Coordination of microbe–host homeostasis by crosstalk with plant innate immunity

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Plants grown in natural soil are colonized by phylogenetically structured communities of microbes known as the microbiota. Individual microbes can activate microbe-associated molecular pattern (MAMP)-triggered immunity (MTI), which limits pathogen proliferation but hampers plant growth, a phenomenon known as the growth–defence trade-off. Here, we report that, in monoassociations, 41% (62 out of 151) of taxonomically diverse root bacterial commensals suppress Arabidopsis thaliana root growth inhibition (RGI) triggered by immune-stimulating MAMPs or damage-associated molecular patterns. Amplicon sequencing of bacterial 16S rRNA genes reveals that immune activation alters the profile of synthetic communities (SynComs) comprising RGI-non-suppressive strains, whereas the presence of RGI-suppressive strains attenuates this effect. Root colonization by SynComs with different complexities and RGI-suppressive activities alters the expression of 174 core host genes, with functions related to root development and nutrient transport. Furthermore, RGI-suppressive SynComs specifically down-regulate a subset of immune-related genes. Precolonization of plants with RGI-suppressive SynComs, or mutation of one commensal–downregulated transcription factor, MYBIS, renders the plants more susceptible to opportunistic Pseudomonas pathogens. Our results suggest that RGI-non-suppressive and RGI-suppressive root commensals modulate host susceptibility to pathogens by either eliciting or dampening MTI responses, respectively. This interplay buffers the plant immune system against pathogen perturbation and defence-associated growth inhibition, ultimately leading to commensal–host homeostasis.

U biquitous interactions within and between microbial communities and their plant hosts often shape host phenotypes and drive community diversification, leading to the conceptualization of plants and their associated microbes as discrete ecological units, or holobionts1. Analysis of A. thaliana grown in different locations has shown that plants accommodate a conserved core microbiota—microbial assemblages that represent a subset of microbes from the surrounding soil seeding inocula2–4. Although most microbiota members are commensals, a small number provide beneficial services for the host5, or become pathogenic under favourable conditions. Recent studies have shed light on how specialized metabolites6–11 and abiotic stresses12,13 influence host-associated microbiota. However, how microbe–host homeostasis is maintained after perturbation remains poorly understood.

Plants have evolved a sophisticated innate immune system to protect themselves against pathogens. One arm of this system is activated by the extracellular perception of microbe-associated molecular patterns (MAMPs)/PAMPs that are recognized by host pattern recognition receptors (PRRs). For example, the bacterial flagellin-derived epitope flg22 is detected by the cognate PRR FLS2. Both pathogenic and beneficial bacteria can carry flg22 epitope variants14, resulting in MTI15,16. MTI effectively restricts pathogen proliferation17 but, if unrestrained, MTI may result in plant growth penalties, a phenomenon known as the growth–defence trade-off18. Pathogens have evolved diverse mechanisms to suppress MTI19; however, this property is not limited to harmful bacteria, as a previous report has shown that commensal Alphaproteobacteria from the Arabidopsis root culture collection (At-RSPHERE)20 can also override flg22-mediated RGI1. Similarly, the beneficial rhizobacterium Pseudomonas simiae suppresses more than half of the MAMP-triggered transcriptional responses in monosociation with Arabidopsis, possibly through acidification of the rhizosphere1,22. However, how plants tolerate a rich diversity of commensals without compromising effective resistance to pathogens is unknown. In this Article, we used a bottom-up approach to show that phylogenetically diverse root commensals can modulate plant immunity, and that their combined interactions in community contexts coordinate commensal–host homeostasis under pathogen challenges23,24.

Results
Taxonomically widespread ability of root commensals to interfere with defence-associated growth inhibition. To facilitate the screening of individual root commensals of the At-RSPHERE culture collection, we took advantage of a flg22-hypersensitive line, pWER::FLS2-GFP25,26, in which the flg22 receptor FLS2 is overexpressed but restricted to the root epidermis. This hypersensitivity leads to an enhanced signal-to-noise ratio for flg22-mediated RGI (Extended Data Fig. 1a). After three weeks of co-culturing with individual bacterial isolates and flg22, 41% of the strains (62 out of 151) were found

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to interfere with RGI. RGI-suppressive activity was detected across all four phyla of the microbiota—Actinobacteria, Proteobacteria, Bacteroidetes and Firmicutes—but was overrepresented among Actinobacteria and Gammaproteobacteria commensals (Fig. 1a).

Fig. 1 | At-RSPHERE root commensals exhibit strain-specific variations to suppress flg22-mediated RGI in pWER::FLS2-GFP plants. a, Phylogenetic tree showing the distribution of strains exhibiting RGI-suppressive activity. The outer rings represent root lengths of plants (aged 3 weeks) germinated on plates supplemented with 1 μM flg22 and individual strains (OD_{600} = 0.0005). n = 2 independent replicates. The threshold for suppressive activity is indicated by the red line, that is, root length > 2 cm. b, The impact of four independent five-member SynComs (Supplementary Table 1), which differed in RGI-suppressive activity, on flg22-mediated RGI. Plants (aged 2 weeks) were germinated on plates supplemented with 1 μM flg22 and the indicated SynCom. Shapes represent three (SynCom NS1 + S1) and four (SynCom NS2 + S2) independent replicates in b. The n values indicate the total number of biological samples. Different letters indicate statistical significance, determined using two-sided Dunn’s Kruskal–Wallis test (P < 0.05). The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within 1.5x the interquartile range.

Although germ-free pWER::FLS2-GFP plants respond to flg22 treatment with enhanced RGI compared with Col-0 on synthetic medium, no growth differences were noted between these two genotypes when grown on natural soil (Extended Data Fig. 2c). Given that root growth in natural soil probably proceeds in the face of chronic exposure to MAMPs and DAMPs, as well as colonization by both suppressive and non-suppressive commensals, we speculated that the aforementioned RGI suppression phenotype may act as a dominant community trait. To test this hypothesis, we composed four independent but taxonomically similar five-member SynComs with contrasting abilities for RGI suppression, that is, non-suppressive SynComs (SynCom NS1 and NS2) and suppressive SynComs (SynCom S1 and S2; Supplementary Table 1). We observed RGI-suppressive activity in plants inoculated with the suppressive SynComs, but not in plants inoculated with the non-suppressive SynComs. Furthermore, full RGI-suppressive activity was retained when these commensals were combined as ten-member SynComs (Fig. 1b). A recent study showed that auxin-mediated RGI could be rescued by *Variorax* commensals26. However, our four tested SynComs did not induce RGI to a level comparable to flg22
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To determine whether plant immunity affects microbiota establishment, we used diverse mechanisms to suppress elicitor-mediated RGI. We found that the addition of diverse individual suppressive strains to a five-member non-suppressive SynCom resulted in only partial RGI suppression (Extended Data Fig. 2d). This result suggests that the identity of suppressive commensals, as well as the input proportion of suppressive to non-suppressive strains, affect RGI-suppression capacity quantitatively.

The ability of specific strains to differentially suppress Atpep1- and flg22-mediated RGI prompted us to investigate the mechanisms that underlie this biological process. Previously, commensal Pseudomonas spp. in monoassociations were shown to acidify the growth medium, rendering plants insensitive to flg22 (ref. 22). To determine whether acidification is responsible for RGI suppression by our SynCom, we measured the growth medium pH of plants that were co-inoculated with different SynComs. We observed average reductions in pH, ranging from pH 5.18 in mock treated plants to pH 4.62 and pH 3.97 in the presence of a SynCom S1 and NS1, respectively. This lack of correlation between RGI suppression and growth medium acidification suggests that this mechanism is unlikely to explain suppression in our community set-up. We next investigated whether type-III secretion, which is a well-characterized virulence mechanism among Gram-negative bacteria pathogens, is required for the suppressive activity of root commensals tested. Interestingly, hrcC—a gene that is essential for a functional type-III secretion system in pathogenic Pseudomonas—is dispensable for RGI suppression mediated by suppressive Pseudomonas strain 569 (Extended Data Fig. 2e,f). We next investigated whether root commensals can target the step upstream of flg22 perception. Only the culture filtrate of Janibacter 101, an Actinobacteria member, but not that of three other suppressive strains, derepressed both flg22 and Atpep1-induced RGI (Extended Data Fig. 3a–c). Heat treatment and filtration of the culture filtrate showed that the molecule(s) responsible for the suppressive activity retained in the supernatant of Janibacter 101 is heat-labile, and is larger than 3 kDa (Extended Data Fig. 3d). Mass spectrometry analysis further revealed that the filtrates of Janibacter 101 and the closely related suppressive Janibacter 563, but not three other tested suppressive commensals, elicited a significant reduction in intact flg22 peptide (Extended Data Fig. 3e). Thus, the ability of these two Janibacter strains to suppress MTI resembles the activity of pathogenic bacteria39, and is associated with an extracellular mechanism that can modify/degrade flg22 peptide. Together, these data reveal that commensals use diverse mechanisms to suppress elicitor-mediated RGI.

