Host preference and invasiveness of commensal bacteria in the Lotus and Arabidopsis root microbiota

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Roots of different plant species are colonized by bacterial communities, that are distinct even when hosts share the same habitat. It remains unclear to what extent the host actively selects these communities and whether commensals are adapted to a specific plant species. To address this question, we assembled a sequence-indexed bacterial culture collection from roots and nodules of Lotus japonicus that contains representatives of most species previously identified using metagenomics. We analysed taxonomically paired synthetic communities from L. japonicus and Arabidopsis thaliana in a multi-species gnotobiotic system and detected signatures of host preference among commensal bacteria in a community context, but not in mono-associations. Sequential inoculation experiments revealed priority effects during root microbiota assembly, where established communities are resilient to invasion by latercomers, and that host preference of commensal bacteria confers a competitive advantage in their cognate host. Our findings show that host preference in commensal bacteria from diverse taxonomic groups is associated with their invasiveness into standing root-associated communities.

Results

Host-species-specific bacterial culture collections. We compared the bacterial communities associated with roots of Lj and At plants grown in the same soil (experiment (exp.) A, Extended Data Fig. 1a and Supplementary Table 2)13,15 and confirmed that both hosts associate with communities that are clearly distinct from those of the surrounding soil (Fig. 1a,b). This shift is characterized by a decrease in alpha diversity (within-sample diversity; Fig. 1a) as well as by a separation between root, rhizosphere and soil samples (beta diversity; Fig. 1b, principal coordinates analysis (PCoA) 1). In addition, Lj and At root samples formed two distinct clusters, indicating host-species-specific recruitment of commensals from identical pools of soil-dwelling bacteria (Fig. 1b, PCoA 2), which is in line with previous studies15,16. This separation (28% of variance, \( P = 0.001 \)) was mainly explained by the different relative abundance of Proteobacteria, Actinobacteria, Bacteroidetes (Flavobacteria and Sphingobacteria) and Firmicutes (Bacilli) in Lj compared to At (Extended Data Fig. 2).

To investigate plant host preference of commensal bacteria, we assembled a collection of culturable bacterial species from the roots and nodules of the model legume Lotus japonicus (hereafter Lj) that is comparable to the collection previously established from Arabidopsis thaliana (hereafter At) roots4 in terms of taxonomic and genomic composition, despite 125 Myr of divergence between Lj and At whose crown groups evolved 65 and 32 Mrya, respectively17. These two collections originate from plants grown in the same soil, enabling us to design SynComs for microbiota reconstitution experiments. Using this setup, we investigated host preference of commensal communities and the role of nitrogen-fixing nodule symbiosis, immunity and root exudation in microbiota establishment.

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Fig. 1 | Lotus and Arabidopsis root-associated bacterial communities. a, Alpha diversity analysis of soil- (n = 8), rhizosphere- (n = 13 for Gifu, n = 15 for Col-0) and root-associated bacterial communities (n = 13 for Gifu, n = 15 for Col-0) from Lj and At plants grown in natural soil (exp. A), assessed using the Shannon index. b, PCoA of Bray–Curtis dissimilarities of the same communities (n = 64). c, e, Rank abundance plots of OTUs found in the Lotus (c) and Arabidopsis (e) natural root communities. Community members captured in the corresponding culture collection are depicted as black while non-recovered OTUs are shown in white. The vertical axis on the right shows the accumulated relative abundance in natural communities of all recovered OTUs. d, f, Percentage of abundant OTUs (≥0.1% RA) associated with Lotus (d) or Arabidopsis (f) roots in nature (natural communities, NatComs) that are captured in the Lotus or the Arabidopsis IRLs (At- and Lj-IRL).
Lj root and nodule microbiota (Extended Data Fig. 1b). A total of 3,960 colony-forming units were obtained and taxonomically characterized by sequencing the bacterial 16S rRNA (Supplementary Data 1), resulting in a comprehensive sequence-indexed rhizobacterial library (IRL) from Lj (Lj-IRL). In parallel, a subset of the root samples was also subjected to amplicon sequencing to obtain culture-independent community profiles for cross-referencing with the Lj-IRL data. In the Lj collection, we were able to recover up to 53% of the most abundant bacterial operational taxonomic units (OTUs, defined by 97% 16S rRNA sequence identity) found in the corresponding natural community profiles, compared with 57% for the At collection (Fig. 1c,e and Supplementary Note). The recovered bacterial taxa in the respective collection accounted for 82% of all sequencing reads from Lj root samples and 59% from At. Approximately 45% of the abundant OTUs found in the natural communities of one host were captured in the culture collection of the other species (Fig. 1d,f), indicating a substantial overlap of the recovered bacterial taxa. Both culture collections include members of the Actinobacteria, Proteobacteria, Bacteroidetes and Firmicutes, the four phyla robustly found in the root microbiota of diverse plant species.  

To establish a core Lj culture collection of whole-genome sequenced strains (Lj-SPHERE), we selected from the Lj-IRL a taxonomically representative subset of bacterial isolates maximizing the number of covered taxa, as previously done for At (Methods). A total of 294 isolates belonging to 20 families and 124 species, including both commensal and mutualistic bacteria, were subjected to whole-genome sequencing (WGS) (Supplementary Data 2). Comparative analyses of all sequenced isolates from both collections revealed an extensive taxonomic and genomic overlap between exemplars derived from Lj and At (Extended Data Fig. 3 and Supplementary Note). This indicates that the observed differences in natural community structures (Fig. 1b) are probably not driven by the presence of host-specific bacterial taxonomic groups. Instead, the distinct root community profiles of the two hosts are possibly due to differences in the relative abundance of shared taxonomic groups (Extended Data Fig. 2).  

