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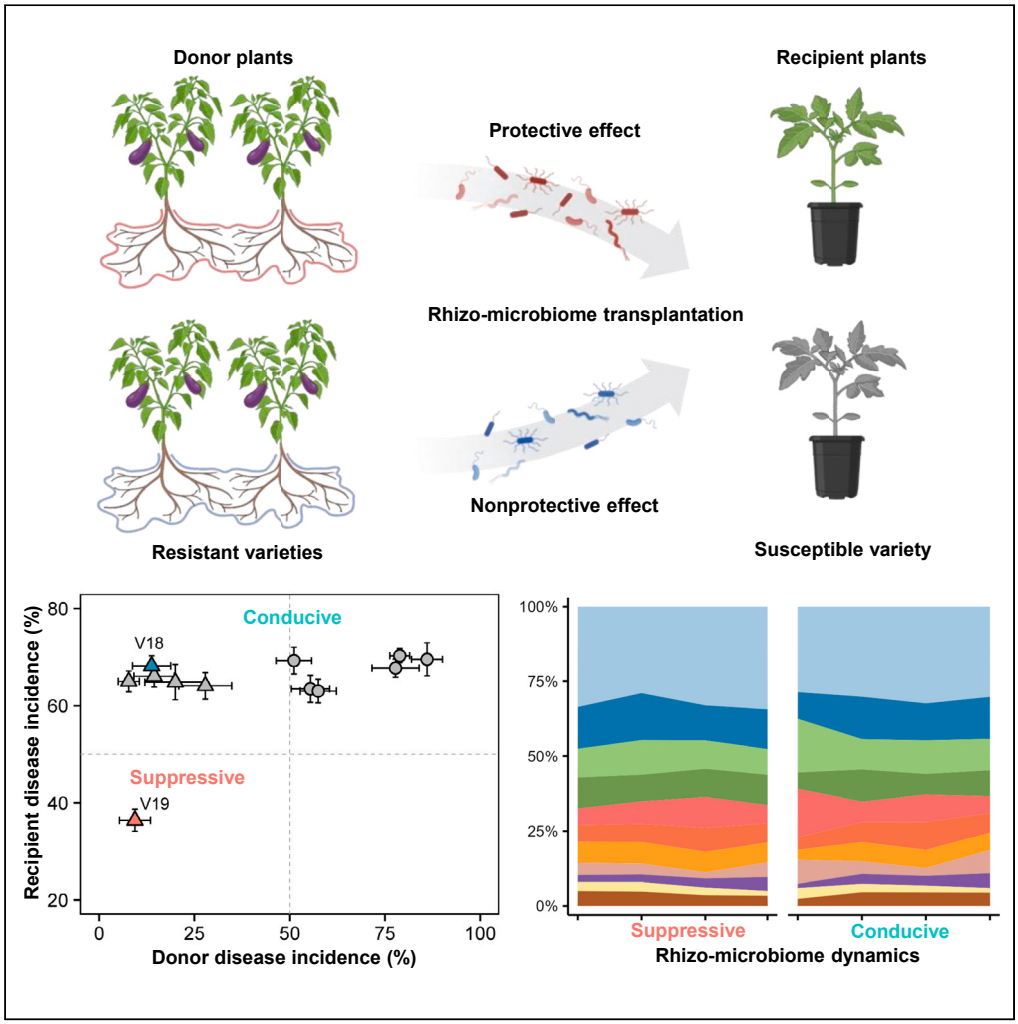

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Highlights

Definition of a suppressive rhizo-microbiome for plant health

Compositional differences between a suppressive and conducive microbiome

Increased stability and taxonomic diversity of the suppressive microbiome over time

Host selection impacts assembly processes that structure a suppressive microbiome

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Exploring the temporal dynamics of a disease suppressive rhizo-microbiome in eggplants

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SUMMARY

The rhizosphere microbiome is important for plant health, yet their contributions to disease resistance and assembly dynamics remain unclear. This study employed rhizosphere microbiome transplantation (RMT) to delineate the impact of the rhizosphere microbiome and the immune response of eggplant (*Solanum melongena*) on resistance to bacterial wilt caused by *Ralstonia solanacearum*. We first identified disease-suppressive and disease-conducive rhizosphere microbiome in a susceptible tomato recipient. Using a non-destructive rhizobox and 16S rRNA amplicon sequencing, we monitored the dynamics of both microbiome types during the eggplant development. Most differences were observed at the early stage and then diminished over time. The suppressive microbiome maintained a higher proportion of initial community members throughout eggplant development and exhibited stronger deterministic processes in the early stage, underscoring the importance of plant selection in recruiting protective microbes for rhizosphere immunity. Our study sheds light on the development of microbiome-based strategies for plant disease management and resistance breeding.

INTRODUCTION

The rhizosphere, the narrow region of soil surrounding plant roots, plays an important role in plant health and resistance to soil-borne pathogens.[1] In this microenvironment, specific microorganisms can inhibit the invasion of pathogens or boost the immune response of plant roots, delaying or even reducing disease severity.[1] This phenomenon and the ability to confer disease inhibition have been proposed as extensions of plant resistance and further defined as 'rhizosphere immunity'.[2] Unlike classical plant immunity, rhizosphere immunity relies on dynamic plant–microbiome interactions, including the selective recruitment of protective microorganisms that naturally suppress pathogen growth. This rhizosphere immune assembly is determined by host plant features.[3–6] Growing evidence suggests that the rhizosphere microbiome composition differs substantially between resistant and susceptible plant varieties. For instance, resistant tomato cultivars harbor more bacterial taxa, such as the antagonistic *Flavobacteriaceae*, than susceptible cultivars.[7] The initial differences in rhizosphere microbiomes are major determinants of plant resistance against soil-borne diseases under field conditions,[8] such as *Ralstonia solanacearum*, the causative agent of bacterial wilt and a top-ten global bacterial phytopathogen.[9] The recruited beneficial microbes can induce plant resistance via the secretion of antimicrobial agents,[10] the emission of volatiles,[11] the induction of immunity,[12] and other defense pathways.[13] Therefore, understanding the contribution of the microbiome to rhizosphere immunity is crucial for designing innovative strategies to maintain plant health.

