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Supplemental Information

Root-Secreted Coumarins and the Microbiota Interact to Improve Iron Nutrition in *Arabidopsis*

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Figure S1

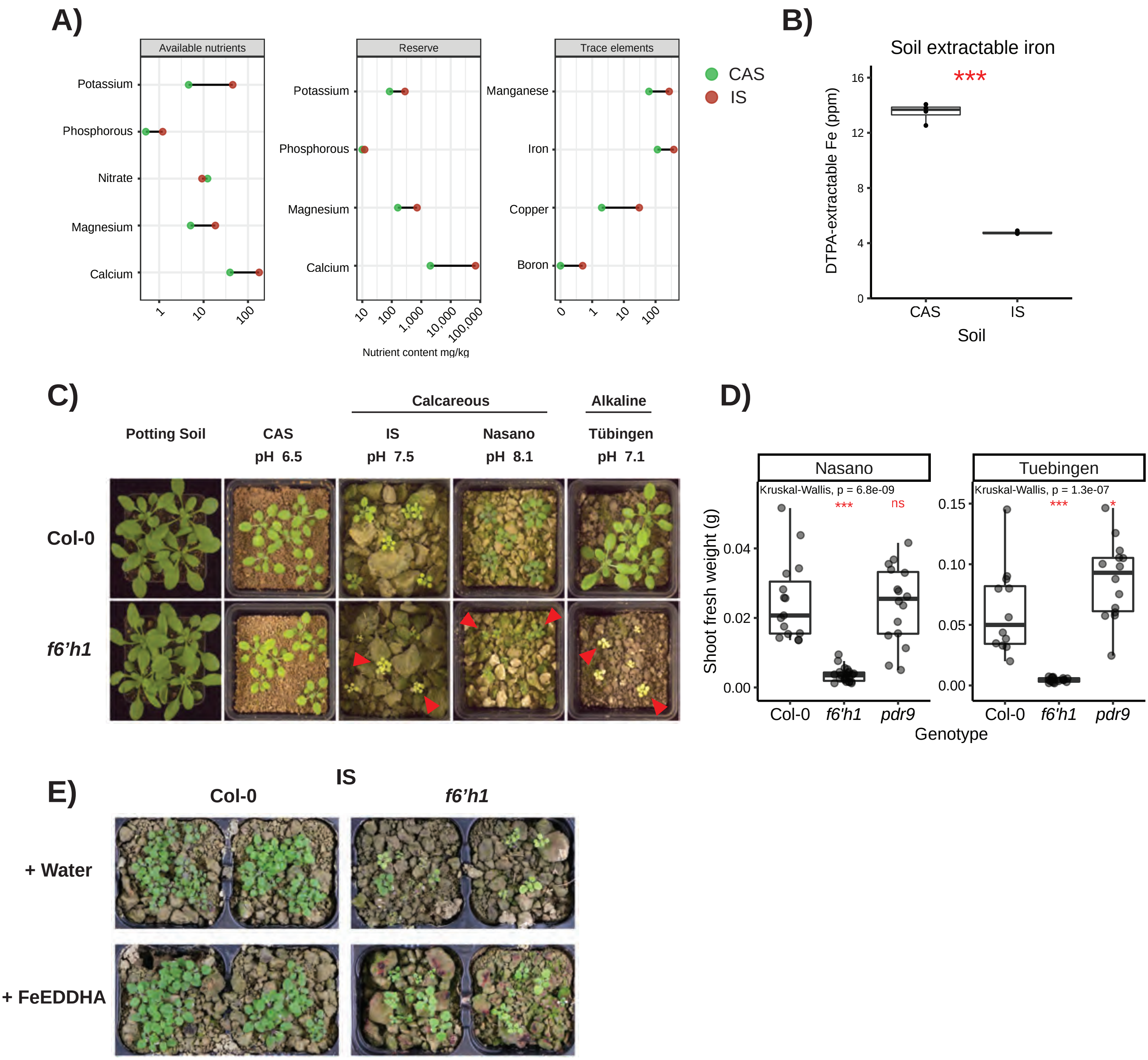
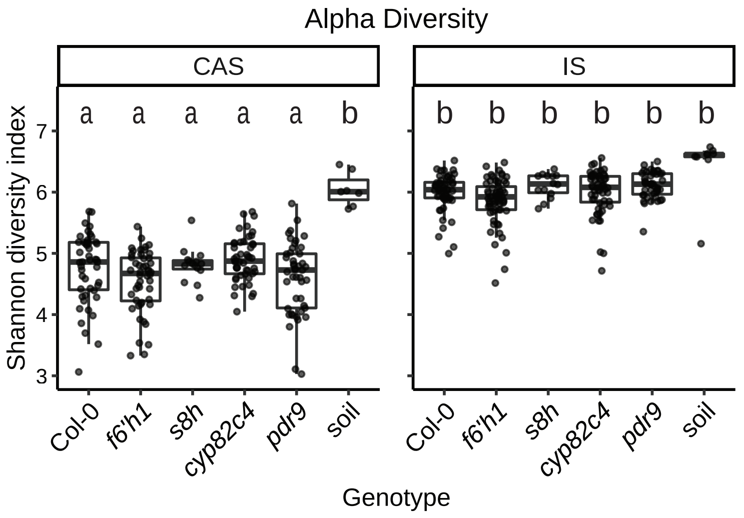


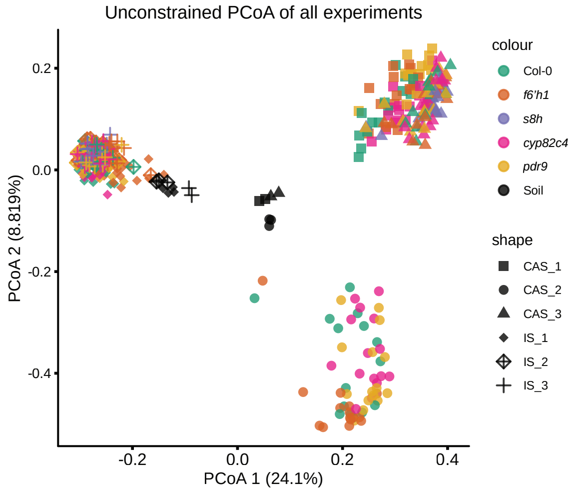
Figure S1, related to Figure 1. *f6'h1* plants exhibit stunted growth on alkaline and calcareous soils due to iron limitation.
A) Soil nutrient analysis of CAS and IS showing plant-available (water soluble) nutrients, reserve (ammonium nitrate EDTA extractable) nutrients, and trace elemental content. Soil analyses were performed by Labor für Boden- und Umweltanalytik, Switzerland. **B)** Plant-available iron content in CAS and IS. Available iron was measured as the amount of DTPA-extractable iron as in **(Lindsay and Norvell, 1978)**. Data are from 4 extractions of a single batch of each soil. Significance was determined by Student's T-test. **C)** *f6'h1* plants grow poorly in multiple alkaline and calcareous soils. Red arrowheads highlight plants displaying stunting and chlorosis. **D)** Shoot fresh weight of Col-0, *f6'h1*, and *pdr9* plants grown on Nasano and Tübingen soils from **C)**. Data are from a single soil batch and 12-24 plants per genotype. Statistical significance was determined by Kruskal-Wallis; each mutant was compared to Col-0 by Wilcoxon Ranked Sum post-hoc. **E)** Poor growth of *f6'h1* on calcareous IS soil is improved by watering with FeEDDHA solution, an iron complex with improved solubility and availability under alkaline conditions. Significance in **B)** and **D)** is indicated by red asterisks (*, **, ***, indicate $p < 0.05$, 0.01, and 0.001, respectively).

