Cover Sheet for Supplementary Information

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Supplementary Information for

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Text S1. Plant growth

Arabidopsis thaliana seeds were surface-sterilized with sodium hypochlorite solution (5%, w/v) for 10 min and then washed with sterile water. Subsequently, the seeds were soaked in sterilized water and kept at 4 °C in dark for 3 days for vernalization. A simplified hydroponic culture of *Arabidopsis* described in the previous study was used in this study.[1] Briefly, the 0.5 mL plastic centrifuge tubes with the open hole in the cap center were filled with 100 μL 0.7% agar media (made with 1/2 Murashige and Skoog medium). After the solidification of agar, each tube was cut off the bottom and filled with 1/2 Hoagland nutrient solution on the solidified agar media. The 24 filled tubes were subsequently transferred to the wells of the rack of the empty 1 mL pipette tip box which was wrapped in aluminum foil and filled with 700 mL 1/2 Hoagland nutrient solution. In this way, total of three pipette tip boxes with 72 filled tubes were prepared. Finally, 2-3 germinant *A. thaliana* seeds were placed onto the agar surface via the hole on each tube cap. The prepared pipette box was closed and darkly incubated in the growth chamber that was set with a 16/8 h light/dark cycle, 40-60 % atmospheric humidity, 20-25 ℃ temperature, and 120 μmol photons $m^{-2}s^{-1}$ light intensity. After 5 weeks of growth, 32 healthy seedlings with similar growth status were selected for the hydroponic experiments.

Text S2. Hydroponic Experiment and Sampling

The hydroponic experiment was divided into two groups according to whether soil bacteria are present, as described in Figure S1. For the soil bacteria-free group, 5 mL sterilized PBS was added in 700 mL the sterilized hydroponic solution, while for the

soil bacteria-amended group, 5 mL extracted soil bacteria suspension was added to make the soil bacteria concentration at 10^7 CFU/mL in the hydroponic solution. Then, 32 aforementioned *A. thaliana* seedlings were transplanted into the prepared culture media in soil bacteria-free and amended groups (n=16 for each group). All the plants were growing in a growth chamber with a controlled condition of 16/8 h light/dark cycle, 20-25 ℃, 40 %-60 % atmospheric humidity, and 120 μmol photons $m^{-2}s^{-1}$ light intensity for 4 days. After that, 1 mL *E. coli* suspension in PBS was carefully added into the hydroponic solutions in both soil bacteria-free and amended groups to make the *E. coli* concentration of 10^7 CFU/mL. Meanwhile, to maintain RP4 plasmid in *E. coli*, tetracycline at concentration of 10 mg/L was also added into the hydroponic solutions. After cultivation for another 8 days under the same condition described above, all plants were removed from the solution with a sterile tweezer. The plant roots and the basal leaves were separated from the stems with sterile scissors for sampling. At the meantime, 50 mL hydroponic solution was also collected with a pre-shaking. The collected samples were then used to extract bacterial pellets, as described in the Text S3. Notably, to prevent potential bacterial contamination from the surrounding environment and possible *E. coli* transmission along with water evaporation, the hydroponic solutions were covered with plastic wrap on the top during the whole cultivation period.

Text S3. Extraction of bacterial community from the hydroponic solution, root surface, root and leaf endosphere

Centrifugation method was used to extract bacterial pellets from the collected

hydroponic solution. Briefly, 50 mL solution samples were sonicated for 7 min and shaken at 180 rpm for 1 h to avoid cell agglomeration. The obtained solution was filtered through 5 μm cellulose membrane, and then the filtrates were again centrifuged at 10000 *g* for 15 min. The obtained cell pellets were washed with sterile PBS solution twice and re-suspended in the PBS.

The bacteria on the root surface were collected as the previous study with slight modification.[2, 3] Briefly, the harvested plant roots were suspended in sterile PBS solution containing 0.05% Tween-80. Then, the solutions with the root samples were sonicated for 7 min and shaken at 180 rpm for 1 h. The obtained solution was filtered through 5 μm cellulose membrane. Then, the filtrates were centrifuged at 10000 *g* for 15 min and the cell pellets were washed with sterile PBS solution twice.

