Root development is maintained by specific bacteria-bacteria interactions within a complex microbiome

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Abstract

Plants grow within a complex web of species interacting with each other and with the plant via a wide repertoire of chemical signals. To model plant-microbe-microbe-environment interactions, we inoculated seedlings with a defined 185-member bacterial synthetic community (SynCom), and manipulated the abiotic environment to enable classification of the SynCom to modules of co-occurring strains. We deconstructed the SynCom based on these modules, identifying a single bacterial genus, *Variovorax*, which reverts phenotypic effects on root development induced by a wide diversity of bacterial strains and by the entire 185-member community. *Variovorax* use mechanisms related to auxin and ethylene manipulation to balance this ecologically realistic root community's effects on root development. We demonstrate metabolic signal interference within a complex model community, defining *Variovorax* as determinants of bacteria-plant communication networks.

Introduction

Plant phenotypes, and ultimately fitness, are influenced by the microbes living in close association with them (*1*–*4*). This plant microbiota assembles based on plant-derived cues (*5*) resulting in myriad plant-microbe interactions. Beneficial and detrimental microbial effects on plants can be direct, via production of chemical signals (*6*, *7*) or modulation of nutrient availability (*1*, *4*, *8*); or they can be an indirect consequence of microbe-microbe interactions (*3*). A potentially significant class of microbe-microbe interactions is metabolic signal interference (*7*, *9*): rather than direct antagonism, microbes interfere with the delivery of chemical signals produced by other microbes, altering plant-microbe signaling (*10*–*12*).

Here, we apply a plant microbiota synthetic community (SynCom) to axenic plants, to ask how microbe-microbe interactions shape plant phenotypes. We used plant colonization patterns across 16 abiotic conditions to guide stepwise deconstruction of the SynCom, leading to the identification of a single bacterial genus that is required for maintaining the root's intrinsically controlled developmental program by tuning its chemical landscape.

Results

We established a controlled plant-microbiota microcosm representing the native bacterial microbiota on agar plates. We inoculated 7-day-old seedlings with a defined 185-member bacterial SynCom (fig. S1 and data S1) composed of genome-sequenced isolates obtained from

Arabidopsis roots (Material and Methods 1). To test the robustness of microbiota assembly to the abiotic environment, we exposed each microcosm to one of 16 different abiotic contexts by manipulating four variables (salinity, temperature, phosphate concentration and pH). We measured SynCom composition in root, shoot and agar fractions 12 days post-inoculation using 16S rRNA amplicon sequencing. Across all abiotic variables, fraction (agar, root, shoot; fig. S2) explained most (40%) of the variance. Abiotic conditions significantly affected both alpha- (fig. S3) and beta-diversity (Fig. 1A). Our SynCom therefore exhibits deterministic niche sorting within the plant across a range of abiotic conditions.

We calculated pairwise correlations in relative abundance across all samples, and identified four well-defined modules of co-occurring strains (A, B, C and D; Fig. 1B and data S2). These modules formed distinct phylogenetically-structured guilds in association with the plant: module A contained mainly Gammaproteobacteria and was predominantly more abundant in the agar than in the seedling; module B contained mainly low-abundance Firmicutes, with no significant seedling enrichment trend; modules C and D were composed mainly of Alphaproteobacteria and Actinobacteria, respectively, and showed seedling-enrichment across all abiotic conditions (Figure 1A and data S2). Both Alphaproteobacteria (module C) and Actinobacteria (module D) are consistently plant-enriched across plant species (*13*), suggesting that they contain plant-association traits that are deeply rooted in their evolutionary histories.

We next asked whether the different modules of co-occurring strains play different roles in determining plant phenotypes (Materials and Methods 2). We inoculated seedlings with SynComs composed of modules A, B, C and D singly, or with all six possible pairwise module combinations (Fig. 2A), and imaged the seedlings 12 days post-inoculation. We observed strong primary root growth arrest in seedlings inoculated with plant-enriched modules C or D (Fig. 2A). This root growth inhibition (RGI) did not occur in seedlings inoculated with modules A or B, which do not contain plant-enriched strains (Fig. 2A, 2C and data S3). To test whether plant-enrichment and RGI are correlated traits, we inoculated seedlings in mono-association with each of the 185 SynCom members (Materials and Methods 3). Surprisingly, we observed that RGI-inducing strains were found in all four modules (Fig. 2B, fig. S4-S5 and data S4). Thus, in line with (*4*), plant-enrichment within a bacterial community does not predict phenotypic effects on seedlings in mono-association.

The mono-association assay confirmed that RGI is a prevalent trait across the plant bacterial microbiota. Thirty-four taxonomically diverse strains, derived from all four modules, induced RGI. However, neither the full SynCom nor derived SynComs of modules A or B exhibited RGI (Fig. 2A), suggesting that microbe-microbe interactions are responsible for RGI attenuation within these SynComs. Furthermore, in seedlings inoculated with module pairs, we observed an epistatic interaction: in the presence of module A, RGI caused by modules C and D was reverted (Fig. 2A). Thus, through deconstructing the SynCom into four modules, we found that bacterial effects on root development are governed by multiple levels of microbe-microbe interactions, exemplified by at least four instances: within modules A and B and between module A and modules C and D. Since three of these interactions involve module A, we predicted that this module contains strains that strongly attenuate RGI, preserving stereotypic root development.

To identify strains within module A responsible for intra- and inter-module RGI attenuation, we reduced our system to a tripartite plant-microbe-microbe system (Materials and Methods 4). We individually screened the 18 non-RGI strains from module A for their ability to attenuate RGI caused by representative strains from all four modules. We found that all strains from a single genus, *Variovorax* (Family Comamonadaceae) suppressed RGI caused by representative RGI-inducing strains from module C (*Agrobacterium* MF224) and module D (*Arthrobacter* CL28; Fig. 2D, fig. S6 and data S5). The strains from modules A (*Pseudomonas* MF48) and B (*Bacillus* MF107), were not suppressed by *Variovorax*, but rather by two closely related *Burkholderia* strains (CL11, MF384). A similar pattern was observed when we screened three selected RGI-suppressor *Variovorax* strains (CL14, MF160) and *Burkholderia* CL11, against a diverse set of RGI-inducers. *Variovorax* attenuated 13 of the 18 RGI-inducers tested (Fig. 2E-F, fig. S6 and data S6).

We further tested if our RGI-suppressor strains maintain their capacity to attenuate RGI in the context of the full 185-member community (Materials and Methods 5). We compared the root phenotype of seedlings exposed to either the full SynCom or to the same community dropping-out all ten *Variovorax* strains and/or all six *Burkholderia* strains (drop-out system, Fig. 3A-C). We found that *Variovorax* are necessary and sufficient to revert RGI within the full community (Fig. 3B-C and data S7). This result was robust across a range of biotic and abiotic contexts (Fig. 3D-E, fig. S7-S8 and data S7; Materials and Methods 6-7).

To ascertain the phylogenetic breadth of the *Variovorax* ability to attenuate RGI, we tested additional *Variovorax* strains from across the genus' phylogeny (fig. S9A and data S1; Materials and Methods 8). All tested *Variovorax* reverted RGI induced by *Arthrobacter* CL28 (Materials and Methods 9). A strain from the nearest plant-associated outgroup to this genus, *Acidovorax* Root219, did not revert RGI (fig. S9A-B and data S8). *Variovorax*-mediated RGI attenuation extended to tomato seedlings, where *Variovorax* CL14 reverted *Arthrobacter* CL28-mediated RGI (fig. S10, data S9, Materials and Methods 10). Thus, a single bacterial genus, *Variovorax,* interacts with a wide diversity of bacteria to enforce stereotypic root development within complex communities, independent of biotic or abiotic contexts, and this ability is general to this genus.

We tested whether *Variovorax* attenuate RGI by inhibiting growth of RGI-inducing strains (Materials and Methods 11). We counted colony forming units of the RGI inducer *Arthrobacter* CL28 from roots in the presence or absence of *Variovorax* CL14 and found that CL28 abundance increased in the presence of *Variovorax* CL14 (fig. S11, data S10). To test whether *Variovorax* modulates bacterial abundances in the whole community, we compared the bacterial relative abundance profiles in seedlings colonized with the full SynCom to that colonized with the *Variovorax* drop-out community (Materials and Methods 6d). We found no changes in the abundances of RGI-inducing strains in response to the *Variovorax* drop-out (Fig. 3F). In fact, *Variovorax* account for <1% of the community on our roots (Fig. 3G), and in natural soils (fig. S12). Notably, while rare, *Variovorax* are root-enriched in two natural soils tested (fig. S12). These results rule out the possibility that *Variovorax* enforce root developmental patterns by antagonizing or outcompeting RGI-inducers. We hypothesized that RGI attenuation by *Variovorax* is likely mediated by signal interference.

To study the mechanisms underlying RGI and RGI attenuation, we analyzed the transcriptomes of seedlings colonized for 12 days with the RGI-inducer *Arthrobacter* CL28 and the RGI-attenuator *Variovorax* CL14, either in mono-association with the seedling or in a tripartite combination (Fig. 2F; Materials and Methods 12). We also performed RNA-Seq on seedlings colonized with the full SynCom (no RGI) or the *Variovorax* drop-out SynCom (RGI; Fig. 3A). Eighteen genes were significantly induced only under RGI conditions across both experiments (Fig. 4A-B and data S11). Seventeen of these are co-expressed genes related to the root apex (*14*) (Fig 4B and fig. S13). The remaining gene is Indole-3-acetic acid-amido synthetase GH3.2, which conjugates excess amounts of the plant hormone auxin and is a robust marker for late auxin responses (*15*, *16*) (Fig. 4C). Production of auxins is a well-documented mechanism by which bacteria modulate

plant root development (*10*). Indeed, the top 12 auxin-responsive genes from an RNA-Seq study examining acute auxin response in Arabidopsis (*15*) exhibited an average transcript increase in seedlings exposed to our RGI-inducing conditions (Fig. 4C and data S12).

Next, we asked whether RGI-attenuation by *Variovorax* is directly and exclusively related to auxin signaling (Materials and Methods 13). Besides auxin, other small molecules cause RGI. These include the plant hormones ethylene (*17*) and cytokinin (*18*); and microbial-associated molecular patterns (MAMPs) including the flagellin-derived peptide flg22 (*19*). We tested the ability of diverse *Variovorax* strains and of the *Burkholderia* strain CL11 to revert RGI induced by auxins (Indole-3-acetic acid [IAA] and the auxin analogue 2,4-Dichlorophenoxyacetic acid [2,4-D]), ethylene (the ethylene precursor 1-Aminocyclopropane-1-carboxylic acid [ACC]), cytokinins (Zeatin, 6-Benzylaminopurine) and flg22 peptide (fig. 4D). All tested *Variovorax* suppress RGI induced by IAA or ACC (Figure 4D and data S13), with the exception of *Variovorax* YR216 which did not suppress ACC-induced RGI and does not contain an ACC deaminase gene (fig. S9A), a plant growth-promoting feature associated with this genus (*17*). *Burkholderia* CL11 was only able to partially revert ACC-induced RGI. None of the *Variovorax* attenuated RGI induced by 2,4-D, by flg22 or by cytokinins, indicating that *Variovorax* revert RGI induction by interfering with auxin and/or ethylene signaling. Furthermore, this function is mediated by recognition of auxin by the bacteria and not by the plant auxin response *per se* since RGI induced by 2,4-D is not reverted. Indeed, we found that *Variovorax* CL14 degrades IAA *in-vitro* (fig. S14; Materials and Methods 14) and quenches fluorescence of the Arabidopsis auxin reporter line *DR5::GFP* caused by the RGI inducer *Arthrobacter* CL28 (Fig. 4E, fig. S15 and data S14; Materials and Methods 15).

We used the auxin-insensitive *axr2-1* mutant (*20*), combined with a competitive inhibitor of ethylene receptors, 1-Methylcyclopropene (1-MCP) (*21*), to examine the roles of plant auxin and ethylene perception in bacterially-induced RGI (Materials and Methods 16). We inoculated wild type seedlings and the *axr2-1* mutants, treated or not with 1-MCP, with the RGI-inducing *Arthrobacter* CL28 or the *Variovorax* drop-out SynCom. We observed in both cases that bacterial RGI is reduced in *axr2-1* and 1-MCP-treated wild type seedlings, and is further reduced in doubly-insensitive 1-MCP-treated *axr2-1* seedlings, demonstrating a synergistic effect of auxin and ethylene (Fig. 4F and data S15) on bacterial RGI. Thus, a complex SynCom can induce severe morphological changes in root phenotypes via both auxin- and ethylene-dependent pathways, and both are reverted when *Variovorax* are present. We conclude that seedlings growing in a realistically diverse bacterial microbiota depend on specific taxa for maintaining a controlled

168 developmental program by tuning the root's response to its microbially-encoded chemical
169 landscape.
170
171 **Discussion**
172 Microbes communicate with the plant and each other by secreting a wide repertoire of secondary
173 metabolites, which have profound effects on plant physiology and development (*10*, *19*). Equally
174 important are the interactions between these signals, as in the case of quorum quenching (*12*) or
175 degradation of MAMPs (*22*). The degradation of bacterially-produced auxin is prevalent in the
176 rhizosphere, including among *Variovorax* (*10*, *11, 23*), but its consequences for the plant have
177 not been demonstrated in a community context. Intriguingly, sequenced *Variovorax* genomes do
178 not contain any canonical auxin degradation operons (Materials and Methods 18) (*23*), suggesting
179 a novel mechanism. This work demonstrates metabolic signal interference from within a complex
180 model community and stages *Variovorax* as key players in bacteria-plant communication
181 networks, demonstrating an ability to block bacterial effects on root development across a wide
182 range of biotic and abiotic contexts.
183
184 Signaling molecules and other secondary metabolites are products of evolution towards
185 increasing complexity that allows microbes to survive competition for primary metabolites. Our
186 results illuminate the importance of an additional trophic layer of microbes that utilize these
187 secondary metabolites for their own benefit, while providing the unselected exaptation (*24*) of
188 metabolic signal interference between the bacterial microbiota and the plant host. This potential
189 exaptation, and the consequent homeostasis of plant hormone signals that emerges from it,
190 allows the plant to maintain its root developmental program within a chemically rich matrix.
191 Moreover, our data suggest that *Variovorax* accomplish this function while not disrupting the
192 colonization of other taxa, some of which likely provide the host additional ecosystem services.
193 As we proceed to design and develop plant probiotics, we need to consider the maintenance of
194 intra-and inter-taxa microbial homeostasis as potential contributors to satisfactory invasion and
195 persistence of probiotic strains into standing heterogeneous microbial communities.