Methodologies have been developed to dissect the intricate relationships between plant resistance and rhizosphere microbial communities. For example, researchers have inoculated sterile soils with single strains or synthetic microbial communities to elucidate the antagonistic effects of specific microbes or microbiomes.[14,15] However, the power of this approach is limited by the lack of unified incubation conditions for culturable microbes and the overlooked functions of many nonculturable microbes in soils. Recently, rhizosphere microbiome transplantation (RMT) opened new avenues for exploring the protective effect of entire rhizosphere microbiomes on bacterial wilt disease.[16,17] RMT has been recognized as a promising approach for revealing the relative contributions of rhizosphere microbiomes and plant immunity to disease inhibition during resistance breeding. However, the understanding of rhizosphere microbiome dynamics and their contributions to disease resistance over time is limited.

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Rhizosphere microbiome assembly varies during plant development.[18–20] For example, the community structure of Bambara groundnut at the maturation stage was distinct from that at other growth stages, with an enrichment of specific bacterial taxa.[21] Similarly, the root-associated microbiota of field-grown rice was highly dynamic during the vegetative growth period and then stabilized at later stages.[20] Thus, the ability to establish a disease-suppressive rhizosphere microbiome is likely influenced by host plant genotype and life cycle.[22] While previous studies have characterized rhizosphere microbiome dynamics, few have explicitly focused on disease-suppressive versus disease-conducive (non-suppressive) microbiomes and compared their differences during assembly processes. It is crucial to elucidate whether deterministic processes (*e.g.*, environmental filtering) or stochastic processes (*e.g.*, ecological drift) predominantly govern the assembly of suppressive microbiomes across plant growth.

In this work, we screened for disease-suppressive and conducive rhizosphere microbiomes of 12 eggplant varieties by transplanting their microbiomes (from plants subjected to RMT) into the disease-susceptible tomato variety Micro-Tom (see pipeline in Figure 1A). Here, we performed time-series greenhouse experiments to determine the temporal variations in two microbiome types during the development of eggplant plants using a non-destructive rhizobox system.[23] We addressed the following questions: How does the suppressive microbiome differ from the conducive microbiome during various plant development stages? What are the differences between the assembly processes of suppressive and conducive rhizosphere microbiomes? Our work aims to explore temporal dynamics of the rhizosphere as a predictor of disease suppression, enabling a new generation of microbiome-based strategies to enhance plant health and designing sustainable agricultural practices.

RESULTS

Screening of the suppressive rhizosphere microbiome

To distinguish between rhizosphere immunity and plant innate immunity, we conducted rhizosphere microbiome transplant (RMT) experiments on 12 eggplant varieties (including 6 resistant and 6 susceptible varieties, see measurements Figure S1) to evaluate the ability of the susceptible tomato variety Micro-Tom to protect against the inoculation of *R. solanacearum* (see pipelines in Figure 1A). No significant effects on tomato disease incidence were detected among the microbiomes transplanted from the six susceptible eggplant rhizospheres (67.74% on average, see results in Figure 1B). Although most RMTs of resistant eggplant varieties also showed no protective effects on Micro-Tom, the microbiome of variety V19 protected this susceptible tomato recipient plant against pathogen infection (30%–45.45%, $p < 0.001$, HSD test, see details in Figure 1B). Therefore, the rhizosphere microbiome of the eggplant variety V19 was selected as the suppressive microbiome. We defined a microbiome with a non-protective effect as conducive. To further determine the effects of temporal changes in the microbiome on plant health, we selected the resistant eggplant varieties V18 and V19 as representative conducive and suppressive microbiomes, respectively, to compare their differences in assembly dynamics during plant growth.

Suppressive and conducive microbiomes differed at the initial stage

To compare the dynamic differences in the suppressive and conducive rhizosphere bacterial communities, we measured the rhizosphere bacterial community of the eggplant during each developmental stage using a non-destructive rhizobox (see details in Figure 2A). Overall, *Proteobacteria* (24.98–44.57%), *Acidobacteria* (6.01–19.94%), and *Gemmatimonadetes* (5.28–14.63%) were the dominant bacterial phyla in the suppressive rhizosphere bacterial community, whereas *Proteobacteria* (22.39–42.82%), *Chloroflexi* (7.31–29.05%), and *Actinobacteria* (2.82–21.91%) were the most abundant phyla in the conducive rhizosphere bacterial community throughout plant development (see detailed composition in Figure 2B). At the initial stage (14 days), significant differences were detected in the relative abundances of *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Actinobacteria*, and *Bacteroidetes* between the suppressive and conducive rhizosphere bacterial communities, while no significant differences were detected at the later development stage (see all details in Figure S2).

