# Coordination between microbiota and root endodermis supports plant mineral nutrient homeostasis

**Isai Salas-González*, Guilhem Reyt*, Paulina Flis*, Valéria Custódio, David Gopaulchan, Niokhor Bakhoum, Tristan P. Dew, Kiran Suresh, Rochus Benni Franke, Jeffery L. Dangl, David E. Salt, Gabriel Castrillo†**

**INTRODUCTION:** All living organisms have evolved homeostatic mechanisms to control their mineral nutrient and trace element content (ionomes). In plant roots and animal guts, these mechanisms involve specialized cell layers that function as a diffusion barrier to water, solutes, and immunoactive ligands. To perform this role, it is essential that the cells forming these layers are tightly sealed together. Additionally, these cells must perform their homeostatic function while interacting with the local microbiota. In animals, resident microbes influence the function of intestinal diffusion barriers and, in some cases, miscoordination of this interplay can cause dysbiosis.

In plants, two types of extracellular root diffusion barriers have been characterized at the endodermis: Casparian strips, which seal cells together, and suberin deposits, which influence transport across the cell plasma membrane. Whether and how these root diffusion barriers coordinate with the microbiota inhabiting the root is unknown. Such coordination could influence plant performance, agronomic yields, and the nutritional quality of crops.

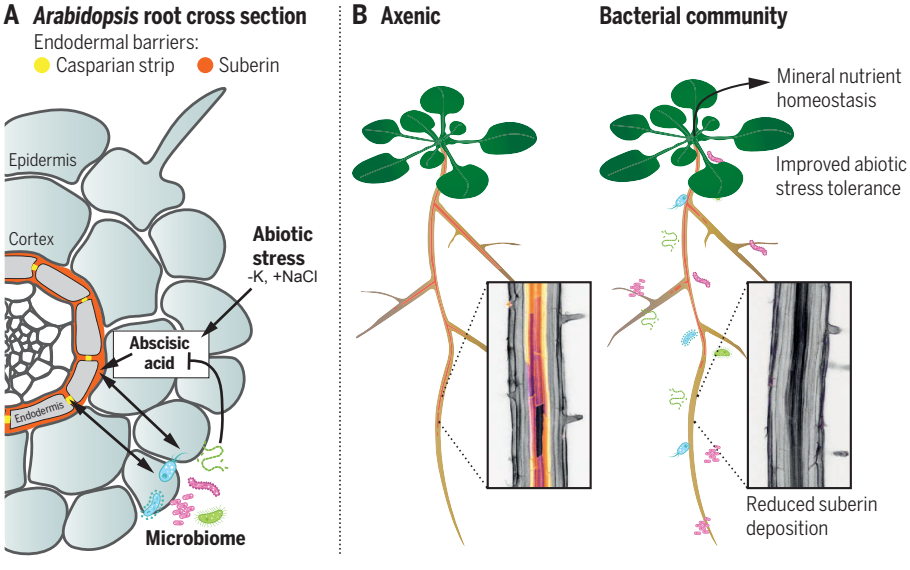
**RATIONALE:** We explored and characterized the interplay between the regulatory networks controlling the performance of the root diffusion barrier and the functionally complex and metabolically dynamic microbiota inhabiting the root. To address this, we explored the presumptive reciprocal nature of this interaction using two complementary approaches.

First, we profiled the microbiome of a collection of plants with a range of specific alterations to the root diffusion barrier to determine whether the regulatory network controlling the synthesis and deposition of the barrier components also controls the structure of the root microbiome. Second, we deployed a collection of bacterial strains isolated from the shoots and roots of plants grown in natural soils to establish the influence of the microbiome over root barrier function. Last, we coupled both approaches to identify the molecular links between the root diffusion barrier and their associated microbiota.

**RESULTS:** We analyzed a nonredundant and diverse collection of 19 root diffusion barrier mutants and overexpression lines to reveal the influence of the root diffusion barrier regulatory network on the assembly of the plant microbiota. We screened 416 individual bacterial strains for their ability to modify the function of the Casparian strip and suberin deposits in the endodermis and uncovered a new role for the plant microbiota in influencing root diffusion barrier functions with an impact on plant mineral nutrient homeostasis. We designed and deployed a bacterial synthetic community combined with ionomics and transcriptomics to discover the molecular mechanisms underlying the coordination between root diffusion barriers and the plant microbiota.

Our research has three main findings: (i) The regulatory network controlling the endodermal root diffusion barriers also influences the composition of the plant microbiota; (ii) individual members of the plant microbiome, bacterial synthetic communities, or natural microbial communities control the development of endodermal diffusion barriers, especially suberin deposition, with consequences for the plant's ionome and abiotic stress tolerance; and (iii) the capacity of the plant microbiome to influence root diffusion barrier function is mediated by its suppression of signaling dependent on the phytohormone abscisic acid.

**CONCLUSION:** Our findings that the plant microbiome influences root diffusion barrier function generalizes the role of the microbiome in controlling cellular diffusion barriers across kingdoms. In addition, we defined the molecular basis of how diffusion barriers in multicellular organisms incorporate microbial function to regulate mineral nutrient balance. This discovery has potential applications in plant and human nutrition and food quality and safety. Microbial-based strategies to control suberization of plant roots presents new opportunities to design more resilient crops, new biofortification strategies, and carbon-sequestration approaches.




**The microbiota influences root diffusion barriers.** (**A**) Model showing the interplay between the microbiota and root diffusion barriers. Microbes influence Casparian strip synthesis and co-opt plant-based abscisic acid signaling to control endodermal suberization. (**B**) Schematic representation of suberin accumulation in plants grown under axenic conditions or with the root microbiota. Root-inhabiting microbes reduce endodermal suberization optimizing mineral nutrient homeostasis and abiotic stress responses in the plant.

