symbionts, as they function as extensions of the plant’s defense system against biotic challenges. The significance of these microorganisms in disease prevention is highlighted by evidence that transferring rhizosphere soil microbiota from resistant plant root soils can enhance disease-suppressive activities in susceptible plants(Kwak et al.,2018). Conversely, the natural resistance properties of suppressive soils often wane following sterilization(Chialva et al.,2018). It is essential to understand that the role of individual microbial strains in plant defense can be limited within soil ecosystems, given the intricate nature of microbial communities. Recent research on plant microbiomes underscores the significance of core consortia and keystone taxa associated with plants, which play a crucial role in providing sustained resistance against pathogens and reducing disease incidence(Jeon et al.,2022). While advancements in the ecological functions of plant microbiomes are increasingly informed by metagenomic approaches, it is still necessary to complement these insights with the functional identification of specific community members.

Bacterial root commensals utilize a multifaceted approach to help plants adapt to adverse environmental changes. These mechanisms range from the direct release of metabolites that confer resistance, modification of the plant's specialized metabolism, and the indirect induction of systemic resistance, to alterations in the microbial community's structure(Etalo et al.,2018; Carlson et al.,2020; Huang et al.,2019). Remarkably, these microbes can trigger a cascade of responses within plants, from

signal perception to metabolic shifts, which mitigate disease pathogenicity and protect against biotic stresses(Rodriguez et al.,2019). Metabolism-mediated beneficial interactions with microbes often trigger the synthesis of a diverse array of metabolites in plants. The functions and significance of individual metabolites in plant-microbe interactions underscore the complexity of these relationships and the intricacies of plant metabolism(Wen et al.,2023). This metabolic process operates as a finely tuned and dynamic system that responds sensitively to external environmental stimuli. The root endophytic bacterium *Enterobacter* sp. SA187 has been shown to reprogram the sulfur regulon in *Arabidopsis* plants under salt stress conditions(Andrés-Barrao et al.,2021). This bacterium orchestrates the regulation of sulfur metabolic pathways, potentially representing a common mechanism utilized by various beneficial microbes to mitigate the negative impacts of abiotic stressors on their host plants. Plant growth-promoting rhizobacteria (PGPR) are known to stimulate a systemic activation of the plant's secondary metabolism through their microbe-associated molecular patterns (MAMPs) (Liu et al.,2020). Notably, *Pseudomonas fluorescens* acts as an effective catalyst for enhancing isoflavone metabolism and accumulation in soybean seedlings, providing protection against the pathogen *Xanthomonas axonopodis*(Algar et al.,2014). This interaction elucidates the link between the isoflavone profile and the systemic response triggered by the bacteria.

Additionally, a study employing a metabolomics approach revealed that the rearrangement of primary and secondary metabolism in sorghum seedlings could indicate a primed state, preparing them for a reaction to fungal infection by *Colletotrichum sublineolum* as a result of *Paenibacillus alvei* inoculations(Tugizimana et al.,2019). Thus, understanding the precise interactions between microbes and plants that modify plant metabolic flexibility could be harnessed to develop effective phyto-disease management strategies.

In this study, pot experiments were conducted under controlled laboratory conditions to explore the tripartite interactions within the rhizosphere soil and the effects of synthetic microbial communities (SynComs) on multi-parametric metabolic reprogramming related to induced systemic resistance (ISR) and plant priming. Thes et al.,2019e experiments aimed to elucidate the mechanisms through which belowground bacterial commensals influence tomato plants' resistance responses, underpinned by extensive metabolomic profiling. Additional analyses sought to clarify how certain elevated metabolites modify and reshape the structure and function of rhizosphere soil bacterial communities, as well as their potential implications for plant health. Key defensive metabolites, along with associated genes, pathways, and microbial biomarkers underpinning the induced defense mechanisms against Fusarium wilt disease, were identified through integrated phenotypic, phytochemical, biochemical, and molecular examinations. These findings may pave the way for the development of future crop protection strategies by harnessing the induction of root defenses through the application of beneficial inducers.

2. Materials and Methods

2.1 Strains, plant material, and growth conditions

The bacterial strains used in this study, including *Bacillus velezensis*, *Bacillus subtilis*, and *Pseudomonas chlororaphis*, were provided by Dalian Polytechnic University. All strains were routinely cultured overnight at 25°C in sterile Luria–Bertani (LB) liquid medium. The fungal pathogen *Fusarium oxysporum* was incubated in PDA medium at 25°C and 150 rpm for one week, and the conidia suspension at the desired concentration of 1×10^6 spores/ml was prepared as previously described(Zhao et al.,2023). Tomato seeds (*Solanum lycopersicum* L. cv Zhongshu No. 4) were surface-disinfected in 3% (v/v) sodium hypochlorite for 10 min and then washed thoroughly with sterile distilled water. Seeds were placed in Petri dishes on filter paper moistened with pure water to germinate at 25°C until the cotyledons appeared. After 2–3 days, the seedlings were transferred to pots filled with sterilized soil and were grown in a greenhouse under constant conditions (12/12 h light /dark cycle, 22–24°C). The plants at different stages of growth (4 to 8 weeks) were grouped for the follow-up analyses of bacterial colonization, metabolite, and gene expression.

2.2 Measurements of bacterial rhizosphere soil colonization ability

In order to evaluate the bacterial colonization efficiency of plant root tissues, the rhizoplane plus rhizosphere soil of five random plants (per treatment) was collected on the 2nd, 4th, and 6th day after pre-inoculation of seedlings with bacteria. 1 g of soil was dissolved in 0.9% NaCl to make a series of dilutions, and 100 µL of each dilution was transferred and spread on a solid LB agar plate. The plates were inverted and incubated overnight at 37°C. The number of bacterial colonies was counted, and the colonization rate was calculated as log CFU·g^{-1} dry weight of rhizosphere soil. Furthermore, the bacterial isolates were characterized and identified by 16S rRNA gene sequencing.

2.3 Bacterial treatment, experimental design, and disease development estimate

Inocula were prepared by harvesting bacterial cells of *B. velezensis*, *B. subtilis*, and *P. chlororaphis*, which were then mixed in a 1:1:1 ratio to create a bacterial suspension. The final concentration of this suspension was approximately 10^7 cells·mL^{-1}. The study was structured with four experimental groups: Group I (CK): Untreated control with sterile water. Group II (BA): Seedlings treated with 20 mL of bacterial suspension. Group III (BF): Plants irrigated with 20 mL of bacterial suspension at the root level and dually inoculated with *F. oxysporum* after 5 days. Group IV (Fol): Infected only with *F. oxysporum*, without prior BA treatment. The root-irrigation method with the bacterial solution was applied to one-month-old tomato plants as a pre-treatment(Zhou et al.,2022). Each treatment was replicated three times, with each replicate consisting of at least five individual seedling pots, and samples were collected at the same time.

After 15 days of inoculation with *F. oxysporum*, various growth parameters and disease indices of tomato plants were assessed, measured/monitored. These included plant height, wet weight, dry weight, and the disease severity index (DSI). Disease incidence was monitored using a graded counting method, with the

following levels defined: Level 0 (L = 0): No foliar chlorosis and no wilting symptoms. Level 1 (L = 1): Less than 10% of the leaf area showing chlorosis. Level 2 (L = 2): 10%-50% of the leaf area with severe chlorosis and initial wilting phenotype. Level 3 (L = 3): 50%-90% of the leaf area with serious chlorosis and wilted symptoms. Level 4 (L = 4): More than 90% of the seedlings withered and were completely necrotic. The disease symptoms level (L) represents the severity of the plant disease. The DSI was calculated using the following formula. For each sample, three plants were harvested, each serving as an independent biological replicate.

Disease severity index(%)=∑(L*The number of infected plants at each level)/(Total plants observed* Representative value at the highest level)*100%

2.4 Metabolomics profiling and quantitative analysis of metabolites by HPLC-MS/MS

Tomato seedlings from each group were harvested at 48 hours post-treatment. Roots stems, and leaves were repeatedly rinsed with deionized water to remove surface impurities. Tissue samples were frozen in liquid nitrogen and ground to obtain powder. Rhizosphere soil was collected from a depth of 5 cm surrounding tomato roots. Metabolites were extracted by adding 1.2 mL of 80% methanol to 50 mg of the sample. The mixture was vortexed for 30 s, and this process was repeated six times. All the extracts were centrifuged at 12,000×g for 3 minutes, filtered using a 0.22 μm filter, and stored at -20°C for subsequent HPLC-MS/MS analysis(Seybold et al.,2020).

Metabolites were detected by Q Exactive Plus high-performance liquid chromatography mass spectrometry using an analytical Unitary C18 column (4.6 × 250 mm, 5 μm). The mobile phase A consisted of 0.1% aqueous formic acid-acetonitrile(5:95 v/v), and the mobile phase B was acetonitrile (0.1% formic acid). The gradient elution procedure was set as follows: the proportion of eluent A decreased from 100% to 72% within 0–12 min, then from 72% to 63% during 12–18 min, and further from 63% to 35% within 18–20 min. The injection volume was 5 μL, and the column temperature was maintained at 35°C. Isocratic elution with eluent A was carried out at a flow rate of 1.0 mL·min⁻¹. All information on MS data was obtained by the ESI-Q-Orbitrap MS in both positive and negative ion modes. The monitoring conditions were as follows: $m \cdot z^{-1}$ range of 50 to 1500 Da, spray voltage: 3.0 kV; Dry gas velocity is 10 $mL \cdot min^{-1}$, ion transport tube temperature is 350 ℃. The relative quantification was carried out by the area normalization method.

2.5 Metabolite identification, metabolic pathway, and network analyses

Metabolite identification was streamlined by exporting data from MarkerLynx to Taverna for PUTMEDID_LCMS Metabolite ID workflows. These workflows, which are designed for the automated and high-throughput annotation and identification of putative metabolites from LC-ESI-MS metabolomic data, include correlation analysis, metabolic feature annotation, and metabolite annotation being performed. The data matrix was tailored to Taverna's specifications, and the Metabolite ID procedure was executed

through three workflows: (i) Pearson correlation analysis to evaluate variable relationships; (ii) metabolic feature annotation, which involved grouping ion peaks by characteristics such as retention time and annotating features with calculated elemental compositions/molecular formulas; and (iii) metabolite annotation, where the calculated molecular formulas were matched against a reference file of metabolites. To enhance annotation accuracy, selected metabolite candidates were manually verified against databases including DNP, Chemspider, PlantCyc, Knapsack, and KEGG, with structural confirmation through MS1 and MSE spectra analysis. Metabolites were annotated to level 2 as per the Metabolomics Standard Initiative, and their presence and abundance were visualized using PCA score plots generated by SIMCA software.

