*Secreted coumarins and the root microbiota interact to improve iron nutrition in Arabidopsis*

**Abstract**

Roots of plants growing in natural soil harbour a diverse community of commensal microbes thought to provide beneficial services to the plant host, including mineral nutrition. The mechanisms by which plants select and instruct this community for these services are poorly understood. We report in *Arabidopsis thaliana* a beneficial plant root-microbiota interaction under iron limitation that is dependent on the malnutrition-inducible biosynthesis and secretion of coumarins. We show that loss of this pathway alters root-associated bacterial communities, impacting especially *Bukholderiaceae*, in an iron-limiting soil, but is inconsequential in iron-replete soil. Reconstitution of the microbiota in a gnotobiotic system revealed that bacterial commensals improve iron-limiting plant performance. This alleviation of iron starvation is a strain-specific, functionally redundant trait across phylogenetic lineages of the core microbiota, and is dependent on the host’s system for reductive import of iron and secretion of the coumarin fraxetin. Root transcriptome analysis revealed that coumarin-dependent interaction with commensals fully alleviates an iron-starvation transcriptional signature. Our work suggests that the root microbiota is an integral mediator of plant edaphic adaptation to iron-limiting soil by the secretion of coumarins, a compound class with known inhibitory activities on bacterial quorum sensing.

**Introduction**

Plant roots are colonized by a diverse community of microbes, collectively termed the root microbiota, originating from the surrounding soil biome1-3. The structure of these communities is shaped by soil edaphic factors, and diverse root-secreted photosynthates and secondary metabolites4,5. The bacterial root microbiota provides indirect protection against soil-borne fungal pathogens6,7 and is thought to provide nutrients to the host by increasing nutrient bioavailability8. However, the extent to which plants can selectively alter their microbiota to harness these beneficial traits in response to nutritional stress is unknown.

Iron is an essential mineral nutrient of plants, acting as a catalyst in many biological processes including photosynthesis and respiration. Although it is an abundant element in most soils, its bioavailability is often limiting due to its extremely low solubility at neutral and alkaline pH, as in calcareous soils containing a high proportion of calcium carbonate. Iron deficiency results in stunted plant growth and leaf chlorosis, decreasing crop yields and nutrient content in ~30% of arable land9. In response to iron-limiting conditions, non-graminaceous plants, such as *A. thaliana,* improve the solubility of iron through rhizosphere acidification by H+-ATPase AHA210 and reduction of iron(III) to more-soluble iron(II) by plasma membrane protein FERRIC REDUCTION OXIDASE 2 (FRO2)11. Iron(II) is then imported to the root epidermis by high-affinity IRON-REGULATED TRANSPORTER1 (IRT1)12. This iron starvation response is coordinated by FER-LIKE IRON DEFICIENCY INDUCED TRANSCRIPTION FACTOR (FIT)13, and a suite of associated basic helix-loop-helix (bHLH) type transcription factors induced under iron-limiting conditions14. It has recently become clear that the secretion of coumarins, phenolic secondary metabolites deriving from the general phenylpropanoid pathway, is also induced by the iron starvation response and is thought to contribute to iron nutrition through direct mobilization of recalcitrant iron pools15-23. Three main coumarin structure types are synthesized in *A. thaliana* in response to iron deficiency (**Fig 1a**)21,23, and are synthesized in a linear pathway. F6’H1 synthesizes scopoletin24, which can be converted to fraxetin by S8H21,23, and further converted to sideretin by CYP82C421. Each of these coumarins can be exported by ABC transporter PDR916, though other efflux pumps may also contribute to export of some coumarin types25. Coumarin secretion was recently shown to influence the structure of root microbial communities in artificially-limed soil26 and synthetic media27. However, the impact of coumarin secretion on the root microbiota in soils with different mineral nutrient availabilities and the consequences for plant productivity remain undefined.

**Results**

**Coumarins are important for growth and root microbiome assembly on an iron-limiting soil**

To assess potential links between coumarin secretion, plant growth, and the root-associated microbiota, we grew *A. thaliana* Col-0wild-type (WT)plants and mutants with defects in coumarin biosynthesis or export into the rhizosphere (**Fig. 1a**) on two soils with contrasting iron availability. Cologne agricultural soil (CAS) was obtained from a local site (GPS code : 50.958 N, 6.856 E) and is slightly acidic with pH 6.4, at which iron is sufficiently available. We also obtained soil from a vineyard (GPS code : 44.292 N, 11.784 E), termed here Italian Soil (IS), which is slightly alkaline and calcareous (pH 7.5, 9.7 % of active CaCO3), conditions which reduce the availability of iron. Further soil nutrient analysis in **Suppl. Fig 1.** We grew the aforementioned *A. thaliana* genotypes in each soil and observed a significant decrease in shoot fresh weight (SFW) and leaf chlorophyll content in *f6’h1* and *s8h* plants grown on IS, whereas the tested performance parameters of all mutants were indistinguishable from WT on CAS (**Fig. 1b and c**). A similar growth deficit was observed in *f6’h1* plants grown on other alkaline soils isolated from geographically diverse sites (**Suppl. Fig. 2**). Additionally, the growth deficit of *f6’h1* plants growing on IS could be rescued by supplementing with solubilized iron in the form of FeEDDHA (**Suppl. Fig. 2**). These results show that coumarin biosynthesis is important for growth in naturally iron-limiting soils. However, unlike germ-free *pdr9* plants on synthetic media16,28 (and **Fig. 4** below), the performance of this mutant was indistinguishable from WT on both CAS and IS; coumarin export via the ABC transporter PDR9 was not growth-limiting in natural iron-limiting soil (**Fig. 1a and b**). These data support that in a soil context, export of coumarins may involve additional members of the diversified and promiscuous ABC transporter family25,29.