Activation of immunity shapes root microbiota establishment. To determine whether plant immunity affects microbiota establishment, we performed reconstitution experiments with gnotobiotic plants grown on an agar matrix. We designed three taxonomically similar SynComs with contrasting RGI suppression abilities for community profiling experiments at a strain-specific resolution (a total of six SynComs; the SynComs used in experiment 1 and 2 differ in two Gammaproteobacteria, and the SynComs used in experiment 3 are composed of entirely different strains; Supplementary Table 1). Principal coordinate analyses (PCoA) of Bray–Curtis dissimilarities revealed that root-associated bacterial communities were distinct from the corresponding unplanted or planted matrix samples (Extended Data Fig. 4), regardless of the SynCom composition and plant genotypes (Col-0 and pWER::FLS2-GFP). Constrained PCoA revealed that flg22 treatment elicited a consistent community shift in plants inoculated with non-suppressive SynComs, while samples from those inoculated with suppressive SynComs remained together. Consistent with a dominant effect of RGI suppression, roots inoculated with ten-member mixed communities (suppressive plus non-suppressive SynComs) were not affected by flg22 treatment (Fig. 2a–c and Extended Data Fig. 5a–c). This is consistent with another report showing that roots of fli2 mutant and Col-0 plants have similar community profiles, consisting of a mixed 32-member SynCom30.

To dissect the contribution of individual strains to the overall community shift, we quantified the relative abundance (RA) of individual strains. The detection of non-suppressive commensals as the most abundant strains in the mixed SynComs suggests that the ability to dominate in a community is not necessarily coupled to RGI suppression (Fig. 2d–f and Extended Data Fig. 5d–f). However, the RA of specific strains in a community was impacted by plant immunity. For example, flg22 treatment led to an altered RA of Pseudomonas 9/68 (increased) and Variovorax 434 (decreased), while Microbacteriaceae 60 was unaltered (experiments 1 and 2). Similarly, flg22 treatment altered the RA of Microbacteriaceae 1293 (increased) and Comamonadaceae 568 (decreased), while Pseudomonas 71 was unaffected (experiment 3; Fig. 2d–f). A similar trend was also detected in Col-0 (Extended Data Fig. 5d–f), although the effect was more pronounced in pWER::FLS2-GFP plants, possibly due to enhanced MTI and/or altered root architecture. Furthermore, we found that flg22 treatment reduced within-sample diversity of non-suppressive SynComs (experiments 1 and 2; Extended Data Fig. 5g), suggesting that immune activation can affect the distribution of specific strains in community contexts.

Root transcriptomic changes and dampening of immunity by suppressive SynComs. Although flg22-mediated RGI is closely associated with immune activation, its role as a bona fide immune output is unclear. Here, we sought to explore how inoculation with suppressive or non-suppressive SynComs affected the root transcriptome of plants treated with flg22 and grown on an agar matrix (Supplementary Tables 2–7). Principal component analyses (PCA) at the transcriptome level revealed distinct expression patterns between Col-0 plants inoculated with live bacteria, compared with germ-free plants (PC1, 20% variance; Fig. 3a). Interestingly, the transcriptional output of roots inoculated with these two taxonomically similar SynComs was clearly distinguishable after two weeks of co-cultivation, even in the absence of flg22 treatment (Fig. 3a, triangles). Furthermore, we observed a separation according to the immune status of the plants, triggered by flg22 exposure, in all of the samples treated with heat-killed bacteria as well as with the non-suppressive SynCom (PC2, 7% variance; Fig. 3a). By contrast, flg22 treatment of plants colonized by the suppressive SynCom did not elicit significant changes. Independent transcriptome experiments using pWER::FLS2-GFP plants confirmed these results (Extended Data Fig. 6 and Supplementary Tables 2–4).

Next, we performed k-means clustering of differentially expressed genes (DEGs) involved in the flg22 response, the SynCom response or both (Fig. 3b and Supplementary Tables 5–7). We observed three large clusters (2,221 DEGs) that were induced (c4 and c5) or suppressed (c8) by live bacteria, independent of flg22 treatment (Fig. 3b,c). Gene Ontology (GO) enrichment analyses showed that the SynCom-responsive clusters were primarily enriched in functions related to detoxification, root development, nutrient transport and response to hypoxia (Extended Data Fig. 7). To determine whether similar GO terms could also be identified in experiments with more complex SynComs, we compared our data with two independent Arabidopsis root transcriptome studies that used SynComs consisting of both suppressive and non-suppressive commensals.
Fig. 2 | Activation of immunity by flg22 affects community establishment. a–c, Constrained coordination of the microbial profile of pWER:FLS2-GFP root samples showing the corresponding community shift of non-suppressive SynCom after treatment with flg22. Ellipses correspond to Gaussian distributions fitted to each cluster (95% confidence interval). P values next to the ellipses indicate statistical significance determined using a permutational analysis of variance (PERMANOVA) test between untreated and flg22-treated samples of each SynCom (permutation = 999, P < 0.005). d–f, The RA of strains after treatment with flg22. Three experiments were performed: experiment 1 (a and d); experiment 2 (b and e); and experiment 3 (c and f). These experiments were conducted using different SynComs and were repeated twice with consistent results. The corresponding strains used in each SynCom are indicated at the top of d–f. Non-suppressive and suppressive strains are indicated by the dashed and solid lines, respectively. The values in parentheses are eigenvalues explained by the principal component. The colours indicate the SynComs used and the shapes indicate flg22 treatment. n values indicate the total number of biological samples collected from three independent replicates. Asterisks (*) indicate statistical significance determined using two-sided ANOVA (P < 0.05) and the P values are provided at the top of each graph. The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within 1.5× the interquartile range.

(35 members[1] and 115 members[3]). Despite differences in technical set-ups and SynCom complexities, we identified 174 common SynCom-responsive DEGs in the absence of flg22 that were related to the same biological functions mentioned above (Extended Data Fig. 8 and Supplementary Tables 8–12).

Importantly, we found a flg22-inducible cluster (c3), which was significantly upregulated by the non-suppressive SynCom, but downregulated by the suppressive community (Fig. 3bc), in a pattern matching the RGI phenotype of the plant (Fig. 1b) and the bacterial community shifts (Fig. 2). As expected, a portion of
defence-related genes were enriched in c3, such as PER5, FRK1 and RBOHD (70 genes; Fig. 3b). However, additional defence-related DEGs were found outside c3 and were upregulated by flg22 treatment, even in the presence of the suppressive SynCom (348 genes; Fig. 3b). Previously characterized examples include regulators of antimicrobial camalexin, for example, MYBS1 (refs. 32,33) (c5);
systemic acquired resistance, for example, FMO1 (c5) and SARD1 (c1)\textsuperscript{42,5}; and endogenous peptides amplifying MTI, for example, PIP1 and PIP2 (ref. \textsuperscript{20}) (c5; Fig. 3b).

Recent research showed that MAMP responsiveness in germ-free roots was gated by the expression of damage-induced PRRs\textsuperscript{37}. However, the sustained expression of FLS2 (c1) in the presence of SynComs indicates that RGI suppression is not due to FLS2 downregulation (Fig. 3b). The ability of diverse root commensals to suppress Atpep1-mediated RGI (Extended Data Fig. 2a) also highlights the interference from FLS2-independent pathway(s). An independent study by Teixeira et al. also identified a cluster of DEGs that was highly induced in axenic Arabidopsis by treatment with flg22, but suppressed by the presence of a 35-member SynCom consisting of suppressive and non-suppressive root commensals\textsuperscript{38} (Extended Data Fig. 9). Remarkably, this cluster showed the largest overlap with our cluster c3, with 58 common DEGs (at least 21 were defence-related) that were downregulated by both SynComs (Extended Data Fig. 9). Even though we have shown that two Janibacter strains can degrade/modify flg22 extracellularly, the downregulation of only a subset of flg22-mediated responses suggests that the direct removal of the flg22 peptide is insufficient to account for the differential suppressive activities observed.

