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Microbe-induced phenotypic variation leads to overyielding in clonal plant populations

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Waseem Raza[1,2,5], Gaofei Jiang[1,5], Nico Eisenhauer[3,4], Yishuo Huang[1], Zhong Wei[1] ✉, Qirong Shen[1], George A. Kowalchuk[2] & Alexandre Jousset[1] ✉

Overyielding, the high productivity of multispecies plant communities, is commonly seen as the result of plant genetic diversity. Here we demonstrate that biodiversity–ecosystem functioning relationships can emerge in clonal plant populations through interaction with microorganisms. Using a model clonal plant species, we found that exposure to volatiles of certain microorganisms led to divergent plant phenotypes. Assembling communities out of plants associated with different microorganisms led to transgressive overyielding in both biomass and seed yield. Our results highlight the importance of belowground microbial diversity in plant biodiversity research and open new avenues for precision ecosystem management.

Biodiversity–ecosystem functioning (BEF) relationships are predominantly ascribed to trait complementarity, that is, when co-existing plants differ in their resource use efficiency, morphology or stress resistance[1,2]. This complementarity allows mixtures of plant species to exploit their environment more efficiently than monocultures[3,4], thereby increasing ecosystem functioning and often resulting in (transgressive) overyielding, that is, higher productivity than the constituent monocultures[5–7]. Plant phenotypic variability can be linked with intra- and interspecific genetic diversity, with phylogenetically distinct plants tending to harbour more divergent traits[5,8]. It is further modulated by environmental conditions, including resources, stressors, symbionts or natural enemies[9,10]. As a result, a given set of plant species may interact in different ways depending on the environmental conditions[4]. There is in particular an increasing awareness of the plant-associated microbiome as one of the major determinants of plant phenotype[11,12]. Plant-associated microorganisms encode thousands of functional genes directly influencing plant phenotype and even expanding it by providing additional enzymatic capabilities[13,14]. This genetic pool co-regulates a range of plant traits, including disease resistance, nutrient use efficiency or morphology[15,16]. Plant- and microbiome-encoded traits show such a level of integration that a growing scientific community approaches the plant as a holobiont combining the plant genome and the microbiome metagenome into a single cohesive unit[17].

The interplay between plants and their environment is often approached under the assumption that all plants in a local population are exposed to a similar environment, including alpha but not beta diversity[18,19]. We surmise that this may lead to an incomplete view of plant–plant interactions. In a natural soil environment, microbiome composition varies over small distances, to the point that two neighbouring clonal plants may experience divergent phenotypes, for instance, disease resistance[20]. As different phenotypes may lead to overyielding, we sought to integrate plant–microbe interactions into BEF relationships and test whether overyielding may arise in clonal populations when co-existing plants interact with distinct microorganisms. Specifically, as different microbial species may induce distinct plant phenotypes[13], we anticipated that mixtures of clonal plants associated with different microorganisms may outperform their respective 'monocultures' due to phenotypic complementarity.

To investigate this hypothesis, we primed plants with microorganisms representative of those found on plant roots. We kept plants and bacteria physically separated while allowing, through a shared headspace, the diffusion of signal molecules such as volatile compounds, which are well-established to potentially rewire key plant hormonal pathways[21,22] and alter several plant traits[23]. The main rationale for this experiment is to manipulate plant–microbe interactions while keeping the plant sterile, preventing downstream cross-contamination

[1]College of Resources and Environmental Science, Key Lab of Organic-Based Fertilizers of China and Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, Nanjing Agricultural University, Nanjing, China. [2]Institute for Environmental Biology, Ecology and Biodiversity, Utrecht University, Utrecht, the Netherlands. [3]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany. [4]Institute of Biology, Leipzig University, Leipzig, Germany. [5]These authors contributed equally: Waseem Raza, Gaofei Jiang. ✉e-mail: weizhong@njau.edu.cn; jousset@njau.edu.cn

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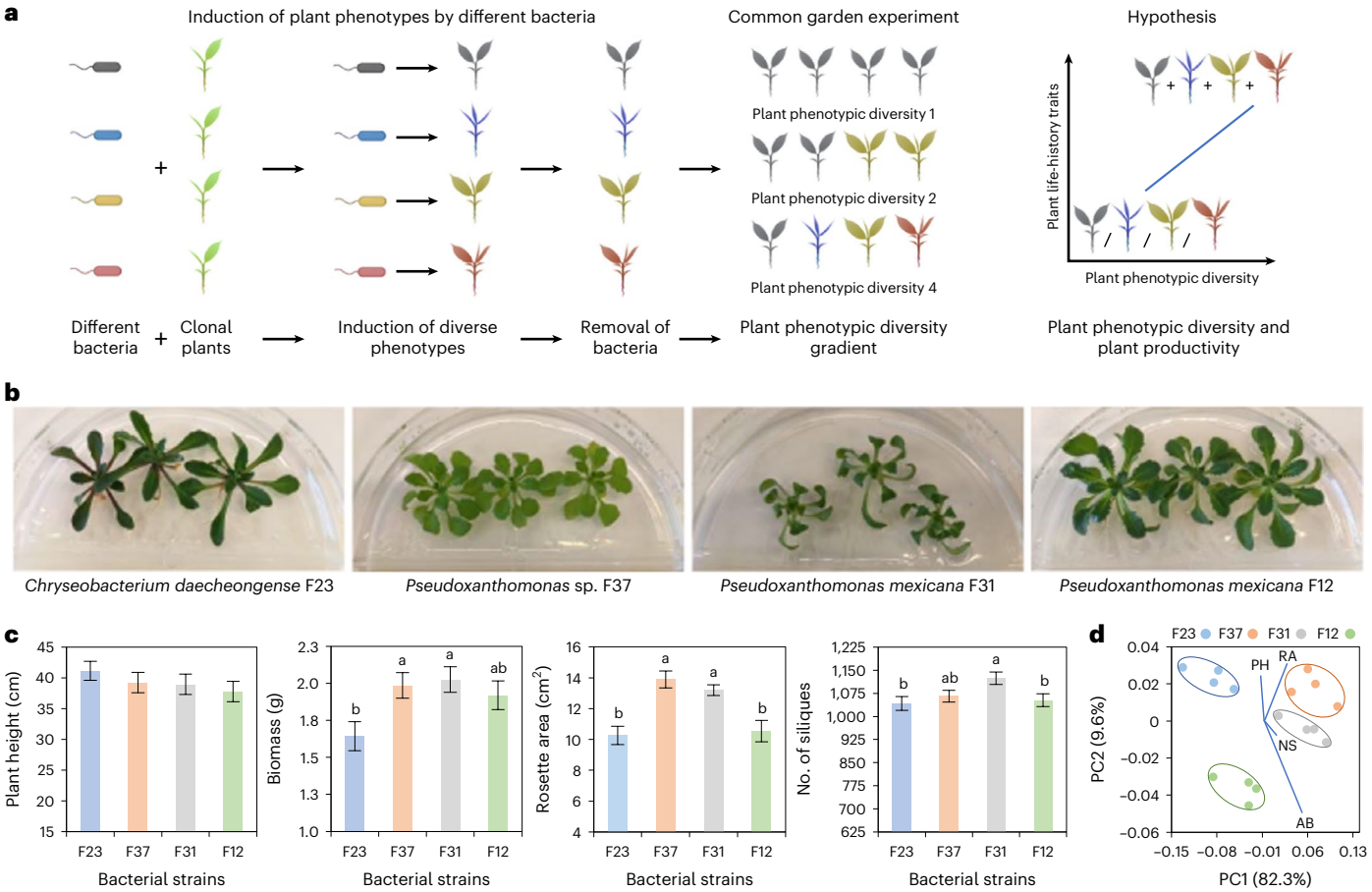