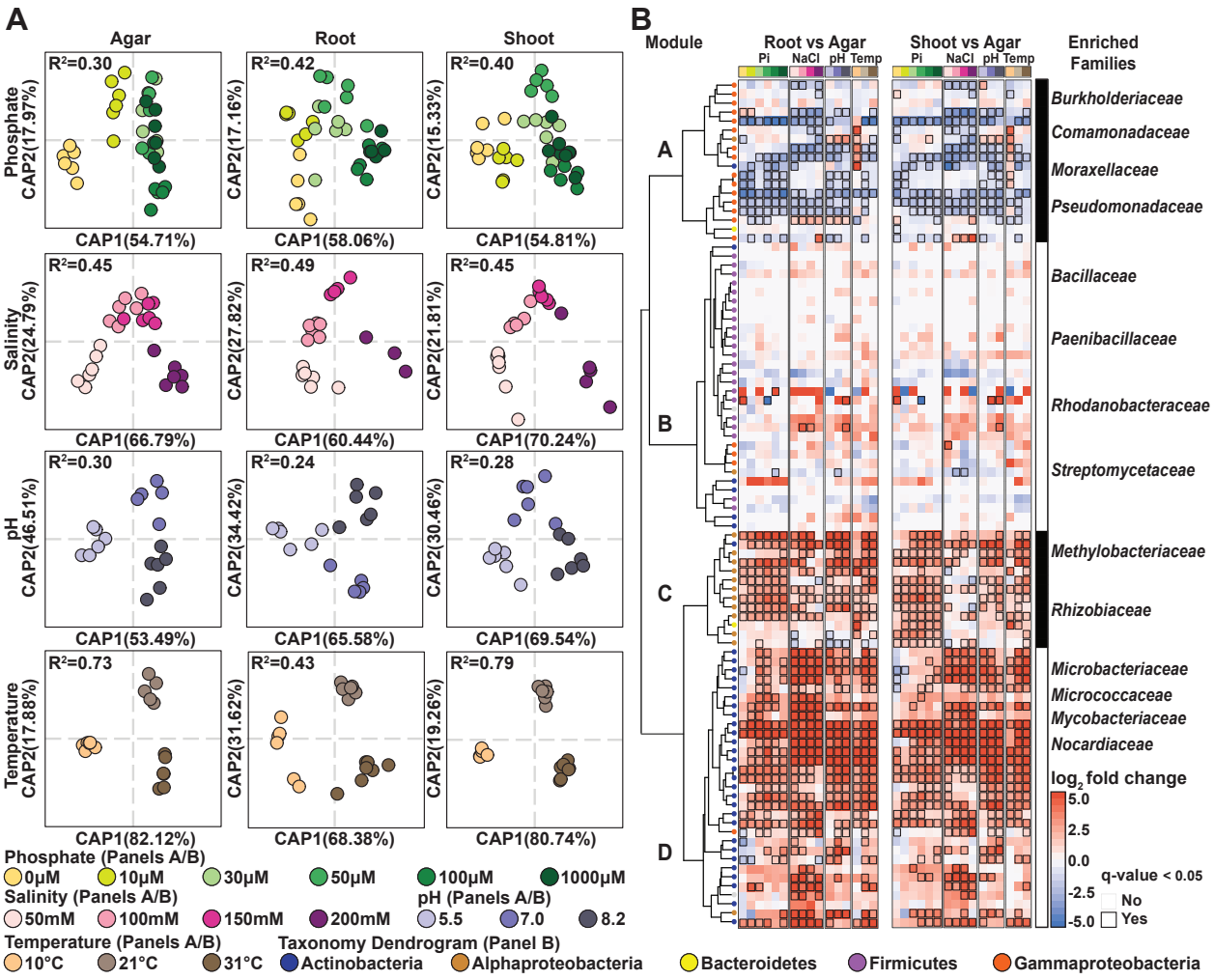

Fig. 1. Reproducible effects of abiotic conditions on the synthetic community assembly.

(A) Canonical analysis of principal coordinates (CAP) scatterplots showing the influence of each of the four abiotic gradients (phosphate, salinity, pH, temperature) within agar, root and shoot fractions. PERMANOVA R^2 values are shown within each plot. **(B)** Fraction enrichment patterns of the SynCom across abiotic gradients. Each row represents a USeq. Letters on the dendrogram represent the four modules of co-occurring strains (A, B, C, D). Dendrogram tips are colored by taxonomy. The heatmaps are colored by log2 fold changes derived from a fitted GLM. Positive fold changes (red gradient) represent enrichments in plant tissue (root or shoot) compared with agar, negative fold changes (blue gradient) represent depletion in plant tissue compared with agar. Comparisons with q-value < 0.05 are contoured in black. Family bar highlights enriched families within each module.

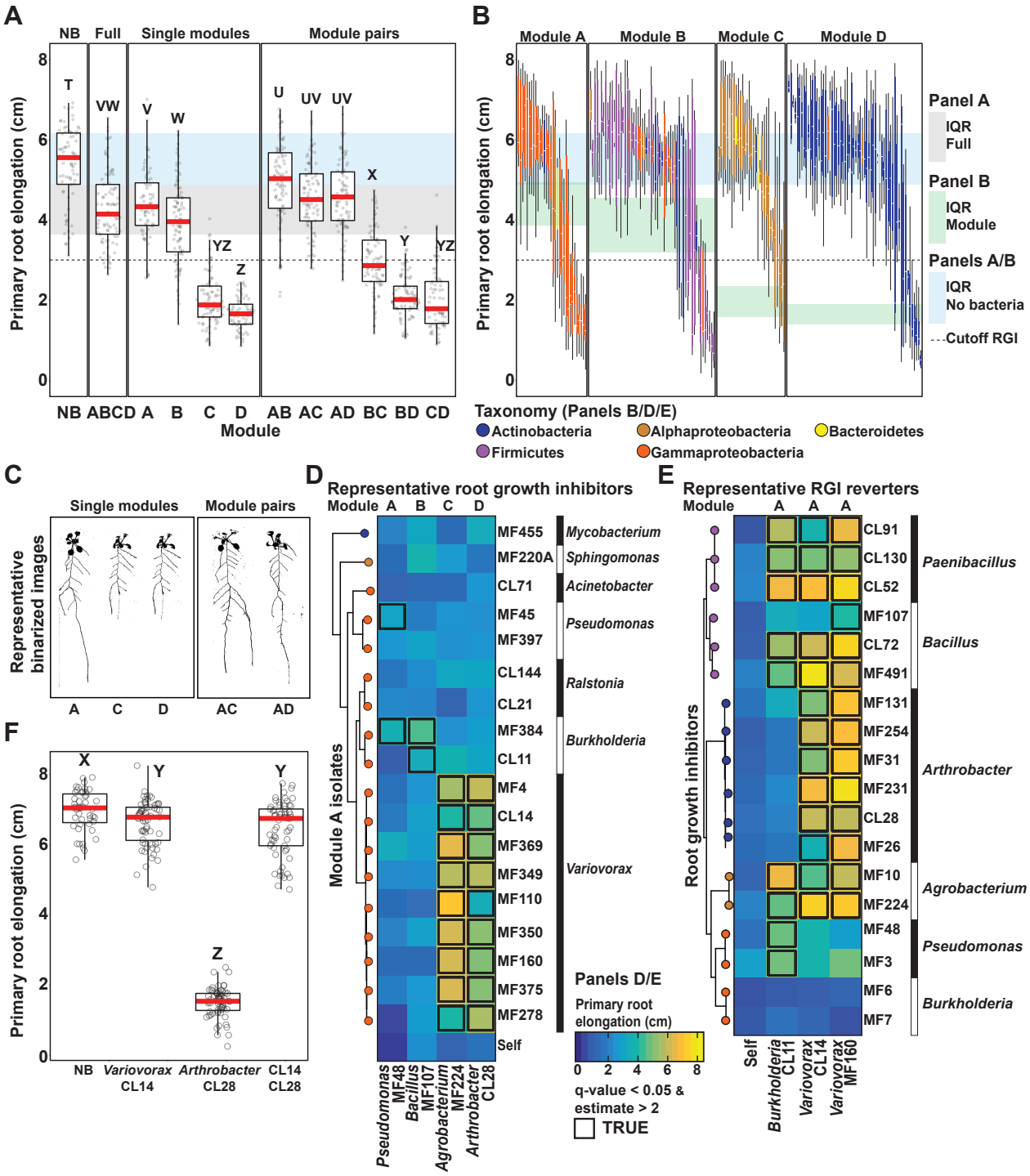

Fig. 2. Arabidopsis root length is governed by multiple bacteria-bacteria interactions within a community.
(A) Primary root elongation of seedlings grown with no bacteria (NB), with the full 185-member SynCom (Full) or with its subsets: Modules A, B, C and D alone (single modules), as well as all six possible pairwise combination of modules (module pairs). Differences between treatments are denoted using the compact letter display. **(B)** Primary root elongation of seedlings inoculated with single bacterial isolates. Isolates are colored by taxonomy and grouped by module membership. The strips across the panels correspond to the interquartile range (IQR) as noted at far right. The dotted line represents the cutoff used to classify isolates as root growth inhibiting (cutoff RGI). **(C)** Binarized image of representative seedlings inoculated with modules A, C and D, and with module combinations AC and AD. **(D, E)** Heatmaps colored by average primary root elongation of seedlings inoculated with different pairs of bacterial isolates: **(D)** with four representative RGI-inducing strains from each module (columns) alone (Self) or in combination with isolates from module A (rows), **(E)** with eighteen RGI-inducing strains (rows) alone (Self) or in combination with *Burkholderia* CL11, *Variovorax* CL14 or *Variovorax* MF160 (columns). Statistically significant RGI reversions are contoured in black. **(F)** Primary root elongation of uninoculated seedlings (NB) or seedlings inoculated with *Arthrobacter* CL28 and *Variovorax* CL14 isolates individually or jointly. Letters indicate post-hoc significance.

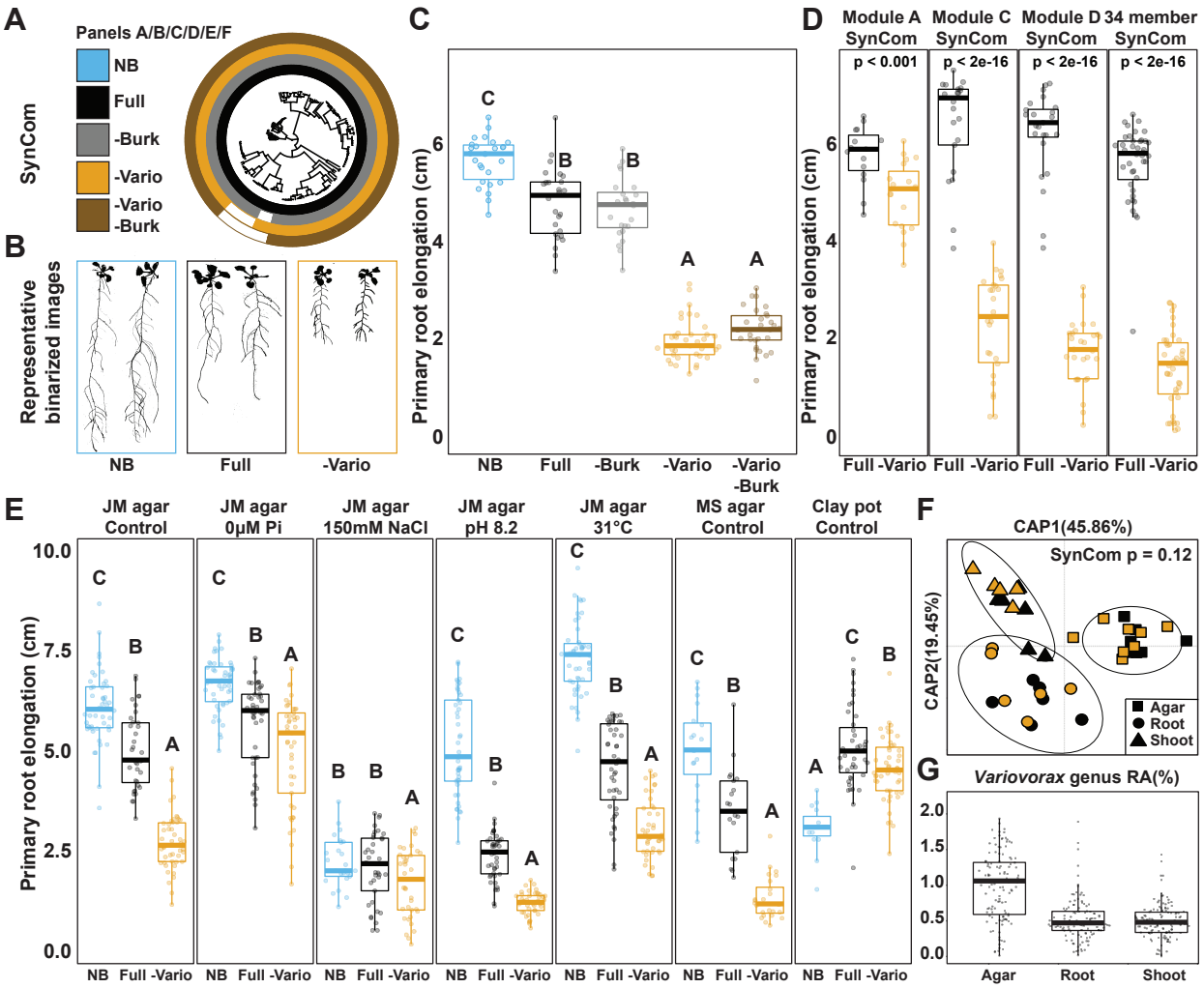

Fig. 3 *Variovorax* are necessary and sufficient to maintain stereotypic root development.

(A) Phylogenetic tree of 185 bacterial isolates. Concentric rings represent isolate composition of each SynCom treatment (-Burk: *Burkholderia* drop-out, -Vario: *Variovorax* drop-out). **(B)** Binarized image of representative uninoculated seedlings (NB), or seedlings with the full SynCom (Full) or the *Variovorax* drop-out SynCom (-Vario) treatments. **(C)** Primary root elongation of uninoculated seedlings (NB) or seedlings with the different SynCom treatments. **(D)** Primary root elongation of seedlings inoculated independently with four compositionally different SynComs (Module A, C, D and 34-member) with (Full) or without (Vario) 10 *Variovorax* isolates. FDR-corrected p-values are shown within each plot. **(E)** Primary root elongation of uninoculatd seedlings (NB), or seedlings with the Full SynCom or with the *Variovorax* drop-out SynCom (-Vario) across different abiotic conditions: unamended medium (JM agar control), phosphate starvation (JM agar 0 μM Pi), salt stress (JM agar 150 mM NaCl), high pH (JM agar pH 8.2) and high temperature (JM agar 31˚ C) and media: Johnson Medium (JM agar control), Murashige and Skoog (MS agar control) and calcined clay (Clay pot control). Letters indicate statistical significance. **(F)** Canonical analysis of principal coordinates scatterplots comparing community full vs *Variovorax* drop-out SynComs across all fractions (agar, root, shoot). PERMANOVA p-value is shown. **(G)** Relative abundance (RA) of the *Variovorax* genus within the full SynCom across the agar, root and shoot fractions.

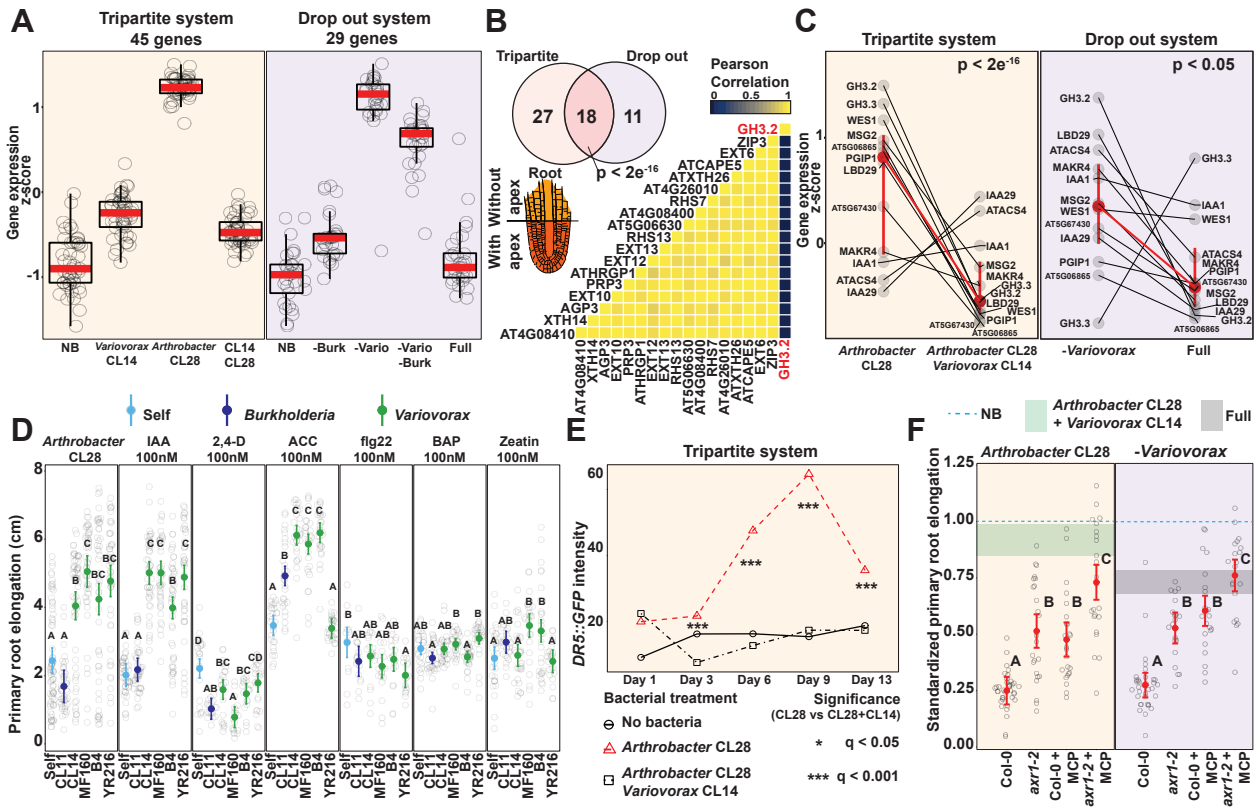

Fig. 4. *Variovorax* attenuation of root growth inhibition is related to auxin and ethylene signaling.