To further assess differential taxa at each developmental stage, we compared differences in taxonomic composition between suppressive and conducive rhizosphere bacterial communities throughout eggplant development (see details in Figure 2C; Figure S3). Overall, a total of 613 differential OTUs were observed at the initial stage of 14 days (see pairwise comparison in Figure S3). The number of observed differential OTUs decreased from 49 at 28 days to <20 at 42 days (see pairwise comparison in Figure S3). In the initial stage, these differential OTUs were mainly Cyanobacteria (0.16% in the suppressive group) and *Acidobacteria* (0.10% in the suppressive group). After the 28th day, the disease-suppressive rhizosphere microbiome exhibited the specific enrichment of *Chloroflexi* (0.18%), *Bacteroidetes* (0.15%), and *Proteobacteria* (0.14%). The number of differential taxa gradually declined over time, and in the last two periods (56 days and 70 days), only *Bacteroidetes* remained specifically enriched in the disease-suppressive rhizosphere microbiome (see details in Figure 2C).

We further evaluated differences in the bacterial alpha diversity and community between the suppressive and conducive rhizospheres. Consistent with the compositional differences, the bacterial alpha diversity was significantly greater in the suppressive rhizosphere microbiome at the initial stage than in the conducive microbiome (Shannon: $p < 0.05$; richness: $p < 0.05$; see results in Figures 3A and 3B). In addition, the coefficients of variation (CVs) of the Shannon richness of the conducive microbiome were higher than those of the suppressive microbiome during four different stages (14, 28, 42, and 56 days) (see comparisons and dynamics Figure S4). On the other hand, the plant developmental stage was the major factor impacting the rhizosphere community structure ($F_{1,69} = 22.53$, $p < 0.001$, $R^2 = 0.24$), followed by eggplant variety ($F_{1,69} = 2.36$, $p = 0.019$, $R^2 = 0.025$) and their interaction ($F_{1,69} = 2.87$, $p = 0.002$, $R^2 = 0.031$, two-way ANOVA; see details in Figure 3C). Consistently, more compositional differences were observed in the rhizosphere microbiome at the initial stage than at the latter developmental stages (see details in Figure 3D; Figure S5). Overall, these results indicate that the two microbiome types display substantial dissimilarity at the initial stage.

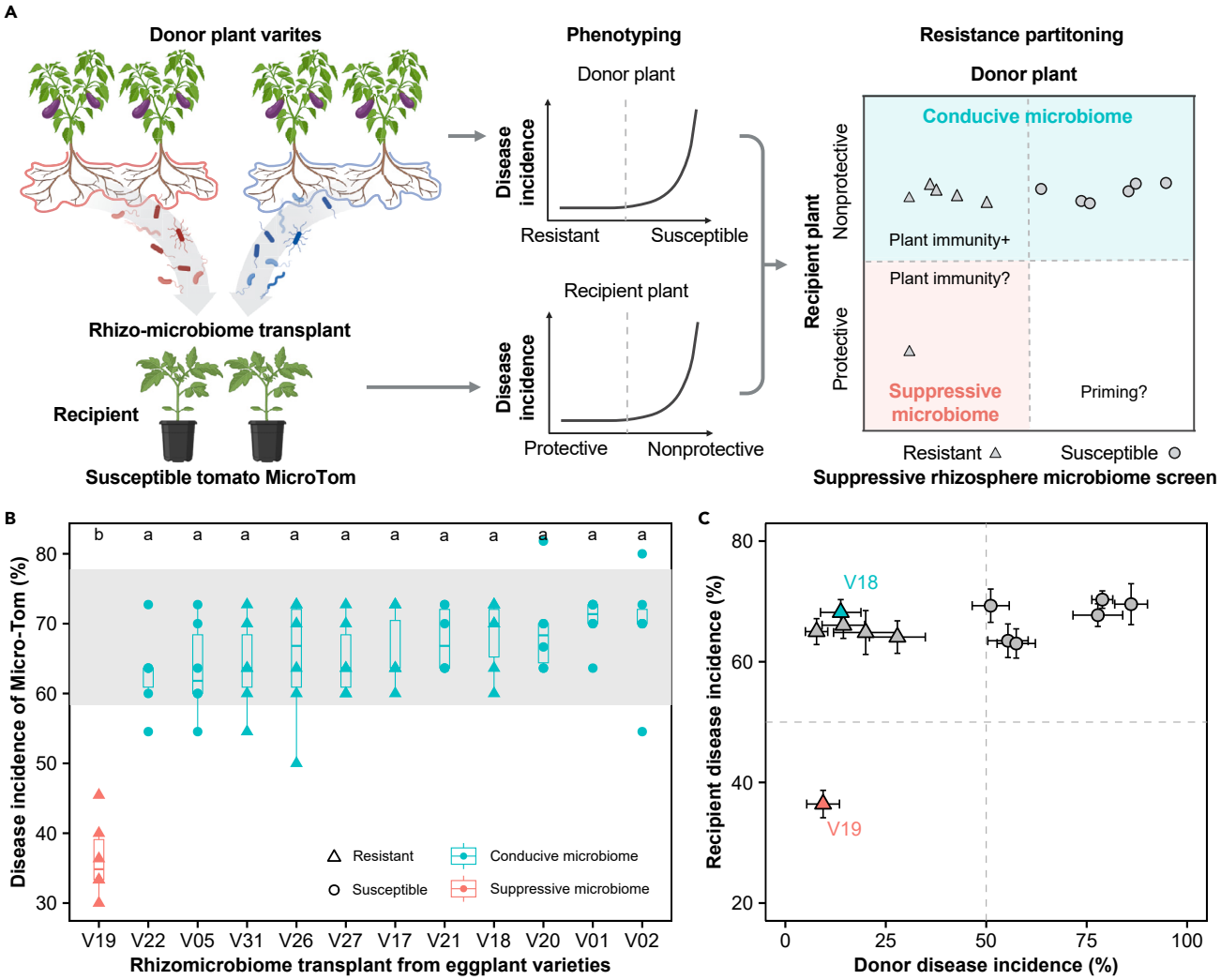

Figure 1. Screening of the suppressive and conducive rhizosphere microbiome

(A) The pipeline used to screen the suppressive/conducive rhizosphere microbiomes of eggplant varieties. The rhizosphere microbiomes of 12 eggplant varieties were subsequently transplanted to the disease-susceptible tomato variety Micro-Tom, and their disease incidence was tested. The triangles and circles represent resistant and susceptible eggplants, respectively. The rhizosphere microbiome of resistant eggplants, which protects susceptible tomato plants against pathogen invasion, is defined as the suppressive microbiome; instead, it is defined as the conducive microbiome.

