Supplementary Materials for

Root hair developmental regulators orchestrate drought triggered microbiome changes and the interaction with beneficial Rhizobiaceae

Author list

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The PDF file include:

Supplementary Fig. 1-11

Supplementary Tables 1-4

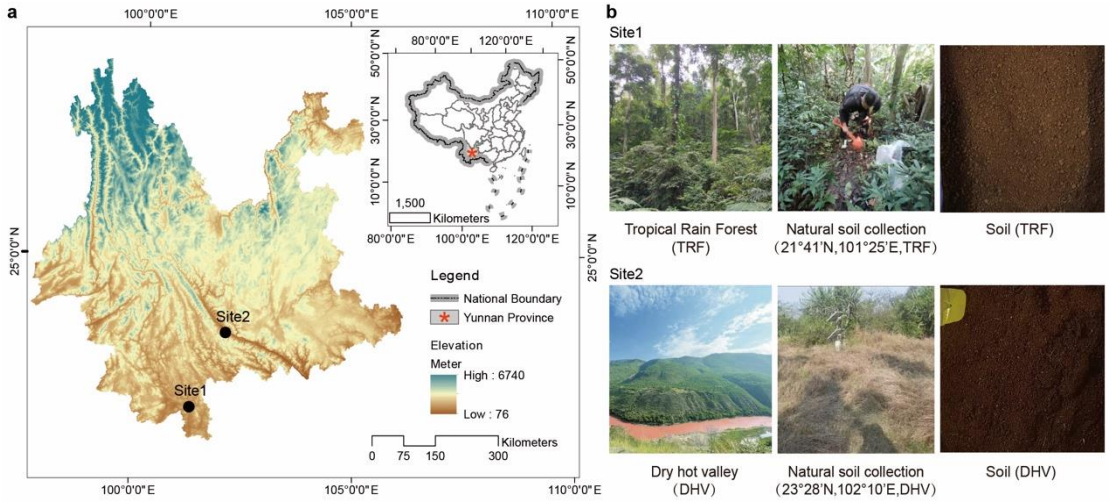
Legend for Supplementary Fig. 1-11 and Supplementary Tables 1-4

Other Supplementary Material for this manuscript includes the following:

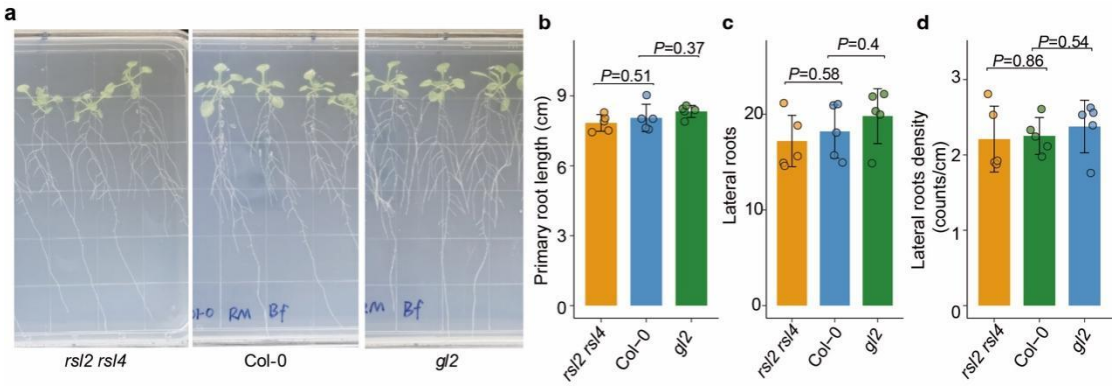
Supplementary Data 1-10

Supplementary Fig. 1 | Geographic location of natural soil sites used in this study.

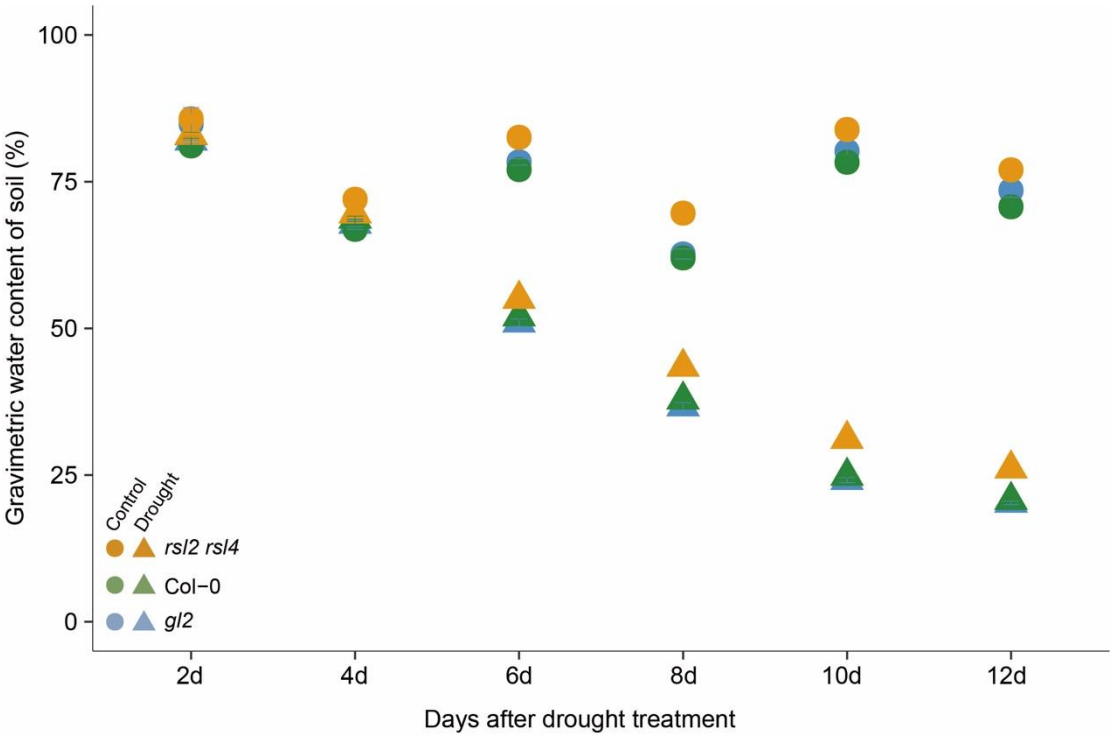
a Locations of 2 natural soil collection sites from different ecosystems, including tropical rain forest (site 1) and Yuanjiang dry-hot valley (Savanna Ecosystem, site 2), Yunnan Province, China. **b** Photos displayed the landscape of different ecosystems, the habitats of the sampling sites, and the soil types.