(A) Boxplots showing the average standardized expression of genes induced in seedlings in response to: Left (Tripartite system) *Arthrobacter* CL28 compared with uninoculated seedlings (NB) or seedlings inoculated with both *Arthrobacter* CL28 and *Variovorax* CL14 (CL14 CL28). Right (Drop-out System) Variovorax drop-out SynCom (-Vario) compared to uninoculated seedlings (NB) and to the full SynCom (Full). **(B)** Venn diagram showing the overlap in enriched genes between the tripartite and drop-out systems. The heatmap shows the pairwise correlation in expression of these 18 genes across tissues (14). **(C)** Standardized expression of 12 late-responsive auxin genes across the tripartite and drop-out systems. Each dot represents a gene. Identical genes are connected between bacterial treatments with a black line. Mean expression (95% CI intervals) of the aggregated 12 genes in each treatment is highlighted in red and connected between bacterial treatments with a red line. **(D)** Primary root elongation of seedlings grown with six hormone or MAMP RGI treatments (panels) individually (Self) or with either *Burkholderia* CL11 or four *Variovorax* isolates. Significance between the bacterial treatments is shown using the confidence letter display. **(E)** GFP intensity of *DR5::GFP* Arabidopsis seedlings grown with no bacteria, *Arthrobacter* CL28 and *Arthrobacter* CL28+*Variovorax* CL14. Significance within time points is denoted with asterisks. **(F)** Primary root elongation, standardized to sterile conditions, of wild type (Col-0) auxin unresponsive (*axr1-2*), ethylene unresponsive (Col-0 + MCP), or auxin/ethylene unresponsive (*axr1-2* + MCP) seedlings inoculated with RGI-inducing *Arthrobacter* CL28 or the *Variovorax* dropout SynCom (-*Variovorax*). The blue dotted line marks the relative mean length of uninoculated seedlings. The horizontal shade in each panel corresponds to the interquartile range of seedlings (all genotypes) grown with: *Arthrobacter CL28*+-*Variovorax* CL14), or the full 185-member SynCom including 10 *Variovorax* isolates (Full SynCom). Differences between treatments are denoted using the compact letter display.

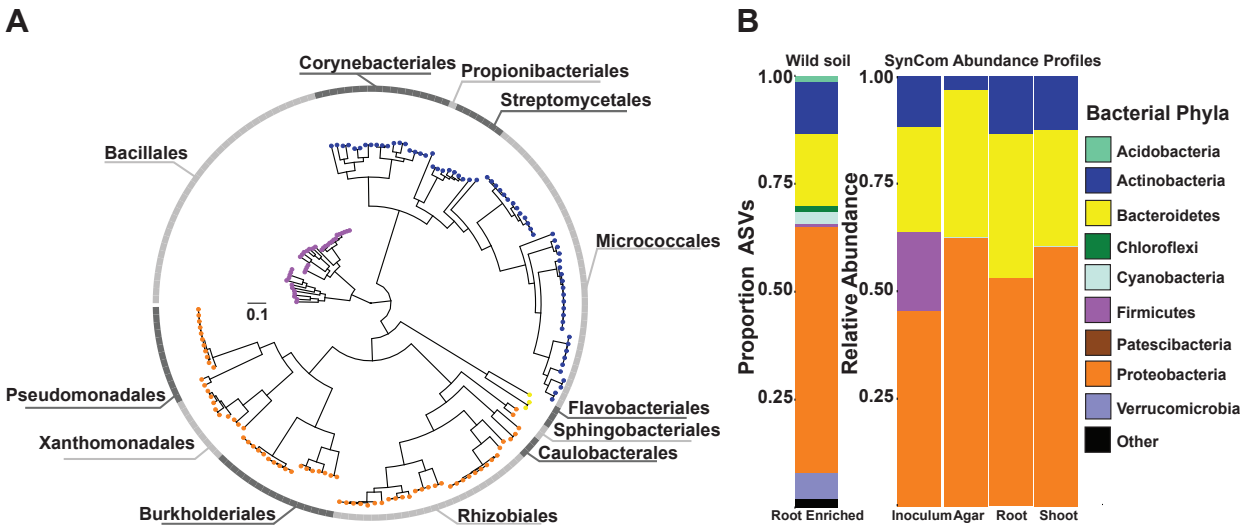

Fig. S1.

Synthetic community resembles the taxonomic makeup of natural communities. **(A)** Phylogenetic tree of 185 bacterial genomes included in the synthetic community (SynCom). The tree tips are colored according to phylum. The outer ring shows the distribution of the 12 distinct bacterial orders present in the SynCom. **(B)** The panel on the left (wild soil) shows the proportion of amplicon sequence variants (ASVs) enriched (q-value < 0.1) in the plant root in comparison to soil in a microbiota profiling study from the same soil that SynCom strains were isolated from (2). In the panel, ASVs are colored according to phylum. The panel on the right (SynCom panel) represents the relative abundance profiles of bacterial isolates across the initial inoculum, planted agar, root and shoot in plant exposed to the full SynCom. Bacterial isolates in the SynCom are colored based on their phylum.

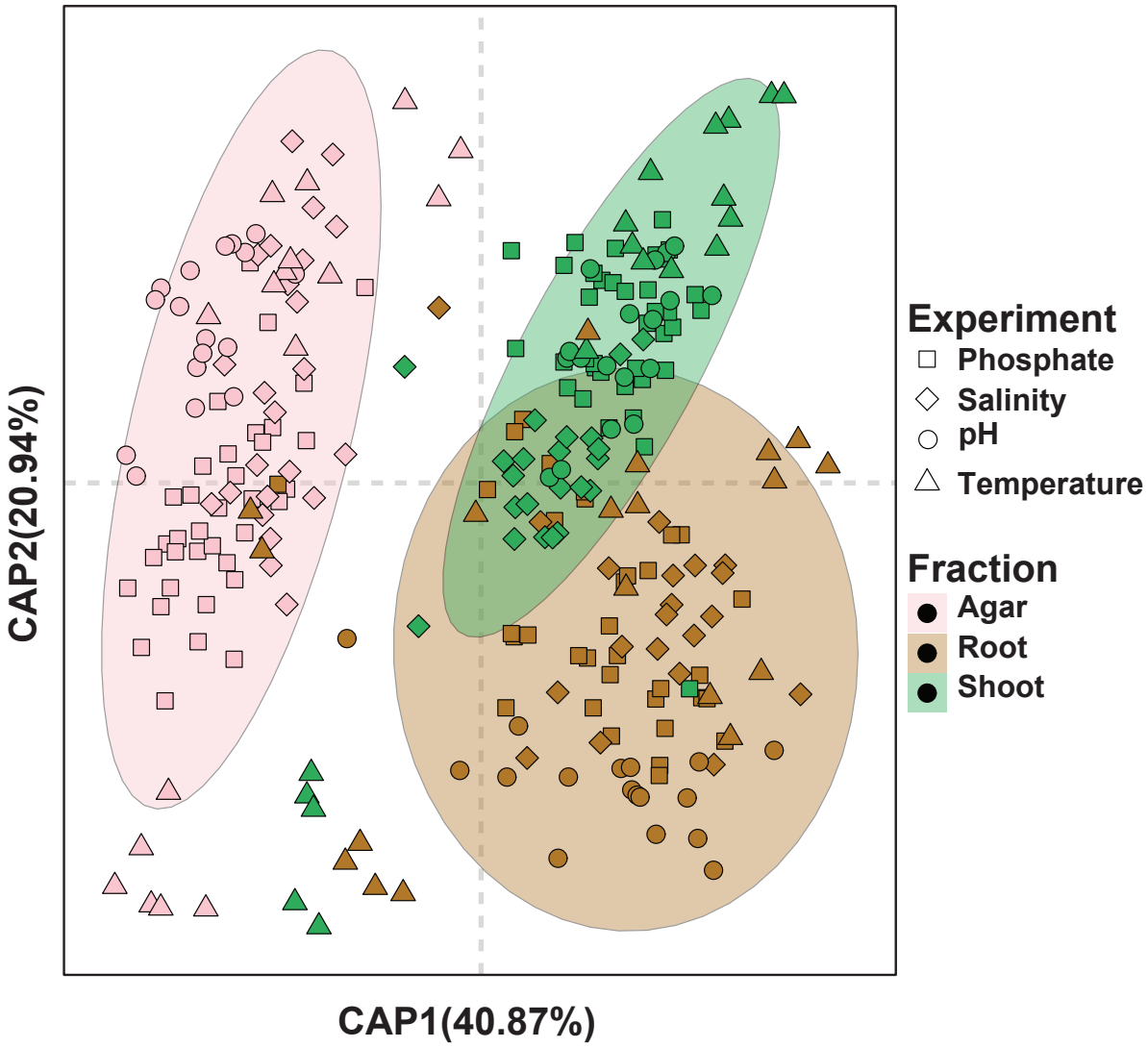

Fig. S2.
Plant microbiota assembly is robust to environmental variations.
Canonical analysis of principal coordinates showing the influence of the fraction (agar, root, shoot) on the assembly of the bacterial synthetic community across the four gradients used in this work (phosphate, salinity, pH, temperature). Different colors differentiate between the fractions and different shapes differentiate between experiments. Ellipses denote the 95% confidence level of each fraction.

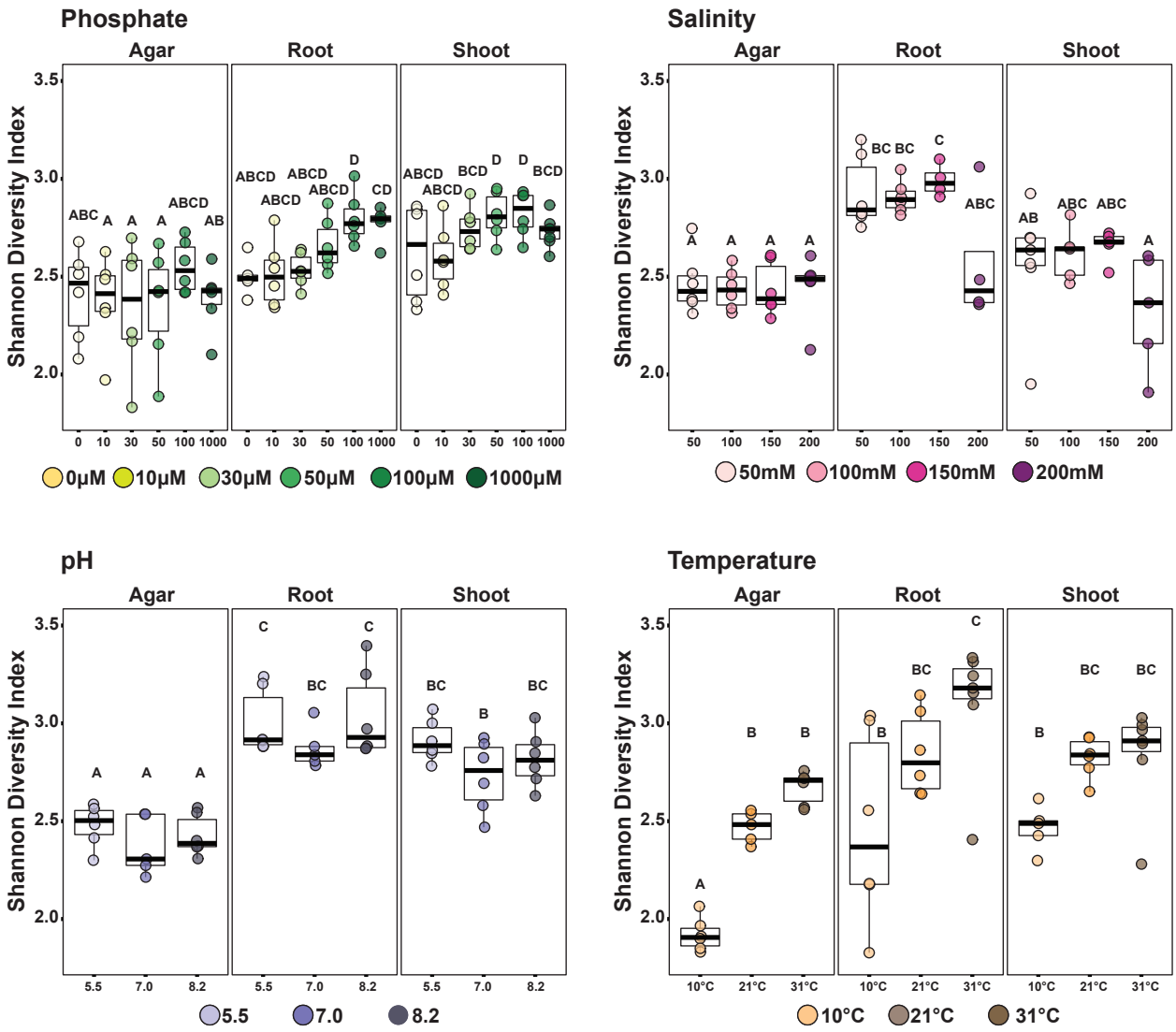

Fig. S3.

Abiotic conditions displayed reproducible effects on alpha-diversity.

Each panel represents the bacterial alpha-diversity across the different gradient conditions (phosphate, salinity, pH, temperature) and the fractions (agar, root, shoot) used in this work. Bacterial alpha-diversity was estimated using Shannon Diversity. Letters represent the results of the post hoc test of an ANOVA model testing the interaction between fraction and abiotic condition.