Ingenuity Pathway Analysis (IPA) was employed for metabolic pathway analysis on metabolites identified by OPLS-DA with MetaboAnalyst's MetPA tool. This analysis leveraged KEGG pathways to identify and visualize affected metabolic pathways(Seybold et al.,2020). Metabolites were assessed for biological roles through enrichment analysis, utilizing a hypergeometric test algorithm and topological analysis based on betweenness centrality. Statistical p-values were adjusted using Holm-Bonferroni and false discovery rate methods. Correlation-network analysis was conducted to explore metabolite associations, with biochemical and chemical similarity networks constructed using MetaMapR and Cytoscape 3.5.0, respectively. Structural similarities were determined by PubChem Substructure Fingerprints, with a Tanimoto coefficient threshold set at 0.7 for network visualization and characteristic mapping to integrate chemometric modeling information.

2.6 Determination of total phenolic and total flavonoid content

The total phenolic content of samples was measured using the Folin-Ciocalteu colorimetric method (Dominguez-López et al.,2024). Initially, the plant extracts were dissolved in absolute methanol to prepare the samples. A 0.2 mL aliquot of the sample, diluted with deionized water, was mixed with 0.2 mL of the Folin-Ciocalteu reagent. The mixture was vortexed and allowed to rest at room temperature for 6 min. Subsequently, 2 mL of a 7% Na_2CO_3 was added. The mixture was vortexed again and then incubated at room temperature in a dark place for 90 min. The absorbance of the reaction mixture was measured at 765 nm using a spectrophotometer. The results were expressed as grams of gallic acid equivalent (GAE) per 100 g of plant extract. The analysis was performed in triplicate for each sample.

The total flavonoid content in the samples was measured using the colorimetric method. Firstly, 0.3 mL of 5% $NaNO_2$ was added to a mixture containing 1 mL of the diluted sample and deionized water. After vortexing, the mixture was incubated at room temperature for 5 minutes. Subsequently, 0.3 mL of 10% $AlCl_3$ was added. The mixture was vortexed again and then incubated at room temperature for an additional 6 minutes. Finally, 4 mL of 1 $mol \cdot L^{-1}$ NaOH was added, followed by the addition of distilled water to adjust the final volume of the mixture to 10 mL. The absorbance was read at 510 nm using a spectrophotometer, and the results were expressed as grams of quercetin equivalents (QE) per 100 g of plant extract. The analysis was performed in triplicate for each sample.

2.7 Expression analysis of defense-related genes in tomato

The total RNA was extracted from tomato tissues (roots, stems, and leaves) harvested at 12, 24, 48, and 72 h after SynComs inoculation. First-strand cDNA was synthesized as described previously(Jin et al.,2022). The relative expression levels of defense-related genes were analyzed by quantitative real-time PCR (qRT-PCR). The gene-specific primers are listed in Supplementary Table 1. The reaction conditions were as follows: 5 min at 95 ◦C; 40 cycles of 20 s at 95 ◦C, 15 s at 50 ◦C, and 15 s at 72 ◦C; and 10 min at 72 ◦C. The ACTIN gene was assayed as the reference gene. All experiments were performed in triplicate. The fold change and relative expression of these genes were calculated by the $2^{-\Delta\Delta CT}$ method.

2.8 Rhizosphere soil microbiome analysis by 16S rRNA amplicon sequencing

Each rhizosphere soil sample was homogenized in a blender for 10 seconds, and total soil DNA was extracted from 0.25 g of the samples using the Power Soil DNA Isolation Kit. The universal primer pair 515F (5'-GTGCCAGCMGCCGCGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') was employed to amplify the V4 regions of bacterial 16S rRNA genes(Zhao et al.,2023). Phusion® High Fidelity PCR Master Mix with GC Buffer was utilized for all PCR amplifications. Following amplification, the PCR products were combined with an equal volume of 1×loading buffer and electrophoresed on 2% agarose gels for detection. Subsequently, these products were equimolarly mixed and purified employing a Gel Extraction Kit. Libraries for sequencing were prepared using the Ion Plus Fragment Library Kit, and their quality was evaluated with a Qubit Fluorometer. High-throughput sequencing was conducted on an Ion S5TM XL platform, yielding single-end reads.

2.9 Data Processing and Analysis

Metabolomic data analysis was conducted using Xcalibur, with the qualitative outcomes visualized as donut charts in Origin 2022 and analyzed using UP-SET with R (V4.31). The quantitative findings were depicted as heatmaps with R (V4.31) and were processed through PCA, principal component analysis in Origin 2022. Sequencing data were demultiplexed according to Barcode sequences, leading to the removal of Barcode and primer sequences, followed by the merging and quality filtering of reads with FLASH (V1.2.7). The detection and elimination of chimera sequences resulted in the final dataset, which was then clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using the Uparse algorithm, with the selection of representative sequences based on their occurrence frequency. These sequences were matched against the SILVA database using Mothur for species annotation at an 0.8 confidence level to ascertain the taxonomic composition of the community. The phylogenetic affiliations of OTUs from soil samples were investigated via multiple sequence alignment with MUSCLE (V3.8.31), and the data were normalized to the sample with the minimal dataset for alpha diversity analysis, with the indices being computed using QIIME (V1.7.0).

3. Results

3.1 Root-colonizing bacterial consortia induce disease resistance in tomato to rescue plants from Fusarium wilt

The effective colonization of bacterial root microbiota and their persistence throughout the plant's growth is critical for enhancing plant fitness and providing protection against various biotic and abiotic threats. Here, a collection of root-**derived dominant** bacteria, which were previously isolated from tomato and other healthy plants' rhizosphere soil by our lab, was used to engineer and construct bacterial synthetic consortia. Two native bacteria of *Bacillus* sp. (*B. velezensis* YY13, *B. subtilis JN*1) and one strain of *Pseudomonas chlororaphis* JN72 were individually inoculated into the rhizosphere soil of tomato seedlings grown in natural potting soil. The functional dynamics of bacterial communities in both inoculated and non-inoculated soils, as well as their correlation with the disease index of tomato wilt, were examined. Firstly, bacterial re-isolation was conducted, and the colonization efficiency of the plant root was estimated quantitatively (CFU) in a time-course experiment that spanned over 6 days post-inoculation (dpi) (Fig. 1A). All three strains were retrieved at high frequencies by the second day, which signifying their successful survival in the tomato rhizoplane and their robust root colonization capabilities. Among them, *B. subtilis* strain ZJ1 demonstrated exceptional adaptation to the rhizosphere soil environment, heavily colonizing the roots and maintaining high levels of presence up to 6 dpi (10^5 CFU·g^{-1} root FW). Following this, the effect of the bacterial inoculum pre-treatment, administered 2 days before pathogen inoculation, on the resistance of tomato seedlings was further confirmed. Figure 1B-E illustrates that the simplified bacterial core consortium moderately alleviated the disease severity index (DPI) of tomato seedlings by 33.49%, concurrent with a strong increase in biomass. Thus, it was revealed that tomato plants colonized by potentially protective commensal bacteria exhibited the most resistant phenotype, which aligns with bacteria-induced extended defence responses.

Plant beneficial microbes, typically drawn from the root- and rhizosphere-inhabiting microbiome, are known for their plant growth-promoting properties and biological control activities to protect the host from environmental stresses. In recent years, research has intensively explored a variety of individual cultured bacterial species, their positive impacts, and their ecological function on host plants. However, it poses challenges to fully exploit the interaction between the microbial populations and plants because of community complexity. To decipher the issues, it is essential to simplify the consortia structure and create surrogate microbiomes by selecting a few representative bacterial isolates for direct evaluation of microbial community function(Zhou et al.,2022; Li et al.,2021). Previous studies have reported that experimental inoculations of *Nicotiana attenuata* with a root-associated bacterial consortium consisting of five strains can reduce symptoms of Fusarium wilt disease(Santhanam et al.,2015). Indigenous soils harboring microbiotas elicit an alert state in tomato plants and hence suppress *Fusarium* pathogens, which reflects that microbial mutualistic associations with plants contribute to improving host stress resilience(Chialva et al.,2018). Meanwhile, it has been demonstrated that initial differences in the composition and function of the tomato root microbiome may predetermine future plant health and

disease progression, and specifically, certain microbial taxa are associated with the suppression of bacteria-mediated phytopathogen(Wei et al.,2019). In the current study, a greatly simplified bacterial community dominated by *Bacillus* spp. and *Pseudomonas* sp. members was shown to partially rescue tomato seedlings from soil-borne disease invasion. This finding underscores the potential of selected beneficial microbes to mitigate the impact of pathogens and highlights the importance of microbial community simplification in understanding and applying plant-microbe interactions for plant health management.

in the tomato rhizosphere at 2, 4, 6 dpi. B-E. The plant growth-promoting abilities and the

suppression effects on Fusarium wilt disease by inoculation of tomato roots with bacterial

consortia. n = 6. BS(*Bacillus subtilis*)、BV(*B. velezensis*)、PC(*Pseudomonas chlororaphis*).

Control (CK); BA (Bacteria); BF (Bacteria and Fusarium); FOL (Fusarium).

Consistent use of abbreviations is maintained throughout this manuscript.