Figure S2

A)



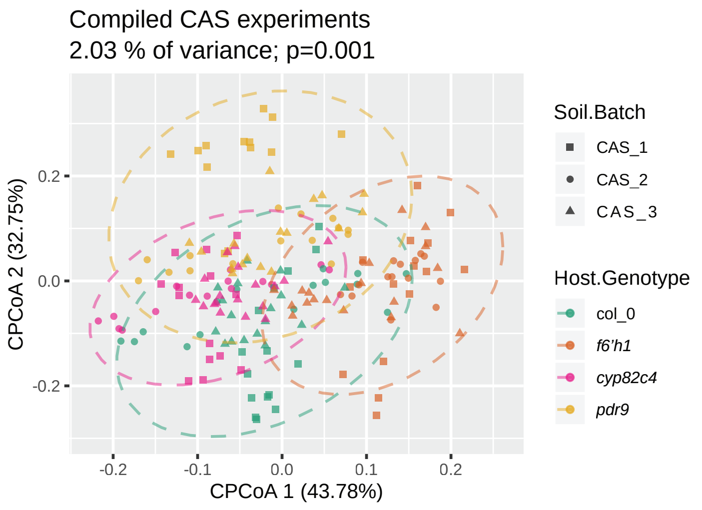
B)



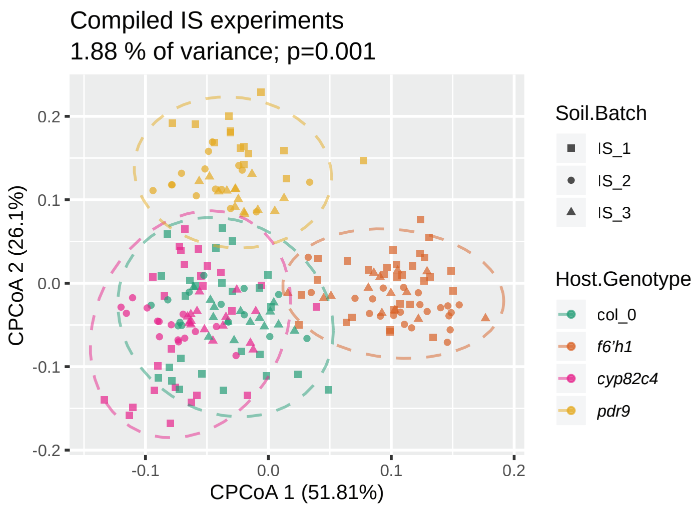
C)

Soil Batch	Col-0	f6'h1	s8h	cyp82c4	pdr9
CAS_1	15	15	0	14	13
CAS_2	15	15	0	15	15
CAS_3	17	18	15	18	17
IS_1	25	29	0	25	14
IS_2	14	22	0	15	15
IS_3	15	14	14	15	14

D)



E)



F)

Pairwise PERMANOVA

	Col-0	*f6'h1*	*cyp82c4*	*pdr9*
f6'h1	0.25	---	---	---
cyp82c4	0.41	0.25	---	---
pdr9	0.25	0.25	0.25	---

Permutations: 999

P value adjustment method: fdr

G)

Pairwise PERMANOVA

	Col-0	*f6'h1*	*cyp82c4*	*pdr9*
f6'h1	**0.002**	---	---	---
cyp82c4	0.653	**0.002**	---	---
pdr9	0.204	**0.002**	**0.204**	---

Figure S2, related to Figures 1 and 2. *f6'h1* plants have an altered root bacterial community on IS.
A) Shannon index (alpha diversity) of root samples from CAS and IS. Soil and root samples from IS have increased diversity compared to CAS samples, but did not vary by genotype. Letters indicate significant pairwise differences between groups ($p \leq 0.05$ by Dunn's pairwise comparison with Bonferroni correction. **B)** Unconstrained principle coordinate analysis (PCoA) of community profiles from three experimental replicates in each soil. The largest driver of beta-diversity is soil type, with CAS and IS samples separating along PCoA1. **C)** Summary of sample and experimental replicates for root bacterial community profiling experiments. Numbers indicate pooled root samples. Constrained PCoA (constrained for Genotype and conditioned on soil batch) displaying root bacterial community beta diversity on three batches of CAS soil (**D**) and IS soil (**E**). Genotype *s8h* was removed from the analysis, as it was only included in one of three experiments (see **Figure 1)**. The genotype effect on Bray-Curtis distances was calculated for compiled batches of CAS (**F**) and IS (**G**) samples by ADONIS test 999 permutations. Pairwise comparisons using PERMANOVA were performed on the distance matrix with 999 permutations. P values were adjusted for multiple comparisons with the FDR method. Significant separation of *f6'h1* samples from Col-0 was observed on IS, but no significant separation was observed on CAS.