We further validated our findings by examining the expression of two flg22-inducible defence marker genes\textsuperscript{22,29,30} in roots of Arabidopsis using quantitative PCR (qPCR) in the presence of other suppressive SynComs. PER5 and FRK1 remained significantly elevated two weeks after co-inoculation with flg22 and a non-suppressive SynCom, but not with a suppressive SynCom (Extended Data Fig. 10a). A non-suppressive SynCom alone also significantly induced the expression of PER5 and FRK1, indicating that non-suppressive commensals stimulate specific root immune responses. As expected, a ten-member mixed SynCom, which was shown to suppress flg22-mediated RGI (Fig. 1b), did not significantly induce the expression of PER5 and FRK1 (Extended Data Fig. 10b). We next examined whether the suppressive SynCom exerted an effect on the root defence response at earlier time points after flg22 stimulation. Intriguingly, we observed a significant induction of PER5, FRK1 and MYB15 1 h after flg22 treatment. However, suppressive SynCom, in contrast to non-suppressive SynCom, specifically downregulated the expression of these three genes after 24 h (Extended Data Fig. 10c), suggesting that SynComs can modulate defence responses as early as 1 d after stimulation.

To determine whether MTI has a direct impact on commensal proliferation independent of any microbe–microbe interactions, we focused on transcription factors (TFs) and investigated the contributions of the top three candidates identified in our dataset—WRKY30, MYB15 and WRKY28 (cluster c3; Extended Data Fig. 10d,e). Null mutants of WRKY30 and WRKY28 have not been reported, and our attempts to knock out these TFs using CRISPR failed, suggesting that these genes are essential for plant viability\textsuperscript{39,40}. We therefore focused on MYB15, a positive regulator of defence against the foliar pathogen PtoDC3000 (ref. \textsuperscript{41}). In myb15-1 plants, elimination of this single TF led to a significantly enhanced proliferation of the detrimental strain Pseudomonas 401, and the commensal Variovorax 434 (P < 0.05; Extended Data Fig. 10f,g), which also showed a reduced RA after flg22 treatment in community contexts (Fig. 2d,e). Together, amplicon sequencing and transcriptome data support the idea that colonization of specific root commensals is affected by host MTI, which can be attenuated by suppressive strains.

Next, we tested whether our suppressive SynCom can also suppress defence responses triggered by a non-proteinaceous elicitor, chitin, which is commonly found as a MAMP in the cell wall of eukaryotic fungi. We performed a time-resolved experiment to follow the expression of the defence marker genes FRK1, PER5 and chitinase (AT2G43620) 1 h, 6 h and 24 h after elicitor application. In contrast to flg22, chitin treatment only marginally induced FRK1 expression after 1 h, whereas PER5 and AT2G43620 were significantly induced up to 6 h (Extended Data Fig. 10h). This is consistent with a previous report showing that flg22 and chitin induce both overlapping and specific root responses\textsuperscript{43}. No stimulation of marker gene expression was detected after 24 h chitin application. Interestingly, our SynComs exerted a cooperative effect on chitin-mediated signalling. For example, chitin-induced PER5 expression was further stimulated by a suppressive SynCom after 1 h but this stimulation was reversed such that the non-suppressive SynCom enhanced PER5 expression after 6 h. After 24 h, both suppressive and non-suppressive SynComs induced PER5 expression, while the chitin-triggered response was no longer detectable (Extended Data Fig. 10h), suggesting that our SynComs interact differently with flg22- and chitin-triggered responses.

**Suppressive and non-suppressive commensals differentially impact plant susceptibility to opportunistic pathogens.** As a subset of commensals dampens root immune responses, we hypothesized that colonization with a suppressive SynCom might render plants more susceptible to opportunistic pathogens. We identified three detrimental strains from At-RSPHERE. In particular, Arabidopsis plants inoculated with Pseudomonas 401 exhibited reduced growth and accumulated pigments in shoots reminiscent of stress-inducible anthocyanins (Extended Data Fig. 1c), which indicates its pathogenic potential in a laboratory environment. Consistent with the fact that Pseudomonas 401 was originally isolated from healthy and asymptomatic Arabidopsis roots colonized by a diverse microbial community, the detrimental effect was attenuated when plants were colonized by our SynComs. Interestingly, the attenuation was stronger when plants were co-colonized with the non-suppressive SynCom, compared with the suppressive SynCom (Fig. 4a).

Recent reports suggest that there is a positive correlation between disease progression in natural Arabidopsis populations and bacterial biomass\textsuperscript{38,44}. To determine whether the virulence of Pseudomonas 401 is related to enhanced plant colonization, we quantified its absolute abundance on pWER:FLS2-GFP precolonized with suppressive or non-suppressive SynComs. Plants already colonized by suppressive SynComs harboured significantly higher Pseudomonas 401 titres compared with plants that were precolonized with non-suppressive SynComs (Fig. 4b,c). Interestingly, this SynCom-dependent difference seemed to be limited to roots, as Pseudomonas 401 growth in shoots was similarly restricted by co-colonization with either community (Fig. 4b,c). Even though we are not excluding an impact of microbe–microbe interactions through niche competition, none of the individual strains antagonized Pseudomonas 401 in vitro (Extended Data Fig. 10i). These data further suggest that the underlying growth differences are probably not the result of antibiosis.

To determine whether SynComs modulate plant susceptibility to a characterized opportunistic pathogen prevalent in natural A. thaliana populations, and exclude the possibility that differential impact of root commensals in roots and shoots is a result due to niche specialization of Pseudomonas 401 on roots only, we inoculated plants with the opportunistic Pseudomonas leaf pathogen OTU5 (isolate p5.e6) (Extended Data Fig. 1c). Plants colonized by suppressive SynComs supported higher growth of Pseudomonas OTU5 compared with plants colonized by non-suppressive SynComs, and this SynCom-specific effect was again observed only in roots and not in shoots (Fig. 4b,c). Together with the RNA-sequencing (RNA-seq) and targeted PCR data, these results suggest that precolonization with non-suppressive SynComs activated root immunity and this correlates with reduced growth of the tested opportunistic pathogens, whereas suppressive SynComs failed to provide pathogen protection.
Fig. 4 | Imbalance of specific bacteria impacts plant susceptibility to opportunistic Pseudomonas pathogens. a, Symptoms of WT plants (aged 3 weeks) germinated with the indicated SynCom and Pseudomonas 401. Scale bars, 1 cm (top and third row, and second and fourth row (left)) and 0.5 cm (second and fourth row (right)). b, c, Bacterial titre of Pseudomonas 401 and OTU5 on the roots (b) and shoots (c) of pWER::FLS2-GFP plants that were precolonized with the indicated SynComs for 2 weeks. No flg22 was used in these experiments. The shapes represent five (SynCom NS5 + S5), four (NS6 + S6) and five (NS7 + S7) biological replicates in b, c.f.u., colony-forming units. n values indicate the total number of biological samples. Different letters indicate statistical significance determined using two-sided Dunn’s Kruskal–Wallis test (P < 0.05). The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within 1.5 × the interquartile range. d, The ‘rheostat model’ proposes that the balance between non-suppressive and suppressive strains integrates with plant innate immunity, and buffers the system against pathogen challenge and defence-associated trade-off.
Discussion
In nature, a subset of soil-dwelling bacteria colonizes roots seemingly without influencing host traits, and are therefore often considered to be commensals. Here, using a bottom-up approach, we show that phylogenetically diverse commensals, representing the core of the Arabidopsis root microbiota, share the ability to suppress host defence responses, a microbial trait that is dominant in our community set-up and is therefore easily overlooked in nature. The functional redundancy of members of the root microbiota to interfere with the host’s immune response is consistent with our observation that mixed communities consisting of non-suppressive and suppressive strains resist flg22-mediated community changes otherwise detected in non-suppressive SynCom-colonized plants (Fig. 2). This finding might explain why immune-related Arabidopsis mutants, tested in natural soil, show relatively mild changes in root microbiota composition. Here we showed that the reduction in apoplastic pH and potential type-III secretion system-dependent mechanisms are insufficient to fully account for our MTI-suppression phenotype. Two closely related Janibacter strains, but not three other tested suppressive commensals, were shown to degrade/modify flg22. Even though we cannot rule out the possible involvement of specific plant metabolites produced after interactions with the suppressive bacteria, our findings suggest that root commensals can use multiple mechanisms to target host immune responses, rendering this community trait difficult to overcome by the plant host.