3.2 Root bacterial microbiota elicit altered metabolomic states of flavonoids and hydroxycinnamic acids in tomato whole plants

Plant metabolism and root exudation are generally affected by environmental factors like biotic stresses of pathogen invasion. However, it remains largely undetermined how root microbial commensals trigger plant metabolome effects and metabolic adjustment to combat plant disease. Furthermore, compared to the intense investigation of above-ground tissue metabolism, very limited information is available on whole-plant metabolic responses and their biological impacts induced by beneficial microbiotas at the systemic level. To address this, the metabolome profiling of hydromethanolic extracts of tomato seedlings growing in potting systems in contrasting treatment groups was analyzed by untargeted HPLC-MS/MS, and a comparative metabolome was conducted to identify potential antifungal metabolites in the whole tomato plant. A total of 39 discrete chromatographic peaks of key compounds were detected, corresponding to 9 flavonoids, 26 hydroxycinnamic acids (HCAs), 3 coumarins, and 1 amino acid in tomato leaf, stem, root, and exudate. Most of them were annotated to plant secondary metabolites based upon the retention times, $m{\cdot}z^{-1}$ values, and fragmentation patterns. The metabolic alterations observed in chemically diverse metabolites encompassed various classes of compounds. Representative components within the biosynthetic pathways of plant phenylpropanoids, flavonoids, and HCA derivatives and their conjugates are detailed in Fig. S2. The divergence of metabolite profiles is minor between bacterized tomato seedlings and negative control (Fig. 2A), except that naringenin, kaempferol-3-rutinoside, feruloyl-quinic acid, 5-caffeoylquinic acid, ferulic acid-hexose, feruloyltyramine, feruloyltyramine glycoside, and caffeoylputrescine glycoside are active in certain treatment groups. The constitutive levels and specialized responses of metabolites are ranked in order of precedence among leaf, stem, root, and exudate. A large part of metabolites differentiate in four plant parts (Fig. S1), and nine compounds of naringenin, quercetin, 7-hydroxycoumarin, 3-caffeoylshikimic acid, N-

caffeoylputrescine, sinapoyl glycoside, chlorogenic acid, and 3-, 4-caffeoylquinic acid are shared by all parts. Only one quercetin derivative of quercetin-hexose particularly exists in the above-ground rather than below-ground, while feruloyltyramine, coumaroyloctopamine isomer, feruloyloctopamine, isorhamnetin pentoside, and myricetin-dimethyl ether are detected in the below-ground. Moreover, one component of HCAs, including feruloylmethoxytyramine, is specifically found in roots. Furthermore, the PCoA score plot, calculated using the Bray-Curtis dissimilarity index, reveals differences in the main chemical compositions among tomato plant treatment groups. The variance percentage indicates that the treatment grouping factor accounts for the metabolites variation in exudate, whereas it explains a certain implication on the metabolome in the leaf, stem, and root (Fig. 2B). Together, despite there being no regular pattern to follow for composition variation, contributions from root consortia cannot be ruled out.

Evidence is gradually accumulating and demonstrates that a community of beneficial rhizospheric microbiota may specifically modulate the production of plant secondary metabolites and defensive compounds. A prime example of this is that the rhizosphere soil microbiome chemically induces reprogramming of root metabolite exudation via root-to-root systemic signaling in tomato. Notably, Pseudomonadales drive ferulic acid hexose accumulation and exudation, contrasting with Bacillales mediate acyl sucrose secretion*(*Korenblum et al.,2020). However, rhizosphere soil chemistry, which mediates plants' defense responses, is still largely ignored. Plant phenylpropanoids comprise one of the major classes of important natural secondary metabolites, including flavonoids, HCA, coumarins, and lignan. Phenylpropanoids and their derivatives play a vital role in crop resistance and adaptation. These metabolites consistently act as stress-inducible antimicrobials and phytoalexins to protect plants against pathogens, and their presence and levels fluctuate in response to specific environmental stimuli(Wang et al.,2022). A study is a manifestation that the differential changes in HCA derivatives and flavonoids, induced by the application of microbial biostimulants, contribute to increased drought tolerance in maize plants(Nephali et al.,2021). On the other hand, metabolites are the end products of gene expression and serve as the ultimate recipients or beneficiaries in the flow of biological information, which can regulate the physiological state and determine the phenotypic traits of plants. Therefore, based on the potential functions of these compounds and the aforementioned metabolome analysis, biomarkers involved in establishing the pre-conditioned or primed state of tomato plants following SynComs treatment are tentatively identified. These biomarkers include two flavonol glycosides, three hydroxycinnamic acid (HCA) derivatives, two amines and their conjugates, and one aliphatic amino acid conjugate. Our observations herein indicate that differential metabolite profiles observed in tomato plants are indicative of organ-specific and treatment-dependent physiological and biological responses to microbial symbionts.

under the four treatment conditions. B. PCoA analysis of metabolites

variation of tomato plants across treated groups and the control group.

3.3 Root bacterial microbiota induce reprogramming of targeted defense-related functional metabolic web

Based on the above qualitative profiling, the relative abundances of core metabolites linked to defensive response-related pathways were further quantitatively assessed. Despite total phenolic level being universally higher in all three experimental groups compared to the control, there were no marked differences observed among the three treated plants, except for an increase in the roots of plants treated with a combination of BF (Fig. 3A-1). The measurements of total flavonoid content revealed that the apparent accumulation of flavonoids at the whole-plant level in tomatoes, especially at the 48-hour mark post-induction by BA bio-stimulation in the presence of a pathogen (Fig. 3A-2). The metabolic state alterations were characterized by the perturbation of an array of compounds. Specifically, the changes were notable in polyphenols, HCAs, and flavonoids, as well as their conjugates. Among these, the antifungal compounds stood out, such as rutin (quercetin-O-rutinoside), naringenin, quercetin, 7-hydroxycoumarin, chlorogenic acid, and caffeoylquinic acid (Fig. 3B). These metabolites showed a differential accumulation, which was noticeable in at least one comparison group (BA or BF), when contrasted with the FOL group. For instance, the levels of quercetin and 7-hydroxycoumarin in the below-ground parts of tomatoes treated with BA/BF were higher than in other comparative objects, while rutin and chlorogenic acid were found to moderately accumulate in the root exudation of BF-treated plants. Conversely, a general up-regulation in the content of iso-quercetin and quercetin hexose was noted in the above-ground parts of the plants. Unexpectedly, the amount of naringenin slightly decreased in both below-ground and above-ground tissues of tomato plants in the beneficial consortia treatment groups compared to the FOL group. The research findings illustrated that a systemic metabolic response to the advantageous microbiota in the rhizosphere soil correlated with increased resistance in tomato plants.

To comprehend how the affected defence-related metabolic processes confer the tomato plant's resistance and tolerance, the global interrelationships and pathways for the identified metabolites were analyzed. Utilizing the MetPA database and the KEGG pathway, this analysis facilitates the identification and visualization of differential metabolic pathways (Fig. 4). It is discovered that the selective modulation of microbiota-mediated metabolic changes primarily affects the restructuring of metabolic pathways. This restructuring involves the phenylpropanoid (sly00940), flavonoid (sly00941), and flavone and flavonol (sly00944) biosynthetic pathways. As a result, there is a differential accumulation of metabolites, including aromatic compounds and precursors to flavonoids. By comparing the metabolomes of BA-activated and fungal-infected tomato plants, it is demonstrated that the rhizosphere soil consortia may not induce a global shift in plant metabolism. Instead, they selectively target specific metabolites. Additionally, the microbiota-induced protective system in tomatoes is based on a multi-component response strategy, which is featured by the formation of a functional metabolic web. Key components in the defense network are mainly secondary metabolites derived from two closely interrelated and interdependent pathways: phenylpropanoid and flavonoid metabolism. These pathways are crucial for mounting a counterattack against pathogens. The most noticeable disturbance is observed in the altered phenylpropanoid pathway, as evidenced by the increased levels of phenylalanine

in the exudates and entirety of BA/BF-activated groups. Indeed, the high accumulation of 5-p-coumaroylquinic acid and feruloyltyramine glycoside, especially in the BA- or BF-treated groups, implies that the metabolic flux is redirected by the consortia induction. This redirection shifts the focus away from lignin biosynthesis and towards the production of flavonoids, flavonols, or isoflavones within the branch of the phenylpropanoid pathway. It is also supported by the enhanced accumulation levels of feruloyl glycoside both in the above-ground and below-ground parts of the tomato plants. The changes in intracellular metabolite flux, which are quantifiable outcomes corresponding to plant phenotypes, further substantiate this. Therefore, our results indicate that the metabolic reprogramming of immune-related pathways in tomato seedlings leads to the altered accumulation of specific responsive compounds. This metabolic shift is in response to bacterial consortia and is pivotal for plant defense.

B

content following microbial treatment. B. Heatmap illustrating the

comparative differential regulation of specific metabolite abundances

across various tissues in tomato plants subjected to four treatments.

of phenylpropanoid-flavonoid is differentially regulated by

Four treatments among various tissues in tomato plants.

3.4 Transcript induction of tomato defense-related genes differs by rhizosphere microbiota

Since the three beneficial functional strains have been demonstrated to have direct antifungal activities against *F. oxysporum in vitro* (the data are not presented here), the systemic transcriptional defence responses elicited by these strains were further evaluated in planta. The transcript induction of 10 defense genes in tomato tissues was determined by qRT-PCR at intervals of 12, 24, 48, and 72 hpi. The gene expression patterns were subsequently analyzed for variance across four experimental groups at distinct time points and within different plant tissues (Fig. 5). In comparison with the CK, the three treatment groups universally led to an enhanced accumulation of transcripts beyond their baseline levels during the later stages of induction, with a few exceptions noted. Data revealed that the relative expression levels of the targeted genes exhibited varying responses to single or combined treatments. However, for the majority of transcripts, the application of BA did not lead to any significant and consistent alterations in gene expression levels in the absence of pathogen challenge. The fold change ratio in the BA group was weaker relative to those treated with BF or FOL. The expression levels and patterns of the relevant genes were associated with the type of inducer and the induction time, and there were variations in expression across different parts of the plant. Various treatments resulted in certain differences in gene expression up-regulation, particularly for genes involved in ROS scavenging and the JA signaling pathways, including *POD*, *LOX*, and *PR2*. This suggests that the induction of systemic acquired resistance (SAR) in tomatoes by *F. oxysporum* primarily relied on the pathogenesis-related

protein PR2 and the jasmonic acid (JA) signaling pathway. In contrast, the induction of induced systemic resistance (ISR) in tomatoes by beneficial microbial agents was mainly dependent on both the JA and ethylene (ET) signaling pathways.

Bacterial pre-inoculation and colonization initiate systemic and localized alterations of gene expression, encompassing the host's innate defense mechanisms. Key enzymes such as PAL, 4CL, CHS, CHI, and F3H, which are pivotal in the biosynthesis of phenylpropanoids and flavonoids, show activity and transcriptional state closely linked to the accumulation of defensive compounds(Wang et al.,2022). In our study, the application of protective commensals reinforces the extent of the transcriptional defense responses and the resistant phenotype, coinciding with more phenolics and flavonoids in the treated groups. Furthermore, our findings reflect that defence genes in the root responded rapidly to the microbial induction than those in the leaf. For instance, resistance genes like *PAL*, *4CL*, *CHS*, and *CHI* begin to up-regulate only after 48 hours in leaves, whereas in roots, these genes show an earlier response. Our analyses reveal that organ-specific defense responses are activated in whole tomato seedlings to some extent, indicating a coordinated system of defense mechanisms tailored to different parts of the plant. The transcriptional responses to bacterial colonization vary in magnitude of gene induction across roots, shoots, and leaves. This variation suggests that different defensive genes and systems may be employed by above-ground and below-ground organs. Overall, this study highlights the differential regulation of tomato disease resistance and antioxidant activity in response to various treatments across different tissues. It underscores the significant role of roots in coordinating whole-plant responses to damage sensing.

disease resistance genes in tomato seedlings post-microbial treatment.