Fig. 1 | Overview of the experimental design and induction of specific plant phenotypes by individual bacteria. a, Sterile clonal plant seedlings were exposed to the volatile compounds of four distinct bacteria during their early life. The experimental design kept plants and bacteria spatially separated, allowing diffusion of volatile compounds while keeping plants sterile. After phenotypic induction, bacteria were removed and plants were placed together in a common-garden experiment at diversity levels of 1, 2 and 4 phenotypes. We hypothesized that the phenotypic diversity in clonal plants induced by different bacteria may lead to complementarity and improve the life-history traits of the community. **b**, Exposure to different bacteria-induced isolate-specific phenotypic variations in clonal plants as illustrated by leaf morphology. **c**, Bacteria altered 4 major plant life-history traits: plant height (PH), aboveground biomass (AB), rosette area (RA) and number of siliques (NS). Error bars show the mean ± s.d. ($n = 4$). Data were analysed using one-way ANOVA separately for plant height ($F_{(3,12)} = 7.99$, $P = 0.141$), aboveground biomass ($F_{(3,12)} = 9.98$, $P = 0.001$), rosette area ($F_{(3,12)} = 43.33$, $P = 2.6 \times 10^{-6}$) and number of siliques ($F_{(3,12)} = 8.46$, $P = 0.003$). Bars with different letters are significantly different based on Tukey's HSD test, $P < 0.05$. **d**, Principal component analysis based on plant life-history traits at the end of the growth period indicates that plants retained distinct phenotypes long after removal of the associated bacteria. Each data point ($n = 16$) is the average of 4 replicates.

in the later common garden experiment. First, we selected four root-associated bacterial strains out of an initial collection (Supplementary Data 1 and Supplementary Table 1) for their ability to induce visually different phenotypic characteristics in sterile *Arabidopsis thaliana* seedlings via the action of volatile compounds they produce (Extended Data Figs. 1 and 2, and Supplementary Data 2). Later, we grew seedlings for 1 week on divided agar plates such that they were exposed to the volatile compounds of one of the four bacterial strains. This design enabled us to induce plant phenotypic variation (representing four different phenotypes; Fig. 1a) while allowing for the physical separation needed to prevent confounding effects due to contamination. Plants were subsequently transplanted to pots to construct a phenotypic diversity gradient of 1, 2 and 4 co-cultivated phenotypes. To disentangle effects due to biotic interactions from those due to plant density, we grew all possible plant combinations at densities of 1, 2 and 4 plants per pot. In total, we built 28 independent treatments (Supplementary Table 2). Community-level functioning and overyielding pattern for measures beyond biomass were evaluated by tracking 4 plant life-history traits: plant height, aboveground biomass, rosette area and number of siliques. We used the relative interaction index (RII)[24] to characterize facilitation or competition between phenotypes. This index shows the normalized performance of each plant phenotype in the presence of different phenotypes relative to its performance in monoculture at the same density level[24]. We hypothesized that phenotypic variation in clonal plants arising from association with different bacteria may lead to complementarity and increase the performance of mixtures (Fig. 1a).

Exposing clonal plants to the four selected bacterial strains induced clear, diverging plant phenotypes after 1 week of co-cultivation on divided agar plates (Fig. 1b). This finding confirms the ability of microorganisms to alter plant phenotype and showcases the importance of volatile compounds as an important class of components involved in microbe-driven plant phenotype induction[23,25]. Microbial volatile exposure impacted a range of key life-history traits, such as plant height, rosette area, aboveground biomass and number of siliques (Fig. 1c,d). Importantly for the experimental design and the broader implications of this work, the differences in plant phenotype persisted for the whole duration of the experiment even after removal of the bacteria and their signalling molecules (Fig. 1d).

When grown in mixtures (common garden experiment), plant density generally had a negative effect on measured life-history traits (Extended Data Fig. 3) and this effect increased with time (Extended

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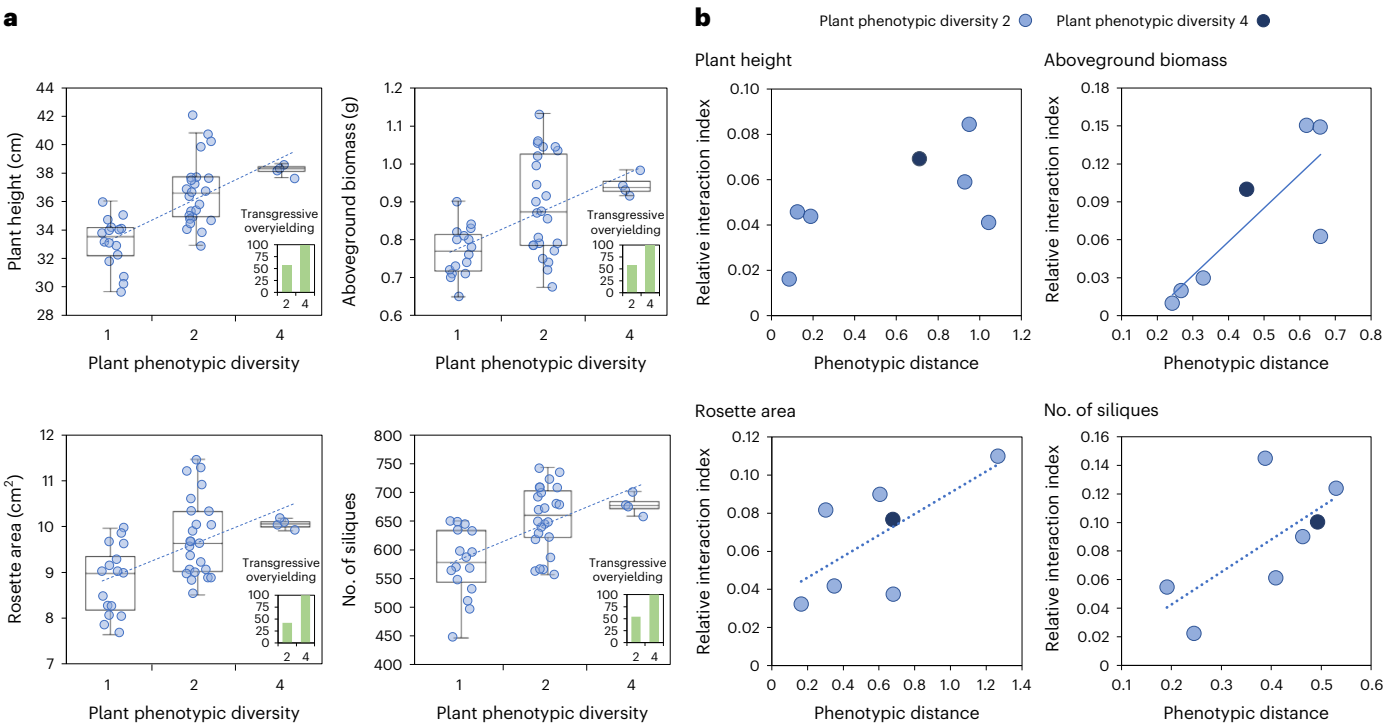

