Differences between species promote stable coexistence in a resource-limited environment. These differences can result from interspecies competition leading to character shifts, a process referred to as character displacement. While character displacement is often interpreted as a consequence of genetically fixed trait differences between species, it can also be mediated by phenotypic plasticity in response to the presence of another species. Here, we test whether phenotypic plasticity leads to a shift in proteome allocation during co-occurrence of two bacterial species from the abundant, leaf-colonizing families Sphingomonadaceae and Rhizobiaceae in their natural habitat. Upon mono-colonizing of the phyllosphere, both species exhibit specific and shared protein functions indicating a niche overlap. During co-colonization, quantitative differences in the protein repertoire of both bacterial populations occur as a result of bacterial coexistence in planta. Specifically, the Sphingomonas strain produces enzymes for the metabolization of xylan, while the Rhizobium strain reprograms its metabolism to beta-oxidation of fatty acids fueled via the glyoxylate cycle and adapts its biotin acquisition. We demonstrate the conditional relevance of cross-species facilitation by mutagenesis leading to loss of fitness in competition in planta. Our results show that dynamic character displacement and niche facilitation mediated by phenotypic plasticity can contribute to species coexistence.
The mechanisms that generate and maintain genetic and phenotypic variation in natural populations are central to ecology and evolution. Character displacement, the evolutionary divergence of competing species, plays a fundamental role in the assembly of diverse communities. Sympatric species compete for the same set of limited resources, and thus natural selection might favor diversification of their use. As a result, phenotypes may emerge in which alternative resources are exploited, thereby reducing competition for nutrients. Once separated in niche space, a species may modify the environment and directly or indirectly enhance its own growth and survival. As a result, competitive interactions are altered among species through mechanisms such as increased access to limiting resources, which in turn has impacts on biodiversity and prevalence.

The nature of the interactions that generate patterns of diversity remains difficult to examine. To provide a mechanistic basis, evolution experiments and evolutionary analysis of bacterial populations have been used to study the processes that lead to diversifications, and these model studies have illustrated how competitive and facilitative interactions between distinct genotypes can evolve and contribute to their coexistence. These and other studies have added to our understanding of microbial community assembly by providing exemplary analysis of the evolutionary processes that drive diversity.

A complementary and non-exclusive perspective on the interaction of microbial species that may contribute to coexistence is their potential to dynamically shift their phenotype in response to the presence of other species. This process is referred to as phenotypic plasticity and has been underappreciated in mediating character displacement and evolution. According to this idea, when two extant species encounter each other and compete for limited resources, they diversify through changes in gene expression and thus become less similar (Fig. 1). The latter can be seen as the result of an ecological interaction, but may be the consequence of an evolutionary process in the past. Such phenotypic diversification could be exhibited by competing species to reduce their niche overlap. In fact, it has been hypothesized that phenotypic plasticity may precede genetic differences among species.

While measuring differences among species is more straightforward, discerning the processes causing character displacement is inherently difficult and requires empirical quantifiable differences with appropriate controls. Here, we set out to test the phenotypic plasticity of two commensal species and to examine whether they become phenotypically more dissimilar when co-occurring compared to being present alone. To provide context for their interaction, we chose two bacterial strains isolated from the phyllosphere of the model plant Arabidopsis and tested their interaction in planta. The phyllosphere, the above-ground parts of plants dominated by leaves, is an important ecosystem due to its contribution to carbon dioxide fixation in terrestrial systems and indigenous bacteria may impact plant growth and health. In addition, the phyllosphere is also a highly suitable habitat to study microbial interactions because it represents a discrete habitat or, more precisely, a sum of discrete habitats and allows quantifying population sizes in a well-defined area. The phyllosphere, a heterogeneously structured and oligotrophic environment, has been shown to maintain high and reproducible microbial biodiversity. As a result, due to limited resources, it provides the ground for species interactions and opportunities to discover rules for community assembly in this habitat.

Culture-independent studies on the composition of the phyllosphere microbiota of different plant species revealed that reoccurring phylogenetic structures can be observed. Bacteria belonging to the phylum Proteobacteria are most commonly found, followed by Actinobacteria, Bacteroidetes, and Firmicutes across distinct plant species. These bacteria are mostly found on the surface of leaves, where they can form aggregates. These bacterial aggregates can be constituted by a single bacterial species or by several species and are of great interest for the elucidation of bacteria-bacteria interactions.

Several studies have addressed the physiological adaptation strategies involved in leaf colonization by individual bacterial species of commensals and plant pathogens. Complementary studies have provided the first insights into the physiological capacity of the coexisting phyllosphere microbiota in planta using metaproteomics. While providing a snapshot of the state of microbial communities in situ, the dynamic processes underlying niche occupancy as a consequence of species competition are currently not well understood. Because interspecies interactions in situ are complicated by the presence of a multitude of species, simplified systems are needed, that allow to dissect dynamic behavior of populations.

In the present study, we use an established gnotobiotic Arabidopsis thaliana model system to test whether two species undergo shifts in the phenotype upon co-colonization. We select two strains representing the most common colonizing proteobacterial families Sphingomonadaceae and Rhizobiacae and apply mass spectrometry-based proteomics to quantify proteome-level changes in each strain upon co-colonization of plants.

**Fig. 1** Conceptional overview of niche occupancy by bacteria colonizing the plant in mono-association and upon coexistence. **a** General niche of two species, A (gray) and B (orange), in case they do not encounter each other in a given habitat. **b** During co-occurrence, the niches of the two species overlap substantially, leading to competition for shared resources (left) but also to potential for a dynamic character displacement (right, indicated by arrows). **c** As a consequence, one species may displace the other species from the niche resulting in competitive exclusion (left). Alternatively, a dynamic character displacement may occur, reducing the original niche overlap and leading to coexistence (right). The size of the circle indicates productivity in the ecological niche. In this study, niche occupancy was measured using a proteomics approach.
compared to mono-colonization. In addition, we identify the specific adaption of each strain to the in planta condition compared to growth on artificial media. As expected, we confirm that both species are generalist bacteria that have the potential to utilize a variety of substrates such as glucose or sucrose as an available, yet limiting carbon source on leaves. In addition to facilitation, we observe a dynamic character displacement that might contribute to the coexistence of the populations. We confirm relevant traits by mutagenesis and testing in planta.