Host preference of commensal synthetic communities. Given the overlap between the Lj- and At-SPHERE culture collections at a high taxonomic and whole-genome level, we speculated that strain-specific phenotypic variation in planta could allow commensal bacteria to preferentially colonize their cognate host. To test this hypothesis, we designed taxonomically paired SynComs for each host, representing 16 bacterial families present in both collections (Fig. 2). We then combined these SynComs into a mixed community composed of 32 strains (Supplementary Table 1). We allowed commensal bacteria to compete for colonization of the host from which they were derived (hereafter referred to as native strains) with strains isolated from the other plant species (non-native strains, Supplementary Fig. 1a). We used a gnotobiotic system to grow wild-type At (Col-0), Lj (Gifu) and a Lj mutant deficient in root nodule symbiosis (Ljnfr5) in the presence of the mixed community (Fig. 3a). After 5 weeks, we performed community profiling via 16S rRNA gene amplicon sequencing of the root, rhizosphere and unplanted soil compartments. Analysis of community diversity revealed a significant separation (P = 0.001) of communities of root samples from those of rhizosphere and soil, which in turn clustered together (exp. B, Fig. 3b). In addition, we observed that the two hosts are colonized by distinct root microbial communities starting from the same input, and that samples from wild-type Lj are differentiable from those of Ljnfr5 (Fig. 3b). These results were confirmed by two independent, full factorial experiments using different mixed communities (exp. C and M, Extended Data Fig. 4a,c). An additional experiment, where strains belonging to families found exclusively in the Lj or At culture collections (two and five families, respectively) were added to the mixed community, resulted in similar patterns of beta diversity (exp. D, Extended Data Fig. 4b). These results recapitulate the community shifts between compartment, host species and plant genotype, which were previously observed in culture-independent community profiles obtained from plants grown in natural soils (Fig. 1b)\cite{15,16,17}, thus validating our comparative reconstitution system to study host-species-specific microbiota establishment.  

Next, we tested whether communities of commensal bacteria would preferentially colonize roots of their cognate host species (that is, from which they were originally isolated) compared to those of the other host. We found that the aggregated relative abundance of strains from the Lj-SPHERE collection was higher in wild-type Lj root samples than in those of At (Fig. 3e and Extended Data Figs. 4d–f). Likewise, strains from the At-SPHERE collection were more abundant on their cognate host than on Lj. Commensal host preference and host species community separation was reduced but still present in the Ljnfr5 mutant (Fig. 3e), suggesting that nodule symbiosis only partially contributes to commensal host preference. Further, sequential in silico removal of individual bacterial families did not alter the observed patterns of host preference at the community level (Extended Data Fig. 5), indicating that host preference was not driven by a single taxonomic group. Mono-association experiments with Lj and At wild-type plants grown on agar plates revealed that most community members maintained their root colonization capacity, but did not show a significant host preference in isolation (exp. E, Extended Data Fig. 6), suggesting that this commensal phenotype requires a community context. Moreover, we found that shoot biomass of both host species was not affected by these strains, confirming their commensal lifestyle in mono-associations (Extended Data Fig. 7).  

We then investigated if the phenotype of commensal host preference was conserved in a plant phylogenetic framework. We selected two additional plant species, Lotus corniculatus and Arabidopsis lyrata, which diverged from Lj and At approximately 12.5 and 13 Mya, respectively\cite{18,19,20}, and are indigenous to the region from which the soil used to isolate these bacterial strains was collected\cite{21,22}. We inoculated these four species with a mixed community of Lj and At commensals and obtained amplicon profiles of root, rhizosphere and unplanted soil samples (exp. F). We observed a significant separation between Lotus and Arabidopsis root communities (Fig. 3c, P = 0.001), and to a lesser extent between samples from the sister species within the same genus (Extended Data Fig. 8), which is in line with similar results obtained from At relatives grown in natural sites\cite{23}. We found that the patterns of host preference observed in Lj and At were retained in their relative species (Fig. 3f), suggesting that this community phenotype might be the result of commensal adaptation to root features conserved in a given host lineage.  

Host factors driving preferred associations in the root microbiota. Previous studies have reported shifts in At leaf or root microbiota structure in mutants impaired in different host immunity pathways\cite{24,25}. We speculated that the plant immune system might also play a role in selecting commensal bacteria in a host-specific manner. We thus tested whether host mutants impaired in perception of ubiquitous microbi-associated molecular patterns (MAMPs) were also preferentially colonized by native commensal strains (exp. G). Community profiles of roots of At and Lj mutants lacking the receptor FLS2, which detects the bacterial flagellin epitope flg22 (Ljfls2 and Athfls2)\cite{26,27,28,29}, were indistinguishable from those of their respective wild types (Extended Data Fig. 9a). Similar results were obtained with an At mutant lacking MAMP coreceptors BAK1 and BKK1 as well as CERK1 receptor kinase, known to play a role in the perception of the bacterial MAMP peptidoglycan (Attbc triple mutant)\cite{30}. In addition, bacterial host preference was retained in those mutants (Extended Data Fig. 9b). A separate experiment using the dde2 ein2
compounds by for glucosinolate biosynthesis, we speculated that secretion of these all three major defence phytohormone signalling pathways (sali-
reconstitution experiments.

At and = 294, blue track) core culture collections. Arrows in the outer rings indicate the strains selected for five mixed communities used in

pad4 sid2 (deps) mutant in At, which is simultaneously defective in all three major defence phytohormone signalling pathways (salicylic acid, jasmonate and ethylene)27, showed comparable results (exp. H, Extended Data Fig. 9c,d). Together, these data indicate that the tested MAMP receptors and immune signalling pathways do not play a crucial role in preferential colonization by native commensal bacteria.