The root-associated bacterial microbiota of plants grown on CAS and IS was assessed by culture-independent *16S rRNA* gene amplicon sequencing and analysis at the amplicon sequence variant (ASV) level. **Supplemental Table 1** summarizes biological and experimental replicates for each genotype and soil type. Alpha-diversity (within-sample diversity) was greater in IS than CAS for both unplanted soil and root samples, but did not vary by genotype (**Suppl. Fig. 3**). Unconstrained principal coordinate analysis (PCoA) of Bray-Curtis distances between samples showed that the soil type and batch were the largest drivers of divergence between samples (**Suppl. Fig. 3**). Plotting beta-diversity on a PCoA constrained (CPCoA) for the interaction between genotype and soil type revealed a significant separation of *f6’h1* and *s8h* plants from other genotypes when grown on IS, but not on CAS (**Fig. 1d**). These bacterial communities were significantly separated from WT plants (p = 0.0075 and 0.0080 by pairwise PERMANOVA, respectively) on IS, but not on CAS (p = 0.36 and 0.43, respectively). Analysis of bacterial community profiles from three batches of each soil type confirms that *f6’h1* plants separate significantly from other genotypes when grown in IS, but not in CAS (**Suppl. Fig. 3**). Together, this indicates that coumarin biosynthesis, especially of scopoletin and fraxetin, is important for plant growth and root microbiota establishment in a naturally iron-limiting calcareous soil but is dispensable in a naturally iron-replete soil. This illustrates how the interaction between soil type and plant genotype can serve as a major determinant of root microbiota structure, explaining 27.7% of community variation (**Fig. 1d**).

**Coumarin production influences the microbiota at the ASV level**

We next determined which ASVs were differentially enriched (deASVs) in each mutant genotype compared to WT on each soil (**Fig. 2a**). For this analysis we pooled the data from three batches of each soil type, and filtered to include only ASVs found in at least three samples above 0.05% relative abundance. The greatest number of deASVs was detected in *f6’h1* plants when grown in IS (255 deASVs; **Fig.2a**). An increase in the number of deASVs was also observed for *s8h* plants grown in IS compared to CAS. The impact of these deASVs on the microbiota structure in terms of relative abundance was also observed in f6’h1 plants grown in IS (**Suppl. Fig 4**).

To determine which taxonomic lineages are affected by coumarin secretion, deASVs were classified using the SILVA database. Multiple families were significantly over- or under-represented within the deASV list compared to the full list of detected ASVs (**Fig. 2c and Suppl. Fig. 5**). *Burkholderiaceae* were the most prevalent family detected within the deASVs (56 deASVs; 1.9-fold enriched in deASVs compared to detected ASVs). However, even at family-level resolution, no consistent enrichment pattern was observed; most families contained deASVs which were more abundant in *f6’h1* plants as well as less abundant compared to WT. Despite containing the most deASVs, the overall relative abundance of *Burkholderiaceae* was not significantly altered in coumarin-deficient plants (**Fig. 2d**). The relative abundance of families *Rhizobiaceae* and *Streptomycetaceae*, the next two most-impacted families, however, were slightly but significantly altered in *f6’h1* plants on IS. This indicates that, at ASV level, lack of coumarin biosynthesis has a profound quantitative impact on multiple root-associated commensal families, but without affecting microbiota structure at higher taxonomic ranks. This suggests the existence of ASV-level compensatory mechanism(s) within bacterial families which, during microbiota establishment, maintain root microbiota structure at higher taxonomic ranks in coumarin-deficient plants on iron-limiting soil.

As various coumarin structure types have been shown to exert antimicrobial activity against diverse bacteria, we examined the coumarin sensitivity of a panel of *Burkholderiales* root commensal strains by quantifying their growth in the presence of 50 µM scopoletin or fraxetin, within the range of physiological concentrations of coumarins observed within roots22,26,30. We observed a range of strain-specific variation in the inhibitory activity of fraxetin, but consistent inactivity of scopoletin (**Fig. 2d)**. This indicates that fraxetin exerts antimicrobial activity on a subset of *Burkholderiales* strains and may explain the ASV-level variability observed between WT and coumarin-deficient plants in iron-limiting soil.

**A gnotobiotic system for iron limitation and microbiota reconstitution**

To assess the impact of root commensals on plant growth under iron-limiting conditions, we employed an agar medium-based gnotobiotic system where we could control both iron mobility and the presence of bacterial commensals. In this system, nutrient medium (1/2 MS) is strongly buffered (10mM HEPES) at an alkaline pH of 7.4, similar to the pH of iron-limiting IS soil. Iron is provided at 100µM in one of two forms: available iron (avFe) FeEDTA, a complex which retains solubility even at alkaline pH, or an unavailable form (unavFe) FeCl3, which is highly insoluble at the buffered pH. Providing unavFe mimics conditions in calcareous and alkaline soils such as IS; iron is present but recalcitrant due to extremely low solubility, and must be actively mobilized for utilization. We also induced iron starvation by supplying only an insufficient amount of soluble iron (1µM FeEDTA).

Using this gnotobiotic system, we reconstituted plants with a synthetic community (SynCom) of bacterial commensals to assess the impact of the microbiota on iron-limiting plant performance. To achieve this, we took advantage of a diverse culture collection of bacterial commensals isolated from *A. thaliana* roots grown in CAS (Bai et al., 2015). We designed a taxonomically-diverse SynCom of 115 members which reflects the root bacterial diversity observed by culture-independent methods at high taxonomic ranks (**Fig 3a, Suppl. Strains file**). Shoot fresh weight and chlorophyll content were measured as readouts of plant performance and as a proxy measurement for iron nutritional status. A growth deficit accompanied by leaf chlorosis (**Fig. 3b and c**), indications of iron starvation, were observed in plants grown on unavFe; furthermore, transcriptomic analysis revealed a repression of ferritins, iron storage proteins, accompanied by up-regulation of iron import components in plants grown on unavFe (**Fig. 5b, Suppl. Table 3,** and **Suppl. Fig. 10**). Together, these data confirm a robust induction of iron starvation in this experimental system. We found that addition of the SynCom strongly improved both SFW and chlorophyll content of plants grown on unavFe. This beneficial interaction was unexpected, given that iron starvation-induced coumarins exert antimicrobial activity against commensals27,31-36 (**Fig. 2d**). Notably, this commensal-mediated improvement of plant performance was not observed in plants grown on media containing avFe or insufficient iron (**Fig. 3c**). These results suggest that bacterial commensals can improve plant performance by increasing their ability to utilize immobile sources of iron.

To survey the ability of various taxa to improve iron-limiting plant growth, we grew plants in mono-associations with bacterial strains on unavFe. Forty-five diverse SynCom strains across taxonomic lineages were tested for their ability to rescue iron-limiting plant growth (**Fig 3a**, red arrows, **Suppl. Table 2**). Within each broader taxonomic lineage, we observed growth-rescuing strains as well as strains lacking this ability (**Fig. 3d**), demonstrating this commensal activity is ubiquitously present but exhibits strain-specific variation within all core taxonomic lineages of the root microbiota. Thus, in a bacterial community context the capacity for plant growth rescue under iron-starved conditions is functionally redundant (**Fig. 3c and d**). Strain-specific variation in growth rescue activity and sensitivity to the antimicrobial activity of iron-starvation induced coumarins (**Fig. 2d)** may be functionally linked, and result in differential enrichment of beneficial community members based on coumarin status.

