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Associations with rhizosphere bacteria can confer an adaptive advantage to plants

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Host-associated microbiomes influence host health. However, it is unclear whether genotypic variations in host organisms influence the microbiome in ways that have adaptive consequences for the host. Here, we show that wild accessions of *Arabidopsis thaliana* differ in their ability to associate with the root-associated bacterium *Pseudomonas fluorescens*, with consequences for plant fitness. In a screen of 196 naturally occurring *Arabidopsis* accessions we identified lines that actively suppress *Pseudomonas* growth under gnotobiotic conditions. We planted accessions that support disparate levels of fluorescent Pseudomonads in natural soils; 16S ribosomal RNA sequencing revealed that accession-specific differences in the microbial communities were largely limited to a subset of Pseudomonadaceae species. These accession-specific differences in *Pseudomonas* growth resulted in enhanced or impaired fitness that depended on the host's ability to support *Pseudomonas* growth, the specific *Pseudomonas* strains present in the soil and the nature of the stress. We suggest that small host-mediated changes in a microbiome can have large effects on host health.

One of the most profound discoveries in biological research of the past decade is the overwhelming importance of host-associated microbial communities for the health of multicellular organisms[1,2]. Microbes growing in association with either an animal gut or plant roots (in the rhizosphere) can affect the health of their host. Because host genotype can shape the associated microbial communities[3–6], it is possible that eukaryotic organisms have evolved the ability to cultivate specific beneficial microbiomes. In plants, the microbial composition of the surrounding soil is the largest determinant of the final rhizosphere community, and the effect of host genotype is minor by comparison[3,4]. As a result, it is unclear whether host genotype-mediated differences are important for host health, or if it is only random environmental encounters that matter. For a microbiome to be a host genotype-dependent adaptive trait (i.e. under natural selection), we reasoned that the following attributes of host-microbe interactions should be apparent in a single host species: first, populations of the host organism should possess genetic variation in traits that allow them to select environmentally adaptive microbes; and second, differences in microbial communities should differentially affect the fitness of the variant host organisms.

The plant rhizosphere is an ideal system to explore these two postulates. First, it is already known that plants shape their rhizosphere through exuded carbon and other metabolites[7,8] and that there is genetic variation in these traits[9]. This variation presumably contributes to an observed variation in rhizosphere communities[10–12]. Second, microbes in the rhizosphere are known to affect plant health by assisting with host nutrient acquisition[13], protecting against biotic and abiotic stress[14,15], and altering plant growth and physiology[16]. Here, we sought to determine whether particular wild accessions of *Arabidopsis thaliana* differ in their ability to associate with particular soil microbes and thereby gain potential adaptive advantages.

To make this question experimentally tractable, we developed an assay (described below) to probe the interaction of *Arabidopsis* with a single-member rhizosphere community consisting of the beneficial root-associated bacterium *Pseudomonas fluorescens*. We used the wild plant *Arabidopsis* because of its small size and collections of naturally occurring inbred lines (accessions). *P. fluorescens* can confer benefits to members of the Brassicaceae including *Arabidopsis* by protecting them from biotic and abiotic stresses and promoting growth[17–21], indicating that *P. fluorescens* is an important member of the indigenous rhizosphere community of *Arabidopsis*. We reasoned that enhanced or impaired ability of *Arabidopsis* to associate with *P. fluorescens* might confer an advantage under certain environmental conditions.

Results

To test whether natural variation in plants can contribute to an adaptive rhizosphere, we first developed a high-throughput assay that allowed us to screen a large number of plant genotypes for altered associations with rhizosphere bacteria. In this root–bacterium association assay, *Arabidopsis* plants are grown hydroponically in 48-well clear-bottom plates with the roots submerged and the shoots separated from the media by a floating mesh disk (Supplementary Fig. 1a). Plant roots are exposed to bacteria expressing green fluorescent protein (GFP), and bacterial fluorescence is measured with a plate reader. The plant growth media contains no added carbon, so bacteria are dependent on plant photosynthate for significant growth (Supplementary Fig. 1b). After testing a number of well-studied commensal *P. fluorescens* isolates in our assay (Supplementary Fig. 1b), we selected *P. fluorescens* WCS365. This strain was originally isolated from potato[22], protects tomato plants from *Fusarium oxysporum* infection[23,24], and has a broad host range[25]. WCS365 consistently grew to $\sim 3 \times 10^7$ colony-forming units (CFU) per root of the *Arabidopsis* accession Col-0 in the hydroponic assay without causing any apparent disease symptoms or stress to the plant (Supplementary Fig. 1a,b).

To determine whether natural variation in *Arabidopsis* affects the levels of *P. fluorescens* WCS365 in the plant rhizosphere, we used the 48-well plate assay to screen a collection of 196 geographically diverse naturally occurring *Arabidopsis* accessions[26]. The 196 accessions supported an approximately 1.5 log range of *P. fluorescens* WCS365 growth, with the reference accession Col-0 supporting a

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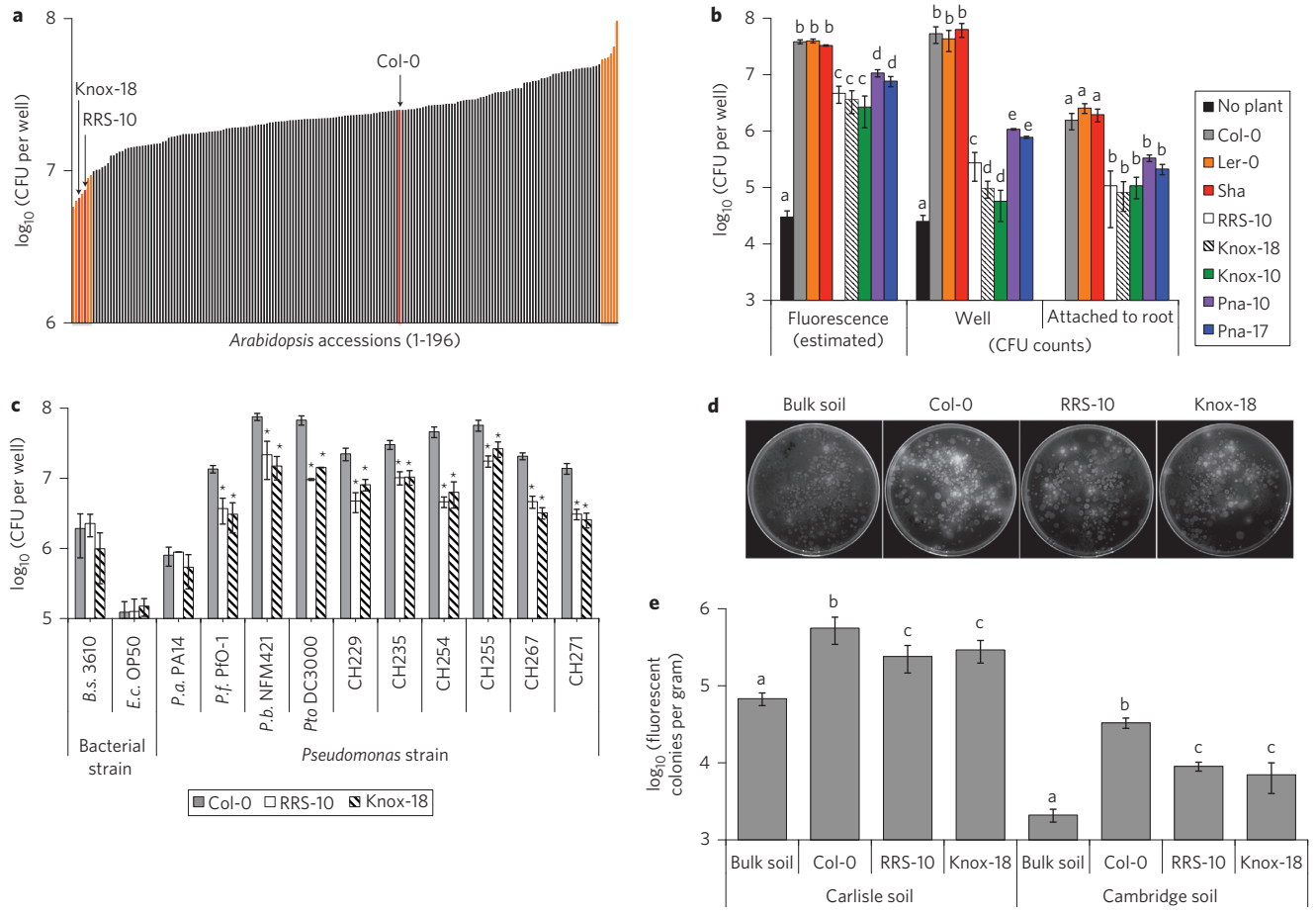