Plant root exudates contain molecular cues that can be differentially metabolized or perceived as signals by root microbiota members35,36. In particular, glucosinolates, a group of nitrogen- and sulfur-containing metabolites found in root exudates throughout the family Brassicaceae, including At, are known to play a role in plant defence and serve as precursor of compounds that inhibit microbial growth31–33. Since legumes such as Lj lack genes required for glucosinolate biosynthesis, we speculated that secretion of these compounds by At might contribute to the observed differences in community structure. We therefore tested whether the At cyp79b2 cyp79b3 double mutant34, which is defective in the production of microbe-inducible and tryptophan-derived metabolites, including indole glucosinolates, was also preferentially colonized by native commensal strains (exp. H). Comparison of bacterial community profiling data indicates that indole glucosinolate had no effect on overall community structure or bacterial host preference in planta (Extended Data Fig. 10). Notably, incubation of bacterial SynComs in root exudates from Lj and At plants in an in vitro millifluidics system (exp. I) resulted in small but significant community separation according to the plant genotype (Supplementary Fig. 1a, 5% of variance, $P = 0.002$). However, in this system, we observed a loss of the host preference phenotype (Supplementary Fig. 1b), indicating that root exudates from axenic plants are not sufficient to recapitulate this phenomenon. This observation prompted the question of whether live root tissue was required for preferential colonization by native commensals. We profiled the bacterial communities associated with dead root material from flowering Lj and At wild-type plants and with inert lignocellulose matrices (softwood birch toothpicks) at 5, 12 and 19 d after inoculation with a mixed community (exp. J). Diversity analyses showed that dead roots and toothpicks harboured distinct microbial communities that were separated from those of soil or detritusphere (soil surrounding dead roots), independently of the timepoint (Fig. 3d). This separation was probably driven by an increase in the relative abundance of Flavobacteria, a taxon associated with the capacity to decompose complex polysaccharides37, and which dominates the dead root communities (53% RA on average). Unlike the large separation between living Lj and At roots (36% of the variance), we observed only a small but significant differentiation between Lj and At dead root communities (6.4% of variance, $P = 0.001$). Additionally, commensal host preference was undetectable in dead roots, where Lj- and At-derived strains reached similar aggregated relative abundances in root material harvested from either host (Fig. 3g). Taken together, these results suggest that a living root and other factors besides root exudates, such as a physical contact with the plant (that is, host-commensal feedbacks) are required for host preference in the root microbiota.

SynCom-specific transcriptional responses of Lj and At roots. Next, we sought to assess whether native, non-native or mixed commensal communities elicited a differential response in either host species. We grew wild-type Lj and At plants in our soil-based gnotobiotic system inoculated with Lj-, At- or mixed SynComs for 5 weeks (exp. K). Assessment of plant performance revealed that treatment with commensal communities led to increased plant biomass and bacterial load compared to axenic controls, but not to differences according to SynCom treatment (Supplementary Fig. 2). Given the observation that a living root is required for commensal host preference, we conducted RNA-sequencing (RNA-Seq) of cross-inoculated Lj and At roots to explore host transcriptional responses that might mediate this process (exp. K). Analysis of these data showed that transcriptional outputs separated according to SynCom treatment in both hosts (Fig. 4). Analysis of k-means clustering of whole transcriptomes revealed gene clusters associated with general response to bacterial colonization, as well as clusters specific to treatment with native or non-native SynComs. Among genes specifically induced by the native SynComs in both plant hosts we found several transcriptional regulators of immunity (for example, WRKY20, WRKY32 and MYB15), well-characterized MAMP receptor kinases (LYK4) and ethylene response factors (for example, ERF34). This conserved pattern of differential response in the two plant species suggests a specific transcriptional response to native commensal communities that involves components of the host immune system. The differentially expressed transcription factors identified here constitute prime candidates for future exploration of the underlying mechanisms of differential microbiota assembly.

Fig. 2 | Whole-genome phylogeny of the Lotus and Arabidopsis core culture collections. Maximum likelihood phylogeny, constructed from a concatenated alignment of 31 conserved, single-copy genes (AMPHORA) showing the taxonomic overlap of the Lj-SPHERE (n=194, red track) core culture collections. Arrows in the outer rings indicate the strains selected for five mixed communities used in reconstitution experiments.
Fig. 3 | Reconstitution experiments recapitulate culture-independent patterns and show signatures of host preference by commensal communities.

**a.** Setup of the competition experiments. **b**–**d**, Constrained PCoA (CPCoA) of Bray–Curtis dissimilarities (constrained by all biological factors and conditioned by all technical variables) of soil, rhizosphere and root samples. **b**, Lj wild-type Gifu, nfr5 mutant and At wild-type Col-0 plants cocultivated with the mixed community LjAt-SC2 (exp. B, n = 155, variance explained 53.8%, P = 0.001). **c**, Gifu, Col-0, A. lyrata MN47 (Al) and L. corniculatus cocultivated with LjAt-SC3 (exp. F, n = 173, variance explained 65.1%, P = 0.001). **d**, Dead roots of Gifu and Col-0, and toothpick cocultivated with LjAt-SC3 (exp. J, n = 250, variance explained 43.9%, P = 0.001). **e**–**g**, Aggregated RA of the 16 Lj-derived and the 16 At-derived strains in the live (e,f) or dead roots (g) of Lotus and Arabidopsis plants inoculated with LjAt-SC2 (n = 66, e) or LjAt-SC3 (n = 72, f) and n = 89, g).
Invasiveness and persistence in the root microbiota. The results obtained from four independent experiments using five different mixed communities (Fig. 3 and Extended Data Figs. 4, 9 and 10) show that native strains have a competitive advantage when colonizing roots of their cognate host. Ecological theory suggests that in the presence of a competitive hierarchy, the order of species arrival does not matter, as better adapted species tend to dominate irrespective of the history of the community38. To investigate the role that priority effects play in root community assembly we designed a series of sequential inoculation experiments using host-specific SynComs (exp. L, Fig. 5a). At and Lj wild-type plants were inoculated with taxonomically paired SynComs derived from Lj (Lj-SC3), At (At-SC3) or a mixed community (LjAt-SC3) for 4 weeks. Subsequently, we challenged the established root communities by adding the complementary SynCom (At-SC3 or Lj-SC3, respectively) to the soil matrix or, in the case of plants initially treated with the mixed community (LjAt-SC3), a mock solution (Fig. 5a).

We then allowed all plants to grow for an additional 2 weeks before harvesting. Amplicon sequencing showed a significant separation of communities by compartment, and, within root samples, according to host species (Fig. 5b, P = 0.001), mirroring the patterns observed in culture-independent community profiles (Fig. 1a).

