Root hairs shape microbime structure and network interactions upon drought stress

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Abstract

Drought is one of the most serious abiotic stresses which also shifts the composition of root associated microbiomes. However, there is a lack of genetic evidence regarding whether and how plant genetic effects positively reshape drought induced microbiome changes. Root hairs play essential roles in water uptaking, but whether root hairs also orchestrate microbiome re-shaping process during drought stress is unknown. By utilizing genetic mutants with enhanced or decreased root hair densities, we detected a significant effect of plant genetic effect on drought induced microbiome changes. In addition, the hairy mutant (gl2) triggers a deterministic process during drought induced microbiome reassembly, which further confirms the involvement of host effects in re-shaping drought induced microbiome changes. Rhizobiaceae strains were detected as key biomarker species positively correlated with root hair densities. Moreover, the gl2 mutant also shapes more complex microbiome co-occurrence networks, with more Rhizobiaceae hubs. Our findings unveil the novel roles of root hairs in shaping microbiome structure and network interactions upon drought stress, particularly through regulating the abundance and network centrality of Rhizobiaceae strains. Root hair related mutants also broadly affect root metabolome upon drought stress. Understanding the physiological and microbial ecological basis of host mediated microbiome re-shaping under drought helps develop microbiome engineering approaches to combat climate changes.

Full Text

Preamble

Root hairs shape microbiome structure and network interactions upon drought stress

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Abstract

Drought is one of the most serious abiotic stresses and profoundly alters the composition of root-associated microbiomes. However, genetic evidence regarding whether and how plant genetic factors positively reshape drought-induced microbiome changes remains lacking. Root hairs play essential roles in water uptake, yet whether they also orchestrate microbiome reshaping during drought stress is unknown. Using genetic mutants with enhanced or decreased root hair densities, we detected significant plant genetic effects on drought-induced microbiome changes. The hairy mutant (*gl2*) triggered a deterministic dominant process during drought-induced microbiome re-assembly, confirming host involvement in reshaping drought-induced microbiome changes. *Rhizobiaceae* strains emerged as key biomarker species positively correlated with root hair density. Moreover, the *gl2* mutant shaped more complex microbiome co-occurrence networks with more *Rhizobiaceae* hubs. Our findings unveil novel roles for root hairs in shaping microbiome structure and network interactions under drought stress, particularly through regulating the abundance and network centrality of *Rhizobiaceae* strains. Root hair-related mutants also broadly affect the root metabolome during drought. Understanding the physiological and microbial ecological basis of host-mediated microbiome reshaping under drought will help develop microbiome engineering approaches to combat climate change.

Introduction

Plant roots are surrounded by highly diverse soil microbial communities, and their co-evolution is crucial for rhizosphere ecosystem function and plant fitness. Different plant ecotypes or cultivars share common and sometimes heritable microbiome features, indicating that hosts can selectively shape a "core microbiome." Additionally, plants can actively reshape the microbiome in response to various stresses to enhance fitness. However, our understanding of the regulatory relationships between host genes and microbiome ecosystems, and their consequences for plant fitness, remains in its infancy.

Drought stress poses a significant threat to global agricultural production, with estimates indicating that over 50% of arable land will be affected by drought by 2050. Drought also profoundly disrupts soil and plant-associated microbiomes across various plant species and ecosystems, and prolonged drought stress even dampens rhizosphere ecosystem resilience after recovery. Currently, how hosts reshape root-associated microbiomes under drought stress remains largely elusive.

Numerous studies suggest that host effects are positively involved in reshaping drought-induced root microbiome changes, though solid genetic evidence is still lacking. Drought stress exerts a much stronger influence on root-associated microbiomes than on bulk soil microbiomes. Furthermore, drought effects on root-associated microbiomes vary across plant developmental stages (e.g., flowering versus non-flowering), indicating that developmental stage impacts drought-induced microbiome reshaping. Integrated multi-omics approaches have identified glycerol-3-phosphate (G3P) and iron as potential metabolic cues affecting drought-induced microbiome shifts. Studies across wild species and crops have demonstrated that drought stress can enrich certain microbes, such as Actinobacteria (especially *Streptomyces*), which may enhance host drought tolerance. Recent research has also shown that tree seedlings inoculated with microbiomes from dry or warmer environments exhibit enhanced fitness over multiple years. These studies suggest that microbiome engineering or "microbiome breeding" approaches hold promise as environmentally friendly strategies to help plants adapt to climate change. Further genetic studies could provide deeper mechanistic understanding of how hosts positively regulate drought-triggered microbiome changes, which is crucial for harnessing the microbiome to combat drought stress.

Plant genetic studies have provided valuable insights into how plants regulate microbiome composition. For example, our previous genetic screening identified the receptor-like kinase FERONIA, which regulates colonization by beneficial *Pseudomonas* species, potentially related to pathogen-triggered recruitment of beneficial *Pseudomonads*. A pioneering genetic study demonstrated that Arabidopsis mutants disrupting several hormone signaling pathways could alter microbiome structure, providing the first solid genetic evidence that plants can shape root-associated microbiomes. Moreover, using a quadruple mutant to dampen multiple plant immune pathways, a previous study revealed the critical roles of innate immunity in maintaining microbiome homeostasis and plant health. Leveraging genetic manipulations in Arabidopsis, researchers have further shown that various plant signaling pathways influence microbiome structure, including epigenetic modifications, small RNA generation, and diverse metabolic pathways. Although plant genetic tools have been extensively employed to dissect microbiome regulation mechanisms, genetic studies addressing how plants positively reshape microbiomes under drought stress are still lacking.

Root hairs are essential for water and nutrient uptake and serve as frontline cells in host-microbiome interactions. Additionally, plants secrete approximately 20-30% of their photosynthetic carbon as root exudates into the rhizosphere, with root hairs playing a vital role in this process. We therefore hypothesized that root hairs might orchestrate microbiome sculpting during drought stress through long-term evolution. The well-characterized genetic regulation pathways of root hair development enable us to obtain mutants with altered root hair densities to study their effects on drought-induced microbiome changes. The master transcription factor GLABRA 2 maintains a non-hair cell fate and negatively regulates root hair initiation and development. In contrast, the basic helix-loop-helix transcription factors ROOT HAIR DEFECTIVE 6 (RHD6) and its homolog RHD6-LIKE 1 (RSL1) positively regulate expression of RSL2-5 genes to promote root hair development. We thus utilized genetic mutants with different root hair densities to dissect the role of root hairs in regulating drought-induced microbiome changes.