(B) Disease incidence of recipient plants following the RMT treatment of different donor plants. A total of 12 donor eggplant varieties were subjected to RMT on disease-susceptible recipient tomato plants. The horizontal gray lines indicate the range of disease incidence in the Micro-Tom self-transplant group (CK). The boxes represent the 25^{th}–75^{th} percentiles of the data, and the lines and dots represent the medians and individuals, respectively. Different lowercase letters indicate significant differences in disease incidence among varieties (HSD post hoc test: $p < 0.05$).

(C) Identification of susceptible and conducive rhizosphere microbiomes in eggplants. The triangles and circles represent resistant and susceptible eggplants, respectively. The resistant eggplant varieties V18 and V19 were selected as representative conducive and suppressive microbiomes, respectively, to examine their temporal changes during plant growth. Each point and error bar indicates mean ± SEM.

High stability of the suppressive rhizosphere bacterial community

To explore the ecological dynamics of microbial communities, we compared the persistence of bacterial species between two types of rhizosphere microbiomes during eggplant growth (see results in Figure 4). Source tracker analysis unveiled that the average of 72% (67%–76%) and 68% (56%–73%) of bacterial species at each substantial stage (sink) was originated from the suppressive and conducive rhizosphere microbiomes of their previous stages (source), respectively (see details in Figure 4A). These percentage values were referred to as the persistent OTUs from its early microbiome compared to the unknown sources (soils). Considering the initial microbiome as the source community, the proportion of persistent bacterial species in the suppressive microbiome decreased from 67.9% at the early stage (28 days) to 32.7% at the maturation stage (70 days), which was 13.4% (7.3%–19.5%) higher than the persistent taxa in the conducive microbiome (see Figure 4A). To investigate the relative influence of assembly processes the structuring of rhizosphere microbial community, the beta nearest taxon index