**Bacteria-mediated plant growth rescue is dependent on plant expression of iron uptake machinery and coumarin biosynthesis**

We utilized Arabidopsis mutant lines deficient in genes involved in iron uptake and homeostasis to determine their importance for bacteria-mediated growth rescue under iron limitation. Mutants in components of the reduction-based iron uptake system (*fro2* and *irt1*), rhizosphere acidification (*aha2*), and a negative regulator of the iron starvation response (*bts*) were grown on unavFe in the absence and presence of a live SynCom. Genotypes *fro2* and *irt1* displayed an exaggerated growth deficit and leaf chlorosis (**Fig. 4a**), consistent with their reported hypersensitivity to iron starvation11,12,37,38. In contrast to WT plants, addition of the bacterial SynCom was unable to improve the phenotype of these iron import mutants. Moreover, *bts* plants, which are tolerant to iron deficiency39-41, were larger than WT plants on unavFe, were not chlorotic, and still displayed slightly improved performance when inoculated with SynCom. No difference was observed between *aha2* and WT controls, indicating that plant-mediated rhizosphere acidification is not rate-limiting for commensal-mediated plant growth rescue in strongly-buffered alkaline conditions. When grown on avFe, the SynCom did not improve growth or chlorophyll content in any genotype (**Suppl. Fig. 6**). These results validate our gnotobiotic system for microbiota reconstitution under iron-limiting conditions, confirm that growth limitation and chlorosis on unavFe is due to iron starvation, and suggests that improved plant performance in the presence of SynCom occurs via the reduction and import of iron.

We next investigated the role of coumarins in this commensal-mediated growth rescue under iron limitation using the aforementioned coumarin pathway mutants (**Fig. 1a**). Addition of SynCom was unable to rescue plant growth or chlorosis of *f6’h1,* *s8h*, or *pdr9* plants grown on unavFe. In contrast, the SynCom robustly improved performance of *cyp82c4* plants similar to WT (**Fig. 4b**). No growth promotion by the SynCom was observed when grown in the presence of avFe (**Suppl. Fig. 7**). These data suggest that plant biosynthesis (F6’H1andS8H) and secretion (PDR9) of scopoletin and/or fraxetin are required for bacteria-mediated growth rescue under iron limitation, while sideretin (CYP82C4) is dispensable. To further dissect the roles of scopoletin and fraxetin in commensal-mediated plant growth rescue, we chemically complemented *f6’h1* plants by supplementing the growth media with each coumarin (**Fig. 4c**). Addition of scopoletin did not improve plant performance. The addition of fraxetin, however, restored the ability of the SynCom to improve both plant growth and leaf chlorophyll content in coumarin-deficient *f6’h1* plants. Enhanced rescue activity was observed when scopoletin and fraxetin were supplied in combination, suggesting a synergistic interaction or conversion of scopoletin into fraxetin by endogenous S8H. Notably, supplementation of neither coumarin significantly improved growth or chlorophyll content of axenically-grown *f6’h1* plants at 50µM, a concentration lower than that used in previous chemical complementation experiments21,23. This indicates that commensal-mediated improvement in iron-limiting plant growth is induced at concentrations lower than is needed for direct mobilization by fraxetin in axenic conditions. Together, these results confirm that secreted (exogenous) fraxetin is both necessary and sufficient for eliciting growth rescue activity from bacterial commensals under iron limitation. These findings argue for an indirect activity of fraxetin in commensal-mediated mobilization of recalcitrant iron pools in addition to the reported direct mobilization of iron20,21,23.

**Iron-limiting plant growth rescue is accompanied by alleviation of iron-starved transcriptional signature**

To identify coumarin- and SynCom-responsive plant pathways under different iron regimes, we performed analysis of the whole root transcriptome. RNA was isolated from roots of WT and *f6’h1* seedlings grown for one week on avFe or unavFe medium inoculated with a live 115-member SynCom or a heat-killed control. Of note, at this time point, SynCom-mediated rescue of iron-limiting plant growth and chlorosis was observed in WT, but growth was still comparable between WT and *f6’h1* plants (**Suppl. Fig 8**).

Plotting sample distances on a PCA (**Fig. 5a**) revealed that the supplied iron form was the largest determinant of transcriptome dissimilarity, separating along PC1(18% of variance), followed by SynCom status (PC2, 9% of variance). When grown on avFe, both Col-0 and *f6’h1* plants clustered together, but separate by SynCom status: plants inoculated with a heat-killed SynCom occupy the upper-right quadrant, while those with live SynCom cluster in the lower-right quadrant. This indicates that a live SynCom impacts host transcriptional responses when iron is available, independently of plant coumarin status. The transcriptomes of plants grown on unavFe, however, were distinct from those on avFe, and further separated based on genotype and SynCom status. When inoculated with heat-killed SynCom, both WT and *f6’h1* on unavFe separated from the avFe cluster along PC1 (upper-left quadrant), with *f6’h1* mutants separating even further than WT. A larger genotype-driven separation was observed between Col-0 and *f6’h1* plants when inoculated with live SynCom on unavFe. As on avFe, live SynCom-inoculated samples separated from heat-killed SynCom-inoculated samples along PC2. Remarkably, WT plants inoculated with live SynCom on unavFe clustered closely with SynCom-inoculated WT plants on avFe (lower-right quadrant), while *f6’h1* plants remained in the lower-left quadrant, clearly separated from the avFe cluster. This pattern indicates that the transcriptional responses to growth on unavFe are more pronounced in *f6’h1* plants than WT; indeed, more iron-responsive differentially-expressed genes (DEGs) were detected in *f6’h1* than WT plants (**Suppl. Fig 9**). Importantly, the iron starvation-induced response is almost fully alleviated by the addition of SynCom in Col-0 but not *f6’h1* plants (**Fig. 5a and Suppl. Fig 9**).