The list of author affiliations is available in the full article online.
*These authors contributed equally to this work.
†Corresponding author. Email: gabriel.castrillo@nottingham.ac.uk
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# Coordination between microbiota and root endodermis supports plant mineral nutrient homeostasis

**Isai Salas-González[1,2]*, Guilhem Reyt[3,4]*, Paulina Flis[3,4]*, Valéria Custódio[5]†, David Gopaulchan[3,4], Niokhor Bakhoum[3,4]‡, Tristan P. Dew[3,4], Kiran Suresh[6], Rochus Benni Franke[6], Jeffery L. Dangl[1,2], David E. Salt[3,4], Gabriel Castrillo[3,4]§**

**Plant roots and animal guts have evolved specialized cell layers to control mineral nutrient homeostasis. These layers must tolerate the resident microbiota while keeping homeostatic integrity. Whether and how the root diffusion barriers in the endodermis, which are critical for the mineral nutrient balance of plants, coordinate with the microbiota is unknown. We demonstrate that genes controlling endodermal function in the model plant *Arabidopsis thaliana* contribute to the plant microbiome assembly. We characterized a regulatory mechanism of endodermal differentiation driven by the microbiota with profound effects on nutrient homeostasis. Furthermore, we demonstrate that this mechanism is linked to the microbiota's capacity to repress responses to the phytohormone abscisic acid in the root. Our findings establish the endodermis as a regulatory hub coordinating microbiota assembly and homeostatic mechanisms.**

Plant roots, analogous to animal guts, selectively absorb mineral nutrients and water from the environment and transport them into the vascular system for long-distance transport to other tissues and organs (*1*, *2*). These processes are tightly controlled by specialized cell layers, the root endodermis and exodermis (when present) in plants and the intestinal epithelium in animals. These cells act as control points for the diffusion of water, solutes, and immune ligands. Diffusion barriers must permit the presence of the metabolically active resident microbiota and still protect homeostatic integrity. In animals, the mechanisms by which the intestinal epithelium functions with the microbiota present have been partially elucidated (*2*), and serious diseases caused by their malfunction have been described (*3*). By contrast, in plants, the mechanisms controlling the deposition of root diffusion barriers have been described only under axenic conditions (*4*), and the integration of the microbiota into their function is unknown. The endodermis has two types of root diffusion barriers, the Casparian strips, consisting of fine bands of lignin that encircle endodermal cells, and the deposition of suberin within the space between the cell wall and the plasma membrane of endodermal cells. Endodermal suberization follows a defined program in which a few cells first suberize in a "patchy" manner that later expands into a zone of continuous suberization (*5*). Suberin deposition changes in response to nutritional stress and is regulated by the plant hormones ethylene and abscisic acid (ABA) (*5*). The activation of a surveillance system to check the integrity of the Casparian strips, controlled by the Schengen pathway, induces lignification and suberization of the endodermis (*6*). The discovery that the endodermis restricts the diffusion of microbe-associated molecular patterns (*7*) important for the establishment of the root microbiome (*8*) suggests its role as a regulatory hub coordinating the plant ionome, plant mineral nutrient and trace element composition (*9*), and assembly of the microbiota. This coordination might influence plant performance under changeable environments, with consequences on agronomic yields and food nutritional quality.

[1]Curriculum in Bioinformatics and Computational Biology, Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. [2]Howard Hughes Medical Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. [3]School of Biosciences, University of Nottingham, Sutton Bonington, UK. [4]Future Food Beacon of Excellence, University of Nottingham, Sutton Bonington, UK. [5]Instituto de Tecnologia Química e Biológica António Xavier, Universidad de Nova de Lisboa, Lisboa, Portugal. [6]Institute of Cellular and Molecular Botany, University of Bonn, Bonn, Germany.
*These authors contributed equally to this work. †Present address: Future Food Beacon of Excellence and the School of Biosciences, University of Nottingham, Sutton Bonington, UK. ‡Université Sine-Saloum El Hadj Ibrahima NIASSE, UFR Environnement Biodiversité Développement Durable (EB2D), Kaolack/Laboratoire Commun de Microbiologie (IRD/ISRA/UCAD), Laboratoire Mixte International Adaptation des Plantes et microorganismes associés aux Stress Environnementaux (LAPSE), BP 1386 Dakar, Senegal.
**§Corresponding author. Email: gabriel.castrillo@nottingham.ac.uk**

## Genes controlling endodermal function influence microbiome assembly

We compared the bacterial community composition of wild-type *Arabidopsis thaliana* (hereafter *Arabidopsis*) (accession Col-0) plants with five groups of root diffusion barrier mutants and transgenic lines mis- or overexpressing relevant genes (fig. S1A and table S1). *Arabidopsis* plants lack an exodermis, so this collection of genotypes represents clean combinatorial impairments in different sectors of the endodermal root diffusion barrier network (fig. S1A). We grew plants in natural soil and determined their shoot area, as well as root, shoot, and soil bacterial community profiles, using 16*S* rRNA amplicon sequencing. We observed that genotypes with strong impairment in the root diffusion barriers (groups 5 and 6) showed a significant reduction in shoot area (fig. S1B) that might be the result of complex interactions among soil properties, the microbiome presence, and the root diffusion barriers.