To date, information on Arabidopsis root transcriptomic changes evoked by commensals is limited to monoassociations, leaving a gap in our understanding of how plant roots respond to commensal communities that can reach a steady-state as early as 13 d after inoculation. We unexpectedly found that, after 2 weeks, root colonization by taxonomically similar commensal SynComs, differing in their ability to suppress RGI, elicited 2,221 DEGs (clusters c4, c5, c8) with remarkable overall similarity. These changes, which are associated with presumably steady-state SynComs, contrast with the subtle response to heat-killed SynComs or flg22 treatment alone, reflecting an impact of active commensal colonization on host transcriptional outputs beyond plant responses to chronic exposure to MAMPs. Furthermore, we observed robust enrichment of specific GO terms related to root development, nutrient transport, response to hypoxia and detoxification across experimental set-ups and SynCom complexities. Indeed, rhizobia alone are known to modulate root traits, leaving a gap in our understanding of how plant roots respond to commensal communities that can reach a steady-state as early as 13 d after inoculation. We unexpectedly found that, after 2 weeks, root colonization by taxonomically similar commensal SynComs, differing in their ability to suppress RGI, elicited 2,221 DEGs (clusters c4, c5, c8) with remarkable overall similarity. These changes, which are associated with presumably steady-state SynComs, contrast with the subtle response to heat-killed SynComs or flg22 treatment alone, reflecting an impact of active commensal colonization on host transcriptional outputs beyond plant responses to chronic exposure to MAMPs. Furthermore, we observed robust enrichment of specific GO terms related to root development, nutrient transport, response to hypoxia and detoxification across experimental set-ups and SynCom complexities. Indeed, rhizobia alone are known to modulate root traits, leaving a gap in our understanding of how plant roots respond to commensal communities that can reach a steady-state as early as 13 d after inoculation.

We hypothesize that the imbalance between non-suppressive and suppressive commensals might reduce plant fitness under stress conditions. Indeed, plants that are precolonized by suppressive SynComs are as susceptible as germ-free plants to opportunistic Pseudomonas pathogens, whereas plants associated with non-suppressive SynComs are more resistant, but prone to MAMP-induced RGI. The observed defence-associated community shifts and potentially reduced alpha-diversity might hinder the provision of microbiota-derived beneficial services, or exert a detrimental impact on the host under dysbiosis. Thus, we propose a rheostat model (Fig. 4d) in which a balance between commensals with contrasting MTI-modulating activities constitutes an integral feature of the holobiont to buffer plant resistance to pathogen perturbation and defence-associated growth reduction.

It remains unclear which factors govern the state of equilibrium and the corresponding ratio between non-suppressive and suppressive strains. However, on the basis of the results obtained using three pairs of SynComs (Fig. 2), the initial input composition has a key role in defining the equilibrium state. The timing of colonization and abiotic factors probably also exert an influence. As a consequence, these complex interactions enable community coexistence, and ultimately establish microbe–host homeostasis. Accordingly, their ratio will impact the amplitude and/or might set the threshold for effective resistance in the zigzag model. Plants in nature are influenced by fluctuating stresses and are colonized by more diverse microbial communities that modulate plant physiology through multiple mechanisms, including the modulation of phytohormone signalling. As our SynComs are constructed on the basis of their ability to suppress flg22-mediated responses, we found that they exert a synergistic effect on chitin-mediated responses. Selective modulation of chitin and flg22-mediated responses—for example, by the cytoplasmic receptor-like kinase PBL27—has been reported. The characterization of the molecular mechanisms that underlie immunosuppression by root commensals may identify immune components that are specific to one or integrate multiple upstream signalling pathways. Another future task will be to test whether the rheostat model also applies to communities with different traits to alleviate abiotic stresses.

Methods
Arabidopsis thaliana ecotype Columbia (Col-0, CS60000) and pep1 pep2 were laboratory stocks. myb15 (ref. 41) (SALK_151976) was a gift from N. Clay (Yale University, USA). The transgenic line pWER::FLS2-GFP (fsb2: SAIL691_C04 background) was provided by N. Geldner (Université de Lausanne, Switzerland). flg22 (QRSTG5RINSAKDAAAGLQIA) and Atpep1 (ATKVKAKQGKEVSSGRGPQQHN) peptides were synthesized by EZbiolab. Chitin was purchased from Sigma–Aldrich (C7952).

Growth conditions for plants. Arabidopsis seeds were surface-sterilized in 70% ethanol twice for 5 min each followed by a brief wash with 100% ethanol. Seeds were then washed three times with sterile water. Cold-stratified seeds were sowed on agar plates (1%, Difco Agar Granulated, BD Biosciences, discontinued) or Bacto agar (BD Biosciences) prepared with half-strength Murashige and Skoog (MS) medium (Duchefa) and 0.1 g l−1 2-(N-morpholino)ethanesulfonic acid (pH 5.7). Sugars were not provided as an additional carbon source unless otherwise specified. Plants were grown under short-day conditions (10 h light–14 h dark) under a 21 °C–19 °C cycle, 65% relative humidity and a light intensity of 120 μmol m−2 s−1. For experiments involving myb15-1, surface-sterilized seeds were sowed on half-strength MS agar plates supplemented with 5 g l−1 sucrose.

Culture conditions for bacteria. Information on individual strains used can be found at At-RSPHERE (http://www.at-sphere.com/) OTUS5 (p5.60) was provided by D. Weigel (Max Planck Institute for Developmental Biology, Tübingen, Germany). Bacterial strains were prepared by taking an aliquot from the glycerol stock, followed by incubation on 50% tryptic soy broth (TSB) agar plates (Sigma–Aldrich) at 25 °C for 1–4 d. Before the start of the experiments, strains were cultured in 50% TSB medium to saturation, and subcultured to log phase with fresh medium at a 1:5 ratio. Bacterial cultures were pelleted by centrifugation at 8,000g for 5 min, followed by two washes with 10 mM MgSO4.
Screening for RGI-suppressive strains in monoassociation. After washing, bacteria were diluted with 10 mM MgSO₄ to an optical density at 600 nm (OD₆₀₀) concentration of about 0.1. A total of 150 μl bacterial suspension was added to still warm 5 ml half-solid (0.5%) agar medium at 95 °C, followed by addition of OD₆₀₀ = 0.0005. A final concentration of 1 μl flg22 was added accordingly. Plates were dried for 2 h before approximately 15 surface-sterilized pWEX-FLS2-GFP seeds were sown on each plate. The expression of the flg22 receptor FLS2 in pWEX-FLS2-GFP is limited to the root epidermis such that potential interorgan shoot-to-root signal after flg22 perception is minimized. The plates were separated with 3M tape and transferred to the phytocrib for incubation. One week after germination, plants with delayed germination were removed and the plates were trimmed to about ten remaining plants. Pictures were taken 3 weeks after incubation and the primary root lengths were quantified using ImageJ. Shoots were separated from the roots and the fresh shoot weight of individual plants was taken. For experiments using 1 μM Ape1, wild-type Col-0 plants were used instead.

A phylogenetic tree of selected strains from Ar-SPHERE was performed previously and visualized using iTOL. Strains leading to a rescue of RGI with a root length longer than 2 cm (average root length of germ-free flg22-treated pWEX-FLS2-GFP plants = 1.53 cm; n = 37) after co-inoculation with 1 μl flg22, and exhibiting consistent suppressive activity across two biological replicates, were considered to be suppressive. Suppressive strains are indicated by a red triangle in Fig. 1a. For the inoculation of SynCom, each bacterium was inoculated to a final concentration of OD₆₀₀ = 0.0005, that is, for a five-member SynCom, the total bacteria added was OD₆₀₀ = 0.0025. The five-member SynCom is composed of five Pseudomonas strains. Fresh half-solid (0.5%) agar medium was added at 95 °C. Fumigicetes were not included in these SynComs as no strains with differential ability to suppress RGI were identified in these two phyla. The composition of SynComs used in this manuscript can be found in Supplementary Table 1.