We performed k-means clustering of all DEGs based on expression pattern to further investigate the interaction between iron availability, SynCom, and genotype (**Fig. 5b**). Ten clusters were identified (plots left of heat map) and showed iron- and SynCom-responsive gene sets. Cluster 4 reveals a set of genes activated in both Col-0 and *f6’h1* plants in response to immobile iron in the absence of a live SynCom. These genes are more strongly induced in *f6’h1* plants. Furthermore, their expression is reduced in Col-0 plants upon addition of SynCom, but remains elevated in *f6’h1* plants. This gene set corresponds to iron-responsive genes that are also responsive to SynCom in a genotype-dependent manner. Gene ontology analysis revealed that this cluster is enriched for genes belonging to the iron starvation response, iron homeostasis, and metal transport (**Fig 5b**, annotations right of heatmap). Genes in cluster 8 display the inverse pattern – downregulated on unavFe and restored in the presence of SynCom in Col-0, but not *f6’h1*. We compared our DEGs to a list of 25 previously identified core iron starvation marker genes42 (**Suppl. Table 3**). Of the 12 genes upregulated under iron starvation, 11 were found in cluster 4, while 7 out of 13 genes down-regulated under iron starvation were present in cluster 8. The cluster assignment (**Fig. 5c**) and expression pattern (**Suppl. Fig. 10**) of selected iron homeostasis regulators and coumarin biosynthesis genes reveal that these genes are iron- and SynCom-responsive in a genotype-dependent manner. Together, these data reveal robust induction of an iron-starvation transcriptional signature in plants grown on unavFe, which is alleviated by commensals in WT, but not *f6’h1* plants.

Analysis of SynCom-responsive genes (**Suppl. Fig 9**) revealed a core set of DEGs common to both genotypes, in addition to many genotype-specific DEGs. Accordingly, clusters 10 and 3 consist of genes that were consistently activated or repressed, respectively, upon addition of live SynCom independently of iron availability and genotype. These clusters were enriched for genes related to defense responses and response to bacteria. Interestingly, genes associated with these terms were also significantly enriched in clusters 2 and 8. Cluster 2 represents genes activated by both unavFe and SynCom, and more strongly in *f6’h1* plants than Col-0, while cluster 8 represents genes activated by SynCom on unavFe in Col-0 but not *f6’h1*. The presence of immune-related genes in these clusters suggests that outside of the core SynCom-responsive genes, a subset of defense genes are regulated by the presence of commensals in a coumarin-dependent manner. Collectively, these results show that the interaction between coumarins and commensals on unavFe not only relieves an iron-starvation transcriptional signature, but extends to the regulation of a subset of immune and defense-related genes.

**Discussion**

Our results reveal unexpected roles of root-secreted coumarins in governing plant-bacteria interactions, including soil type-dependent microbiota assembly, a novel mechanism of root commensal-mediated alleviation of iron starvation, and regulation of a subset of defense genes. We show that *s8h* plants, like fully coumarin-deficient *f6’h1*, assemble an altered root bacterial community, potentially driven by loss of fraxetin, which exhibits strain-specific antimicrobial activity. Individual members of the *Burkholderiaceae*, core members of the plant root microbiota43 often displaying plant-beneficial activities44, are selectively impacted by fraxetin. This strain-specific variation in sensitivity to coumarins may explain ASV-level changes in abundance and potentially result in selection of plant-beneficial members. The greater impact on the microbiota observed in *f6’h1* plants suggests that both scopoletin and fraxetin modulate bacterial microbiota assembly. Indeed, upon transplantation of axenically-grown WT and coumarin-deficient *A. thaliana* plants into artificially limed soil, a metagenome analysis indicated an altered microbial multi-kingdom assemblage and provided evidence for selective scopoletin anti-fungal activity against soil-borne fungal pathogens *in vitro26,45-47*. Given that *A. thaliana* can modulate the production of various coumarin structure types in response to biotic and abiotic factors20,23,46,48,49, the host may thus selectively impact root-associated fungal and/or bacterial communities directly, and indirectly through their respective inter-kingdom interactions.

The robustness of our gnotobiotic system as a model of iron limitation is supported by the phenotypic recapitulation of iron starvation hypersensitive and insensitive *A. thaliana* genotypes, and iron starvation-induced transcriptional reprogramming. Plant performance data, coupled with transcriptomic analysis, confirms that performance benefits under iron limitation conferred by bacterial commensals occur via improved iron nutrition. To the best of our knowledge, experimental evidence for such clear plant nutritional benefits from commensals in a community context has not been reported before. On the contrary, under low phosphate conditions, *A. thaliana* must compete with a bacterial SynCom for access to the macronutrient, requiring integration of phosphate starvation and defence responses50. Of note, the performance of plants with and without commensals has not been tested in the presence of unavailable forms of phosphate, as found in many soils. Importantly in our system, growth promotion by microbes is observed only when iron is present but immobile, conditions present in most iron-limiting soils. Thus, our results highlight the importance of studying plant nutritional phenotypes in systems mimicking natural conditions as closely as possible, including the presence of commensals and defined forms of soil minerals that are unavailable to the plant host.

Plant-secreted fraxetin is both necessary and sufficient for the described beneficial interaction. Strain-specific variation of this commensal trait within and presence of this beneficial activity across all core taxonomic lineages of the *A. thaliana* bacterial microbiota suggest the underlying molecular mechanism(s) evolved independently rather than by common descent. As the observed growth rescue depends on plant expression of the iron reductive import machinery, this mechanism(s) must function upstream of reduction and import at the root surface. Root-secreted coumarins are inducible under iron starvation and mediate an interaction between the host and commensals that improves host iron nutrition. This genotype-environment interaction strongly suggests the root microbiota is an integral component of plant edaphic adaptation to growth in iron-limiting soil. Quantitative variation in coumarin production has been demonstrated among *A. thaliana* accessions23,30, and was shown to correlate with performance on axenic iron-limiting media23. Importantly, our results were obtained using a culture collection isolated from *A. thaliana* roots grown in CAS3. That taxonomically diverse commensals from an iron replete soil are capable of rescuing iron-limiting plant growth suggests a function ubiquitous across soil types whose activity can be elicited by fraxetin. For instance, bacterial siderophores (and PQQ) are potential mediators of microbially-assisted iron nutrition51,52.

How fraxetin indirectly elicits this activity from the root microbiota remains a question. In addition to antimicrobial activities27,33,35,36, a number of coumarin structure types act as inhibitors of bacterial quorum sensing (QS)32,53, a cell-to-cell communication mechanism that allows cell-density-dependent bacterial gene expression. By inhibiting QS, coumarins could modulate bacterial proliferation, pathogenic activity, and siderophore production, which are under the control of QS across bacterial phyla32,54-57. In addition, coumarin pathway intermediates are utilized by some soil-borne bacteria as substrates for the biosynthesis of an environmental quorum sensing signal, *p*-coumaroyl-HSL58. We find homologs of genes encoding the signal receptor that responds to p-coumaroyl-HSL to regulate global bacterial gene expression in x out of y strains of the *A. thaliana* root-derived commensal culture collection. This suggests root commensals have repurposed coumarin-type QS inhibitors for sensing of the rhizosphere environment. These findings, together with the ubiquitous production and chemical diversification of coumarins among flowering plants21,59,60, provide an ecological framework to examine their evolutionary diversification in the context of microbiota-mediated mineral nutrition of plant hosts.