Fig. 2 | Phenotypic diversity induced by microorganisms generates transgressive overyielding in clonal plant populations. a, Effect of phenotypic diversity on selected life-history traits at the highest tested density of 4 plants per pot. Clonal plants were treated with volatile compounds emitted by different bacteria and the sterile plants assembled into a total of 11 communities covering diversity levels of 1, 2 and 4 phenotypes. Plant height and rosette area were measured after 35 d, and aboveground biomass and number of siliques were determined after 45 d of transplantation. All tested plant life-history traits (measured at community level) increased significantly with phenotypic diversity. Each point corresponds to an individual community ($n = 44$), with 4 replicates per treatment. Data were analysed using linear regression analysis for plant height ($F_{(1,42)} = 23.7$, $P = 1.6 \times 10^{-5}$), aboveground biomass ($F_{(1,42)} = 11.4$, $P = 0.002$), rosette area ($F_{(1,42)} = 10.8$, $P = 0.002$) and number of siliques ($F_{(1,42)} = 13.8$, $P = 0.001$). Boxplots represent median (middle line), 25%–75% quantiles (box), minimum and maximum values within 1.5× interquartile range (whiskers), and outliers (points beyond whiskers). The inset bar plots indicate the percentage of assembled plant communities at 2- and 4-phenotype diversity levels showing transgressive overyielding. **b**, Community-level facilitation increases with phenotypic divergence. Community-level facilitation was determined as the relative interaction index for each of the assembled communities at the highest plant density (4-plant density) with the diversity levels of 2 and 4 phenotypes. Community-level RII was computed using the averaged performance of phenotypes in a given community relative to their averaged performance in monocultures at the same plant density. The phenotypic distance was defined as the average Euclidean distance between the base-normalized life-history trait values of co-assembled plants. Both RII and phenotypic distance were calculated separately for each life-history trait and Pearson correlation coefficient (r) was used to determine their correlation separately for plant height ($r^2 = 0.61$, $P = 0.154$), aboveground biomass ($r^2 = 0.81$, $P = 0.025$), rosette area ($r^2 = 0.70$, $P = 0.077$) and number of siliques ($r^2 = 0.71$, $P = 0.074$). Each data point ($n = 7$) shows the mean of 4 replicates of each community at 4-plant density and diversity levels of 2 and 4 phenotypes. Solid and dashed regression lines indicate a correlation at $P < 0.05$ and $P < 0.10$, respectively.

Data Fig. 4), confirming that neighbouring plants in a community were competing for resources in our experimental system. Plants previously exposed to bacteria showed higher values for all four life-history traits (plant height, rosette area, aboveground biomass and number of siliques) compared with untreated control plants, indicating a positive effect of phenotypic induction on measured traits (Extended Data Fig. 3). The life-history traits of phenotypes growing in mixtures further increased with the diversity of co-existing phenotypes and with time, pointing to the alleviation of competition as an underlying mechanism (Extended Data Figs. 3 and 4, and Supplementary Table 3); this was also evident in the decrease in pairwise competitive interactions from 40% to 21% and increase in pairwise facilitative interactions from 45% to 62% with the increase in plant density from 2 plants per pot to 4 plants per pot, respectively (Extended Data Fig. 5).

Phenotypic diversity induced by different bacteria had further profound consequences for plant–plant interactions. At the highest plant density (4 plants per pot), different bacteria-induced phenotypes facilitated each other, an effect largely attributable to phenotypic complementarity (Fig. 2a). These positive interactions were prominent enough to induce transgressive overyielding in plant height, rosette area, aboveground biomass and number of siliques (Fig. 2a). As a result, 48–58% of the 2-phenotype and the 4-phenotype treatments outperformed their best-performing constituent (Fig. 2a and Supplementary Table 4). These higher life-history trait values in 4-plant mixtures could be ascribed to the prevalence of facilitative interactions between phenotypes (determined as positive relative interaction index; Fig. 2b)[24]. This facilitation at the trait level was correlated with phenotypic distance between competing phenotypes for the measured plant traits (Fig. 2b), indicating that microbe-induced phenotypic complementarity can drive overyielding in mixtures. The sampling effect had a minor contribution to the life-history traits of the community (Extended Data Fig. 6), with the high prevalence of transgressive overyielding in 2- and 4-phenotype combinations demonstrating that the observed effects are the result of complementarity or facilitation rather than the presence of a specific phenotype.

Assessing the functioning of biodiverse ecosystems requires a deep understanding of interspecific interactions shaping community function. Often, the type and distribution of interactions surpass individual species' characteristics in their ability to shape community functioning[6,26]. The main rationale is that co-existing plants with divergent phenotypes[27,28] may complement or even facilitate each other in mixtures[7,29]. Biodiversity effects on community performance are often seen as driven by plant genetic diversity, which is further modulated by biotic and abiotic environmental conditions and determines phenotypic

diversity[9,10,26,30]. This study shows that microscale heterogeneity of plant–microbe interactions, here produced through the controlled association of neighbouring plants with different bacteria, also generates phenotypic diversity, which in turn leads to higher values of life-history traits.

Past studies have shown that genetic alterations in plant-associated microorganisms and plant mutations can result in a convergent plant phenotype[15,16]. We extend the concept to the community level by demonstrating that a higher beta diversity of soil microorganisms leads to higher phenotypic diversity and productivity at the population level. The current experiment relied on an extremely simplified setup with microbial induction of the plant phenotype by volatile compounds only. Under natural conditions, the microbiome is complex with the additional contribution of species diversity and their interactions[18,19] and may have a larger or smaller contribution to plant phenotype. However, this setup was essential to keep plants sterile and prevent downstream confounding effects due to mutual contamination of neighbouring plants with their associated bacteria. At the same time, this design allowed for keeping results representative of general patterns of plant–microbe interactions affecting plant phenotype in natural systems. Even if volatiles represent only a small fraction of the palette of bioactive molecules produced by plant-associated bacteria[31,32], their effect on plants is representative of the broader range of plant–microbe interactions, owing to their potential to modulate all main plant hormonal cascades that ultimately determine phenotype[22,33].

By approaching BEF relationships through the lens of plant–microbe interactions, we highlight the importance of belowground microbial biodiversity in shaping plant community dynamics. Crucially, we demonstrate that a positive BEF and even transgressive overyielding can emerge in the absence of any plant genetic diversity if neighbouring plants are associated with distinct bacteria, providing an additional explanation complementing other sources of overyielding[30,34]. Soil microorganisms are known to impact plant dynamics and interspecific interactions[35]. The soil microbiome being highly heterogeneous[36], metre-scale changes in the soil microbiome can lead to huge differences in the development of neighbouring plants[20,37]. Therefore, akin to diversity at the level of plant genetics or traits, spatial variation of microbial interactions can also contribute to an increase in community function to a level similar to overyielding stemming from plant diversity or traits plasticity[4,30]. In this study, we observed a clear plant phenotypic diversity effect on both vegetative biomass and seed yield. These effects were strong enough to lead to transgressive overyielding[5] at 4-plant density, outperforming the best-included plant phenotype in monoculture[7]. We ascribe this overyielding to increase in facilitation and reduction in competition between different phenotypes (Extended Data Fig. 5), which in turn were well explained by the phenotypic divergence between neighbouring plants (Fig. 2b). These are conservative estimates of transgressive overyielding that probably underestimate it, considering the sampling bias described in ref. 5.