General microbiome characteristics were consistent with previous findings (*10*, *11*) (fig. S1, C and D). Canonical analysis of principal coordinates (CAP) showed significant differences in bacterial community compositions across the root diffusion barrier genotypes (Fig. 1A and fig. S1E). As expected from a plant-derived mechanism, we consistently observed these differences in root and shoot but not in the soil fraction [root and shoot permutational multivariate analysis of variance (PERMANOVA), $p < 1 \times 10^{-4}$; soil PERMANOVA, $p = 0.25$] (Fig. 1A and fig. S1, E and F). Genotypes bearing significantly different bacterial communities represented most of the root diffusion barrier plant groups analyzed (Fig. 1A and fig. S1, E to G), indicating that certain genes broadly distributed across the root diffusion barrier regulatory network contribute to the composition of the plant microbiome.

To further understand the interaction between root diffusion barriers and the plant microbiome, we built a bacterial synthetic community consisting of 41 taxonomically diverse bacteria isolated from the roots and shoots of *Arabidopsis* grown in natural soils (*12*, *13*). This synthetic community approximates the endophytic compartment community composition observed in natural *Arabidopsis* populations (fig. S2A). We inoculated seedlings of wild-type plants and a selection of seven root diffusion barrier genotypes representing the different functional groups grown on agar plates. We recapitulated, in all genotypes, microbiome composition differences observed in natural soil (Fig. 1B and fig. S2, B to D) regardless of the differences observed in the root metabolome (fig. S2, E and F) and the root diffusion barrier hormonal control in some of them (fig. S2G). Thus, we confirmed that plants with atypical root diffusion barriers assemble an altered microbiota even on agar plates that minimize developmental and physiological differences among the root diffusion barrier genotypes (fig. S2, H and I).

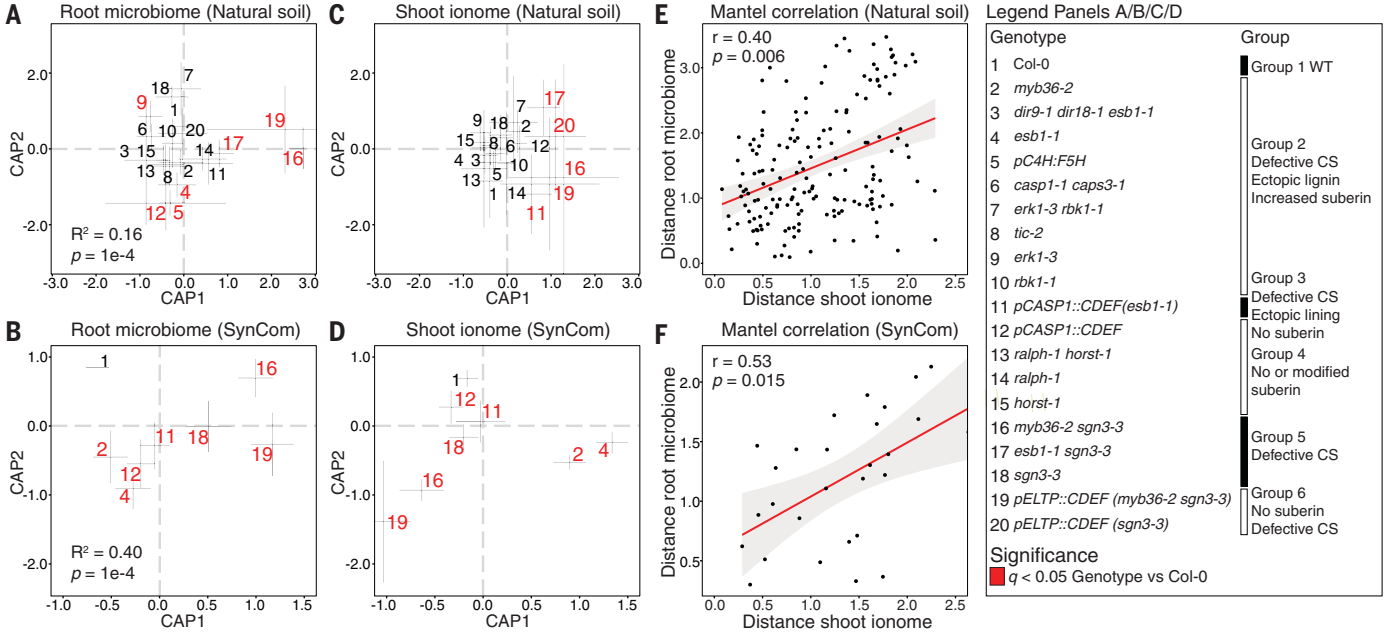


**Fig. 1. Plants with modified root diffusion barriers assemble a distinct microbiome.** (**A** and **B**) CAP analysis of root microbiome composition showing the projected microbiome assembly of the root diffusion barrier genotypes (numbers) in plants grown in (A) natural soil and (B) agar plates inoculated with a bacterial synthetic community (SynCom). (**C** and **D**) CAP analysis of shoot mineral nutrient composition (ionome) showing the projected ionomic profiles of plant genotypes (numbers) in plants grown in (C) natural soil and (D) agar plates inoculated with a bacterial SynCom. Numbers in red are statistically significant compared with Col-0 ($q < 0.05$). (**E** and **F**) Pairwise correlation analysis between the shoot ionome and the root microbiome composition in plants grown in (E) natural soil and (F) agar plates inoculated with a bacterial SynCom. Panel shows the Mantel *r* statistic and its *P* value.