Figure 1 | Natural variation in *Arabidopsis* affects growth of *Pseudomonas* in the rhizosphere. a, Using hydroponically grown *Arabidopsis* plants in 48-well plates, a collection of 196 naturally occurring accessions was screened for *P. fluorescens* growth in the rhizosphere. $n = 6$ plants per accession; $P < 0.05$ by ANOVA and Tukey's HSD test (compared to Col-0) are shown in orange. **b**, Five accessions had consistently lower levels of *P. fluorescens* whether bacterial fluorescence, number of CFUs in the well, or number of CFUs attached to roots were measured; $n \geq 24$ plants per treatment. **c**, Hydroponically grown plants were inoculated with *B. subtilis* 3610, *E. coli* OP50, previously sequenced *Pseudomonas* strains, or *Pseudomonas* isolates identified in this study. CFUs were either counted directly by plating bacteria in wells, or approximated by measuring bacterial fluorescence; $n \geq 12$ plants per treatment. **d**, The culturable microbiome of Col-0 is enriched for fluorescent Pseudomonads relative to bulk soil and the microbiomes of RRS-10 and Knox-18. **e**, Fluorescent colonies per g of Cambridge or Carlisle soil or roots grown in these soils. **b**,**c**,**e**, Averages ± s.e.m. are shown; $^*P < 0.01$ by *t*-test; letters designate $P < 0.05$ by ANOVA and Tukey's HSD.

slightly higher level than the average accession (Fig. 1a). We retested accessions that supported reduced levels of WCS365 in the primary screen ($P < 0.05$ by analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test relative to Col-0) and identified five 'incompatible' accessions, RRS-10, Knox-10, Knox-18, Pna-10 and Pna-17, that consistently exhibited 0.5 to 1.5 logs less WCS365 growth than 'compatible' accessions Col-0, Sha and Ler-0 as measured by fluorescence, or by counting CFUs by plating the hydroponic media or the bacteria physically stuck to the plant roots (Fig. 1b).

Accessions RRS-10 and Knox-18 were chosen for further study because they had the greatest consistent reduction in growth of *P. fluorescens* WCS365 relative to Col-0. To determine whether the reduced growth of *P. fluorescens* WCS365 in the RRS-10 and Knox-18 rhizospheres was a general rule for the growth of any bacteria in the rhizosphere of these *Arabidopsis* genotypes, we tested RRS-10, Knox-18 and Col-0 for their association with several other bacterial species. In the rhizospheres of all three genotypes, we found equivalent growth of the human *P. aeruginosa* isolate PA14, the non-pathogenic strain *Escherichia coli* OP50, and the *Arabidopsis*-colonizing *Bacillus subtilis* 3610 (Fig. 1c). This indicates that the reduced level of *P. fluorescens* growth associated with RRS-10 and Knox-18 is not a general rule, but is specific to certain bacterial taxa.

To determine whether the reduced level of *P. fluorescens* WCS365 in the RRS-10 and Knox-18 rhizospheres is specific to WCS365 or whether other *Pseudomonas* strains behave similarly, we isolated fluorescent *Pseudomonas* strains from the roots of *Arabidopsis* plants growing naturally at several sites in eastern Massachusetts, USA (see Methods and Supplementary Table 1; accession codes for full-length 16S rRNA sequences from *Pseudomonas* strains WCS365, CH229, CH235, CH254, CH255, CH267 and CH271 are KP253039-45). We selected six fluorescent *Pseudomonas* strains from different plant roots representing three ecological sites and found that all showed less growth in the RRS-10 and Knox-18 rhizospheres than in the Col-0 rhizosphere (Fig. 1c). We also tested the sequenced *Pseudomonas* strains *P. fluorescens* Pf0-1 and *P. brassicacearum* NFM421 and the well-studied plant pathogen *P. syringae* pv. *tomato* DC3000 (*Pto* DC3000) and found that similarly to WCS365, RRS-10 and Knox-18 support lower rhizosphere levels of all three of these *Pseudomonas* strains relative to Col-0 (Fig. 1c). These data indicate that compared to Col-0, Knox-18 and RRS-10 support less growth of a diverse repertoire of *P. brassicacearum*, *P. fluorescens* and *P. syringae* strains.