Results

Assessment of the interaction of two phyllosphere strains based on population sizes and proteome allocation in planta. To study the interaction between phyllosphere microbiota strains, we selected two representative strains from the Alphaproteobacteria, the most abundant bacterial class found on A. thaliana leaves. Among Alphaproteobacteria the bacterial families of Sphingomonadaceae and Rhizobiaceae are the most common and include strains with plant beneficial functions. Previous experiments have shown that the genera of Sphingomonas and Rhizobium have the potential to affect community assembly and structure. Furthermore, both genera have overlapping metabolic capacities and occur in the spatially defined, oligotrophic environment of the A. thaliana phyllosphere. Specifically, we chose Sphingomonas Leaf257 and Rhizobium Leaf68 and confirmed their consistent colonization capacities in monoculture using a gnotobiotic plant growth system (see Methods). Both strains were isolated from leaf washes and colonize leaves as epiphytes. We also ensured that both strains colonized the phyllosphere upon co-colonization. In addition, we confirmed their co-occurrence during colonization at the microscale (Supplementary Fig. 1).

We then conducted four independent large-scale colonization experiments in which we inoculated the plants with either Rhizobium Leaf68 and Sphingomonas Leaf257 or a mixture of both. In addition, we determined the bacterial population sizes upon mono- and co-colonization. Both strains colonized the phyllosphere, with Sphingomonas Leaf257 reaching 1.6 × 10^9 CFUs per gram fresh weight (CFUs g\(^{-1}\) FW) (Fig. 2a) and Rhizobium Leaf68 of 2.6 × 10^8 CFUs g\(^{-1}\) FW (Fig. 2b). During co-colonization of the phyllosphere, both strains significantly changed their colonization capacity (Kruskal–Wallis, P-value <0.01). Sphingomonas Leaf257 was reduced to 7.5 × 10^8 (0.46-fold) (Fig. 2a) and Rhizobium Leaf68 increased to 1.2 × 10^9 CFUs g\(^{-1}\) FW (4.6-fold) (Fig. 2b). The population sizes indicated an interaction between the two strains during co-colonization in the phyllosphere, as both populations changed in number relative to mono-colonization. Because commensal bacteria can elicit plant responses and may indirectly affect colonization of other bacteria, we tested the interaction of both strains using a selection of plant immunity mutants (Methods). We found that the bacterial interaction was robust in different plant backgrounds (Supplementary Fig. 2).

We determined the proteomes of the bacterial populations in planta using a washing protocol (Methods) (i.e., Rhizobium Leaf68, Sphingomonas Leaf257 and both upon co-colonization) from the corresponding experiments enumerated above from four biological replicates. For protein determination, we used liquid chromatography-mass spectrometry (LC-MS/MS) operated in data-dependent acquisition mode (Methods, Supplementary Fig. 3a). Notably, to improve protein quantification and to compensate effects of sample complexity of bacteria during mono-colonization compared to co-colonization, we also measured an artificial mixture from bacteria grown in mono-colonization in planta after cell lysis and protein digestion. In addition to the biological samples of bacteria grown in planta, we harvested the two strains grown each on solidified artificial medium on agar plates incubated under the same light and temperature regime in plant growth chambers in four independent replicates. Our MS-based approach allowed the detection of up to 2500 proteins per strain with normalized protein abundances spanning more than six orders of magnitude (Supplementary Fig. 3b–d) and the quantification of about 2000 proteins detected with two or more unique peptides for each strain. We identified proteins that were induced or reduced when the bacteria encountered each other in planta, confirming that both bacteria interacted with each other (Fig. 2c, d), as already suggested by the population sizes (Fig. 2a, b).

Individualized and common adaptation strategies to colonize the phyllosphere. Before investigating the interaction between both bacteria, we first analyzed the physiological adaptation to the in planta conditions of each strain compared to growth on artificial media using proteomics. For Sphingomonas Leaf257, we identified 184 proteins that were induced in planta (Supplementary Fig. 4a, Supplementary Data 1). Among these, a number of proteins indicate an adaptation to environmental stress in terms of desiccation, oxidative stress and light in line with earlier reports on leaf strains. Exemplarily, these included catalase counteracting oxidative stress as well as deoxyribozyme-related protein (COG3046) and UmuC as SOS induced DNA polymerase enabling DNA damage repair. We also identified a glycosyltransferase (ASF14_08960) as a highly induced protein (Log\(_2\) fold-change >35) and its paralog (ASF14_03660, >4-fold) that was also induced upon plant colonization in Sphingomonas melonis Fr1, another phyllosphere commensal. Glycosyltransferases might be involved in lipopolysaccharide and biofilm synthesis and their strong induction might highlight their relevance in phyllosphere colonization. In addition, fourteen distinct TonB-dependent receptors were induced upon phyllosphere colonization that might be involved in uptake of iron, carbohydrates or amino acids.

For Rhizobium Leaf68, we found 356 proteins induced in planta (Supplementary Fig. 4b, Supplementary Data 2). The most strongly induced protein (about 1000-fold) in planta was an alcohol dehydrogenase (ASF03_19755), closely followed by several hypothetical proteins. We also identified two proteins that are involved in the detoxification of reactive oxygen species, a peroxidases (ASF03_20400) and a superoxide dismutase (ASF03_03695) with fold changes higher than 10 indicating that the bacterium has to cope with ROS-stress during phyllosphere colonization. In addition, we found a predicted biotin sulfoxide reductase (ASF03_18405, 5.5-fold) and a biotin synthase (ASF03_03440, 5.2-fold) to be induced, which may suggest the need of Rhizobium Leaf68 to scavenge a biotin derivative. Two proteins (ASF03_19470 and ASF03_19480) involved in the synthesis of succinoglycan, an exopolysaccharide, were also strongly induced with fold changes of more than an order of magnitude (Supplementary Data 2). Succinoglycans are exopolysaccharides (EPS) known for their thermal stability, high water retention and viscosifying properties in many soil bacteria, including Rhizobium. EPS production is a major factor in the formation of bacterial aggregates and might therefore benefit Rhizobium Leaf68 to colonize and form aggregates in the phyllosphere. Another phyllosphere-specific response of Rhizobium Leaf68 was the induction of two sulfate monoxygenases (ASF03_18445, 20.6-fold; ASF03_18375, 5.4-fold) and several ABC transporter binding proteins (CysA, 7.5-fold; CysP, 13.5-fold; SsuA, 32-fold; SsuB, 27-fold), proteins involved in sulfur acquisition during sulfur limiting conditions. Furthermore, we found the two subunits of sulfate adenyltransferase
(ASF03_03950, >20-fold; ASF03_03955, 5.4-fold), an enzyme involved in sulfate assimilation, significantly induced, supporting the hypothesis that sulfur is limiting in the phyllosphere congruent with earlier studies44,63,64.