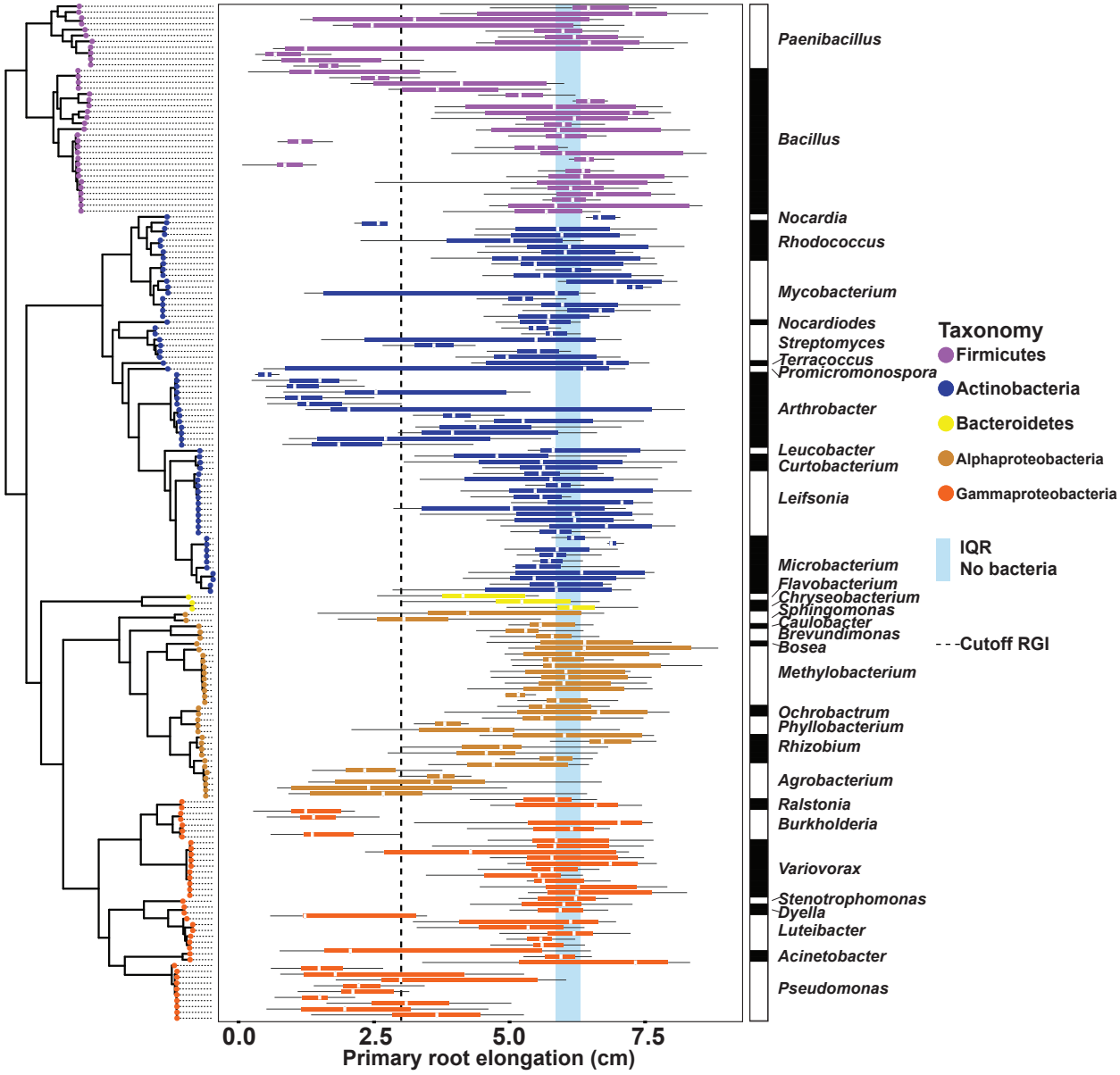

Fig. S4.

Root growth inhibition trait is distributed across bacterial phylogeny.

Primary root elongation of plants inoculated with single bacterial isolates (one boxplot per isolate). Isolates are ordered according to the phylogenetic tree on the left side of the panel and colored based on their genome-based taxonomy. The vertical blue strips across the panel corresponds to the interquartile range (IRQ) of plants grown in sterile conditions. The vertical dotted line represents the 3 cm cutoff used to classify strains as root growth inhibiting strains. The bar on the right side of the panel denotes the genus classification of each isolate.

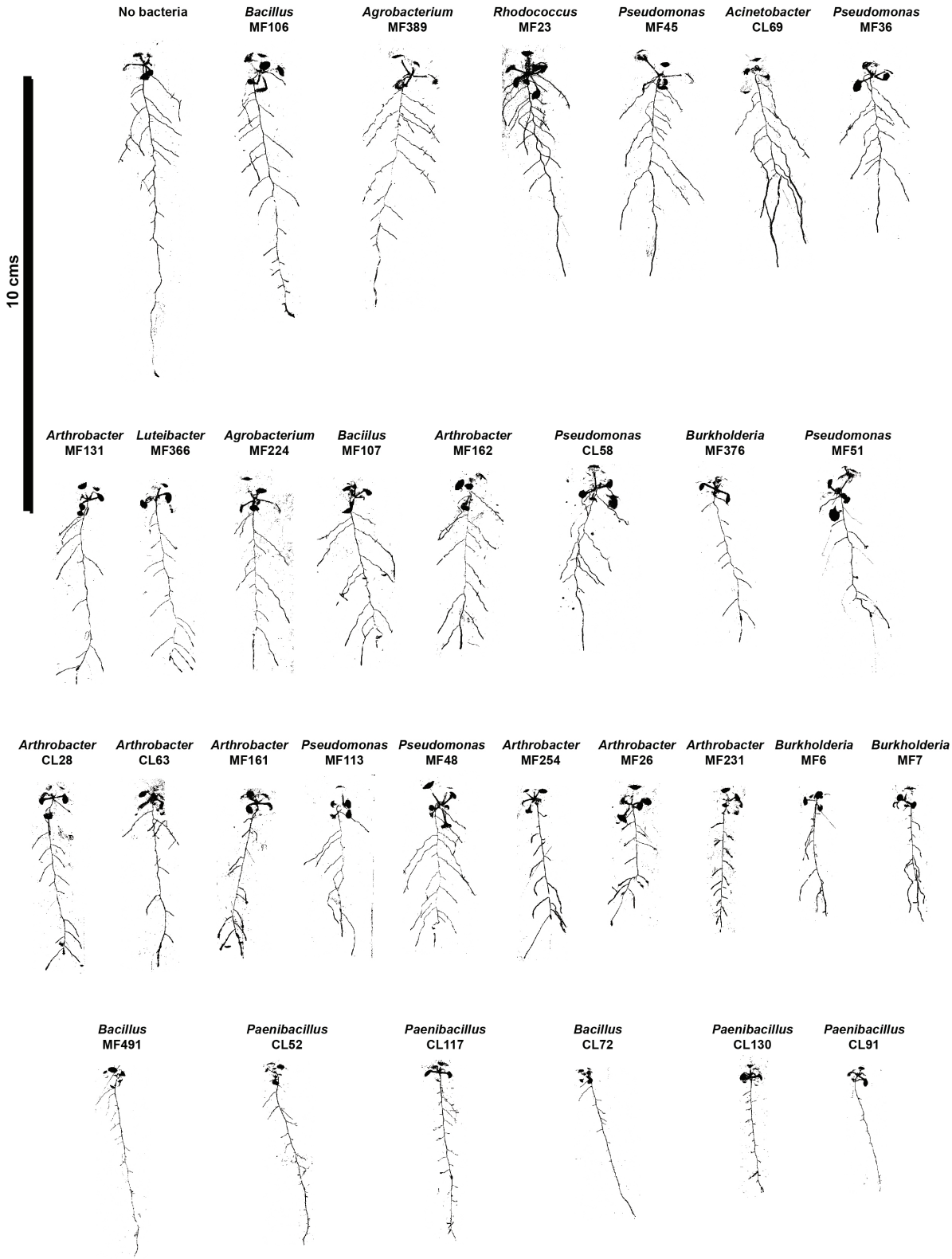

Fig. S5.

Root growth inhibition phenotypes comprise variable root architectures.

Binarized image of representative seedlings grown axenically (no bacteria) or with thirty-four root growth inhibiting (RGI) strains individually.

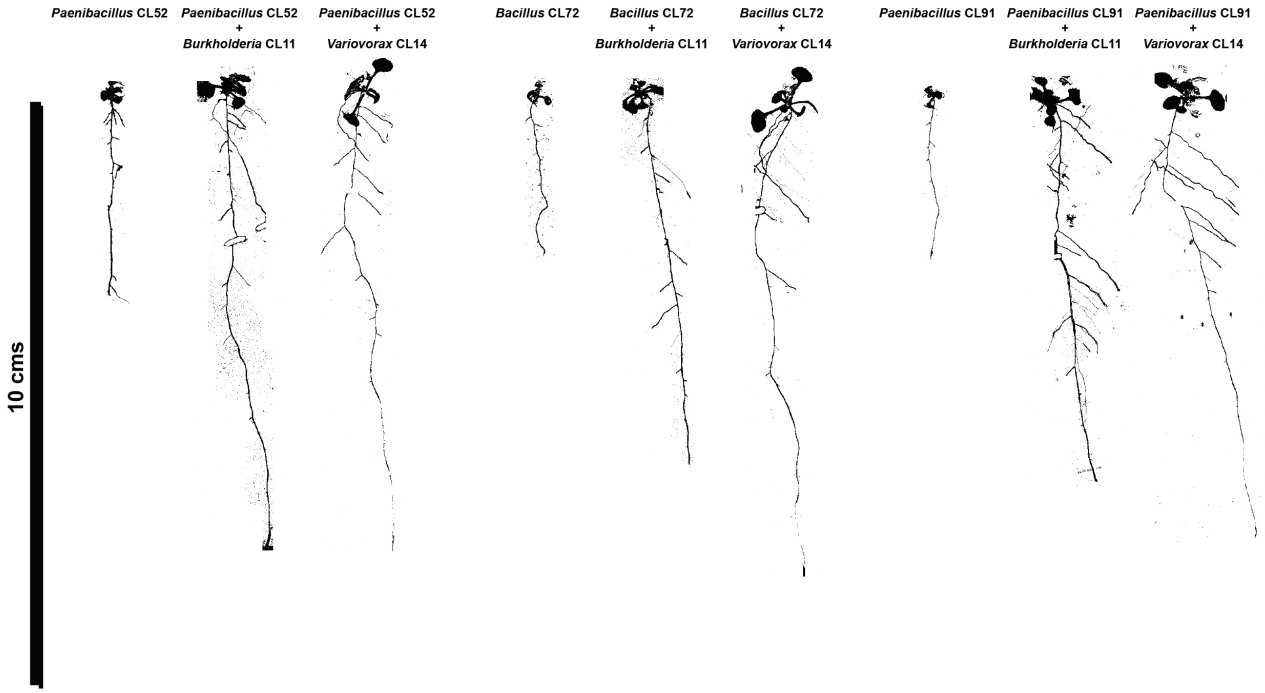

Fig. S6.
Example of strains that attenuate severe root and growth inhibition phenotypes. Binarized image of representative seedlings grown with three severely growth inhibiting isolates (*Paenibacillus* CL52, *Bacillus* CL72 and *Paenibacillus* CL91) individually or jointly with *Burkholderia* CL11 or *Variovorax* CL14.

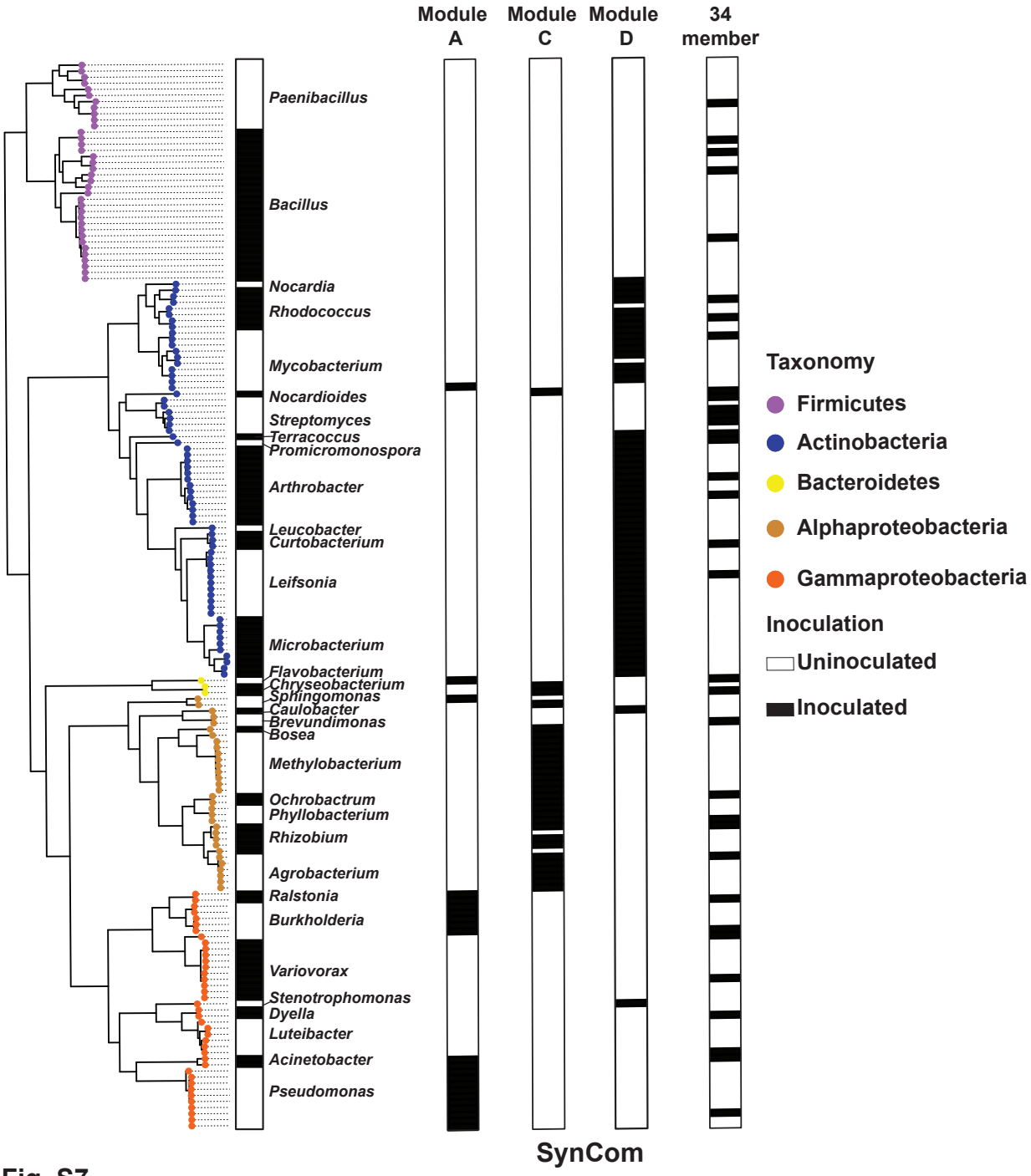

Fig. S7.

Taxonomic composition of the SynCom used in Figure 3D.

Bar graphs showing the isolate composition of SynComs composed by module A (Module A), module C (Module C), module D (Module D) and a 34-member synthetic community (34 member) (2). Isolates are ordered according to the phylogenetic tree on the left side of the panel. The tips of the phylogenetic tree are colored based on the genome-based taxonomy of each isolate. Presence of an isolate across the different SynComs is denoted by a black filled rectangle.