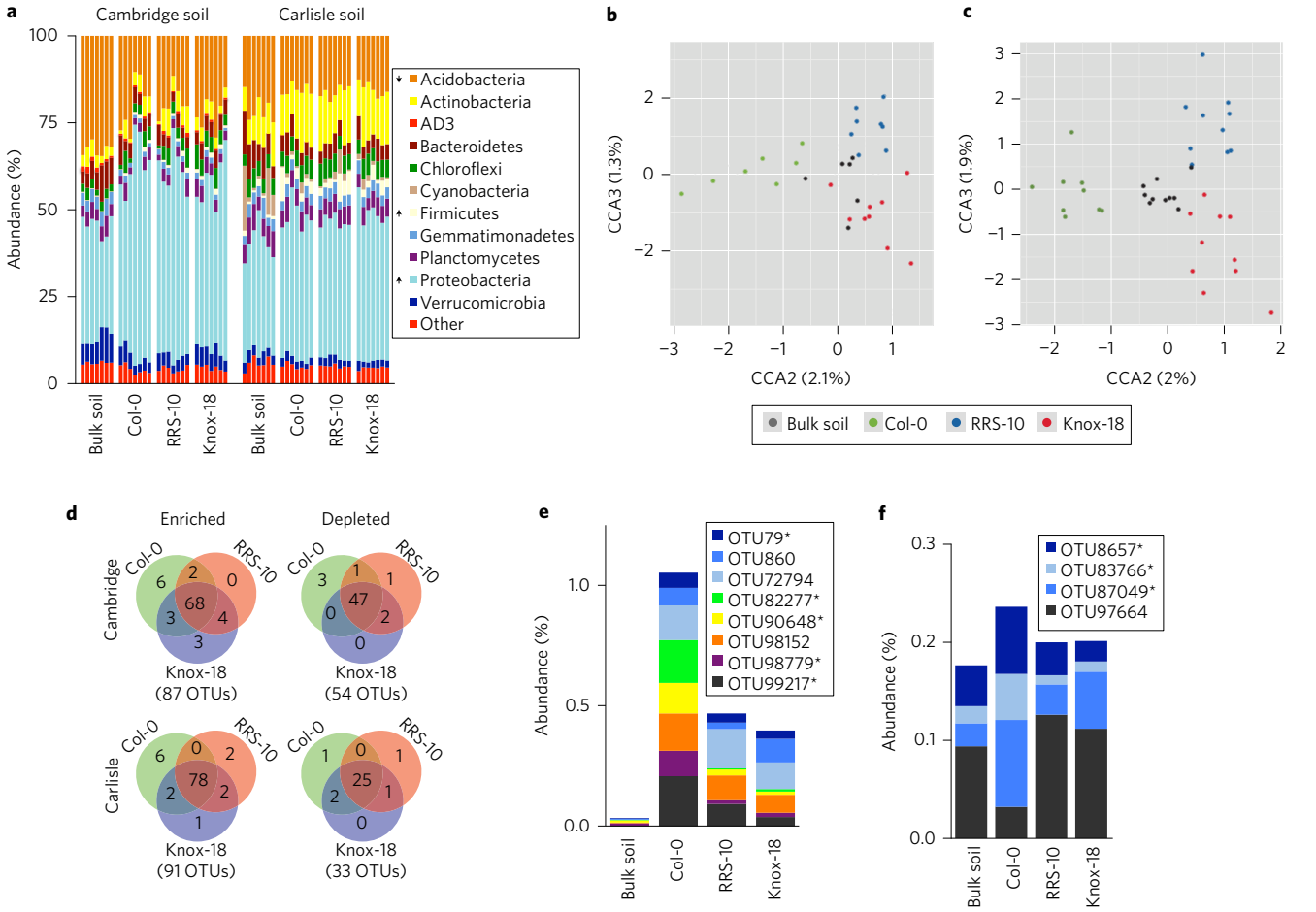

Figure 2 | The effect of *Arabidopsis* genotype is largely limited to OTUs in the family Pseudomonadaceae. a, Abundance of 16S rRNA genes from major bacterial phyla in bulk soil and the rhizospheres of Col-0, RRS-10 and Knox-18. Arrows denote phyla that are significantly enriched or depleted in the rhizosphere of all three accessions in both soils. **b**,**c**, Genotype-constrained principal component analysis of bulk soil and rhizosphere samples from Cambridge (**b**) and Carlisle (**c**) soils. **d**, Venn diagrams showing the number of OTUs that are enriched or depleted in the rhizosphere of an individual plant genotype relative to the other two plant genotypes. In Cambridge soil, 60% (15/25) of genotype-dependent differences were Col-0-specific ($P = 0.02$ by chi-squared test); in Carlisle soil, 55.6% (10/18) of genotype-dependent differences were Col-0 specific ($P = 0.03$ by chi-squared test). **e**,**f**, OTUs in the Pseudomonadaceae that are significantly enriched in the rhizosphere of at least one plant genotype grown in soil from Cambridge (**e**), or Carlisle (**f**). *OTUs that are enriched in the Col-0 rhizosphere relative to RRS-10 and Knox-18; $P < 0.01$ by moderated *t*-test.

To establish whether the *Arabidopsis* or the *P. fluorescens* genotype has a greater effect on *P. fluorescens* abundance in the rhizosphere, we tested WCS365, and the *Arabidopsis* rhizosphere isolates CH229 and CH267 on a set of nine *Arabidopsis* accessions spanning a range of compatibility with *P. fluorescens* WCS365. We found a strong correlation between the growth of *P. fluorescens* WCS365 with the growth of CH229 and CH267 ($R^2 = 0.95$ for WCS365 vs. CH229 and $R^2 = 0.89$ for WCS365 vs. CH267; Supplementary Fig. 2). Although the full-length 16S rRNAs from these strains are highly similar, geographical isolation combined with functional differences described below suggest that these are genotypically distinct bacterial strains. Collectively, these data suggest that *Arabidopsis* genotype rather than *P. fluorescens* genotype primarily determines the levels of certain *Pseudomonas* strains in the rhizosphere.

We also tested whether Col-0 supports higher levels of *P. fluorescens* than Knox-18 and RRS-10 in the context of an intact microbial community. We planted Knox-18, RRS-10 and Col-0 in two distinct soils collected from Cambridge and Carlisle, Massachusetts (soil chemistry and site locations are described in the Methods, Supplementary Table 2 and Supplementary Figs 3 and 4). The former is an unamended site with relatively low levels of endogenous fluorescent Pseudomonads (2.5×10^3 fluorescent CFU g^{-1}), whereas the latter is a cultivated soil with a relatively high level of endogenous fluorescent Pseudomonads (6×10^4 fluorescent CFU g^{-1}). In both soils, we found that relative to bulk soil, the culturable microbiome from Col-0 was enriched in fluorescent colonies from both soils when plated on King's B (Fig. 1d,e). We also found enrichment in the number of fluorescent Pseudomonads in Knox-18 and RRS-10, but to a significantly reduced level relative to Col-0 (Fig. 1e). This indicates that the results obtained in our gnotobiotic hydroponic system are reflective of indigenous levels of fluorescent Pseudomonads in an intact microbiome.

To determine how the endogenous bacterial microbiomes differ between Col-0, RRS-10 and Knox-18, we planted these three genotypes in the Cambridge soil and Carlisle soils described above and sequenced partial 16S rRNAs to determine bacterial diversity in the rhizosphere and in bulk soil (Methods and Supplementary Methods). Using a 97% identity threshold, we identified a total of 15,891 species defined as operational taxonomic units (OTUs) that were represented by at least 25 reads in any five samples (Methods and Supplementary Methods; alpha diversity for each sample type is shown in Supplementary Fig. 5). Consistent with previous reports, we found that (1) the microbial community in the starting soil has the largest influence on the rhizosphere