We determined the leaf ionomic profiles of the different genotypes grown in natural soil and on agar plates. We observed that some of the genotypes with an atypical shoot ionome also assembled a distinct root and shoot microbiome (Fig. 1, C and D, and fig. S3, A and B). We found a significant correlation (Mantel test $p < 0.05$) between the root bacterial community dissimilarity and the shoot ionome dissimilarity (Fig. 1, E and F) in both natural soil and agar systems. This correlation was less obvious in the shoot microbiome of plants grown in natural soil (fig. S3C) and did not exist in the case of the soil microbiome (fig. S3C) and the shoot and agar microbiome of plants grown on agar plates (fig. S3D). As a control, we repeated the same analysis with soil elemental profiles that were different from the plant shoot ionome (fig. S3, E and F) and did not detect a significant correlation with the microbiome (fig. S3G).

Our results (Fig. 1) indicate that endodermal root diffusion barrier components regulate plant microbiome configuration in *Arabidopsis* plants. This effect suggests that the same mechanisms that maintain mineral nutrient homeostasis also contribute to microbiome composition.

### Individual bacterial strains modify root diffusion barriers

To explore the interplay between root diffusion barriers and the plant microbiome, we analyzed the microbiota's ability to influence the deposition of root diffusion barriers in the endodermis. We determined how the deposition of the Casparian strips and suberin synthesis changed in response to a collection of 416 individual bacterial strains (fig. S4, A and B) isolated from the roots and shoots of *Arabidopsis* grown in natural soil (*12*, *13*). We individually screened the bacterial strains for their ability to modify the function of the Casparian strip in blocking the diffusion of propidium iodide, a fluorescent apoplastic tracer, into the root tissue layers (*14*) (fig. S4C). We found that 25 and 1.9% of the isolates analyzed induced a significant early and late block in the diffusion of propidium iodide, respectively (Fig. 2A). Using a representative subset ($n = 41$) of the bacterial strains (Fig. 2B), we showed that these effects were not a mere consequence of root growth modification (Fig. 2A and fig. S5, A to D). Indeed, we observed that some bacteria had the capacity to induce changes in the endodermal lignification independently of the appearance of the first root hair, a marker of root development (fig. S5, C to F). These results indicate that members of the root microbiome have the capacity to modify Casparian strip formation.

To test whether this bacterial effect also occurs in the deposition of endodermal suberin, we analyzed the expression of the suberization reporter *pGPAT5::mCITRINE-SYP122* (*15*) in plant roots in response to 416 individual bacterial isolates (fig. S4, A to C). Most of the bacteria analyzed (71%) significantly expanded the root zone where *GPAT5* expression follows a patchy pattern (Fig. 2A). Accordingly, the root zone with continuous activation of *GPAT5* was reduced (Fig. 2A). We ruled out that the bacterial effect on endodermal suberization was exclusively linked to the bacterial capacity to induce changes in root growth (Fig. 2A and fig. S5, A, G, and H). This suberin deposition phenotype showed a strong phylogenetic signal (Pagel's $\lambda = 0.78$, $p = 4.3 \times 10^{-40}$), highlighting that closely related strains exhibit similar effects on root suberization (Fig. 2A).

We demonstrated that the bacterial effects on Casparian strip function and endodermal suberization are not linked because we found no correlation ($r = -0.07$, $p = 0.13$) between these two parameters (fig. S5I). The small variation found in Casparian strip function does not explain the large effect detected in the case of suberin deposition in response to the bacterial collection (fig. S5J). These results indicate that Casparian strip synthesis is more resilient to the effect of individual bacteria than endodermal suberization, and that members of the plant microbiome can modify suberin deposition independently of the Casparian strip.

Next, we used the representative subset ($n = 41$) of the bacterial strains in plant-association assays to test whether their effect on suberization regulates plant mineral nutrient homeostasis