3.5 The additions of flavonoids alter the structure of the rhizosphere microbiome

To examine the influence of flavonoids present in root exudates on the bacterial communities within the tomato plant's rhizosphere soil, a controlled experiment was conducted (Fig. 6). In this study, pot soil was treated with specific concentrations of rutin, naringenin, and quercetin, at levels of 763, 340, and 378 μg g soil^{-1}, respectively. These compounds were introduced every two days for 14 days. Subsequently, the bacterial alpha diversity within the flavonoid-treated soils was assessed through the analysis of 16S rRNA amplicon sequences. The findings revealed that the application of all tested flavonoids led to an increase in both Shannon diversity and overall α-diversity indices of the soil bacterial communities. Remarkably, naringin demonstrated the most significant impact on enhancing the diversity of the rhizosphere soil microbial communities. However, it was found that the introduction of rutin to the environment led to a substantial reduction in bacterial community richness. In contrast, the exogenous additions of quercetin and naringin were correlated with an enhancement in the richness of bacteria in the rhizosphere soil. Furthermore, the relative abundance of bacteria from the phylum Actinobacteria was particularly enriched in the soils treated with the three compounds, while the abundance of the Proteobacteria and Bacteroidetes exhibited a certain degree of decline following the treatment. Genus-

level species cluster analysis revealed that the plant-protective rhizosphere soil taxa were specifically recruited by flavonoid additions, including *Bacillus* sp., *Pseudomonas* sp., *Sphingomonas* sp., *Acinetobacter* sp., and *Lysobacter* sp. However, differential enrichment of predominant bacterial species was observed among the three compound applications. These results proved that the potential role of flavonoids, and the underlying mechanism, in mediating plant-microbiota interactions, which could endorse plant disease resistance. This was achieved by uplifting the structure and functionality of the bacterial community.

Since soil is a reservoir of diverse microorganisms, including both pathogenic and beneficial types, it is necessary to identify specific metabolites that can effectively distinguish between these two groups. Flavonoids, which are stress-inducible plant metabolites, play a significant role in plant-microbe interactions(Wang et al.,2022). Naringenin, a flavonoid that functions as a signaling molecule, has been found to boost the colonization of *Azorhizobium caulinodans* in rice roots(Nouwen et al.,2019). While their role in initiating nodulation with rhizobia in legumes is well-documented, the extent to which flavonoids might also contribute to plant stress resistance by influencing non-nodulating bacteria remains largely unexplored. Flavonoids released by *Panax* roots are essential for attracting and shaping beneficial bacterial populations, which in turn alleviate and suppress soilborne root rot disease(Fang et al.,2024). Additionally, the existence of root-exuded coumarins has been demonstrated to influence root microbial diversity by either stimulating or inhibiting the proliferation of specific microorganisms(Stringlis et al.,2019). Here, the metabolite-dependent microbiome profiling was conducted, and it demonstrated that plant flavonoids were broadly conducive to the diversity of the tomato rhizosphere soil microbiome. Our findings indicate that these compounds have a preferential attraction for protective microbiota and mediate the assembly of a disease-suppressive rhizosphere soil microbiome. Consequently, it is proposed that the deployment of synthetic microbial communities (SynComs) in a synergistic manner may trigger the beneficial activity of root-secreted flavonoids, which serve as a keystone modulator recognized for its extensive connections to microbial taxa. This orchestrated interaction is believed to optimize the microbial community structure, consequently leading to an enhancement in plant and soil health, especially when facing biotic stress.

A C

diversity (B) and functional profile (C) of the bacterial microbiome

in the tomato rhizosphere.

3.6 Beneficial microbes prime plant systemic resistance and defensive pathways against *Fusarium*

Root-colonizing *Bacillus-Pseudomonas* consortia orchestrate a tripartite defense network in tomato plants through metabolic-immune-microbiota crosstalk. Upon rhizosphere soil colonization (Step 1), the synthetic community firstly triggers localized phenylpropanoid pathway activation in roots via upregulation of PAL/4CL/CHS genes (Step 2a). This metabolic reprogramming redirects carbon flux

towards antifungal phenolic biosynthesis (naringenin, chlorogenic acid) while suppressing lignin deposition (Step 2b). Systemic signaling induces leaf-specific accumulation of flavonol glycosides (quercetin-rutinoside) through F3H-mediated branch pathway activation (Step 3). Concurrently, root-exuded flavonoids (naringenin, quercetin) reshape rhizosphere soil microbiota composition by enriching Actinobacteria and recruiting plant-protective genera (*Bacillus*, *Pseudomonas*) (Step 4). The restructured microbiome may amplify defense priming through JA/ET-mediated transcriptional reinforcement of PR genes (*POD*, *LOX*) in both roots and shoots (Step 5), establishing a positive feedback loop between metabolic fortification and microbial symbiosis. The proposed tripartite interaction and defense mechanism is well-supported by empirical evidence, yet requires further refinement. The consortia likely prime systemic resistance through a phased strategy. Initial root colonization involves bacterial secretion systems (e.g., T7SS in *Bacillus amyloliquefaciens*) and iron-scavenging metabolites that enhance rhizocompetence, as demonstrated by Liu et al. (Liu et al.,2023) showing YukE protein-mediated root exudate modulation. The localized phenylpropanoid activation aligns with PAL/4CL upregulation patterns observed in root *Pseudomonas fluorescens* PTA-CT2-treated grapevine(Gruau et al.,2015), where transcriptional reprogramming shifts flux toward flavonoids while lignin biosynthesis is suppressed via CCoAOMT downregulation(Yu et al.,2025). Crucially, this metabolic trade-off strategy is corroborated by independent evidence from maize QTL studies linking phenylpropanoid gene variants to *Fusarium* resistance(Yao et al.,2020). The leaf-specific flavonol glycoside accumulation may involve F3H-mediated spatial regulation, analogous to *Arabidopsis* MYB12-driven transcriptional partitioning observed in rhizobacteria-primed *Arabidopsis* plants(Zamioudis et al.,2015). Root-exuded flavonoids exhibit dual functionality: Naringenin directly inhibits *Fusarium* hyphal growth, while quercetin enriches Actinobacteria through chemotaxis receptor activation, and enhances metabolic adaptation, as demonstrated in rhizosphere soil microbiome remodeling studies(Bag et al.,2022*).* The JA/ET-PR gene amplification mirrors *Pseudomonas simiae* WCS417-induced ISR, though the purported positive feedback loop necessitates validation—recent work on AI-2-mediated *Bacillus* biofilms suggests quorum-sensing molecules may stabilize microbial-plant metabolic dialogues(Pieterse et al.,2021; Sun et al.,2024). Key knowledge gaps persist regarding temporal coordination between metabolic reprogramming and microbiome restructuring, warranting time-resolved multi-omics approaches to disentangle causal relationships in this defense network.

defensive pathways primed by rhizospheric bacterial Syncoms

and induced systemic resistance against *Fusarium oxysporum*.

Conclusion

To elucidate mechanisms of microbe-mediated plant disease resistance, this study simultaneously investigated the comprehensive chemical and molecular defense responses triggered systemically in tomato plants following rhizobacterial inoculation. Our work demonstrates that beneficial root commensals not only modulate local root metabolism but also elicit systemic alterations in aboveground tissues, reprogramming distant leaf metabolism and defense pathways. This indicates that

root bacterial microbiota enhance plant resilience against soil-borne fungi disease by activating host-specific defense mechanisms throughout the entire plant. Collectively, the induced whole-plant resistance phenotype arises from both direct defense responses and primed resistance mechanisms, integrating local and systemic adaptations. This systemic response is characterized by coordinated transcriptional reprogramming and the accumulation of defense-related metabolites, notably polyphenols and flavonoids. Our results establish that aboveground responses to belowground microorganisms are orchestrated along a microbiota-root-shoot axis, boosting plant resistance. Future research challenges involve the identification of putative long-distance mobile metabolites and elucidating their roles in the overall resistance of the plant. Furthermore, flavonoid exudation modulates the structure of soil bacterial communities, preferentially attracting plant-protective rhizosphere soil taxa and enhancing the diversity and richness of the rhizosphere soil microbiome. This research highlights the potential of using simplified bacterial consortia to enhance plant disease resistance and underscores the intricate interactions between plant metabolites and soil microbiota in mediating plant health.

Declarations

CRediT authorship contribution statement

Zhaoxia Jin (First Author and Corresponding Author): Conceptualization, Methodology, Supervision, Formal Analysis, Writing-Original Draft; Validation, Supervision, Funding Acquisition; Xinyuan Chen: Data Curation, Investigation, Methodology; Binyan Li, Xuan Liu, Long Chen: Data Curation, Formal Analysis, Software; Fang Yu: Supervision, Funding Acquisition; Yanyan Wang, Ping Kou: Formal Analysis, Validation; Finally, we guarantee that our research papers are free of plagiarism and copyright disputes.

Acknowledgments

This study was funded by the National Natural Science Foundation of China under Grant No. 42177112.