The objectives of this study are: (1) to decipher how genetic mutations affecting root hairs influence microbiome composition under drought stress; (2) to identify keystone taxa or microbes influenced by root hairs under drought stress; (3) to profile potential metabolic cues related to root hair-mediated community assembly; and (4) to validate the effects of root hair-regulated microbes on plant growth and drought tolerance. This study will enhance our understanding of the genetic and physiological mechanisms involved in plant-mediated microbiome reshaping under drought.

Results

Mutants with Different Root Hair Densities Shift Microbiome Composition Under Drought Stress

To provide genetic evidence for the role of root hairs in shaping drought-induced microbiome changes, we employed the *rsl2 rsl4* double mutant (complete loss of root hairs), the *gl2* mutant (significantly increased root hair density), and wild-type Arabidopsis for microbiome profiling analysis (Fig. 1a [Figure 1: see original paper]). To mimic a highly diverse natural soil microbiome and enrich drought-adapted microbes, we mixed natural soils from a tropical rainforest (expected to have high microbial diversity) and a dry-hot valley in southwest China (expected to contain drought-adapted microbes) as our experimental soil. To comprehensively capture microbiome changes across compartments, we sampled both root (thoroughly washed) and rhizosphere (soil closely attached to the root surface) microbiomes for each group.

A total of 15,332,970 reads were obtained from 70 samples, including rhizosphere, root, and bulk soil (soil under the same treatment conditions but without plants) samples (Supplementary Table 1). After filtering, denoising, chimera removal, and taxonomic annotation using the SILVA database with a pre-trained naive Bayes classifier in DADA2, we obtained 5,174 amplicon sequence variants (ASVs) from all samples (Supplementary Table 2). We observed significant differences in microbiome composition between root-associated microbiomes and bulk soil samples at both phylum and family levels, indicating a clear rhizosphere effect (Supplementary Fig. 1a, b). The *gl2* mutant showed enhanced alpha diversity in root microbiomes under control conditions (Supplementary Fig. 1c), suggesting a positive role of root hairs in maintaining microbial diversity.

Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities revealed that samples from different compartments (bulk soil, root, and rhizosphere) clustered in distinct groups (Fig. 1b; $R^2 = 0.4757$, $P < 0.001$), with significant separation among treatments (Fig. 1b; $R^2 = 0.039$*, $P < 0.05$). Both drought stress and root compartments had substantial effects on microbiome composition, consistent with previous reports. Interestingly, plant genotypes also exerted significant influence on microbiome composition (Fig. 1b; R^2 = 0.207***, $P < 0.001$). These results suggest that both root hair-related mutants (genotypes) and drought stress (treatments) jointly shape root-associated microbiome changes during drought stress.

Fig. 1 Root hair mutants shift microbiome composition under drought stress. (a) Photos and quantification of root hair densities in 5-day-old roots of Col-0, *rsl2 rsl4*, and *gl2*. Data represent mean (bar) ± standard error of mean (error bar). Experiments were repeated twice with consistent results. Different letters indicate significant differences among genotypes ($p < 0.05$ corrected using Bonferroni method, one-way ANOVA followed by LSD test; N = 40 biological replicates). (b) Principal coordinates analysis based on Bray-Curtis dissimilarity calculated from relative abundance matrices at ASV level (PERMANOVA by adonis, n = 5 replicates per group). (c) β-nearest taxon indexes (βNTI) in different genotypes. Each dot represents the βNTI calculated from each pairwise sample in each genotype and treatment condition. The dotted line represents the cutoff for determining deterministic ($|\beta\text{NTI}| > 2$) and stochastic ($|\beta\text{NTI}| < 2$) processes. Box plots show median (horizontal bar), 25th (bottom of boxes) and 75th (top of boxes) quartile range (QR), and non-outlier data values (upper and lower whiskers) of βNTI values in each group. (d) Percentage of relative influence of each community assembly cue, defined as the proportion of pairwise samples governed by each process. The horizontal line in the legend represents the boundary between main ecological processes driven by deterministic processes (above) and stochastic processes (below).

gl2 Mutant Drives a Deterministic Microbiome Assembly Process During Drought Stress

Understanding community assembly cues helps elucidate factors influencing community changes. To assess whether plant root hair-related genetic effects contribute to deterministic processes in microbiome assembly, we calculated β-nearest taxon index (βNTI) values ($|\beta\text{NTI}| > 2$ indicates a deterministic process). For rhizosphere samples, a stochastic process ($-2 < \beta\text{NTI} < 0$) governed genotype-specific bacterial community assembly in both control and drought-treated groups (Fig. 1c), suggesting a relatively weak rhizosphere effect on microbiome changes. In contrast, we detected a stronger genotype effect on root microbiome assembly. With increasing root hair density, root microbiome assembly shifted from a pattern co-governed by stochastic and deterministic processes (in *rsl2 rsl4* and Col-0) to one primarily governed by deterministic processes (in *gl2*). Importantly, bacterial community assembly in *gl2* roots was completely governed by a deterministic process (βNTI $<$ -2) under drought conditions (Fig. 1c), indicating that enhanced root hair density exerts strong deterministic influence on drought-induced root microbiome changes.

We further calculated the Bray-Curtis-based Raup-Crick index (RCbray) to quantify contributions of different community assembly cues. This index allowed us to assess the proportion of pairwise community comparisons dominated by each process. In rhizosphere samples, dispersal limitation (βNTI $<$ -2 and RCbray $>$ 0.95) governed the stochastic process across all genotypes under drought (Fig. 1d). For root samples, deterministic processes were dominated by homogeneous selection (Fig. 1d; βNTI $<$ -2). Importantly, the relative influence of homogeneous selection in bacterial community assembly was much higher (75–100%) in *gl2* than in *rsl2 rsl4* (below 50%) and Col-0 (approximately 50%). Our results provide genetic evidence for plant involvement in deterministic microbiome assembly under drought stress.

Rhizobiaceae Are Key Taxa Regulated by Root Hairs Under Drought Stress

To identify key taxa influenced by root hairs under drought conditions, we analyzed relative abundances at different taxonomic levels among genotypes. Mutants exhibited clear shifts at the family level (Supplementary Fig. 1d). *Rhizobiaceae* abundance was significantly higher in *gl2* than in *rsl2 rsl4* under drought stress (Supplementary Fig. 2b [Figure 2: see original paper]). Using RandomForest prediction, we identified *Rhizobiaceae* as the most effective family for distinguishing the three genotypes (Fig. 2a) and as the most abundant among all biomarker families identified (Fig. 2b).