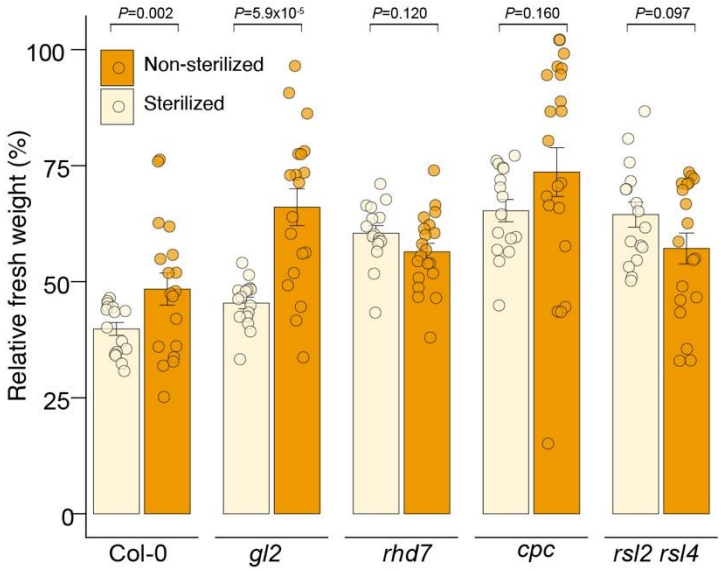
Supplementary Fig. 2 | Root hair related mutants do not affect primary and lateral root growth on plates. a Phenotype of the roots (9 days after germination) of all root hair mutans and Col-0 grow on the 1/2 MS plates. **b** Primary root length, **c** number of lateral roots, and **d** lateral roots density (number of lateral roots/primary root length) were assessed for all genotypes (two-sided Student's t-test). Data represent mean (bar) ± standard error of mean (error bar). *n*=5 biological replicates for individual group.