Figure S3

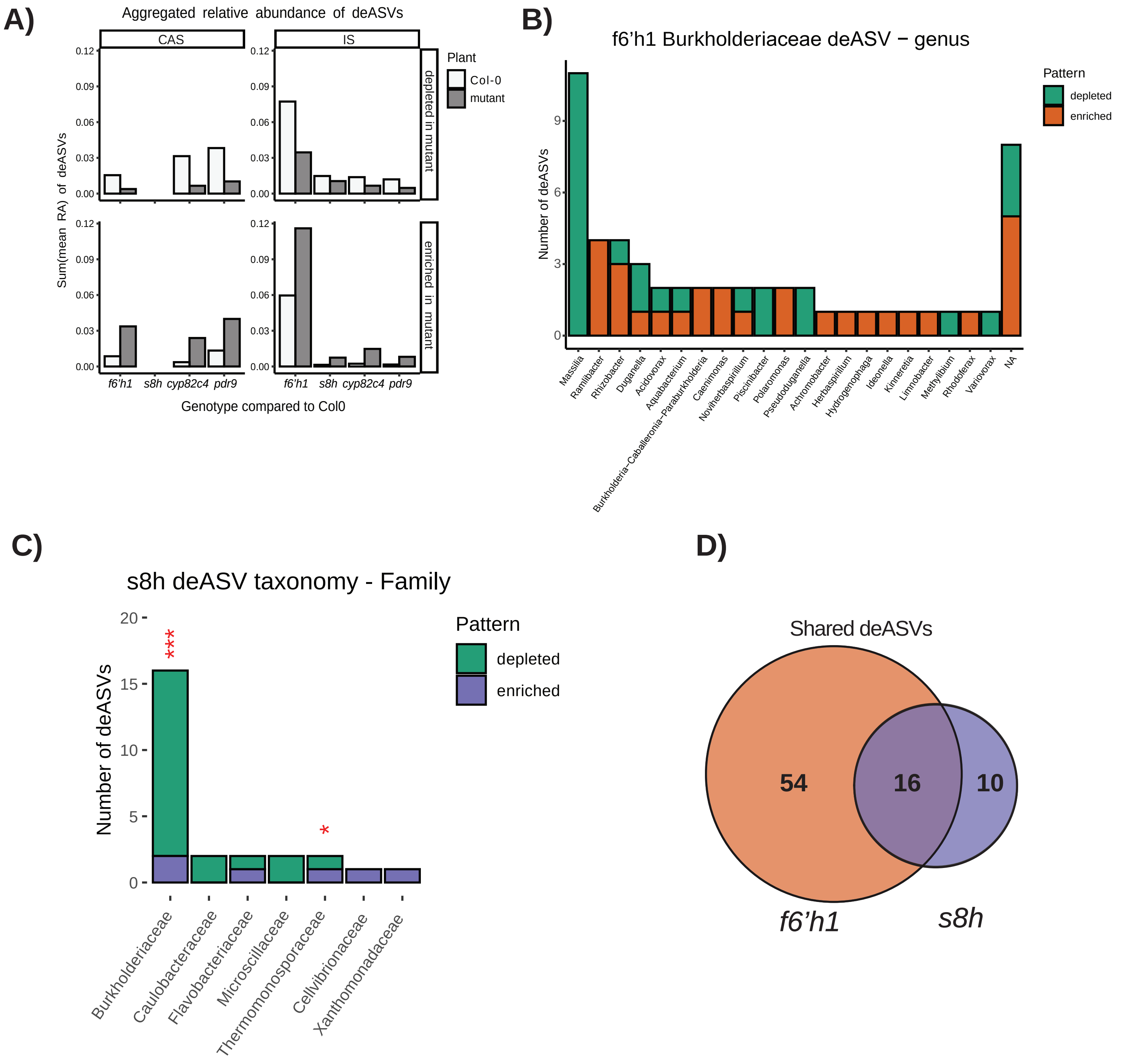


Figure S3, related to Figure 2. Burkholderiaceae ASVs are significantly impacted by coumarin biosynthesis. **A)** For the deASVs identified in each indicated mutant-Col-0 comparison (**Figure 2A**), the mean RA was calculated in each genotype and soil type. To quantify the magnitude of change in community composition attributable to differential enrichment of deASVs, the aggregated RA was calculated as the sum of the mean RA of all deASVs. Each paired bar set displays the aggregated relative abundance in Col-0 (white) and the indicated mutant (grey) of deASVs identified from the indicated Col-0-mutant comparison. **B)** Taxonomy of Burkholderiaceae deASVs from **Figure 2B** at genus-level resolution. **C)** Taxonomy of deASVs detected on *s8h* in IS. The hypergeometric enrichment test was performed for deASVs identified in **Figure 2A**. All significant deASVs detected in *s8h* vs Col-0 growing in IS were compiled at the family level and were compared to all ASVs detected in these samples. Positive enrichment denotes families present in the deASV subset more frequently than expected if ASVs were selected at random; negative enrichment indicates underrepresentation in deASVs. Data are from a single experiment (see **Figure 1**). **D)** Venn diagram showing the overlap of deASVs detected on *f6'h1* and *s8h* in IS from a single experiment containing both genotypes.

