Microbes to support plant health: understanding bioinoculant success in complex conditions

Sanne WM Poppeliers*, Juan J Sánchez-Gil* and
Ronnie de Jonge

A promising, sustainable way to enhance plant health and productivity is by leveraging beneficial microbes. Beneficial microbes are natural soil residents with proven benefits for plant performance and health. When applied in agriculture to improve crop yield and performance, these microbes are commonly referred to as bioinoculants. Yet, despite their promising properties, bioinoculant efficacy can vary dramatically in the field, hampering their applicability. Invasion of the rhizosphere microbiome is a critical determinant for bioinoculant success. Invasion is a complex phenomenon that is shaped by interactions with the local, resident microbiome and the host plant. Here, we explore all of these dimensions by cross-cutting ecological theory and molecular biology of microbial invasion in the rhizosphere. We refer to the famous Chinese philosopher and strategist Sun Tzu, who believed that solutions for problems require deep understanding of the problems themselves, to review the major biotic factors determining bioinoculant effectiveness.

Address
Plant-Microbe Interactions, Department of Biology, Science for Life, Utrecht University, Utrecht, the Netherlands

Corresponding author: de Jonge, Ronnie (r.dejonge@uu.nl)
* These authors contributed equally to this manuscript.

## Introduction: ponder and deliberate before you make a move

Past and present anthropogenic disturbances are diminishing soil quality worldwide and decreasing crop yields through an intense demand for food production. At the same time, there is a joint effort to develop sustainable agricultural techniques that can maintain or enhance productivity while reducing synthetic chemical inputs. One of the most promising methods to enhance plant health is by leveraging the benefits of the vast diversity of microbes that form the plant microbiome. Many pivotal features in plant fitness are dependent on the ensembled microbiome, such as nutrient homeostasis [1], resilience to drought and salt stress [2,3] or natural protection against phytopathogens [4••,5]. Therefore, the scientific literature brims with examples of beneficial microbes and the responsible traits that can support plant health. These biological inoculants, or bioinoculants for short, are composed of single or multiple microbial species that are applied to soil and are the most common form of plant microbiota-engineering efforts.

The benefit associated with bioinoculants is commonly provided through their colonisation of the host plant roots, and most often, bioinoculant density in the rhizosphere environment greatly determines the eventual efficacy [6,7•]. Whether the bioinoculant manages to engraft — that is, successfully invade a microbial community — depends on the composition and interactions with and within the locally adapted resident microbiota [8•,9], edaphic factors [10], host genotype [11••], root architecture and tissue-specific expression [12,13], invader density and timing of invasion events [14] and the inherent ability of the bioinoculant to circumvent these barriers and establish itself in the microbiome [15] (Figure 1). All of this commonly makes the design of bioinoculants strongly dependent on trial-and-error assays, and ultimately the most promising bioinoculants in the lab show variable efficacy in real-world agricultural conditions [16].

Some studies have already shown that global responses in the microbiome can be explained as an ensemble of the local interactions [17,18]. Therefore, optimising plant health through the design and application of beneficial microbes in agricultural conditions requires a deep mechanistic comprehension of all relevant interactions that take place during engraftment into the resident microbiome. In this review, by intertwining ecological theory with molecular research, we root for