**Figure Captions**

**Figure 1: Coumarin biosynthesis mutants display symptoms of iron deficiency and assemble an altered root microbiota on a naturally calcareous soil.**

**a,** Diagram of coumarin biosynthesis and export, and reductive uptake of iron in Arabidopsis. F6’H1 synthesizes scopoletin from phenylpropanoid pathway-derived precursors. Scopoletin can be converted to fraxetin by S8H, and fraxetin to sideretin by CYP82C4. Each of these coumarins can be exported to the rhizosphere by PDR9. Root surface-expressed FRO2 reduces iron(III) to more-soluble form iron (II), which is imported by transporter IRT1. **b,** Shoot fresh weight and **c,** total chlorophyll content of coumarin pathway mutants grown in a non-calcareous (CAS) and a calcareous (IS) natural soil. Statistical significance was determined by Kruskal-Wallis; each mutant was compared to Col-0 wildtype by Wilcoxon Ranked Sum post-hoc. Significance is indicated by red asterisks (\*, \*\*, \*\*\*, indicate p < 0.05, 0.01, and 0.001, respectively). SFW values represent individual plants from three experiments in each soil, except *s8h* which was included in only one experiment, (Col-0 n= 171, 204, *f6’h1* n = 168, 272, *s8h* n = 93, 113, *cyp82c4* n = 164, 209, and *pdr9* n = 172, 169 in CAS and IS, respectively). Chlorophyll content was measured from pooled leaf samples, (Col-0 n= 35, 29, *f6’h1* n = 34, 36, *s8h* n = 19, 14, *cyp82c4* n = 34, 30, and *pdr9* n = 35, 30 in CAS and IS, respectively). **d,** Constrained ordination of root bacterial community composition of coumarin pathway mutants, constrained for the interaction between soil type and genotype. Samples separate by soil type on the first axis, CPCoA 1, and by genotype on the second axis, CPCoA 2, when grown in calcareous soil. Ellipses delineate the multivariate normal distribution at 95% confidence. Data are from one representative experiment of three, (Col-0 n= 17, 15, *f6’h1* n = 18, 14, *s8h* n = 15, 14, *cyp82c4* n = 18, 15, and *pdr9* n = 17, 14 in CAS and IS, respectively).

**Figure 2: Coumarin secretion impacts microbiota members at the ASV level**

**a**, Number of deASVs detected in indicated mutants compared to Col-0 in each soil type. Data was pooled from three experiments (except *s8h*, which was included in only one), and filtered for ASVs found in at least three samples at a relative abundance (RA) > 0.05%. Differential enrichment was calculated using a negative binomial generalized log-linear model at an FDR-adjusted p value of 0.05. **b,** Family-level taxonomic classification of deASVs in *f6’h1* plants growing on IS. ASV sequences were taxonomically classified using the SILVA database. Colors indicate if deASVs were enriched or depleted in *f6’h1*. Hypergeometric enrichment test was performed to determine if each family was over- or under-represented in deASV list compared to all detected ASVs. Red asterisks indicate significance (\*, \*\*, \*\*\* denote FDR-adjusted p value < 0.05, 0.01, 0.001, respectively). **c,** Sample-wise aggregated relative abundance of the top 3 families most significantly over-represented in deASVs: *Burkholderiaceae*, *Rhizobiacea*, and *Streptomycetaceae*. Each data point represents the average RA of the indicated family in a single sample. **d,** Overnight growth of *Burkholderiales* bacterial strains in the presence of scopoletin or fraxetin. Optical density (OD) of cultures at 18-20 hours post inoculation was normalized to the OD of each strain in the absence of coumarins. Data are averages of 2-4 biological replicates, each with 2-3 technical replicates, per strain.

**Figure 3: Reconstitution of root commensals rescues plant growth on unavailable iron substrate**

**a,** Phylogenetic tree showing taxonomy of 115-strain SynCom used for microbiota reconstitution. Red arrows indicate strains used in **d**. **b,** Representative images of plants grown for two weeks on media containing available (avFE) and unavailable (unavFE) forms of iron inoculated with live SynCom or heat-killed control. **c,** Shoot fresh weight and total chlorophyll quantification of Col-0 plants after two weeks growth in indicated iron form and concentration. Data are pooled from 3 experiments with avFe and unavFe: n=42-54 plants per condition, and chlorophyll measured in pooled samples, n=13-15 per group. Insufficient iron data from 1 experiment, n = 18 plants. Asterisks indicate significance between Heat-killed- and Live-SynCom-inoculated groups assessed by Wilcoxon Ranked Sum test (\*, \*\*, \*\*\*, indicate p < 0.05, 0.01, and 0.001, respectively). **d,** Iron-limiting growth rescue activity of SynCom strains in monoassociation. SFW was measured and plotted as percent growth rescue of bacteria-inoculated plants on unavFe compared to the growth deficit between sterile plants on avFe vs unavFe ([SFWinoculated-unavFe – SFWsterile-unavFe]/ [SFWSterile-avFe – SFWSterile-unavFe]). Black and red lines indicate 0% (sterile-unavFe growth) and 100% (sterile-avFe growth), respectively.

**Figure 4: Bacteria-mediated growth rescue requires plant expression of the iron reductive-import system and secretion of fraxetin.**

**a,** SFW (left) and leaf chlorophyll content (right) of indicated mutants in reductive-import of iron pathway grown on unavFe media inoculated with Heat-killed or Live SynCom. Total chlorophyll content was measured in samples pooled from 6 plants. Data are from two independent experiments per genotype (n = 36 plants, 6 chlorophyll samples). Each experiment included Col-0 control (n = 90 plants, 18 chlorophyll samples). **b,** SFW and chlorophyll content of indicated coumarin pathway mutants grown on unavFe media inoculated with Heat-killed or Live SynCom. SFW data are from two experiments (n = 36 plants), and chlorophyll content from one experiment (3 samples per genotype). **c,** SFW and chlorophyll content of Col-0 plants, and *f6’h1* plants grown on unavFe supplemented with 50µM scopoletin and/or fraxetin and inoculated with heat-killed or live SynCom. Data are from one experiments (n = 18 plants, 3 chlorophyll samples per genotype). 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. n=18 plants, 3 chlorophyll samples per condition).