We performed differential abundance analysis of ASVs between each mutant and Col-0. Differentially abundant ASVs (DA-ASVs) from rhizosphere samples were distributed across multiple families in both drought and control groups (Supplementary Fig. 2a-d). However, DA-ASVs in root samples were enriched in a few families, particularly under drought conditions (Supplementary Fig. 2e, f; Fig. 2c, d). We detected five significantly enriched ASVs in *gl2*, three of which belonged to *Rhizobiaceae* (Fig. 2c). Only one ASV (belonging to *Comamonadaceae*) was enriched in *rsl2 rsl4* root microbiomes (Fig. 2d). Collectively, our machine learning-based biomarker prediction and differential abundance analysis at the family level both support *Rhizobiaceae* as major biomarker taxa regulated by root hairs under drought stress.

Fig. 2 Biomarker taxa and species between mutants and Col-0. (a-b) Seven marker families were identified under drought conditions by Kruskal-Wallis rank sum test and random forest classification (a) with their corresponding relative abundances (b) in each genotype. In bar plots, data represent mean (bar) ± standard error of mean (error bar). (c-d) Manhattan plots showing differentially abundant ASVs enriched or depleted in roots of *gl2* (c) and *rsl2 rsl4* (d) compared to Col-0 (Wilcoxon rank sum test, unadjusted $p < 0.05$) under drought conditions ($n = 5$ biological replicates per genotype and treatment). Each dot and triangle represents an ASV, colored by taxonomic family. Dot/triangle size represents relative abundance. Solid upward triangles indicate ASVs enriched in mutant roots; hollow downward triangles indicate ASVs depleted in mutants.

gl2 Mutant Affects the Network Importance of *Rhizobiaceae* Nodes

Complex microbe-microbe interactions substantially affect network structure and stability, which in turn influence community function. Network analysis is widely used to identify keystone taxa that play important roles in shaping microbial community structure and function. Since we observed deterministic assembly processes only in root but not rhizosphere microbiomes (Fig. 1c, d), we focused on network interactions within root microbiomes. To explore whether and how root hairs affect network interactions, we constructed co-occurrence networks based on Spearman's correlations (correlation coefficient threshold of 0.7, $PFDR < 0.05$) between paired ASVs in root microbial communities within each genotype.

Compared to Col-0 (Fig. 3a [Figure 3: see original paper]; 89 nodes under control; Fig. 3d, 118 nodes under drought), the hairy mutant *gl2* had much larger and more complex microbiome networks under both control (Fig. 3c; 287 nodes) and drought (Fig. 3f; 147 nodes) conditions. Network connectivity (total edges) and average connectivity (average links/degree per node) were also higher in *gl2* compared to Col-0 and *rsl2 rsl4* (Fig. 3a-f; Supplementary Table 3). In contrast, no significant differences in network size or connectivity were observed between *rsl2 rsl4* and Col-0 networks (Fig. 3a, b, d, e). Our data strongly support that increased root hair density leads to increased complexity in root microbiome networks under both control and drought conditions.

Differences in network topological properties among nodes (ASVs) determine each node's importance. Degree centrality (number of edges connected to a node) and closeness centrality (average length of shortest paths between a node and all other nodes) are commonly used to describe node importance. ASVs from the top six families (overlapping top 10 abundance-ranked and top 10 network-node-count-ranked families across all genotypes) showed significantly higher mean degree centrality in *gl2* networks under control conditions compared to Col-0 and *rsl2 rsl4* (Fig. 3g; ANOVA followed by Fisher's LSD test, $P < 0.05$ corrected using Bonferroni method). ASVs belonging to *Rhizobiaceae*, *Comamonadaceae*, and *Oxalobacteraceae* showed significant differences in mean degree centrality in *gl2* compared to Col-0 under drought conditions. The mean degree centrality of *Rhizobiaceae* ASVs consistently increased from *rsl2 rsl4* to Col-0 to *gl2*, indicating a crucial role for root hairs in maintaining the network importance of *Rhizobiaceae* nodes (Fig. 3h).

Hub nodes (ASVs) were identified based on degree centrality, closeness centrality, and betweenness centrality. Two hub ASVs were identified in the *gl2* network under drought treatment, both belonging to *Rhizobiaceae* (Supplementary Fig. 3). We also detected increased interactions with *Rhizobiaceae* nodes in *gl2* under drought conditions. In the *gl2* co-occurrence network, edges interacting with *Rhizobiaceae* ASVs increased from 16.2% to 24.7% from control to drought conditions (Supplementary Table 3). Collectively, our results suggest that increased root hair density enhances network complexity and the importance of *Rhizobiaceae* ASVs in the root microbiome.

Fig. 3 Changes in microbial co-occurrence networks of root-associated samples from root hair mutants and Col-0. (a-c) Co-occurrence networks of root microbiomes from *rsl2 rsl4* (a), Col-0 (b), and *gl2* (c) under control conditions. Red dots indicate *Rhizobiaceae* nodes. (d-f) Co-occurrence networks of root microbiomes from *rsl2 rsl4* (d), Col-0 (e), and *gl2* (f) under drought conditions. Exact node and edge numbers are indicated below each graph. Arrows indicate hub nodes within each network. (g-h) Distributions of degree centrality for ASVs from the top six families across microbiome networks in roots of different genotypes under control (g) and drought (h) conditions. Different letters indicate significant differences among genotypes ($p < 0.05$ corrected using Bonferroni method, one-way ANOVA followed by LSD test). Box plots show median (horizontal bar), 25th (bottom of boxes) and 75th (top of boxes) quartile range (QR), and non-outlier data values (upper and lower whiskers) of ASV degree centrality within each family.

gl2 Mutant Broadly Shifts Root Metabolome Composition and Enriches Flavonoids

Root exudates play a pivotal role in shaping genotype- or stress-specific microbiomes. To investigate potential metabolic cues related to *gl2*-mediated microbiome reshaping under drought stress, we conducted non-targeted metabolomics analysis of root chemical compositions in *gl2*, *rsl2 rsl4*, and Col-0. A total of 3,920 compounds were identified across root samples from different genotypes. Principal component analysis (PCA) revealed significant differences in root metabolite biochemical composition among genotypes ($R^2 = 0.58$, $P < 0.05$, PERMANOVA by adonis) (Fig. 4a [Figure 4: see original paper]). Flavonoid content was significantly higher (7.1%) in *gl2* roots compared to Col-0 (4.5%) and *rsl2 rsl4* (2.1%) (Fig. 4b). Conversely, relative ketone content was higher in *rsl2 rsl4* roots (51.4%) compared to Col-0 (32.6%) but lower in *gl2* roots (11.7%) (Fig. 4b). Indole and its derivatives, as well as organic acids and their derivatives, were higher in *gl2* roots compared to Col-0 but lower in *rsl2 rsl4*. These metabolome profiling results demonstrate that mutations in root hair development significantly alter root metabolite composition under drought.