Figure S4

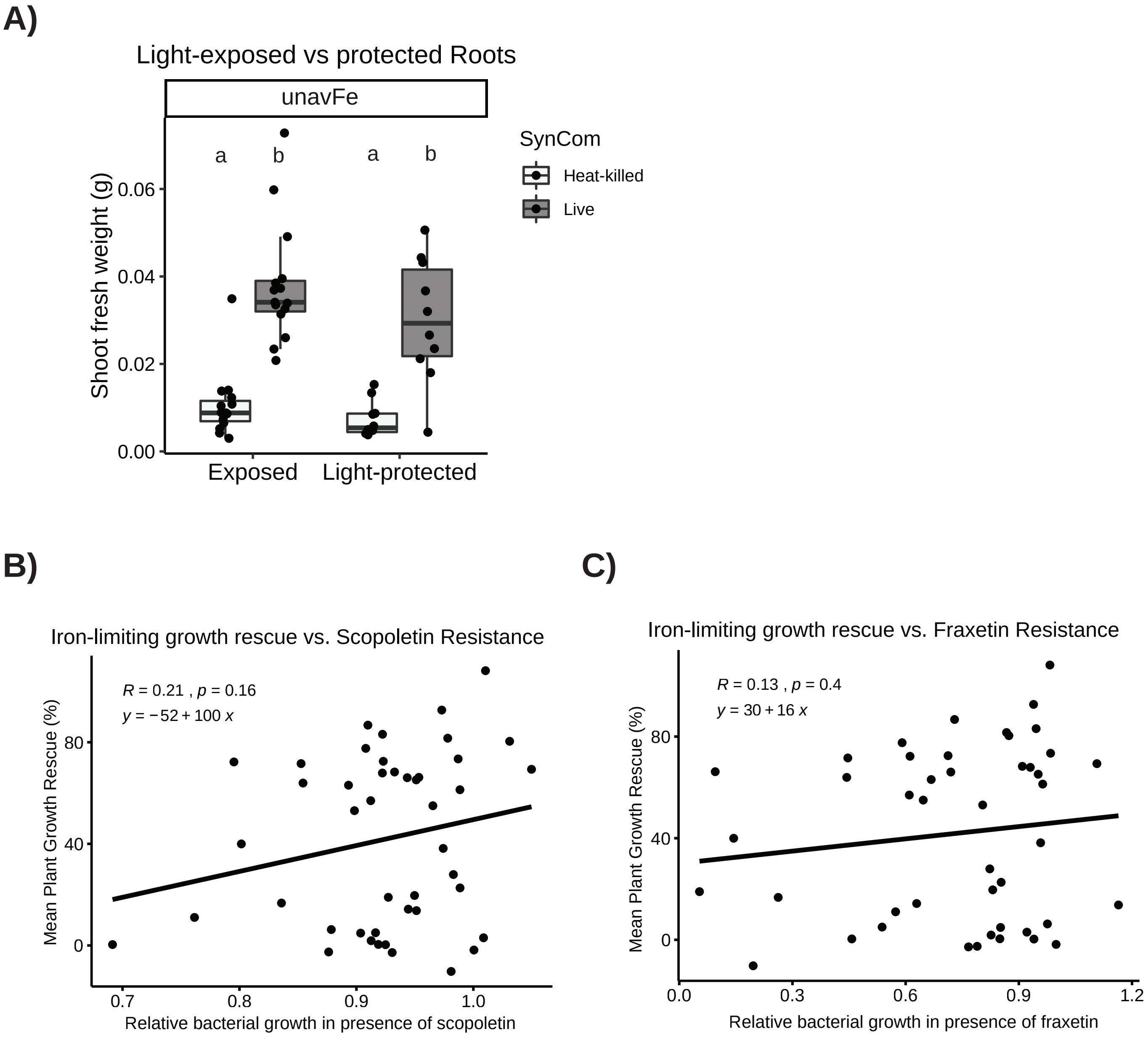
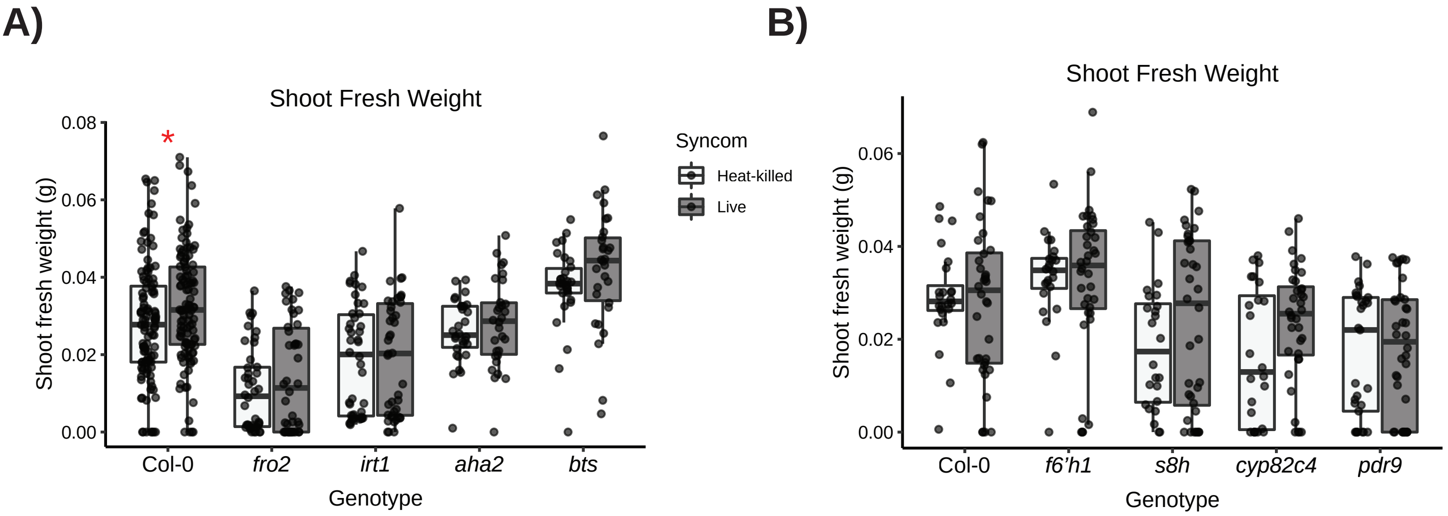


Figure S4, related to Figure 3. Iron-limiting plant growth rescue by bacterial SynCom is not affected by root exposure to light, and is independent of susceptibility to coumarin antimicrobial activity.
A) Shoot fresh weight of WT plants grown for two weeks on unavFe in vertical agar plates. Light-exposed roots were not shielded from light during growth; light-protected samples were grown in completely opaque black plastic boxes with only the shoots exposed to light as described in (Silva-Navas et al., 2015). Data are from a single experiment (n=15 and 10 plants for light-exposed and light-protected groups, respectively). Letters indicate significant pairwise differences between groups (p-adj ≤ 0.05 by Dunn's pairwise comparison with Bonferroni correction). **B)** Plant growth rescue activity of strains tested in mono-association in **Figure 3D** correlated with their growth in the presence of 50μM scopoletin, and **C)** fraxetin. Sensitivity to coumarins was assessed by overnight culture optical density as in **Figure 2D**.

Figure S5



C)

	Shoot Fresh Weight			
	avFe		unavFe	
	Heat-killed	**Live**	**Heat-killed**	**Live**
Col-0	de	dg	ab	de
fro2	ab	abf	ac	ac
irt1	bef	bef	ac	c
aha2	defg	deg	abc	def
bts	dg	dg	deg	g

D)

	Shoot Fresh Weight			
	avFe		unavFe	
	Heat-killed	**Live**	**Heat-killed**	**Live**
Col-0	ef	ef	abcd	aef
f6'h1	ef	ef	bcd	cd
s8h	abdef	aef	bcd	cd
cyp82c4	abcdef	aef	abcd	abef
pdr9	abcdf	abdf	c	cd

E)

Chlorophyll Content	
	Anova results
Iron	***
Genotype	***
SynCom	***
Genotype:Iron	
Genotype:SynCom	
Iron:SynCom	***
Genotype:Iron:SynCom	*

	avFe		unavFe	
	Heat-killed	**Live**	**Heat-killed**	**Live**
Col-0	fgh	efgh	a	defgh
fro2	abcd	abcdefgh	abc	ab
irt1	abcdefg	abcde	a	abcdef
aha2	bcdegfh	cdefgh	abc	abcdefgh
bts	h	gh	bcdefgh	efgh

F)

Chlorophyll Content	
	Anova results
Iron	***
Genotype	***
SynCom	***
Genotype:Iron	***
Genotype:SynCom	***
Iron:SynCom	***
Genotype:Iron:SynCom	

	avFe		unavFe	
	Heat-killed	**Live**	**Heat-killed**	**Live**
Col-0	c	c	b	c
f6'h1	c	c	ab	ab
s8h	c	c	ab	a
cyp82c4	c	c	ab	c
pdr9	c	c	a	ab

Figure S5, related to Figures 4 and 5. Plant growth promotion by bacterial SynCom is not observed in iron homeostasis or coumarin pathway mutants on avFe.
SFW of **A**) iron homeostasis pathway mutants, and **B**) coumarin biosynthesis and secretion mutants grown on avFe with and without live SynCom. Data in **A)** are from two independent experiments per genotype (See **Figure 4**, **n** = 36 plants. Each experiment included Col-0 control (n = 90 plants). Data in **B)** are from two experiments (See **Figure 5**, n = 36 plants). Asterisks indicate significance between heat-killed- and live SynCom-inoculated groups by Wilcoxon Ranked Sum test (*, **, ***, indicate p < 0.05, 0.01, and 0.001, respectively). No significant differences were detected in **B. C)** Results of Dunn's pairwise comparison with Bonferroni correction for SFW of all groups in **Figures 4**, and **D)** results for **Figure 5**. Letters indicate significance groups (p-adj ≤ 0.05). **E)** Results of three-factor ANOVA for effects of Genotype, Iron, and SynCom, and significance letters from Tukey's HSD *post-hoc* corrected for multiple comparisons for leaf chlorophyll content in **Figure 4**, and **F)** for **Figure 5** (*, **, ***, indicate p-adj < 0.05, 0.01, and 0.001, respectively; letters indicate significance groups at p-adj ≤ 0.05).