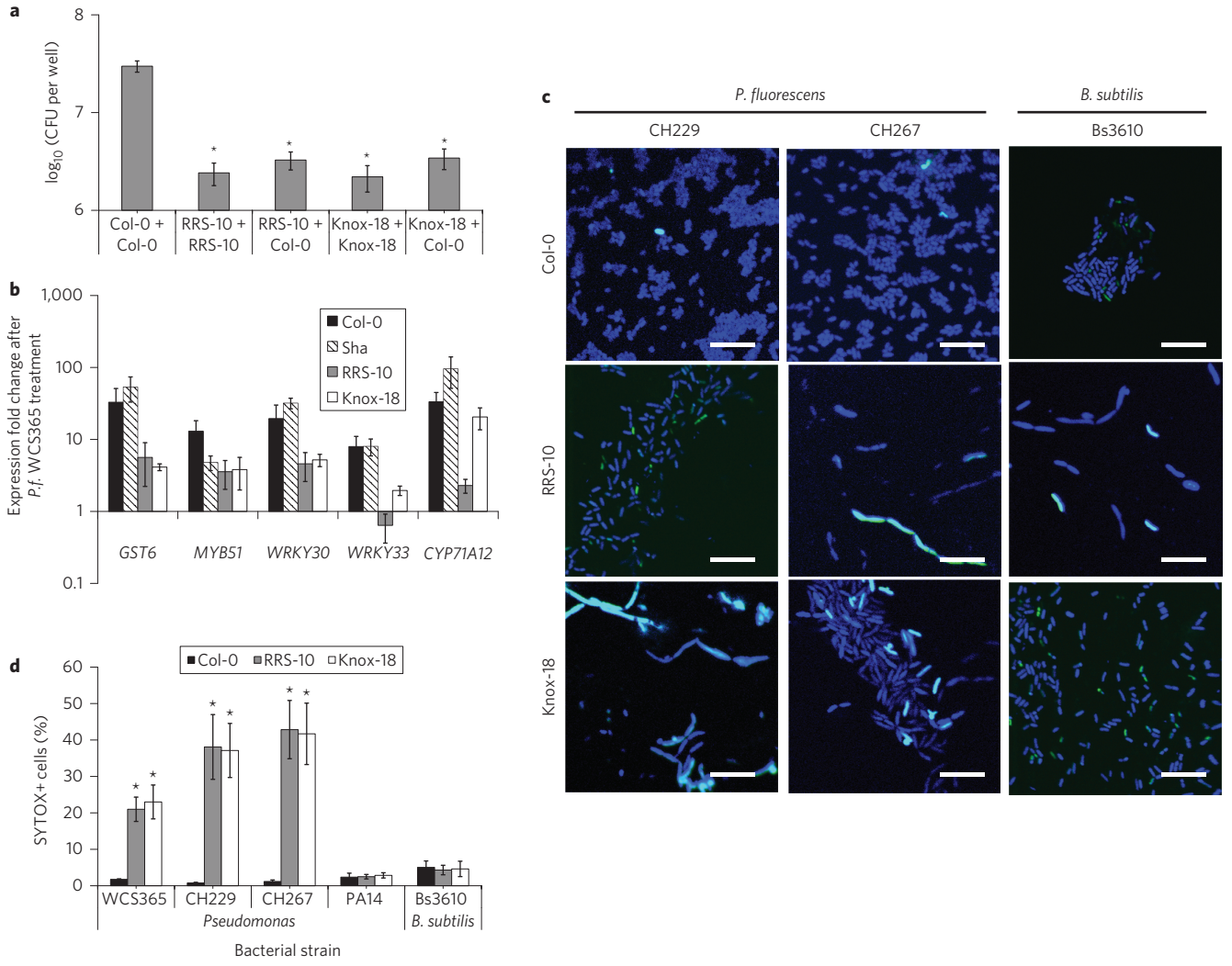

Figure 3 | Incompatible *Arabidopsis* accessions actively inhibit growth of *Pseudomonas*. **a**, Knox-18 and RRS-10 plants grown in the same wells as Col-0 can inhibit growth of bacteria in trans; $n = 18$ plant pairs per treatment. **b**, Defence gene induction 24 h after treatment with *P. fluorescens* WCS365 measured by qRT-PCR. Averages ± s.d. of three biological replicates are shown; $n = 24$ plants per replicate. **c**, Bacteria grown in 48-well plates with Col-0, RRS-10 or Knox-18 were stained with DAPI (blue) and SYTOX (green); Scale bar: 5 µm. **d**, Quantification of SYTOX+ positive cells in the *Arabidopsis* rhizosphere. **a**,**d**, $^*P < 0.001$ compared to Col-0 by *t*-test; Averages ± s.e.m. are shown.

microbiome[3,4,27]—24.4% of the variation between samples (Supplementary Fig. 6); (2) that the rhizosphere is enriched for the phyla Proteobacteria and Firmicutes[3,4,27] and depleted for Acidobacteria[3,4] (Fig. 2a); and (3) that host genotype had a minor but significant effect on rhizosphere community[3,4,28,29] (Fig. 2b–f).

Host genotype accounted for 1–2% of the difference in community structure depending on the soil and plant genotype (Fig. 2b,c). About 2% of the variation distinguished the Col-0 rhizosphere from both the Knox-18 and RRS-10 rhizospheres (constrained component 2 for both soils; Fig. 2b,c). The majority of the host genotype-mediated differences were observed at the OTU level, and of the OTU-specific differences, the majority distinguished Col-0 from RRS-10 and Knox-18 (Fig. 2d and Supplementary Figs 7 and 8). Of the OTUs that were specifically enriched in the Col-0 rhizosphere, a significant proportion was in the family Pseudomonadaceae. In the Cambridge soil, of the OTUs that were enriched to a greater degree in the Col-0 rhizosphere than in the Knox-18 and RRS-10 rhizospheres, 83.3% (5/6) were in the family Pseudomonadaceae; this is a significant enrichment relative to the 9.2% (8/87) Pseudomonadaceae OTUs that were enriched in association with all three plant genotypes ($P = 8 \times 10^{-7}$ by chi-squared test). Carlisle soil showed a similar trend where of the OTUs enriched to a greater degree in association with Col-0, 50% (3/6) were Pseudomonadaceae compared to 3.3% (3/91) of the total enriched OTUs that were Pseudomonadaceae ($P = 0.02$ by chi-squared test; Fig. 2e,f). The remaining OTUs that were more significantly enriched in association with Col-0 than RRS-10 and Knox-18 were all members of the Proteobacteria (Supplementary Table 4 and Figs 7 and 8). In Cambridge soil, there was a significant enrichment in the genus *Pseudomonas* and family Pseudomonadaceae in the rhizospheres of all three plant genotypes (Supplementary Fig. 9). Surprisingly, we did not find a significant enrichment of the genus *Pseudomonas* and family Pseudomonadaceae in rhizosphere samples from Carlisle soil (Supplementary Fig. 9). This is due to the presence of a single OTU in the family Pseudomonadaceae that was depleted in the Col-0 rhizosphere relative to bulk soil (Fig. 2f). Collectively, these data indicate that the mechanism by which Knox-18 and RRS-10 limit growth of *Pseudomonas* in the rhizosphere is largely limited to a subset of species (defined as OTUs with 97% identity) in the family Pseudomonadaceae.

To determine whether Knox-18 and RRS-10 fail to support bacterial growth, for instance by not providing a nutrient the bacteria require, or if these genotypes actively suppress bacterial growth we

co-cultured Col-0 and RRS-10 or Col-0 and Knox-18 (by growing two plants in the same well) and measured *P. fluorescens* growth. We found that bacterial growth associated with the mixed genotype co-cultured plants was more similar to two RRS-10 or two Knox-18 plants than to two Col-0 plants (Fig. 3a). We also measured the growth of bacteria in root exudates and found that RRS-10 and Knox-18 exudates supported less growth of several *P. fluorescens* strains relative to Col-0 exudate after 1 day of bacterial growth; by 2 days, bacterial growth was equivalent in the exudates of all three accessions (Supplementary Fig. 10). These findings indicate that Knox-18 and RRS-10 actively inhibit bacterial growth by a mechanism that appears to be inducible.