The first implication of these findings is that we may have to rethink the baseline definition of a plant species as a meaningful unit. Microscale variation in microbiome composition[36] can generate a hidden layer of phenotypic biodiversity independent of plant genetic diversity or other forms of trait plasticity. Therefore, clonal monocultures may already harbour facilitative interactions, even before plant diversity is increased. We anticipate that soil heterogeneity may be an overlooked driver behind the context-dependence of biodiversity effects[35,38]. Intraspecific competition is a major hurdle for ecosystem restoration and agricultural yield[39]. Our findings suggest that such competition may be alleviated by increasing the diversity of microbial associations at a metre-scale level, fomenting facilitative interactions between neighbouring plants. In conclusion, by bridging BEF and microbe-mediated plant phenotypic variability, we demonstrate that the belowground pool of microbial species is one of the major determinants of net BEF relationships, which should be considered to maintain and restore well-functioning ecological communities in a rapidly changing world.

Methods

Bacterial strains

We screened a total of 191 bacterial strains isolated from the tomato rhizosphere at the flowering stage[40] to evaluate their potential for volatile compounds-mediated increase in plant growth (Supplementary Data 1). The bacterial strains stored at −80 °C were obtained, plated on nutrient agar (NA) medium at 30 °C to select a single colony and then routinely grown on NA medium at 30 °C. For experiments, bacterial cultures were prepared by growing bacterial strains in nutrient broth overnight at 30 °C and 170 r.p.m., washed with sterilized water twice and adjusted to a final concentration of 1×10^7 colony-forming units (c.f.u.s) ml^{-1}. The bacterial strains were also stored at −80 °C in nutrient broth containing 20% glycerol for further use.

Rationale for the use of microbial volatiles as a representative mechanism

The choice of volatile compounds as a mechanism is justified by the desire to control plant–microbe interactions while keeping plants spatially separated from bacteria. Keeping plants sterile in the phenotype induction phase is crucial to prevent confounding effects due to cross-contamination in the common-garden experiment. Further, volatile compounds are representative mechanisms for the interaction between plants and their associated microorganisms. Virtually all plant-associated microorganisms emit a specific blend of bioactive volatile compounds that can impact plant physiology by affecting key hormonal pathways regulating the plant phenotype (for example, auxin, ethylene, cytokinin or abscisic acid)[21,22]. Given the importance of both the identity and composition of volatile blends for plant phenotype[25], volatile compounds have the potential to generate a whole range of plant phenotypes depending on the microorganisms associated with them. Further, volatiles are thoroughly documented drivers of plant–microbiome interactions under field conditions[21,22], thus building a realistic model for natural plant–soil feedback.

Initial screening for volatile compounds-mediated effect of bacteria on plant growth

For the first screening experiment, each of the bacterial strains in our collection was evaluated for their potential to promote or reduce plant growth through the production of volatile compounds. For this, we used a divided Petri plate assay, in which the plant was placed in one compartment and the bacteria in another. As the two compartments are physically separated, only gases can be exchanged between them (Extended Data Fig. 1). For the experiment, seeds of *Arabidopsis thaliana* Col-1 were surface-sterilized in 70% (v/v) ethanol for 1 min and then in 5% (v/v) NaOCl for 5 min. Later, seeds were washed with sterilized water three times and spread on Petri plates containing ½ strength Murashige and Skoog (MS) agar (0.8%) medium[41]. The seeds were first vernalized for 3 d at 4 °C and then placed in the growth chamber set at 21 °C, 40 W fluorescent light and 16 h light:8 h dark photoperiod. After the emergence of seedlings, three equal-sized seedings per plate were placed in one compartment of a divided Petri plate containing 15 ml ½ strength MS agar medium with 0.5% sucrose and 0.8% agar. Later, Petri plates were sealed with parafilm and placed again in the growth chamber. After the emergence of the third true leaf, the other compartment of the divided Petri plate was filled with 15 ml minimal salt agar medium containing 1.5% agar, 1.5% sucrose and 0.4% tryptic soy broth (w/v). After the solidification of minimal salt agar medium, three 5 µl drops of bacterial culture (1×10^7 c.f.u.s ml^{-1}), prepared as described above in 'Bacterial strains', were added at a distance of 3 cm from each other (Extended Data Fig. 1). After bacterial inoculation, the plates were sealed with a double layer of parafilm and placed in the growth chamber. Each bacterial strain was evaluated in triplicate and Petri plates without bacterial inoculation on minimal salt agar medium were used as control. After 1 week, plants were gently removed from the divided Petri plate and washed with water to remove the remaining agar-medium substances. Later, plants were blot-dried and plant growth

was estimated as fresh weights. Out of 191 bacterial strains, 71 strains led to >200% increase in plant fresh weight compared with the control and were selected for the second screening experiment.

Second screening for volatile compounds-mediated effect of bacteria on plant phenotype

A second screening experiment was conducted to evaluate the volatile compounds-mediated effect of 71 selected bacterial strains on *Arabidopsis* plant phenotype in divided Petri plates. The *Arabidopsis* plants' growth conditions and methods, and bacterial strains' growth and inoculation conditions and methods were the same as described in 'First screening for volatile compounds-mediated effect of bacteria on plant growth', except that here we were not testing for an increase in plant fresh weight but instead for changes in the phenotypic characteristics of plants. Each bacterial strain was evaluated in triplicate and Petri plates without bacterial inoculation on minimal salt agar medium were used as the control for comparison. After the second screening experiment, we selected four bacterial strains: F23, F31, F37 and F12, whose volatile compounds-mediated interaction induced visually different leaf phenotypic characteristics in plants (Fig. 1b). The whole genomes of four selected bacterial strains were sequenced by Nanopore sequencing followed by genome assembly using the Trycycler pipeline[42] and submitted to the NCBI genome database (Supplementary Table 1). Genome-to-genome distance of selected strains was calculated using the Genome-to-Genome Distance Calculator Tool (3.0)[43] (Extended Data Fig. 2a).

Analysis of volatile compounds of selected bacterial strains

The volatile compounds produced by the four selected bacterial strains were identified in triplicate using gas chromatography–mass spectrometry (GC–MS) as we previously described in detail[32]. Briefly, bacterial cultures were prepared as described in 'Bacterial strains' and three spots (5 µl each) inoculated on minimal salt agar medium (15 g agar per litre) in a 100-ml vial. Later, vials were sealed with Parafilm and incubated at 30 °C for 48 h. Vials without the inoculation of bacteria on minimal salt agar medium were used as control. After incubation, 10 µl 5 mM (Z)-3-hexenyl acetate was added into the vial as an internal standard and then a solid-phase microextraction (SPME) fibre (Supelco) stable flex divinylbenzene/carboxen/polydimethylsiloxane (DCP, 50/30 µm) was inserted into the vial. The adsorption of volatile compounds from the headspace of the bacterial culture was done for 30 min at 50 °C. The SPME fibre was then inserted into the GC–MS instrument and chromatographs were obtained[32]. Later, the mass spectra of peaks were deconvoluted using AMDIS 2.73 (US National Institute of Standards and Technology) and compared with those in the NIST/EPA/NIH Mass Spectrometry Library with respect to the spectra in the Mainlib and/or Replib databases (Agilent). The alkane calibration mix (C6-C27) was then used to calculate the Kovats retention indexes (RI) for each compound and compared with those found in the NIST/EPA/NIH Mass Spectrometry Library. The identification of volatile compounds, except for the peaks similar to the control, was done on the basis of mass spectra match with the listed compounds, >750 match factor and ≤5 difference in RI between detected and listed compounds for a semi-standard non-polar column. The production of 37 out of 58 identified volatile compounds was further confirmed by comparing with standard compounds (Sigma, Tokyo Chemical Industry and Aladdin Reagent Database). The peak area was noted and expressed as the peak area relative to (Z)-3-hexenyl acetate (internal standard) in arbitrary units (a.u.). To visualize the overall differences in volatile compounds among the four bacterial strains, principal component analysis was computed on relative peak area data (Extended Data Fig. 2b).