Differentially abundant metabolites (DMs) were identified based on variable importance in projection scores ($VIP > 1.0$) and fold changes ($logFC > 0$) in relative abundances ($P < 0.05$). A total of 383 DMs were identified between *gl2* and Col-0 (Supplementary Table 4), and 964 DMs were identified between *rsl2 rsl4* and Col-0 (Supplementary Table 5). Enrichment analysis based on DMs in *rsl2 rsl4* and *gl2* revealed that *gl2* DMs were enriched in flavonoid biosynthesis pathways, as well as caffeine metabolism, purine metabolism, and nucleotide metabolism pathways (Fig. 4c). In contrast, *rsl2 rsl4* DMs were mainly enriched in C5-branched dibasic acid metabolism, plant hormone signal transduction, butanoate metabolism, and monobactam biosynthesis pathways (Supplementary Fig. 4a).

A previous study showed that flavonoid accumulation in roots can induce chemotaxis of *Aeromonas* and enhance plant dehydration resistance. Furthermore, flavonoids (e.g., naringenin) are well known for inducing rhizobia nod gene expression in leguminous plants and chemoattracting rhizobia toward roots. In the present study, tangeretin (a flavonoid) in *gl2* roots exhibited the highest VIP value (Supplementary Table 3; $P < 0.001$) among DMs with more than 3-fold enrichment compared to Col-0 (Fig. 4d, Supplementary Fig. 4b). These results comprehensively reveal metabolome changes in *gl2* during drought stress, including enrichment of flavonoid compounds previously reported to be associated with rhizobia colonization in leguminous plants.

Fig. 4 Metabolomic profiling of root samples from different genotypes under drought stress. (a) Principal component analysis (PCA) of metabolites detected in roots of Col-0, *gl2*, and *rsl2 rsl4* ($p < 0.001$, permutational multivariate analysis of variance (PERMANOVA) by Adonis). N = 3 biological replicates. (b) Relative content of different metabolite classes in roots of Col-0, *gl2*, and *rsl2 rsl4*. (c) Enriched pathways of differentially abundant metabolites (DMs) in *gl2* compared to Col-0 under drought conditions. The differential abundance (DA) score represents the number of changed DMs relative to total metabolites within each pathway. Colors indicate p-values; dot size indicates metabolite number. (d) Variable importance of projection (VIP) scores of all DMs (fold change > 2 displayed) in *gl2* roots. Colors indicate p-values; circle size represents fold changes of relative content in *gl2* versus Col-0.

Discussion

In the era of the host-microbiome holobiont, understanding how host genetics facilitate recruitment of beneficial microbes under stress is fundamental for engineering plant microbiomes. This requires integrating genetic approaches, multi-omics, and reductionist-based validation. Thanks to powerful and cost-effective plant genetics, previous studies have established that plant genetic effects positively contribute to recruiting disease-suppressive microbes. In contrast, while drought is the most serious abiotic stress that drastically disturbs root-associated microbiomes, whether and how plant genetic effects positively recruit stress-alleviating microbes during drought remains largely unknown. By leveraging elaborately selected genetic mutants with a gradient of root hair densities, we confirmed the crucial role of root hairs in positively reshaping drought-triggered microbiome changes. Our microbiome composition and network analyses revealed *Rhizobiaceae* as the major family regulated by root hairs. Our work supports the essential role of plant genetic effects in positively reshaping a drought-alleviating microbiome and advances our understanding of the "cry for help" phenomenon during abiotic stress.

Similar to gut microvilli, plant root hairs substantially increase root surface area and play essential roles in nutrient and water uptake. Conventional crop breeding programs typically prioritize high root hair density and length as desirable traits, primarily from a nutrient uptake perspective. However, our work reveals a critical novel role for root hairs in orchestrating beneficial interactions with root-associated microbiomes. This is reminiscent of a recent study surveying root cell type-specific transcriptome responses to beneficial *Pseudomonas simiae* WCS417, which found that root hairs show special immune responsiveness to WCS417. Root hair-related mutants also dampen immune responses and compatible interactions with WCS417, highlighting the crucial role of root hairs in sensing and regulating interactions with beneficial microbes. Furthermore, our recent single-nucleus RNA-seq analysis revealed that beneficial microbes (*Pseudomonas simiae* WCS417) and pathogenic microbes (*Ralstonia solanacearum* GMI1000) trigger very distinct transcriptome responses in root hairs (trichoblasts) (only 11.84% overlap). Beneficial WCS417 promotes expression of growth-related GO terms associated with ribosome functions, while pathogenic GMI1000 triggers senescence and phosphorelay signaling-related stress responses in root hairs. This further supports the specialized role of root hairs in differential responses to beneficial and pathogenic microbes. Since both root hairs and gut microvilli are continually exposed to extremely diverse microbiomes, these cell types may share similar microbial ecological roles in mediating host-microbiome interactions. Further molecular and microbial ecological studies in plant systems will advance our understanding of the rules governing host-microbiome interactions in these "frontline" hair cells.

Although *Rhizobiaceae* is one of the most well-studied plant symbiotic bacterial families in legumes, our work suggests they confer fitness benefits and act as microbiome network hubs in the non-legume plant Arabidopsis. A previous study surveying core microbiome compositions across diverse plant lineages (31 species) characterized *Bradyrhizobium* and *Rhizobium* as universally plant-enriched core microbiome taxa. Phylogenetic analysis of 1,314 Rhizobiales genomes suggested that both nodulating and non-nodulating strains share common genes related to root colonization. The evolutionarily conserved associations between *Rhizobiaceae* and roots indicate their crucial roles in root-associated microbiome structure and function. Moreover, a previous study in *Medicago* suggested that *Rhizobiaceae* are critical hub species in the root microbiome and that genetic mutants losing association with Rhizobiales show altered microbiome structure. Interestingly, in our non-legume Arabidopsis, enhanced root hair density was associated with increased colonization levels and more edges connected to *Rhizobiaceae* nodes in the microbial co-abundance network, especially under drought stress. This further suggests potentially conserved roles for *Rhizobiaceae* in mediating microbe-microbe interactions within root microbiome networks.