References

1. Algar, E., Gutierrez-Mañero, F. J., Garcia-Villaraco, A., García-Seco, D., Lucas, J. A., Ramos-Solano, B., 2014. The role of isoflavone metabolism in plant protection depends on the rhizobacterial MAMP that triggers systemic resistance against *Xanthomonas axonopodis* pv. *glycines* in *Glycine max* (L.) Merr. Cv. Osumi. Plant Physiology and Biochemistry 82, 9-16. https://doi.org/10.1016/j.plaphy.2014.05.001
2. Andrés-Barrao, C., Alzubaidy, H., Jalal, R., Mariappan, K. G., de Zélicourt, A., Bokhari, A., Artyukh, O., Alwutayd, K., Rawat, A., Shekhawat, K., Almeida-Trapp, M., Saad, M. M., Hirt, H., 2021. Coordinated bacterial and plant sulfur metabolism in *Enterobacter* sp. SA187-induced plant salt stress tolerance. Proceedings of the National Academy of Sciences 118, e2107417118. https://doi.org/10.1073/pnas.2107417118

3. Bag, S., Mondal, A., Majumder, A., Mondal, S. K., Banik, A., 2022. Flavonoid-mediated selective cross-talk between plants and beneficial soil microbiome. Phytochemistry Reviews 21, 1739-1760. https://doi.org/10.1007/s11101-022-09806-3
4. Carlson, R., Tugizimana, F., Steenkamp, P. A., Dubery, I. A., Hassen, A. I., Labuschagne, N., 2020. Rhizobacteria-induced systemic resilience in *Sorghum bicolor* (L.) Moench against *Fusarium pseudograminearum* crown rot under drought stress conditions. Biological Control 151, 104395. https://doi.org/10.1016/j.biocontrol.2020.104395
5. Chialva, M., Salvioli di Fossalunga, A., Daghino, S., Ghignone, S., Bagnaresi, P., Chiapello, M., Novero, M., Spadaro, D., Perotto, S., Bonfante, P., 2018. Native soils with their microbiotas elicit a state of alert in tomato plants. New Phytologist 220, 1296-1308. https://doi.org/10.1111/nph.15014
6. Dominguez-López, I., Pérez, M., Lamuela-Raventós, R. M., 2024. Total (poly)phenol analysis by the Folin-Ciocalteu assay as an anti-inflammatory biomarker in biological samples. Critical Reviews in Food Science and Nutrition 64, 10048-10054. https://doi.org/10.1080/10408398.2023.2220031
7. Etalo, D. W., Jeon, J.-S., Raaijmakers, J. M., 2018. Modulation of plant chemistry by beneficial root microbiota. Natural Product Reports 35, 398-409. https://doi.org/10.1039/c7np00057j
8. Fang, H., Guo, C., Mei, X., Hao, M., Zhang, J., Luo, L., Liu, H., Liu, Y., Huang, H., He, X., Zhu, Y., Yang, M., Zhu, S., 2024. Light stress elicits soilborne disease suppression mediated by root-secreted flavonoids in *Panax notoginseng*. Horticulture Research 11, uhae213. https://doi.org/10.1093/hr/uhae213
9. Gómez-Lama Cabanás C, Mercado-Blanco J., 2025. Groundbreaking technologies and the biocontrol of fungal vascular plant pathogens. J Fungi (Basel) 11, 77. https://doi.org/10.3390/jof11010077
10. Gruau, C., Trotel-Aziz, P., Villaume, S., Rabenoelina, F., Clément, C., Baillieul, F., Aziz, A., 2015. *Pseudomonas fluorescens* PTA-CT2 triggers local and systemic immune response against *Botrytis cinerea* in grapevine. Molecular Plant-Microbe Interactions® 28, 1117-1129. https://doi.org/10.1094/MPMI-04-15-0092-R
11. Huang, A. C., Jiang, T., Liu, Y.-X., Bai, Y.-C., Reed, J., Qu, B., Goossens, A., Nützmann, H.-W., Bai, Y., Osbourn, A., 2019. A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. Science 364, eaau6389. https://doi.org/10.1126/science.aau6389
12. Jeon JS., Rybka D., Carreno-Quintero N., De Vos R., Raaijmakers JM., Etalo DW.,2022. Metabolic signatures of rhizobacteria-induced plant growth promotion. Plant Cell Environ45, 3086-3099. https://doi.org/10.1111/pce.14385
13. Jin, X., Jia, H., Ran, L., Wu, F., Liu, J., Schlaeppi, K., Dini-Andreote, F., Wei, Z., Zhou, X., 2024. Fusaric acid mediates the assembly of disease-suppressive rhizosphere microbiota via induced shifts in plant root exudates. Nature Communications 15. https://doi.org/10.1038/s41467-024-49218-9
14. Jin, Z., Zhu, Q., Guo, Y., Xing, R., Wang, Y., Zhang, Y., Gao, X., Yu, F., 2022. Functional characterization of secologanin synthase-like homologous genes suggests their involvement in the biosynthesis of diverse metabolites in the secoiridoid biosynthetic pathway of *Camptotheca acuminata* Decne.

International Journal of Biological Macromolecules 222, 2594-2602. https://doi.org/10.1016/j.ijbiomac.2022.10.042

15. Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Massalha, H., Meir, S., Rogachev, I., Aharoni, A., 2020. Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. Proceedings of the National Academy of Sciences 117, 3874-3883. https://doi.org/10.1073/pnas.1912130117
16. Kwak, M.-J., Kong, H. G., Choi, K., Kwon, S.-K., Song, J. Y., Lee, J., Lee, P. A., Choi, S. Y., Seo, M., Lee, H. J., Jung, E. J., Park, H., Roy, N., Kim, H., Lee, M. M., Rubin, E. M., Lee, S.-W., Kim, J. F., 2018. Rhizosphere microbiome structure alters to enable wilt resistance in tomato. Nature Biotechnology 36, 1100-1109.https://doi.org/10.1038/nbt.4232
17. Li, Z., Bai, X., Jiao, S., Li, Y., Li, P., Yang, Y., Zhang, H., Wei, G., 2021. A simplified synthetic community rescues *Astragalus mongholicus* from root rot disease by activating plant-induced systemic resistance. Microbiome 9,217. https://doi.org/10.1186/s40168-021-01169-9
18. Liu, H., Brettell, L. E., Qiu, Z., Singh, B. K., 2020. Microbiome-mediated stress resistance in plants. Trends in Plant Science 25, 733-743. https://doi.org/10.1016/j.tplants.2020.03.014
19. Liu, Y., Shu, X., Chen, L., Zhang, H., Feng, H., Sun, X., Xiong, Q., Li, G., Xun, W., Xu, Z., Zhang, N., Pieterse, C. M. J., Shen, Q., Zhang, R., 2023. Plant commensal type VII secretion system causes iron leakage from roots to promote colonization. Nature Microbiology 8, 1434-1449. https://doi.org/10.1038/s41564-023-01402-1
20. Nephali, L., Moodley, V., Piater, L., Steenkamp, P., Buthelezi, N., Dubery, I., Burgess, K., Huyser, J., Tugizimana, F., 2021. A metabolomic landscape of maize plants treated with a microbial biostimulant under well-watered and drought conditions. Frontiers in Plant Science 12,676632. https://doi.org/10.3389/fpls.2021.676632
21. Nouwen, N., Gargani, D., Giraud, E., 2019. The modification of the flavonoid naringenin by *Bradyrhizobium* sp. strain ORS285 changes the nod genes inducer function to a growth stimulator. Molecular Plant-Microbe Interactions® 32, 1517-1525. https://doi.org/10.1094/mpmi-05-19-0133-r
22. Pieterse, C. M. J., Berendsen, R. L., de Jonge, R., Stringlis, I. A., Van Dijken, A. J. H., Van Pelt, J. A., Van Wees, S. C. M., Yu, K., Zamioudis, C., Bakker, P. A. H. M., 2021. *Pseudomonas simiae* WCS417: star track of a model beneficial rhizobacterium. Plant and Soil 461, 245-263. https://doi.org/10.1007/s11104-020-04786-9
23. Rodriguez, P. A., Rothballer, M., Chowdhury, S. P., Nussbaumer, T., Gutjahr, C., Falter-Braun, P., 2019. Systems biology of plant-microbiome interactions. Molecular Plant 12, 804-821. https://doi.org/10.1016/j.molp.2019.05.006
24. Russ, L., Andreo Jimenez, B., Nijhuis, E., Postma, J., 2024. *Rhizoctonia solani* disease suppression: addition of keratin-rich soil amendment leads to functional shifts in soil microbial communities. FEMS Microbiology Ecology 100,24. https://doi.org/10.1093/femsec/fiae024
25. Santhanam, R., Luu, V. T., Weinhold, A., Goldberg, J., Oh, Y., Baldwin, I. T., 2015. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous

cropping. Proceedings of the National Academy of Sciences 112,5013-5020. https://doi.org/10.1073/pnas.1505765112

26. Seybold, H., Demetrowitsch, T. J., Hassani, M. A., Szymczak, S., Reim, E., Haueisen, J., Lübbers, L., Rühlemann, M., Franke, A., Schwarz, K., Stukenbrock, E. H., 2020. A fungal pathogen induces systemic susceptibility and systemic shifts in wheat metabolome and microbiome composition. Nature Communications 11, 1910. https://doi.org/10.1038/s41467-020-15633-x
27. Stringlis, I. A., de Jonge, R., Pieterse, C. M. J., 2019. The age of coumarins in plant-microbe interactions. Plant and Cell Physiology 60, 1405-1419. https://doi.org/10.1093/pcp/pcz076
28. Sun, J., Nie, L., Xie, W., Zheng, X., Zhou WW.2024. Potentiation effect of the AI-2 signaling molecule on postharvest disease control of pear and loquat by *Bacillus amyloliquefaciens* and its mechanism. Food Chemistry 441, 138373. https://doi.org/10.1016/j.foodchem.2024.138373
29. Teixeira, P.J.P., Colaianni, N.R., Fitzpatrick, C.R., Dangl, J.L., 2019. Beyond pathogens: microbiota interactions with the plant immune system. Current Opinion in Microbiology 49, 7-17. https://doi.org/10.1016/j.mib.2019.08.003
30. Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hamonts, K., Anderson, I. C., Singh, B. K., 2017. Keystone microbial taxa regulate the invasion of a fungal pathogen in agro-ecosystems. Soil Biology and Biochemistry 111, 10-14. https://doi.org/10.1016/j.soilbio.2017.03.013
31. Tugizimana, F., Steenkamp, P.A., Piater, L.A., Labuschagne, N., Dubery, I.A.,2019. Unravelling the metabolic reconfiguration of the post-challenge primed state in *Sorghum bicolor responding* to *colletotrichum sublineolum* infection. Metabolites 9,194. https://doi.org/10.3390/metabo9100194
32. Wang, L., Chen, M., Lam, P.-Y., Dini-Andreote, F., Dai, L., Wei, Z., 2022. Multifaceted roles of flavonoids mediating plant-microbe interactions. Microbiome 10,233. https://doi.org/10.1186/s40168-022-01420-x
33. Wei, Z., Gu, Y., Friman, V.-P., Kowalchuk, G. A., Xu, Y., Shen, Q., Jousset, A., 2019. Initial soil microbiome composition and functioning predetermine future plant health. Science Advances 5, eaaw0759. https://doi.org/10.1126/sciadv.aaw0759
34. Wen, T., Xie, P., Liu, H., Liu, T., Zhao, M., Yang, S., Niu, G., Hale, L., Singh, B. K., Kowalchuk, G. A., Shen, Q., Yuan, J., 2023. Tapping the rhizosphere metabolites for the prebiotic control of soil-borne bacterial wilt disease. Nature Communications 14, 4497. https://doi.org/10.1038/s41467-023-40184-2
35. Yao, L., Li, Y., Ma, C., Tong, L., Du, F., Xu, M., 2020. Combined genome-wide association study and transcriptome analysis reveal candidate genes for resistance to *Fusarium* ear rot in maize. Journal of Integrative Plant Biology 6, 1535-1551. https://doi.org/10.1111/jipb.12911
36. Yin, J., Zhang, Z., Zhu, C., Wang, T., Wang, R., Ruan, L., 2022. Heritability of tomato rhizobacteria resistant to *Ralstonia solanacearum*. Microbiome 10, 1-18. https://doi.org/10.1186/s40168-022-01413-w
37. Yu, K., Yang, Y., Duan, Y., You, J., Guo, J., 2025. Integrated multi-omics reveals flavonoid-dominated defense strategies in *Coptis chinensis* under *Fusarium* root rot infection. BMC Plant Biology 25, 842.