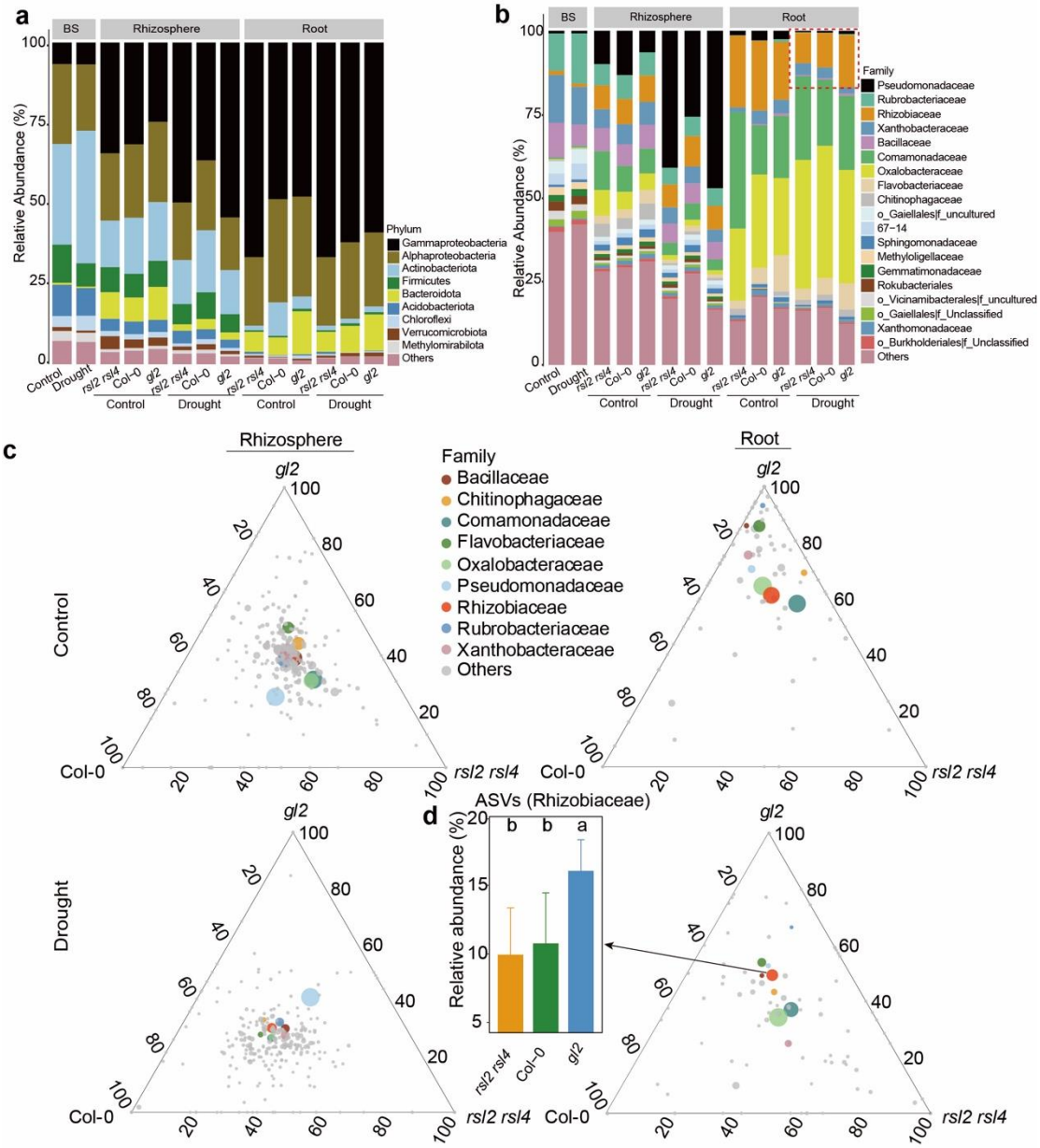
Supplementary Fig. 3 | Measurements of soil gravimetric water content in control and drought treated groups. Measurements of soil gravimetric water content in control and drought treated groups. *n*=5 for each genotype in each group. Different colors indicate genotypes and shapes indicate treatments. Data represent mean (bar) ± standard error of mean (error bar). Gravimetric soil water content (%) = [(mass of moist soil (g) − mass of oven-dried soil (g))/mass of oven-dried soil (g)] × 100.



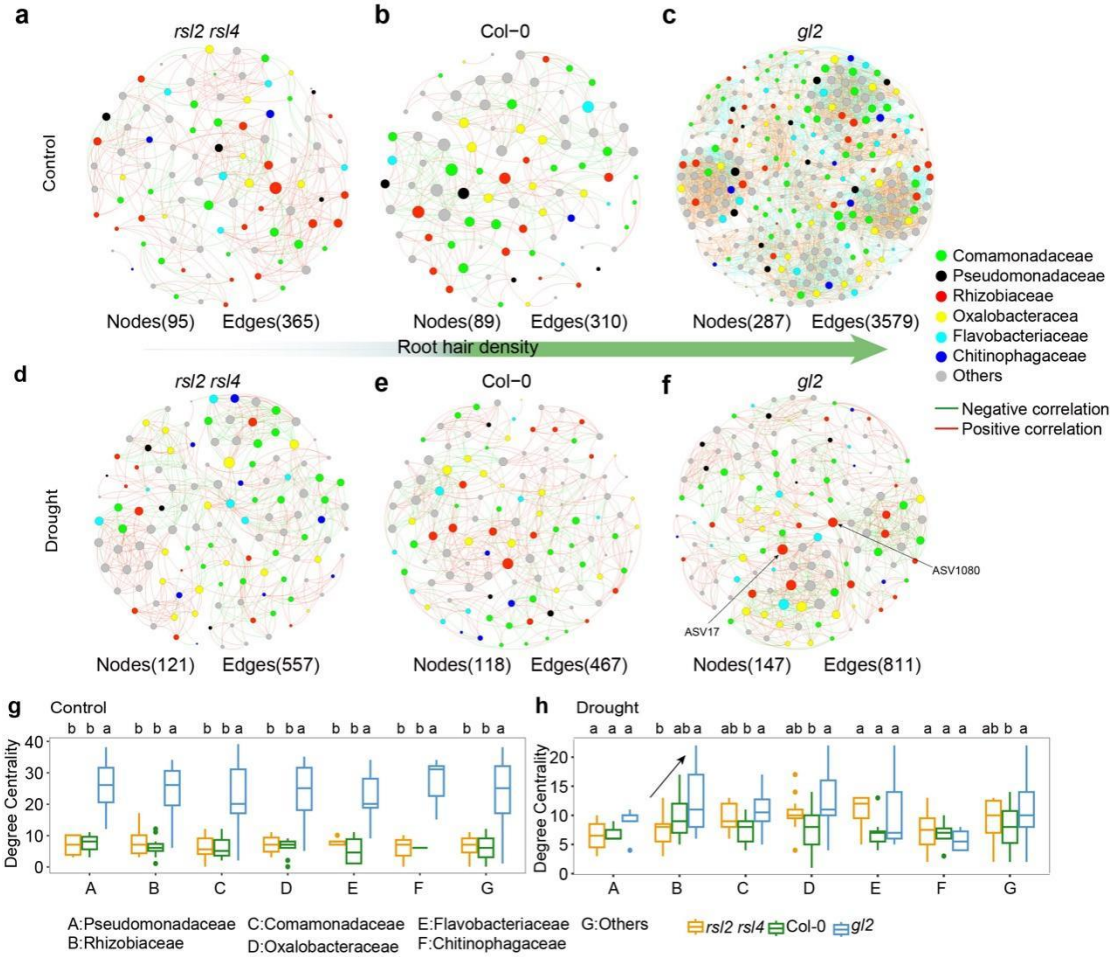
Supplementary Fig. 4 | Root hair related genetic mutants affect microbiome mediated drought protecting effect in a farm soil harvested in Guangzhou, China. The relative fresh weight (fresh weight of plant shoots grown in drought treated group relative to the average shoot fresh weight in the control group) between sterilized treatment and non-sterilized treatment (two-sided Student's t-test) were assessed for each genotype (n=20). Data represent mean (bar) ± standard error of mean (error bar).