Most mechanistic studies of root-*Rhizobiaceae* interactions have been conducted in legumes. *Rhizobiaceae* can enter root tissues not only in legumes but also in non-legumes like rice, suggesting they may be endophytes for both plant types. A recent study systematically surveyed transcriptome responses to *Rhizobiaceae* in roots and found that NAC060 is a key regulator of Rhizobiales-specific responses. More importantly, they identified that host sulfated peptide (phytosulfokine) signaling is essential for *Rhizobiaceae*-mediated growth-promoting effects, providing critical insights into the molecular mechanisms underlying root-Rhizobiales interactions. Given the powerful genetic and genomic tools available for Arabidopsis and *Rhizobiaceae*, this interaction system will likely facilitate new discoveries about root-commensal interactions.

Materials and Methods

Plant Material and Growth Conditions

All seeds were surface-sterilized for 20 minutes with chlorine gas (exposure to 100 ml bleach plus 5 ml concentrated hydrochloric acid) to eliminate potential endophytes. Sterilized seeds were soaked in 0.1% agar solution and stored at 4°C in darkness for 2 days before use. Seeds were germinated on 1/2× Murashige and Skoog (MS) agar plates with 1% sucrose (12 h light/12 h dark). Seven-day-old seedlings were transplanted into pots filled with soil (described below) in a greenhouse. Plants were grown under 10 h light (100 mol m^{-2} s^{-1})/14 h dark at 22°C.

Natural Soil Growth Substrates

Natural soils were collected from Yuanjiang Savanna Ecosystem Research Station (E102°10 , N23°28) and Xishuangbanna Tropical Botanic Garden (E101°27 , N21°92) of the Chinese Academy of Sciences. Visible stones, plant debris, and litter were removed before harvesting. Soil was sieved through a 2 mm mesh, and soils from different locations were thoroughly homogenized and mixed 1:1. We prepared a mixed substrate composed of equal volumes (1:1:1:1) of mixed natural soil, commercial growth room soil, vermiculite, and perlite for all natural soil experiments. Soil was placed in 6 cm × 6 cm pots in the greenhouse for plant transplantation. To sterilize natural soil, we autoclaved the substrate twice (121°C for 20 minutes each) with at least 24 hours between autoclaving sessions (which thoroughly kills any germinated microbe spores after the first autoclaving). Fertilizer was added once per week for natural soil growth substrates.

Drought Treatments

Plants were transplanted from plates into natural soil growth substrates 7 days after germination. After normal watering for three weeks, plants within each genotype were randomly assigned to drought and control treatments. Drought treatment was conducted by completely withholding water, with all plants rotated randomly each day.

Sample Collection

Whole plants were removed from pots, and soil not closely adhered to roots was removed by shaking. Root tissue from four plants was immediately cut below the shoot-root junction into a 50 ml Falcon tube containing 25 ml sterile PBS (10 mM/L). After shaking at 180 rpm for 20 minutes, roots were transferred to a new 10 ml Falcon tube with fresh PBS. The washing buffer was centrifuged at 4,000g for 20 minutes at 16°C, and the resulting pellet was defined as the rhizosphere sample. For root sample collection, roots in fresh PBS were thoroughly washed and sonicated (40 Hz for 30 s) twice to remove remaining soil. Pot soil without plants, after removing the top 2 cm, was defined as bulk soil. All samples were transferred to new 2 ml tubes, immediately frozen in liquid nitrogen, and stored at -80°C before DNA extraction.

DNA Extraction and Microbiome Sequencing

DNA was extracted using the PowerSoil DNA Isolation Kit (Qiagen, Germany) following the manufacturer's protocol. Samples with DNA concentration >20 ng/ l were used for microbiome sequencing. For bacterial amplicon sequencing, the V3-V4 region of the 16S rRNA gene was amplified with primers 349F (5 -ACTCCTACGGGAGGCAGCA-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3). Amplification was performed under thermal conditions: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 minutes. Libraries were prepared using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). Library concentrations were determined using a Qubit 4.0 Fluorometer. Paired-end 250 bp sequencing was performed on the Illumina NovaSeq 6000.

Microbiome Data Processing

Raw sequencing reads were filtered using fastp v.0.14.1. Adapter sequences and primers were removed using cutadapt v.4.0. Sequencing reads were processed using QIIME2 v.2022.2. Briefly, DADA2 was used to generate a table of unique amplicon sequence variants (ASVs) and their counts per sample. For taxonomic annotation, representative sequences of each ASV were assigned to the SILVA database (release 138) using a pre-trained naive Bayes classifier. Unassigned sequences and those annotated as chloroplast or mitochondrial (considered host contamination) were removed. ASVs present in fewer than 3 samples were also removed. Retained ASVs were used for downstream analysis.

Microbial Community Assembly Processes

Null model analysis was performed to evaluate microbial community assembly processes by calculating β-nearest taxon index (βNTI). First, we calculated observed abundance-weighted β-mean-nearest taxon distance (βMNTDobs) of pairwise communities using the comdistnt function in the "picante" R package. By randomly shuffling phylogenetic tree tips, null model expectations and distributions of βMNTD (βMNTDnull) were generated through 999 randomizations. βNTI values were then calculated to quantify standard deviations of βMNTDobs from the βMNTDnull distribution. When $|\beta\text{NTI}| > 2$, community assembly is interpreted as governed by deterministic processes. Conversely, $|\beta\text{NTI}| < 2$ indicates stochastic-dominated assembly. Heterogeneous and homogeneous selection in deterministic processes were estimated by $\beta\text{NTI} > 2$ and $\beta\text{NTI} < -2$, respectively.

To further assess contributions of stochastic and deterministic processes ($|\beta\text{NTI}| < 2$) in community assembly, we calculated the Raup-Crick index (RCbray) as previously described. When pairwise community comparisons had $|\text{RCbray}| > 0.95$, dispersal was indicated to drive community turnover. $\text{RCbray} > 0.95$ indicated dispersal limitation-dominated turnover, while $\text{RCbray} < -0.95$ indicated homogenizing dispersal. When pairwise comparisons had $|\beta\text{NTI}| < 2$ and $|\text{RCbray}| < 0.95$, community turnover was estimated to be affected by drift alone. βNTI and RCbray values were integrated to assess deterministic and stochastic processes in microbial community assembly and the relative influence of each specific process.

Metabolome Profiling

For sample collection, fresh roots from 16 plants grown in natural soil were harvested as one biological replicate. Root collection steps were identical to those for microbiome sequencing. After sampling, root samples were quickly (within 5 minutes) and thoroughly washed in 200 ml sterile deionized water, then immediately frozen in liquid nitrogen. Fresh tissue weight was recorded before storage at -80°C until processing.

Non-targeted metabolomics was performed to investigate impacts of root hair mutation on root metabolites under drought conditions. Metabolite detection and identification were conducted using ultra-performance liquid chromatography (LC-30A, Shimadzu, Japan)–tandem mass spectrometry (TripleTOF 6600+, SCIEX) (UPLC-MS/MS) at Wuhan Metware Biotechnology Co., Ltd. (Wuhan, China).