Figure 1
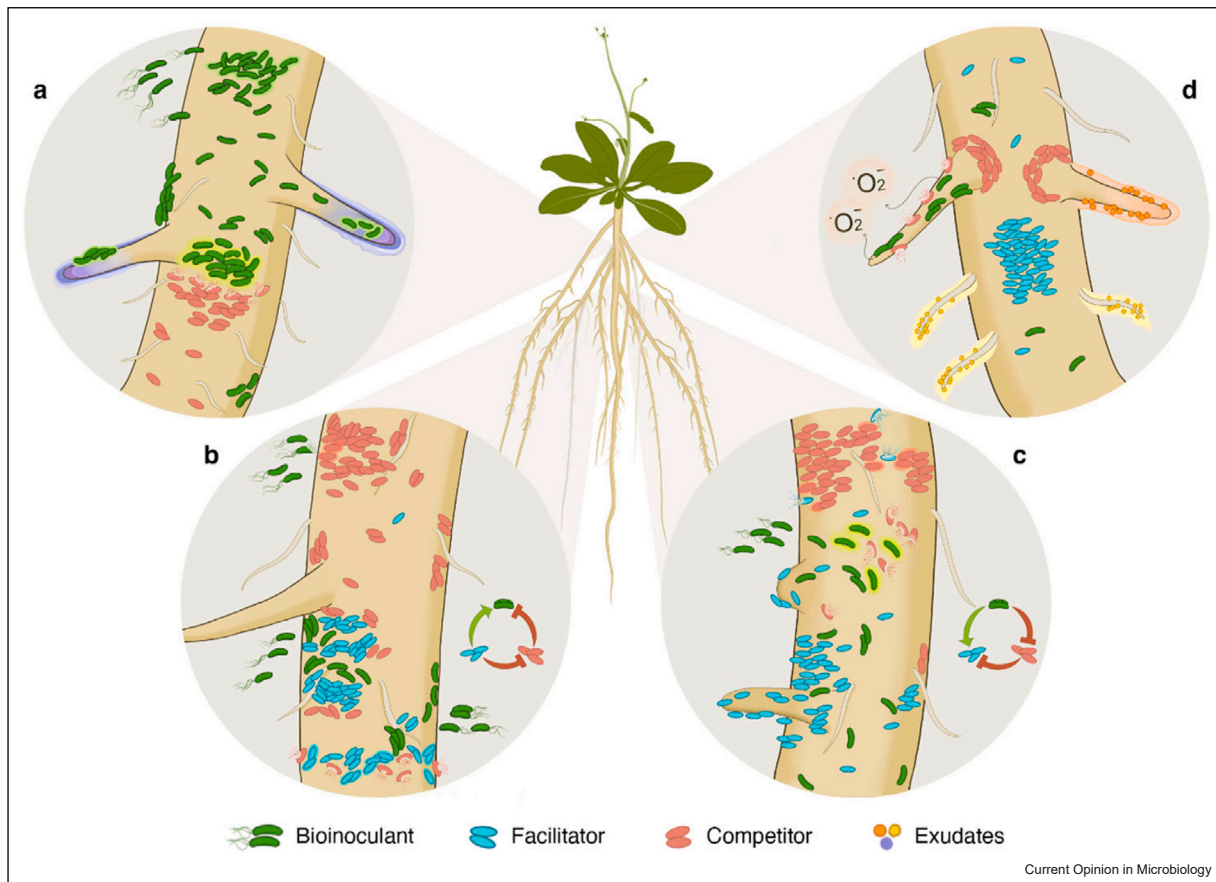
Processes and mechanisms influencing bioinoculant engraftment into the rhizosphere microbiome. **(a)** The activity of a bioinoculant depends on its physiological state and on interactions with other microbes. Top: relevant traits are only expressed in specific states and not in others. Middle: some traits are only expressed in interaction with specific microbes. Bottom: the interaction-triggered expression of traits is commonly of competitive nature, and is dependent on microbial relatedness. **(b)** Positive interactions with the residential microbiome can be used to favour invasion of an inoculated microbe. Top: a competitor is already present on the root, but without facilitator (or co-inoculated helper) invasion cannot occur. Middle: invasion can happen because a facilitator is present that benefits the invader directly. Bottom: the facilitator antagonises the competitor, leaving space for the invader to engraft. **(c)** Regardless of invasion success, the invader can induce effects on the resident microbiome that lead to positive results for plant health. Top: on the root, some beneficial residential microbes (blue) are restricted by the presence of competitors (red). Middle: invasion by a bioinoculant can benefit the lowly abundant microbes directly or kill the competitors. Bottom: the promoted residential can promote plant fitness regardless of what happens to the bioinoculant in future stages. **(d)** The plant host can control its microbiome through its immune system and by exuding different metabolites. The way the bioinoculant communicates with or is adapted to the host matters in predicting its success during invasion. Top: the plant host controls its microbiome through immune system activity, such as production of ROS, which benefit some microbes (green) but kill others (red). Middle: in other regions, different exudation profiles create different niches that select for different microbial communities. Bottom: the joint action of all processes, together with root architecture and soil properties, creates specific microhabitats for microbes.