**Figure 5: A bacterial SynCom alleviates iron starvation signature and modulates a subset of defense genes in an F6’H1-dependent manner.**

**a**, PCA ordination of sample distances between root transcriptional profiles of Col-0 and *f6’h1* plants grown for 1 week on avFe or unavFe media inoculated with a live SynCom or heat-killed control. Data are from two pooled experiments (n = 6 samples pooled from 6 plant roots each). **b,** Selected genes related to iron homeostasis and coumarin production and their corresponding cluster assignment. DEGs were sorted into 10 clusters by expression pattern (selected plots left of heat map in **c**). **c**, Heat map of scaled counts for \_\_\_\_\_\_\_ DEGs identified across samples, arranged by K-means clustering. Significantly-enriched iron homeostasis-related and defense-related GO terms of pertinent clusters are indicated right of heat map. GO analysis was performed by comparing the indicated DEG cluster to the whole transcriptome (padj ≤ 0.05).

**Supplemental Figures**

**Supplemental figure 1: Soil mineral and nutrient analysis of CAS and IS soils.** Soil analyses were performed by Labor für Boden- und Umweltanalytik, Switzerland.

**Supplemental figure 2: *f6’h1* plants display stunted growth on alkaline and calcareous soils due to iron limitation**

**a)** *f6’h1* plants grow poorly in multiple alkaline and calcareous soils. Red arrowheads highlight plants displaying stunting and chlorosis. **b)** Poor growth of *f6’h1* on calcareous soil is improved by watering with FeEDDHA solution, an iron complex with improved solubility and availability under alkaline conditions.

**Supplemental Table 1: Sample and experimental replicates for root bacterial community profiling experiments**

Numbers indicate pooled root samples. All roots were collected from a single pot per sample. One batch of CAS and one batch of IS were included in each of 3 experiments.

**Supplemental Figure 3:** ***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. **b)** Unconstrained principle coordinate analysis (PCoA) of community profiles from all 3 experimental replicates in both soil. The largest driver of beta-diversity is soil type. **c)** Constrained PCoA (CPCoA, constrained for Genotype) analysis of pooled CAS experiments. **d)** CPCoA analysis, constrained for Genotype, of pooled IS experiments. *s8h* genotype was removed from the analysis, as it was only included in 1/3 experiments. Significant separation of *f6’h1* samples from Col-0 was observed on IS (p = 0.002 by pairwise PERMANOVA), but no significant separation was observed on CAS (p = 0.25 by pairwise PERMANOVA).**e) and f)** Bray-Curtis distances between indicated genotype samples and Col-0 for CAS and IS samples. Col0-Col0 distance represents intra-genotype distance, whilte mutant-Col0 distance represent inter-genotype distances. Sample distance is only significantly increased in *f6’h1* samples on IS.

**Supplemental Figure 4: Aggregated relative abundance of deASVs.**

For each deASV found in **a**, the mean RA was calculated in each genotype and soil type. The aggregated RA was then calculated as the sum of the mean RA of all deASVs, to quantify the magnitude of change in community composition attributable to differential enrichment of deASVs.

**Supplemental Figure 5: Bacterial families impacted by coumarin secretion**

**a)** Enrichment pattern of families in deASV subset. Hypergeometric enrichment test was performed for deASVs identified in **Figure 2**. All significant deASVs detected in f6’h1 vs Col0 growing on IS were compiled at the family level and were compared to all ASVs detected in these samples. Positive enrichment means the family was present in the deASV list more frequently than expected if ASVs were merely selected at random, negative enrichment indicates underrepresentation in deASVs. Thus, these families represent those containing significantly more or significantly fewer ASVs that are impacted by coumarin status.

**Supplemental Table 2: SynCom member taxonomy and growth rescue activity.**

Full taxonomy of strains included in 115-member SynCom and their percent growth rescue activity (**Fig. 3d**). *nt* = not tested for growth rescue activity.

**Supplemental Figure 6: Addition of Syncom does not impact plant growth or chlorosis of iron homeostasis mutants on avFe**

**a)** SFW and **b)** chlorophyll measurement of plants grown on avFe with and without live SynCom. Data are from two independent experiments (same experiments as in **Figure 4)** per genotype (n = 36 plants, 6 chlorophyll samples. Each experiment included Col-0 control (n = 90 plants, 15 chlorophyll samples)

**Supplemental Figure 7: Addition of SynCom does not impact plant growth of coumarin biosynthesis and export mutants when iron is available.**

**a)** SFW of plants grown on avFe with and without live SynCom (same experiments as in **Figure 5)**. Data are from two experiments (n = 36 plants, 3 chlorophyll samples per genotype).

**Supplemental Figure 8: Plant growth and chlorophyll content of plants used in RNASeq experiment (Figure 6.)**

SFW (**a**) and chlorophyll content (**b**) were measured after 8 days growth in experimental conditions. Growth rescue activity by SynCom is already observed in Col0 plants at this timepoint, but growth deficit between genotypes is less notable. (n = 36 plants for SFW, and 6 pooled plant samples for chlorophyll content).

**Supplemental Figure 9:** **Number and overlap of iron-responsive and SynCom-responsive DEGs in each Col-0 and f6’h1 plants.** Venn diagrams showing the overlap between genotypes of identified iron-responsive genes in 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.

**Supplemental table 3: Cluster assignment of iron starvation response marker genes.** Genes identified in *(Kai/Bauer paper citation)* as iron starvation responsive and their indicated clusters in DEG heat map in **Figure 6**.

**Supplemental Figure 10: Expression patterns of selected iron homeostasis-related genes from RNA-seq experiment**. Normalized count data for indicated genes and conditions. Count are rld log2-transformed normalized counts. All genes were assigned to cluster 8 (top row, down-regulated under iron starvation) or cluster 4 (lower 4 rows, up-regulated under iron-starvation), except for F6’H1 (cluster 7) during K-means clustering. Expression pattern is consistent with iron starvation being induced on unavFe in both genotypes, but alleviated by addition of SynCom in Col-0, but not *f6’h1* plants.

**Methods**

**Soils**

Cologne agricultural soil (CAS) was obtained from a local site (GPS code : 50.958 N, 6.856 E) that has not been exposed to agriculture for \_\_\_ years. Italian soil (IS) was obtained from a vineyard in Tebano, Italy (GPS code : 44.292 N, 11.784 E). Soil was homogenized and stored at 4°C until used in experiments. Soil nutrient analysis was performed by Labor für Boden- und Umweltanalytik (Switzerland).