Analysis of beta diversity of root samples at strain-level resolution revealed an effect of the treatment on community structure (Fig. 5c), demonstrating that the order of arrival of strains affects community assembly. Examination of aggregated relative abundances showed that, in a competition context (that is, initial inoculation with the mixed community LjAt-SC3), commensal SynComs preferentially colonized roots of their cognate host (Fig. 5d), in line with results from the previous competition experiment shown in Fig. 3. However, in an invasion context, early-arriving SynComs invariably reached higher proportions in the output communities compared to the late-arriving SynComs (Fig. 5d,e). Notably, estimation of absolute bacterial abundances showed that a secondary inoculation with an invading SynCom did not result in a significant increase in total bacterial load (Supplementary Fig. 3). Together, the results from our sequential inoculation experiments (Fig. 5c,d) are indicative of the existence of priority effects in the root and rhizosphere microbiota, a well-known phenomenon in microbial community assembly39. These effects could be explained by niche preemption, where early-arriving community members reduce the number of resources available (for example, nutrients, space) for latecomers40; alternatively, they could be the result of a feedback process between the host and the early-arriving commensals.

We proposed that commensal bacteria would be less affected by priority effects when colonizing their cognate host, given their competitive advantage with respect to non-native strains. To test this, we examined aggregated relative abundances of Lj- and At-derived SynComs in the root and rhizosphere communities. We found that host-specific SynComs were better able to invade a resident community in the roots of their cognate host compared to those of the other plant species (Fig. 5d), thus reducing the strength of the priority effects. However, in the rhizosphere compartment of either plant species, host-specific SynComs showed neither host preference in a competition context nor differences in their ability to invade standing communities (Fig. 5e). However, it is also possible that root communities did not reach equilibrium 2 weeks after invasion, and that the observed patterns could change over time.

We then tested if host preference was directly linked to invasiveness and to what extent these traits were found in individual community members. First, we quantified the strength of host preference by calculating the ratio between the relative abundance of each strain in their cognate host compared to the other plant species (host preference index, Methods). Notably, although Lj root samples did not include nodules, but possibly contained incipient symbiotic events, the strains with the highest host preference index were the nitrogen-fixing Lj strains belonging to the Phyllobacteriaceae family (Fig. 6a), indicating that host preference of mutualistic rhizobia is not limited to nodule tissue. In addition, multiple other commensal strains showed significant host preference, with members of the families Pseudomonadaceae, Oxalobacteriaceae, Rhizobiaceae and Microbacteriaceae robustly displaying a high host preference index. Members of these last two bacterial families also had an impact on community structure during invasion in a recent study with phyllosphere bacteria41. Next, we calculated an invasiveness index by comparing the ability of each strain to invade a standing community on their cognate host compared to the other plant species (Methods). We found a strong correlation between host preference and invasiveness of commensal bacteria, which is independent of their relative abundance (r = 0.89, P = 4.3 × 10⁻10; Fig. 6b). In contrast, this correlation was absent in the rhizosphere samples (Fig. 6c), indicating that the link between these two bacterial traits is mediated by host attributes that do not extend to the rhizosphere. Together, our data show that host preference is prevalent in commensal bacteria from diverse taxonomic groups and that this trait is tightly linked to invasiveness and together play a role during root microbiota assembly.

Discussion

The current concept of host specificity in plant–microbe interactions was originally developed based on studies using microorganisms with either pathogenic or mutualistic lifestyles. Recently, it has been shown that soilborne, nitrogen-fixing Ensifer melloti mutualists can adapt to local host genotypes in only five plant generations and proliferate to greater abundances in hosts with shared evolutionary histories42. We show here that in the Lj and At root microbiota, there is a gradient of host preference among commensals belonging to diverse taxonomic lineages. Maintenance of host preference in the sympatric relative species L. corniculatus and A. lyrata raises the possibility that these commensals might have adapted to host features conserved in the respective plant genera. Alternatively, the observed host preference patterns might be the consequence of other ecological processes, such as ecological fitting, whereby organisms are able to colonize and persist in a new environment using traits that they already possess43. Diversification of plant traits as a result of adaptation to edaphic or other environmental factors is expected to result in new host features that constitute new root niches for microbial colonization. It is also possible that host diversification is partly driven by the adaptation of plants to commensal microbiota in soils with contrasting properties. However, the observation that in our experimental conditions colonization by native or non-native bacterial SynComs had no impact on plant growth suggests that host preference is the result of microbial adaptation to host features instead of coevolution. However, it is possible that a significant impact on host fitness might be observed in long-term experimentation, or in the presence of biotic or abiotic stresses, which were absent in the tested conditions, or in direct competition with other plant species. This latter hypothesis is supported by the observation that similarity between the root microbiota of different species affects competitive plant–plant interactions and has an impact on host performance through plant–soil feedback44. Future experimentation using multi-species gnotobiotic systems and varying environmental conditions will serve to test these hypotheses.

In aquatic and terrestrial ecosystems, microbial traits such as growth rate, antagonistic activity or resource use efficiency are known determinants of invasiveness45,46. In microbial communities associated with eukaryotic organisms, the ability to interact with the host might also be required for successful invasion. Our results indicate that native commensals have a competitive advantage when invading standing communities in the root but not in soil or rhizosphere. One possibility is that increased invasiveness by native bacteria is enabled by the existence of unfilled host-species-specific
root niches that can be occupied by latecomers. Alternatively, direct interaction of commensals with their host may be required to trigger the formation of host-species-specific root niches, which could be linked to the specific transcriptional reprogramming in roots observed during colonization by native SynComs. This latter hypothesis is further supported by the observation that bacterial...
Fig. 5 | Invasion and persistence of commensal bacteria. a, Setup of the sequential inoculation experiment. Lj Gifu and At Col-0 plants were cocultivated with the mixed community LjAt-SC3, or individual SynComs Lj-SC3 and At-SC3, followed by inoculation with the contrasting SynCom (exp. L).

b, c, Constrained PCoA (CPCoA) of Bray–Curtis dissimilarities (constrained by all biological factors and conditioned by all technical variables; n = 267; variance explained 14.7%, P = 0.001) of soil, rhizosphere and root samples (b), and PCoA of root samples only (n = 137, c). d, e, Aggregated RA of the 16 Lj-derived and the 16 At-derived strains in Lotus and Arabidopsis root (d) (n = 120) and rhizosphere (e) (n = 120) samples in the indicated treatments. Different letters above boxes indicate different significance groups according to a Kruskal–Wallis test, followed by a Dunn’s post hoc.
SynComs colonizing dead roots or incubated in root exudates in vitro showed no significant host preference. Our study provides a framework to test these hypotheses and to investigate the molecular basis of host preference in multiple taxa of the bacterial root microbiota in comparison with host adaptation mechanisms in plant pathogens and mutualists.