The induced proteins upon plant colonization in mono-colonization indicated that unique but also shared adaptation might occur. To compare the induced proteome systematically, we analyzed the data for orthologous groups (OGs) of proteins and determined which OGs were significantly regulated and shared between both strains (q-value < 0.05 and Log2 fold-change >1.5 or <−1.5). From a total of 62 shared OGs, 24 were induced and 38 reduced when comparing in planta regulated proteins compared to in vitro conditions (Supplementary Fig. 4c, Supplementary Data 3). Next, we grouped all OGs by their functional category to see which functions are shared between both bacteria during phyllosphere colonization (Supplementary Fig. 4d, Supplementary Data 3). Some of the most induced shared protein orthologues are two glycosyltransferases (COG0438, COG0463), proteins involved in the molybdenum cofactor and thiamine biosynthesis, catalases, and one of the key enzymes involved in the glyoxylate cycle, isocitrate lyase (COG2224). The other key enzyme in the glyoxylate cycle, malate synthase
(COG2225), was also induced in both strains but only 1.3-fold in *Rhizobium* Leaf68. The induction of the glyoxylate cycle upon plant colonization was previously observed in another phyllosphere commensal, *S. melonis* Frl144. Additionally, a pyruvate dehydrogenase (COG0028) was induced in both strains with similar fold changes as well as the histidine kinase sensor protein EnvZ. The EnvZ histidine kinase is involved in the response to changes in osmolality in *E. coli*45, a stress that bacteria also face during phyllosphere colonization46. Furthermore, we observed a glutathione transferase, an alcohol dehydrogenase, acetolactate and glutamine synthetase, and several outer membrane proteins. Interestingly, D-3-phosphoglycerate dehydrogenase was induced in both strains, which is the committing and rate limiting step in the phosphorylated pathway of L-serine biosynthesis, consistent with findings in *S. melonis* Frl144. Furthermore, both strains downregulated three OGs involved in translation, ribosomal structure and biogenesis, and transcription respectively, which could be due to lower growth rates in planta compared to artificial media. Notably, we also found flagellin (COG1334) reduced in line with earlier studies44,46. Whether or not this strategy serves to minimize detection by the plant immune system68,69 remains to be shown. Common OGs downregulated by both strains were a glycerol-3-phosphate dehydrogenase (COG0578), an alpha-L-arabinofuranosidase (COG3534) and several hypothetical proteins of which one is potentially a glycosyl transferase (COG0463).

In summary, the majority of shared regulated OGs were associated with intracellular stress responses as well as membrane-, cell wall- and envelop-biogenesis, indicative of the importance of cell-barrier remodeling in bacterial adaptation to the rather hydrophobic and water depleted environment of the phyllosphere in accordance with previous studies26,36. Importantly also, shared induced OG related to nutrient uptake and metabolism suggested common adaptation to the oligotrophic phyllosphere environment and potential niche overlap.

**Proteome changes during co-colonization of the phyllosphere.**

Next, we analyzed the dynamic niche occupancy upon coexistence based on the proteomes of the two strains. To do so, we compared the proteomes generated upon co-colonization with the ones determined under mono-colonization conditions (upon artificial mixture, see above). *Sphingomonas* Leaf257 significantly changed 55 proteins upon co-colonization (q-value < 0.05 and Log, fold-change >1.5 or < −1.5) of which 32 were induced and 23 were reduced (Fig. 2c, Supplementary Data 4). *Rhizobium* Leaf68 significantly altered 177 proteins (q-value < 0.05 and Log2 fold-change >1.5 or < −1.5) of which 56 were induced and 121 reduced (Fig. 2d, Supplementary Data 5). These changes indicate a reciprocal interaction between the two strains in the phyllosphere.

For *Sphingomonas* Leaf257, one of the most induced proteins upon co-colonization with *Rhizobium* Leaf68 was dipetidyl peptidase-4 that cleaves X-proline dipeptides from the N-terminus of polypeptides (ASF14_01870, 6.4-fold) (Supplementary Data 4)70. Proline-rich proteins are a common building block in the glycoproteins found in plant cell walls, where they are used as anchor points for glycan chains71. In agreement with this, several polysaccharide-degrading enzymes were also strongly induced, namely a xyllosidase/arabinosidase (ASF14_10230, 5.2-fold), a xylan 1,4-beta-xyllosidase (ASF14_16825, 5.2-fold), a glucan 1,4-beta-glucosidase (ASF14_10240, 4.2-fold), and another xylanase (ASF14_16480, 3.1-fold) (Fig. 3a, Supplementary Data 4). In addition, the induction (2.4-fold) of an ABC transporter (ASF14_10270) genomically encoded close to a gene for a predicted xylosidase suggested an increased metabolization of sugar monomers obtained from xylan degradation, highlighting a possible degradation of plant cell walls. We also found 6-phosphogluconolactonase (ASF14_10560) induced (2.3-fold), which is required, for example, to metabolize glucuronic acid, which forms side chains of xylan, thus supporting the notion of a sugar metabolism from xylan. In addition, *Sphingomonas* Leaf257 induced the iron transporter FeoB (2.9-fold) as well as a predicted ferrichrome-iron receptor (2.5-fold) indicating increased iron limitation during co-colonization of the plant under competitive conditions. Interestingly, less strongly but consistently induced were several enzymes involved in the Shikimate pathway (Supplementary Data 4). Isochorismate pyruvate-lyase (*ASF14_15595*) catalyzes the reaction from isochorismate to pyruvate and salicylic acid, the latter being observed in other bacteria under iron-limiting conditions72,73. The most downregulated proteins by *Sphingomonas* Leaf257 under co-colonization conditions compared to mono-association were a Zn-dependent dipeptidase (*ASF14_12990*, 18-fold), a putative phosphatase (*ASF14_09200*, 12-fold) and several hypothetical proteins (7-fold). In addition, a predicted low-affinity phosphate transporter (*ASF14_05655*, 3.4-fold) and a phosphate ABC transporter substrate-binding protein (*ASF14_05925*, 2.4-fold) were downregulated upon co-colonization that were induced in planta upon mono-colonization (Supplementary Data 1 and 4). Thus, our results suggest that co-colonization with *Rhizobium* Leaf68 results in increased iron- and phosphate shortage for *Sphingomonas* Leaf257 and drives the latter to exploit additional carbon sources by metabolizing components of the plant cell wall, most prominently xylan.