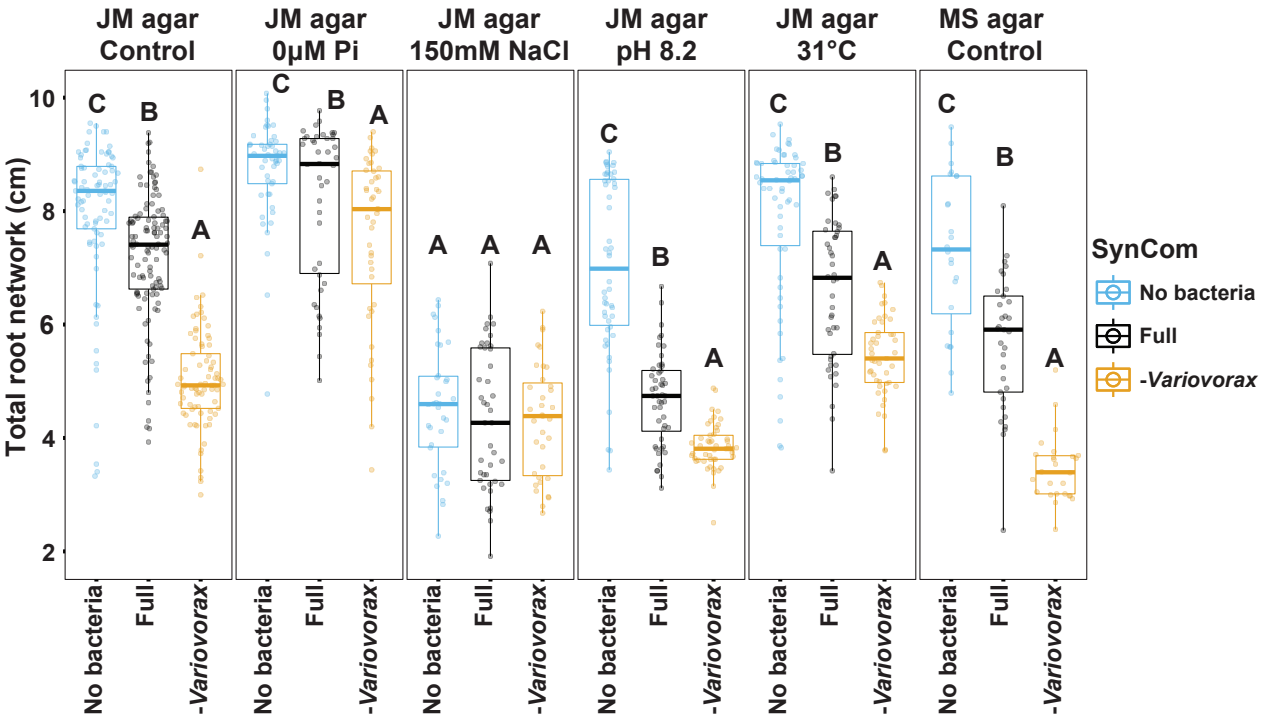

Fig. S8.
***Variovorax* increase total root network length.**
Total root network of seedlings grown axenically (No Bacteria), with the full SynCom (Full) or with the full SynCom excluding *Variovorax* (*-Variovorax)* across different abiotic conditions: full medium (JM agar control), phosphate starvation (JM agar 0 µM Pi), salt stress (JM agar 150 mM NaCl), high pH (JM agar pH 8.2) and high temperature (JM agar 31° C) and media: Johnson medium (JM agar control) and Murashige and Skoog (MS agar control) medium. Letters indicate statistical significance using ANOVA performed within each experimental condition.

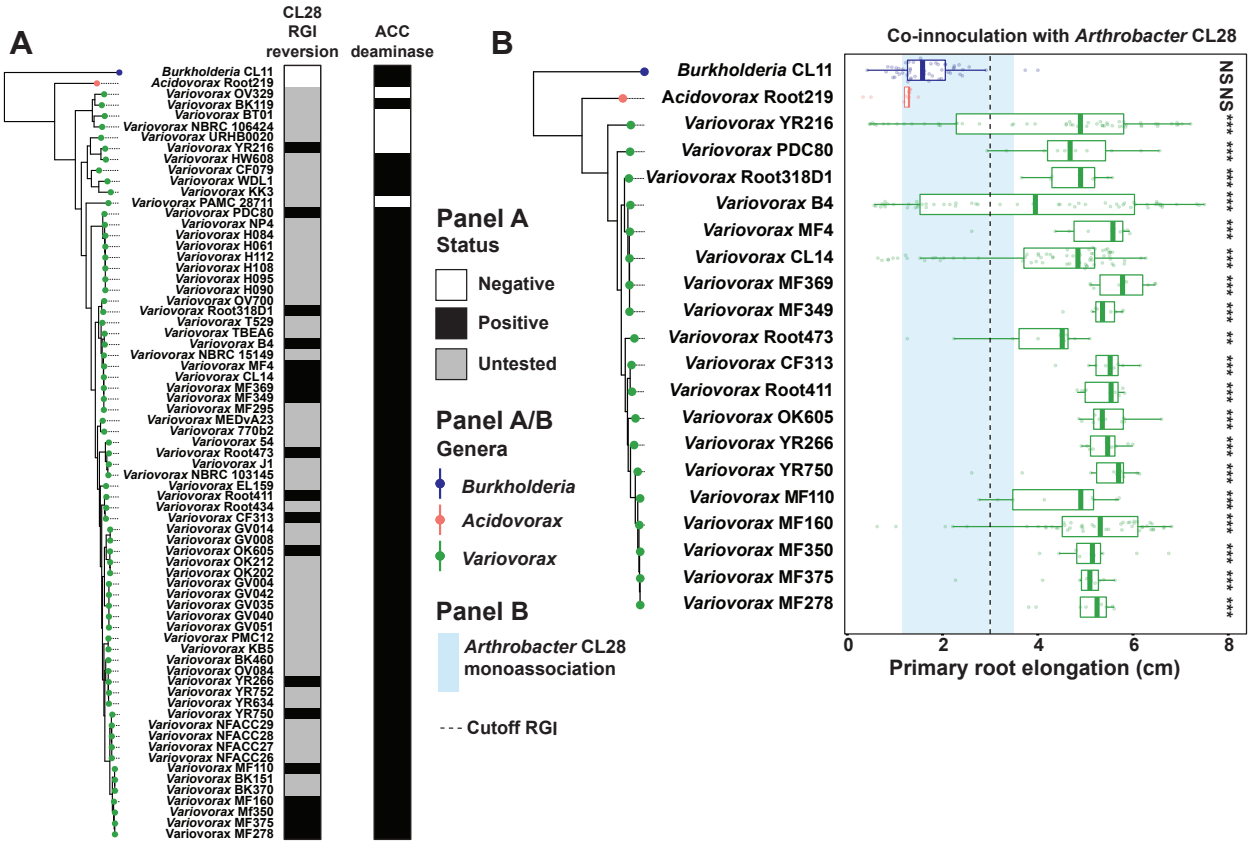

Fig. S9.

Reversion of root growth inhibition is prevalent across the *Variovorax* phylogeny. **(A)** Phylogenetic tree of 54 publicly available *Variovorax* genomes and two outgroup isolates, *Acidovorax* Root219 and *Burkholderia* CL11. The CL28 RGI reversion bar binarizes (positive, negative, untested) the ability of each isolate in the phylogeny to revert the root growth inhibition caused by *Arthrobacter* CL28. The ACC deaminase bar denotes the presence of the KEGG orthology term KO1505 (1-aminocyclopropane-1-carboxylate deaminase) in each of the genomes. **(B)** Phylogenetic tree of 19 *Variovorax* genomes along with two outgroup isolates, *Acidovorax* Root219 and *Burkholderia* CL11 that were tested for their ability to revert the root growth inhibition (RGI) imposed by *Arthrobacter* CL28. The blue vertical strip across the panel denotes the interquartile range of plants treated solely with *Arthrobacter* CL28. The dotted vertical line across the panel denotes the 3 cm cutoff used to classify a treatment as a root growth inhibitor (RGI). Each boxplot is colored according to the genus classification of each isolate. Statistical significance is denoted on the top of each boxplot.

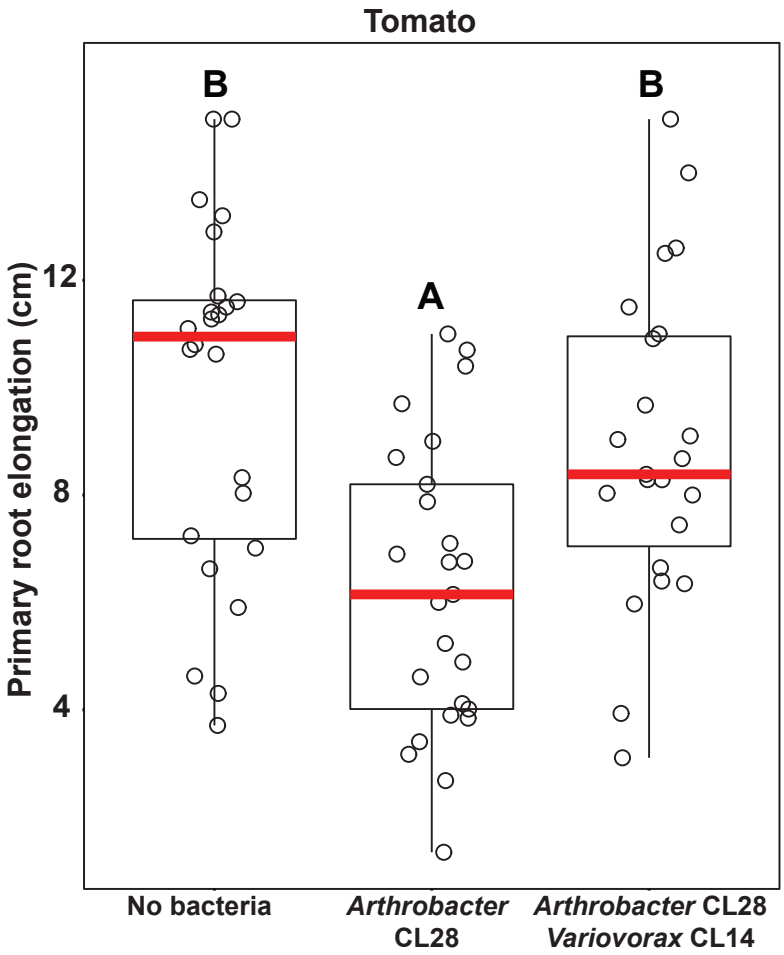

Fig S10.
Reversion of root growth inhibition in tomato seedlings
Primary root elongation of uninoculated seedlings (No bacteria) or seedlings inoculated with the *Arthrobacter* CL28 individually or along with *Variovorax* CL14. Letters indicate post hoc significance.

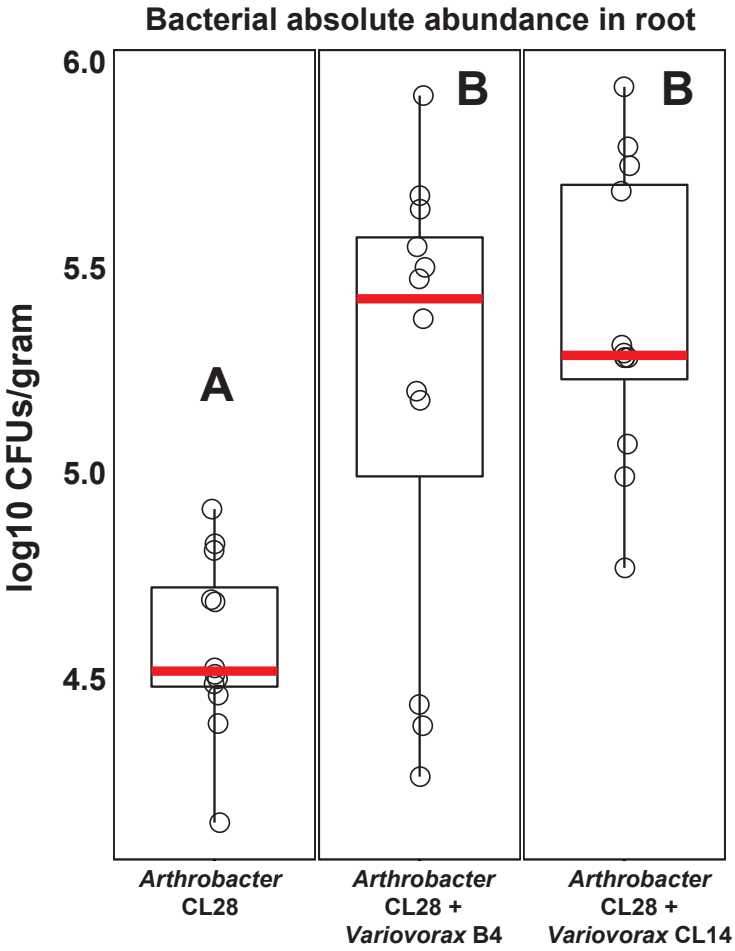

Fig. S11.
***Variovorax* does not inhibit the growth of an RGI strain in Arabidopsis roots.**
In planta colony forming units of *Arthrobacter* CL28 when inoculated alone or with two *Variovorax* representatives: *Variovorax* B4 and *Variovorax* CL14. Log-transformed-Colony forming units (CFU) of *Arthrobacter* CL28 normalized to root weight are shown. To selectively grow *Arthrobacter* CL28, CFUs were counted on Luria Bertani (LB) agar plates containing 50 µg/ml of Apramycin, on which neither *Variovorax* B4 and *Variovorax* CL14 grow.

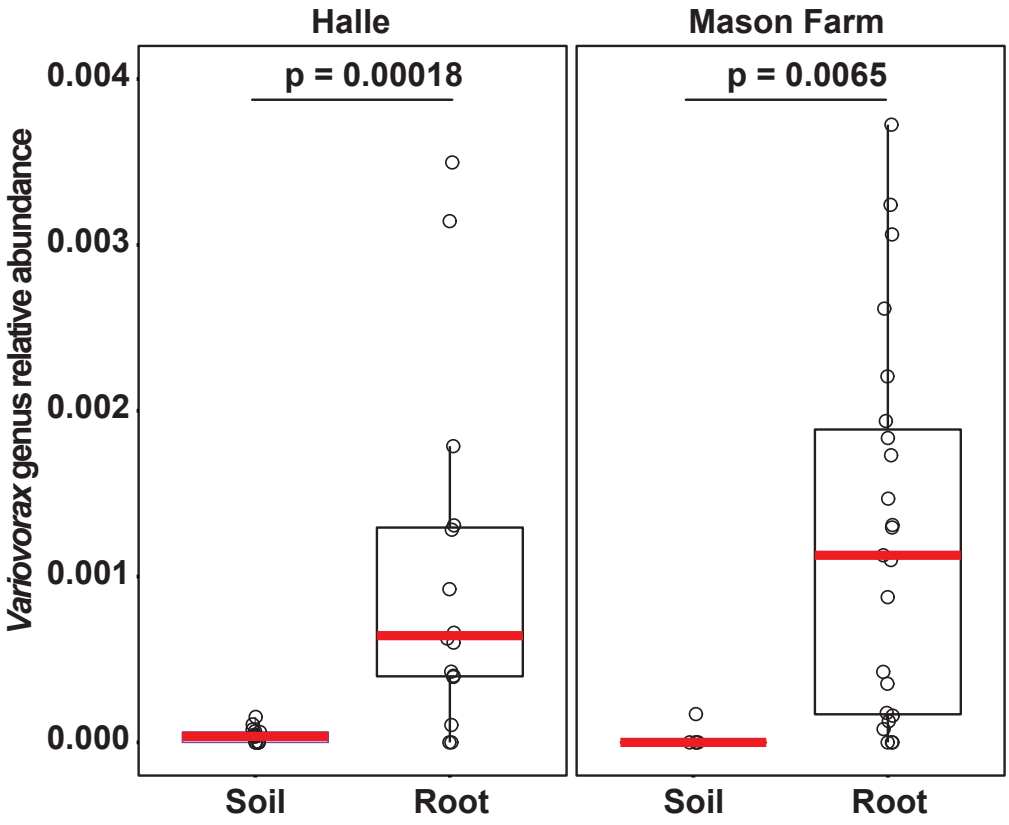

Fig. S12.
***Variovorax* is a root-enriched taxon.**
Cumulative relative abundance of Amplicon Sequence Variants (ASVs) classified as *Variovorax* across the soil and root fractions of *Arabidopsis thaliana* plants sampled across two independent studies (2,25). The cumulative abundance between fractions was compared using Mann-Whitney U test inside each of the two data sets independently.