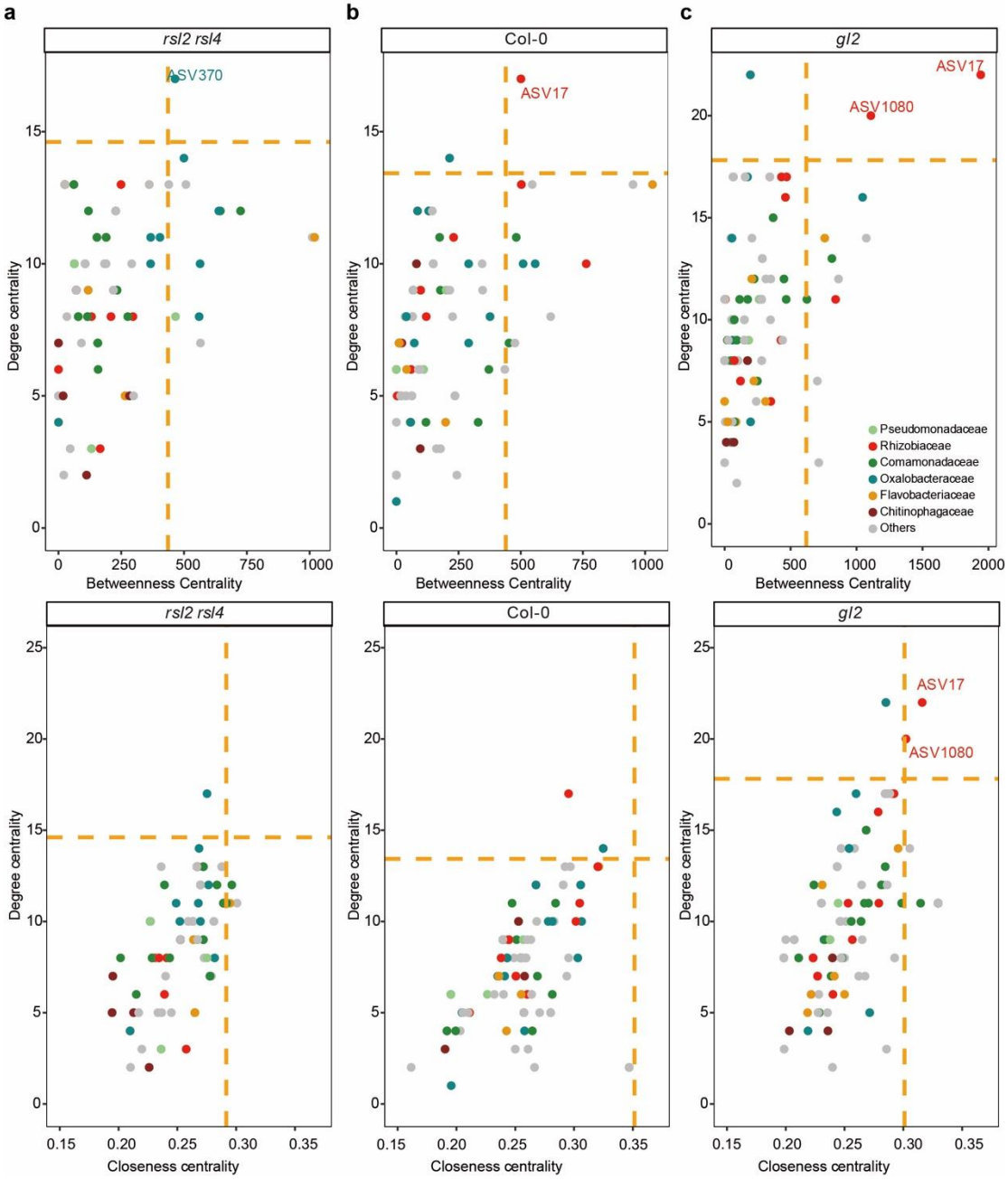
Supplementary Fig. 5 | Relative abundance distribution and differences of root-associated microbial community among all genotypes. a-b Relative abundance of phyla (a) and family (b) level compositions in the root, rhizosphere and bulk soil (BS) microbiomes under drought and control. **c** Ternary plot of different bacterial families detected in the roots of Col-0, *gl2*, and *rsl2 rsl4*. Different colors represent different families. **d** The relative abundance of ASVs belonging to Rhizobiaceae in different genotypes. Data are represented as mean (bar) ± standard error of mean (error bar). Different letters represent the significant (one-way ANOVA followed by LSD test) differences among genotypes.