For *Rhizobium* Leaf68, one of the most induced proteins upon co-colonization with *Sphingomonas* Leaf257 was an alpha-galactosidase (ASF03_16130, 9-fold). Another alpha-galactosidase (ASF03_16155) was induced 4-fold, and we observed a small but significant induction for an alpha-galactoside-binding protein (ASF03_1635), suggesting adaptation towards the digestion and uptake of alpha-galactosyl moieties that might be derived from polysaccharides of the plant cell wall (Supplementary Data 5)74,75. Several hypothetical proteins were also among the most induced proteins. Interestingly, isocitrate lyase (ASF03_04985; 4-fold) and a glycylate dehydrogenase (ASF03_04840; 6-fold) were induced during co-colonization, suggesting an increased flux towards the glyoxylate cycle as an anabolic pathway. Consistent with this finding, we observed an induction of several enzymes involved in the beta-oxidation of fatty acids (ASF03_04360, 7-fold; ASF03_12360, 2.1-fold; ASF03_12365, 3.4-fold; ASF03_20960, 4.5-fold). Several TonB-dependent receptors, RND efflux systems and other transporters were also induced in response to co-colonization, some of which were predicted ABC transporter for glycerol-3-phosphate (ASF03_16050, 6.4-fold; ASF03_21060, 3.9-fold; ASF03_21075, 3.4-fold; ASF03_18705, 3.3-fold; ASF03_18690, 3.2-fold). In line with this observation, we measured a strong reduction (5-fold) of the glycerol-3-phosphate regulon repressor GlpR (ASF03_11310) indicating an adaptation towards the acquisition of glycerol-3-phosphate through de-repression of the underlying regulon. Glycerol-3-phosphate uptake is notable in the context of fatty acid degradation as this may suggest an adaptation of *Rhizobium* Leaf68 towards growth on lipids. Furthermore, two proteins involved in phosphonate utilization were significantly induced (ASF03_14895, 8-fold; ASF03_14885, 5.8-fold), suggestive of phosphate starvation during co-colonization. Further highlighting an association with phosphate starvation, the PhoB transcriptional regulator (ASF03_21155) associated with phosphate and other nutrient stress networks76 was induced (Fig. 4a). Taken together, this indicated that in response to co-colonization with *Sphingomonas* Leaf257,
Rhizobium Leaf68 adapted to phosphate scarcity by engaging global regulatory mechanisms. One of the most reduced proteins during co-colonization was alcohol dehydrogenase (ASF03_19755; Log2 fold-change $< -6.8$), which was striking as the enzyme was highly induced in the mono-colonization compared to artificial media (Fig. 4a, Supplementary Data 2 and 5). Another observation was that biotin synthase (ASF03_03440), the last enzyme in the biotin biosynthesis, was significantly reduced in Rhizobium Leaf68 upon co-colonization with Sphingomonas Leaf257.
**Phosphateregulon transcriptional regulatory protein (PhoB)** of total population wild type versus each mutant respectively. A 1:1 mixture is indicated at 50% with a red dotted line. For statistical analysis a two- and 95% confidence interval are shown. For both graphs each data point represents the values obtained from a single plant with a total of 12 plants for each condition.

In summary, our results suggest that both strains react to each other, encounter enhanced competition for phosphorus and iron and shift towards alternative metabolic pathways.

**Sphingomonas Leaf257, a plant-derived xylan degrader and sugar generalist.** Our proteomics data suggested that *Sphingomonas Leaf257* explores new niches when experiencing species competition. In particular, the data for co-colonization compared to mono-colonization indicated an increased utilization of xylan from the cell wall (Fig. 3a and Supplementary Data 4), a polysaccharide predominantly made up of xylose. To verify the ability of *Sphingomonas Leaf257* to metabolize plant-derived xylan, we performed growth assays in a minimal medium containing xylan as the sole carbon and energy source. Indeed, we observed growth both in liquid culture as well as on plates, confirming the ability of *Sphingomonas Leaf257* to degrade xylan (Fig. 3b). Growth on xylan requires the secretion of xylan-degrading enzymes. To test for the presence of such proteins, we sampled the spent medium from liquid cultures with xylan, concentrated it and analyzed it using SDS-PAGE. We observed prominent bands at around 55 kDa upon growth on xylan, but not when glucose was the carbon source (Fig. 3c). LC-MS analysis resulted in the identification of several proteins, six of which were enzymes involved in the degradation of larger polysaccharides such as xylan and its subsequent metabolism (Fig. 3d). One of these enzymes was already identified as significantly induced during co-colonization (ASF14_10230) (Fig. 3a) and three others were detected in planta (ASF14_10220, ASF14_10225, ASF14_10250) (Fig. 3d). The three xylan-degrading enzymes, ASF14_10230, 10250, and 10340, contained a signal peptide, indicating that they are indeed secreted to degrade the xylan polysaccharide. In contrast, both the xylene isomerase and xylulose kinase as well as the glycoside hydrodase did not contain a predicted signal peptide. The presence of these enzymes in culture supernatants might thus be due to partial cell lysis and accumulation in the medium. Nonetheless, their abundance in a minimal medium with xylan is further suggestive of the metabolism of xylose derived from xylan through the pentose phosphate pathway.

The degradation of xylan results in the release of mostly xylose but also additional carbohydrates, including C6 sugars that form side chains on the β-1,4-xylose backbone as mentioned above. To test if *Sphingomonas Leaf257* prefers certain sugars, we determined the growth on different substrates and their combinations in liquid culture. In addition, we took samples of the supernatant for HPLC analysis. We found that *Sphingomonas Leaf257* is able to grow on xylose, glucose, and galactose and co-consumes these sugars (Supplementary Fig. 5). We did not observe evidence for the accumulation of incompletely metabolized intermediates such as pyruvate, acetate, or other byproducts during growth on the different sugar substrates. Our data therefore indicate that *Sphingomonas Leaf257* is a sugar generalist able to consume the carbohydrates derived from xylan degradation.
Rhizobium Leaf68 requires PhoB for phyllosphere fitness and is facilitated by Sphingomonas Leaf257 to replete its biotin pool.