Next, we assessed whether the mechanism by which Knox-18 and RRS-10 inhibit *Pseudomonas* growth has signatures of characterized defence responses. We examined the expression of defence responsive genes including the flagellin-inducible genes *MYB51*[30] and *CYP71A12*[30] and the flagellin and salicylic acid-inducible genes *GST6*[31], *WRKY30*[32] and *WRKY33*[33] (we also tested expression of *PR1* and *PR2* but could not detect transcripts in roots under any conditions). We found that all defence genes tested had similar or higher induction levels in compatible interactions (in Col-0 and Sha) than in incompatible interactions (RRS-10 and Knox-18) (Fig. 3b). This indicates that the magnitude of defence responses do not correlate with bacterial growth inhibition but rather appear to be a signature of a compatible interaction.

Based on the observation that Knox-18 and RRS-10 actively inhibit bacterial growth, we hypothesized that these accessions are secreting an antimicrobial compound. A combination of bacterial vital dyes can be used to assess the mode of action of unknown antimicrobial compounds[34]. We found that *Pseudomonas* strains WCS365, CH229 and CH267 growing in the Knox-18 and RRS-10 rhizospheres exhibited an increase in membrane permeability as indicated by a 30- to 40-fold increase in the fraction of cells that took up the membrane-impermeable DNA-binding dye SYTOX (Fig. 3c,d). We also observed an increase in bacterial cell size and bacterial filament formation associated with Knox-18 and RRS-10 roots. No change in cell shape or the number of SYTOX-positive *B. subtilis* or *P. aeruginosa* cells was observed in association with RRS-10 and Knox-18 roots (Fig. 3c,d). The observed changes in *P. fluorescens* cell morphology are reminiscent of those induced by inhibitors of cell wall synthesis or inhibitors of cell division[34]. These data are consistent with Knox-18 and RRS-10 actively suppressing growth of some *Pseudomonas* strains through the production of an antimicrobial compound.

Because we found evidence for active suppression of bacterial growth in the rhizosphere, we tested whether Knox-18 and RRS-10 can also suppress growth of the pathogenic *Pseudomonas* strain *Pto* DC3000 in a standard foliar pathogenicity assay. RRS-10 and Col-0 exhibited no significant difference in susceptibility to *Pto* DC3000, and Knox-18 was significantly more susceptible than Col-0 (~5-fold higher bacterial growth relative to Col-0, $P < 0.05$; Supplementary Fig. 11). This indicates that the differences in *Pseudomonas* growth associated with RRS-10 and Knox-18 are rhizosphere-specific.

The results described thus far establish that natural variation in *Arabidopsis* affects *Pseudomonas* abundance in the rhizosphere. Our next aim was to determine whether *Arabidopsis* genotype-mediated differences in *Pseudomonas* result in differences in plant health. *P. fluorescens* is generally known to promote plant growth and increase lateral root formation[35–37]. We observed growth promotion and increased lateral root formation on Col-0 grown vertically on solid agar plates (with no added carbon source) by *P. fluorescens* WCS365 and the majority of Massachusetts *Pseudomonas* isolates tested (four of six) (Supplementary Fig. 12). In contrast, RRS-10 and Knox-18 did not show increased biomass or numbers of lateral roots in the presence of *P. fluorescens* WCS365 or *Pseudomonas* isolates CH229 and CH267 (Fig. 4a–c) indicating that in our assays, a compatible host genotype is required for plant growth promotion by beneficial bacteria. We also observed growth inhibition of Knox-18 in the presence of strain CH229, indicating that some *P. fluorescens* strains might have a detrimental effect on incompatible accessions. This observation suggests a possible selection pressure for the evolution of a mechanism that limits *Pseudomonas* growth in the *Arabidopsis* rhizosphere.

To determine whether host genotype confers a selective advantage when a more complex microbiome is present, we assessed the ability of *Pseudomonas* to promote *Arabidopsis* growth in both natural and commercial soils. For a natural soil, we chose the soil from Cambridge, Massachusetts, that has low levels of indigenous fluorescent Pseudomonads ($2.5 \pm 0.6 \times 10^3$ CFU g^{-1} soil; growth promotion and pathogen protection occur at *P. fluorescens* levels upwards of 10^5 CFU g^{-1} root[38]). We reasoned that this soil type would allow us to obtain a baseline plant weight that captures the plant genotype–environment and genotype–microbe interactions that are largely independent of fluorescent Pseudomonads. In this soil, the shoot weights of RRS-10, Knox-18 and Col-0 were not statistically significantly different (Fig. 4d). When the soil was inoculated with 10^5 CFU g^{-1} *Pseudomonas* strain CH267 (isolated from this soil), but not with equivalent amounts of WCS365 or CH229 (from different soils), we observed significant growth promotion only on Col-0 but not on RRS-10 or Knox-18 (Fig. 4d and Supplementary Fig. 13a). This indicates that both *Arabidopsis* and *Pseudomonas* genotype contribute to whether the *Arabidopsis* microbiome provides a benefit to its host.

In commercial soil, we were only able to observe *P. fluorescens*-dependent growth promotion on Col-0 under nutrient-limiting conditions (when plants were grown in very small cells and soil was diluted with vermiculite). Under these conditions, we did not observe growth promotion on RRS-10 or Knox-18 (Fig. 4e and Supplementary Fig. 13b). Growth promotion of all accessions in both natural and commercial soils was observed with fertilizer addition indicating that plants were indeed nutrient limited (Fig. 4e). We found that growth promotion in soil and on plates did not always correlate (Fig. 4). These results indicate that host genotype-mediated differences in microbiome community may not be important for growth under nutrient-rich soil conditions. However under conditions of stress (such as nutrient limitation), a host's ability to cultivate beneficial microbes may provide an advantage.

To determine how the plant rhizosphere changes upon artificial addition of *Pseudomonas*, we compared 16S rRNAs from the rhizosphere of plants grown in Cambridge soil with or without CH267 added. We found that addition of *Pseudomonas* CH267 had a minor but significant effect on the microbial community and explained 1.6% of the differences in microbiome composition (treatment-constrained CCA; Fig. 5). We found that upon addition of *Pseudomonas* CH267 (which likely corresponds to OTU79; Fig. 5b), both bulk soil and the Col-0 rhizosphere showed a significant enrichment of this OTU while the RRS-10 and Knox-18 rhizospheres did not (Fig. 5b). Addition of CH267 also resulted in a significant increase in the abundance of multiple genera within the Firmicutes in both bulk soil and in the Col-0 rhizosphere, but not in RRS-10 and Knox-18 rhizospheres (Fig. 5c). We found a reciprocal change in a single genus within the Bacteroidetes (*Flavobacterium*; Fig. 5d); a significant decrease in abundance of *Flavobacterium* was found in the Col-0 rhizosphere and in bulk soil, while an increase in abundance of *Flavobacterium* was observed in the rhizospheres of Knox-18 and RRS-10. We identified 46 OTUs that had distinct behaviours in the plant rhizospheres in untreated soil compared to CH267-treated soil (that is, enriched in the rhizosphere of plants grown in untreated soil and depleted in the rhizospheres of plants grown in soil treated with