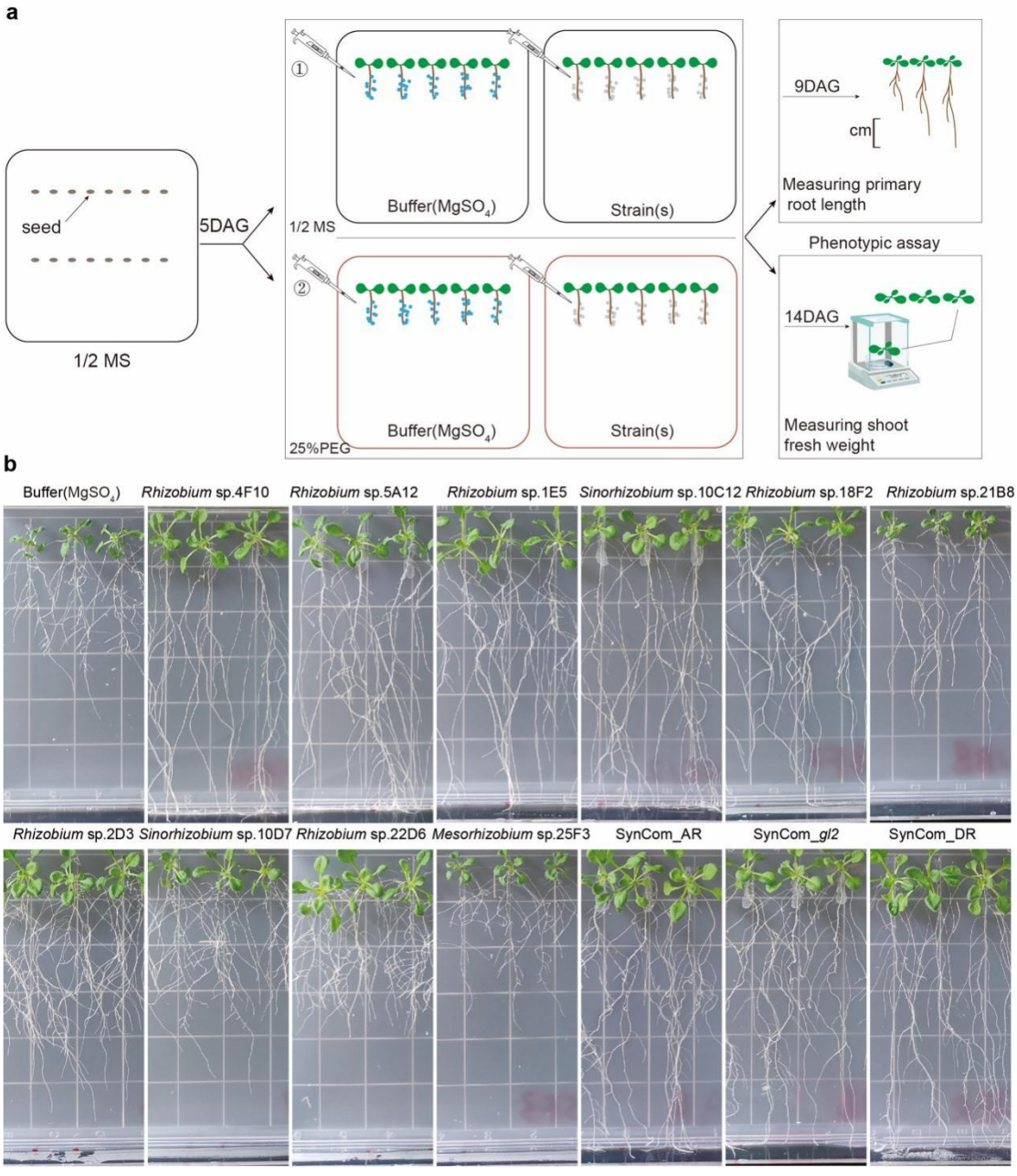
Supplementary Fig. 6 | Changes in the microbial co-occurrence networks in root-associated microbiomes. a-c Co-occurrence networks of microbiomes in the root samples of *rsl2 rsl4* (**a**), Col-0 (**b**), *gl2* (**c**) under control. Red dots indicate nodes belonging to Rhizobiaceae. **d-f** Co-occurrence networks in the root microbiome in *rsl2 sl4* (**d**), Col-0 (**e**), and *gl2* (**f**) under drought condition. The exact numbers of nodes and edges were indicated below each graph. The arrows indicate the hub nodes within each network. **g-h** The distributions of degree centrality of ASVs from the top 6 families across microbiome networks in the root microbiome under control (**g**) and drought condition (**h**). The different letters represent the significant (one-way ANOVA followed by LSD test) differences among genotypes. Box plots show the median (horizontal bar), 25th (bottoms of boxes) and 75th (tops of boxes) quartiles range (QR), and non-outlier data value (upper and lower whiskers) of ASV's degree centrality belonging to each family within each network.