understanding of the following questions: (1) how is efficacy determined by interactions with other microbes, (2) how can we benefit from positive interactions with residential microbes to promote engraftment, (3) can we leverage bioinoculant-induced modifications on the community regardless of invasion outcome and (4) how does the interaction with the host determine effectiveness. We address these questions using Sun Tzu's philosophy from *The Art of War* to highlight the strategic nature behind optimisation of bioinoculant invasion and success.

Know thyself, know thy enemy: bioinoculant physiology and local interactions determine efficacy

Microbial traits such as growth rate, carbon utilisation, production of antibiotic compounds or the ability to form biofilms, are considered relevant for bioinoculant success. The expression of these traits, however, depends on the physiological state of the microbe. In the case of the *Pseudomonas* genus, a vastly divergent clade that comprises many potential bioinoculants [19], most of the secondary metabolites that are relevant for their

beneficial activity are produced during the biofilm state [20]. At the same time, the production of these metabolites can be dramatically altered by other surrounding microbes, affecting bioinoculant efficacy. For example, production of the antimicrobials pyoluteorin and 2,4-diacetylphloroglucinol (DAPG) is crucial for the biocontrol activity of *Pseudomonas protegens*, and production of pyoluteorin can be enhanced upon sensing of a secreted metabolite from a related pseudomonad. In turn, antibiosis of pyoluteorin towards this related pseudomonad results in the release of intracellular compounds that inhibit production of DAPG in *P. protegens* [21•]. In *Bacillus velezensis*, sensing pseudomonads' siderophores enhances the production of secondary metabolites, increasing its survival and triggering a broad antimicrobial activity [22••,23]. Microbe–microbe interactions frequently enhance production of antimicrobial compounds across diverse environments, including the plant phyllosphere [24]. Conversely, the very same study shows that isolates from the soil produced antimicrobials constitutively, probably as a result of adaptation to an environment with much higher microbial density. In a previous study, however, 42% of isolates from soil exhibit antimicrobial activity only when in interaction with other specific isolates [25]. These findings are in line with studies showing that most of microbial biosynthetic gene clusters (BGC) remain silent until their expression is induced by an external stimulus, commonly antimicrobial compounds [26].

Generally, direct competition is more frequent among closely related organisms and commonly leads to strain exclusion [27]. For instance, commensal pseudomonads were able to protect host plants against a closely related *Pseudomonas* pathogen via hitherto unknown mechanism [7•,28•], and closely related *P. protegens* strains can inhibit each other via the release of tailocins, bactericidal protein complexes [29]. In contrast, closely related strains of *Bacillus subtilis* can essentially merge swarms and co-colonise surfaces, while kin discrimination leads to exclusion of distantly strains from swarms [30], a phenomenon also observed during biofilm development [31].

On the contrary, some microorganisms engraft in a fully independent manner, almost no matter what the resident community looks like. Such organisms carry the potential to be more broadly applicable in agriculture due to their reduced dependency on local conditions. Only few examples are described of such potentially superior bioinoculants, and one of them is *Pseudomonas putida* strain IsoF. This strain is able to aggressively invade biofilms, thanks to a horizontally acquired type-IVB secretion system that confers a broad contact-dependent toxicity ensuring *P. putida* engraftment into the rhizosphere community and enabling robust biocontrol activity [15].