The endophytic bacteria from the roots and the basal leaves were collected using a previously reported method.[4, 5] Plant tissue samples (either roots or leaf) were immersed in 70% ethanol for 3 min, and then washed with fresh sodium hypochlorite solution (2.5% available Cl^-) for 5 min. After further washed with sterilized PBS for three times, the samples were again treated with 70% ethanol for 1 min and washed in sterilized PBS, this step was repeated twice. To confirm the success in removing plant tissue surface-associated bacteria, the last wash solution was incubated in LB medium, at 30 °C, 180 rpm, for 5 days to observe the potential bacterial growth. No visible OD_{600} change after 5 days confirmed no surface-associated bacteria were remained. After successful surface sterilization, the roots (or leave) were homogenized by sterilizing mortar for extracting endophytic bacteria. The obtained homogenate was re-suspended

by 100 mL sterilized PBS and shaken at 180 rpm at 30 °C for 1 h, which were used to release bacterial cells from the plant cells and debris.[6] Then, the endophytic bacteria of roots (or leave) were obtained by orderly filtering through 40 μm, 20 μm, and 5 μm cellulose membrane to remove the plant debris and chloroplasts.[5] Subsequently, the filtrates were centrifuged at 10000 *g* for 15 min and the cell pellets were washed with sterile PBS solution twice and re-suspended in the PBS.

The above obtained bacterial suspensions were evenly divided into two parts. One part was used to extract DNA for quantifying target genes by quantitative polymerase chain reaction (qPCR), the other part was performed to sort the RP4 plasmid carrying bacteria by flow cytometry.

Table S1. Primer sets for qPCR used in this study.

Gene	Forward Primer	Reverse Primer	Annealing temperature (℃)
gfp	TCCGTTCAACTAGCAGACCAT	TCATCCATGCCATGTGTAATCC	60
mCherry	ACGGCGAGTTCATCTACA	GAGGTGATGTCCAACTTGAT	60