Figure S6

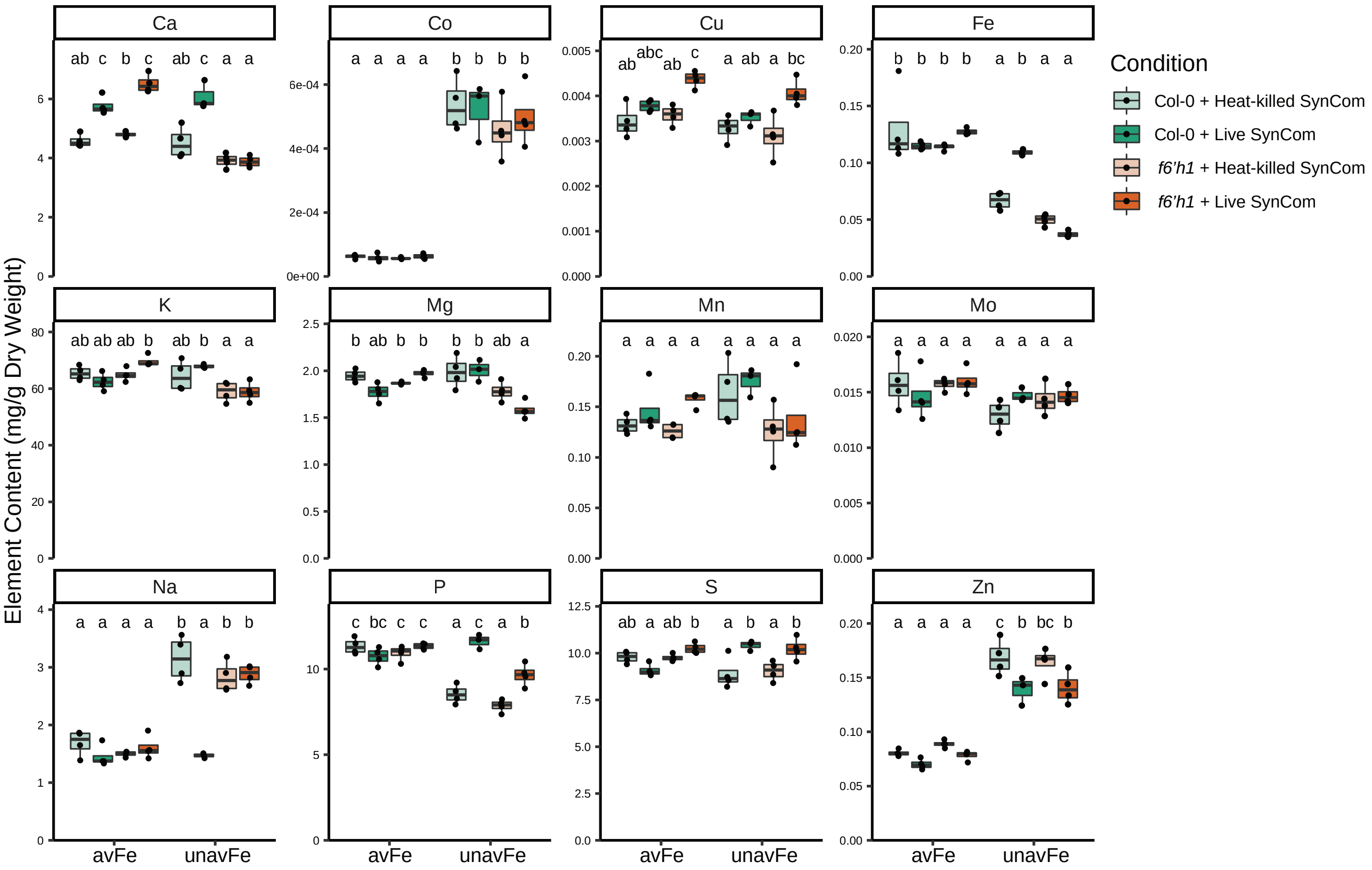
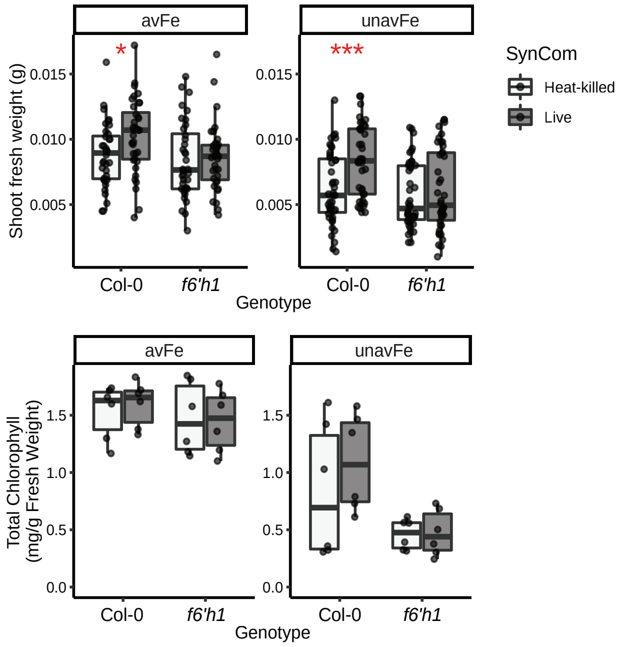


Figure S6, related to Figure 7. Effects of iron availability, coumarin biosynthesis, and bacterial SynCom on plant elemental content.