The wise warrior avoids the battle: positive interactions with residential microbes to promote engraftment

Interactions between bioinoculants and other microorganisms do not necessarily have to be competitive, instead, they can also be of a facilitative nature. Specific members of the host microbiome can help the bioinoculant invade, both through direct and indirect mechanisms. Additionally, there are ample examples where bioinoculants that are co-inoculated with certain 'helper' strains are better able to establish and persist in a new environment, and consequently enhance plant performance. In bacilli, plant growth-promoting effects are dependent on biofilm formation in the rhizosphere, and this process is influenced by interactions with other microbes. For example, co-inoculation with helper strains that were not necessarily rhizosphere-competent themselves increased *B. subtilis* adherence on *Arabidopsis thaliana* roots [32]. In the cucumber rhizosphere, dual-species inoculation of *B. velezensis* and *Pseudomonas stutzeri* promoted plant growth more efficiently than individual species, and both inoculants reached higher abundances when co-inoculated in comparison with singular application. Intriguingly, such synergism was only evident under static nutrient-rich conditions, implying that spatial structure and nutrient availability are key to their collaborative nature [33•]. In general, compared with single-strain inoculation, consortia often have a larger effect on the resident microbial community [34,35], and possibly therefore better stimulate plant growth [36] and protect plants against different stresses [37].

Understanding the mechanisms of pathogen invasion can help elucidate bioinoculant success or failure, since similar ecological principles apply. Many microorganisms might act as helpers of pathogens in the rhizosphere. Although the molecular mechanisms behind pathogen invasion remain elusive, the compatibility of siderophores [38••] and tolerance to antimicrobials [24,39] produced by the resident community seem to play important roles. Facilitative interactions promote invasion of the pathogen *Ralstonia solanacearum in vitro* and *in vivo* due to loss of pathogen inhibition [40]. Mathematical modelling of experimentally validated, pairwise interactions predicts that inhibition of pathogen helper strains can reduce pathogen density in the rhizosphere more than direct targeting [41]. In general, more and more evidence confirms that the interactions between co-occurring species, as opposed to the presence or absence of a given species and its functional traits, are the most important determinant of disease suppression [40,42,43].

Interactions between members of a community and an invader can transition from antagonistic to facilitative or vice-versa, when let to co-evolve. It has been recently

reported that facilitation by a resident community enables invader survival and growth in a toxic medium where without these facilitative interactions the invader would not survive. However, the community became resistant to invasion after it co-evolved, possibly caused by increased growth of community members inhibiting invader survival [44]. A transition from antagonism to coexistence was also observed in two well-known plant-beneficial microbes: *Pseudomonas chlororaphis* and *Bacillus amyloliquefaciens*. Mutual antagonism is conferred by the production of secondary metabolites with antimicrobial activity. Coevolution, however, drove the emergence of spontaneous mutants in both species that provide resistance against the competitor molecules by modifying the respective targets. Although the mutations came at the cost of pleiotropic effects, they facilitated the coexistence between both organisms that apparently outweigh these effects [45]. Together, both studies provide valuable insight into the adaptive mechanisms underlying simple and more complex microbial interactions that need to be considered when designing future bioinoculants.

All warfare is based on deception: leveraging invasion-induced effects on the resident community