Common-garden experiment on plant–plant phenotypic complementarity

The four plants whose phenotypes were induced by different bacteria were placed in the common-garden experiment in different monoculture and polyculture diversity combinations to evaluate whether phenotypic diversity can induce plant–plant phenotypic complementarity. For the common-garden experiment, four clonal plants (*Arabidopsis*) with different phenotypes were generated using the same methods as described in 'First screening for volatile compounds-mediated effect of bacteria on plant growth'. At the emergence of the third true leaf, *Arabidopsis* seedlings were exposed to the volatile compounds of four bacterial strains: F23, F31, F37 and F12, in divided Petri plates separately. For each phenotype generation, a total of 25 divided Petri plates were prepared and plates without bacteria were used as control. After 1 week, plants were gently removed from the divided Petri plates and washed with sterilized water to remove the remaining agar-medium substances. Later, plants were transplanted to pots containing 35% potting mixture (Primasta) and 65% sand at plant densities of 1, 2 and 4, and phenotypic diversities of 1, 2 and 4, respectively. Note that not all combinations of plant density and phenotypic diversity are possible (Supplementary Table 2), which is why we performed different sets of statistical analyses accounting for this design. The potting mixture contained 28% organic C, 1% organic N and 50% organic matter on dry weight basis. A total of 28 treatments representing different phenotypic diversities and plant densities were designed as described in Supplementary Table 2. After transplanting, plants were watered with 100 ml per pot of ½ strength MS medium for the first time and later, every third day with 75 ml tap water per pot until the end of the experiment. The pots, plant growth medium (potting mixture and sand) and irrigation water were not sterilized. Each treatment contained 4 replicates and the whole experiment was repeated twice.

Plant life-history traits

For the common garden experiment, we evaluated four plant life-history traits connected with both vegetative and reproductive growth[44]. We selected plant height, rosette area and aboveground biomass as plant life-history traits representative of vegetative growth. We chose the number of siliques, a trait highly correlated with seed production[45], as a representative trait for reproductive growth. To evaluate changes in plant life-history traits with time, plant height and rosette area were first determined after 10 d of transplantation and then after 25 d and 35 d, respectively, while the aboveground biomass and number of siliques were determined after 45 d of transplantation. Plant height was determined from ground level to the highest point of plant bolting with a ruler. To measure the rosette area, plant boltings were cut from the bottom, the rosette was photographed and the area was determined using the smartphone app 'Easy Leaf Area'[46]. Siliques were counted manually and for the aboveground biomass, the plant was cut from the ground level and dried at 70 °C until constant weight. Aboveground vegetative biomass was determined as plant dry weight without siliques.

RII

We used RII[24] to evaluate competitive or facilitative interactions. To prevent confounding effects due to density, pairwise RII between bacteria-induced phenotypes was determined separately at 2- and 4-plant density levels using only a diversity level of 2 phenotypes. RII was computed separately for each of the 4 life-history traits. Pairwise RII was defined as the performance of each phenotype in the presence of another phenotype relative to its performance in monoculture at the same plant density. The pairwise RII was calculated using the following formula[24]:

$$\mathrm{RII} = (\mathrm{PW} - \mathrm{PS})/(\mathrm{PW} + \mathrm{PS}) \quad (1)$$

where PW is the performance of a plant phenotype in the presence of another phenotype and PS is the performance of a plant phenotype in monoculture. RII ranges from −1 to +1, with negative values indicating competition and positive values net facilitative interactions.

Later, community level RII was calculated separately for all 4 life-history traits of bacteria-induced phenotypes at the highest plant density (4-plant density) with the diversity levels of 2 and 4 phenotypes.

RII at the community level was defined as the averaged performance of phenotypes in a given community relative to their averaged performance in monocultures at the same plant density and calculated using the formula described above[24], where PW is the performance of a phenotype in a given community and PS is the performance of a phenotype in monoculture.

Phenotypic distance among bacteria-induced plants in a community

To determine the phenotypic distance among bacteria-induced plants in each community of 4-plant density at the diversity levels of 1, 2 and 4 phenotypes, all 4 plant life-history trait values were standardized separately and their Euclidean distance among plants in each pot was computed[47]. The standardization of plant life-history trait values was done using the formula:

$$\text{Standardized PTV} = (\text{PTV} - \text{NPTV})/(\text{XPTV} - \text{NPTV}) \quad (2)$$

where PTV is the plant trait value, NPTV is the minimum observed value of that plant trait and XPTV is the maximum observed value of that plant trait.

Statistical analyses

After the common-garden experiment, we first compared four selected bacteria-induced plants individually at 1-plant density to evaluate whether phenotypic induction could last for the whole growth period of the bacteria-induced plants. For this, we used one-way analysis of variance (ANOVA) on every single life-history trait (plant height, rosette area, aboveground biomass, number of siliques) and further differences among bacteria-induced plant phenotypes were computed using Tukey's HSD test ($P < 0.05$). To visualize the overall differences among four bacteria-induced plants at 1-plant density, we also performed principal component analysis on the data of all life-history traits.

Later, for the whole common-garden experiment with different plant density and phenotypic diversity levels (Supplementary Table 2), we used a generalized linear mixed model (GLMM) to investigate the effect of experiment repetition (fixed factor, two repetitions), phenotypic diversity (fixed factor, factor with three levels) and plant density (fixed factor, factor with three levels) on each of the plant life-history traits, using treatment identity as a random factor to account for pseudoreplication[48].

The positive interactions among bacteria-induced plant mixtures were prominent enough that transgressive overyielding was determined at 4-plant density. Thus, to get a better insight into the effect of phenotypic diversity on plant life-history traits at the 4-plant density level, a separate GLMM analysis was conducted to investigate the effect of experiment repetition (two repetitions) and phenotypic diversity (factor with three levels) on each of the plant life-history traits, using treatment identity as a random factor.

Furthermore, we calculated separate RII between pairs of bacteria-induced plants, and networks of net facilitative and competitive interactions were prepared (Extended Data Fig. 5). The statistical significance of RII values between bacteria-induced plant pairs was determined using a two-tailed t-test at $P < 0.05$. Pearson correlation analysis was conducted between phenotypic distance and RII to determine whether the phenotypic distance of plant life-history traits drives the RII in bacteria-induced plant communities of 4-plant density at the diversity levels of 2 and 4 phenotypes.

In addition, the sampling effect[49] was computed for all 4 life-history traits separately at the highest plant density (4-plant density) with the diversity levels of 2 and 4 phenotypes. The sampling effect was defined as the performance of communities in the presence of a focal phenotype compared to the performance of communities in the absence of that focal phenotype, and the differences were evaluated for statistical significance using a two-tailed t-test at $P < 0.05$. All statistical analyses were performed using SPSS v.19.0 statistical software.

Reporting summary

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

Data availability

All data that support the findings of this study are available via Figshare at https://figshare.com/s/1094376bbb9259e1b18e.