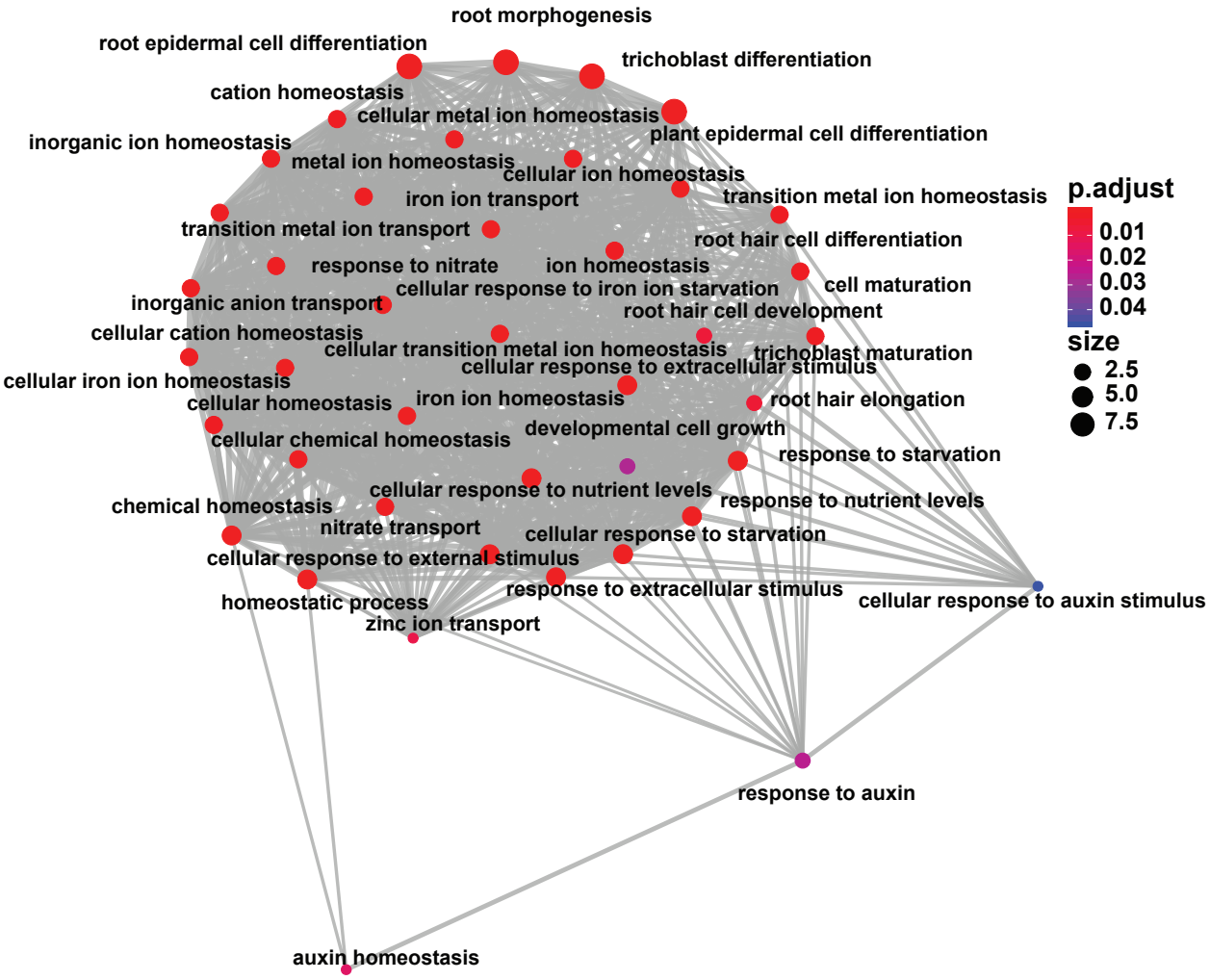

Fig. S13.

Root growth inhibition-related genes are interconnected.

Network of statistically significant gene ontology terms contained in the 18 genes cohesively overexpressed in RGI treatments in the tripartite and dropout systems. See Figure 4A and 4B. The network was computed using the emapplot function from the package clusterProfiler in R. A p-value for terms across the gene ontology was computed using a hypergeometric test, additionally the size of each point (Gene ontology term) denotes the number of genes mapped in that particular term.

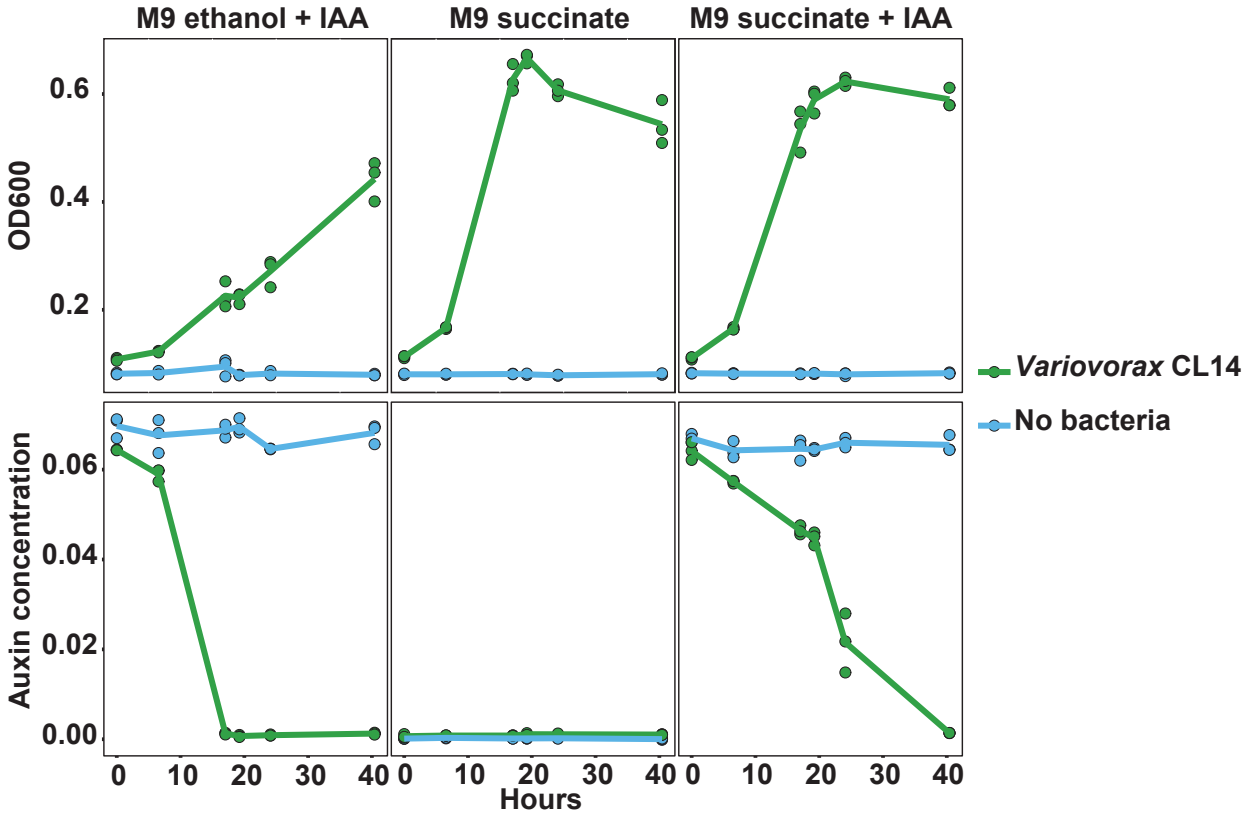

Fig. S14.
***Variovorax* degrades Indole-3-acetic acid.**
Growth curves showing optical density (OD600) (top) and Indole-3-acetic acid (IAA) concentrations (mg/mL) (bottom) in *Variovorax* CL14 cultures grown in M9 media with auxin (left), in M9 media with succinate (center) and M9 media with succinate and auxin (right).

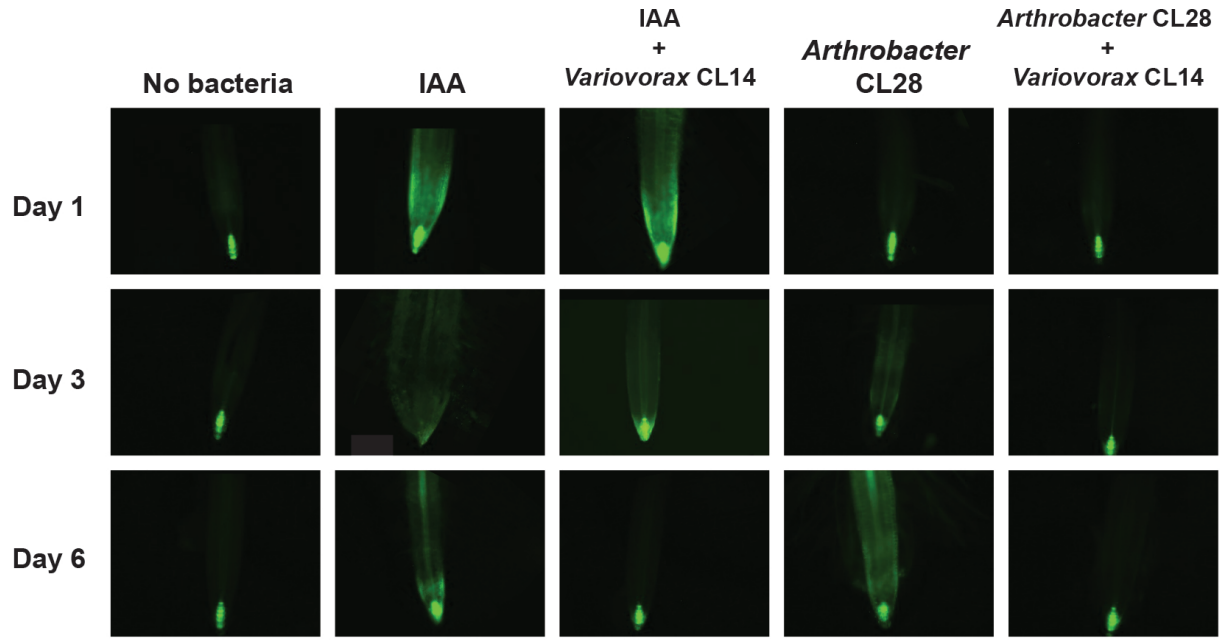

Fig. S15.
***Variovorax* quenches *DR5::GFP* induction.**
Main root tips of plants grown with different Indole-3-acetic acid (IAA) and bacterial treatments. *DR5::GFP* plants were treated with the tripartite system (*Arthrobacter* CL28, *Variovorax* CL14, CL28+CL14), IAA and IAA+*Variovorax* CL14, and GFP fluorescence was imaged 1, 3 and 6 days post inoculation. Fluorescence was quantified in the root elongation zone.

Materials and Methods

1. Arabidopsis with bacterial SynCom microcosm across four stress gradients (Fig. 1, fig. S2-S3, data S2)

a. Bacterial culture and plant-inoculation

The 185-member bacterial synthetic community (SynCom) used here contains genome-sequenced isolates obtained from *Brassicaceae* roots, nearly all *Arabidopsis thaliana*, planted in two North Carolina, US, soils. A detailed description of this collection and isolation procedures can be found in (*25*). One week prior to each experiment, bacteria were inoculated from glycerol stocks into 600 µL KB medium in a 96 deep well plate. Bacterial cultures were grown at 28 °C, shaking at 250 rpm. After five days of growth, cultures were inoculated into fresh media and returned to the incubator for an additional 48 hours, resulting in two copies of each culture, 7 days old and 48 hours old. We adopted this procedure to account for variable growth rates of different SynCom members and to ensure that non-stationary cells from each strain were included in the inoculum. After growth, 48-hour and 7-day plates were combined and optical density of cultures was measured at 600 nm (OD_{600}) using an Infinite M200 Pro plate reader (TECAN). All cultures were then pooled while normalizing the volume of each culture to OD_{600}=1. The mixed culture was washed twice with 10 mM $MgCl_2$ to remove spent media and cell debris and vortexed vigorously with sterile glass beads to break up aggregates. OD_{600} of the mixed, washed culture was then measured and normalized to OD_{600}=0.2. 100 µL of this SynCom inoculum was spread on 10 X 10 cm vertical square agar plates with amended Johnson medium (JM; (*2*)) without sucrose prior to transferring seedlings.

b. In vitro plant growth conditions

All seeds were surface-sterilized with 70% bleach, 0.2% Tween-20 for 8 min, and rinsed three times with sterile distilled water to eliminate any seed-borne microbes on the seed surface. Seeds were stratified at 4 °C in the dark for two days. Plants were germinated on vertical square 10 X 10 cm agar plates with JM containing 0.5% sucrose, for 7 days. Then, 10 plants were transferred to each of the SynCom-inoculated agar plates. The composition of JM in the agar plates was amended to produce environmental variation. We added to the previously reported phosphate concentration gradient (0, 10, 30, 50, 100, 1000 µm Pi) (*26*) three additional environmental gradients: Salinity (50, 100, 150, 200 mM NaCl), pH (5.5, 7.0, 8.2), Pi concentration and incubation temperature (10, 21, 31˚C). Each gradient was tested separately, in two independent replicas. Each condition included three SynCom+plant samples, two no plant controls and one no bacteria control. Plates were placed in randomized order in growth chambers and grown under a

16-h dark/8-h light regime at 21 °C day/18 °C night for 12 days. Upon harvest, DNA was extracted from roots, shoots and agar.

c. DNA extraction

Roots, shoots and agar were harvested separately, pooling 6-8 plants for each sample. Roots and shoots were placed in 2.0 ml Eppendorf tubes with three sterile glass beads. These samples were washed three times with sterile distilled water to remove agar particles and weakly associated microbes. Tubes were stored at -80 ˚C until processing. Root and shoot samples were lyophilized for 48 hours using a Labconco freeze dry system and pulverized using a tissue homogenizer (MPBio). Agar from each plate was stored in 30 ml syringes with a square of sterilized Miracloth (Millipore) at the bottom and kept at -20 °C for one week. Syringes were then thawed at room temperature and samples were squeezed gently through the Miracloth into 50 ml tubes. Samples were centrifuged at max speed for 20 min and most of the supernatant was discarded. The remaining 1-2 ml of supernatant, containing the pellet, was transferred into clean microfuge tubes. Samples were centrifuged again, supernatant was removed, and pellets were stored at -80 °C until DNA extraction. DNA extractions were carried out on ground root and shoot tissue and agar pellets using 96-well-format MoBio PowerSoil Kit (MOBIO Laboratories; Qiagen) following the manufacturer's instruction. Sample position in the DNA extraction plates was randomized, and this randomized distribution was maintained throughout library preparation and sequencing.

d. Bacterial 16S sequencing

We amplified the V3-V4 regions of the bacterial 16S rRNA gene using the primers 338F (5′-ACT CCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Two barcodes and six frameshifts were added to the 5' end of 338F and six frameshifts were added to the 806R primers, based on the protocol in (*27*). Each PCR reaction was performed in triplicate, and included a unique mixture of three frameshifted primer combinations for each plate. PCR conditions were as follows: 5 µl Kapa Enhancer, 5 µl Kapa Buffer A, 1.25 µl 5 µM 338F, 1.25 µl 5 µM 806R, 0.375 µl mixed chloroplast rRNA gene-blocking peptide nucleic acids (PNAs; 1:1 mix of 100 µM plastid PNA and 100 µM mitochondrial PNA (*27*)), 0.5 µl Kapa dNTPs, 0.2 µl Kapa Robust Taq, 8 µl dH2O, 5 µl DNA; temperature cycling: 95°C for 60 s, 24 cycles of 95°C for 15 s, 78°C (PNA) for 10 s, 50°C for 30 s, 72°C for 30 s, 4°C until use. Following PCR cleanup, the PCR product was indexed using 96 indexed 806R primers with the same reaction mix as above, and 9 cycles of the cycling conditions described in (*2*). PCR products were purified using AMPure XP

magnetic beads (Beckman Coulter) and quantified with a Qubit 2.0 fluorometer (Invitrogen). Amplicons were pooled in equal amounts and then diluted to 10 pM for sequencing. Sequencing was performed on an Illumina MiSeq instrument using a 600-cycle V3 chemistry kit. DNA sequence data for this experiment is available at the NCBI bioproject repository (accession PRJNA543313). The abundance matrix, metadata and taxonomy are available at https://github.com/isaisg/variovoraxRGI.

e. 16S amplicon sequence data processing

SynCom sequencing data were processed with MT-Toolbox (*28*). Usable read output from MT-Toolbox (that is, reads with 100% correct primer and primer sequences that successfully merged with their pair) were quality filtered using Sickle (*29*) by not allowing any window with Q-score under 20. The resulting sequences were globally aligned to a reference set of 16S rDNA sequences extracted from genome assemblies of SynCom members. For strains that did not have an intact 16S rDNA sequence in their assembly, we sequenced the 16S rRNA gene using Sanger sequencing. The reference database also included sequences from known bacterial contaminants and Arabidopsis organellar sequences. Sequence alignment was performed with USEARCH v7.1090 (30) with the option 'usearch_global' at a 98% identity threshold. On average, 85% of sequences matched an expected isolate. Our 185 isolates could not all be distinguished from each other based on the V3-V4 sequence and were thus classified into 97 unique sequences (USeqs). A USeq encompasses a set of identical (clustered at 100%) V3-V4 sequences coming from a single or multiple isolates.