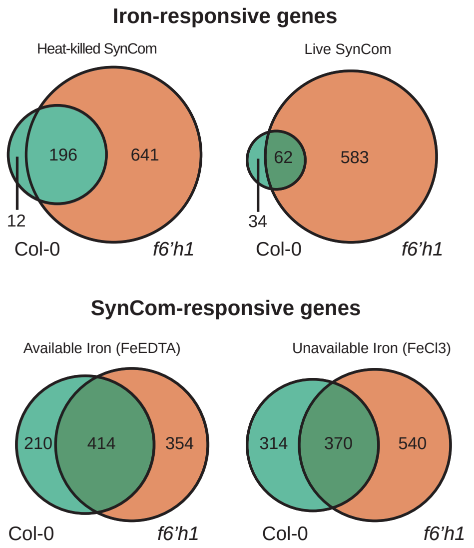
Leaf elemental content of Col-0 and *f6'h1* plants grown on avFe and unavFe, inoculated with a live bacterial SynCom or heat-killed control. Plants were grown for two weeks in indicated conditions before harvest, desiccation, and analysis by ICP-MS. Letters indicate significance groups (p-adj ≤ 0.05) by Tukey's HSD corrected for multiple comparisons (n = 3-4 pooled plant samples per group).

Figure S7

A)



B)



C)

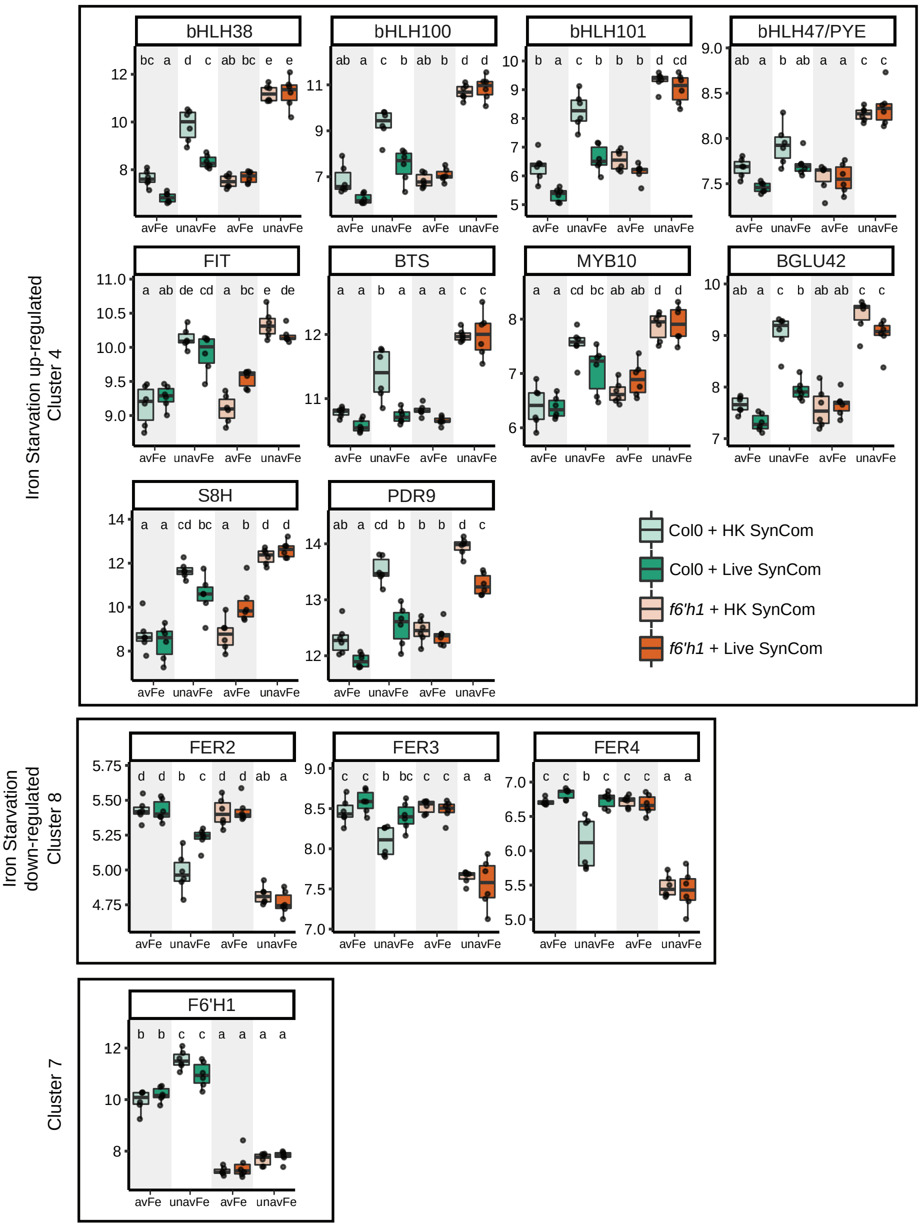
Genes upregulated by iron starvation

Genes upregulated by iron starvation	TAIR_ID	Cluster
Kelch repeat family protein	AT3G07720	4
MTPA2	AT3G58810	4
IRT1	AT4G19690	4
2OG	AT3G12900	4
unknown	AT3G61930	4
GLP5	AT1G09560	4
Unknown	AT3G06890	4
COPT2	AT3G46900	2
UGT72E1	AT3G50740	4
bHLH039	AT3G56980	4
MYB72	AT1G56160	4
Cation efflux family protein	AT3G58060	4

Genes downregulated by iron starvation

Genes downregulated by iron starvation		
FER1	AT5G01600	8
ATABC1	AT4G04770	8
unknown	AT2G36885	n.s.
PSAF	AT1G31330	5
uncharacterized protein (UPF0016)	AT1G68650	8
PER21	AT2G37130	5
FER4	AT2G40300	8
LAC7	AT3G09220	n.s.
SAPX	AT4G08390	5
unknown	AT5G59400	8
HEMA1	AT1G58290	8
FSD1	AT4G25100	8
peroxidase, putative	AT5G64100	10

D)



E)

Cluster	DEG transcripts
1	28
2	306
3	263
4	366
5	314
6	90
7	175
8	306
9	55
10	687

Figure S7, related to Figure 7. Bacterial SynCom alleviates iron starvation-associated transcriptional signature in a coumarin-dependent manner.

A) SFW and chlorophyll content in plants used for RNASeq experiment after eight days of growth in experimental conditions. (n = 36 plants for SFW, and 6 pooled-plant samples for chlorophyll content). Asterisks indicate significance between heat-killed- and live SynCom-inoculated groups by Wilcoxon Ranked Sum test for SFW and Student's T-test for chlorophyll content (*, **, ***, indicate $p \leq 0.05$, 0.01, and 0.001, respectively). **B)** Venn diagrams showing the overlap of identified iron-responsive genes between Col-0 and *f6'h1* plants inoculated with heat-killed or live SynCom. Iron-responsive DEGs were defined as having ≥ 2-fold change in expression in unavFe vs avFe samples with fdr-adjusted p-value ≤ 0.05. SynCom-responsive DEGs were defined as having ≥ 2-fold change in expression in live vs heat-killed SynCom samples with FDR-adjusted p-value ≤ 0.05. **C)** Cluster assignment of iron starvation response marker genes. Iron starvation-responsive genes (Mai et al., 2016) and their indicated clusters in DEG heat map in **Figure 5**. **D)** Expression patterns of selected iron homeostasis-related genes from RNA-Seq experiment. Data are log2-transformed counts after regularized logarithm normalization (rld normalization in *DESeq2*). Expression pattern is consistent with iron starvation being induced on unavFe in both genotypes and alleviated by addition of SynCom in Col-0, but not *f6'h1* plants. Letters indicate significant pairwise differences between groups (p-adj≤0.05 by Tukey's HSD corrected for multiple comparisons). **E)** Number of DEGs sorted into each cluster.