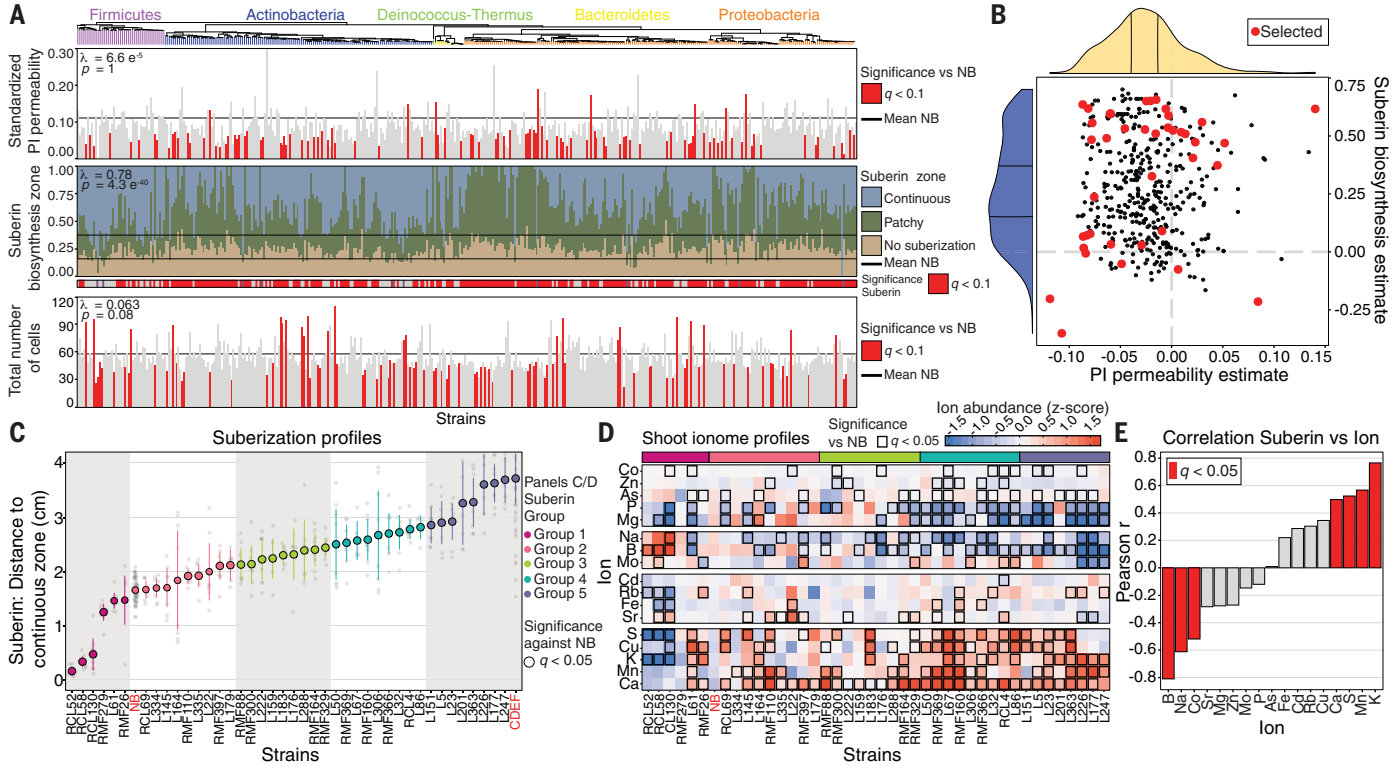


**Fig. 2. Bacterial isolates modify endodermal function.** (**A**) Bar graphs representing bacterial isolates average effect on propidium iodide (PI) permeability, suberin biosynthesis, and the total number of cells in the root. Significantly different values from the no bacteria (NB) control (horizontal lines in each panel) are in red. For suberin biosynthesis, this information is in the bottom bar ("Significance Suberin"). The data are sorted according to the bacterial collection phylogeny, as indicated by the tree (phylum) on the top. The *P* and λ values from Pagel's λ test for phylogenetic signal are also shown. (**B**) Selection of a representative number of bacterial strains. At the top of each axis is the corresponding data distribution divided into tertiles. Red dots represent the selected bacteria. (**C**) Distinct suberization (distances from the root tip to the continuous zone of suberization) profiles in Col-0 exposed to the bacterial isolates. Controls used, plants grown in axenic conditions (NB) and the line *pCASP1::CDEF1* are in red. Colors represent groups of bacteria with a differential effect on suberization. (**D**) Heatmap showing the standardized mineral nutrient concentrations in plant inoculated or not (NB) with the bacterial strains. The columns have been ordered to match the bacterial effect on endodermal suberization (C). Significant ($q$ < 0.05) values in relation to NB are outlined in black. (**E**) Bar graph showing the correlation coefficient calculated between each mineral nutrient abundance and the suberization in plant exposed to bacterial strains (fig. S7A). Bars in red are significant ($q$ < 0.05).

(Fig. 2B and fig. S6, A and B). We observed that root suberin, stained with Fluorol yellow (*16*) and quantified as the distance from the root tip to the continuously suberized zone, recapitulated the gradient of suberization found in our previous screening (Fig. 2C and fig. S6, A, C, D, and E). We identified strains that increased deposition of suberin (Fig. 2C and fig. S6C) and strains that inhibited suberization to levels found in the line *pCASP1::CDEF1* expressing the cuticle destructing factor1 (*CDEF1*) that degrades endodermal suberin (*16*) (Fig. 2C and fig. S6C). This suggests that members of the plant microbiota might interfere with the mechanisms controlling endodermal suberization, such as hormonal control (*5*) or immune system activation (*17*). Several controls validated that, in general, the bacterial effect on suberization is not due to an indirect effect on plant development (figs. S5G and S6, D to F). These results indicate that strains from the plant microbiome can modify suberin accumulation in the endodermis over a wide accumulation range.

We also investigated whether bacterially induced changes in the root diffusion barriers function affect plant mineral nutrient homeostasis. Analyses of shoots from plants inoculated with the selected strains showed strong perturbation in the ionome (Fig. 2D). We identified clusters of mineral nutrients with concentrations that significantly increased, decreased, or were not changed across the bacterial strain treatments (Fig. 2D). The variations in suberin accumulation induced by single isolates were highly correlated with the accumulation of a significant number of nutrients in the shoot (Fig. 2E and fig. S7A). Various controls excluded that the differences observed were an indirect fertilizing effect caused by the bacteria present in the leaves (fig. S7, B to D). These findings strongly suggest that the mechanisms that influence suberin deposition mediated by members of the plant microbiota also influence mineral nutrient homeostasis in the plant.

We next investigated whether bacterial abundance could explain the observed suberin phenotypes and found a positive correlation between root bacterial colonization capacity and suberin deposition in the plant (figs. S6D and S7E). This correlation suggests that bacterial colonization might be a predictor of a positive bacterial effect on suberin deposition.

### A bacterial synthetic community modifies suberin plasticity

To investigate the role of a more complex plant microbiome in regulating suberin deposition, we used a bacterial synthetic community consisting of 41 taxonomically diverse bacteria (Fig. 2B and figs. S2A and S4A) able to colonize the rhizoplane and the endophytic compartment of the root (fig. S8A). We grew wild-type plants axenically or inoculated with the synthetic community under nutritional stresses known to induce obvious perturbations in suberin deposition (*5*) (fig. S6B). We recapitulated the suberin plasticity found in response to nutritional stresses in plants growing axenically (*5*) (Fig. 3, A and B, and fig. S8B).