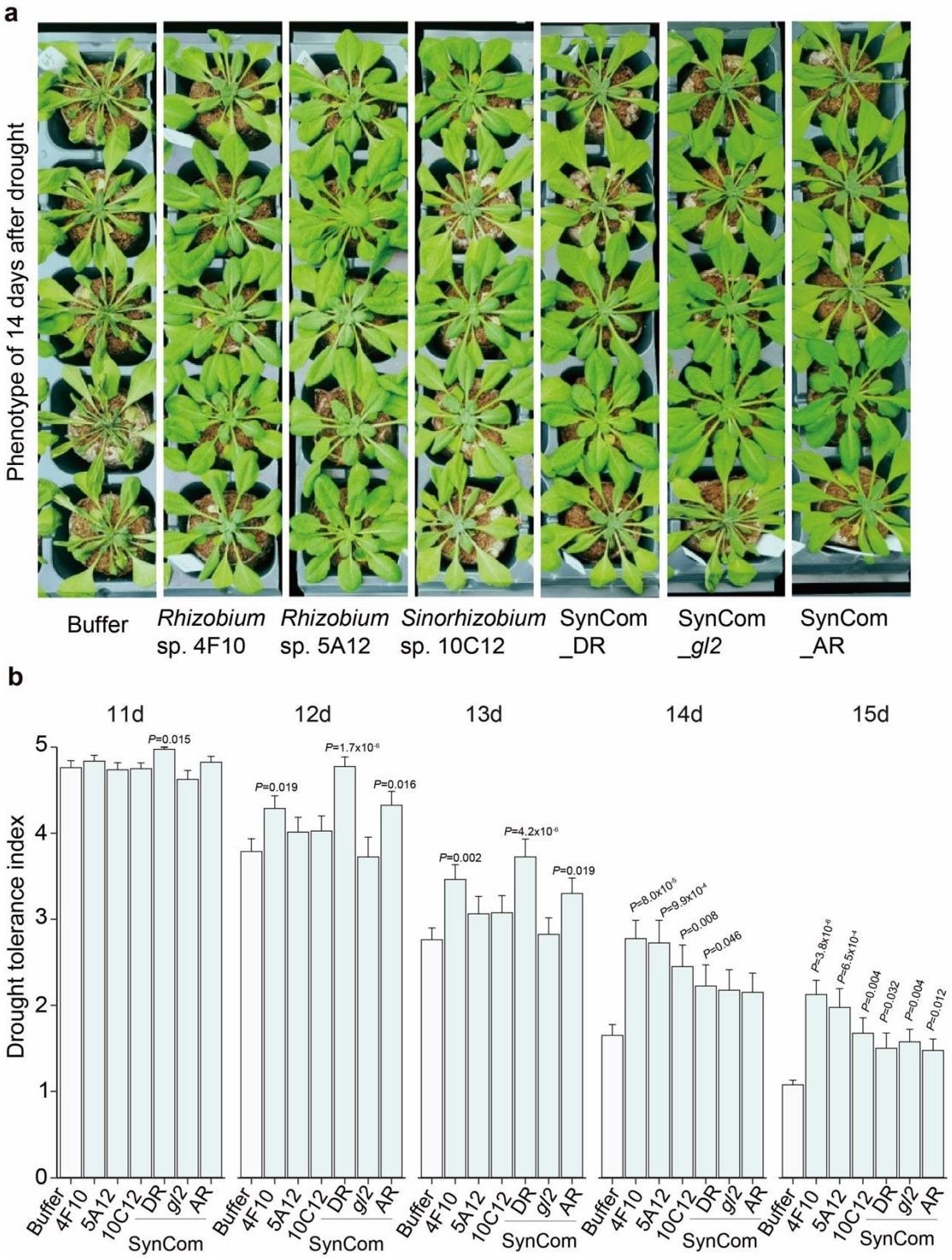
Supplementary Fig. 7 | Microbial hub nodes (ASVs) in the community co-occurrence networks. Hub nodes were identified based on all three cutoffs of centrality indexes, including degree centrality, closeness centrality, and betweenness centrality. Different colors represent nodes belonging to different families. The hub ASVs were labeled. Yellow line: p = 0.1 based on a log-normal distribution fit.



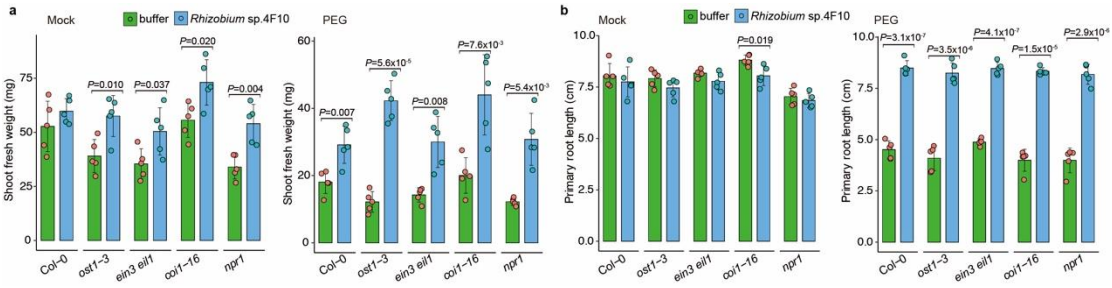
Supplementary Fig. 8 | Experiment design of osmotic stress alleviating experiment. a The design of PEG plate based osmotic stress treatment system for testing the effects of different isolates on plant fitness. Seedlings were grown on mock (1/2 MS) plates or polyethylene-glycol (PEG) plates (1/2 MS + 25% PEG). Buffer represents 10 mM $MgSO_4$ buffer treated group. DAG, days after germination. **b** Phenotype of plants (Col-0) inoculated with different isolates grown on mock plates and 25% PEG plates (corresponding to Fig. 4b, c). Three independent experiments showed similar results.