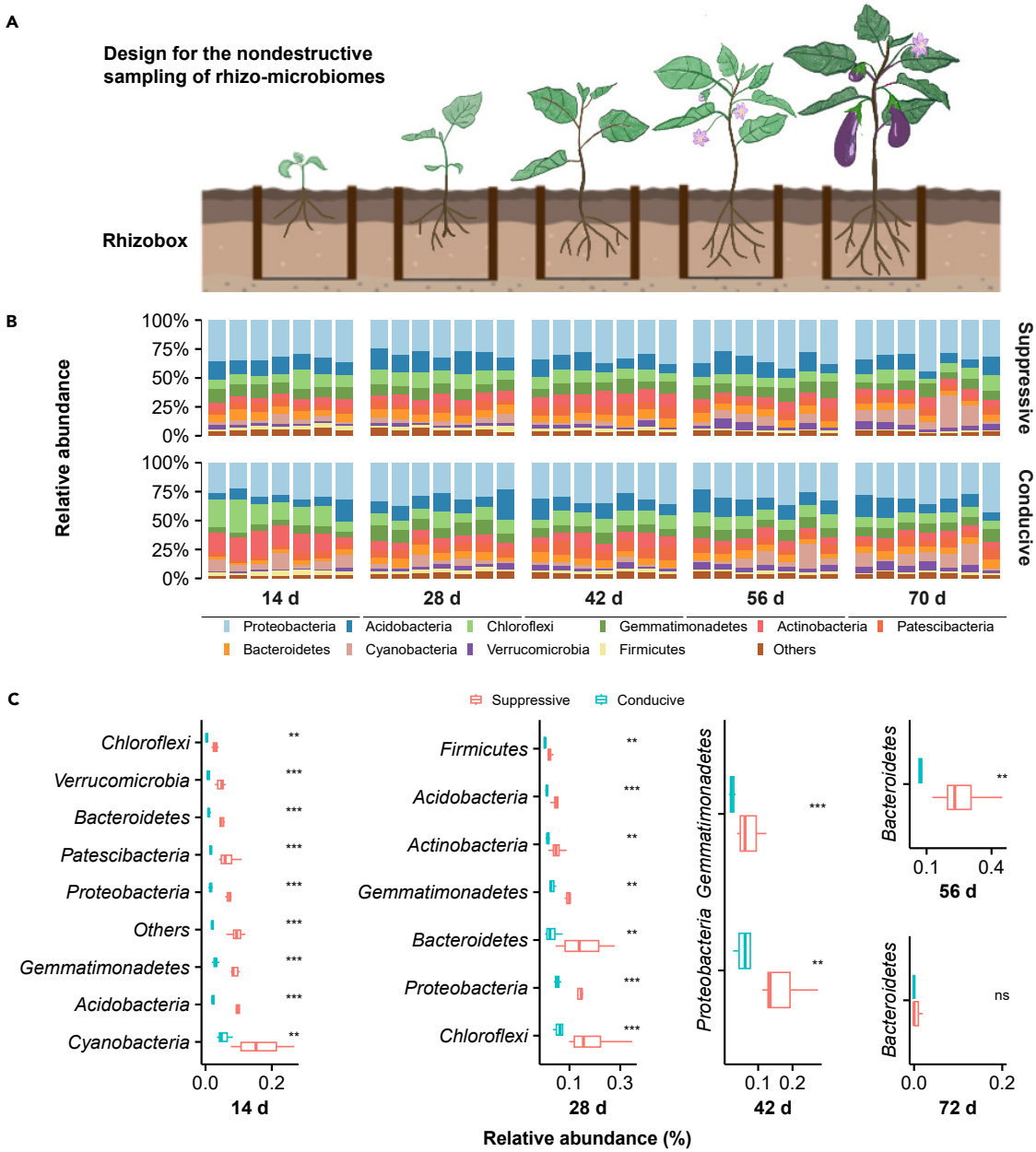

Figure 2. The dynamics of the suppressive and conducive rhizosphere bacterial community composition during plant development

(A) Non-destructive sampling of eggplant rhizosphere soil during plant growth.

(B) Relative abundance of bacterial phyla in the suppressive and conducive rhizosphere bacterial communities at different growth stages.

(C) Differences in the relative abundances of bacterial phyla based on discriminating OTUs ($|\log_2$-fold change$| \geq 1$ and adjusted $p < 0.05$) in the suppressive and conducive microbiomes during plant growth. The red and blue colors indicate the discriminant phyla enriched in the suppressive (red) but depleted in the conducive (blue) rhizosphere microbiome. The boxes represent the 25th–75th percentiles of the data, and the lines and dots represent the medians and individuals, respectively. *p* values were calculated using pairwise Student's t tests (ns$p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

(βNTI) analysis was performed on suppressive and conducive microbiomes over time. The assembly processes of both rhizosphere microbiomes were affected by different growth stages of eggplant (see details in Figures 4B and 4C). In general, stochastic processes dominated most of the development periods (86.2% in the suppressive and 93.8% in the conducive; see details in Figures 4B and 4C). More deterministic processes were observed in the suppressive microbiome than in the conducive microbiome at 28 days (18.4% in suppressive and 2.0% in conducive) and 42 days (28.6% in suppressive and 2.0% in conducive). Deterministic processes of the suppressive microbiome decreased at the late growth stage of eggplant, while the conducive microbiome was increasingly shaped by deterministic processes at the plant

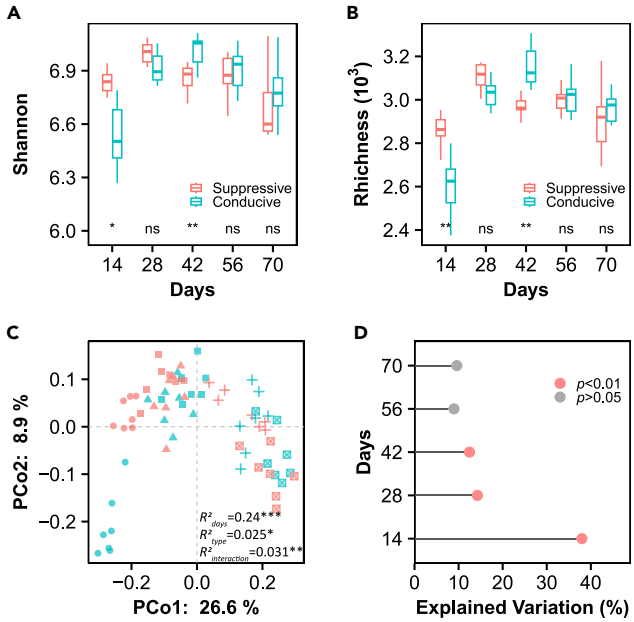

Figure 3. Differences in the diversity and composition of the suppressive and conducive microbiomes during plant development

(A) Shannon and (B) Richness indices of the suppressive and conducive rhizosphere bacterial communities (n = 7).

The boxes represent the 25th–75th percentiles of the data, and the lines and dots represent the medians and individuals, respectively. p values were calculated using pairwise Student's t tests (ns$p$ > 0.05;*p < 0.05; **p < 0.01; ***p < 0.001).

(C) Principal coordinate analysis (PCoA) based on Bray–Curtis distances displaying a significant difference between microbiome type ($F_{1,69}$ = 2.362, R^2_{varity} = 0.025, p = 0.019) and growth stage ($F_{1,69}$ = 22.53, R^2_{day} = 0.240, p < 0.001). p values were calculated using the PERMANOVA test (*p < 0.05; **p < 0.01; ***p < 0.001).

(D) Summary of microbial compositional variation explained by microbiome type (suppressive or conducive) during plant growth stages (in days). p values were calculated using the PERMANOVA test based on the Bray–Curtis distances of PCoA analyses.

maturation stage. Taken together, these results indicate that the suppressive rhizosphere bacterial community was more stable than the conducive one during plant growth (see details in Figure 4).