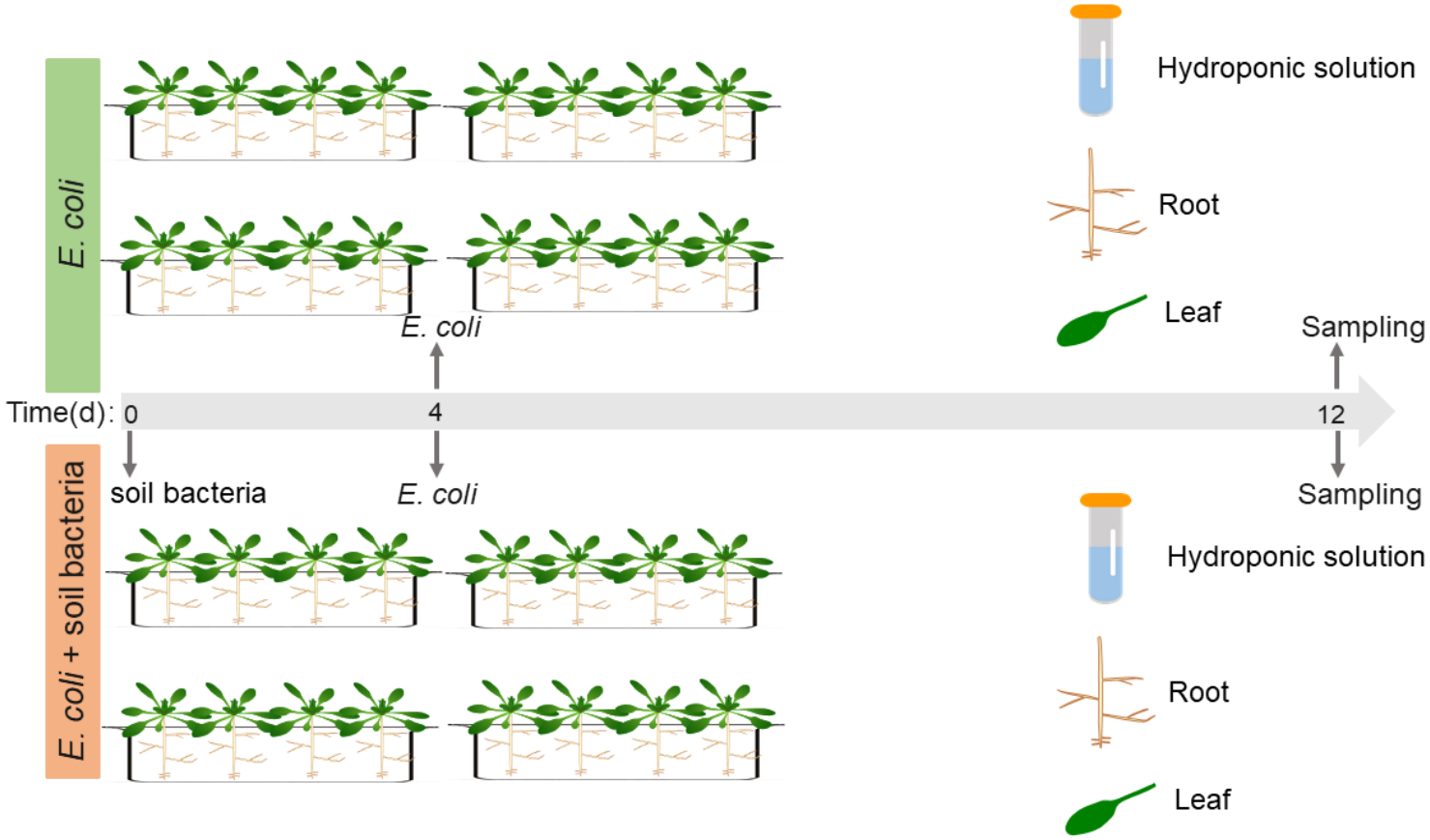


Figure S1. Hydroponic experimental design and sampling.

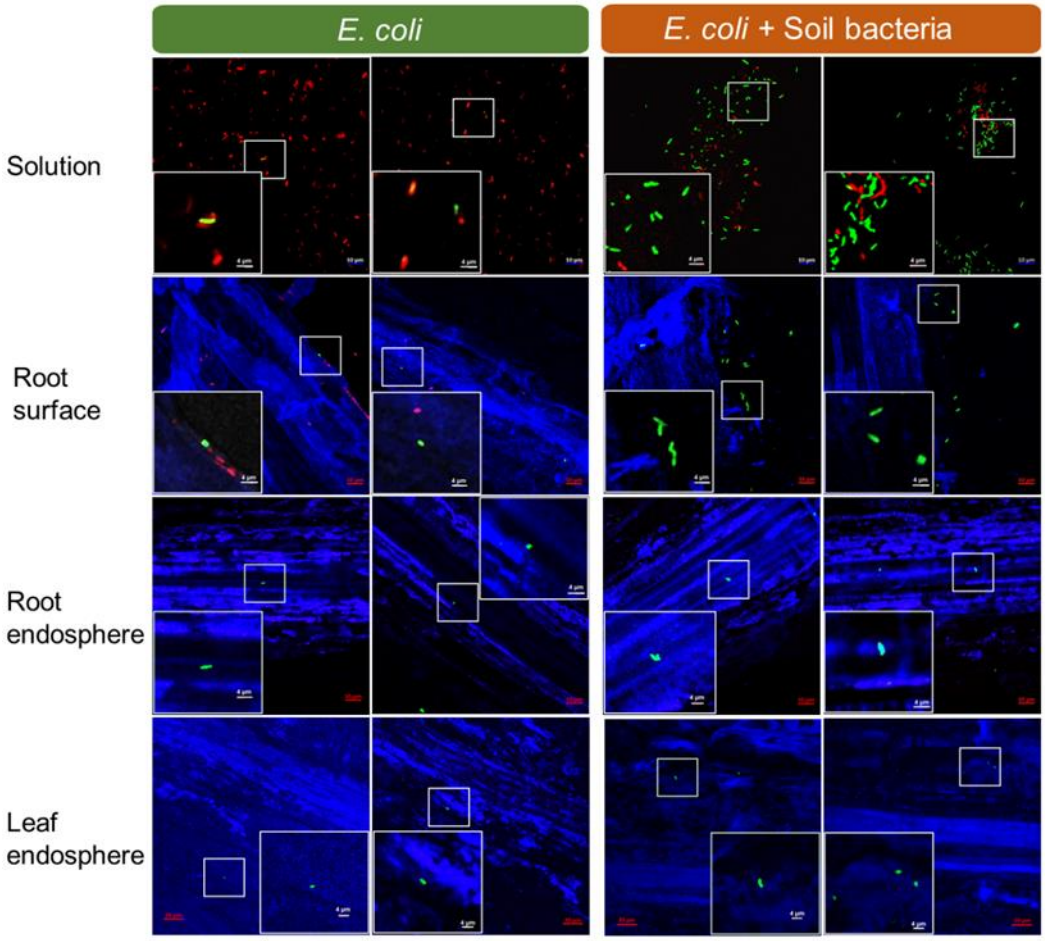
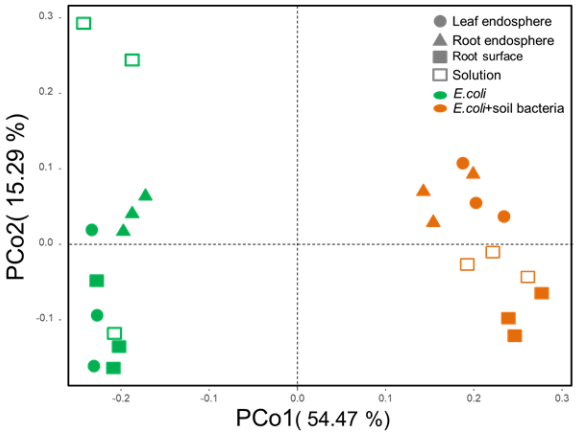