Table S1

Name	**Primer Sequence**	**Size**
799F	**AACMGGATTAGATACCCKG**	**20**
1192R	**ACGTCATCCCCACCTTCC**	**18**
B5-F	**AATGATACGGCGACCACCGAGATCTACACGACTGCGACTGGCGAACMGGATTAGATACCCKG**	**62**
B5-1	CAAGCAGAAGACGGCATACGAGATTCCCTTGTCTCCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-2	CAAGCAGAAGACGGCATACGAGATACGAGACTGATTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-3	CAAGCAGAAGACGGCATACGAGATACCGGTATGTACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-4	CAAGCAGAAGACGGCATACGAGATTGCATACACTGGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-5	CAAGCAGAAGACGGCATACGAGATTGGTCAACGATACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-6	CAAGCAGAAGACGGCATACGAGATATCGCACAGTAACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-7	CAAGCAGAAGACGGCATACGAGATGTCGTGTAGCCTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-8	CAAGCAGAAGACGGCATACGAGATTACAGCGCATACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-9	CAAGCAGAAGACGGCATACGAGATATCCTTTGGTTCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-10	CAAGCAGAAGACGGCATACGAGATAGTCGAACGAGGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-11	CAAGCAGAAGACGGCATACGAGATACCAGTGACTCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-12	CAAGCAGAAGACGGCATACGAGATCCAATACGCCTGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-13	CAAGCAGAAGACGGCATACGAGATGCAACACCATCCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-14	CAAGCAGAAGACGGCATACGAGATAGTCGTGCACATCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-15	CAAGCAGAAGACGGCATACGAGATAGTTACGAGCTACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-16	CAAGCAGAAGACGGCATACGAGATTTGCGTTAGCAGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-17	CAAGCAGAAGACGGCATACGAGATTACGAGCCCTAACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-18	CAAGCAGAAGACGGCATACGAGATTGTCGCAAATAGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-19	CAAGCAGAAGACGGCATACGAGATACAATAGACACCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-20	CAAGCAGAAGACGGCATACGAGATTCTCTACCACTCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-21	CAAGCAGAAGACGGCATACGAGATCGATCGAACACTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-22	CAAGCAGAAGACGGCATACGAGATATTGCAAGCAACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-23	CAAGCAGAAGACGGCATACGAGATAGCGCTCACATCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-24	CAAGCAGAAGACGGCATACGAGATTCGACCAAACACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-25	CAAGCAGAAGACGGCATACGAGATTGTGTTACTCCTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-26	CAAGCAGAAGACGGCATACGAGATTGCACAGTCGCTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-27	CAAGCAGAAGACGGCATACGAGATTTCTAGAGTGCGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-28	CAAGCAGAAGACGGCATACGAGATACACCTGCGATCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-29	CAAGCAGAAGACGGCATACGAGATATTCCTCTCCACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-30	CAAGCAGAAGACGGCATACGAGATCATCGACGAGTTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-31	CAAGCAGAAGACGGCATACGAGATCACCACAGAATCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-32	CAAGCAGAAGACGGCATACGAGATGGTCTTAGCACCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-33	CAAGCAGAAGACGGCATACGAGATTATCGCGATACACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-34	CAAGCAGAAGACGGCATACGAGATCTCTACGAACAGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-35	CAAGCAGAAGACGGCATACGAGATCTCCTCCCTTACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-36	CAAGCAGAAGACGGCATACGAGATCGTGTTATGTGGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-37	CAAGCAGAAGACGGCATACGAGATATTAGCAGCGTACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-38	CAAGCAGAAGACGGCATACGAGATCAAGTTTCCGCGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-39	CAAGCAGAAGACGGCATACGAGATCCTTGTTCACCTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-40	CAAGCAGAAGACGGCATACGAGATAACCAGCAGATTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-41	CAAGCAGAAGACGGCATACGAGATCTAGAGCTCCCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-42	CAAGCAGAAGACGGCATACGAGATCACGCAGTCTACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-43	CAAGCAGAAGACGGCATACGAGATACAAACATGGTCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-44	CAAGCAGAAGACGGCATACGAGATTCGAAACATGCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-45	CAAGCAGAAGACGGCATACGAGATTTCCCACCCATTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-46	CAAGCAGAAGACGGCATACGAGATAGCAGAACATCTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-47	CAAGCAGAAGACGGCATACGAGATGAAACATCCCACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-48	CAAGCAGAAGACGGCATACGAGATCTGTCAGTGACCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-49	CAAGCAGAAGACGGCATACGAGATCGGATCTAGTGTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-50	CAAGCAGAAGACGGCATACGAGATTTCTCCATCACACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-51	CAAGCAGAAGACGGCATACGAGATATTTAGGACGACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-52	CAAGCAGAAGACGGCATACGAGATGGTTTAACACGCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-53	CAAGCAGAAGACGGCATACGAGATAGACAGTAGGAGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-54	CAAGCAGAAGACGGCATACGAGATGCAGATTTCCAGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
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B5-56	CAAGCAGAAGACGGCATACGAGATTATCACCGGCACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-57	CAAGCAGAAGACGGCATACGAGATCCAGATATAGCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
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B5-59	CAAGCAGAAGACGGCATACGAGATACAGCTCAAACACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-60	CAAGCAGAAGACGGCATACGAGATATAGCGAACTCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-61	CAAGCAGAAGACGGCATACGAGATAACCGCATAAGTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-62	CAAGCAGAAGACGGCATACGAGATCTTGAGAAATCGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-63	CAAGCAGAAGACGGCATACGAGATCAGTCGTTAAGACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-64	CAAGCAGAAGACGGCATACGAGATCTTCCAACTCATCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69