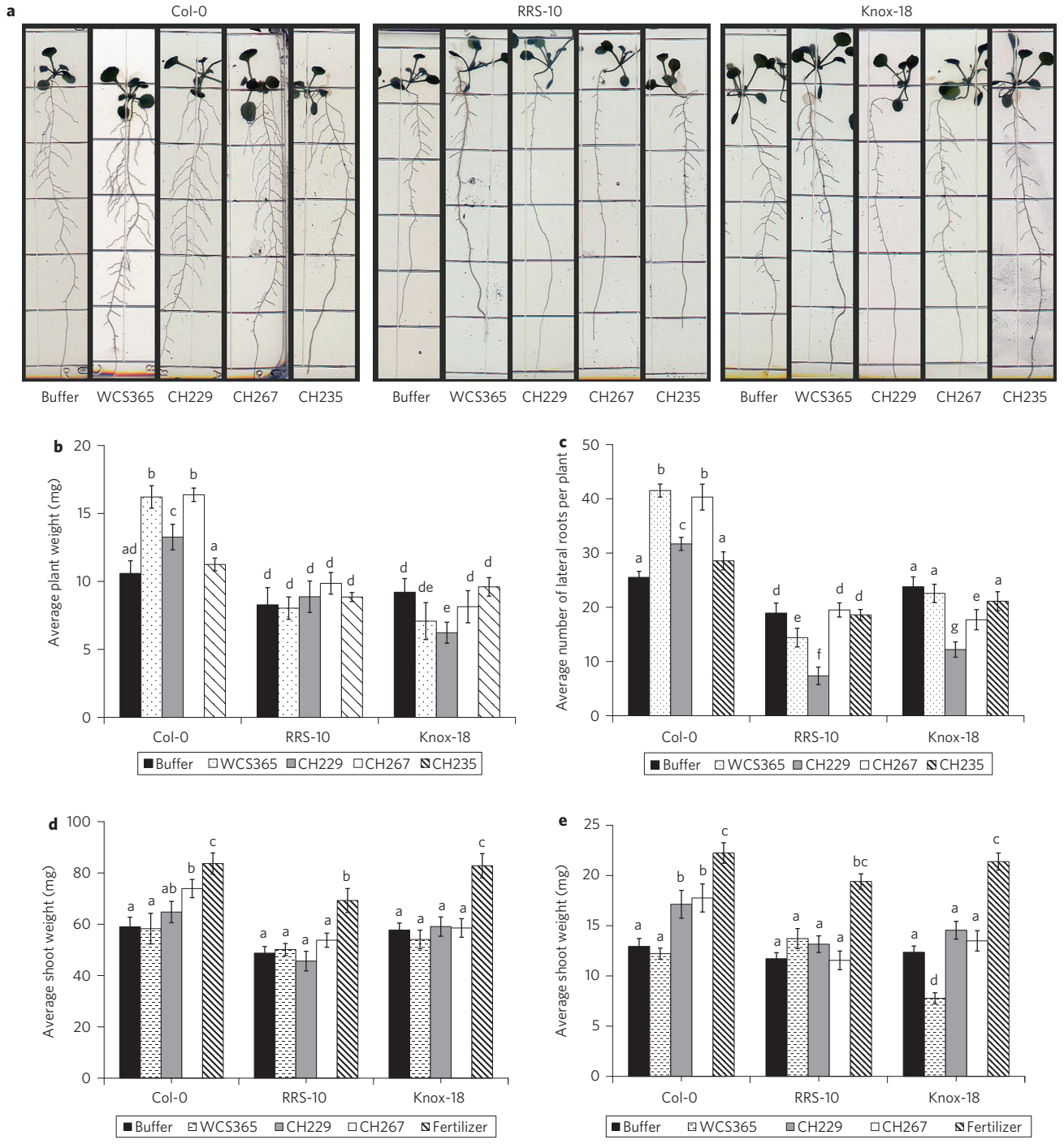

Figure 4 | *Pseudomonas* strains do not promote the growth of incompatible accessions. a, Col-0, RRS-10 and Knox-18 grown on agar plates with no carbon source and inoculated with buffer or the indicated *Pseudomonas* strains. **b**, Quantification of fresh plant weight. **c**, Number of lateral roots on plants shown in **a**; $n = 15$ plants per treatment. **d**, Fresh shoot weight of plants grown in Cambridge soil and treated with 10 mM $MgSO_4$, bacteria resuspended to an A_{600} of 0.01 in 10 mM $MgSO_4$ or fertilizer. **e**, Fresh shoot weight of plants grown in a commercial soil/vermiculite mixture and treated as described in **d**; in **b**–**d** letters designate $P < 0.05$ by ANOVA and Tukey's HSD tests; $n \geq 12$ plants; averages ± s.e.m. are shown.

Pseudomonas CH267; Supplementary Table 4); the majority of these changes could be explained by differences in OTU abundance in treated versus untreated bulk soil (Supplementary Table 4). Because there were changes to the overall community composition, we cannot rule out the possibility that plant growth promotion in soil is an indirect result of the effect of *Pseudomonas* on the microbial community. These data indicate that addition of *Pseudomonas* CH267 to Cambridge soil at a level that is sufficient to confer plant growth promotion (1) results in significant changes to a small number of bacterial taxa and (2) has little effect on the overall rhizosphere microbiome community.

To study the effects of host genotype-mediated *P. fluorescens* levels on pathogen stress, we developed a hydroponic infection assay to study susceptibility to the fungal pathogen *Fusarium oxysporum*. We found that Col-0 had reduced susceptibility to *F. oxysporum* in the presence of *P. fluorescens* WCS365 and several