Figure S2. Supplemented confocal microscopy images of red fluorescent *E. coli* and green fluorescent bacteria (i.e., bacteria receiving RP4 plasmid from *E. coli*) in the hydroponic solution and different plant compartments of *A. thaliana*.

a



b

Summary of tests for differences in bacterial community, using PERMANOVA based on Bray-Curtis distances

Treatment	Community comparisons	*p*
E. coli	Solution *vs* Root surface	0.2
	Root ensoaphere *vs* Leaf endosphere	0.33
	Root surface *vs* Root ensoaphere *vs* Leaf endosphere	0.053
E. coli + soil bacteria	Solution *vs* Root surface	0.1
	Root ensoaphere *vs* Leaf endosphere	0.1
	Root surface *vs* Root ensoaphere *vs* Leaf endosphere	0.006

Figure S3. Comparison of RP4 plasmid carrying bacterial communities in all treatments based on (a) PCoA analysis and (b) associated PERMANOVA test result.

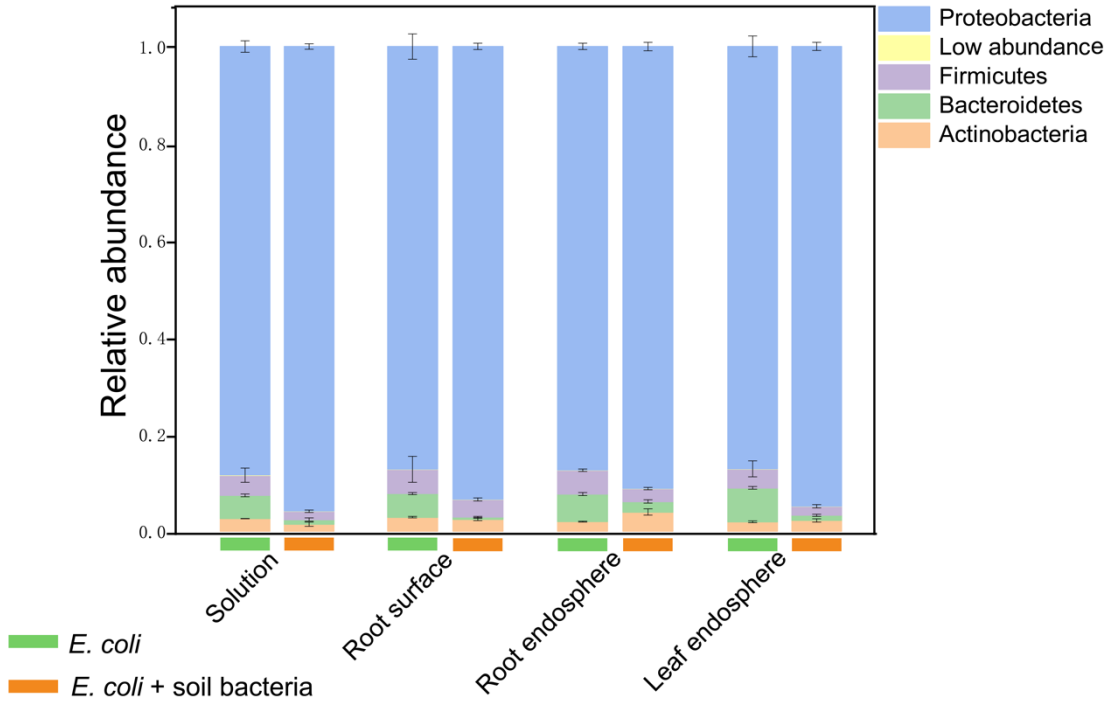


Figure S4. Identification of bacterial phylum with obtained RP4 plasmid in different plant compartments of *A. thaliana*.