Methods

Bacterial and plant material. Bacterial strains were grown in tryptic soy broth (15 g l$^{-1}$, TSB, Sigma-Aldrich) liquid medium or on agar plates containing 15 g l$^{-1}$ of Bacto Agar (Difco) at 25°C. Mesorhizobium strains LjNodule210, LjNodule215 and LjNodule218, isolated from Lj root nodules, were cultured in TY medium (5 g l$^{-1}$ tryptone, 3 g l$^{-1}$ yeast extract) supplemented with 10 mM CaCl$_2$ or in YMB medium (5 g l$^{-1}$ mannitol, 0.5 g l$^{-1}$ yeast extract, 0.5 g l$^{-1}$ K$_2$HPO$_4$, 3 g l$^{-1}$H$_2$O, 0.2 g l$^{-1}$ MgSO$_4$·7H$_2$O, 0.1 g l$^{-1}$ NaCl). The composition of synthetic bacterial communities (SynComs) is listed in Supplementary Table 1. Lj ecotype Gifu B-129 was used as wild-type. Symbiosis-deficient mutant nfr5-2 (ref. 36) and flagellin receptor-deficient mutant fls2 (LORE1-30003492) were derived from the Gifu B-129 genotype. For At, ecotype Columbia-0 was used as wild-type. Mutant genotypes fls2 (ref. 35), bbe$^{c}$, depp$^{c}$ and cyp79c2 cyb79c3 (ref. 36) were available in our seed stock. L. corniculatus seeds, cultivated in the North-Western German lowland, were retrieved from Rieger-Hofmann GmbH, Blaustädten-Raboldshausen, Germany. A. lyrata MN47 seeds were a gift from J. de Meaux, University of Cologne.

Establishment of the Lj bacterial culture collection. The Lj culture collection combines strains isolated during three independent isolation events. Bacterial isolation, DNA isolation and identification using Illumina sequencing were performed as previously described. Wild-type Lj (ecotype Gifu B-129) plants were grown in natural soil (Cologne agriculture soil (CAS)), batch 10 from spring 2014 and batch 11 from spring 2015) in the greenhouse and harvested after 4 or 8 weeks to cover different developmental stages. Root systems of 20 plants were subjected to DNA isolation and culture-independent community

Fig. 6 | Host preference is linked to invasiveness. a, Analysis of host preference of individual commensal strains across gnotobiotic experiments (n = 366). Each strain is represented by a dot, whose colour corresponds to its host preference index and whose size to its average relative abundance. A significant host preference (Mann–Whitney test, false-discovery-rate corrected) is depicted by a black circle around a dot. NS, not significant. b, c, Correlation between host preference and invasiveness index for each strain in root (n = 115) (b) and rhizosphere samples (n = 119) (c), respectively, obtained from the sequential inoculation experiment (exp. L). The colour of each point designates the host of origin of each strain and the size denotes its mean relative abundance (log$_{2}$ transformed). Each point is labelled with a numeric identifier that corresponds to the strains in a (LjAt-SC3). At, A. thaliana; Lj, L. japonicus; Al, A. lyrata; Lc, L. corniculatus.

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Bacterial and plant material. Bacterial strains were grown in tryptic soy broth (15 g l$^{-1}$, TSB, Sigma-Aldrich) liquid medium or on agar plates containing 15 g l$^{-1}$ of Bacto Agar (Difco) at 25°C. Mesorhizobium strains LjNodule210, LjNodule215 and LjNodule218, isolated from Lj root nodules, were cultured in TY medium (5 g l$^{-1}$ tryptone, 3 g l$^{-1}$ yeast extract) supplemented with 10 mM CaCl$_2$ or in YMB medium (5 g l$^{-1}$ mannitol, 0.5 g l$^{-1}$ yeast extract, 0.5 g l$^{-1}$ K$_2$HPO$_4$, 3 g l$^{-1}$H$_2$O, 0.2 g l$^{-1}$ MgSO$_4$·7H$_2$O, 0.1 g l$^{-1}$ NaCl). The composition of synthetic bacterial communities (SynComs) is listed in Supplementary Table 1. Lj ecotype Gifu B-129 was used as wild-type. Symbiosis-deficient mutant nfr5-2 (ref. 36) and flagellin receptor-deficient mutant fls2 (LORE1-30003492) were derived from the Gifu B-129 genotype. For At, ecotype Columbia-0 was used as wild-type. Mutant genotypes fls2 (ref. 35), bbe$^{c}$, depp$^{c}$ and cyp79c2 cyb79c3 (ref. 36) were available in our seed stock. L. corniculatus seeds, cultivated in the North-Western German lowland, were retrieved from Rieger-Hofmann GmbH, Blaustädten-Raboldshausen, Germany. A. lyrata MN47 seeds were a gift from J. de Meaux, University of Cologne.