16S amplicon sequencing and community profiling. For 16S community profiling, all harvested shoots and leaves were processed according to previously a published protocol. In brief, plants were germinated with the indicated SynCom in the presence or absence of 1 μM flg22, and inoculated for 14 d before harvesting. Plants were inoculated with SynCom NS1 and S1 for experiment 1 (Fig. 2a,d and Extended Data Fig. 5a,d); SynCom NS3 and S3 for experiment 2 (Fig. 2b,e and Extended Data Fig. 5e,h); and SynCom NS4 and S2 for experiment 3 (Fig. 2c,f and Extended Data Fig. 5c,f). Plant roots were separated from the shoots and pooled from three plates from each biological replicate. Roots were washed briefly with sterile water and blotted dry before being transferred into Lysing Matrix E tubes (MP Biomedicals) at ~80 °C until processing. Samples were homogenized using a Precellys 24 homogenizer (Bertin Technologies). Total root and bacteria DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's instructions, eluted in 80 μl elution buffer and quantified using the Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific). Samples were diluted to 3.5 ng μl⁻¹, and 3 μl samples were used in a three-step PCR amplification protocol as follows:

Step 1: The V5V7 region of the bacterial 16S rRNA gene was amplified in triplicate reactions using the primers 799F and 1192R in a 25 μl reaction volume containing 2 U DFS–Taq DNA polymerase (Bioron), 1× incomplete buffer, 2 mM MgCl₂, 0.3% bovine serum albumin, 0.2 mM dNTPs (Life Technologies) and 0.3 μM forward and reverse primers. The same PCR program was used for each primer pair (initial denaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, repeat steps 2–4 for 25 cycles, and final extension at 72 °C for 10 min). Primers and primers were digested by adding 1 μl of Antarctic phosphatase, 1 μl exonuclease 1 and 2.44 μl Antarctic phosphatase buffer (New England Biolabs) to 20 μl of the pooled replicate reactions at 37 °C for 30 min, followed by enzyme deactivation at 85 °C for 15 min. Reactions were centrifuged for 15 min at 4,000 r.p.m. and 3 μl supernatant was used for the second PCR step in triplicate reactions.

Step 2: PCR reactions were performed as stated above with the number of cycles reduced to ten using primer pairs 799F and 1192R in a 25 μl reaction volume containing 2 U of Taq DNA polymerase (Bioron), 1× incomplete buffer, 2 mM MgCl₂, 0.3% bovine serum albumin, 0.2 mM dNTPs (Life Technologies) and 0.3 μM forward and reverse primers. The same PCR program was used for each primer pair (initial denaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, repeat steps 2–4 for 25 cycles, and final extension at 72 °C for 10 min). Primers and primers were digested by adding 1 μl of Antarctic phosphatase, 1 μl exonuclease 1 and 2.44 μl Antarctic phosphatase buffer (New England Biolabs) to 20 μl of the pooled replicate reactions at 37 °C for 30 min, followed by enzyme deactivation at 85 °C for 15 min. Reactions were centrifuged for 15 min at 4,000 r.p.m. and 3 μl supernatant was used for the second PCR step in triplicate reactions.

Step 3: Gel-purified DNA was used as a template for the third PCR using forward barcoded primers and p7_pad_R with a total of ten cycles. PCR reactions were loaded onto a 1.5% agarose gel and DNA bands with the correct size were cut out and purified using the QIAquick Gel Extraction Kit (QIAGEN).

Double-barcode DNA was purified and concentrated using Agencourt AMPure XP beads. The concentration of the purified DNA was determined using Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific). PCR error from each sample was estimated by loading 5 μl of the pooled replicate reactions on a 1.5% agarose gel. Approximately similar amounts of DNA sample from the same biological replicate were pooled, and the mixtures were loaded onto a 1.5% agarose gel. DNA bands with the correct size were cut out and purified using the QIAquick Gel Extraction Kit (QIAGEN).

Pseudomonas strain 569. The Pseudomonas strain 569 deletion mutant was generated using a homologous recombination protocol. In brief, PCR fragments flanking the upstream and downstream region of the hrcC gene were PCR-amplified and cloned into pkBlmobsBac. The FRT-flanked cassette from pCP5209 was inserted between the two hrcC fragments. The resultant pkBlmobsBac::ΔhrcC construct was electroporated into log-phase-grown Pseudomonas 569, which was prewashed and resuspended in 0.3 mM sucrose, at 2.5 kV and 1500 μF using the Biorad electroporator. The transformant was selected on half of a TSA plate supplemented with 25 mg μl⁻¹ gentamycin. The double-crossover deletion mutant was further confirmed by colony PCR and Sanger sequencing.

Mass spectrometry. For in vitro detection of flg22, 1 μl flg22 was co-incubated with 1 μl supernatant for 1 h at room temperature. Half-strength MS medium without sugar was used as a control. Sample aliquots (100 μl) were mixed with 200 μl UA (8 M urea in 100 mM Tris-HCl pH 8.5) and adjusted to 10 mM dithiothreitol using 1 M stock. Samples were loaded onto 30 kDa spin filters (Vivaco 500, Sarciortus) at 14,000 g for 30 min. Samples were centrifuged and loaded onto 2 kDa spin filters (Vivaco 500, Sarciortus) and centrifuged at 14,000 g for 30 min, after which 300 μl UA was added and the samples were centrifuged again (14,000 g for 45 min, or until most liquid had passed through the filter). Next, 100 μl 55 mM chloroacetic acid was added to the filter and samples were incubated for 30 min in the dark, after which they were centrifuged at 14,000 g for 20 min. UA (300 μl) was added and the samples were centrifuged at 14,000 g for 45 min. The samples were washed twice with 300 μl 100 mM Tris-HCl, pH 8.5, by centrifugation (14,000 g for 45 min). For elution, 200 μl Tris-HCl was added, and the inverted spin filters were centrifuged at 2,000 g for 2 min to collect eluate into a fresh tube. The eluates were desalted using StageTips with C18 Empore disk membranes (3M)61, and a final elution was performed using 40% ACN in 0.1% trifluoroacetic acid. The samples were dried in a vacuum evaporator, and dissolved in 10 μl 2% acetonitrile (ACN) and 0.1% trifluoroacetic acid for analysis.

Liquid chromatography coupled with tandem mass spectrometry data acquisition and data analysis. Samples were analysed using the EASY-nLC 1000 system (Thermo Fisher Scientific) coupled to a QExactive mass spectrometer (Thermo Fisher Scientific). Peptides were separated on 16 cm frit-less silica emitters (New Objective, 0.75 μm inner diameter), packed in-house with reversed-phase ReproSil-Pur C18 AQ 3 μm resin (Dr. Maisch). Peptides were loaded to 50 μl column and eluted using an isocratic gradient of 5% to 95% solvent B (0 min, 5% B; 5–0 min, 5% B; 5–25 min, to 20% B; 25–35 min, to 35% B; 35–40 min, to 95% B, 40–50 min, 95% B) (solvent A, 0% ACN and 0.1% formic acid; solvent B, 80% ACN and 0.1% formic acid) at a flow rate of 300 nl min⁻¹. Mass spectra were acquired in data-dependent acquisition mode using a TOP10 method. Mass spectra were acquired in the Orbitrap analyzer with a
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mass range of 300–1,500 m/z at a resolution of 70,000 full width at half maximum, and a target value of 3 × 10^10 ions. Precursors were selected with an isolation window of 2.0 m/z. HCD fragmentation was performed at a normalized collision energy of 25. MS/MS spectra were acquired with a target value of 5 × 10^10 ions at a resolution of 17,500 full width at half maximum, a maximum injection time of 85 ms and a fixed first mass of 100 m/z. Peptides with a charge of 1, greater than 6 or with an unassigned charge state were excluded from fragmentation for MS^2; dynamic exclusion for 20 s prevented repeated selection of precursors.

Raw data were directly analysed at the MS1 level using Skyline (https://skyline-md) against the sequence of the flg22 peptide. LysC specificity was required, and a maximum of two missed cleavages was allowed. The minimum peptide length was set to 7 amino acids and the maximum length was set to 25 amino acids. Carbamylation of cysteine, oxidation of methionine and protein N-terminal acetylation were set as modifications. The results were filtered for precursor charges of 2 and 3. Peaks of the intact flg22 peptide precursor were integrated manually, and peak areas were exported for further processing.