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Author contributions

W.R., Z.W. and A.J. designed the experiments. W.R. carried out the experiments. W.R., G.J., Y.H. and A.J. analysed the data and wrote the manuscript. G.J., Z.W., G.A.K., Q.S. and N.E. revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Wei Zhong or Alexandre Jousset.

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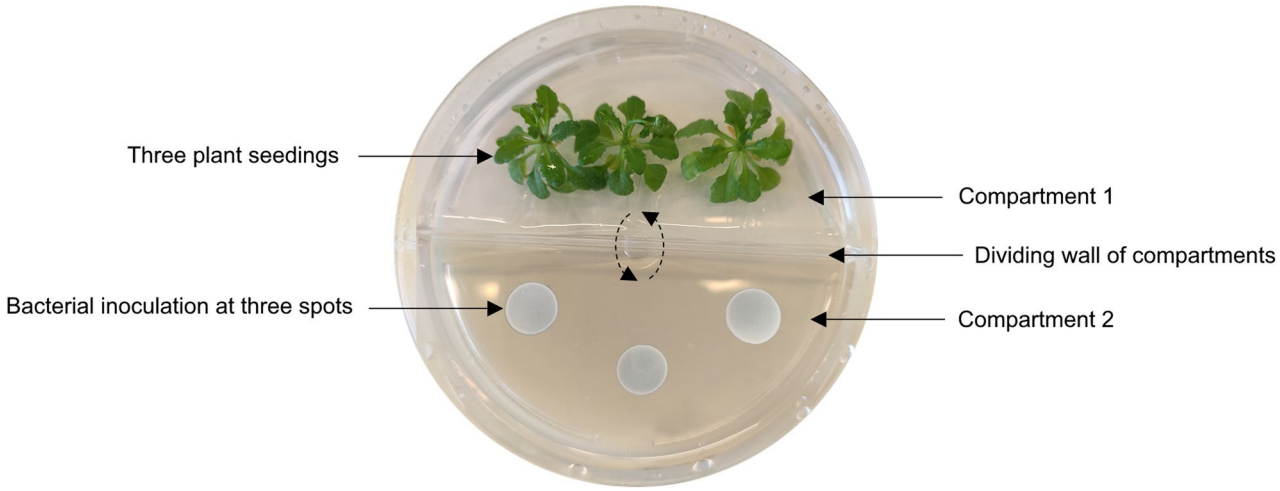

Extended Data Fig. 1 | Experimental setup of the divided Petri plate for the exposure of clonal plants to bacterial volatiles. Petri plate has two compartments divided by a wall, which provides physical separation of both compartments but allows the exchange of gases. In one compartment containing Murashige and Skoog agar medium, three seedlings of *Arabidopsis* were placed, while in the second compartment containing minimal salt agar medium, three 5 µl drops of bacterial culture were added at a distance of 3 cm from each other. Later, the plate was covered with a lid, sealed with parafilm and incubated for one week.

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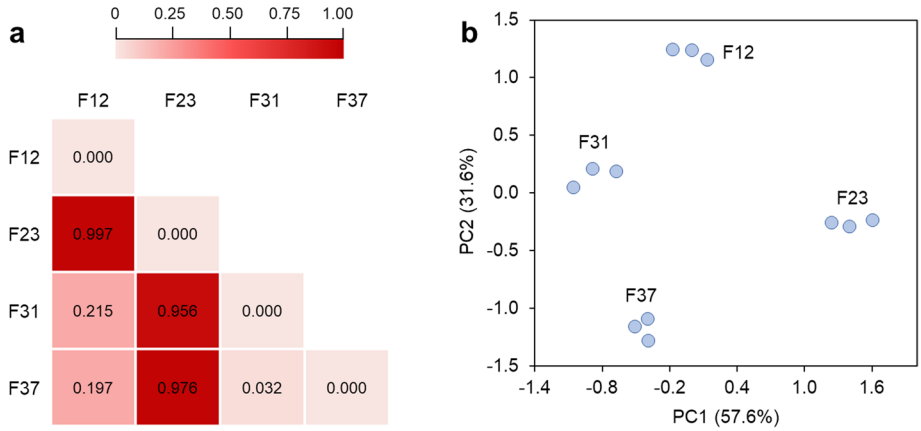

Extended Data Fig. 2 | Comparison of genome sequences and volatile compounds produced by four bacterial strains. (**a**) Genome to genome distance of four bacterial strains used for volatile compounds-mediated phenotypic induction of *Arabidopsis thaliana* Col-0. In the heatmap, higher intensity of red color represents higher genome-to-genome distance. (**b**) Principal component analysis (PCA) based on the relative peak area of volatile compounds produced by four bacterial strains in triplicate ($n = 12$). The volatile compounds analysis was conducted using gas chromatography-mass spectrometry (GC-MS). The four bacterial strains used in the study are *Pseudoxanthomonas mexicana* F12, *Chryseobacterium daecheongense* F23, *Pseudoxanthomonas mexicana* F31 and *Pseudoxanthomonas* sp. F37.

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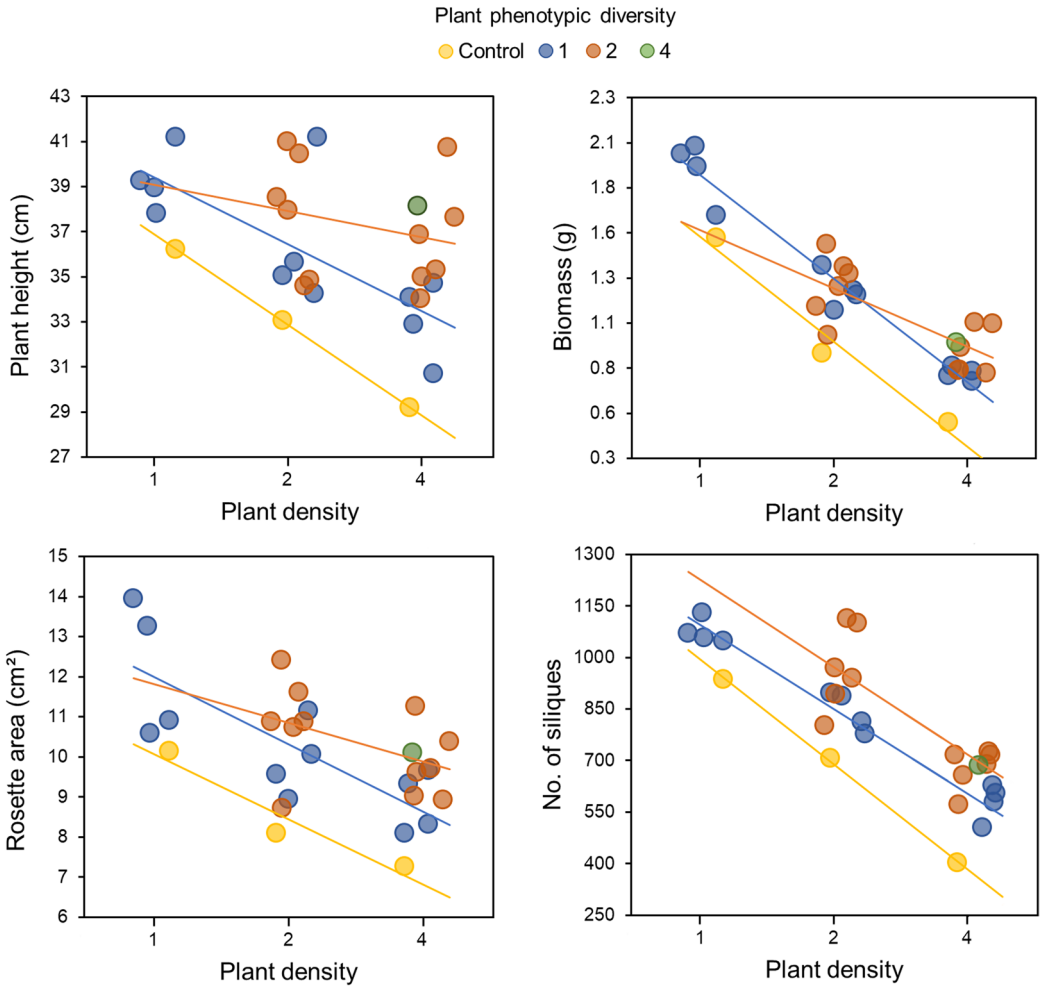