For metabolome data processing, principal component analysis (PCA) was implemented using the prcomp function in the R "stats" package to assess differences in root metabolites among root hair mutants and Col-0. Differentially abundant metabolites (DMs) between each mutant and Col-0 were determined by variable importance in projection ($\text{VIP} > 1$), P-value ($P < 0.05$, Student's t-test), and fold change ($|\log_2\text{FC}| > 0$). VIP values were extracted from Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) results generated using the R package MetaboAnalystR. Heatmaps showing relative abundance of differential metabolites between mutants and Col-0 were generated using the "ComplexHeatmap" R package. Differential metabolites were annotated using the KEGG compound database and mapped to the KEGG Pathway database.

Statistical Analysis and Data Visualization

All statistical analyses were conducted in R v.4.1.3. Before selecting statistical tests, normality was assessed using the Shapiro-Wilk test. Bartlett's test was used to test for homogeneity of variances using the "stats" package. The "vegan" package was used to assess alpha and beta diversity of root-associated microbial communities. Species richness and Shannon diversity were calculated using the diversity function. Differences between root hair mutants and Col-0 were assessed using one-way ANOVA followed by LSD test for multiple comparisons using the LSD.test function in the "agricolae" package. Bray-Curtis distance matrices were calculated using the vegdist function, and PCoA plots were generated accordingly. Permutational multivariate analysis of variance (PERMANOVA) was performed with the "adonis" function to assess effects of genotype (Col-0, *gl2*, and *rsl2 rsl4*), water regime (drought and control), and compartment (bulk soil, root, and rhizosphere) on microbiome composition variation. The "randomForest" and "microeco" packages were used to perform differential abundance tests and identify potential marker families among genotypes. Wilcoxon rank sum tests were used to detect differential ASVs (FDR-adjusted $p < 0.05$) between each root hair mutant and Col-0.

For network analysis, ASVs with relative abundance >0.01% and present in at least two samples were used for co-occurrence network construction. Spearman correlation analysis (correlation coefficient > 0.7, PFDR < 0.05) was conducted between paired ASVs using the "ggClusterNet" package. Network topological parameters, including node and edge counts, positive and negative correlations, and node properties (degree centrality, closeness centrality, betweenness centrality), were calculated using the "igraph" package. Hub nodes were identified based on all three centrality measurements using a log-normal distribution fit. Except for network visualization conducted in Gephi 0.10, all plots were generated using the "ggplot2" package.

Data Availability

All raw 16S amplicon and shotgun metagenomic sequencing data reported in this study will be deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) upon publication.

Author Contributions

Y.S. designed the project. Z.W. and Z.L. conducted experiments with help from J.Z., X.T., and K.G. Z.W. analyzed data. Y.S. and Z.W. wrote the manuscript.

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References

1. Wippel, K. et al. Host preference and invasiveness of commensal bacteria in the *Lotus* and *Arabidopsis* root microbiota. *Nat Microbiol* 6, 1150-1162 (2021). https://doi.org:10.1038/s41564-
2. Steinauer, K. et al. Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment. *Ecology* 96, 99-112 (2015). https://doi.org:10.1890/14-0088.1
3. Wang, Z. et al. Land use intensification in a dry-hot valley reduced the constraints of water content on soil microbial diversity and multifunctionality but increased CO_2 production. *The Science of the Total Environment* 852, 158397 (2022). https://doi.org:10.1016/j.scitotenv.2022.158397
4. Allsup, C. M., George, I. & Lankau, R. A. Shifting microbial communities can enhance tree tolerance to changing climates. *Science* 380, 835-840 (2023). https://doi.org:10.1126/science.adf2027
5. Walters, W. A. et al. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. *Proceedings of the National Academy of Sciences of the United States of America* 115, 7368-7373 (2018). https://doi.org:10.1073/pnas.1800918115
6. Lundberg, D. S. et al. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488, 86-90 (2012). https://doi.org:10.1038/nature11237
7. Haney, C. H., Samuel, B. S., Bush, J. & Ausubel, F. M. Associations with rhizosphere bacteria can confer adaptive advantage to plants. *Nature Plants* (2015). https://doi.org:10.1038/nplants.2015.51
8. Wang, Z. & Song, Y. Toward understanding the genetic bases underlying plants mediated "cry for help" to the microbiota. *iMeta* 1, e8 (2022). https://doi.org:10.1002/imt2.8
9. Zhang, H., Sun, X. & Dai, M. Improving crop drought resistance with plant growth regulators and rhizobacteria: Mechanisms, applications, and perspectives. *Plant Commun* 3, 100228 (2022). https://doi.org:10.1016/j.xplc.2021.100228
10. Naylor, D., DeGraaf, S., Purdom, E. & Coleman-Derr, D. Drought and host selection influence bacterial community dynamics in the grass root microbiome. *The ISME Journal* 11, 2691-2704 (2017). https://doi.org:10.1038/ismej.2017.118
11. Santos-Medellin, C., Edwards, J., Liechty, Z., Nguyen, B. & Sun-

daresan, V. Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes. *mBio* 8 (2017). https://doi.org:10.1128/mBio.00764-17

12. Santos-Medellín, C. et al. Prolonged drought imparts lasting compositional changes to the rice root microbiome. *Nature Plants* 7, 1065-1077 (2021). https://doi.org:10.1038/s41477-021-00967-1
13. Xu, L. et al. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 115, E4284-e4293 (2018). https://doi.org:10.1073/pnas.1717308115
14. Xu, L. et al. Genome-resolved metagenomics reveals role of iron metabolism in drought-induced rhizosphere microbiome dynamics. *Nature Communications* (2021). https://doi.org:10.1038/s41467-021-23553-7
15. Fitzpatrick, C. R. et al. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America* 115, E1157-E1165 (2018). https://doi.org:10.1073/pnas.1717617115
16. Xu, L. et al. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 115, E4284-E4293 (2018). https://doi.org:10.1073/pnas.1717308115
17. Naumann, G. et al. Global changes in drought conditions under different levels of warming. *Geophysical Research Letters* 45, 3285-3296 (2018). https://doi.org:10.1002/2017gl076521
18. de Vries, F. T., Griffiths, R. I., Knight, C. G., Nicolitch, O. & Williams, A. Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science* 368, 270-274 (2020). https://doi.org:10.1126/science.aaz5192
19. Song, Y. et al. FERONIA restricts *Pseudomonas* in the rhizosphere microbiome via regulation of reactive oxygen species. *Nature Plants* 7, 644-654 (2021). https://doi.org:10.1038/s41477-021-
20. Lebeis, S. L. et al. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349, 860-864 (2015). https://doi.org:10.1126/science.aaa8764
21. Chen, T. et al. A plant genetic network for preventing dysbiosis in the phyllosphere. *Nature* (2020). https://doi.org:10.1038/s41586-020-2185-0
22. Pfeilmeier, S. et al. The plant NADPH oxidase RBOHD is required for microbiota homeostasis in leaves. *Nature Microbiology* 6, 852-864 (2021). https://doi.org:10.1038/s41564-021-00929-5
23. Vilchez, J. I. et al. DNA demethylases are required for myo-inositol-mediated mutualism between plants and beneficial rhizobacteria. *Nature Plants* (2020). https://doi.org:10.1038/s41477-020-0707-2
24. Lv, S. et al. Dysfunction of histone demethylase IBM1 in Arabidopsis causes autoimmunity and reshapes root microbiome. *The ISME Journal* (2022). https://doi.org:10.1038/s41396-022-01297-6
25. Kaushal, R. et al. Dicer-like proteins influence Arabidopsis root microbiota independent of RNA-directed DNA methylation. *Microbiome* 9, 57 (2021).