**Infection experiments.** To detect any *Pseudomonas*–401-dependent symptoms and the corresponding attenuation by SynComs, plants were germinated with *Pseudomonas* 401 alone (p5.e6) or co-inoculated with a SynCom for 2 weeks (Fig. 4a). For viable plate-counting experiments, plants were pregerminated on half-strength MS agar plates inoculated with the indicated strain or SynCom for 14 d (Fig. 4b,c). Plants were then flood inoculated with a bacterial suspension of *Pseudomonas* strain 401 or OTU5 (p5.e6) OD_{600}=0.001 in 10 ml TSB buffer. The inoculated plant was incubated with 0.05% PVP solution for 5 min after flood inoculation, and plants were transferred to a new half-strength MS agar for further incubation. After 1 d or 2 d, shoots and roots were separated, roots and shoots from five and three individual plants were pooled after brief washing and blotted dry with sterile filter paper. The samples were homogenized with metal beads in 500 µl MgSO_{4} with a Precellys 24 homogenizer (6,200 rpm, twice with 15 s pauses in between).

*Pseudomonas* strain 401 and OTU5 (p5.e6) were transformed with pBBR1-MCS5 carrying a gentamycin-resistance cassette. Serial dilution was performed, and bacterial dilutions were spread on 50% TSB plates supplemented with 25 µg·m^{-1} gentamycin to select for the strain of interest until single colonies appeared.

**In vitro halo of-inhibition assay.** Washed *Pseudomonas* strain 401 (100 µl) was inoculated into 50 ml warm 25% TSB medium with an initial OD_{600}=0.1. After solidification, 10 µl prewashed bacterial suspension prepared from 1 ml saturated overnight bacterial culture from individual strains was spotted onto the *Pseudomonas*-401-preinoculated plates. Any halo-of-inhibition was recorded up to 5 d after incubation at 25 °C.

**Transcriptome experiments.** Plants were germinated with the indicated SynCom in the presence or absence of 1 µM flg22 and inoculated for 1 d before harvesting. For transcriptome experiments, Col-0 plants were inoculated with SynCom NS5 and SynCom S3 (Fig. 3); pWER-FLS2-GFP plants were inoculated with SynCom NS5 and SynCom S1 (Extended Data Fig. 6). For transcriptome experiments, plants were not transferred to minimize induced damage. Roots from three plates (minimum of 15 plants) were combined as one replicate and a total of three replicates were sampled for each condition. Roots were washed briefly with sterile water, cut into small pieces before being transferred into Lysing Matrix E tubes (MP-Biomedicals) at −80 °C until processing. Roots were homogenized with Lysing Matrix E using a prechilled adapter and the Tissuelyser II (QIAGEN, 20 pulses per 1 min). RNA was extracted using the Plant RNAeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. RNA quality was determined using a 2100 Bioanalyzer (Agilent Technologies). Preparation of Illumina sequencing libraries was conducted by the Max Planck Genome Center using an input of 1 µg total RNA. Sequences were generated using the Illumina HiSeq2500 platform. Approximately 6 million paired-end reads and 20 million single-end reads per sample with a length of 150 bp were generated for Col-0- and pWER-FLS2-GFP-based experiments.

**RNA-seq data analysis.** Raw Illumina RNA-seq reads were preprocessed using fastp (v0.19.10) with the default settings for paired-end (Col-0 experiment) or single-end reads (pWER-FLS2-GFP experiment). For single-end reads, low-quality sequence fragments from the head (8 bases) and tail (2 bases) were trimmed. High-quality reads were pseudolonged to the TAIR 10 Arabidopsis thaliana transcriptome reference *Ensemble*^2^ using Kallisto (v0.46.1). On average, 6.7 million paired-end and 18.1 million single-end reads per sample were mapped to the reference *Arabidopsis* transcriptome. After removing low-abundant transcripts that were absent 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^2^.

First, raw counts were normalized with respect to the library size (log function) and log-transformed. We tested for sample batch effects by surrogate variable (SV) analysis using the sva package. Significant SVs were automatically detected and integrated into the model for differential analyses. PCA (prcomp function) on the basis of whole transcripts was performed and plotted to visualize the cluster and variance of biological replicates under each condition. The abundance of *Arabidopsis* latent virus-1 reads did not correlate with sample variances and therefore removed from downstream analyses. Pairwise comparisons were done before (1) flg22 treatment effect only; (2) pWER-SynCom treatment and suppressive SynCom effect only; (3) flg22 treatment plus SynCom effects; and (4) living versus heat-killed bacteria. Transcripts with fold changes of >1.5 and adjusted P values corrected for multiple comparisons (Benjamini–Hochberg method) equal to or below 0.05 were considered to be significant.

The log2-scaled counts were normalized by the identified SVs using the limma package (removeBatchEffect function), and transformed as median-centred z score by transcripts (scaled counts, scale function). Then, z scores were used to conduct k-means clustering for all transcripts. The cluster number (k=10) was determined by the sum of squared error and Akaike information criterion. Next, confirmed transcripts with similar expression patterns were grouped in the same cluster. Differentially expressed transcripts were selected (3,718 in pWER-FLS2-GFP and 4,450 in Col-0 experiments) and cluster results were visualized using heat maps generated using the ComplexHeatmap package.

GO enrichment for each cluster using the whole *Arabidopsis* transcriptome as background were performed using the goseq package with the consideration of transcript length. GO annotations were retrieved from the Gene Ontology Consortium^3^ (September 2019). Significantly changed biological process GO terms (adjusted P < 0.05) were visualized in dot plots using the clusterProfiler package.

Defence-related genes were enriched on the basis of the GO-term annotation with manual curation. These genes were marked in Supplementary Table 7.

**Statistical analysis.** Analyses were performed using the R environment. t-tests, Dunns Kruskal–Wallis tests, Dunn's tests and ANOVA were used to test for statistical significance. Unless otherwise indicated, P < 0.05 was considered to be significant.

**Data availability.** Raw transcriptome and 16S rRNA amplicon sequencing data from this project were deposited at NCBI under the accession number GSE157128. Mass spectrometry data have been deposited to Panorama Public (https://panoramaweb.org/flg22_RGI.url) and the ProteomeExchange (PDX020452). Source data are provided with this paper.
26. Emonet, A. et al. Spatially restricted immune responses are required for a plant community of commensal bacteria. Proc. Natl Acad. Sci. USA https://doi.org/10.1073/pnas.2100678118 (2021).

27. Frerigmann, H., Glawischnig, E. & Gligošavlihi, T. The role of MYB34, MYB51 and MYB122 in the regulation of camalexin biosynthesis in Arabidopsis thaliana. Front. Plant Sci. 6, 654 (2015).

28. Frerigmann, H. et al. Regulation of pathogen-triggered tryptophan metabolism in Arabidopsis thaliana by MYB transcription factors and indole glucosinolate conversion products. Mol. Plant 9, 682–695 (2016).

29. Zheng, Y. et al. Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc. Natl Acad. Sci. USA 107, 18220–18225 (2010).

30. Hartmann, M. et al. Flavin monooxygenase-generated $N$-hydroxypropionic acid is a critical element of plant systemic immunity. Cell 173, 456–469 (2018).

31. Houl, S. et al. The secreted peptide PIP1 amplifies immunity through receptor-like kinase 7. Plant Pathog. 10, e104331 (2014).

32. Zhou, F. et al. Co-incidence of damage and microbial patterns controls localized immune responses in roots. Cell 180, 440–453 (2020).

33. Millet, Y. A. et al. Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. Plant Cell 22, 973–990 (2010).

34. Zhao, L. et al. KLU suppresses necroptotic cell fate through SWR1-mediated activation of WRKY28 expression in Arabidopsis. Plant Cell Physiol. 115, E526–E535 (2018).

35. Zou, L. et al. Transcription factor WRKY30 mediates resistance to Cucumber mosaic virus in Arabidopsis. Biochem. Biophys. Res. Commun. 517, 118–124 (2019).

36. Guerin, W. R., Memon, A., Li, F. S., Wenz, J. K. & Clay, N. K. SG2-type R2R3-MYB transcription factor MYB15 controls defense-induced lignification and basal immunity in Arabidopsis. Plant Cell 29, 1907–1926 (2017).

37. Karasov, T. L. et al. Arabidopsis thaliana and Pseudomonas pathogens exhibit stable associations over evolutionary timescales. Cell Host Microbe 24, 168–179 (2018).

38. Karasov, T. L. et al. The relationship between microbial biomass and disease in the Arabidopsis thaliana phyllosphere. Preprint at bioRxiv https://doi.org/10.1101/288141 (2019).

39. Edwards, J. et al. Structure, variation, and assembly of the root-associated microbiome. Nat. Ecol. Evol. 3, 825–837 (2019).

40. Zou, L. et al. The root microbiota of Arabidopsis thaliana is highly dynamic and maintains stable associations. Proc. Natl Acad. Sci. USA 116, 5871–5876 (2019).