Establishment of the Lj bacterial culture collection. The Lj culture collection combines strains isolated during three independent isolation events. Bacterial isolation, DNA isolation and identification using Illumina sequencing were performed as previously described. Wild-type Lj (ecotype Gifu B-129) plants were grown in natural soil (Cologne agriculture soil (CAS)), batch 10 from spring 2014 and batch 11 from spring 2015) in the greenhouse and harvested after 4 or 8 weeks to cover different developmental stages. Root systems of 20 plants were subjected to DNA isolation and culture-independent community
profiling via amplicon sequencing. From 45 plants, a 4-cm section of the roots was collected and rigorously washed three times with phosphate-buffered saline (130 mM NaCl (7.6 g l⁻¹), 7 mM Na₂HPO₄·12H₂O, 1.246 g l⁻¹, 3 mM NaH₂PO₄, 0.814 g l⁻¹, pH 7.0) and three times with sterile water. Nodules were separated and homogenized independently. Homogenized roots from each individual plant were allowed to sediment for 15 min and the supernatant was diluted (1:20,000, 1:80,000 and 1:60,000) with four different media: 3g l⁻¹ TS, 50% TY, Caustine yeast for enrichment of Moraxellaceae (3g l⁻¹ Caustine, 1.36 g l⁻¹ Casitone, 2H₂O and 1 g l⁻¹ yeast extract; pH adjusted to 7.2) and yeast agar van Niel’s, for enriching of Burkholderiales (10 g l⁻¹ yeast extract, 1 g l⁻¹ K₂HPO₄, and 0.5 g l⁻¹ MgSO₄·7H₂O). Bacterial dilutions cultivated in 96-well microtitre plates. Homogenized nodules from each individual plant were directly diluted (1:20,000, 1:40,000 and 1:60,000) and cultivated in 96-well microtitre plates. This procedure was carried out for individual plants to obtain bacterial isolates from different plant roots. After 10–20 d at room temperature, plates that showed visible bacterial growth in around 30 wells were chosen for high-throughput sequencing. Bacterial isolates were identified with a two-step barcoded PCR protocol described previously⁴, with the difference that at the first step of the PCR, the v5-v7 fragments of the 16S rRNA gene were amplified by the degenerate primers 799F (5′-AACMGGATTAGATACCCKG-3′) and 1192R (5′-ACGTCATCCCCACCTTCC-3′), followed by indexing was done using Illumina-barcoded primers. The indexed 16S rRNA amplicons were pooled, purified and sequenced on the Illumina MiSeq platform. Strains isolated from nodules were tested for their ability to form functional nodules in Lj Gifu plants grown on agar plates.

Cross-genus sequences with culture-independent profiles was used to identify candidate strains for further characterization, purification and WGS. Two main selection criteria were used: maximum taxonomic coverage, selecting candidates from as many taxa as possible and priority to strains whose 16S sequences were highly abundant in the natural communities. Whenever multiple candidates from the same phylum were identified, we aimed to obtain multiple independent strains, if possible, coming from separate biological replicates to ensure they represented independent isolation events. After validation of selected strains, 294 (including nine isolated from nodules) were successfully subjected to WGS for Illumina MiSeq sequencing.

For WGS, DNA was isolated from strains using the QiAmp Micro DNA kit (Qiagen). Culture-independent community profiling. Bacterial communities were profiled by amplicon sequencing of the variable v5-v7 regions of the bacterial 16S rRNA gene. Library preparation for Illumina MiSeq sequencing was performed as described previously⁴. In all experiments, multiplexing of samples was performed by double-indexing (barcoded forward and reverse oligonucleotides for 16S rRNA gene amplification).

Greenhouse experiment. Lj Gifu and At Col-0 were grown for 5 weeks in CA5 soil (batch 15 from January 2020) in 7.7 cm pots alongside uninfected controls pots under short-day conditions. Pots were watered with sterile water from the bottom as needed. Root, rhizosphere and soil samples were harvested and processed as described previously⁴. 15, 13, 18 and 8 replicates were sampled for Col-0, Gifu and uncontrolled roots, respectively. DNA was isolated from those samples using the MP Biomedicals FastDNA Spin Kit for Soil. Culture-independent community profiling.

Multi-species microbiota reconstitution experiments. We used the gnotobiotic FlowPot system⁵ to grow At and Lj plants with and without bacterial SynComs. In brief, the system allows for even inoculation of each growth pot with microbes and indexing was done using Illumina-barcoded primers. The indexed 16S rRNA amplicons were pooled, purified and sequenced on the Illumina HiSeq3000 platform. The Max Planck Genome Centre, Cologne, Germany (https://mpgc.mpimp.mpg.de/home/). Sequencing depth was 5 million reads per sample.

Collection of root exudates. Arabidopsis and Lotus plants were grown in a customized hydroponic system (original design by M. Peukert, University of Cologne, unpublished). This sterile growth setup consists of glass jars filled with glass beads and a stainless-steel mesh on top. Nutrient solution (modified 0.25x B&D medium, Fe-EDTA instead of Fe-citrate) was poured into the jars until the beads were covered in liquid and the liquid touched the metal mesh. We used the same medium for both plant species to allow for direct comparison of exudate composition, and to minimize differential effects on the bacterial community originating from different media types. We chose the Lotus B&D medium since Arabidopsis grew reasonably well in it. Sterilized and pregerminated seeds were placed onto the mesh, jars were put into sterile plastic boxes with filter lids (SacO2 medium, www.wisewaxes.com) for 3-5 weeks. The medium containing root exudates was removed from the jars in the clean bench using a sterile metal needle and plastic syringe. After transfer to 50 ml Falcon tubes, exudates were frozen at −80°C, freeze-dried until a volume of 2–3 ml was left, thawed and adjusted with sterile water to 5 ml. Exudates were kept at −80°C until further usage.

Millifluidics experiment. Bacterial inoculation in root exudates was performed in a millifluidics system (MilliDrop Analyzer, MilliDrop, www.millidrop.com). This drop-based system allows inoculation of bacteria in very small volumes of root exudates or growth medium. In brief, bacteria and exudates or growth medium are combined in wells of a 96-well plate using a pipetting robot. Freedom Evo 153 (Eppendorf) was used for 20 ml exudate drops, and then sucked in from the wells of the loading plate by a tip on the robotic arm of the MilliDrop Analyzer, generating hundreds of droplets within an oil-filled tube, separated by air spacers. During inoculation, the droplet ‘train’ moves back and forth, so that during each round, each droplet passes a detector that counts the droplets. Culture droplets are collected after the experiment and subjected to community profiling.
The mixed community LjAt-SC1 was used and was essentially prepared as described above for the in planta experiments, adjusted to OD₆₀₀ of 0.1 and used as input for preparation of the loading plate. Pure exudates (pH between 7.0 and 8.0) were transferred to squared petri dishes containing 0.25× TSB agar (Gifu) of sterile water. Serial dilutions of 10⁻⁶ to 10⁻¹ were transferred to squared petri dishes containing 0.25× TSB agar. The 32 strains of the mixed community LjAt-SC3 were grown individually in liquid medium, harvested and adjusted to an OD₆₀₀ of 0.02. Seedlings were inoculated by adding 500 µl of bacterial culture to the roots. Plants were grown for 14 d under long-day conditions (16/8 day-night cycles) at 21 °C. DNA concentration of plants grown in FlowPots (experiments K and L). DNA was isolated from roots of plants grown in CAS soil in the greenhouse, along with unplanted CAS soil. DNA from each sample was extracted by using the Quick-DNA Miniprep Kit (Thermo Fisher Scientific). For colony counts (exp. E), roots were harvested, washed, weighed and crushed in 500 µl (Col-0) or 750 µl (Gifu) of sterile water. Serial dilutions of 10⁻⁶ to 10⁻¹, 10⁻² to 10⁻¹, and 10⁻⁶ to 10⁻⁵ of the crushed roots were prepared in sterile water. Then 10 µl of each were spotted onto 10% TSB agar square plates. Single colonies were counted after 1–3 d.