Sequence mapping results were used to produce an isolate abundance table. The remaining unmapped sequences were clustered into Operational Taxonomic Units (OTUs) using UPARSE (*31*) implemented with USEARCH v7.1090, at 97% identity. Representative OTU sequences were taxonomically annotated with the RDP classifier (*32*) trained on the Greengenes database (*33*) (4 February 2011; Supplementary Data set 1). Matches to Arabidopsis organelles were discarded. The vast majority of the remaining unassigned OTUs belonged to the same families as isolates in the SynCom. We combined the assigned USeq and unassigned OTU count tables into a single count table. In addition to the raw count table, we created rarefied (1000 reads per sample) and relative abundance versions of the abundance matrix for further analyses.

The resulting abundance tables were processed and analyzed with functions from the ohchibi package (https://github.com/isaisg/ohchibi). An alpha diversity metric (Shannon diversity) was

calculated using the diversity function from the vegan package v2.5-3 (*34*). We used ANOVA to test for differences in alpha diversity between groups. Beta diversity analyses (Principal coordinate analysis, and canonical analysis of principal coordinates) were based on Bray-Curtis dissimilarity calculated from the relative abundance matrices. We used the capscale function from the vegan R package v.2.5-3 (*34*) to compute the canonical analysis of principal coordinates (CAP). To analyze the full dataset (all fraction, all abiotic treatments), we constrained by fraction and abiotic treatment while conditioning for the replica and experiment effect. We explored the abiotic conditions effect inside each of the four abiotic gradients tested (phosphate, salinity, pH and temperature). We performed the Fraction:abiotic interaction analysis within each fraction independently, constraining for the abiotic conditions while conditioning for the replica effect. In addition to CAP, we performed Permutational Multivariate Analysis of Variance (PERMANOVA) using the adonis function from the vegan package v2.5-3 (*34*). We used the package DESeq2 v1.22.1 (*35*) to compute the enrichment profiles for USeqs present in the count table.

We estimated the fraction effect across all the abiotic conditions tested by creating a group variable that merged the fraction variable and the abiotic condition variable together (e.g Root_0Pi, Agar_0Pi). We fitted the following model specification using this group variable:

Abundance ~ Rep + Experiment + group

From the fitted model, we extracted, for all levels within the group variables, the following comparisons: Agar vs Root and Agar vs Shoot. A USeq was considered statistically significant if it had a false discovery rate (FDR) adjusted *p*-value < 0.05.

All scripts and dataset objects necessary to reproduce the synthetic community analyses are deposited in the following github repository: https://github.com/isaisg/variovoraxRGI

f. Co-occurrence analysis

The relative abundance matrix (USeqs X Samples) was standardized across the USeqs by dividing the abundance of each USeq in its sample over the mean abundance of that USeq across all samples. Subsequently, we created a dissimilarity matrix based on the Pearson correlation coefficient between all the pairs of strains in the transformed abundance matrix, using the cor function in the stats base package in R. Finally, hierarchichal clustering (method ward.D2, function hclust) was applied over the dissimilarity matrix constructed above.

g. Heatmap and family enrichment analysis

We visualized the results of the GLM model testing the fraction effects across each specific abiotic condition tested using a heatmap. The rows in the heatmap were ordered according to the dendrogram order obtained from the USeqs co-occurrence analysis. The heatmap was colored based on the log2FoldChange output by the GLM model. We highlighted in a black shade the comparisons that were significant (q-value < 0.05). Finally, for each of the four modules we computed for each family present in that module a hypergeometric test testing if that family was overrepresented (enriched) in that particular module. Families whose FDR p-value < 0.1 were visualized in the figure.

2. Deconstructing the SynCom to four modules of co-occurring strains (Fig. 2A, 2C and data S3).

a. Bacterial culture and plant-inoculation

Strains belonging to each module (A, B, C and D, Materials and Methods 1f) were grown in separate deep 96-well plates and mixed as described above (Materials and Methods 1a). The concentration of each module was adjusted to OD=0.05 (1/4 of the concentration of the full SynCom). Each module was spread on the plates either separately, or in combination with another module. In addition, we included a full SynCom control and an uninoculated control, bringing the number of SynCom combinations to 12. We performed the experiment in two independent replicates and each replicate included five plates per SynCom combination.

b. In vitro plant growth conditions

Seed sterilization and germination conditions were the same as Materials and Methods 1b. Plants were transferred to each of the SynCom-inoculated agar plates containing JM without sucrose. Plates were placed in randomized order in growth chambers and grown under a 16-h dark/8-h light regime at 21 °C day/18 °C night for 12 days. Upon harvest, root morphology was measured.

c. Root and shoot image analysis

Plates were imaged twelve days post-transferring, using a document scanner. Primary root length elongation was measured using ImageJ (*36*) and shoot area and total root network were measured with WinRhizo software (Regens Instruments Inc.).

d. Primary root elongation analyses

Primary root elongation was compared across the No Bacteria, full SynCom, single modules and pairs of modules treatments jointly using an ANOVA model controlling for the replicate effect.

366 Differences between treatments were indicated using the confidence letter display (CLD) derived
367 from the Tukey's *post hoc* test implemented in the package emmeans (*37*).
368
369
370 **3. Inoculating plants with all SynCom isolates separately (Fig. 2B, fig. S4 and data S4)**
371 a. Bacterial culture and plant-inoculation.
372 Cultures from each strain in the SynCom were grown in KB medium and washed separately, and
373 $OD_{600}$was adjusted to 0.01 before spreading. We performed the experiment in two independent
374 replicates and each replicate included one plate per each of the 185 strains. In vitro growth
375 conditions were the same as in Materials and Methods 2b. Upon harvest, root morphology was
376 measured (Materials and Methods 2c). Isolates generating an average main root elongation of <3
377 cm were classified as RGI-inducing strains.
378
379 **4. Tripartite plant-microbe-microbe experiments (Fig. 2D-F and data S5-S6)**
380 a. Experimental design
381 To identify strains that revert RGI (Fig. 2D and data S5), we selected all 18 non-RGI inducing
382 strains in module A and co-inoculated them with each of four RGI inducing strains, one from each
383 module. The experiment also included uninoculated controls and controls consisting of each of
384 the 22 strains inoculated alone, amounting to 95 separate bacterial combinations.
385
386 To confirm the ability of *Variovorax* and *Burkholderia* to attenuate RGI induced by diverse bacteria
387 (Fig. 2E and data S6), three RGI attenuating strains were co-inoculated with a selection of 18 RGI
388 inducing strains. The experiment also included uninoculated controls and controls consisting of
389 each of the 21 strains inoculated alone. Thus, the experiment consisted of 76 separate bacterial
390 combinations. We performed each of these two experiments in two independent replicates and
391 each replicate included one plate per each of the strain combinations.
392
393 b. Bacterial culture and plant-inoculation
394 All strains were grown in separate tubes, then washed, and OD_{600} was adjusted to 0.02 before
395 mixing and spreading. In vitro growth conditions were the same as in Materials and Methods 2b.
396 Upon harvest, root morphology was measured (Materials and Methods 2c) and plant RNA was
397 harvested and processed from uninoculated samples, and from samples with *Variovorax* CL14,
398 *Arthrobacter* CL28 and the combination of both (Materials and Methods 4d).
399

c. Primary root elongation analysis.

We fitted ANOVA models for each RGI-inducing strain tested. Each model compared the primary root elongation with the RGI inducing strains alone against root elongation when the RGI inducing strain was co-inoculated with other isolates. The *p*-values for all the comparisons were corrected for multiple testing using false discovery rate.

d. RNA extraction

RNA was extracted from *A*. *thaliana* seedlings following (*38*). Four seedlings were harvested from each sample and samples were flash frozen and stored at -80 ˚C until processing. Frozen seedlings were ground in liquid nitrogen, then homogenized in a buffer containing 400 µl of Z6-buffer; 8 M guanidine HCl, 20 mM MES, 20 mM EDTA at pH 7.0. 400 µL phenol:chloroform:isoamylalcohol, 25:24:1 was added, and samples were vortexed and centrifuged (20,000 g, 10 minutes) for phase separation. The aqueous phase was transferred to a new 1.5 ml tube and 0.05 volumes of 1 N acetic acid and 0.7 volumes 96% ethanol were added. The RNA was precipitated at -20 °C overnight. Following centrifugation (20,000 g, 10 minutes, 4°C), the pellet was washed with 200 µl sodium acetate (pH 5.2) and 70% ethanol. The RNA was dried and dissolved in 30 µL of ultrapure water and stored at -80 °C until use.

e. Plant RNA sequencing

Illumina-based mRNA-Seq libraries were prepared from 1 µg RNA following (*4*). mRNA was purified from total RNA using Sera-mag oligo(dT) magnetic beads (GE Healthcare Life Sciences) and then fragmented in the presence of divalent cations (Mg^{2+}) at 94°C for 6 minutes. The resulting fragmented mRNA was used for first-strand cDNA synthesis using random hexamers and reverse transcriptase, followed by second-strand cDNA synthesis using DNA Polymerase I and RNAseH. Double-stranded cDNA was end-repaired using T4 DNA polymerase, T4 polynucleotide kinase, and Klenow polymerase. The DNA fragments were then adenylated using Klenow exo-polymerase to allow the ligation of Illumina Truseq HT adapters (D501–D508 and D701–D712). All enzymes were purchased from Enzymatics. Following library preparation, quality control and quantification were performed using a 2100 Bioanalyzer instrument (Agilent) and the Quant-iT PicoGreen dsDNA Reagent (Invitrogen), respectively. Libraries were sequenced using Illumina HiSeq4000 sequencers to generate 50-bp single-end reads.

f. RNA-Seq read processing

Initial quality assessment of the Illumina RNA-Seq reads was performed using FastQC v0.11.7

(*39*). Trimmomatic v0.36 (*40*) was used to identify and discard reads containing the Illumina adaptor sequence. The resulting high-quality reads were then mapped against the TAIR10 Arabidopsis reference genome using HISAT2 v2.1.0 (*41*) with default parameters. The featureCounts function from the Subread package (*42*) was then used to count reads that mapped to each one of the 27,206 nuclear protein-coding genes. Evaluation of the results of each step of the analysis was performed using MultiQC v1.1 (*43*). Raw sequencing data and read counts are available at the NCBI Gene Expression Omnibus accession number GSE131158.

5. *Variovorax* drop-out experiment (Fig. 3A-C and data S7)

a. Bacterial culture and plant-inoculation.

The entire SynCom, excluding all 10 *Variovorax* isolates and all five *Burkholderia* isolates was grown and prepared as described above (Materials and Methods 1a). The *Variovorax* and *Burkholderia* isolates were grown in separate tubes, washed and added to the rest of the SynCom to a final OD_{600} of 0.001 (the calculated OD_{600} of each individual strain in a 185-Member SynCom at a total of OD_{600} of 0.2), to form the following five mixtures: (i) Full community – all *Variovorax* and *Burkholderia* isolates added to the SynCom; (ii) *Burkholderia* drop-out – only *Variovorax* isolates added to the SynCom; (iii) *Variovorax* drop-out – only *Burkholderia* isolates added to the SynCom; (iv) *Variovorax* and *Burkholderia* drop-out – no isolates added to the SynCom; (v) Uninoculated plants – no SynCom. The experiment consisted of six plates per SynCom mixture, amounting to 30 plates. Upon harvest, root morphology was measured and analyzed (Materials and Methods 1c,4c); and Bacterial DNA (Materials and Methods 1d) and plant RNA (Materials and Methods 4d-e) were harvested and processed.

6. *Variovorax* drop-out under varying abiotic contexts (Fig. 3E and data S7)

a. Bacterial culture and plant-inoculation.

The composition of JM in the agar plates was amended to produce abiotic environmental variation. These amendments included salt stress (150 mM NaCl), low Phosphate (10 µm Phosphate), high pH (pH 8.2) and high temperature (plates incubated at 31 ˚C), as well as an un-amended JM control. Additionally, we tested a different media (1/2-strength Murashige and Skoog [MS]) and a soil-like substrate. As a soil-like substrate, we used calcined clay (Diamond Pro), prepared as follows: 100 ml of clay was placed in Magenta GA7 vessels. The vessels were then autoclaved twice. 40 ml of liquid JM was added to the vessels, with the corresponding bacterial mixture spiked into the media at a final OD_{600} of 5E-4. Four 1-week old seedlings were transferred

to each vessel, and vessels were covered with Breath-Easy gas permeable sealing membrane (Research Products International) to maintain sterility and gas exchange.

The entire SynCom, excluding all 10 *Variovorax* isolates was grown and prepared as described above (Materials and Methods 1a). The *Variovorax* isolates were grown in separate tubes, washed and added to the rest of the SynCom to a final OD_{600} of 0.001 (the calculated OD_{600} of each individual strain in a 185-Member SynCom at an OD_{600} of 0.2), to form the following five mixtures: (i) Full community – all *Variovorax* isolates added to the SynCom; (ii) *Variovorax* drop-out – no isolates added to the SynCom; (iii) Uninoculated plants – no SynCom.

We inoculated all three SynCom combinations in all seven abiotic treatments, amounting to 21 experimental conditions. We performed the experiment in two independent replicates and each replicate included three plates per experimental conditions, amounting to 63 plates per replicate. Upon harvest, root morphology was measured (Materials and Methods 2c); and Bacterial DNA (Materials and Methods 1c-e) and plant RNA (Materials and Methods 4d-f) were harvested and processed, excluding the clay treatment.

b. Root image analysis

For agar plates, roots were imaged as described above (Materials and Methods 2c). For calcined clay pots, four weeks post-transferring, pots were inverted, and whole root systems were gently separated from the clay by washing with water. Root systems were spread over an empty petri dish and scanned using a document scanner.

c. Primary root elongation and total root network analysis.

Primary root elongation was compared between SynCom treatments within each of the different abiotic contexts tested independently. Differences between treatments were indicated using the confidence letter display (CLD) derived from the Tukey's *post hoc* test implemented in the package emmeans.

d. Bacterial 16S data analysis

To be able to compare shifts in the community composition of samples treated with and without the *Variovorax* genus, we *in silico* removed the 10 *Variovorax* isolates from the count table of samples inoculated with the Full community treatment. We then merged this count table with the count table constructed from samples inoculated without the *Variovorax* genus (*Variovorax* drop-

out treatment). Then, we calculated a relative abundance of each USeq across all the samples using the merged count matrix. Finally, we applied Canonical Analysis of Principal Coordinates (CAP) over the merged relative abundance matrix to control for the replica effect. In addition, we utilized the function adonis from the vegan R package to compute a PERMANOVA test over the merged relative abundance matrix and we fitted a model evaluating the fraction and SynCom (presence of *Variovorax*) effects over the assembly of the community.

7. *Variovorax* drop-out under varying biotic contexts (Fig. 3D, fig S7 and data S7)

a. Bacterial culture and plant-inoculation.