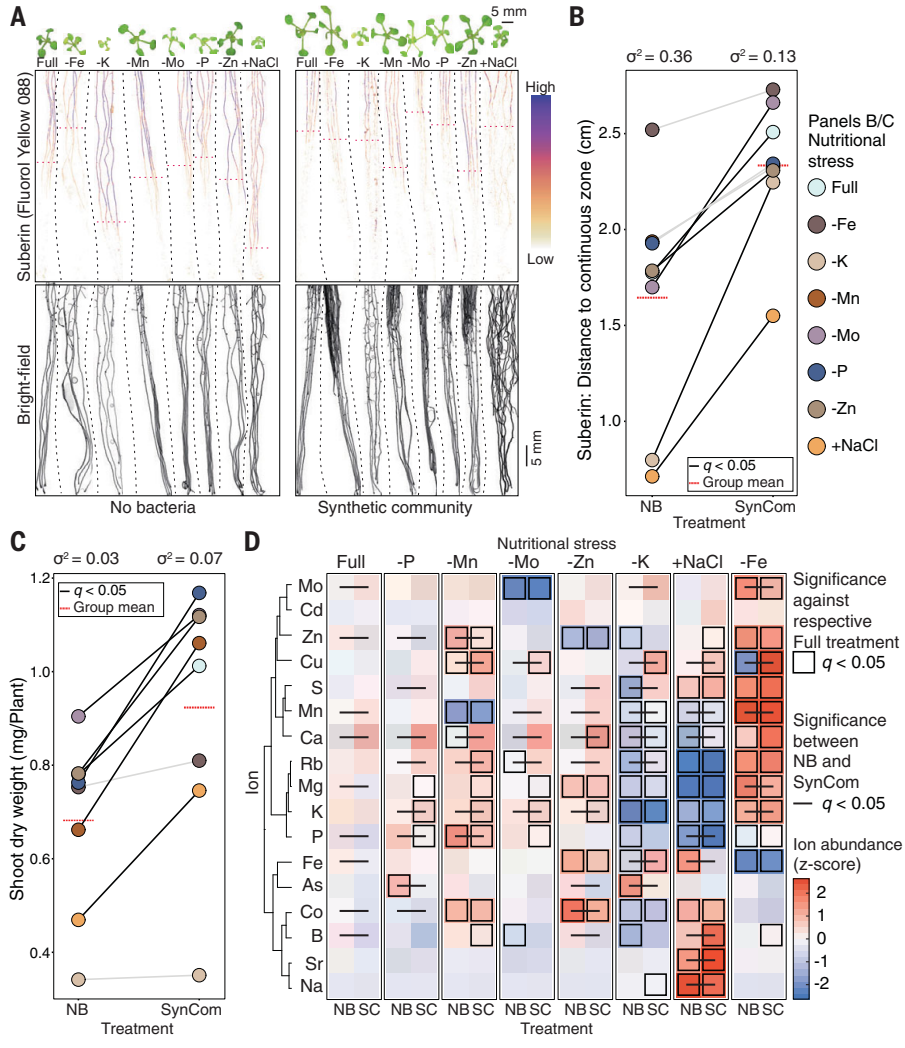


**Fig. 3. A bacterial SynCom controls endodermal suberization to enhance plant adaptation to nutrient stresses.** (**A**) (Top) Endodermal suberization in plants inoculated or not with the SynCom and exposed to nutritional stresses. As a control, we used replete nutrient conditions ("Full"). The red line highlights the initiation of the continuous zone of suberization. (Bottom) Root systems from the same plants ("Bright-field"). Representative plant rosettes are in the upper part of the figure. (**B** and **C**) Comparative analysis of suberin deposition (B) and shoot dry weight (C) in plants inoculated with the SynCom or grown axenically (NB) under nutrient stresses. Colored points represent the average value for each condition. The line connecting both points is the difference between NB and SynCom treatments. A black line indicates statistical significance ($q < 0.05$). The average values (horizontal red line) and variance (top) are depicted for each bacterial treatment. (**D**) Heatmap showing the standardized mineral nutrient concentrations in plants inoculated or not (NB) with the bacterial SynCom (SC) and exposed to the nutrient stresses. Significant intrastress comparisons (stress versus full) are outlined in black ($q < 0.05$), and significant comparisons between no bacteria and SynCom treatment within stress are represented with a horizontal black line ($q < 0.05$).

By contrast, nutrient-stressed plants inoculated with the synthetic community showed significant reductions in the levels of plasticity of suberin deposition, as evidenced by a longer distance to the continuous zone (Fig. 3, A and B, and fig. S8B). This was particularly evident in the case of low K and high NaCl (Fig. 3, A and B, and fig. S8B). This effect was robust over a wide range of synthetic community inoculum concentrations (fig. S8C). We confirmed, using direct chemical quantification, that the synthetic community reduces the suberin content in the root and introduces minor changes in the suberin polyester composition (fig. S8D). Thus, we hypothesize that this microbiome effect on root suberization could be a regulatory component of the root diffusion barriers with consequences for mineral nutrient homeostasis and plant performance during nutritional stress. Indeed, we found that plants inoculated with the synthetic community coped better with nutritional stresses (Fig. 3, A and C, and fig. S8E). Inoculated stressed plants had larger rosettes with a significantly greater dry weight compared with axenically stressed plants (Fig. 3, A and C, and fig. S8E). We linked this beneficial microbiome effect to endodermal suberization. Different *CDEF1*-expressing lines lacking the ability to accumulate suberin were insensitive to the microbiome effect (fig. S8, F and G).