Invasion by a bioinoculant has the ability to alter microbial communities, and these alterations may be leveraged to influence plant health, regardless of the outcome for the bioinoculant. The magnitude of this effect depends on the overlap between the ecological niche, in particular resource use, of the bioinoculant and that of the resident taxa. The presence of such overlap could affect competitive activities within the community and consequently alter its composition. For instance, transient invasion by bacilli induced changes in bacterial diversity in soil that persisted for long periods of time, regardless of invader persistence. Invasion primarily affected copiotrophs — fast-growing organisms found in nutrient-rich environments — during the early phases of community assembly that likely shared a niche with the invader, and as a result oligotrophs — slow-growing organisms found in nutrient-sparse environments — appear to have benefitted at later stages of community assembly [46]. *Escherichia coli* caused a similar effect in soil that persisted after its disappearance. Notably, changes in community composition following invasion correlate with community resource use [47]. A comparable shift in community composition occurred after the introduction of *B. subtilis* into a soil-derived synthetic community. As a result, the closest relatives in the community were repressed [48]. These examples corroborate the idea that bioinoculants can affect the microbiome in a way that is dependent on bioinoculant and community functionality, but the question remains if such effects result in an alternative stable state, or if the community will convert back to its initial state with corresponding consequences for plant health. Alternative states, supported by changes in the abundance and/or presence of keystone microbial species or functionalities, could be responsible for the observed changes in plant health and the resistance against invasion by selected pathogens [49]. Nevertheless, the legacy of invasion might facilitate future invasion attempts and recurrent inoculations, and might be one of the determinants of successful bioinoculant application (Box 1). Therefore, we conclude that it is important to track the effect of bioinoculant application over extended periods of time and the possible alternative states it might induce to know whether invasion causes long-lasting functional changes in the microbial community.

Indirect effects on resident communities can be used to tap into a 'new' resource of plant-beneficial microbes that are rare in soil and/or the rhizosphere under current conditions, via the addition of bioinoculants that do not necessarily need to be beneficial for the plant directly themselves. Introduction of consortia of plant-beneficial *Pseudomonas* strains in the tomato rhizosphere increased the frequency of rare taxa in the resident community, possibly through direct competition for resources with more abundant taxa. Notably, rather than invader consortia-specific beneficial traits, changes in community composition best explained the observed beneficial effects on plant growth, but how these changes resulted in improved plant growth remains to be answered. The effect was larger with more diverse inocula, suggesting synergistic effects between certain strains [35]. Similarly, introduction of the beneficial microbe *B. amyloliquefaciens* into the tomato rhizosphere resulted in increased suppressiveness towards *R. solanacearum*, irrespective of inoculant abundance, possibly due to an enrichment of microbes carrying antimicrobial BGCs in the invaded communities [39].

In addition to invasion-induced effects on the resident microbial community, bioinoculants can also permanently alter host physiology, even after the bioinoculant is eliminated from the community. For example, bioinoculant-induced differential DNA methylation in pokeweed roots resulted in growth promotion, while functional-level variation in the rhizosphere microbiome induced by the inoculum was only detected at an early phase of microbial community assembly [50•].

Knowing the battlefield ensures victory: invasion depends on interaction with the host

Bioinoculants ultimately need to interact with a host plant, which imposes a genotype-dependent selection that determines microbial assembly [65,66]. Host-driven selection eventually enables emergence of compatibility

Box 1 Timing in rhizosphere invasions.

The rhizosphere microbiome and the functionalities it provides change with plant developmental stage. High biodiversity, as often seen in more mature rhizospheres, is often seen as a buffer to invasion [51–53]. Biodiversity can influence resistance to invasion through niche restriction/occupancy — that is, no available space for invader — [42,54], direct or indirect competition — for example, toxicity, activation of host immunity and niche displacement — phenotypic differentiation in space and time [46]. For example, in rice, *Enterobacter asburiae* was only able to promote rice shoot dry weight in the least diverse rich clay soils, likely because there were fewer keystone species in the most diluted microcosms that acted as generalist competitors of the bioinoculant [10].

On young roots, pioneering opportunistic bacterial species occur that invest little in the production of toxic secondary metabolites, while at later stages, bacteria with various life strategies can coexist [55], facilitated by niche differentiation [56]. Stochasticity of microbiome assembly, mainly during the initial stages, leads to differential local configurations, which in turn leads to variability in the density of pathogen-suppressive members. This variability, although imperceptible at the global community scale, translates into differential antagonist densities and therefore an increase in community invasibility. This was sufficient to explain invasion and disease onset by *R. solanacearum* [4••,57,58]. However, assembled communities from mature rhizospheres could prevent its invasion due to increased functional diversity, toxicity and niche restriction [42].