B5-65	CAAGCAGAAGACGGCATACGAGATAATAGCATGTCGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-66	CAAGCAGAAGACGGCATACGAGATAAGTCACACACACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-67	CAAGCAGAAGACGGCATACGAGATCACACAAAGTCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-68	CAAGCAGAAGACGGCATACGAGATGTTCCTCCATTACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-69	CAAGCAGAAGACGGCATACGAGATCATCAAGCATAGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-70	CAAGCAGAAGACGGCATACGAGATCAAGCCCTAGTACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-71	CAAGCAGAAGACGGCATACGAGATCCTCTGAGAGCTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-72	CAAGCAGAAGACGGCATACGAGATACAAGAACCTTGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-73	CAAGCAGAAGACGGCATACGAGATTCATTCCACTCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-74	CAAGCAGAAGACGGCATACGAGATACCATCCAACGACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-75	CAAGCAGAAGACGGCATACGAGATATGCCGGTAATACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-76	CAAGCAGAAGACGGCATACGAGATTCAACCCGTGAACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-77	CAAGCAGAAGACGGCATACGAGATTCTGTAGAGCCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-78	CAAGCAGAAGACGGCATACGAGATTCGGATCTGTGACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-79	CAAGCAGAAGACGGCATACGAGATACTACCTCTTCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-80	CAAGCAGAAGACGGCATACGAGATCTATCCAAGTGGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-81	CAAGCAGAAGACGGCATACGAGATAGCCAGTCATACCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-82	CAAGCAGAAGACGGCATACGAGATGAGTTAGCATCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-83	CAAGCAGAAGACGGCATACGAGATTAAGACTACTGGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-84	CAAGCAGAAGACGGCATACGAGATGTCTCCTCCCTTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-85	CAAGCAGAAGACGGCATACGAGATCTTAGCTACTCTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-86	CAAGCAGAAGACGGCATACGAGATATCGAATCGAGTCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-87	CAAGCAGAAGACGGCATACGAGATCGCGTCAAACTACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-88	CAAGCAGAAGACGGCATACGAGATGATCAACCCACACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-89	CAAGCAGAAGACGGCATACGAGATAGTGTCGATTCGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-90	CAAGCAGAAGACGGCATACGAGATTCCGAGTCACCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-91	CAAGCAGAAGACGGCATACGAGATAGTCTGTCTGCGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-92	CAAGCAGAAGACGGCATACGAGATAGACAAGCTTCCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-93	CAAGCAGAAGACGGCATACGAGATGATCTAATCGAGCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-94	CAAGCAGAAGACGGCATACGAGATTAAACGCGACTCCAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-95	CAAGCAGAAGACGGCATACGAGATTCACGAGTCACACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-96	CAAGCAGAAGACGGCATACGAGATTTGAACAAGCCACAGCCATTTAGTGTCACGTCATCCCCACCTTCC	69
B5-R1	**ACGACTGCGACTGGCGAACMGGATTAGATACCC**	**33**
B5-R2	**CAGCCATTTAGTGTCACGTCATCCCCACCTTCC**	**33**
B5-Index	**GGAAGGTGGGGATGACGTGACACTAAATGGCTG**	**33**

Table S1, related to Figures 1 and 2: Primers.

Primer names and sequences used for amplification of V5-V7 region of the 16S rRNA gene and addition of sample barcodes and Illumina adaptors for root microbiota profiling.

Table S2

Strain	Phylum	Class	Order	Family	Genus	SynCom_Member
Root100	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aminobacter	Yes
Root112D2	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae		Yes
Root1203	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root1212	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root122	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Root123D2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		Yes
Root1240	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root1257	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Root1272	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae		Yes
Root1279	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	Yes
Root1280	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Yes
Root1290	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	Yes
Root1293	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	Yes
Root1294	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Yes
Root1295	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Yes
Root1304	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Yes
Root131	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Yes
Root1312	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Ensifer	Yes
Root135	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Yes
Root136	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Nocardia	Yes
Root1444	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Pelomonas	Yes
Root1464	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces	Yes
Root147	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Yes
Root1471	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium	Yes
Root1480D1	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Pseudoduganella	Yes
Root149	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root151	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Root154	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	Yes
Root157	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Phyllobacterium	Yes
Root166	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	Yes
Root16D2	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter	Yes
Root170	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Achromobacter	Yes
Root172	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium	Yes
Root179	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	Yes
Root181	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Terrabacter	Yes
Root189	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Noviherbaspirillum	Yes
Root190	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Root209	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Hydrogenophaga	Yes
Root22	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Oerskovia	Yes
Root227	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	Yes
Root231	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root236	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium	Yes
Root275	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	Yes
Root318D1	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Variovorax	Yes
Root322	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	Yes
Root329	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Yes
Root381	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea	Yes
Root4	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	Yes
Root402	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	Yes
Root404	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter	Yes
Root405	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Pelomonas	Yes
Root456	Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Angustibacter	Yes
Root472D3	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium	Yes

Root473	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Variovorax	Yes
Root480	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	Yes
Root482	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root483D2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root491	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root495	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium	Yes
Root53	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	Yes
Root552	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aminobacter	Yes
Root562	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Yes
Root563	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Phycicoccus	Yes
Root564	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root565	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Achromobacter	Yes
Root569	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Yes
Root604	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	Yes
Root61	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	Yes
Root65	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Yes
Root670	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea	Yes
Root68	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Yes
Root682	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Root690	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	Yes
Root695	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium	Yes
Root70	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	Yes
Root708	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	Yes
Root79	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Root81	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces	Yes
Root83	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Achromobacter	Yes
Root9	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Yes
Root918	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Oerskovia	Yes
Root920	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Yes
Root930	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Yes
Root935	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	Yes
Root96	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	Yes
Soil522	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Yes
Soil531	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Yes
Soil535	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces	Yes
Soil538	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Yes
Soil724D2	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Yes
Soil728	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Janibacter	Yes
Soil729	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Knoellia	Yes
Soil736	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	Yes
Soil745	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Yes
Soil748	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Phycicoccus	Yes
Soil750	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Yes
Soil756	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Tetrasphaera	Yes
Soil761	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	Yes
Soil762	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	Yes
Soil763	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	Yes
Soil764	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	Yes
Soil766	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Yes
Soil768D1	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Yes
Soil772	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae		Yes
Soil773	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	Yes
Soil774	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Soil777	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes

Soil782	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	Yes
Soil796	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Soil797	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Soil802	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Phycicoccus	Yes
Soil803	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Phycicoccus	Yes
Soil805	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Yes
Soil810	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Terrabacter	Yes
Soil811	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Terrabacter	Yes
Root1221	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter	No
Root267	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	No
Root217	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	No
Root335	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Massilia	No
Root434	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Variovorax	No
Root568	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	No
Root268	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	No
Root71	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	No

Table S2, related to Figure 3: Bacterial strains.

Name and taxonomy of bacterial strains used in this study. All strains are derived from the At-RSPHERE culture collection (Bai et al., 2015) and were isolated from *A. thaliana* roots growing in CAS. Strains included in the 115-member SynCom used for root microbiota reconstitution are indicated.