Next, we enquired fitness traits of Rhizobium Leaf68 and the molecular basis for its enhanced growth upon co-colonization by Sphingomonas Leaf257. Rhizobium Leaf68 (unlike Sphingomonas Leaf257) was amenable to genetic manipulation and we selected five genes for mutagenesis (Fig. 4a). We chose the gene encoding the PhoB transcriptional regulator (ASF03_21155) and the gene encoding the GlpR repressor (ASF03_11310) to test whether the underlying regulons are important for phyllosphere colonization in general or play a role in the interaction with Sphingomonas Leaf257. We also mutated adh encoding for a predicted alcohol dehydrogenase (ASF03_19755). To investigate the relevance of the glyoxylate cycle in planta we generated a mutant in aceA encoding isocitrate lyase (ASF03_04985). In addition, we tested BioB (ASF03_03440), an enzyme involved in biotin biosynthesis that was induced in planta in mono-colonization but reduced in presence of Sphingomonas Leaf257. With the exception of the phoB mutant the generated Rhizobium Leaf68 mutants grew similarly compared to the wild type during growth on minimal medium with glucose (Supplementary Fig. 6). As expected, the isocitrate lyase mutant was unable to grow on acetate (Supplementary Fig. 6g) confirming the essentiality of aceA for the growth on the substrate78,79. Next, we tested whether each mutant retained the ability to colonize the plant and achieved comparable population sizes compared to Rhizobium Leaf68 wild-type, which was the case (Fig. 4b) (Kruskal–Wallis, P-value = ns). Although cell numbers after plant colonization were comparable, strains might still be at a growth disadvantage when competing with the parental strain. To test this, we conducted in planta assays in which we competed the mutants against the wild-type strain using an initial 1:1 ratio in the inoculum. While the isocitrate lyase (ASF03_04985) mutant showed no apparent fitness defect, the alcohol dehydrogenase and GlpR mutants showed a small competitive disadvantage when challenged against the wild type (Fig. 4c). The bioB and phoB mutants showed a strong decrease in competitiveness against the wild type (Fig. 4c). Thus, our data underscore that BioB and PhoB, which are known to be involved in environmental adaptation of Salmonella80,81, are required for fitness during phyllosphere colonization of Rhizobium Leaf68.

Biotin synthase (BioB) catalyzes the last step in biotin biosynthesis. To determine whether the strain has the potential to produce biotin, we searched the genome of Rhizobium Leaf68 for genes for mutagenesis (Fig. 4a). We chose the gene encoding ATP-dependent dethiobiotin synthase was absent, suggesting that the strain is an auxotroph for biotin - or the precursor dethiobiotin. We confirmed that Rhizobium Leaf68 was unable to grow on minimal medium containing glucose without vitamin addition (Supplementary Fig. 6a–c). We observed that Leaf68 wild-type was able to grow with biotin and dethiobiotin alike, and that the bioB mutant required biotin for growth (Supplementary Fig. 6d, e). We then examined whether the bioB mutant can be rescued in competition against the wild type in planta upon external biotin supplementation compared to mock treatment. Indeed, the supplementation with biotin rescued the bioB mutant during competition with the wild type strain (Fig. 5a), further confirming the relevance of this trait during plant colonization.

The findings described above suggested that dethiobiotin is available in planta and may induce bioB expression to catalyze the last step in biotin biosynthesis. BioB is an instable enzyme that is partially consumed during the reaction82,83. It was also shown that upon addition of dethiobiotin a significant fraction of the protein is degraded84. Therefore, the reduction in BioB in Rhizobium Leaf68 upon co-colonization with the Sphingomonas Leaf257 (Supplementary Data 5; Fig. 4a) may reflect a higher degradation of BioB due to an increase in the dethiobiotin pool available to Rhizobium Leaf68. We thus set out to investigate whether the bioB mutant was impaired in its interaction with Sphingomonas Leaf257. Specifically, we sought to test whether the Rhizobium Leaf68 bioB mutant mitigates the negative interaction towards Sphingomonas Leaf257 and the failure of the latter to benefit the Rhizobium strain. Remarkably, we observed a significant loss of the interaction with the Rhizobium Leaf68 bioB mutant (Fig. 5b), indicating that an increased dethiobiotin pool provided by Sphingomonas Leaf257 is responsible for the significant increase in the Rhizobium Leaf68 population during co-colonization (Fig. 2b). In addition, and to complete the analysis of the Rhizobium Leaf68 with respect to its interaction with Sphingomonas Leaf257, we also tested the Rhizobium Leaf68 glpR, phoB, aceA and adh mutants. For the glpR mutant, we observed a significant reduction in population size during co-colonization with Sphingomonas Leaf257 (Supplementary Fig. 7a). This suggests that GlpR is relevant for the fitness of Rhizobium Leaf68 in competition with the wild type or Sphingomonas Leaf257 in planta (Fig. 4c, Supplementary Fig. 7a). In contrast, the Rhizobium Leaf68 phoB mutant showed no effect on the interaction between Rhizobium Leaf68 and Sphingomonas Leaf257, suggesting that PhoB is relevant for fitness in planta compared to the wild type but not for the biotic interaction between Leaf68 and Leaf257 (Fig. 4c, Supplementary Fig. 7b). Neither of the other two Rhizobium mutants, aceA and adh, showed an effect when tested in competition (Supplementary Fig. 7c, d).

After establishing the dethiobiotin dependence in the interaction of Rhizobium Leaf68 with Sphingomonas Leaf257, we tested whether Sphingomonas Leaf257 was able to complement Rhizobium Leaf68 in vitro in minimal medium (Supplementary Fig. 8c, d). Indeed, the prototroph Sphingomonas Leaf257 was able to sustain growth of Rhizobium Leaf68 wild-type in vitro in the absence of biotin supplementation (Supplementary Fig. 8c, d). Finally, we tested whether exogenous application of biotin to the phyllosphere was sufficient to recover the interaction of the Rhizobium Leaf68 bioB mutant and Sphingomonas Leaf257. Notably, exogenous application of biotin restored the phenotype of the Rhizobium mutant interacting with Sphingomonas Leaf257 to wild-type levels (Fig. 5c). In the control treatment (mock) the interaction between Rhizobium Leaf68 wild-type and Sphingomonas Leaf257 remained unchanged (Fig. 5b). The addition of biotin and its precursor dethiobiotin significantly increased the abundance of Rhizobium Leaf68 wild-type during monocolonization of the plant (Supplementary Fig. 8a), while Sphingomonas Leaf257 was unaffected by the treatments (Supplementary Fig. 8b). As expected, the Leaf68 bioB mutant increased its abundance significantly upon the addition of biotin but remained unchanged during dethiobiotin addition (Supplementary Fig. 8a).

Taken together, our results indicate that in the presence of Sphingomonas Leaf257, the available dethiobiotin pool on the leaf surface is increased such that it enables Rhizobium Leaf68 to reach a higher colonization capacity. They highlight that the two-sided interaction of Rhizobium Leaf68 and Sphingomonas Leaf257 is triggered by vitamin supplementation in planta and manifests in dynamic niche displacement of the latter toward exploiting an additional substrate, namely xylan as revealed by protein allocation (Fig. 3).