DISCUSSION

Disease-suppressive soil is a particular ecosystem that provides an inhospitable environment for soil-borne pathogens due to the antagonistic activities of the root-associated suppressive microbiome. Here, we introduced the concept of the "suppressive rhizosphere microbiome" and "rhizosphere immunity," and provided a pipeline for screening beneficial microbiomes through a microbiome transplantation experiment (RMT). By performing disease resistance assessments of 12 eggplant varieties via field trials and RMTs, we identified the rhizosphere microbiomes of the V19 variety (*Solanum melongena* subsp. *ovigerum*) and the V18 variety (*S. melongena* subsp. *melongena*) as suppressive and conducive, respectively. Both eggplant varieties grow similarly and are used as resistant rootstocks for grafting susceptible tomato seedlings, enhancing field tolerance against bacterial wilt disease in tomato production.[24,25] However, only the transplant of suppressive microbiome from the V19 variety endow the disease resistance on the susceptible tomato plants to pathogen exposure in this work. Thus, the plant resistance of the V19 variety is potentially due to the synergistic effect of the recruitment of suppressive microbiomes[16] via root exudation,[4] and the plant innate or effect-triggered immune responses.[4,6,26] In contrast, the V18 variety might allocate more metabolic resources to activate the expression of immune related genes,[24,27] increasing the fitness cost of plant growth upon pathogen infection.[28]

Our results revealed significant differences in microbial composition and diversity between the suppressive and conducive rhizosphere microbiomes, particularly at the early stages of plant development.[29,30] The improved disease resistance of tomato plants caused by the transplantation of a suppressive microbiome suggests the advantages of early microbiomes in sustaining disease suppression, as the early establishment of disease suppressiveness is strongly linked to plant health.[23] Furthermore, specific taxa, including *Proteobacteria, Acidobacteria* and *Gemmatimonadetes,* were found to be consistently enriched in suppressive microbiomes, corroborating previous reports of their roles in disease suppression, such as banana *Fusarium* wilt.[31–33] Compositional differences between the two microbiome types became less pronounced as the plant matured, suggesting that other factors, such as soil properties, may alter the rhizosphere microbiome over time.[34–36] This observation supports the profound impact of soil type and other environmental factors on microbial community assembly in the rhizosphere.[14,37,38]

We revealed a relatively stable microbial community in the suppressive rhizosphere microbiome. Compared to the conducive type, the suppressive microbiome maintained a higher proportion of its initial microbial species across different plant growth stages. Furthermore, ecological drift (*e.g.*, stochastic processes) was found to be dominant overall in the community assembly processes of the suppressive

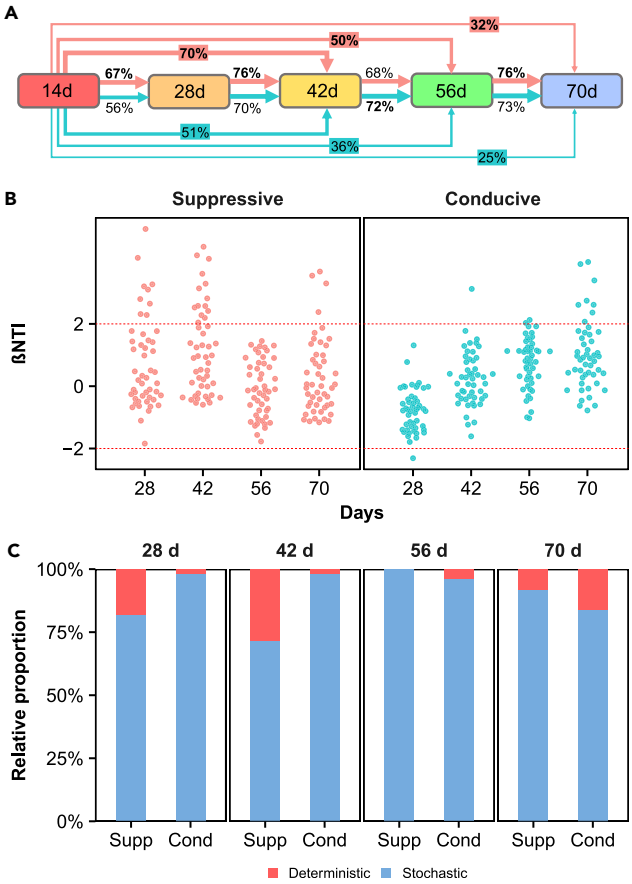

Figure 4. Temporal dynamics and assembly processes of the rhizosphere bacterial community in eggplant varieties during plant growth

(A) Source proportions for the growth period between the suppressive and conducive rhizosphere bacterial communities; the arrow direction indicates the potential transfer of the bacterial community, and the arrow width varies with proportion. The red arrows represent the suppressive rhizosphere bacterial community, and the blue arrows represent the conducive rhizosphere bacterial community.

(B) Patterns of βNTI across different periods in the suppressive and conducive rhizosphere bacterial communities.

(C) Relative proportion of deterministic and stochastic OTUs during rhizosphere microbiome assembly via null model analysis. Supp: suppressive rhizosphere bacterial community; Cond: conducive rhizosphere bacterial community. $|\beta NTI| \geq 2$ indicates the predominance of deterministic processes, and $|\beta NTI| < 2$ indicates the predominance of stochastic processes.

and conducive microbiomes. However, environmental filtering/host selection (*e.g.*, deterministic processes) plays a more substantial role in shaping the suppressive microbiome than in shaping the conducive microbiome. This finding suggests that the host plant exerts a stronger selective force in recruiting specific microbes that contribute to disease suppression. This process could be achieved through the modulation of root exudates.[39,40] For instance, flavonoids are associated with the abundance of protective species, such as *Bacillus* spp.[40] These recruited microbes can inhibit pathogen growth through multiple mechanisms. For instance, certain rhizosphere bacteria, such as *Bacillus amyloliquefaciens*, can produce volatile organic compounds that directly inhibit pathogen growth.[11] Other microbes may outcompete pathogens for nutrient competition in the rhizosphere[10] or target and inhibit helper bacteria (which facilitate pathogen invasion) to protect plants indirectly.[41] Furthermore, the suppressive microbiome can also resist invaders by priming/inducing plant defense responses, such as pattern- or effector-triggered immunity pathways.[42]

In conclusion, our study highlights the importance of a suppressive rhizosphere microbiome and its dynamics to plant health. The disease-suppressive rhizosphere microbiome exhibits increased stability and is determined by host plant selection for the recruitment of beneficial microbes. Our findings open novel avenues for developing microbiome-based strategies to improve plant resistance against soil-borne diseases for sustainable agriculture.