Processing of 16S rRNA gene amplicon data. Amplicon sequencing data from LjAtSPHERE and At-SPHERE collections. Raw Illumina RNA-Seq reads were prepared using fastp (v0.19.10) with default settings for pair-end reads. High-quality reads were pseudo-aligned to the Lj Gifu or Col-0 transcriptomic reference genome using kallisto (v0.46.1). After removal of low abundant transcripts that were not present in at least two replicates under each condition, count data were imported using the tximport package. Differential expression analyses were performed using the DESeq2 package. First, raw counts were normalized with respect to the library size (rlog function) and then transformed into log₂ (TPM) units. We then generated a differential expression table using the sva package. Significant surrogate variables were automatically detected and integrated into the model for differential analyses. Principal component analysis based on whole transcripts were then conducted and plotted to visualize the cluster and variance of biological replicates under each condition. Transcripts with fold-changes >1.5 and adjusted P value for multiple comparisons (Benjamini-Hochberg method) to below 0.05 were considered significant.

The log, scaled counts were normalized by the identified surrogate variables using the limma package (‘removematchEffect’ function), and transformed as
median-centred z-score (by transcripts, 'scale' function). Then z-scores were used to conduct k-means clustering for all transcripts. The cluster number \( k = 10 \) was determined by sum of squared error and Akaike information criterion. Differential expressed transcripts and cluster results were visualized using heatmaps generated by ComplexHeatmap package\(^1\).

Gene ontology enrichment for each cluster using the whole Lotus and Arabidopsis transcripts as backgrounds were performed with the goseq package\(^2\), which considers the transcripts length bias in RNA-Seq data. Gene ontology annotations were retrieved from the Gene Ontology Consortium (September 2019)\(^3\)\(^4\). Significant changes in biological process Gene ontology terms (adjusted \( P < 0.05 \)) were visualized in dot plots using the clusterProfiler package\(^5\).

**Statistics and reproducibility.** All experiments were performed with full factorial (biological and technical) replication. Competition experiments using SynComs were in addition repeated multiple times (Extended Data Fig. 1) using independent bacterial communities. Whenever bacterial abundances or plant growth parameters were compared, we used a two-sided, non-parametric Mann–Whitney test or, in the case of multiple comparisons, a Kruskal–Wallis test, followed by a Dunn's post hoc. Whenever appropriate, values were adjusted for multiple testing using the Benjamini–Hochberg method (\( \alpha = 0.005 \)). Statistical tests on beta-diversity analyses were performed using a PERMANOV A test with multiple testing using the Benjamini–Hochberg method (\( \alpha = 0.005 \)). Statistic tests on beta-diversity analyses were performed using a PERMANOVA test with 5,000 random permutations. Whenever boxplots were used in figures, data were represented as median values (horizontal line), Q1 – 1.5x interquartile range (boxes) and Q3 + 1.5x interquartile range (whiskers).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The strains of the *Lj*-SPHERE collection will be deposited at and will be available on request from the Leibniz Institute DSMZ in Braunschweig, Germany. Raw 16S rRNA amplicon reads have been deposited in the European Nucleotide Archive under the accession number PRED37695. Similarly, sequencing reads and genome assemblies of the *Lj*-SPHERE core collection have been uploaded to the same database with the accession number PRED37696. Source data are provided with this paper.

**Code availability.** The scripts used for the computational analyses described in this study are available at http://www.github.com/garridoo/ljsphere, to ensure replicability and reproducibility of these results.

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Author contributions

K.W., K.T., S.R., P.-L. and R.G.-O. conceived the research and designed the experiments. K.W., K.T., R.Z. and D.B.J. established the *Lj*-SPHERE culture collection. K.W., K.T. and N.K. performed the gnotobiotic competition experiments. K.W. and E.L. conducted the in planta invasion and millifluidics SynCom experiments. R.G. and R.G.-O. analysed culture-independent amplicon data. E.D. and R.G.-O. analysed the *Lj*-IRL data. P.Z. and R.G.-O. processed bacterial whole-genome data from the *Lj*-SPHERE collection. Y.N. and R.G.-O. analysed the transcriptome data. K.W., R.G.-O. and N.K. analysed sequencing data from the SynCom experiments. K.W., K.T., S.R., P.-L. and R.G.-O. interpreted data and wrote the paper.

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Competing interests

The authors declare no competing interests.