Strains belonging to modules A (excluding *Variovorax*), C and D were grown in separate wells in deep 96-well plates and mixed as described above (Materials and Methods 1a). The concentration of each module was adjusted to OD=0.05 (1/4 of the concentration of the full SynCom). The *Variovorax* isolates were grown in separate tubes, washed and added to the rest of the SynCom to a final OD_{600} of 0.001.

In a separate experiment, the 35-member SynCom used in (*2*) was grown, excluding *Variovorax* CL14, to create a taxonomically diverse, *Variovorax*-free subset of the full 185 community. The concentration of this SynCom was adjusted to OD_{600}=0.05. The *Variovorax* isolates were grown in separate tubes, washed and added to the rest of the SynCom to a final OD_{600} of 0.001.

These two experiments included the following mixtures (fig S7 and data S7): (i) Module A excluding *Variovorax*; (ii) Module C; (iii) Module D; (iv) Module A including *Variovorax*; (v) Module C + all 10 *Variovorax*; (vi) Module D + all 10 *Variovorax*; (vii) 35-member SynCom excluding *Variovorax*; (viii) 34-member SynCom + all 10 *Variovorax*; (ix) uninoculated control. The experiment with modules A, C and D was performed in two independent experiments, with two plates per treatment in each. The experiment with the 34-member SynCom was performed once, with 5 plates per treatment. Upon harvest, root morphology was measured (Materials and Methods 2c).

b. Primary root elongation analysis.

We directly compared differences between the full SynCom and *Variovorax* drop-out treatment using a *t*-test and adjusting the *p*-values for multiple testing using false discovery rate.

8. Phylogenetic inference of the SynCom and *Variovorax* Isolates (Fig 2A, fig. S1A, S4, S7

and S9A-B)

To build the phylogenetic tree of the SynCom isolates, we used the super matrix approach previously described in (*25*). We scanned 120 previously defined marker genes across the 185 isolate genomes from the SynCom utilizing the hmmsearch tool from the hmmer v3.1b2 (*44*). Then, we selected 47 markers that were present as single copy genes in 100% of our isolates. Next, we aligned each individual marker using MAFFT (*45*) and filtered low quality columns in the alignment using trimAl (*46*). Then, we concatenated all filtered alignments into a super alignment. Finally, FastTree v2.1 (*47*) was used to infer the phylogeny utilizing the WAG model of evolution. For the *Variovorax* relative's tree, we chose 56 markers present as single copy across 124 Burkholderiales isolates and implemented the same methodology described above.

9. Measuring how prevalent is the RGI attenuation trait across the *Variovorax* phylogeny (fig. S9A-B, data S1 and data S8)

a. Bacterial culture and plant-inoculation.

Fifteen *Variovorax* strains from across the genus' phylogeny were each co-inoculated with the RGI inducer *Arthrobacter* CL28. All 16 strains were grown in separate tubes, then washed, and $OD_{600}$was adjusted to 0.01 before mixing. Pairs of strains were mixed in 1:1 ratios and spread onto agar prior to seedling transfer. The experiment also included uninoculated controls and controls consisting of each of the 16 strains inoculated alone. Thus, the experiment consisted of 32 separate bacterial combinations. We performed the experiment one time, which included 3 plates per bacterial combination. Upon harvest, root morphology was measured (Materials and Methods 2c). Primary root elongation was analyzed as described above (Materials and Methods 4c).

10. Measuring root growth inhibition in tomato seedlings (fig. S10 and data S9)

a. Experimental design

This experiment included the following treatments: (i) No bacteria, (ii) *Arthrobacter* CL28, (iii) *Variovorax* CL14 and (iv) *Arthrobacter* CL28 + *Variovorax* CL14. Each treatment was repeated in three separate agar plates with five tomato seedlings per plate. The experiment was repeated in two independent replicates.

b. Bacterial culture and plant-inoculation

All strains were grown in separate tubes, then washed, and OD_{600} was adjusted to 0.01 before mixing and spreading. 400 µL of each bacterial treatment was spread on 20 X 20 agar plates containing JM agar with no sucrose.

c. In vitro plant growth conditions

We used Heinz 1706 seeds. All seeds were soaked in sterile distilled water for 15 min, then surface-sterilized with 70% bleach, 0.2% Tween-20 for 15 min, and rinsed five times with sterile distilled water to eliminate any seed-borne microbes on the seed surface. Seeds were stratified at 4 °C in the dark for two days. Plants were germinated on vertical square 10 X 10 cm agar plates with JM containing 0.5% sucrose, for 7 days. Then, 5 plants were transferred to each of the SynCom-inoculated agar plates. Upon harvest, root morphology was measured. (Materials and Methods 2c).

c. Primary root elongation analysis.

Differences between treatments were indicated using the confidence letter display (CLD) derived from the Tukey's *post hoc* test from an ANOVA model.

11. Enumeration of *Arthrobacter* CL28 colony forming units from roots (fig. S11 and data S10)

Arabidopsis seedlings were inoculated with (i) *Arthrobacter* CL28 alone, (ii) *Arthrobacter* CL28 + *Variovorax* CL14 or (iii) *Arthrobacter* CL28 + *Variovorax* B4, as described above (Material and Methods 4b). Each bacterial treatment included four separate plates, with nine seedlings in each plate. Upon harvest, all seedlings were placed in pre-weighed 2.0 ml Eppendorf tubes containing three glass beads, three seedlings per tube (producing 12 data points per treatment). Roots were weighed, then crushed using a bead beater. The resulting suspension was plated on Luria Bertani agar plates containing 50 µg/ml of Apramycin, in a dilution series, and colonies were counting after incubation of 48 hours at 28˚ C.

12. RNA-Seq analysis (Fig. 4A-C, fig. S13 and data S11-12)

a. Detection of RGI-induced genes (Fig. 4A-B)

To measure the transcriptional response of the plant to the different SynCom combinations, we used the R package DESeq2 v.1.22.1 (*35*). The raw count genes matrixes for the dropout and

tripartite experiments were used independently to define differentially expressed genes (DEGs). For the analysis of both experiments we fitted the following model specification:

Abundance Gene ~ SynCom

From the fitted models we derived the following contrasts to obtain differentially expressed genes (DEGs). A gene was considered differentially expressed if it had a *q*-value < 0.1. For the tripartite system (Materials and Methods 4), we performed the following contrasts: *Arthrobacter* CL28 vs No Bacteria (NB) and *Arthrobacter* CL28 vs *Arthrobacter* CL28 co-inoculated with *Variovorax* CL14. The logic behind these two contrasts was to identify genes that were induced in RGI plants (*Arthrobacter* CL28 vs NB) AND repressed by *Variovorax* CL14. For the dropout system (Materials and Methods 5), we performed the following contrasts, *Variovorax* drop-out vs NB, and *Variovorax* drop-out vs full SynCom. The logic behind these two contrasts was identical to the tripartite system: to identify genes that are associated with the RGI phenotype (*Variovorax* drop-out vs NB contrast) AND repressed when *Variovorax* are present (*Variovorax* drop-out vs full SynCom contrast).

For visualization purposes, we applied a variance stabilizing transformation to the raw count gene matrix. We then standardized each gene expression (z-score) along the samples. We subset DEGs from this standardized matrix and calculated the mean z-score expression value for each SynCom treatment.

To identify the tissue specific expression profile of the 18 intersecting genes between the tripartite and dropout systems, we downloaded the spatial expression profile of each gene from the Klepikova atlas (*14*) using the Bio-analytic resource of plant biology platform. Then, we constructed a spatial expression matrix of the 18 genes and computed pairwise Pearson correlation between all pairs of genes. Finally, we applied hierarchical clustering to this correlation matrix.

b. Comparison with acute auxin response dataset (Figure 4C)

We applied a variance stabilizing transformation to the raw count gene matrix. We then standardized each gene expression (z-score) along the samples. From this matrix, we subset 12 genes that in a previous study (*15*) exhibited the highest fold change between auxin treated and untreated samples. Finally, we calculated the mean z-score expression value of each of these 12 genes across the SynCom treatments. We estimated the statistical significance of the trend of these 12 genes between a pair of SynCom treatments (Full SynCom vs *Variovorax* drop-out,

635 *Arthrobacter* CL28 vs *Arthrobacter* CL28 plus *Variovorax* CL14) using a permutation approach:
636 we estimated a *p*-value by randomly selecting 12 genes 10000 times from the expression matrix
637 and comparing the mean expression between the two SynCom treatments (e.g Full SynCom vs
638 *Variovorax* drop-out) with the actual mean expression value from the 12 genes reported as robust
639 auxin markers.
640
641 **13. Measuring the ability of *Variovorax* to attenuate RGI induced by small molecules**
642 **(Figure 4D and data S13)**
643 a. Bacterial culture and plant-inoculation.
644 We embedded each of the following compounds in JM plates: 100 nM IAA, 1µM IAA, 100 nM
645 ACC, 100 nM 2,4-d, 100 nM flg22, 100 nM BAP and 100 nM Zeatin. Plates with each compound
646 were inoculate with one of the *Variovorax* strains CL14, MF160, B4 or YR216 or with *Burkholderia*
647 CL11. These strains were grown in separate tubes, then washed, and OD_{600} was adjusted to 0.01
648 before spreading 100µL on plates. In addition, we included uninoculated controls for each
649 compound. We also included unamended JM plates inoculated with the RGI inducer *Arthrobacter*
650 CL28 co-inoculated with each of the *Variovorax/Burkholderia* strains or alone. Thus, the
651 experiment included 42 individual treatments. The experiment was repeated twice, with three
652 independent replicates per experiment. Upon harvest, root morphology was measured (Materials
653 and Methods 2c).
654
655 b. Primary root elongation analysis.
656 Primary root elongation was compared between bacterial treatments within each of root growth
657 inhibition treatments tested. Differences between treatments were estimated as described above
658 (Materials and Methods 4c). We plotted the estimated means with 95% CI of each bacterial
659 treatment across the different RGI treatments.
660
661
662 **14. In vitro growth of *Variovorax* (fig. S14)**
663 *Variovorax* CL14 was grown in 5mL cultures for 40 hours at 28 ˚C in 1x M9 minimal salts media
664 (Sigma M6030) supplemented with 2mM $MgSO_4$, 0.1mM $CaCl_2$, 10µM $FeSO_4$, and a carbon
665 source: either 15mM succinate alone, 0.4mM Indole-3-acetic acid (IAA) with 0.5% Ethanol from
666 IAA solubilization, or both. Optical density at 600 nm and IAA concentrations were measured at
667 six time points. IAA concentrations were measured using the Salkowski method modified from
668 (*48*). 100uL of Salkowski reagent (10mM $FeCl_3$ in 35% perchloric acid) was mixed with 50uL

669 culture supernatant or IAA standards and color was allowed to develop for 30 min prior to
670 measuring the absorbance at 530nm.
671
672 **15. Measuring plant Auxin response *in-vivo* using a bioreporter line (Fig. 4E-F and data**
673 **S14)**
674 a. Bacterial culture and plant-inoculation.
675 7-day old transgenic Arabidopsis seedlings expressing the *DR5::GFP* reporter construct (*49*) were
676 transferred onto plates containing: (i) 100 nM IAA, (ii) *Arthrobacter* CL28, (iii) 100 IAA + *Variovorax*
677 CL14, (iv) *Arthrobacter* CL28 + *Variovorax* CL14, (v) the *Variovorax* drop-out SynCom, (vi) the
678 full SynCom, (vii) uninoculated plates. For treatments ii,iii, Bacterial strains were grown in
679 separate tubes, then washed, and OD_{600} was adjusted to 0.01. For treatment iv, OD-adjusted
680 cultures were mixed in 1:1 ratios and spread onto agar prior to seedling transfer. Cultures for
681 treatments v and vi were prepared as described above (Materials and Methods 6a).
682 b. Fluorescence microscopy.
683 GFP fluorescence in the root elongation zone of 8-10 plants per treatment were visualized using
684 a Nikon Eclipse 80i fluorescence microscope at days 1, 3, 6, 9 and 13 post inoculation. The
685 experiment was performed in two independent replicates.
686 From each root imaged, 10 random 30 X 30 pixel squares were sampled and average GFP
687 intensity was measured using imageJ (*36*). Treatments were compared within each time point
688 using ANOVA tests with Tukey's post hoc in the R base package emmeans. For visualization
689 purposes we plotted the estimated means of each bacterial across the different timepoints.
690
691 **16. Measuring the dual role of auxin and ethylene perception in SynCom-induced RGI (Fig.**
692 **4F and data S15)**
693 a. Bacterial culture and plant-inoculation.
694 We transferred four 7-day old wild type seedling and four *axr1-2* seedlings to each plate in this
695 experiment. The plates contained one of five bacterial treatments: (i) *Arthrobacter* CL28, (ii)
696 *Arthrobacter* CL28 + *Variovorax* CL14, (iii) *Variovorax* drop-out SynCom, (iv) Full SynCom, (v)
697 uninoculated, prepared as described above (Materials and Methods 15a) Plates were placed
698 vertically inside sealed 86 X 68 cm Ziploc bags. In one of the bags, we placed an open water
699 container with 80 2.5 gram sachets containing 0.014% 1-MCP (Ethylene Buster, Chrystal
700 International BV). In the second bag we added, as a control, an open water container. Both bags
701 were placed in the growth chamber for 12 days. After 6 days of growth, we added 32 additional

sachets to the 1-MCP-treated bag to maintain 1-MCP concentrations in the air. Upon harvest, root morphology was measured (Materials and Methods 2c).

b. Primary root elongation analysis.

Primary root elongation was standardized to the No bacteria control of each genotype, and compared between genotype/1-MCP treatments within the *Arthrobacter* CL28 treatment and the *Variovorax* drop-out SynCom treatment, independently. Differences between treatments were estimated as described above (Materials and Methods 4c). We plotted the estimated means with 95% CI of each bacterial treatment across the four genotypes. We calculated the interquartile range for the Full and *Arthrobacter* CL28/*Variovorax* CL14 treatments pooling the four genotypes/treatments.

17. Preparation of binerized plant images (Fig 2C, 3B and fig. S5-S6)

To present representative root images, we increased contrast and subtracted background in imageJ, then cropped the image to select representative roots. Neighboring roots were manually erased from the cropped image.

18. Mining *Variovorax* genomes for auxin degradation operons and ACC-deminase genes (Discussion).

We used local alignment (BLASTp) to search for the presence of the10 genes (*iacA*, *iacB*, *iacC*, *iacD*, *iacE*, *iacF*, *iacG*, *iacH*, *iacI*, and *iacY*) of a previously characterized auxin degradation operon (*50*) across the 10 *Variovorax* isolates in our bacterial synthetic community and searched for hotspots of clustered hits within genomes (<10kb between genes). Across the 10 *Variovorax* isolates scanned, we could not reconstruct any hotspot that recapitulated the previously described operon. Additionally to the *iac* operon, we scanned the auxin degradation operon described in (*51*) and could not identify it across the *Variovorax* isolates. Another piece of evidence that supports the fact that *Variovorax* lack these degradation operons was the weak (< 30%) identity between the spurious hits we did find to some of the components of these operons across the *Variovorax* genomes.

We searched for the ACC deaminase gene by looking for the KEGG orthology id K01505 (1-aminocyclopropane-1-carboxylate deaminase) across our genomes.

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Competing interests: J.L.D. is a co-founder of, and shareholder in, AgBiome LLC, a corporation whose goal is to use plant-associated microbes to improve plant productivity

Data and materials availability:

The 16S amplicon sequencing data associated with this study is deposited in the NCBI SRA archive under the project accession PRJNA543313. The raw transcriptomic data is deposited in the Gene Expression Omnibus (GEO) under the accession GSE131158. In addition to the supplementary tables, we deposited all scripts and additional data structures required to reproduce the results of this study in the following GitHub repository: https://github.com/isaisg/variovoraxRGI