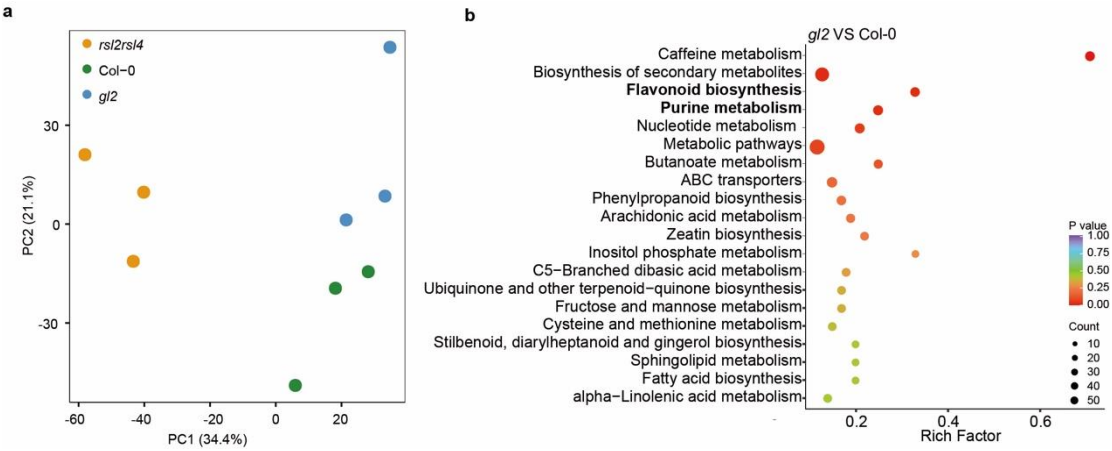
Supplementary Fig. 9 | Validating of drought protecting effects in soil. a Phenotype of plants (Col-0) inoculated with different Rhizobiaceae strains grown in the peat soil. **b** Quantification of drought tolerance indexes after inoculation of different Rhizobiaceae strains. *n*=20 biological replicates for individual group. Three independent experiments showed similar results. Only the significant differences (marked with *P* value) between plants inoculated with inoculated and uninoculated plants (two-sided Student's t-test) are listed. Data are represented as mean (bar) ± standard error of mean (error bar).



Supplementary Fig. 10 | Phenotypic analysis of *Rhizobium sp.* 4F10 mediated osmotic stress tolerance in hormone signaling related mutants and wild type (Col-0). a Shoot fresh weight and **b** Primary root length were assessed for each genotype inoculated with *Rhizobium* sp. 4F10 on mock (1/2 MS) plates and PEG plates. Data are represented as mean (bar) ± standard error of mean (error bar). *n*=5 for each genotype in each group. Buffer represents the plant inoculated with $MgSO_4$ solution (10mM). Statistical significance was determined by two-sided Student's t-test. Only the significant differences (marked with *P* value) between inoculation treatments are listed (two-sided Student's t-test).



Supplementary Fig. 11 | Metabolomic profiling of root samples from different genotypes under drought stress. a Principal component analysis (PCA) of metabolites detected in the roots of Col-0, *gl2* and *rsl2 rsl4* ($P < 0.001$, permutational multivariate analysis of variance (PERMANOVA) by Adonis). n=3 biological replicates. **b** Enriched pathways of differentially abundant metabolites (DMs) in *gl2* compared to Col-0 under drought condition. Rich factor represents ratio of differentially abundant metabolites in a specific pathway relative to the total annotated metabolites in that pathway. A higher value (size of dot) indicates a higher degree of enrichment. The *P* value represents the probability value from the hypergeometric test.



Supplementary Table 1 Topological properties of co-occurrence network of the root microbial communities of each genotype. The values in brackets represent the number of nodes and edges interacting with Rhizobiaceae ASVs.

Properties	Drought			Control		
	rsl2 rsl4	Col-0	*gl2*	*rsl2 rsl4*	Col-0	*gl2*
Nodes	121(12)	118(13)	147(16)	95(18)	89(12)	287(24)
Edges	557(94)	467(116)	811(200)	365(135)	310(79)	3579(579)
Rhizobiaceae related edges (%)	16.9%	24.8%	24.7%	37.9%	25.5%	16.2%
average degree	9.207	7.915	11.304	7.684	7.045	24.941
Average degree of ASVs belonging to Rhizobiaceae	8.167	9.165	13.125	8.389	7.417	25.125
Modularity	0.746	0.684	0.699	0.718	0.676	0.73
Average clustering coefficient	0.725	0.652	0.684	0.725	0.751	0.704
Average path length	3.975	3.937	3.946	4.063	3.993	3.289
Positive correlations	68.22%	67.88%	63.01%	73.70%	55.45%	63.34%
Negative correlations	31.78%	31.22%	36.99%	26.30%	43.55%	36.66%

Supplementary Table 2 Designing the inoculation of cultivated microbes with different strategies, including the synthetic community (SynCom) approaches. The √ symbol represents the member that was used in different inoculation strategies.