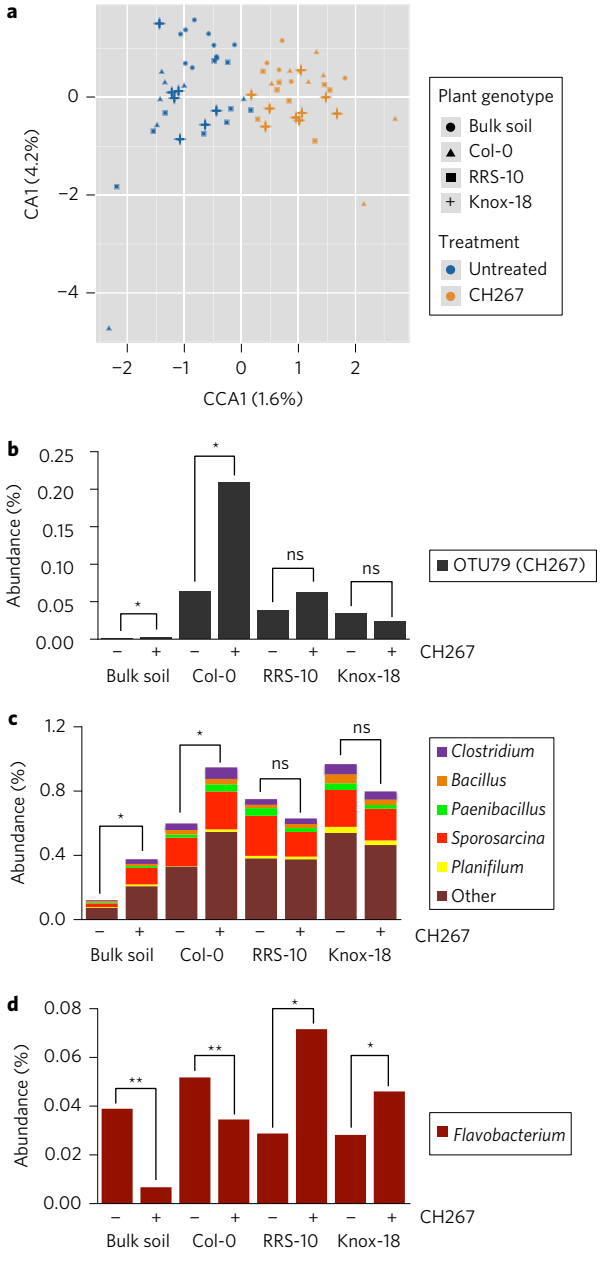

Figure 5 | Addition of *Pseudomonas* has a minor but significant effect on soil and rhizosphere community composition. a, Treatment-constrained principal component analysis of bulk soil and rhizosphere samples with or without the addition of *Pseudomonas* CH267. **b**, Relative abundance of OTU79 (which probably corresponds to *Pseudomonas* CH267) in rhizosphere and soil samples with and without CH267 added. **c**, Abundance (%) of genera in the Firmicutes in Cambridge soil with or without the addition of *Pseudomonas* CH267. Upon addition of CH267, all genera listed showed significant enrichment in bulk soil and the Col-0 rhizosphere and no significant difference in the rhizospheres of RRS-10 and Knox-18. **d**, Abundance (%) of the genus *Flavobacterium* with or without addition of CH267. In **b**–**d** *indicates a significant increase; **indicates a significant decrease by ANOVA and moderated *t*-test ($P < 0.01$); ns, not significant; averages ± s.e.m. are shown.

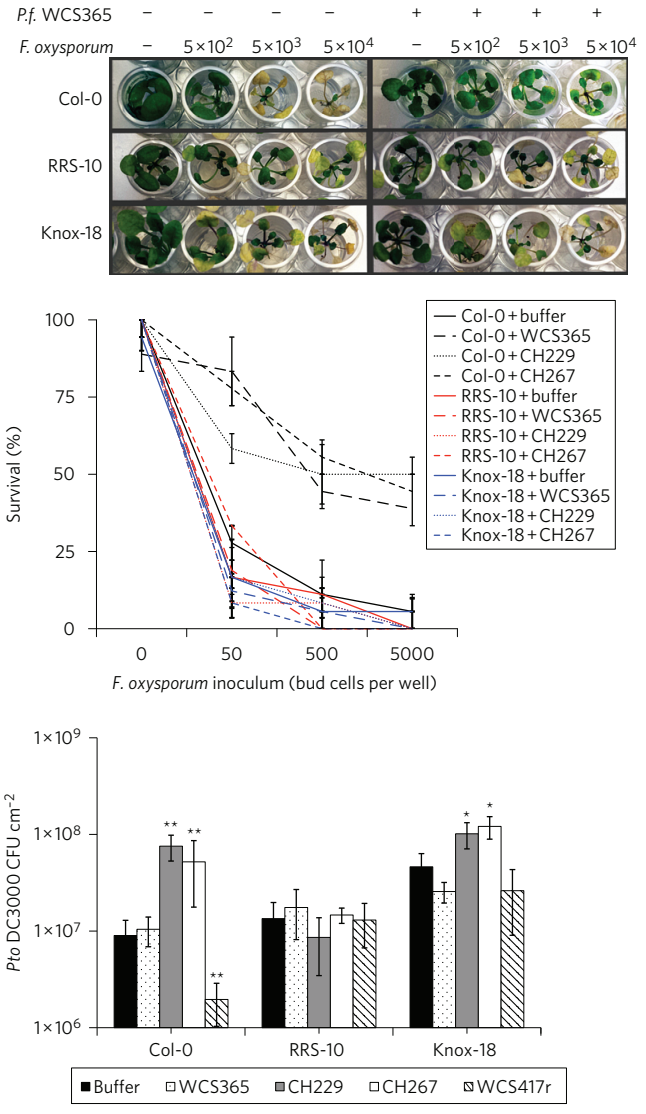

Figure 6 | Disease outcome depends on host genotype, the *Pseudomonas* strain present and the pathogen. a, Accessions were grown hydroponically in 48-well plates and treated with buffer, *P. fluorescens* and/or *F. oxysporum* (*For* 815) as indicated. Representative images 14 days post inoculation with WCS365 are shown. **b**, Percentage survival of plants inoculated with *P. fluorescens* WCS365, CH229 or CH267 and *For* 815 after 21 days. **a**,**b**, Average ± s.d. of three replicates with $n = 6$ plants per treatment per rep. **c**, Growth of *Pto* DC3000 in the leaves of adult soil-grown plants grown with *P. fluorescens* in the rhizosphere. $n = 12$ leaves from six plants; *$P < 0.05$; **$P < 0.01$ by Tukey's HSD test; averages ± s.e.m. are shown.

Massachusetts *Pseudomonas* strains than in their absence (Fig. 6a,b and Supplementary Fig. 14a). In contrast to Col-0, incompatible accessions RRS-10 and Knox-18 did not exhibit *P. fluorescens*-dependent protection from *F. oxysporum* (Fig. 6a,b and Supplementary Fig. 14). This trend was consistent when measured as the percentage of plants that survived after 3 weeks (Fig. 6b), *F. oxysporum* levels in the rhizosphere (Supplementary Fig. 14b), or the number of infection events (Supplementary Fig. 14c,d). Not all rhizosphere-competent strains were able to protect Col-0, indicating that *F. oxysporum* growth is not simply being limited by depletion of nutrients from the medium (Supplementary Fig. 14a). These results indicate that *Arabidopsis* genotypes that curate rhizosphere colonization by *Pseudomonas* have a selective advantage in the presence of beneficial *P. fluorescens* and *F. oxysporum*.

Some strains of *P. fluorescens* in the rhizosphere can induce systemic resistance (ISR) to foliar pathogens including the leaf pathogen *Pto* DC3000[39]. Consistent with previous studies, we found that *P. fluorescens* WCS417r could induce ISR and protect Col-0 from *Pto* DC3000[40] (Fig. 6c). We also tested two of the Massachusetts *Pseudomonas* isolates for their ability to induce ISR and found

unexpectedly that they actually induced systemic susceptibility (ISS) to *Pto* DC3000 (Fig. 6c). Under conditions where we observed *P. fluorescens*-dependent ISR or ISS, we observed no effect on RRS-10 and a marginally significant effect on Knox-18 (Fig. 6c). This indicates that there may be a complex evolutionary trade-off to supporting *P. fluorescens* in the rhizosphere; depending on the *P. fluorescens* strain present in the soil, and the particular pathogen stresses, there may be advantages or disadvantages to generally supporting or inhibiting *Pseudomonas* growth.