Discussion

Gnotobiotic model systems provide the opportunity to address fundamental ecological questions and dissect the mechanism of
**Fig. 5** In planta biotin complementation assays and interaction with *Sphingomonas* Leaf257. a Competition assay depicting the percentage (%) of total population of *Rhizobium* Leaf68 wild type (light gray) versus Leaf68 bioB::KmR (dark gray) without treatment (control), mock treatment (ddH2O) and biotin treatment post inoculation (p.i) (see methods). For statistical analysis a two-sided paired t-test was performed. A 1:1 mixture is indicated at 50% with the red dotted line. b Depiction of the bacterial colonization (CFUs per gram fresh weight) for Leaf68 wild type (light gray), Leaf68 bioB::KmR (dark gray), *Sphingomonas* Leaf257 (orange) during mono- and co-colonization and mock (ddH2O) treatment of the plant. c Bacterial colonization (CFUs per gram fresh weight) of Leaf68 (light gray), Leaf68 bioB::KmR (dark gray) and Leaf257 (orange) during mono- and co-colonization treatment with either ddH2O, dethiobiotin or biotin *in planta* (see "Methods"). Each data point represents the CFUs of a single plant with a total of 12 plants for this experiment, except for the mock (ddH2O) in A due to a contamination. In all graphs, the median and 95% confidence interval are shown. For (b, c), statistical analysis was performed using the Kruskal-Wallis test (*P*-values: *p*-value < 0.05, **p*-value < 0.01, ***p*-value < 0.001, and ****p*-value < 0.0001). Source data are provided as a Source data file.
bacterial interactions in native-like conditions in a controlled environment.\cite{27,28} Omics approaches such as transcriptomics, proteomics and metabolomics offer the possibility to record specific phenotypic states at given time points.\cite{25,44,47,49,85,86} Here, we characterized the in situ phenotype and interaction of two genome-sequenced bacterial isolates of prevalent bacterial taxa from the At-LSPIHERE\cite{29} by mass spectrometry-based proteomics and revealed their ecology and adaptive strategies underlying their coexistence in the phyllosphere.

We first investigated both bacteria colonizing the plant in mono-colonization to establish their general ecological niche occupancy. The proteomics data of the bacteria, Rhizobium Leaf68 and Sphingomonas Leaf257, in planta compared to artificial media as reference indicated adaptive traits that were in line with previous observations in the phyllosphere.\cite{25,42,44,47,49,67} We observed the expression of species-specific but also shared adaptive strategies towards the phyllosphere environment (Supplementary Fig. 4). Overlapping ecological niches makes the pair interesting to study their coexistence with regard to niche separation to evade competition, here referred to as dynamic character displacement. Indeed, both bacteria reacted to each other upon co-colonization, observable in changes in absolute cell abundance and differential expression in the proteome (Fig. 2). Niche separation can be observed in Sphingomonas Leaf257, which allocates its proteome towards the degradation of plant-derived xylan and simultaneous utilization of the derived monosaccharides. The niche versatility of Sphingomonas Leaf257 is further underscored by the induction of numerous TonB-dependent receptors and efflux systems also observed in other Sphingomonas under competitive environmental conditions.\cite{25} The tendency of Sphingomonadaceae to utilize a wide plant-associated substrate range might be an evolutionary result of their consistent phyllosphere colonization across different plant species.\cite{20,26,87,88} Especially, the ability to degrade plant-derived xylan, which is a common resilience trait, enabling Sphingomonas to explore new niches during co-colonization with other phyllosphere microbiota members. While the accessibility of xylan to leaf epiphytes is not fully clear, the leaf contains areas where the cuticle thickness can vary greatly,\cite{89} and accessibility might be higher at veins, leaf margins and the basis of trichomes.

During co-colonization, Rhizobium Leaf68 induces the glyoxylate cycle and allocates proteome resources towards the beta-oxidation of fatty acids. Notably, fatty acids are important structural components and precursors of the plant cuticle making it a potentially resource for nutrients. Overall, the proteomes of both species suggest that both bacteria undergo a niche separation to evade competition. Since changes were inducible and occurred specifically upon coexistence of the two species we refer to dynamic character displacement rather to extend the concept of ecological character displacement that refers to the genetic difference of strains.\cite{2}

We found that facilitation by vitamin sharing contributes to the increase of the Rhizobium Leaf68 cell numbers in the presence of Sphingomonas Leaf257 (Fig. 2b). We identified the biotin precursor dethiobiotin to be crucial for this population gain: In contrast to wild-type cells, the Rhizobium Leaf68 bioA8 mutant did not profit from the presence of Sphingomonas Leaf257 nor from dethiobiotin addition but showed an increase in cell numbers after addition of biotin to the phyllosphere (Fig. 5c, Supplementary Fig. 8a). To understand the nutritional and molecular basis facilitating microbe-microbe interactions, further studies are needed to elucidate the metabolic profile of the phyllosphere regarding free vitamin pool sizes on leaves. However, the finding that the addition of biotin or its precursor dethiobiotin to plants increases the population of Rhizobium Leaf68 indicates that the vitamin is growth limiting, but that some biotin or precursor is available. The microbiota, or as tested here, Sphingomonas Leaf257 helps to supply the biotin precursor dethiobiotin rather than biotin as revealed by the importance of biotin synthase BioB in the interaction and fitness gain of Rhizobium Leaf68. Passive leakage might occur for dethiobiotin as observed for biotin.\cite{90} It is currently unclear why dethiobiotin rather than biotin becomes available. An explanation might be the chemical stability of dethiobiotin compared to biotin. Under mild conditions and in oxic environments, such as the phyllosphere, biotin is readily oxidized to biotin sulfoxide, while dethiobiotin is not, due to the absence of sulfur. In line with this conclusion is the observation that Rhizobium Leaf68 induced a biotin sulfoxide reductase during mono-colonization but did not change its expression during co-colonization with Leaf257 (Supplementary Data 2 and 5). This suggests that Rhizobium Leaf68 scavenges biotin sulfoxide that could become available in the phyllosphere but relies on the dethiobiotin obtained from Sphingomonas Leaf257.