https://doi.org/10.1186/s12870-025-06866-3

38. Zamioudis, C., Korteland, J., Van Pelt, J. A., van Hamersveld, M., Dombrowski, N., Bai, Y., Hanson, J., Van Verk, M. C., Ling, H., Schulze-Lefert, P., Pieterse, C. M. J., 2015. Rhizobacterial volatiles and photosynthesis-related signals coordinate *MYB72* expression in *Arabidopsis* roots during onset of induced systemic resistance and iron-deficiency responses. The Plant Journal 84, 309-322. https://doi.org/10.1111/tpj.12995
39. Zhao, Y., Jin, Z., Hong, Y., Zhang, Y., Lu, Z., Li, Y., 2023. Root Exudates Modulate Rhizosphere Microbial Communities during the Interaction of *Pseudomonas chlororaphis*, β-Aminobutyric Acid, and *Botrytis Cinerea* in Tomato Plants. Journal of Plant Growth Regulation 43, 701-714. https://doi.org/10.1007/s00344-023-11128-3
40. Zhou, X., Wang, J., Liu, F., Liang, J., Zhao, P., Tsui, C. K. M., Cai, L., 2022. Cross-kingdom synthetic microbiota supports tomato suppression of Fusarium wilt disease. Nature Communications 13, 7890. https://doi.org/10.1038/s41467-022-35452-6
41. Zhou, Y., Yang, Z., Liu, J., Li, X., Wang, X., Dai, C., Zhang, T., Carrión, V. J., Wei, Z., Cao, F., Delgado-Baquerizo, M., Li, X., 2023. Crop rotation and native microbiome inoculation restore soil capacity to suppress a root disease. Nature Communications 14, 8126. https://doi.org/10.1038/s41467-023-43926-4

Figures

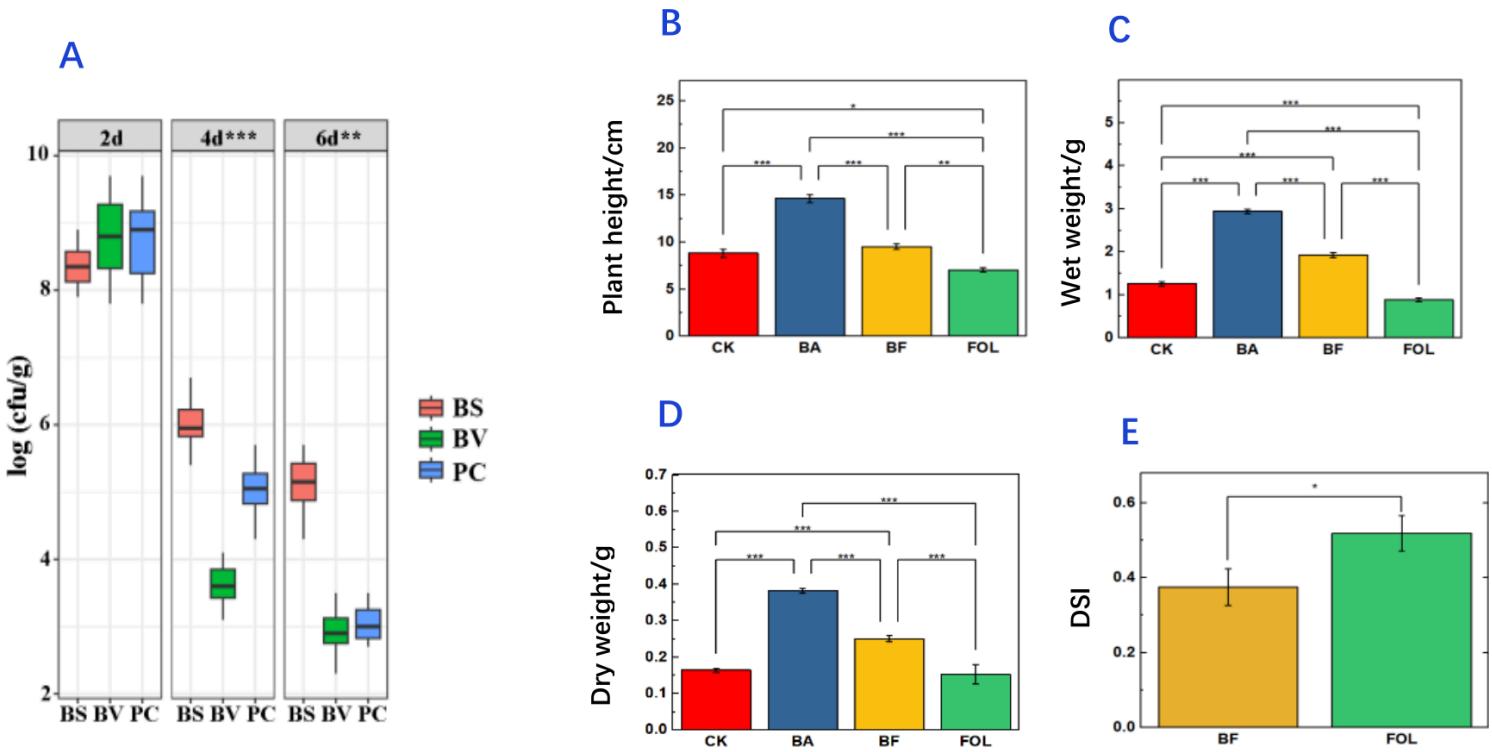


Figure 1

A. The colonization characteristics of *Bacillus* sp., *Pseudomonas* sp. (log **CFU**·g^{-1})

in the tomato rhizosphere at 2, 4, 6 dpi. B-E. The plant growth-promoting abilities and the

suppression effects on Fusarium wilt disease by inoculation of tomato roots with bacterial consortia. n=6. BS(*Bacillus subtilis*)、BV(*B. velezensis*)、PC(*Pseudomonas chlororaphis*).

Control (CK); BA (Bacteria); BF (Bacteria and *Fusarium*); FOL (*Fusarium*).

Consistent use of abbreviations is maintained throughout this manuscript.

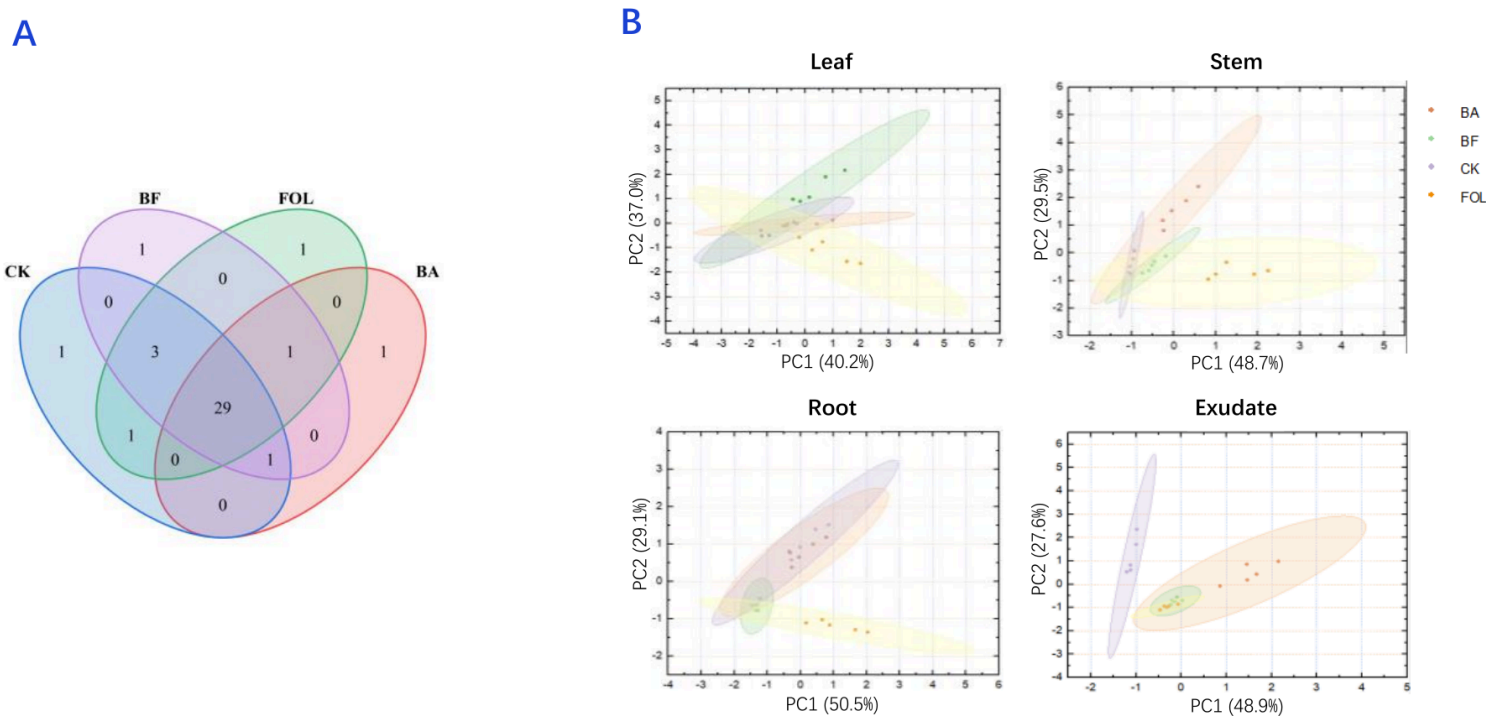


Figure 2

A. Venn diagram of leaf metabolite number and compositions

under the four treatment conditions. B. PCoA analysis of metabolites

variation of tomato plants across treated groups and the control group.