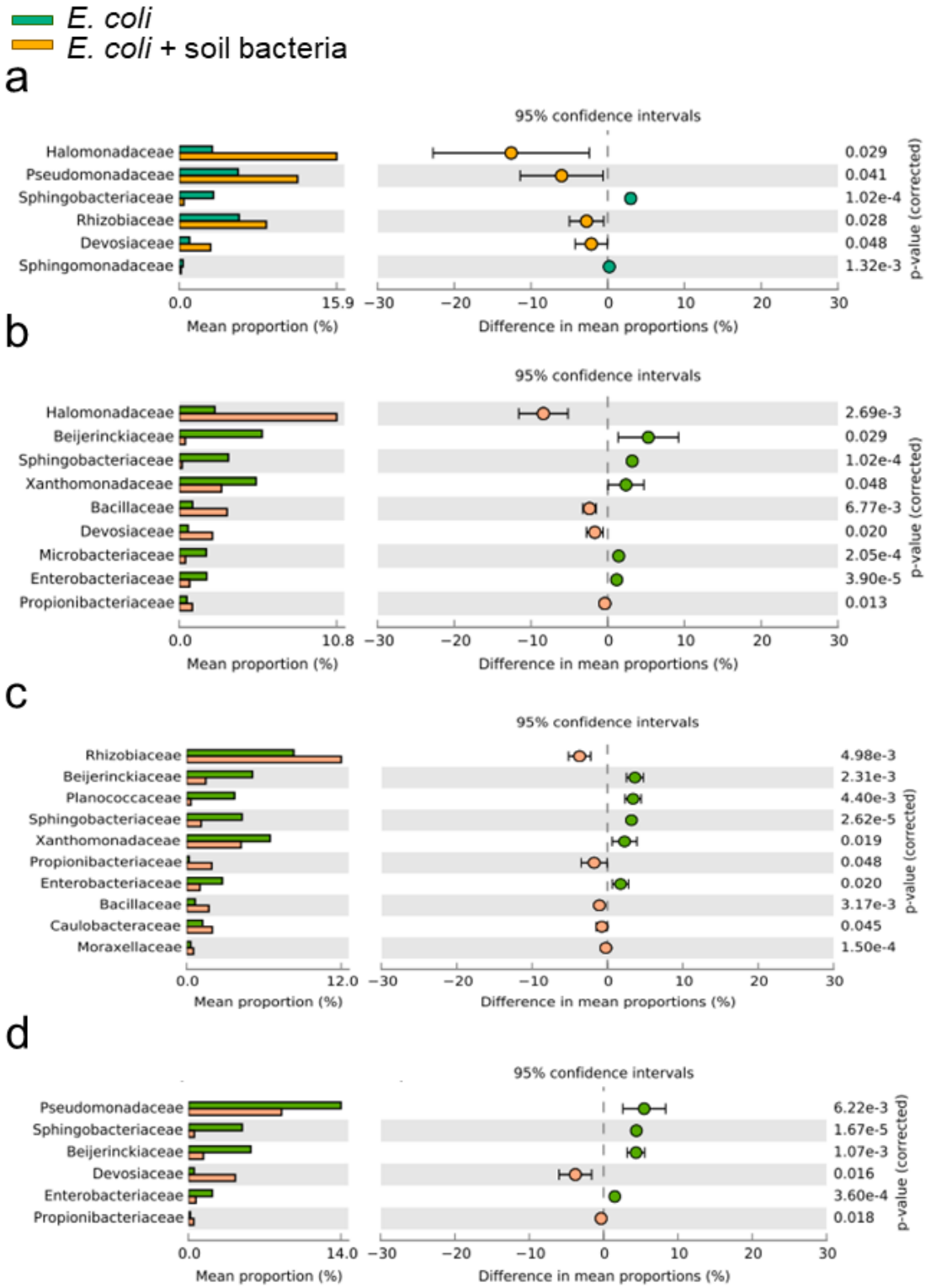


Figure S5. Pairwise comparisons of the dominant bacterial families (relative abundance ≥ 0.1%) in (a) the hydroponic solution and different plant compartments including (b) root surface, (c) root endosphere, and (d) leaf endosphere. Families significantly ($p < 0.05$) enriched in soil bacteria-free treatments have a positive value, and those in soil bacteria-spiked treatments have a negative value.