We then analyzed elemental profiles of plant leaves grown under nutritional stresses (fig. S6B). All tested stress conditions induced significant changes in the plant ionome (Fig. 3D and fig. S9, A and B). In accordance with our previous results (Fig. 2D), plants inoculated with the synthetic community exhibited distinct ionomes compared with axenic plants (Fig. 3D and fig. S9, A and B), indicating a bacterial effect on mineral nutrient homeostasis. We confirmed that the ionomic differences observed were not due to a bacterial fertilizing effect (fig. S9, A and B). The bacterial synthetic community induced a significant reconfiguration of the plant ionome even under replete nutrient conditions (Fig. 3D). We verified that this bacterial effect on the plant ionome is linked to root suberization. Consistent with our previous results (fig. S8, F and G), we observed that lines expressing *CDEF1* grown axenically with a constitutively lower amount of endodermal suberin produced changes in a sector of the plant ionome that recapitulated the synthetic community effect on wild-type plants (fig. S9C). Furthermore, the synthetic community's ability to induce changes in this sector of the plant ionome was reduced in these lines (fig. S9C). This finding, in conjunction with the beneficial microbiome effect (Fig. 3, A and C), indicates that microbiome-mediated suberin deposition optimizes a sector of the plant ionome facilitating plant acclimation to nutrient stresses.

### The microbiome modulates suberization through ABA response repression

To understand how the microbiota modulates suberin deposition, we analyzed the transcriptome of plants grown with the synthetic community. We contrasted sets of differentially expressed genes in roots of wild-type plants and the mutant *myb36-2*, which displays an enhanced accumulation of suberin because of the constitutive activation of the Schengen pathway (*18*). We identified differentially expressed genes that responded to the synthetic community, to the Schengen pathway, or to both (Fig. 4A and fig. S10A). We found that clusters C1 and C2 contained genes with a synthetic community effect (Fig. 4A and fig. S10A). Consistent with microbiome influence on suberization, these clusters are enriched with genes related to defense, ion