41. Leart, A. et al. The root microbiome of Arabidopsis thaliana is highly dynamic and maintains stable associations. Proc. Natl Acad. Sci. USA 116, 5871–5876 (2019).

42. Saleem, M., Hu, J. & Jousset, A. More than the sum of its parts: microbiome composition and function of the root microbiome under abiotic stress. Plant Microbe Interact. 32, 364–376 (2019).

43. Hernandez, M. et al. Different bacterial growth-promoting effects of multiple bacterial strains. FEMS Microbiol. Lett. 369, 10–17 (2019).

44. Edwards, J. et al. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl Acad. Sci. USA 112, E991–E9920 (2015).

45. Zamudiois, C., Mastronesti, P., Dohnukse, P., Blilou, I. & Pieterse, C. M. Unraveling root developmental programs initiated by beneficial Pseudomonas spp. bacteria. Plant Physiol. 162, 304–318 (2013).

46. Lopez-Bucio, J. et al. Bacillus megaterium rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in Arabidopsis thaliana. Mol. Plant Microbe Interact. 20, 207–217 (2007).

47. Hernandez, M., Dumont, M. G., Yuan, Q. & Conrad, R. Different bacterial populations associated with the roots and rhizosphere of rice incorporate plant-derived carbon. Appl. Environ. Microbiol. 81, 2244–2253 (2015).

48. Alderson, L., Demoling, E. & Bann, E. Rapid method of determining factors limiting bacterial growth in soil. Appl. Environ. Microbiol. 67, 1830–1838 (2001).

49. Levy, A. et al. Genomic features of bacterial adaptation to plants. Nat. Genet. 50, 158–150 (2017).

50. Saleem, M., Hu, J. & Jousset, A. More than the sum of its parts: microbiome biodiversity as a driver of plant growth and soil health. Annu. Rev. Ecol. Evol. Syst. 50, 145–168 (2019).

51. Chen, T. et al. A plant genetic network for preventing dysbiosis in the plant microbiome. Curr. Opin. Microbiol. 34, 162–169 (2013).

52. Hartman, K. & Tringe, S. G. Interactions between plants and soil shaping the root microbiome. New Phytol. 206, 3230–3239 (2015).

53. Edwards, J. et al. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl Acad. Sci. USA 112, E991–E992 (2015).

54. Shinya, T. et al. Selective regulation of the chitin-induced defense response by receptor-like cytoplasmic kinase PBL27. Biochem. J. 456, 444–462 (2018).

55. Wippel, K. et al. Host preference and invasiveness of commensals in the Lotus and Arabidopsis root microbiota. Preprint at bioRxiv https://doi.org/10.1101/2021.01.12.426357 (2021).

56. Trichera, P. I. et al. Specific modulation of the root immune system by a plant community of commensal bacteria. Proc. Natl Acad. Sci. USA https://doi.org/10.1073/pnas.2100678118 (2021).
Acknowledgements
We thank A. L. Roth and C. Philipp for technical support; B. Huettel from the Genome Centre of MPI-PZ for the preparation and sequencing of RNA-seq libraries; and A. Harzen from the core proteome facilities for the preparation of mass spectrometry samples; N. Clay and D. Weigel for providing Arabidopsis myb-15-1 seeds and the OTUS strain, respectively; J. Dangl and P. Teixeira for sharing their unpublished data; T. Nürnberger and R. Panstruga for discussions about chitin-related experiments; and N. Donnelly and R. Kardakaris for reading and editing the manuscript. This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy—EXC-2048/1—project 390686111 and SPP 2125 DECryPT to R.G.-O. and P.S.-L.; K.-W.M. was supported by postdoctoral fellowships from EMBO (ALTF 1144–2017) and the Alexander von Humboldt Foundation.

Author contributions
K.-W.M. and P.S.-L. conceptualized the initial project. K.-W.M., R.G.-O. and P.S.-L. designed the experiments. K.-W.M. performed the initial screening of RGI-suppressive members 16S amplicon sequencing with the help of C.C., J.O. and Y.J.; RNA-seq experiments; and inoculation experiments involving Pseudomonas strains 401, 434 and OTUS with the help of J.O. and Y.J.; Y.N. performed RNA-seq data analyses and developed all related pipelines for GO term enrichment, network studies and cross-study comparisons. K.-W.M., R.G. and R.G.-O. analysed the 16S amplicon sequencing data. K.-W.M., Y.J., P.S.-L., H.N. and S.C.S. designed the mass spectrometry experiments. Y.J. performed the experiments. S.C.S and H.N. developed the protocol to detect flg22 peptide and S.C.S. analysed the data. A.E. and N.G. phenotyped the growth of pWER:FLS2-GFP transgenic line in natural soil. K.-W.M., Y.N., R.G.-O. and P.S.-L. wrote the manuscript with input from all co-authors.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41477-021-00920-2.
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41477-021-00920-2.
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Peer review information Nature Plants thanks Jack Gilbert, Susannah Tringe and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.
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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | pWER::FLS2-GFP transgenic Arabidopsis plants are hypersensitive to flg22. 

a, Representative images of 3-week-old plants treated with 1μM flg22 grown on agar plate. 

b, Bacteria load on 3-week-old WT roots inoculated with the indicated commensal. Boxes with filling background represent strains with RGI suppressive activity and colors correspond to phylogeny. n=total number of biological samples collected from one replicate. Asterisks indicate statistical significance (two-sided ANOVA, *p* < 0.05). The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within the range of 1.5x the interquartile range. 