https://doi.org:10.1186/s40168-020-00966-y

26. Huang, A. C. et al. A specialized metabolic network selectively modulates Arabidopsis root microbiota. *Science* 364 (2019). https://doi.org:10.1126/science.aau6389
27. Stringlis, I. A. et al. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proceedings of the National Academy of Sciences of the United States of America* 115, E5213-E5222 (2018). https://doi.org:10.1073/pnas.1722335115
28. Ruiz, S. et al. Significance of root hairs at the field scale—modelling root water and phosphorus uptake under different field conditions. *Plant and Soil* (2020). https://doi.org:10.1007/s11104-019-04308-2
29. Zhang, X. et al. The spatial distribution of rhizosphere microbial activities under drought: water availability is more important than root-hair-controlled exudation. *New Phytologist* (2022). https://doi.org:10.1111/nph.18409
30. Holz, M., Zarebanadkouki, M., Kuzyakov, Y., Pausch, J. & Carminati, A. Root hairs increase rhizosphere extension and carbon input to soil. *Annals of Botany* 121, 61-69 (2018). https://doi.org:10.1093/aob/mcx127
31. Lin, Q. et al. GLABRA2 directly suppresses basic helix-loop-helix transcription factor genes with diverse functions in root hair development. *The Plant Cell* 27, 2894-2906 (2015). https://doi.org:10.1105/tpc.15.00607
32. Pires, N. D. et al. Recruitment and remodeling of an ancient gene regulatory network during land plant evolution. *Proceedings of the National Academy of Sciences of the United States of America* 110, 9571-9576 (2013). https://doi.org:10.1073/pnas.1305457110
33. Yi, K., Menand, B., Bell, E. & Dolan, L. A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. *Nature Genetics* 42, 264-267 (2010). https://doi.org:10.1038/ng.529
34. Alonso, J. M. et al. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* 301, 653-657 (2003). https://doi.org:10.1126/science.1086391
35. Callahan, B. J. et al. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13, 581-583 (2016). https://doi.org:10.1038/nmeth.3869
36. Xu, L. et al. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 115, E4284-E4293 (2018). https://doi.org:10.1073/pnas.1717308115
37. Gao, C. et al. Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. *Nature Communications* 13 (2022). https://doi.org:10.1038/s41467-022-31343-y
38. Stegen, J. C., Lin, X., Konopka, A. E. & Fredrickson, J. K. Stochastic and deterministic assembly processes in subsurface microbial communities. *The ISME Journal* 6, 1653-1664 (2012). https://doi.org:10.1038/ismej.2012.22
39. Liu, C., Cui, Y., Li, X. & Yao, M. microeco: an R package for data mining in microbial community ecology. *FEMS Microbiology Ecology* 97 (2020).

https://doi.org:10.1093/femsec/fiaa255

40. Faust, K. & Raes, J. Microbial interactions: from networks to models. *Nature Reviews Microbiology* 10, 538-550 (2012). https://doi.org:10.1038/nrmicro2832
41. Banerjee, S., Schlaeppi, K. & van der Heijden, M. G. A. Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology* 16, 567-576 (2018). https://doi.org:10.1038/s41579-
42. Barberan, A., Bates, S. T., Casamayor, E. O. & Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *The ISME Journal* 6, 343-351 (2012). https://doi.org:10.1038/ismej.2011.119
43. Agler, M. T. et al. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLOS Biology* 14, e1002352 (2016). https://doi.org:10.1371/journal.pbio.1002352
44. Schafer, M. et al. Metabolic interaction models recapitulate leaf microbiota ecology. *Science* 381, eadf5121 (2023). https://doi.org:10.1126/science.adf5121
45. O'Banion, B. S. et al. Plant myo-inositol transport influences bacterial colonization phenotypes. *Current Biology* (2023). https://doi.org:10.1016/j.cub.2023.06.057
46. He, D. et al. Flavonoid-attracted *Aeromonas* sp. from the Arabidopsis root microbiome enhances plant dehydration resistance. *The ISME Journal* (2022). https://doi.org:10.1038/s41396-022-01288-7
47. Hassan, S. & Mathesius, U. The role of flavonoids in root-rhizosphere signalling: opportunities and challenges for improving plant-microbe interactions. *Journal of Experimental Botany* 63, 3429-3444 (2012). https://doi.org:10.1093/jxb/err430
48. Bakker, P., Pieterse, C. M. J., de Jonge, R. & Berendsen, R. L. The Soil-Borne Legacy. *Cell* 172, 1178-1180 (2018). https://doi.org:10.1016/j.cell.2018.02.024
49. Brown, L. K., George, T. S., Dupuy, L. X. & White, P. J. A conceptual model of root hair ideotypes for future agricultural environments: what combination of traits should be targeted to cope with limited P availability? *Annals of Botany* 112, 317-330 (2012). https://doi.org:10.1093/aob/mcs231
50. Saengwilai, P. et al. Root hair phenotypes influence nitrogen acquisition in maize. *Annals of Botany* 128, 849-858 (2021). https://doi.org:10.1093/aob/mcab104
51. Verbon, E. H. et al. Cell-type-specific transcriptomics reveals that root hairs and endodermal barriers play important roles in beneficial plant-rhizobacterium interactions. *Molecular Plant* 16, 1160-1177 (2023). https://doi.org:10.1016/j.molp.2023.06.001
52. Yang, Q. et al. Comparative single-nucleus RNA-seq analysis captures shared and distinct responses to beneficial and pathogenic microbes in roots. *bioRxiv*, 2023.08.03.551619 (2023).
53. Yeoh, Y. K. et al. Evolutionary conservation of a core root microbiome across plant phyla along a tropical chronosequence. *Nature Communications* (2017). https://doi.org:10.1038/s41467-017-00262-8
54. Garrido-Oter, R. et al. Modular traits of the Rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host &*