Discussion

Our data indicate that accessions of *Arabidopsis* actively inhibit growth of some species within the Pseudomonadaceae while leaving the majority of the microbiome intact. While reminiscent of a defence response, this is distinct from the paradigm of well-characterized defence mechanisms in plants (that is, Resistance (*R*) genes) that achieve specificity at the perception level[41], but where the inputs from perception of diverse pathogens converge on largely overlapping downstream pathways[42]. Known downstream defence mechanisms are largely blunt force antimicrobial instruments such as plant cell death, reactive oxygen species production, or production of broad-spectrum antimicrobials[43,44] that would not be expected to limit the growth of a narrow group of bacterial taxa. We also did not detect increased expression of defence genes in incompatible accessions; in fact, defence gene induction was more closely correlated with compatibility (Fig. 2b). Together, these data indicate that the mechanism by which Knox-18 and RRS-10 limit growth of *Pseudomonas* strains in the rhizosphere may be distinct from well-characterized defence responses in plants.

We conclude that plant genotype is an essential determinant of whether a microbiome community provides benefit or harm to its host. Random encounters with beneficial microbes are not sufficient to explain the health benefits of the plant microbiome; if the plant genotype is incompatible with growth of the beneficial microbe, the plant will not benefit from its presence in the environment. We found that a particular microbiome will provide a benefit or detriment to the host depending on (1) the particular biotic stress and (2) the individual *Pseudomonas* strains present in the rhizosphere. This indicates that there may be an evolutionary trade-off to inhibiting growth of *Pseudomonas*. Our results indicate that plants within a single species have varied rhizosphere microbiomes, which result in divergent outcomes for plant health and physiology depending on precise biotic and abiotic stresses. Thus, multicellular organisms possess the machinery for ongoing microbiome-mediated adaptation in the face of changing nutrient, environmental and pathogen stresses.

Methods

Rhizosphere assay in 48-well plates. Eight-millimetre Teflon mesh disks (McMaster-Carr #1100t41) were sterilized by autoclaving and placed into sterile clear-bottom 48-well tissue culture plates (BD Falcon) with 250 µl Murashige and Skoog (MS) media containing 2% sucrose. Sterile imbibed seeds were placed individually at the centre of each disk. Plates were placed in the dark for 3 days so that the seedlings etiolated. Plates were subsequently transferred to a shelf with 16 h light/8 h dark (at a light fluence of 100 µE) at 22 °C. Ten days after plating, the media was removed and replaced with 270 µl 1/2X MS media with no sucrose. Two days after the media change, 30 µl of bacteria were added to a final absorbance A_{600} = 0.00002 (~3,000 CFU per well). Plates were read from the bottom using a SpectraMax M3 fluorescent plate reader (Molecular Devices) with 418/515(20) nm excitation/emission. Any plants with submerged leaves or showing symptoms of water stress were discarded. The collection of 196 accessions used in the primary screen was described previously[26]. For bacterial growth assays, a minimum of 24 plants was assayed per plant genotype per bacterial strain. For bacterial strains expressing GFP, CFUs per well were approximated by generating a standard curve for each strain. For strains not expressing GFP, media from the well was removed and serially diluted to determine CFUs. To measure bacterial growth in plant root exudate, plants were removed 2 days after the media change and bacteria were added to the wells as described above. Statistics were performed with StatPlus (AnalystSoft).

Natural soil collection, *Arabidopsis* population sites and isolation of new strains of *P. fluorescens*. 'Cambridge' soil was collected near the Weld Boathouse in Cambridge, MA (42 °22′10″ N, 71 °7′18″ W). 'Carlisle' soil was isolated from a vegetable garden at GPS coordinates 42 °31′38″ N, 71 °21′4″ W in Carlisle, Massachusetts (Supplementary Fig. 4). Soil chemistry and texture was assayed by the University of Massachusetts soil and plant tissue testing laboratory and is summarized in Supplementary Table 2. Additional site information and soil collection methods are present in the Supplementary Methods. New strains of *Pseudomonas* were isolated from the roots of plants growing at these and other sights around Massachusetts (Supplementary Methods and Supplementary Table 1).

Microbiome assays. Microbiome samples included the plant root and soil that was firmly adhered to the root. Seven-day-old seedlings were transferred from plates (MS media with 2% sucrose) to pots. Plants were grown in 12 h days and 12 h nights at a fluence of 100 µE. Four seedlings were planted per 7.5 cm pot, ten pots were planted per genotype per soil type, and all four plants were pooled for microbiome analysis. An equivalent number of unplanted pots were used for 'bulk soil' samples. Rhizosphere and bulk soil samples (8 mm cores) were harvested 21 days after transplanting.

16S rRNA microbiome sample preparation, sequencing and analysis. DNA was extracted using the MoBio Powerlyser Power soil kit (MoBio). DNA from seedlings grown sterilely on MS plates (prior to transfer) was also included to confirm that there was no contamination and that the seeds did not harbour any entophytic microbes that survived sterilization. Samples were prepared for sequencing as described in the Earth Microbiome Project (http://www.earthmicrobiome.org/) using primers 515F and 806R and sequenced using MiSeq v3 600 cycles kit (Illumina).

The fastq files from sequencing reads were processed with the QIIME software package[45] (Supplementary Methods). Taxa that were significantly enriched or depleted were determined using the R software package (http://www.r-project.org/) as described[3] using ANOVA and a Bayesian model-based moderated *t*-test[46] that takes into account the variance of all OTUs when determining significance.

Plant growth promotion plate assays. For plate assays, ten seeds were germinated on small square plates containing MS media with no sucrose. Five days after planting, seeds were thinned to five seedlings per plate and inoculated with 1 µl of 10 mM $MgSO_4$ or bacteria diluted to A_{600} = 0.001 (~10^3 CFU per plant or ~10^5 CFU g^{-1} of root) in $MgSO_4$. Ten days after inoculation plants were imaged on an Epson V750 Pro scanner and then removed from plates and fresh weight was determined.

For commercial soil experiments, seeds were sown directly in a 1:1 mix of Sunshine MVP (SunGro Horticulture) and vermiculite in 1.8 cm diameter cells. Eight days after sowing, plants were treated with 1 ml bacteria (A_{600} = 0.01 in 10 mM $MgSO_4$; 10^5 CFU g^{-1} soil), 1 ml 10 mM $MgSO_4$ or 1/4X Hoagland solution. Plants were imaged and weighed 2 weeks after inoculation.

For natural soil experiments, plants were grown as described for microbiome experiments (above) and treated with 5 ml bacteria (A_{600} = 0.01 in 10 mM $MgSO_4$; 10^5 CFU g^{-1} soil), 5 ml 10 mM $MgSO_4$ or 1/4X Hoagland solution. Plants were imaged and weighed 3 weeks after inoculation.

Infection assays. *Pto* DC3000 infection assays[47] and *Fusarium* infection assays[48] were performed as described with modifications (Supplementary Methods).

Data availability. *Pseudomonas* full-length 16S rRNA from new strains have been deposited in GenBank: WCS365, CH229, CH235, CH254, CH255, CH267 and CH271 (accession codes: KP253039, KP253040, KP253041, KP253042, KP253043, KP253044 and KP253045). Illumina HiSeq microbiome data are available on request from C.H.H. (haney@molbio.mgh.harvard.edu).

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Author contributions

C.H.H. and F.M.A. conceived experiments and discussed results. C.H.H. and J.B. designed assays and performed experiments. B.S.S. and C.H.H. analysed data. C.H.H. wrote the manuscript with input from F.M.A., J.B. and B.S.S.

Additional information

Supplementary information is available online. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to F.M.A.

Competing interests

The authors declare no competing financial interests.