Extended Data Fig. 3 | Effect of plant density on the four measured life-history traits (plant height, rosette area, aboveground biomass, number of siliques) with and without previous exposure to the volatile compounds produced by four different bacteria. Plants were exposed to the volatile compounds of four different bacteria during early life for one-week (representing four different phenotypes); later, bacteria were removed, and plants were placed together in pots at 1-, 2-, and 4-plants density (PD) and 1-, 2-, and 4-plants phenotypic diversity (HD) levels, respectively. In scatterplot panels, each data point represents the mean value of quadruplicates ($n = 28$). The colors of data points and regression lines correspond to the plant phenotypic diversity levels.

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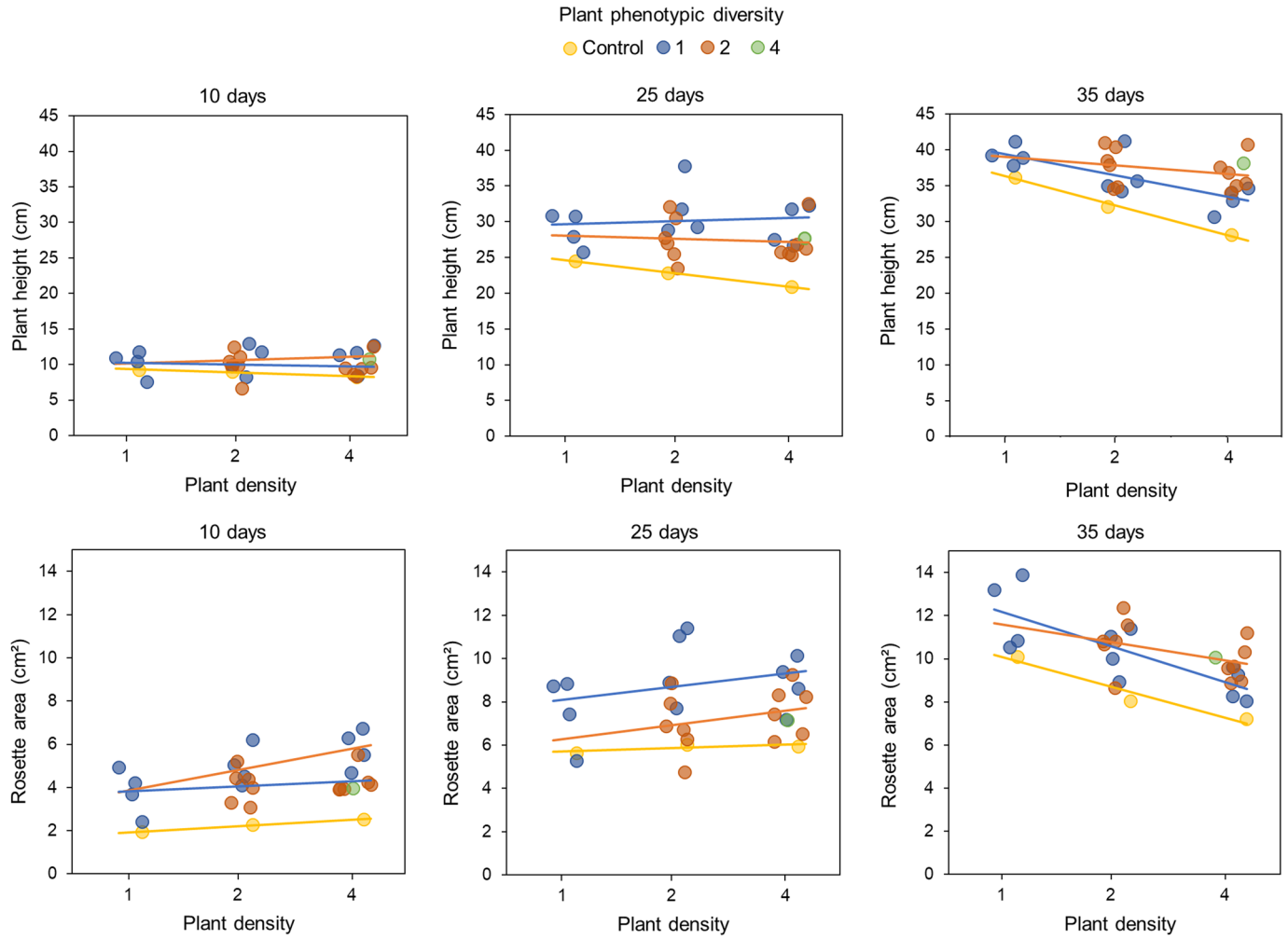

Extended Data Fig. 4 | Development of plant height and rosette area after 10 days, 25 days and 35 days with and without previous exposure to the volatile compounds of four different bacteria. Plants were exposed to volatile compounds of four different bacteria during early life for one-week (representing four different phenotypes); later, bacteria were removed, and plants were placed together in pots at 1-, 2- and 4-plants density and 1- 2- and 4-plants phenotypic diversity levels, respectively. In scatterplot panels, each data point represents the mean value of quadruplicates ($n = 28$). The colors of data points and regression lines correspond to the plant phenotypic diversity levels.