Metabolic dependencies are thought to strengthen the interaction among bacteria by sharing metabolites, leading to adaptive gene loss.\cite{91,96} Although vitamin sharing by Sphingomonas Leaf257 was shown to be sufficient to explain the growth facilitation of Rhizobium Leaf68, also the release of extracellular enzymes for xylan degradation by Sphingomonas Leaf257 could be used as a shared public good helping other species through active niche construction, making Sphingomonas Leaf257 a potential key stone species. In a previous study Rhizobium Leaf68 was not impacted by the presence or absence of one specific strain in a 62 member community.\cite{27} However, many strains might share vitamins, making the strain the most commonly detected one in a higher complexity microbiota. While Sphingomonas Leaf257 was not tested in this particular complex community, another Sphingomonas strain, Leaf231, was identified as a keystone strain.

In summary, we used MS-based proteomics to characterize metabolic shifts and identified the phenotypic plasticity of interacting species, here referred to as dynamic character displacement. While our data demonstrate that both bacteria respond to each other and become more dissimilar in their respective proteome constitution under mono- versus co-colonization, investigations on the microscale level will remain to be conducted. Specifically, the heterogeneous nature of leaves\cite{22,41,97–99} will give rise to subpopulations with each potentially expressing different adaptations to their local microenvironment. While our bulk approach captured changes upon co-colonization, further investigation at the single-cell level will be of interest. Here, microscopy-based approaches such as FISH to visualize bacterial populations in situ,\cite{40,41,100} and fluorescent reporters to monitor expression with spatial resolution\cite{22,41,97} or par-seqFISH\cite{101} could be applied. Our ability to combine in situ phenotyping and relate it to the genetic potential of phyllosphere colonizing bacteria leads us to speculate that the observed dynamic character displacement, as described here, is an important mechanism during the formation of stable microbial communities, and supports the notion that both genetic diversity and phenotypic diversity promotes coexistence, which remains underexplored.\cite{10,17,18} This study contributes to our understanding about how bacteria coexist in complex, oligotrophic environments. Such understanding is a prerequisite for the design of stable, biologically relevant synthetic communities. Due to redundant functions of microbiota members, a bottom-up approach of studying pairs of interactions under controlled conditions represents a way to uncover bacteria-bacteria interactions and together with higher complexity synthetic communities and environmental studies will contribute to a better understanding of the dynamics and structure of...
biological diverse communities, their assembly and ultimately their astonishing persistence.

Methods

Strains and growth conditions. The two bacterial strains Sphingomonas Leaf257 and Rhizobium Leaf68 were selected from the At-LSPHERE collection based on their colonization capacity and previous data collected25,26. Both strains were grown on R-2A-agar (Sigma-Aldrich, Buchs, Switzerland) supplemented with 0.5% v/v methanol (R2A + M) for 3 days at 22 °C prior usage.

Mutant strain construction in Rhizobium Leaf68. The list of primers used is provided in Supplementary Data 6. For the generation of deletion mutants of Leaf68 genomic regions of ~650 bp were amplified using the upstream (HR1) and downstream (HR2) primers for each mutant respectively (Supplementary Data 6) followed by digestion with MnlI/KpnI (upstream) and KpnI/NsiI (downstream) and inserted into the pREDuX vector102. The construction was cleaved with KpnI between the two flanking regions of the respective gene and a kanamycin-resistance cassette (KmR) was inserted which has been cut from pPGD-KmR102, with KpnI. The orientation of the cassette was confirmed by PCR with primers binding the flanking region (OR/OF, this study) and kanamycin cassette (Kan-2/-4 described previously by Ledermann et al., 2016102). Both possible insertion directions were selected, and electroporated into Rhizobium Leaf68. For this, 100 µg/pellet competent cells were pulsed with 0.5 µg plasmid DNA at 2.2 kV in a 1-mm-gap cuvette (MicroPulser BIO-RAD). After 5 h of recovery in half LB medium at 28 °C cells were plated onto R2A agar plates containing 50 µg mL−1 kanamycin. The insertion deletion mutants of all clones were confirmed by PCR using primers inside (IFTIR) and outside (OF/OR) the flanking region, respectively.

Growth assay in micro-titer plates. Overnight pre-cultures were grown in 20 mL minimal medium containing 0.5% glucose and 1% v/v methanol (MM + M) (G™) in 100 mL baffled shake flasks at 28 °C. For Rhizobium Leaf68 50 µM of biotin, pantothenic acid and niacin were added to complement the auxotrophies. For the validation of the auxotrophies (Supplementary Fig. 5a-e) another pre-culture in MM + G without vitamins was inoculated. After 2-3 doublings cells were collected. To remove the remaining substrate, cells were spun down at 3220 × g at room-temperature for 10 min. The pellets were washed with two volumes equivalents of 10 mM MgCl2 and re-suspended in 10 mM MgCl2. The OD600 was measured every 10 min using a Tecan Infinite M200 Pro spectrophotometer (Tecan) with a bandwidth of 9 nm and 25 °C. The growth rate was calculated and estimated the bacterial growth as described above and plotted as a function of the bacterial fraction. For each biological replicate the enriched cell pellet corresponding to 20 plants was pooled, snap frozen and stored at −80 °C until further processing.

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Proteomics of bacteria recovered from the phyllosphere. In total, four biological independent replicates were generated for MS-based proteomics analysis. As a reference to the in planta conditions, both Rhizobium Leaf68 and Sphingomonas Leaf257 were each grown on R2A + M-agar (see above) in the plant growth chamber at the settings described above. Bacterial cell pellets were dissolved and lysed using a lysis buffer containing 100 mM ammonium bicarbonate, 8 M urea and 1% v/v Complete EDTA-free protease inhibitor cocktail (Sigma-Aldrich, Buchs, Switzerland) and indirect sonication (3 x 1 min, 100% amplitude, 0.8 cycle time) in a Misonix Sonicator (HIFU, Hielscher, Teltow, Germany). Proteins were removed by centrifugation at 13,000 g for 15 min at 4 °C. Protein concentration of supernatant was determined using the Pierce BCA assay kit (Thermo Fisher Scientific, Reinch, Switzerland) according to the manufacturer’s instructions. Protein disulide bonds were reduced and cysteine residues were alkylated as described previously using 5 mM 2-mercaptoethanol and 10 mM iodoacetamide respectively (IAA, Sigma-Aldrich, Buchs, Switzerland) and 10 mM iodoacetamide respectively (IAA, Sigma-Aldrich, Buchs, Switzerland). Prior to protein digestion, samples were diluted 1 to 5 with freshly prepared 50 mM ammonium bicarbonate buffer to reduce the urea concentration below 2 M. Sequencing grade modified trypsin (Promega AG, Dübendorf, Switzerland) was added at an trypsin to protein ratio of 1:50 and protein digestion was carried out overnight at 37 °C with shaking at 300 rpm. After incubation, trypsin was inactivated using heat incubation in a waterbath shaker at 95 °C for 5 min followed by acidification through addition of formic acid to an approximate final concentration of 1%. Insoluble parts were removed by centrifugation at 20,000 g for 10 min and the supernatant was desalted using Sep-Pak Vac C18 reversed phase columns (Waters Corporation, Baden-Dättwil, Switzerland) as previously described109 and dried under vacuum. The samples were
re-solubilized in 3% acetonitrile (ACN) and 0.1% formic acid (FA) to a final concentration of 0.1–1.0 mg mL$^{-1}$.