The order of arrival of (invading) strains affects community assembly and determines the final microbial community composition. In *A. thaliana* and *Lotus japonicus*, strains that arrived later in the resident communities reached lower proportions in the output communities, which could be due to niche pre-emption where early-arriving strains reduce the number of resources available. Nevertheless, strains that invaded communities on the host that they were isolated from were better able than strains from a different host plant, negating these priority effects. This effect was host-specific, that is, strains were better able to invade communities on the host from which they were isolated, thereby overcoming these priority effects [11••] (see also *Knowing the battlefield ensures victory: invasion depends on interaction with the host*). A similar effect was observed *in vitro* where early invaders were more successful than those trying to engraft in co-evolved communities at a later timepoint [44]. In the maize rhizosphere, the correlation between AMF colonisation and soil biodiversity was time-dependent, suggesting that once plants get established in the soil, positive interactions between AMF and the resident microbiota might not be as important anymore for AMF colonisation ability [59]. Another example comes from the tomato phyllosphere. Pseudomonads belonging to the *fluorescens* clade might pre-empt as much niche space as possible on tomato leaves in an early stage of community assembly, and benefit from this later on even at the expense of a decreased maximal growth rate [60]. In this case, competitiveness was optimised by shorter lag phases and lower growth rates, suggesting that prompter growth and dispersal are more determinant for bioinoculant efficacy than overall growth rate. However, recent estimations of growth rate across rhizosphere microbiome datasets show that the rhizosphere is dominated by genera with a high growth rate potential [61]. Together, these studies suggest a calibrated balance between the time of exit from the lag phase and growth rate potential in order to succeed in complex communities, and they nuance the classical dichotomy of 'slow and stable' (*K*-strategist or oligotroph) versus 'fast and fluctuating' (*r*-strategist or copiotroph) life histories.

While inoculation of different plant-beneficial strains or combinations thereof in soil resulted in only temporary changes in resident bacterial families, repeated inoculations permanently altered community succession in a strain-specific manner. Yet, beneficial effects of repeated inoculation on nutrient availability in the soil did decrease over time [34]. Repeated inoculations might thus be a solution for countering bioinoculant abundance decline and priority effects, although the efficacy of these measures can be time-dependent.

For a recent detailed account on the ecological mechanisms of invasion and the legacy on indigenous soil microbial communities in an agricultural context, we refer to [62,63], and for relevant insights for bioinoculant application from macroecology please see [64].

and cooperation with its microbiome [67], and this translates into microbial host preference [11••]. Therefore, compatibility and interaction with the host plant are of high relevance in designing future effective bioinoculants.

Evolutionary studies show examples of how to optimise bioinoculant compatibility with the target host. In pseudomonads and bacilli in mono-association with *Arabidopsis*, cooperative and mutualistic microbial traits emerge rapidly [68,69]. Evolved isolates showed improved root colonisation ability, in part driven by mutations in master regulators of bacterial behaviour, such as the GacA/S two-component system that induced major physiological changes [69–71]. Also another, master regulatory system in *Pseudomonas*, the ColR/S two-component system, has been linked to host adaptation at the strain level [72], and is at the same time required for protection against other pathogenic pseudomonads [7•]. This host-driven evolution suggests that master regulatory systems are key targets for optimising bioinoculant compatibility and should be exploited in the future.

An important determinant of compatibility is the interaction with the host immune system. In some beneficial microbes such as *B. velezensis*, host-derived reactive oxygen species (ROS) are required to stimulate bacterial secretion of auxin, which subsequently induces lateral root formation and protects against fungal pathogens [73]. In contrast, ROS production is known to widely restrict colonisation in the rhizosphere [74], and consequently, many successful root-colonising organisms, especially Actinobacteria and Gammaproteobacteria, have developed strategies to circumvent this by suppressing immune responses either locally or systemically [75,76,77].