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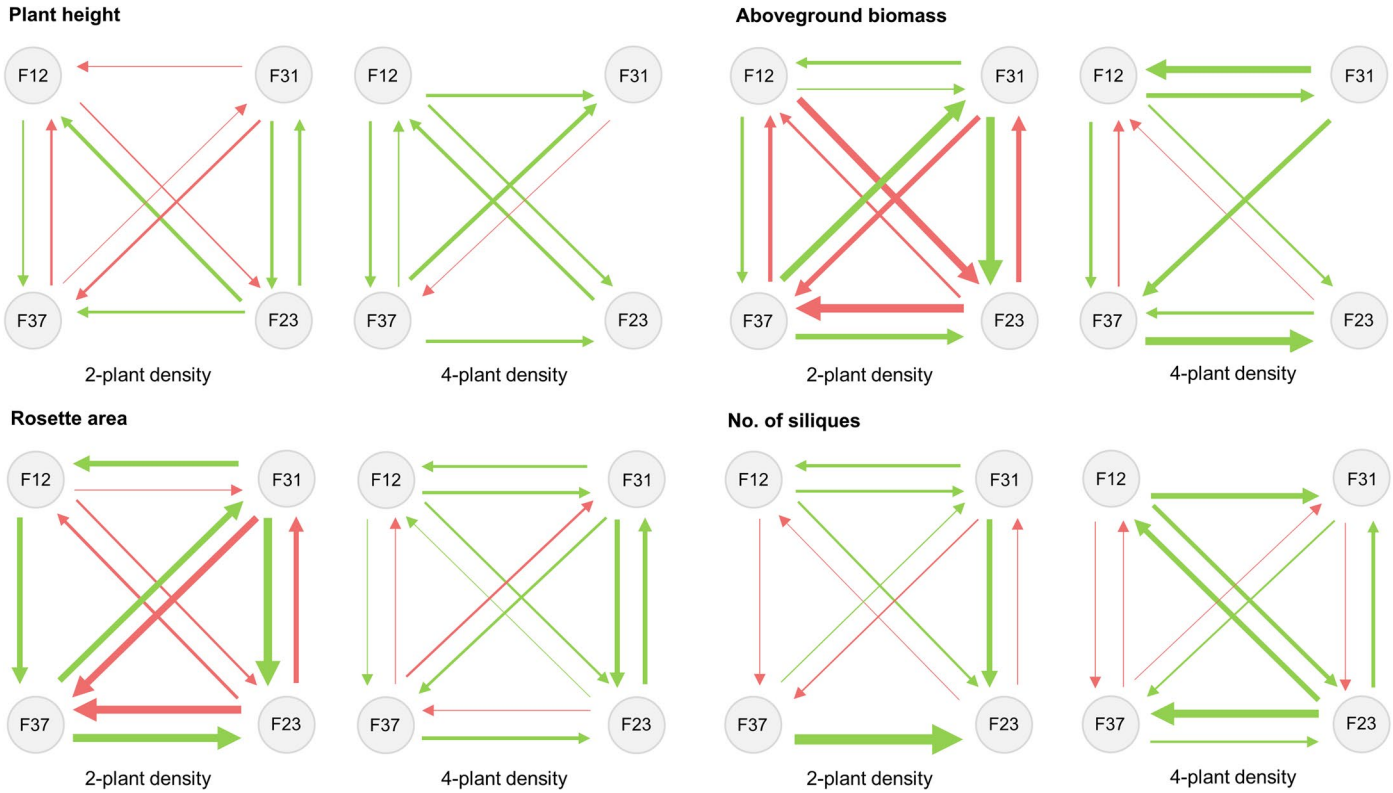

Extended Data Fig. 5 | Pairwise interactions between plant phenotypes for the four-plant life-history traits (plant height, rosette area, aboveground biomass, number of siliques). Pairwise interactions between plant phenotypes were determined as the relative interaction index (RII) separately at 2- and 4-plants density levels using only a diversity level of 2-phenotypes for each of the four life-history traits. Pairwise RII was defined as the performance of each phenotype in the presence of another phenotype, relative to its performance in monoculture at the same plant density. Red arrows stand for competition and green arrows stand for facilitation. Arrow breadth is proportional to |RII|. Only arrows showing significant differences according to the two-tailed T-test at $P < 0.05$ are included.

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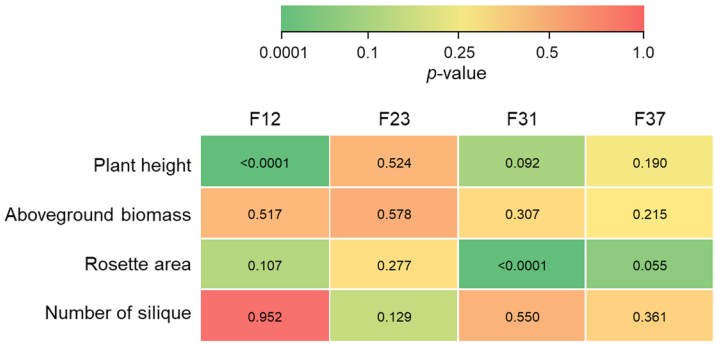

Extended Data Fig. 6 | Sampling effect of plant phenotypes on life-history traits (plant height, rosette area, aboveground biomass, number of siliques). Sampling effect was determined at the highest tested density of four plants per pot, covering diversity levels of 2- and 4-phenotypes. It was calculated by carrying out two-tailed T-test comparing the performance of communities containing the phenotype relative to those lacking it. In the heatmap, the higher intensity of green color represents a significant sampling effect while the higher intensity of red color represents a nonsignificant sampling effect based on *P* values provided. The four bacterial strains used to induce plant phenotypes are *Pseudoxanthomonas mexicana* F12, *Chryseobacterium daecheongense* F23, *Pseudoxanthomonas mexicana* F31 and *Pseudoxanthomonas* sp. F37.

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Corresponding author(s): Alexandre Jousset
Zhong Wei

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Software and code

Policy information about availability of computer code

Data collection	The whole genome of four bacterial strains was sequenced by Nanopore sequencing technology followed by genome assembly using the Trycycler pipeline. Genome-to-genome distance of selected strains was calculated using Genome-to-Genome Distance Calculator Tool 3.0. The volatile compounds produced by bacterial strains were identified using proton transfer reaction-mass spectrometry (PTR-MS; Ionimed Analytik, Innsbruck, Austria).
Data analysis	All statistical analyses was performed using SPSS version 19.0 statistical software (SPSS, Inc., Chicago, IL, USA) and details are provided in the Methods section.

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The whole genome sequences of four bacterial strains were submitted to the NCBI genome database (Accession numbers CP095184-CP095187) and the rest of the data presented in this paper are available in the article and in its online supplementary material.

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Study description	The holobiont biodiversity can lead to significant biodiversity-ecosystem functioning relationships, despite of a clonal plant genetic background. We built a set of minimal holobionts out of clonal plants and individual soil bacteria, resulting in a range of plant phenotypes. After fully removing the bacteria, plants were assembled into a phenotypic diversity gradient of one to four plants. We found that microbially-induced phenotypically diverse clonal plants complemented each other, leading to transgressive over-yielding in both vegetative growth and seed yield.
Research sample	A total of 28 treatments representing 1, 2 & 4 plant density and 1, 2 & 4 plant phenotypic diversity levels, respectively, were designed as described in Supplementary Table 2. Each treatment had four replicates and the whole experiment was repeated twice. All plants (Arabidopsis thaliana Col-1) in each pot were used for data collection and analysis.
Sampling strategy	Plant samples were collected from all 28 treatments representing different phenotypic diversities and plant densities as described in Supplementary Table 2.
Data collection	Plant height was determined from ground level to the highest point of plant bolting with a ruler. To measure the rosette area, the rosette was photographed, and the area was determined using the smartphone app 'Easy Leaf Area'. Siliques were counted manually and for the aboveground biomass at harvest, the plant was cut from the ground level and dried at 70°C until constant weight. All data were collected by WR.
Timing and spatial scale	The experiments were conducted and data were analysed in 2021-22. For the data collection during the experiment, first plant

Timing and spatial scale	height and rosette area were determined after ten days of transplantation and then after 25 days and 35 days, respectively, while the aboveground biomass and number of siliques were determined at the time of harvest.
Data exclusions	No data were excluded from the analysis.
Reproducibility	The whole experiment was repeated twice with four replicates for each treatment to ensure reproducibility and the experiment repetition was used as a fixed factor in GLMM analysis.
Randomization	Plants were exposed to the volatile compounds of four bacteria; later, plants were transplanted to pots in the common-garden experiment at different monoculture and polyculture diversity combinations (Supplementary Table 2) and then placed randomly in the growth chamber. The pots were also randomly relocated in the growth chamber every seven days.
Blinding	Blinding is not relevant to our study.

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Materials & experimental systems

n/a	Involved in the study
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☒	☐ Animals and other organisms
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Methods

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