Limitations of the study

This study is conceived as an initial exploration of microbiome temporal dynamics. While we anticipate the results to be generalizable to several crops, we would like to emphasize a couple of limitations that should be reckoned with. The study tested only one resistant and one conducive microbiome. This limitation is bound to the presence of one single suppressive microbiome in the several tested ones. Further

studies with higher replication will enable disentangling idiosyncratic from generalizable results. Further, the mechanisms linking the presence of specific biomarkers species and assembly processes with disease suppression were not experimentally verified. Understanding these mechanisms, such as nutrient competition or immune priming, will provide the reduction in dimensionality needed to assist large-scale screenings of microbiomes as target for resistance breeding.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Materials availability
  - Data and code availability
- METHOD DETAILS
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  - Non-destructive continuous sampling assays of rhizo-microbiomes
  - DNA extraction and 16S rRNA amplicon sequencing
  - Statistical analyses

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110319.

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AUTHOR CONTRIBUTIONS

Author contributions following the CRediT taxonomy (https://credit.niso.org) are as follows: conceptualization: G.J., Y.W., and Z.W.; resources: G.G., W.L., Y.J., and Y.W.: methodology: Y.Z., G.G., G.J., and W.R.: data curation: Y.Z., G.G., W.L., Y.J., and P.W.: formal analysis: G.J., Y.Z., J.H., N.W., Y.L., Y.X., P.H., and A.J.: funding acquisition: A.J., Y.Z., Q.S., Z.W., G.J., Y.W., and Z.W.: investigation: G.J., Y.W., Y.Z., Y.X., P.H., N.W., X.Q., J.L., and G.G.: project administration: G.J., Y.W., Y.X., Q.S., and Z.W.: supervision: G.J., Y.W., Y.X., Q.S., and Z.W.: software, G.J. and Y.Z.: visualization, G.J. and Y.Z.: writing—original draft, Y.Z., G.G., G.J., J.H., and W.R.: writing—review and editing, G.J., Y.W., Q.S., A.J., P.H., and Z.W.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
16S rRNA gene sequencing files and metadata	This paper	NGDC: PRJCA024819
All data of the OTU feature tables and metadta	This paper	Zenodo [https://doi.org/10.5281/zenodo.11450835]
Oligonucleotides		
The forward primer sequence is 563F (5′-AYTGGG YDTAAAGVG-3′) and the reverse primer sequence is 802R (5′-TACNVGGGTATCTAATCC-3′)	This paper	N/A
Software and algorithms		
Qiime2 v 2022.8	Caporaso et al.[43]	https://doi.org/10.1016/j.soilbio.2017.11.012
USEARCH 11.0.667	Edgar[44]	https://doi.org/10.1093/bioinformatics/btq461
R version 4.1.1	R software	https://www.r-project.org
Vegan v 2.5–7 (R package)	Dixon[45]	https://cran.r-project.org/web/packages/vegan/index.html
ape v 5.5 (R package)	Paradis et al.[46]	https://cran.r-project.org/web/packages/ape/index.html
DESeq2 v 1.32.0 (R package)	Love et al.[47]	https://github.com/mikelove/DESeq2
SourceTracker	Knights et al.[48]	https://doi.org/10.1038/nmeth.1650
Hmisc v 4.5–0 (R package)	Harrell	https://cran.r-project.org/web/packages/Hmisc/index.html
fdrtool v 1.2.16 (R package)	Strimmer[49]	https://cran.r-project.org/web/packages/fdrtool/index.html
igraph v 1.2.6 (R package)	Csardi[50]	https://cran.r-project.org/web/packages/igraph/index.html
β nearest taxon index	Stegen et al.[51]	https://doi.org/10.1038/ismej.2012.22
MUSCLE v5	Edgar[52]	https://doi.org/10.1093/nar/gkh340
picante v 1.8.2 (R package)	Kembel et al.[53]	https://cran.r-project.org/web/packages/picante/index.html
Custom code and script used in this study	This paper	https://doi.org/10.5281/zenodo.11450835

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Gaofei Jiang (gjiang@njau.edu.cn).

Materials availability

This study did not generate any new unique reagents.

Data and code availability

All data underlying this study are available at Zenodo: [https://doi.org/10.5281/zenodo.11450835], and all the raw sequencing data are deposited at the National Genomics Data Center of China: [https://ngdc.cncb.ac.cn/](Project ID: PRJCA024819). All codes used for data analyses and figure visualization in this work are available at Zenodo: [https://doi.org/10.5281/zenodo.11450835].

METHOD DETAILS

Rhizosphere microbiome transplantation (RMT) assays

Tomato rootstock from the Solanaceae family is commonly used for grafting to increase the resistance of tomato plants to biotic stress, with extensive applications in the control of soil-borne diseases.[54] In the rhizosphere microbiome transplant experiment,[23] we used a susceptible tomato (*Solanum lycopersicum* cv. Micro-Tom) as the recipient plant and 12 eggplant varieties as the donor plant. To acquire soil with the rhizosphere microbiome of eggplants, we initially cultivated seedlings of the 12 eggplant varieties in sterilized jiffy substrates (Huaian Agricultural Technology Development Ltd.) until they reached the four-leaf stage. Subsequently, the seedlings were transplanted into pots containing 5 kg of soil in the greenhouse at the Baima Teaching Scientific Research Base of Nanjing Agricultural University, Nanjing, China (119.18° E, 31.61° N). The soil used in the greenhouse experiments was collected from a local area where the pathogen *Ralstonia solanacearum* was not detected. Four weeks after transplanting for the transplant assay, 10 g of rhizosphere soil was collected for microbiome suspension from all eggplant varieties. The collected soil was mixed with 95 mL of sterile ddH_2O and placed in a shaker for 5 min at 170 r/min to obtain a 100 mL rhizosphere soil suspension.