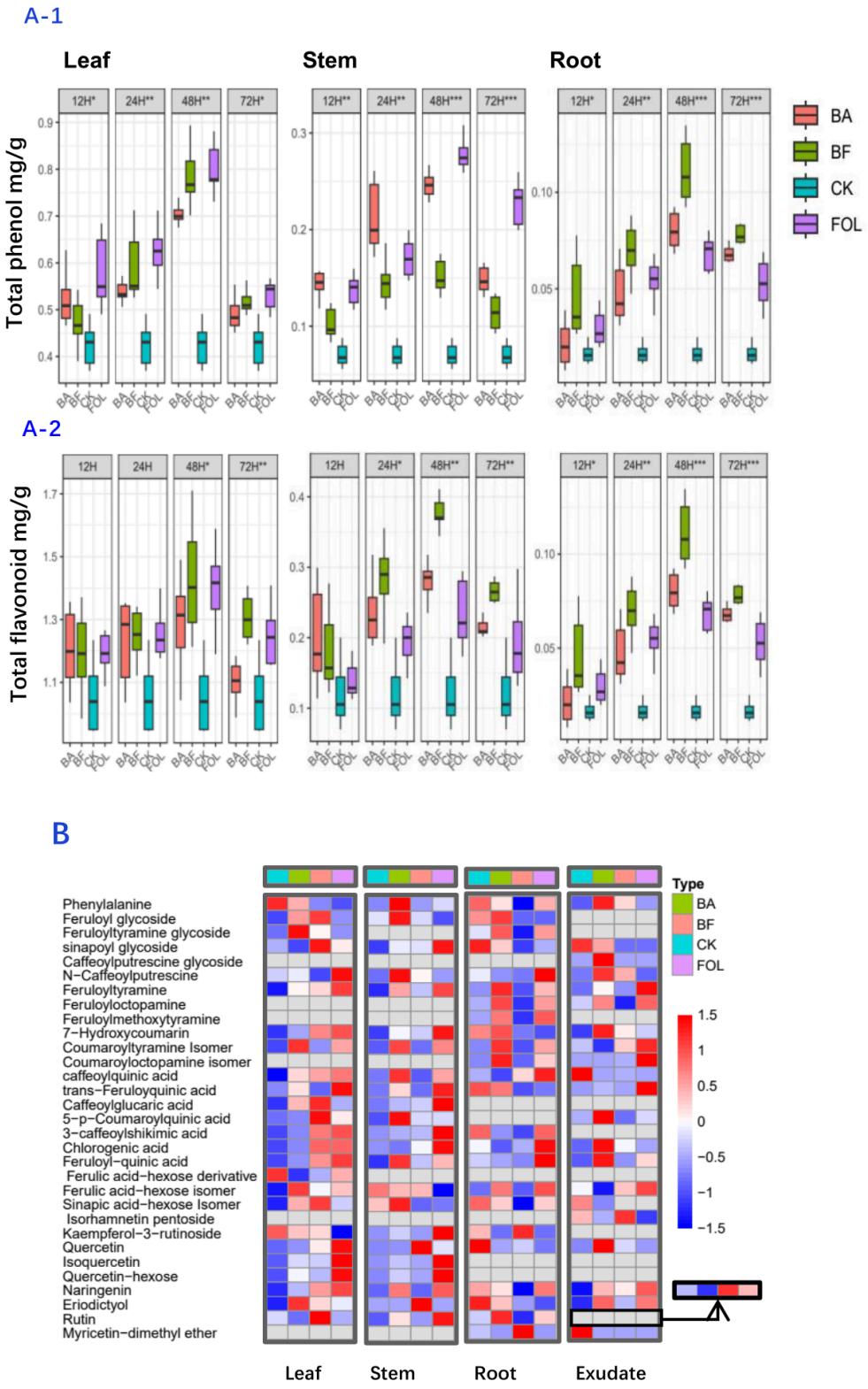


Figure 3

A. The changes of total phenolic (A-1) and total flavonoid (A-2) content following microbial treatment. B. Heatmap illustrating the comparative differential regulation of specific metabolite abundances

across various tissues in tomato plants subjected to four treatments.

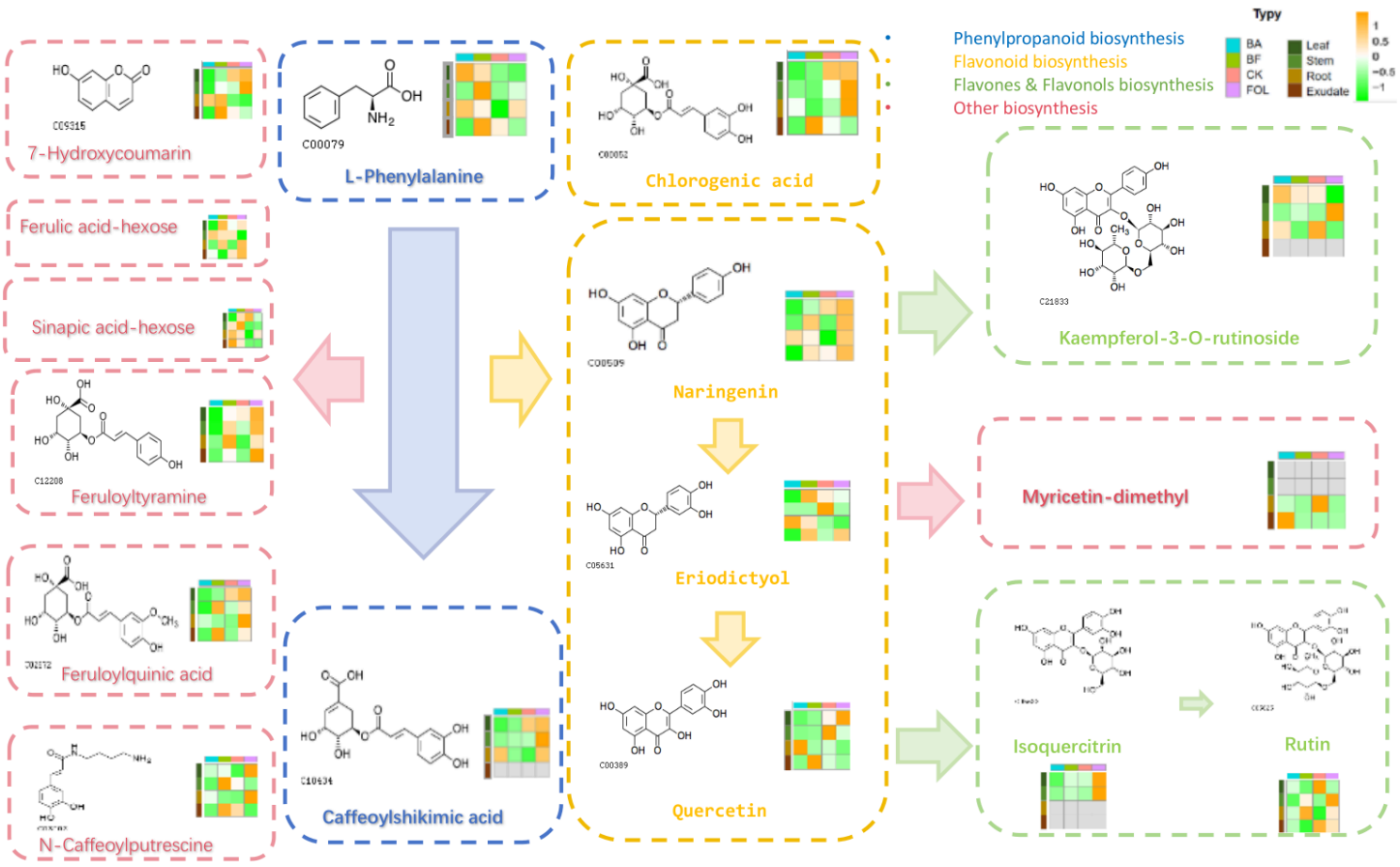


Figure 4

Biosynthesis pathways (modified from KEGG map00940)

of phenylpropanoid-flavonoid are differentially regulated by

four treatments among various tissues in tomato plants .