**MS analysis.** Mass spectrometry analyses were performed on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fischer Scientific) equipped with a digital PicoView source (New Objective, Littleton, USA) and coupled to a M-Class ultra-performance liquid chromatography (UPLC) system using an ACN/water solvent system containing two channels with 0.1% FA (v/v) in water for channel A and 0.1% FA (v/v), 99.9% ACN (v/v) for channel B. For chromatographic separation, 2 µL peptide sample (at a concentration of 0.5 µg µL$^{-1}$) was loaded on a nanoEase M/Z Symmetry C18 trap column (100 A, 5 µm; 180 µm × 20 mm, Waters) followed by a nanoEase M/Z HSS T3 column (100 A, 1.8 µm; 75 µm × 250 mm, Waters). Peptide samples were separated at a flow rate of 300 nL·min$^{-1}$ using the following gradient; 2% to 5% B in 2 min, 25% B in 93 min, 35% B in 10 min, and 100% B in 10 min. The MS was operated in data-dependent acquisition and top-speed mode with a maximum cycle time set to 3 s. Full-scan MS spectra were acquired in the Orbitrap analyzer with a mass range of 300–1500 m/z and a resolution of 120k with an automated gain control (AGC) target value of $5 \times 10^5$. Precursor ions were isolated with a window of 1.6 m/z and fragmented using higher energy collisional dissociation (HCD) with a normalized collision energy of 30%. For fragmentation only precursor ions with charge states from +2 to +7 and a signal intensity of at least $5 \times 10^5$. Fragment ion spectra (MS/MS) were acquired in the ion trap operated in rapid scan mode with an AGC value of 8000 using a maximum injection time of 30 ms. The dynamic exclusion was set to 25 s. Quantitative measurements were acquired using internal lock mass calibration on m/z 371.10124 and 445.12003.

**Database search and quantitative analysis.** Label-free precursor (MS1) intensity-based quantification was performed using Progenesis QI for proteomics (Nonlinear Dynamics, v.4.0) as previously described45,105. Briefly, for all samples the automatic alignment was reviewed and manually adjusted before normalization. From each Progenesis peptide ion (default sensitivity in peak picking) a maximum of the top five tandem mass spectra were exported as mascot generic files (*.mgf) using the charge deconvolution and deisotoping option and a maximum number of 200 peaks per MS/MS. The mascot generic files were searched using the Mascot Server (Matrix Science, v.2.6.2) against a decoyed and reversed protein mixture. We only considered precursor ions specific for proteins from Leaf68 (NCBI Taxon ID: 1736321) and the 4024 annotated proteins of Sphingomonas Leaf257 (NCBI Taxon ID: 1736309) contained in the 4341 annotated proteins of the Mascot Server (Matrix Science, v.2.6.2) against a decoyed and reversed protein mixture we only considered precursor ions specific for proteins from Leaf68 for normalization and vice versa. For protein quantification, the three most abundant peptide ions (Hi-3 approach) were used for protein quantification. Only proteins with two or more unique peptides detected were considered for quantification. For statistical testing, one-way ANOVA was applied and the resulting corrected using the Benjamini-Hochberg procedure directly in Progenesis QI for proteomics.

**Comparison of orthologous protein groups in Leaf257 and Leaf68.** The annotated genomes for both strains were obtained from the PATRIC database114. Subsequently, the genomes were annotated with orthologous groups (OGs) using eggNog v.4.5.15,12. In order to identify shared OGs, the proteomics data of both bacteria were used, using the same filtering strategy during multi-protein database searching. The scaffold spectrum reports were imported into Progenesis QI normalization was performed on all precursor ions of the corresponding strain. Notably, in the mixture and artificial mixture condition normalizations was performed on all precursor ions from Leaf68 or Leaf257 respectively, i.e. to quantify the Leaf68 proteome in the mixture we only considered precursor ions specific for proteins from Leaf68 for normalization and vice versa. For protein quantification, the three most abundant peptide ions (Hi-3 approach) were used for protein quantification. Only proteins with two or more unique peptides detected were considered for quantification. For statistical testing, one-way ANOVA was applied and the resulting P-values were corrected using the Benjamini-Hochberg procedure directly in Progenesis QI resulting in q-values. If not indicated otherwise the general cutoffs for significantly regulated proteins were q-value $< 0.05$ and Log2 fold-change (FC) $>1.5$ or $<-1.5$.

**Experimental investigation of sugar preferences in Sphingomonas Leaf257 using HPLC.** The sugar utilization characterization of Leaf257 was performed in a volume of 20 mL in 100 µL baffeled shake flasks at 22 ± 2°C and 160 rpm on a Minotron Incubator (Infors HT). The minimal media contained either of the three sugars (10 mM), glucose, xylose and galactose (Sigma-Aldrich, Buchs, Switzerland) alone as sole carbon source or binary combinations of each. To estimate bacterial growth OD$_{600}$ of all conditions was measured over eight-time points and simulated using HPLC following media after five tandem mass spectra were exported as mascot generic files (*.mgf) for all samples. The data obtained from the MS-based proteomics approach has been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository115 with the dataset identifier PXD026619 and 10.6019/ PXD026619 (https://www.ebi.ac.uk/pride/archive/projects/PXD026619). A list of all identified protein is available from the PRIDE dataset. The data generated in this work are provided in the Supplementary Information and Source Data files.

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Author contributions

L.H., M.A. and J.A.V. designed the study; L.H. led the experimental work; B.A.M. and M.B.-M. contributed to performing the plant experiments; M.B.-M. helped with the mutant generation in *Arabidopsis* K12; S.C. contributed to the characterization of the auxotrophs in *Rhizobium* Leaf68; C.B. performed the microscopy analysis; I.H. performed the bioinformatics analysis; I.H. and J.A.V. wrote the manuscript with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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