	Differential abundant ASVs			**Hub ASV**	**Isolates belonging to Rhizobiaceae**					
	ASV31 (*Sinorhizobium* sp. 10D7)	ASV35 (*Rhizobium* sp. 22D6)	ASV60 (*Sinorhizobium* sp. 10C12)	ASV17 (*Rhizobium* sp. 2D3)	*Rhizobium* sp. 1E5	*Rhizobium* sp. 5A12	*Rhizobium* sp. 4F10	*Rhizobium* sp. 21B8	Rhizobiaceae Unassigned sp. 25F3	*Rhizobium* sp. 18F2
Single strain inoculation	√	√	√	√	√	√	√	√	√	√
SynCom_DR	√	√	√							
SynCom_*gl2*	√	√	√	√						
SynCom_AR	√	√	√	√	√	√	√	√	√	√

Supplementary table 3 Differential analysis of functional genes (based on metagenomic data) between *gl2* and Col-0. Differential analysis with a two-sided test was used for the statistical analysis (DeSeq2, adjusted $P < 0.05$).

ID	baseMean	log2FoldChange	p value	p.adj	sig_level	gene	compare
K00897	386.322	7.578	5.87E-24	2.08E-20	Up	*aphA*	*gl2* VS Col-0
K01750	851.811	2.883	1.82E-32	9.71E-29	Up	*ocd*	*gl2* VS Col-0
K03111	503.628	2.325	2.07E-18	4.41E-15	Up	*ssb*	*gl2* VS Col-0
K07480	155.798	3.831	5.94E-19	1.58E-15	Up	*insB*	*gl2* VS Col-0
K08151	404.859	1.861	8.43E-12	1.28E-08	Up	*tetA*	*gl2* VS Col-0
K15269	159.196	2.620	1.13E-16	2.00E-13	Up	*pecM*	*gl2* VS Col-0
K18476	826.100	6.275	8.67E-33	9.23E-29	Up	*tetR*	*gl2* VS Col-0
K19299	384.655	8.573	1.09E-09	1.44E-06	Up	*aph3-III*	*gl2* VS Col-0

Supplementary table 4 Differential analysis of functional genes (based on metagenomic data) between *rsl2 rsl4* and Col-0. Differential analysis with a two-sided test was used for the statistical analysis (DeSeq2, adjusted $P < 0.05$).

ID	baseMean	log2FoldChange	*P* value	*P* adj	sig_level	gene	compare
K00699	26.378	-1.847	6.99E-05	2.36E-02	Down	*UGT*	*rsl2 rsl4* VS Col-0
K02144	37.167	8.680	8.59E-09	7.59E-06	Up	*ATPeV1H*	*rsl2 rsl4* VS Col-0
K03014	6.892	6.251	4.40E-06	2.22E-03	Up	*RPABC2*	*rsl2 rsl4* VS Col-0
K05666	14.654	5.448	3.35E-06	1.92E-03	Up	*ABCC2*	*rsl2 rsl4* VS Col-0
K05670	13.654	7.236	7.02E-05	2.36E-02	Up	*ABCC13*	*rsl2 rsl4* VS Col-0
K06515	24.720	-1.957	2.78E-05	1.05E-02	Down	*SLC44A1*	*rsl2 rsl4* VS Col-0
K08504	60.541	9.384	1.09E-10	1.28E-07	Up	*BET1*	*rsl2 rsl4* VS Col-0
K13126	28.057	4.550	2.25E-11	3.98E-08	Up	*PABPC*	*rsl2 rsl4* VS Col-0
K13192	6.240	6.106	2.42E-05	1.01E-02	Up	*RBM26*	*rsl2 rsl4* VS Col-0
K15712	5.884	6.023	1.44E-05	6.34E-03	Up	*TTC3*	*rsl2 rsl4* VS Col-0
K15920	22.153	3.230	3.21E-08	2.27E-05	Up	*XYL4*	*rsl2 rsl4* VS Col-0
K18698	69.608	6.390	9.21E-19	2.17E-15	Up	*blaTEM*	*rsl2 rsl4* VS Col-0
K18795	69.608	6.390	9.21E-19	2.17E-15	Up	*blaCARB-1*	*rsl2 rsl4* VS Col-0
K19217	69.608	6.390	9.21E-19	2.17E-15	Up	*blaCARB-17*	*rsl2 rsl4* VS Col-0
K21444	54.695	9.238	7.12E-11	1.01E-07	Up	*PCBP3_4*	*rsl2 rsl4* VS Col-0
K21911	16.002	-2.192	1.51E-04	4.64E-02	Down	*eryI*	*rsl2 rsl4* VS Col-0