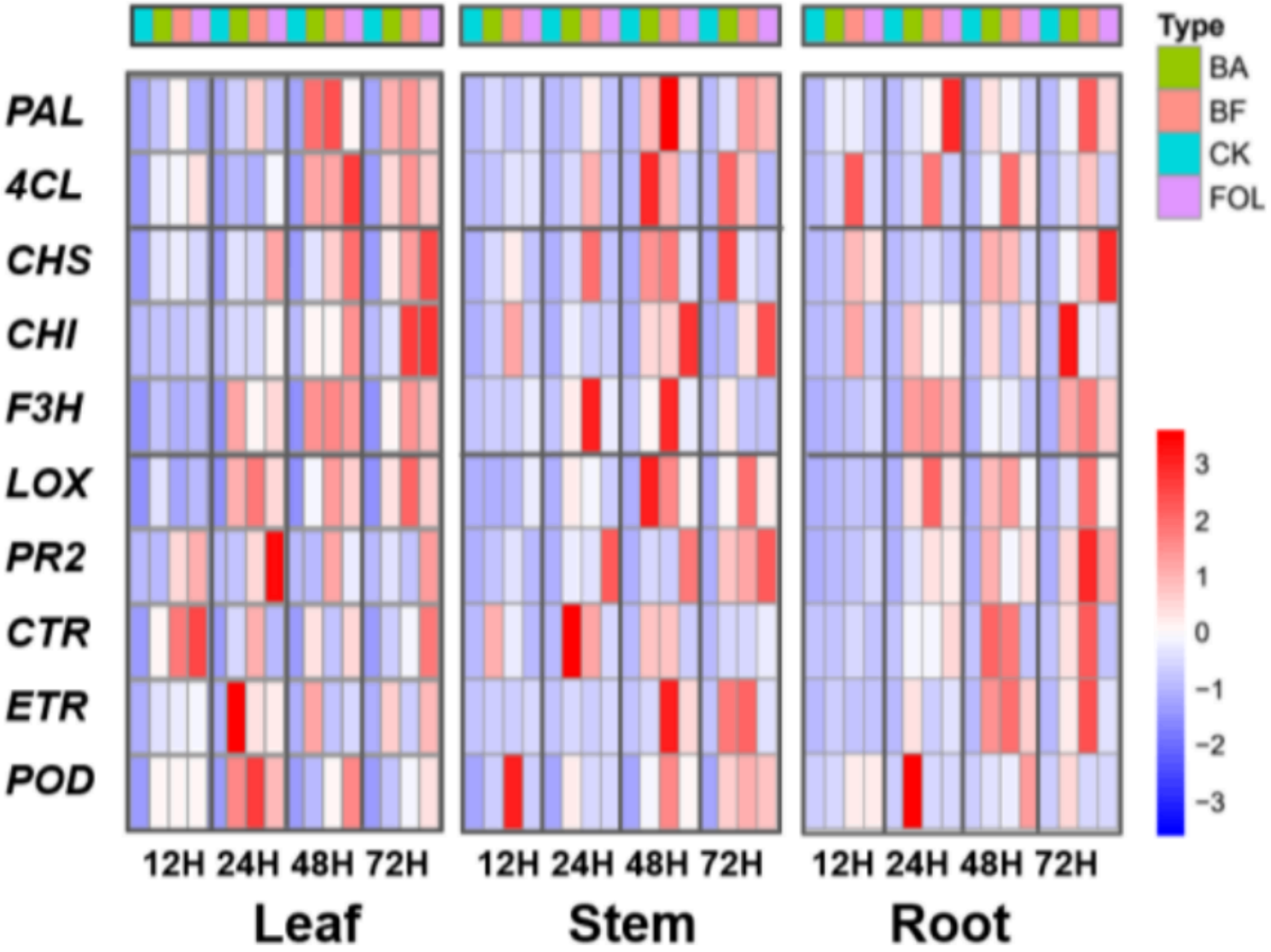


Figure 5

Variations in the expression levels of key metabolic enzymes and

disease resistance genes in tomato seedling post-microbial treatment.

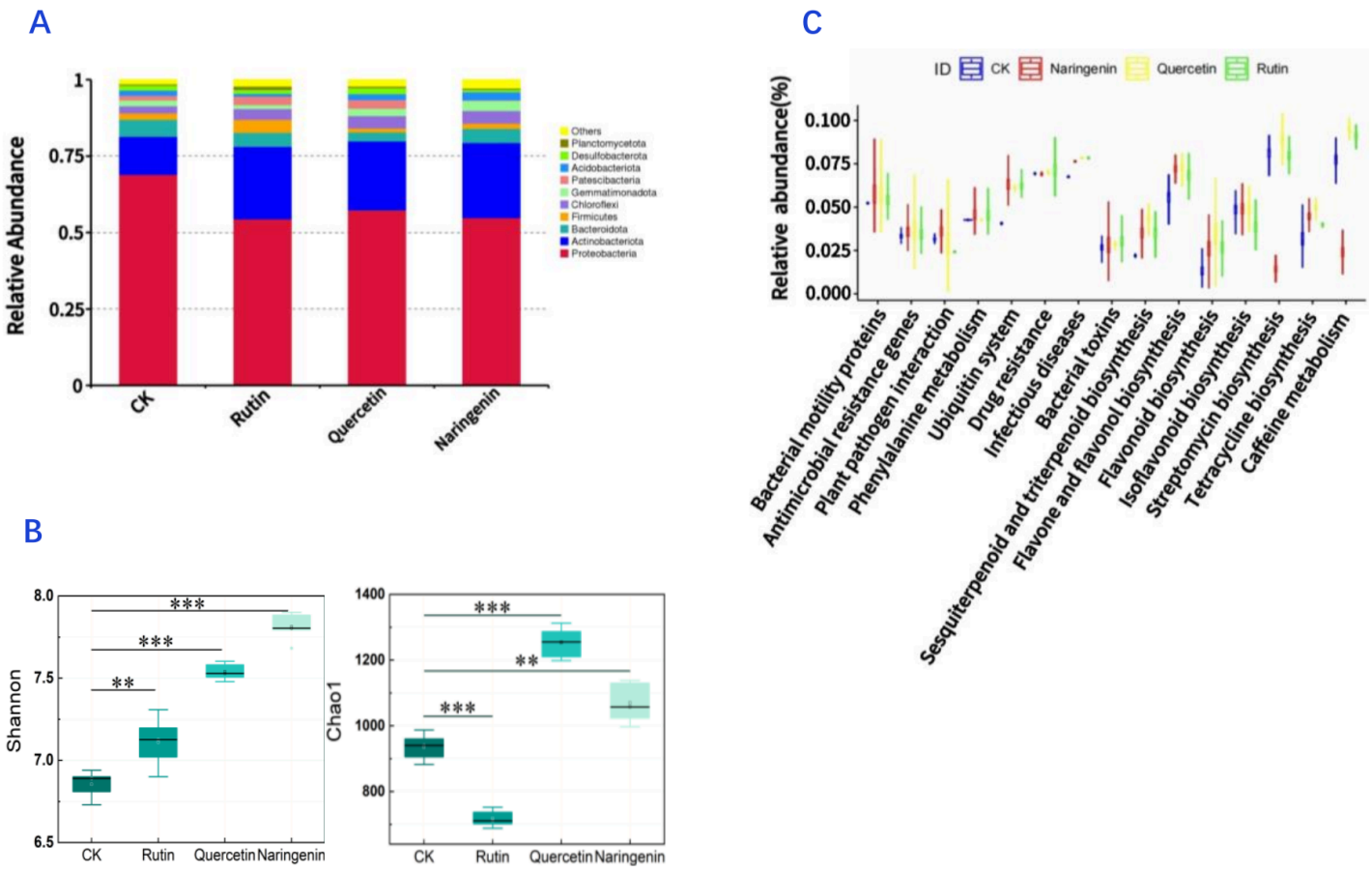


Figure 6

The effects of flavonoids on the abundance composition (A), diversity (B) and functional profile (C) of the bacterial microbiome in the tomato rhizosphere.

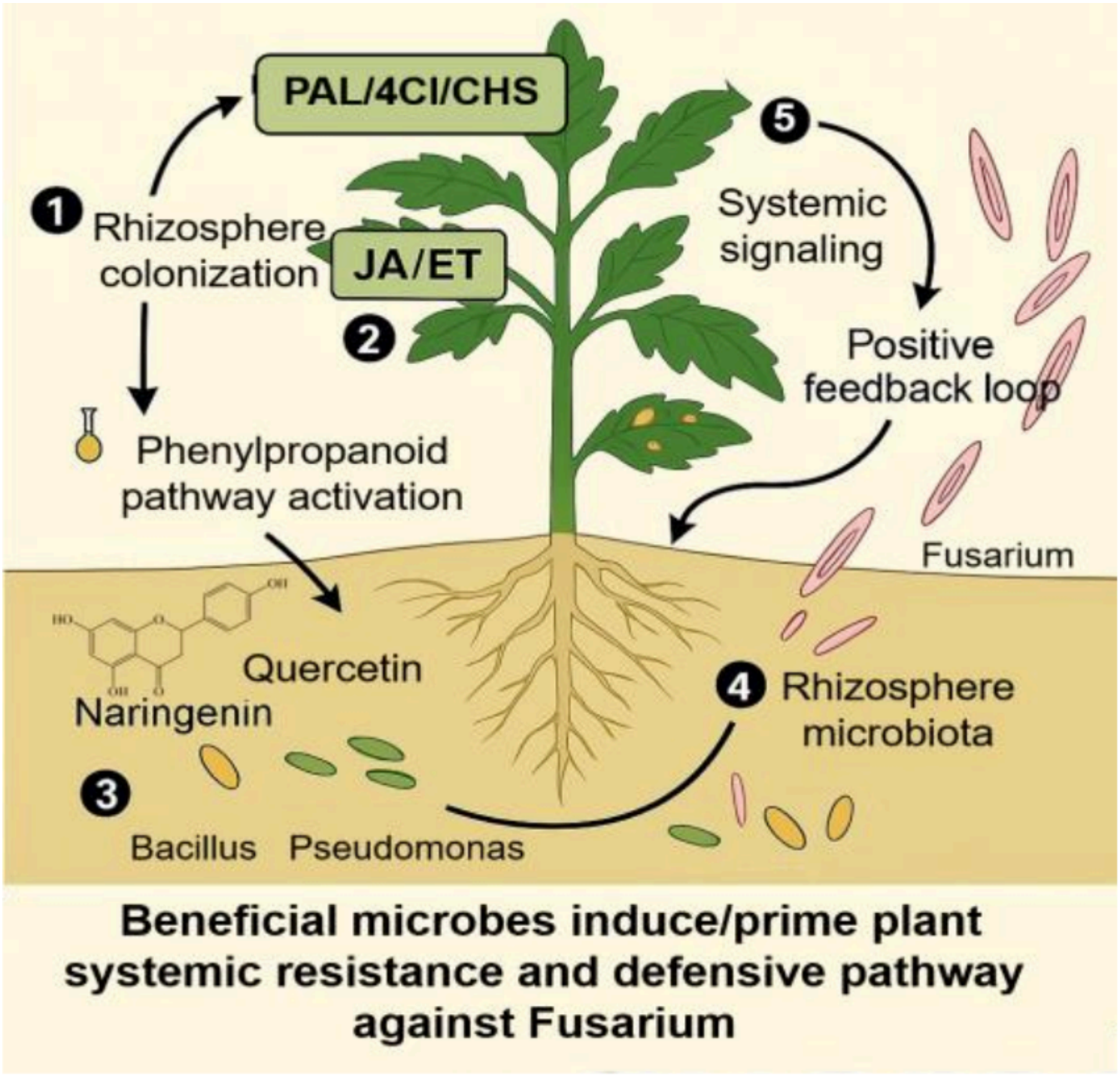


Figure 7

Proposed response model summarizing tomato plant

defensive pathways primed by rhizospheric bacterial Syncoms

and induced systemic resistance against *Fusarium oxysporum.*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure.docx
- renamed973a2.xlsx