**Plant model**

All *A. thaliana* genotypes used in this study were in the Columbia wildtype (Col-0, N60000) background. We employed mutants related to coumarin biosynthesis (*f6’h1-1*, *s8h-2*, *cyp82c4-1*)and export (*pdr9-2*), regulation of the iron starvation response (*bts-1*), and iron reductive import (*aha2-4*, *fro2 (frd1-1)*, and *irt1-1*). Each of these genes are expressed in roots.

**Bacterial strains**

The bacterial strains used in this study were previously described3 and summarized in **Suppl. File: Bacterial strains**. Each of these strains was originally isolated from *A. thaliana* roots grown in CAS soil. Strains were stored in 20% glycerol stocks in single tubes and/or 96-well format and handled by standard microbial culture methods.

**Plant growth conditions**

Seeds were routinely surface sterilized with 70% ethanol for 15 minutes under agitation, followed by 2x washes with 70% ethanol, 1x with 100% ethanol, and 3x with sterile distilled water. Sterilized seeds were stratified at 4˚C in the dark for 2-3 days either imbibed in water (for soil experiments) or on agar media plates (for agar-media experiments) before transfer to growth conditions.

Soil: Surface-sterilized, stratified seeds were germinated in (7\*7 or 9\*9cm?) square pots filled with CAS or IS. Pots were watered from the top with non-sterile distilled water twice per week. Plants were grown in the greenhouse (long day or short day?). Pots were distributed at random within trays. Trays and pots were randomly shuffled periodically to minimize edge and location effects.

Agar-media: Surface-sterilized seeds were were sown on plates containing 1% agar (Agar, granulated, Difco) in 50% Murashige and Skoog (MS) medium with vitamins (2.2 g/L, Duchefa Biochemie) supplemented with 0.5% sucrose. After 2 days of stratification at 4°C, plates were positioned vertically in a climate chamber (Panasonic, MLR-352) and grown for 6 days (10 hours light, 21 ˚C; 14 hours dark, 19 ˚C). Uniform seedlings were then transferred to experimental condition plates prepared fresh on the day of seedling transfer.

Gnotobiotic system for iron limitation

Stock solutions were prepared of Ethylenediaminetetraacetic acid ferric sodium salt (Fe(III)EDTA, Sigma) in distilled water, and 100mM Fe(III)Cl3 (Merck) in 10mM HCl (to prevent precipitation), sterile filtered and stored at 4˚C protected from light. HEPES buffer (Roth) stock solution (2M, 200x) was prepared, and the pH was adjusted with KOH until a dilution to 10mM in 50% MS resulted in a pH of 7.4 (approximately pH 8.2 for stock solution) and stored at 4˚C.

As a base medium, modified 50% MS media without iron or pH buffer (750 μM MgSO4, 625 μM KH2PO4, 10.3 mM NH4NO3, 9.4 mM KNO3, 1.5 mM CaCl2, 55 nM CoCl2, 53 nM CuCl2, 50 μM H3BO3, 2.5 μM KI, 50 μM MnCl2, 520 nM Na2MoO4, 15 μM ZnCl2, and 9.4mM KCl) was prepared from individual stock solutions. Base media with 1% agar was autoclaved and cooled to 50˚C before adding iron source (final 100 µM) and HEPES (final 10mM, pH 7.4) with constant stirring. Media was allowed to cool to ~45˚C, and 45 ml were measured into a conical tube. Live or heat-killed bacteria or SynCom (preparation see below) were added to a final OD600nm = 0.0001, corresponding to approximately 105 cells/ml. For coumarin complementation experiments, scopoletin and fraxetin were added to final concentration of 50 µM, or equivalent DMSO-only control. Media was mixed thoroughly by inverting, poured into 12\*12cm square petri dishes, dried with an open lid for 30 minutes then allowed to solidify. Seedlings were transferred to experimental plates (6 plants per plate, three replicate plates per experiment). Plates were returned to the growth chamber and grown vertically with random shuffling and re-distribution every 2-3 days for uniformity. After 2 weeks, SFW was measured and chlorophyll and root samples were collected.

**Leaf chlorophyll measurement**

Chlorophyll extraction and quantification was adapted from61. Samples were prepared from 20-40mg of leaf tissue pooled from ~6 plants per sample and weighed. Samples were either processed immediately or frozen at -80˚C until processing. Chlorophyll was extracted by adding 1ml DMSO per ~30mg tissue and incubating samples at 65˚C with shaking for 45-60m until plant tissue was transparent and chlorophyll completely extracted. Absorbance of tissue-free chlorophyll extract was measured at 652nm on a spectrophotometer (NanoDrop One, Thermo Scientific) and transformed to 1-cm pathlength absorbance equivalent. Alternatively, absorbance of 100 µl of samples was measured in 96-well microtiter plate in a microplate reader (Infinite M200 PRO, Tecan) and transformed to 1-cm pathlength absorbance equivalent using a regression curve of standard dilution samples measured on both the spectrophotometer and microplate reader. Absorbance652nm cm-1 was converted to total chlorophyll per ml and normalized to input sample tissue mass with the following formula:

**16S profiling of the root microbiota**

**Masayoshi, can you add details – plant age, root sample collection, and check below is correct?**

For 16S profiling, we processed libraries as in43. Total root DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals). Samples were homogenized in Lysing Matrix E tubes using the Precellys 24 tissue lyzer (Bertin Technologies) at 6,200 r.p.m. for 30 s. DNA was eluted in 60 μl of nuclease-free water and quantified using Quant-iT PicoGreen dsDNA Assay (ThermoFisher). Samples were diluted to 3.5 ng µl−1 and used in a two-step PCR amplification protocol. In the first step, the V5–V7 region of the bacterial 16S rRNA gene (primers 799F–1192R) was amplified in triplicate reactions for each sample. Amplification was performed in a 25-μl reaction volume containing 2 U DFS-Taq DNA polymerase, 1× incomplete buffer (both Bioron), 2 mM MgCl2, 0.3% bovine serum albumin, 0.2 mM dNTPs (Life Technologies) and 0.3 μM forward and reverse primers. The same PCR parameters were used for each primer pair (94 °C for 2 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and 72 °C for 10 min for 25 cycles). Single-stranded DNA and proteins were digested by adding 1 μl of Antarctic phosphatase, 1 μl Exonuclease I and 2.44 μl Antarctic Phosphatase buffer (New England Biolabs) to 20 μl of the pooled replicate reactions. Digestion was performed at 37 °C for 30 min, followed by enzyme deactivation at 85 °C for 15 min. Samples were centrifuged for 10 min at 4,000 r.p.m., and 3 μl of supernatant was used for the second PCR step to add barcodes and illumine adapters. Reactions were prepared with barcode primer pairs (Primer table) and performed as above with the number of cycles reduced to ten. PCR quality was controlled by loading 5 μl of each reaction on a 1% agarose gel. The remaining reaction volume was loaded on a 1.5% agarose gel and run at 80 V for 2 h; bands with the correct size of ∼500 base pairs were cut out and purified using the QIAquick Gel Extraction Kit (Qiagen). Concentration of the purified DNA was determined, and 30 ng DNA from each of the barcoded amplicons was pooled into one library per experiment, then purified and re-concentrated twice with Agencourt AMPure XP beads. Paired-end Illumina sequencing was performed in-house using the MiSeq sequencer and custom sequencing primers (Primer table).