Tomato plants were cultivated and transplanted into pots containing 200 g of soil after they reached the four-leaf stage in a greenhouse at Nanjing Agriculture University, College of Resource and Environment Science, Nanjing, China (118.85° E, 32.02° N). The soil used in the greenhouse was collected from the Baima Teaching Scientific Research Base of Nanjing Agricultural University, Nanjing, China (119.18° E, 31.61° N), where the pathogen *R. solanacearum* was not detected. At the four-leaf stage, each Micro-Tom plant was drenched separately with a 20 mL inoculation of rhizosphere soil suspensions of 12 eggplant varieties (18 replicates per treatment). After RMT for one week, a suspension of the pathogen *R. solanacearum* (strain QL-Rs1115) was inoculated in the tomato rhizosphere, which included all RMT treatments and a non-transplanting positive control treatment, through the root irrigation method, resulting in a final concentration of 5.0×10^6 CFU g^{-1} of soil.[55] Tomato wilting was recorded 10 weeks after pathogen inoculation, and disease incidence was calculated as previously described[16] (Field trial to identify bacterial wilt disease-resistant eggplant varieties). The success of rhizosphere microbiome transplantation was indicated by a significant reduction in disease incidence compared with that of the non-transplanting control treatment, while no significant difference in disease incidence between rhizosphere microbiome transplantation and the control indicated failure. The tomato and eggplant plants were cultivated as described previously.[16]

Non-destructive continuous sampling assays of rhizo-microbiomes

According to the test results (Figure 1), representative eggplant varieties with a suppressive rhizosphere microbiome (varieties V19) and conducive rhizosphere microbiome (varieties V18) were grown in a non-destructive rhizobox (a height of 136 mm and a diameter of 110 mm) after four leaf stages for temporal dynamic assays of their rhizosphere microbiome. Rhizosphere soil samples (10 g) from each plant of both eggplant varieties were collected from small nylon bags (see rhizobox design for details[56]) during eggplant growth (i.e., 14, 28, 42, 56, and 70 days after seedling transplantation, with 14 days after seedling transplantation representing the early development stage of the eggplant). At each sampling time point, seven replicates of the rhizosphere soil of each variety of eggplant were collected. A total of 70 rhizosphere soil samples were transported to the laboratory and stored at −80°C until DNA extraction.

DNA extraction and 16S rRNA amplicon sequencing

Rhizosphere soil DNA was extracted from each sample using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). The DNA quality and concentration were checked using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The DNA was subjected to bacterial 16S rRNA amplicon sequencing to determine the composition and diversity of the bacterial communities. PCR amplification was performed as follows: 95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. PCRs were performed in triplicate in a 20 μL mixture containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. Sequencing was carried out at Shanghai Biozeron Biological Technology Co., Ltd., based on the V4 hypervariable region of the 16S rRNA gene using the primer pair 563F (5′-AYTGGGYDTAAAGVG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′). All sequences were processed using a pipeline in QIIME.[43] The nucleotide identity of the bacterial OTUs was set at 97% using USEARCH.[44] The taxonomic affiliation of the OTUs was determined using the RDP database.

Statistical analyses

The statistical analyses were performed in R version 4.1.1. The Shannon index, species numbers representing richness, Bray–Curtis distance for principal coordinate analysis (PCoA) and dissimilarity calculations were performed with the *vegan* package.[45] PCoA based on the Bray–Curtis distance was conducted using the '*pcoa*' function from the *ape* package.[46] Permutational multivariate analysis of variance (PERMANOVA) was performed using the '*adonis*' function in the R package *vegan*. A negative binomial model approach provided by the R package *DESeq2*[47] was used to determine differences in microbiota composition among the eggplant varieties with adjusted p values <0.05 and an absolute value of log2-fold change >1. The SourceTracker model was constructed using the SourceTracker approach.[48]

OTUs with an average relative abundance >0.01% and occurrence frequency ≥ 50% across all samples were selected to construct the general co-occurrence network of all samples. Then, OTUs with an average relative abundance >0.01% across each growth stage and each

treatment were divided from the general co-occurrence network into sub-co-occurrence networks. OTUs that appeared in all growth stages in each treatment were defined as 'stable species'. The pairwise Spearman correlation matrix was calculated by the R package *Hmisc*, and these correlations were then adjusted to the corresponding *p* value by the package *fdrtool*.[49] Spearman's rho >0.75 and adjusted *p* value <0.05 were used for the analysis of the general co-occurrence network. Network modules were clustered according to the Newman greedy algorithm by the function '*cluster_fast_greedy*' in the R package *igraph*.[50]

The community assembly processes of the eggplant rhizosphere bacterial community undergoing growth were estimated using the β nearest taxon index (βNTI).[51] First, a phylogenetic tree was generated based on the multiple sequence alignment of OTUs using the maximum likelihood method in MUSCLE v5.[52] Then, the βNTI calculated under the "*taxa.lables*" null model with 999 permutations by the '*ses. MNTD*' and '*comdistnt*' functions in the R package *picante*.[53] $|\beta NTI| \geq 2$ indicates the predominance of deterministic processes, and $|\beta NTI| < 2$ indicates the predominance of stochastic processes.