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Extended Data Fig. 1 | Flow chart of experimental procedures. a, Overview of experiments and type of analyses performed on root, rhizosphere, and soil samples, or millifluidic droplets. b, Culture collection establishment of *Lotus japonicus* root and nodule bacterial isolates. Image created with BioRender.com.
Extended Data Fig. 2 | Culture-independent diversity analysis of root-associated bacterial communities from Lotus and Arabidopsis. Rank abundance plot of bacterial communities from Lotus or Arabidopsis roots, aggregated to the class level, including all families with a mean accumulated relative abundance of > 0.1% on either host. Statistical differences were assessed using a two-sided, non-parametric Mann-Whitney test. Asterisks represent significant values after multiple testing correction using the Benjamini-Hochberg method (P < 0.05).
Extended Data Fig. 3 | Functional overlap of Lotus and Arabidopsis culture collection genomes. PCoA of functional distances of genomes from bacterial isolates of the Lotus (Lj-SPHERE; *n* = 294) and the Arabidopsis (At-SPHERE; *n* = 194) culture collections.
Extended Data Fig. 4 | Host-species specific bacterial root communities and commensal host preference is confirmed using independent mixed communities. a and c, Constrained PCoA of Bray-Curtis dissimilarity (constrained by all biological factors and conditioned by all technical variables) of soil, rhizosphere, and root samples from L. japonicus wild-type Gifu, nfr5 mutant, and A. thaliana wild type Col-0 plants co-cultivated with the mixed community LjAt-SC1 (a, experiment C, n = 155, variance explained 53.8%, P = 0.001), from Gifu and Col-0 co-cultivated with LjAt-SC4 (b, experiment D, n = 87, variance explained 60%, P = 0.001), or from Gifu and Col-0 co-cultivated with LjAt-SC5 (b, experiment M, n = 100, variance explained 67%, P = 0.001). d, e and f, Aggregated RA of the 16 Lj-derived and the 16 At-derived strains in the roots of Lotus and Arabidopsis plants inoculated with LjAt-SC1 (d; n = 68), LjAt-SC4 (e; n = 34), or LjAt-SC5 (f; n = 40). n refers to biologically independent samples.
Extended Data Fig. 5 | Host preference is retained after in silico removal of individual bacterial families. Aggregated relative abundance of *Lotus*-(a, n = 20) and *Arabidopsis*-derived (b, n = 16) strains in roots from plants inoculated with the mixed community LjAt-SC3 (experiment L). Host preference was assessed using a Mann-Whitney non-parametric test after in silico removal of each family. The x-axis labels indicate each depleted family. n refers to biologically independent samples.
Extended Data Fig. 6 | Bacterial abundance in mono-association with host plants. Bacterial abundances of commensal bacteria (strain IDs indicated at x-axis) colonizing Col-0, Gifu, and nfr5 roots, assessed by counting of colony forming units (CFUs) after extraction from root tissue. Plants were grown for two weeks on agar plates in mono-association with the indicated Lj-SPHERE (a) and At-SPHERE (b) strains (exp. E). n = 6 (3 biologically independent samples x 2 technical replicates). Statistical differences were assessed using a two-sided, non-parametric Mann-Whitney test. Asterisks represent significant values after multiple testing correction using the Benjamini-Hochberg method (P < 0.05).
Extended Data Fig. 7 | Plant performance in mono-associations. Shoot fresh weight of Gifu (a) and Col-0 (b) plants grown for two weeks on agar plates in mono-association with the indicated Lj-SPHERE and At-SPHERE strains (exp. E). n = 30 (3 biologically independent samples × 10 technical replicates).
Extended Data Fig. 8 | Sister species of *L. japonicus* and *A. thaliana* establish distinct bacterial root communities. Constrained PCoA of Bray-Curtis dissimilarity (constrained by all biological factors and conditioned by all technical variables) of root samples from *L. japonicus* wild type Gifu and *L. corniculatus* (a; *n* = 87; variance explained 58.7%; *P* = 0.001), and or root samples from *A. thaliana* wild type Col-0 and *A. lyrata* MN47 (b; *n* = 86; variance explained 65%; *P* = 0.001), inoculated and grown with the mixed community *LjAt-SC3*, and of the corresponding rhizosphere and bulk soil communities (exp. F).
Extended Data Fig. 9 | Tested plant immune receptors and signaling pathways do not affect host preference of commensals. **a**, Constrained PCoA of Bray-Curtis dissimilarity (constrained by all biological factors and conditioned by all technical variables; \(n = 98\); variance explained 24.6\%, \(P = 0.001\)) of root samples from *L. japonicus* wild type Gifu, *Ljfls2* mutant, *A. thaliana* wild type Col-0, *Atfls2* mutant, and *Atbbc* mutant inoculated and grown with the mixed SynCom *LjAt*-SC1 (exp. G), and of the corresponding bacterial input communities. **b**, Aggregated relative abundance of the 16 *Lj*-derived and the 16 *At*-derived strains in the roots of *Lotus* and *Arabidopsis* plants. \(n = 21\) for Gifu, \(n = 16\) for Col-0, \(n = 20\) for *Ljfls2* and *Atfls2*, \(n = 18\) for *Atbbc*. **c**, Constrained PCoA of Bray-Curtis dissimilarity (constrained by all biological factors and conditioned by all technical variables; \(n = 64\); variance explained 42.2\%, \(P = 0.001\)) of soil and root samples from Gifu, Col-0, and *Atdeps* mutant inoculated and grown with the mixed SynCom *LjAt*-SC3 (exp. H). **d**, Aggregated relative abundance of the 16 *Lj*-derived and the 16 *At*-derived strains in the roots of *Lotus* and *Arabidopsis* plants. \(n = 14\) for Gifu, \(n = 20\) for Col-0 and *Atdeps*. \(n\) refers to biologically independent samples.
Extended Data Fig. 10 | Effect of secreted indole glucosinolates on host preference of commensals. a, Constrained PCoA of Bray-Curtis dissimilarity (constrained by all biological factors and conditioned by all technical variables; \( n = 50 \); variance explained 47.2%, \( P = 0.001 \)) of soil and root samples from *L. japonicus* wild type Gifu, *A. thaliana* wild type Col-0, and *Arabidopsis cyp79b2 cyb79b3* mutant inoculated and grown with the mixed SynCom *LjAt*-SC3 (exp. H). b, Aggregated relative abundance of the 16 *Lj*-derived and the 16 *At*-derived strains in the roots of *Lotus* and *Arabidopsis* plants. \( n = 20 \) for Col-0, \( n = 14 \) for Gifu, \( n = 6 \) for *Atcyp79b2b3*. \( n \) refers to biologically independent samples.
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Sample size
Sample size was based on experience from previous experiments using the same gnotobiotic system to allow for confident statistical analyses. Sample size was indicated as "n" in the corresponding figures. No statistical methods were used to determine sample size.

Data exclusions
No data was excluded.

Replication
Experiments B and C included 3 independent preparations of the corresponding SynComs with at least 18 biological replicates of planted pots each. All other experiments were performed with one SynCom preparation and at least 18 biological replicates of planted pots. Experiment M was performed in 3 biological and 6 technical replicates.

Randomization
Position of boxes with planted pots was randomized in the growth chamber during the course of the experiments. Position of pots with different plant genotypes within a box was randomized.

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