**Analysis of 16S profiling data**

ASV table generation

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Alpha and beta diversity:

Analyses and visualization were all performed in the R statistical environment. Analysis was performed on samples with a sequencing depth of at least 2000 high-quality reads. Alpha and beta diversity were calculated on ASV count tables that were rarefied to 2000 reads. Alpha diversity (Shannon index) was calculated with using the “diversity” function in vegan62 (R package version 2.5-6) and differences were tested with ANOVA.

Bray-Curtis dissimilarity index was calculated using the “vegdist” function in vegan and used for unconstrained ordination by Principal Coordinate Analysis (PCoA). Constrained PCoA (CPCoA) was performed with the “capscale” function in vegan, using the square-root distances of Bray-Curtis dissimilarity index. The model formula for genotype-constrained ordination of compiled experiments on single soils (**Suppl. Fig 3**) was “distance.matrix ~ Host.Genotype”. For ordination constrained on the interaction between genotype and soil type (**Fig. 1c**), the formula used was “distance.matrix ~Host.Genotype\*Soil”. Statistical significance of genotype separation was performed by pairwise PERMANOVA with 999 permutations using the RVAideMemoire package (ref).

Analysis of deASVs:

Analyses and visualization were all performed in the R statistical environment. Analysis was performed on samples with a sequencing depth of at least 2000 high-quality reads. Analysis was performed on relative abundance (RA) calculated using non-rarefied ASV count data. To calculate deASVs between coumarin pathway mutants and Col-0 WT, data was pooled from three experiments (except *s8h*, which was included in only one experiment), and filtered for ASVs found in at least three samples at a RA > 0.05%. Statistically-significant differential enrichment was determined with the edgeR package63 (version 3.24.3) using pair-wise genotype comparisons in a negative binomial generalized log-linear model at an FDR-adjusted p value of 0.05.

Family-level taxonomic classification of deASVs in *f6’h1* plants growing on IS was performed using the Silva database. Hypergeometric enrichment test was performed using the “dhyper” function in R. Each family was tested for over- or under-represented in deASVs set by comparing to the list of all detected ASVs. Red asterisks indicate significance at an FDR-adjusted p value of 0.05.

**Coumarin antimicrobial activity**

The antimicrobial activity of scopoletin and fraxetin (Sigma Aldrich) against single bacterial strains was assayed in liquid culture in 50% tryptic soy broth (TSB, 15g/L; Sigma Aldrich). Scopoletin and fraxetin stocks were prepared in sterile DMSO (Sigma Aldrich) and stored at -80˚C. Bacteria colonies were picked from TSA plates into liquid TSB and grown for 2-3 days at 25˚C with 180 rpm agitation. Liquid cultures were subcultured by diluting 1:100 into fresh TSB and incubated 1-2 hours. In a clear flat-bottom 96-well microtiter plate, 100 µl of subculture was added to 100 µl of fresh TSB media supplemented with scopoletin or fraxetin for final 50μM concentration, or equivalent DMSO negative control. The microtiter plate was sealed with a clear adhesive film to prevent evaporation. Growth was monitored kinetically in a microplate reader (Infinite M200 PRO, Tecan) with 30 seconds of shaking followed by measurement of optical density (OD) at 600 nm in 4 locations per well every 30 minutes for 18-20 hours. The OD in each experiment was expressed as the average of triplicate wells per condition. Relative growth (**Fig 2d**) was calculated by dividing the average final OD measurement of each strain and indicated condition by the average OD in the coumarin-free control.

**Preparation of bacteria for microbiota reconstitution**

For single strains, individual colonies were picked from TSA plates and grown in liquid TSB at 25˚C with 150 RPM agitation for 5 days. Strains were subcultured 1:10 in fresh TSB for 2 hours, washed twice with sterile 10mM MgCl2 (Merck), resuspended and adjusted to OD600 = 0.1. For SynCom reconstitution, glycerol stocks of 115 SynCom member strains were prepared and frozen in 96-well format. Cultures were picked directly from glycerol stocks into 1ml TSB in 96-well deep-well plates using a 96-well format microplate tip replicator and sealed with breathable plate sealer (AeraSeal, Sigma Aldrich). Cultures were grown at 25˚C with 180 RPM agitation for 5 days and controlled to ensure that a majority of strains grew successfully. Fresh TSB (500µl per well) was added and cultures were grown overnight, 12-18 hours to harvest metabolically active cells. Cultures were then centrifuged (4000 g, 20 min), washed once with MgCl2, and resuspended in 300µl/well. Cultures were combined, washed and resuspended in MgCl2 and adjusted to OD600 = 0.1. Heat-killed SynCom was prepared by incubating an aliquot of SynCom suspension at 99˚C for 30 minutes. Heat-killed or Live suspensions were used 1000x to inoculate media (final OD600 = 0.0001).

**RNA extraction and RNA-seq analysis**

For transcriptomic analysis, 6-day-old *A. thaliana* seedlings were transferred to avFe or unavFe media with live or heat-killed SynCom as above and grown for 8 days. Roots from 6 plants (one plate) were combined for one replicate and a total of three replicates were sampled for each condition in each of two experiments. Roots were homogenized with Lysing Matrix E and TissueLyser II (30 beats per second for 2x30 s; Qiagen) and RNA was extracted with the miRNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. RNA quality was determined using a 2100 Bioanalyzer (Agilent Technologies). Preparation of Illumina sequencing libraries was conducted by the Max Planck Genome Center. Sequences were generated using the Illumina HiSeq2500 platform. Approximately 20M paired-end reads with a length of 250 bp were obtained per sample in one experiment, and 8M per sample in the second.

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