Microbe 24, 155-167.e155 (2018). https://doi.org:10.1016/j.chom.2018.06.006

55. Wang, X. et al. Mycorrhizal symbiosis modulates the rhizosphere microbiota to promote rhizobia-legume symbiosis. *Molecular Plant* 14, 503-516 (2021). https://doi.org:10.1016/j.molp.2020.12.002
56. Yang, J. et al. Mechanisms underlying legume-rhizobium symbioses. *Journal of Integrative Plant Biology* 64, 244-267 (2022). https://doi.org:10.1111/jipb.13207
57. Perrine-Walker, F. M., Prayitno, J., Rolfe, B. G., Weinman, J. J. & Hocart, C. H. Infection process interaction of rice roots with rhizobia. *Journal of Experimental Botany* 58, 3343-3350 (2007). https://doi.org:10.1093/jxb/erm181
58. Hucklenbroich, J. et al. Rhizobiales commensal bacteria promote *Arabidopsis thaliana* root growth via host sulfated peptide pathway. *bioRxiv*, 2021.05.25.444716 (2021).
59. Haney, C. H., Samuel, B. S., Bush, J. & Ausubel, F. M. Associations with rhizosphere bacteria can confer adaptive advantage to plants. *Nature Plants* (2015). https://doi.org:10.1038/nplants.2015.51
60. Bulgarelli, D. et al. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* 488, 91-95 (2012). https://doi.org:10.1038/nature11336
61. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884-i890 (2018).
62. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17, 3 (2011). https://doi.org:10.14806/ej.17.1.200
63. Bolyen, E. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37, 852-857 (2019). https://doi.org:10.1038/s41587-019-0209-9
64. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41, D590-596 (2013). https://doi.org:10.1093/nar/gks1219
65. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73, 5261-5267 (2007). https://doi.org:10.1128/aem.00062-07
66. Kembel, S. W. et al. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463-1464 (2010). https://doi.org:10.1093/bioinformatics/btq166
67. Jizhong, Z. & Daliang, N. Stochastic Community Assembly: Does It Matter in Microbial Ecology? *Microbiology and Molecular Biology Reviews* (2017). https://doi.org:10.1128/MMBR
68. Stegen, J. C. et al. Quantifying community assembly processes and identifying features that impose them. *The ISME Journal* 7, 2069-2079 (2013). https://doi.org:10.1038/ismej.2013.93
69. Chong, J. & Xia, J. MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data. *Bioinformatics* (2018). https://doi.org:10.1093/bioinformatics/bty528
70. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and

correlations in multidimensional genomic data. *Bioinformatics* 32, 2847-2849 (2016).

71. Jari Oksanen et al. vegan: Community Ecology Package, R package version 2.5-7 (2020).
72. de Mendiburu, F. & de Mendiburu, M. F. Package ‘agricolae’. R Package, version 1.3-5 (2021).
73. Liu, C., Cui, Y., Li, X. & Yao, M. microeco: an R package for data mining in microbial community ecology. *FEMS Microbiology Ecology* 97 (2020). https://doi.org:10.1093/femsec/fiaa255
74. Wen, T. et al. ggClusterNet: An R package for microbiome network analysis and modularity-based multiple network layouts. *iMeta* 1 (2022). https://doi.org:10.1002/imt2.32
75. Csárdi, G. & Nepusz, T. The igraph software package for complex network research. *InterJournal, Complex Systems* 1965, 1-9 (2006).
76. Bastian, M., Heymann, S. & Jacomy, M. Gephi: an open source software for exploring and manipulating networks. *Proceedings of the International AAAI Conference on Web and Social Media* 3, 361-362 (2009). https://doi.org:https://doi.org/10.1609/icwsm.v3i1.13937
77. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis.* Springer-Verlag New York (2016).

Supplementary Information

Supplementary Fig. 1. (a-b) Relative abundance of phylum- (a) and family-level (b) compositions in root, rhizosphere, and bulk soil microbiomes under control and drought conditions. (c) Shannon index of microbial communities in root and rhizosphere samples. Lowercase letters indicate significant differences among genotypes ($P < 0.05$ corrected using Bonferroni method, one-way ANOVA followed by LSD test). (d) Ternary plot of relative abundance-based families detected in roots of Col-0, *gl2*, and *rsl2 rsl4*. Each circle represents a family. (e) Accumulation of relative abundance of ASVs belonging to *Rhizobiaceae* increased with root hair density. Data represent mean (bar) ± standard error of mean (error bar). Different letters indicate significant differences among genotypes ($p < 0.05$ corrected using Bonferroni method, one-way ANOVA followed by LSD test).

Supplementary Fig. 2. Manhattan plots showing differentially abundant ASVs (DA-ASVs) enriched (Wilcoxon rank sum test, dotted line indicates unadjusted $P < 0.05$) in rhizosphere samples (a-d) or roots (f-g) of *rsl2 rsl4* and *gl2* versus Col-0 under control and drought conditions ($n = 5$ replicates per group). Each dot and triangle represents an ASV, colored by taxonomic family. Size represents relative abundance. Solid upward triangles indicate ASVs enriched in mutant roots; hollow downward triangles indicate ASVs depleted in mutants.

Supplementary Fig. 3. Microbial hub nodes (ASVs) within root microbial communities identified in each co-occurrence network under drought stress (related to Fig. 2d-f). Hub nodes were identified based on all three centrality measurements (degree, closeness centrality, and betweenness centrality). Different colors represent nodes belonging to different families. Hub ASVs are labeled. Yellow line: p = 0.1 based on log-normal distribution fit.

Supplementary Fig. 4. Metabolome profiling of mutants affecting root hair densities. (a) Enriched pathways of DMs between *rsl2 rsl4* mutant and Col-0 roots under drought conditions. The differential abundance (DA) score represents changes in DMs within each pathway in *rsl2 rsl4* versus Col-0. Scores of -1 and 1 represent that all DMs in the pathway were downregulated and upregulated, respectively. (b) Heat map showing differences in relative content (log) of all differential flavonoids between root hair mutants and Col-0.

Note: Figure translations are in progress. See original paper for figures.

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