c, Representative images of 2-week-old Col-0 germinated with the indicated strains with detrimental impact.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Microbiota members modulate root growth inhibition (RGI) induced by Atpep1 and function quantitatively in community contexts. a, Root lengths of WT and pepr1pepr2 mutants (left panel) or WT plants (right panel) inoculated with 1μM Atpep1 alone or with individual strain. b, Caulobacter 342 exhibits differential ability to suppress flg22- or Atpep1-mediated RGI. c, Representative images of WT, fls2 and pWER::FLS2-GFP plants grown in natural Lausanne soil and their corresponding shoot fresh weight without any flg22 treatment. d, Root lengths of pWER::FLS2-GFP plants inoculated with different combination of non-suppressive and suppressive strains in the presence of 1μM flg22. n=total number of biological samples collected from four (a), two (b), two (c) and three (d) biological replicates. Asterisks indicate statistical significance (two-sided Dunn’s Kruskal Wallis or two-sided Dunnett’s test, p < 0.05). The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within the range of 1.5x the interquartile range. e, Gel blot (Source ED Fig. 2e) showing the result of PCR genotyping of 569ΔhrcC generated by homologous recombination. Lane from left to right, GeneRuler 1kb plus ladder, four individual colonies and control. f, Root lengths of pWER::FLS2-GFP plants coinoculated with flg22 and 569 or 569ΔhrcC.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Commensals can degrade/modify flg22 in vitro. a, Schematic diagram showing how bacterial supernatants are collected. Root lengths of plants coinoculated with the supernatant of different bacteria and b, 1μM flg22 (pWER::FLS2-GFP) or c, 1μM Atpep1 (Col-0). All strains used in these experiments exhibited RGI suppressive activity as shown in Fig. 1. d, Root lengths of Col-0 plants treated with the supernatant of Janibacter 101 after different treatments shown in (a). e, Mass spectrometry analyses detecting the intact flg22 peptide (QRLSTGSRINSAKDDAAGLQIA [M + 4H] 568.8056) by estimating the peak area generated by Skyline. All strains used in this experiment exhibited suppressive activity on flg22-mediated RGI. n=total number of biological samples collected from three biological replicates (ED Fig. 3b–e). Asterisks or different letters indicate statistical significance (two-sided Dunn’s Kruskal Wallis or two-sided Dunnett’s test, p < 0.05). The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within the range of 1.5x the interquartile range.
Extended Data Fig. 4 | Compartment specific effect on community establishment. Unconstrained PCA plots showing the compartment-specific effect of bacteria community two weeks after inoculation on agar plates. The root samples include both Col-0 and pWER::FLS2-GFP plants.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Activation of immunity by flg22 affects community establishment in WT Col-0 plants. a-c, Constrained coordination of the microbial profile of Col-0 root samples showing the effect of flg22 and SynCom. Ellipses correspond to Gaussian distributions fitted to each cluster (95% confidence interval). p-values next to ellipses indicate statistical significance based on a PERMANOVA test between untreated and flg22-treated samples of each SynCom (permutation = 999, p < 0.05). d-f, Relative abundance of strains upon flg22 treatment. Experiment 1: (a,d); experiment 2: (b,e); experiment 3: (c,f). The corresponding strains used in each SynCom were indicated on top of (d-f). These experiments were conducted using different SynComs and repeated twice with consistent result. Values in bracket are eigenvalues explained by the Principal Component (PC). Colors indicate SynComs used and shapes indicate flg22 treatment. g, Simpson diversity index of the corresponding experiments performed with pWER::FLS2-GFP plants. n= total number of biological samples collected from three biological replicates. Asterisks indicate statistical significance (two-sided ANOVA, p < 0.05) and the p values are provided on top of each graph. The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within the range of 1.5x the interquartile range.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | SynCom colonization and flg22 treatment induce global root transcriptomic changes of pWER::FLS2-GFP plants. **a**, PCA plot separating samples inoculated with taxonomically similar SynComs (SynCom NS1 and S1) treated with 1μM flg22. **b**, Heat map (middle) and DEGs (Supplementary Table 2–4) obtained by pairwise comparison under flg22 treatment (right). k-means clusters (k=10) with hieratical tree are marked on the left. **c**, Scaled counts of transcripts in six clusters and their expression patterns upon treatments. n=total number of biological samples collected from three replicates. The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within the range of 1.5× the interquartile range. **d**, A subset of significantly changed GO terms (adjusted p < 0.05) associated with specific clusters. Size of pie charts corresponds to number of DEGs. Linked GO terms indicate shared genes (Jaccard similarity > 0.2).
Extended Data Fig. 7 | DEG-linked GO network. Network showing the association of significantly changed biological function GO terms (pie charts; adjusted \( p < 0.05 \)) with different DEGs (small nodes) obtained by pairwise comparison in Col-0 transcriptome experiments. Size of pie charts corresponds to number of DEGs. Linked GO terms indicate shared genes (Jaccard similarity > 0.2). Closely gathered GO terms form clusters in the network showing similar functions (indicated with dash lines), and heatmaps for GO clusters corresponding to the average scaled counts of transcripts for each treatment are drawn on the sides. The position of clusters is arranged according to topology. Clusters and DEGs correspond to Fig. 3b.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Live SynComs induce common host transcriptomic response. a, Heat map summarizes the root transcriptomic changes from three independent studies (Supplementary Table 8–12, ①: current study; ②: Harbort and Hashimoto et al., 2020 (ref. 13); ③: Teixeira et al., 2021 (ref. 31)). The data obtained from pWER::FLS2-GFP plants was shown alongside Col-0 dataset indicating conserved trends of gene expression across different genotypes. DEGs were obtained by pairwise comparisons between live and heat-killed (HK) SynCom treatments (adjusted p-value < 0.05 and fold-change > 1.5). The corresponding data extracted from Harbort and Hashimoto et al., 2020 (ref. 13) and Teixeira et al., 2021 (ref. 31) were shown on the right. b, Venn diagrams showing the overlapping SynCom-responsive DEGs (live vs. HK SynComs) of each cluster. Most SynCom-responsive DEGs were found in c1, c4, c5, c6 and c8. c, Venn diagram summarizing the overlap of SynCom-responsive DEGs of these independent studies. The core 174 SynCom-responsive host DEGs were highlighted in purple. d, Enrichment of selected GO terms for the 174 core SynCom-responsive DEGs. Numbers in parentheses indicate the total number of DEGs with GO annotations.
Extended Data Fig. 9 | A subset of flg22-induced genes is downregulated by SynComs. a, Pairwise comparison of Jaccard similarity between clusters from two root transcriptome studies (this study: 10 clusters by row; and 8 clusters by column: Teixeira et al., 2021 (ref. 31)). Significantly enriched GO terms associated with cluster 1 (b, root development related) and cluster 3 (c, defense related) showing highest similarity between studies were shown. 58 common DEGs were downregulated by SynComs across these two studies, and 21 out of 44 GO annotated DEGs shown in (c) were defense-related. Defense-related GOs were highlighted by a star. Numbers in parentheses indicate the total number of DEGs with GO annotations.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | MYB15 is a positive regulator of defense against specific commensals. a, b, Expression of flg22-induced marker genes, PERS and FRK1, in roots of 14-day-old pWER::FLS2-GFP plants upon treatment with the indicated SynCom. c, Expression of FRK1, MYB15 and PERS of Col-0 plants 1 hr and 24 hr post flg22 treatment. Plants were precolonized by the indicated SynCom for two weeks. Expression patterns of WRKY28, WRKY30 and MYB15 in roots of 14-day-old (d) Col-0 and (e) pWER::FLS2-GFP transcriptome experiments. myb15-1 mutant enhances proliferation of Pseudomonas 401 (f) and Variovorax 434 (g). h, Expression of FRK1, PERS and chitinase (AT2G43620) upon coinoculation between chitin (1 mg/ml) and SynComs at the indicated time points. Plants were precolonized by the indicated SynCom for two weeks. i, Individual strains or combined SynComs do not antagonize 401 growth in vitro. Individual strain was spotted on a background lawn of 401 and halo-of-inhibition (if any) was noted 5 dpi. n=total number of biological samples collected from three (a-c, g, 1 and 6 hr), four (c) or two biological replicates (g, 24 hr). Asterisks and different letters indicate statistical significance. One-sided student’s t-test (a-c), two-sided Dunn’s Kruskal Wallis (d-e), two-sided Dunnett’s test (f-g) and two-sided ANOVA (h) were used, p < 0.05). The box plots centre on the median and extend to the 25th and 75th percentiles, and the whiskers extend to the furthest point within the range of 1.5x the interquartile range.
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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about: availability of computer code

Data collection: RNA-Seq raw data was generated by sequencing using the Illumina HiSeq2500 platform. 16S amplicon data was collected using a Illumina MiSeq sequencer.
Data analysis

Software being used in this study were stated explicitly in the method and summarized below:
1. fastp v0.19.10 https://github.com/OpenGene/fastp
2. kallisto v0.46.1 https://pachterlab.github.io/kallisto/about
3. transport R package 1.26.1 https://bioconductor.org/packages/release/bioc/html/transport.html
4. DESeq2 R package 1.28.1 https://bioconductor.org/packages/release/bioc/html/DESeq2.html
5. sva R package 3.36.0 https://bioconductor.org/packages/release/bioc/html/sva.html
6. limma R package 3.44.3 https://bioconductor.org/packages/release/bioc/html/limma.html
7. ComplexHeatmap R package 2.4.3 https://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html
8. goseq R package 1.40.0 https://bioconductor.org/packages/release/bioc/html/goseq.html
9. clusterProfiler R package 3.16.1 https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html
10. Cytoscape 3.8.0 https://cytoscape.org/
11. metascape 3.5 https://metascape.org/gp/index.html#main/step1
12. Arabidopsis thaliana gene ontology datasets retrieved on September 2019 http://geneontology.org/
13. QiME http://qiime.org/
14. usearch v10 https://www.drive5.com/usearch/
15. ggplot2 R package 3.3.2 https://ggplot2.tidyverse.org/
16. vegan R package 2.5-6 https://cran.r-project.org/web/packages/vegan
17. R language 3.6.0 https://www.r-project.org/
18. Custom codes were used to analyze the data and were made available on https://github.com/YulongNiu/MPIP2_microbe-host_homeostasis.

For manuscripts utilizing custom algorithms or software that are not central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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Raw transcriptome and 16S rRNA amplicon sequencing data from this project were deposited at the Gene Expression Omnibus (GEO) under the accession number GSE157128 (project PRJNA660185). Mass spectrometry data has been deposited to Panorama Public (https://panoramaweb.org/fig22_RGI.url) and ProteomeXchange (PDX020452). We also make all data and scripts available to the reviewers and the editorial office at https://github.com/YulongNiu/MPIP2_microbe-host_homeostasis. Source data associated with Figs 1,4 and extended data Fig 1-3,10 were available.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size was determined based on preliminary trials that 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
All amplicon sequencing data were used except for biological replicate c of experiment 1 due to potential contamination issue or PCR error.

Replication
Different biological replicates were indicated as different shapes as reported in the corresponding figures.

Randomization
Experiments involving plants grown on agar plates were randomized in the chamber during the course of the experiments.

Blinding
Researchers were not blinded to group allocation, partly due to the obvious phenotype difference associated with a treatment. In general, experiments were independently validated or performed by different researchers to ensure reproducibility of the data.

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