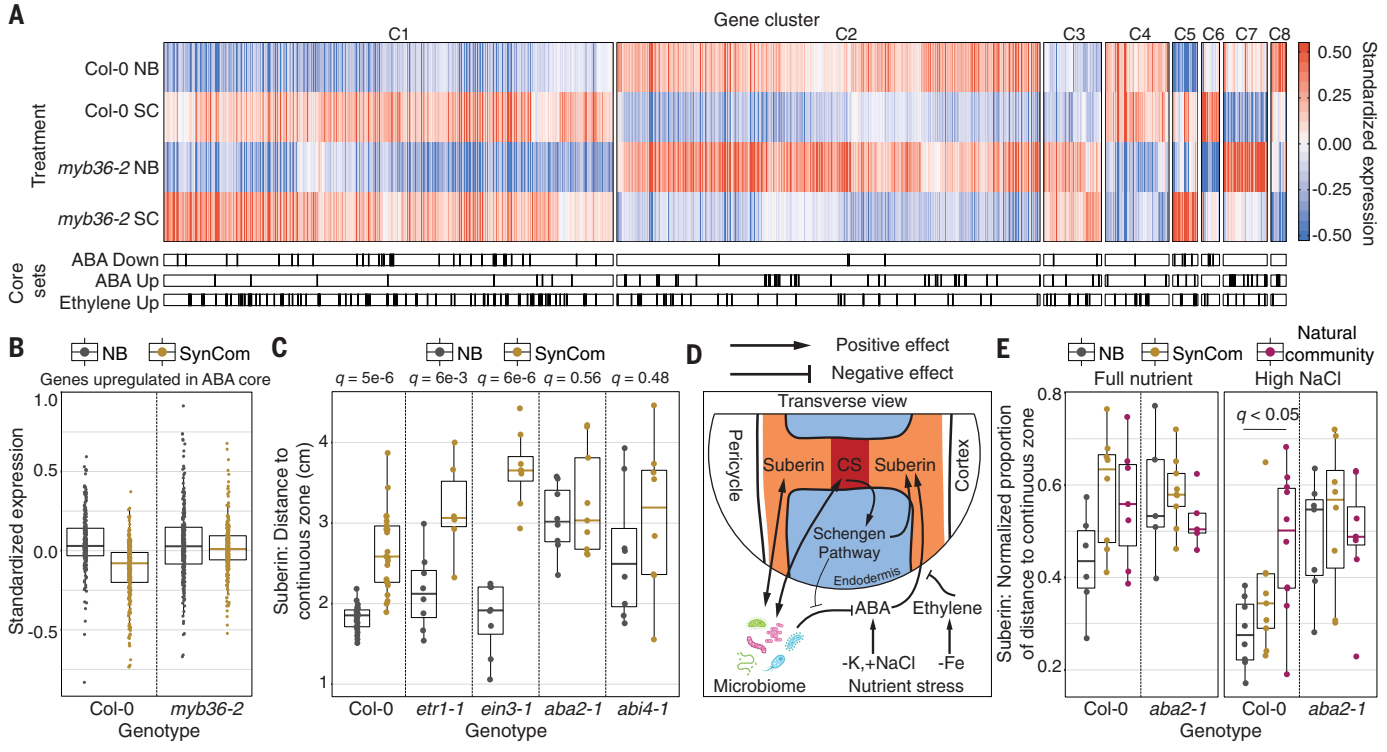


**Fig. 4. Microbiome effect on suberization represents an uncharacterized regulatory pathway.** (**A**) Heatmap of the 4538 differentially expressed genes identified by RNA sequencing in Col-0 and *myb36-2* roots uninoculated (NB) or inoculated with the SynCom. Clusters ("C") are designated with numbers. Bars on the bottom indicate the representation of the ABA ("ABA Up" and "ABA Down") and ethylene core genes identified from the literature (*26*, *27*). (**B**) Boxplots displaying the standardized expression of the ABA-induced core genes extracted from the literature (*26*) in the RNA sequencing. (**C**) Suberin quantification in Col-0, ethylene (*etr1-1* and *ein3-1*), and ABA (*aba2-1* and *abi4-1*) mutants, exposed or not (NB) to the SynCom. Statistical significance was determined using intragenotype *t* tests. *q* values are shown. (**D**) Schematic overview of the microbiome regulatory branch integration within the endodermal suberization regulatory network. (**E**) Suberin quantification in Col-0 and *aba2-1* inoculated with the SynCom, a natural community, or grown axenically (NB) in the perlite system under full nutrient conditions or 70 mM NaCl.

transport, and nutrient responses (fig. S10B). Consistent with previous observations (Fig. 3, A and B), genes related to phenylpropanoid metabolism and fatty acid elongation, which are critical for the synthesis of suberin (*19*), were repressed by the synthetic community (fig. S10, C and D). Further, the synthetic community suppressed the transcriptional response to ABA (cluster C2), a hormone known to induce suberin accumulation (*5*) (Fig. 4, A and B, and fig. S10, E and F). We therefore hypothesized that the microbiome modulates suberization through an ABA-dependent pathway. Indeed, we found that the ABA mutants *aba2-1* (*20*) and *abi4-1* (*21*) mimicked the bacterially induced reduction in suberin observed in wild-type plants (Fig. 4C and fig. S10, G and H) and did not respond to the microbiome effect on suberization and plant growth (Fig. 4C and fig. S10, G and H). We observed similar results when we used the line *pCASP1::abi1-1*, which is impaired in endodermal ABA signaling (*5*) (fig. S10I). Furthermore, we found that the synthetic community suppressed fluorescence in the root of the two ABA reporter lines *6xABRE_A::erGFP* and *6xABRE_R::erGFP* (*22*) in two different nutritional conditions (fig. S10, J and K). Therefore, we conclude that the microbiome controls endodermal suberization through the inhibition of the ABA signaling pathway in the plant and locally in the endodermis. Reinforcing this conclusion, we did not observe a synthetic community effect on the transcriptional response to ethylene, another hormone controlling suberization (*5*), and the ethylene mutants analyzed did respond to the microbiome's effect on suberization (Fig. 4C and fig. S10, E to H).

We found that ABA mutants had different leaf ionomes than plants grown axenically (fig. S11A). This supports the idea that the microbiota also controls other non–suberin-based ionomic mechanisms (*10*, *23*). We hypothesized that the microbiome effect on suberization could represent an uncharacterized regulatory branch of endodermal suberization that is independent of the Schengen pathway. Indeed, the *sgn3-3* mutant, which has an impaired Schengen pathway (*24*), is responsive to the synthetic community (figs. S10H and S11, A and B). We confirmed these results in the *sgn3-3 myb36-2* double mutant, in which we observed less suberization and larger rosettes in synthetic community–inoculated plants compared with axenic plants (figs. S10H and S11, A and B). Therefore, the root microbiota's effect on suberin is a signaling branch of endodermal suberization that affects ABA signaling and is independent of the Schengen pathway (Fig. 4D).

We observed that the Schengen pathway exerted an epistatic effect on the microbiome-controlled branch of suberization (Fig. 4D). We identified genes with repression by the synthetic community that could be overridden by Schengen pathway activation in *myb36-2* (fig. S11, C and D). We also observed that the transcriptional response to ABA in *myb36-2* was not repressed by the synthetic community (Fig. 4B). The synthetic community failed to reduce the suberin levels in the mutants *myb36-2* and *esb1-1,* both expressing constitutive activation of the Schengen pathway (*25*) (figs. S10H and S11B).

Finally, we tested whether the synthetic community's control over suberization translates to a natural microbial community. Wild-type plants inoculated with both synthetic and natural microbiomes had larger leaves (fig. S12, A and B) and less suberin compared with axenic plants in response to salinity

stress (Fig. 4E and fig. S12C). We did not observe any microbiome effect on the ABA mutant *aba2-1* (Fig. 4E and fig. S12, A to C). These results demonstrate that the plant microbiota is an essential component of the root diffusion barrier regulatory network in natural conditions.

## Conclusions

We demonstrate that the genes regulating root diffusion barriers influence the composition of the plant microbiota and, reciprocally, that microbes colonizing the root influence root diffusion barrier function. We also establish that suberization of the endodermis, which is important for plant adaptation to nutritional stresses under axenic conditions (*5*), is reduced by the root microbiome by repression of the plant's ABA transcriptional response. We reveal that coordination between root diffusion barriers and the microbiome leads to a balancing of the plant ionome that allows the plant to successfully absorb environmental perturbations such as low iron or high salinity. Our findings define a mechanism that allows plants to cope with fluctuations in the mineral nutrient supply in nature and generalize the role of the microbiome in controlling diffusion barrier functions across kingdoms. These results improve our understanding of how diffusion barriers in multicellular organisms integrate microbial function to maintain mineral nutrient homeostasis (Fig. 4D). We envision future applications of microbial-based strategies for the modulation of suberin production in crops. We anticipate the opening of unexplored avenues leading to the development of plants more adapted to extreme environmental conditions, with more capacity for carbon sequestration, higher content of beneficial mineral nutrients, and fewer toxic elements.

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## SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S12
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MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.


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