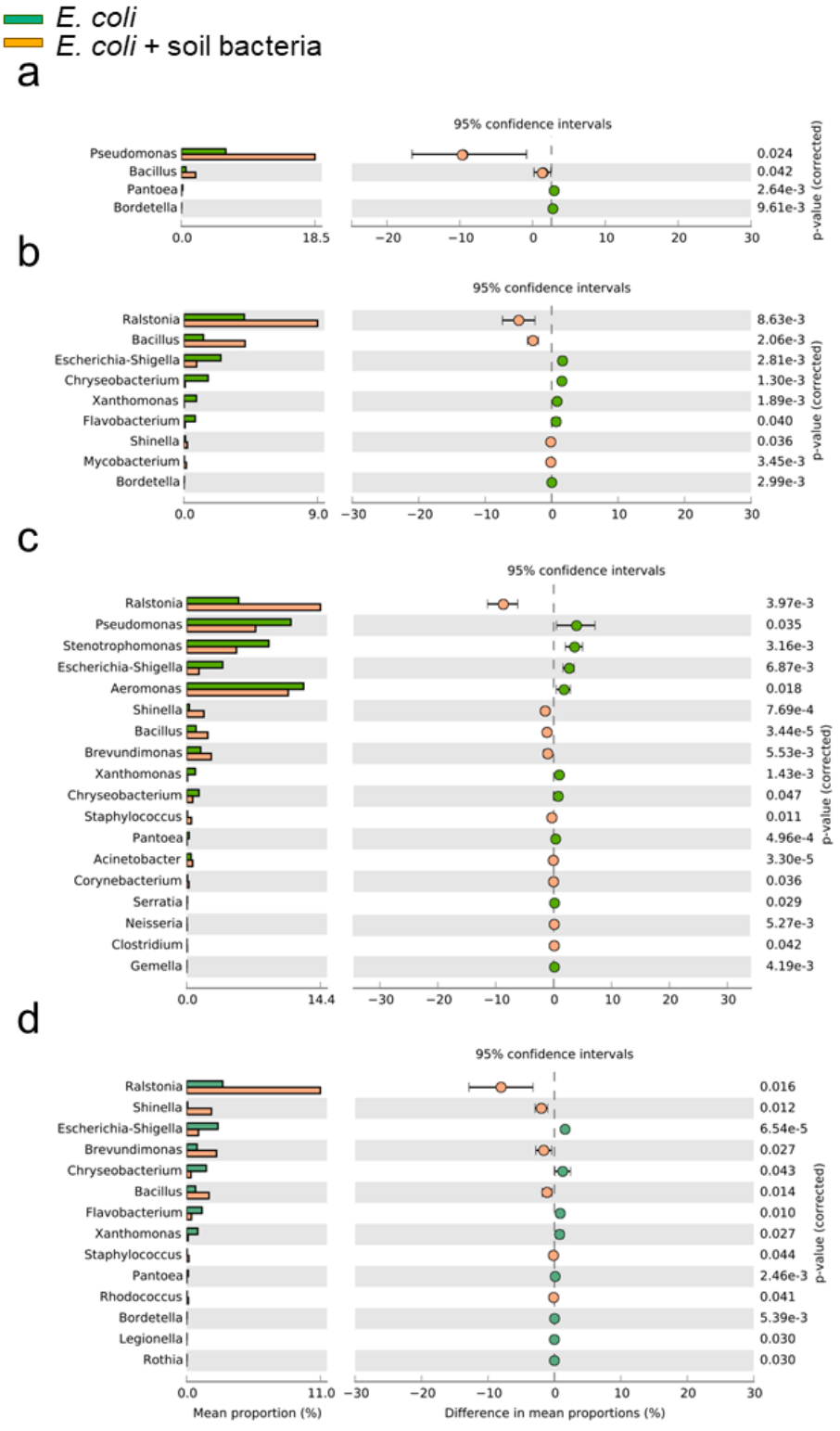


Figure S6. Pairwise comparisons of potential pathogenic genera in (a) the hydroponic solution and different plant compartments including (b) root surface, (c) root endosphere, and (d) leaf endosphere. Genera significantly ($p < 0.05$) enriched in soil bacteria-free treatments have a positive value, and those in soil bacteria-spiked treatments have a negative value.

References:

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