Besides host immune activity, root exudate exploitation is the main driver of microbial compatibility. For instance, exuded inositol from Arabidopsis and tomato roots enhances chemotaxis and biofilm production of *Priestia megaterium* (formerly *Bacillus megaterium*) on their respective roots, resulting in growth promotion [78]. In *B. subtilis* and in pseudomonads, sucrose exudation enhances colonisation, which leads to enhanced protection against the phytopathogens *Fusarium* and *Botrytis* [79].

Furthermore, immune activity and exudation profiles are not homogeneous throughout the root system. The diverse set of exuded metabolites accumulates in different regions of the root [80], and the strength and scope of immune responses are cell-layer- and cell-type-specific and dependent on local damage [81–84]. Moreover, experiments on the colonisation of Firmicutes and Proteobacteria isolates in leaves with different morphology showed that the spatial organisation of the colonised surface alone plays a key role in determining the success of an inoculant [85]. The combination of these three factors creates specific microhabitats on the root where the bioinoculant will eventually interact with its host [12,86]. For example, epidermal trichoblasts mediate the beneficial interaction with *Pseudomonas simiae* WCS417 through exudation of coumarins [13], and many organisms colonise preferentially the root tip and elongation zone [87•].

These studies underline the importance of the bioinoculant-host compatibility, but also the relevance of the precisely invaded microhabitat for anticipating the local determinants for compatibility and eventual bioinoculant engraftment.

In the midst of chaos, there is also opportunity: towards the bioinoculants of the future

In the previous sections, we discussed relevant sources of variability underlying bioinoculant engraftment and success. The current state of the topic would be probably defined by Sun Tzu as: '*one may know how to conquer without being able to do it*'. Yet, although we are technically able to apply bioinoculants in soil, conquering the complexity of microbial invasions requires more knowledge about the physiology of beneficial microbes in soil and the local-scale interactions that govern the microbiome [18]. For example, microbial biosynthetic capacities remain largely underexplored [88], and the role of known metabolites in microbial physiology is still being discovered, even in well-studied microbes [79,89•]. Motility in soil, for example, is another crucial trait for a soil bioinoculant, and is only now being characterised. In *B. subtilis*, Engelhardt and co-authors described recently a collective form of chemotactic motility in soil. When a community of *B. subtilis* cells sense the presence of a root in the vicinity, the cells move like flocks through the soil particles and arrange dynamically around the root tips [87•]. This finding suggests that we need dedicated experiments to understand these mechanisms in current and future bioinoculants, as well as their relevance for successful colonisation.

Knowing the genetic and physiological characteristics from the bioinoculant and resident microbes allows for the prediction of genome-scale interactions, which in turn, allows for the engineering of the community in the long run. Recent advances, including single-cell sequencing techniques such as Microbe-seq [90] and Live-seq [91], could give more detailed information on the physiology of rhizosphere microbes, especially as improvements in sequencing and in sequence analysis software allow better reconstruction of genomes from complex metagenomes [92] and more thorough retrieval of information from existing ones [93]. The interactions with the host and other microbes can benefit from new techniques in spatial metatranscriptomics [94], together with the dissection of microbial transcriptional activity at single-cell resolution [95•], and new combinations with expansion microscopy and sequencing [96]. Other studies opt for overcoming the variability in efficacy with bold innovations such as the use of encapsulated microbes that allow engineering of cooperative interactions [97].

However, sometimes, even 'failed' bioinoculants might have beneficial effects and simply remain unseen in many experiments. A plant growth-promoting *Bacillus* isolate that can promote *A. thaliana* growth *in vitro* did not have any effect on shoot biomass in artificial soil, but its application increased the total number of siliques [98]. This suggests that productivity-based parameters should be regarded in experimental conditions when evaluating bioinoculant effects.

For Sun Tzu, a deep knowledge of oneself and of the opponent was equivalent to avoiding war. Commonly, efforts in understanding and applying microbes as bioinoculants come from apparently opposite perspectives, ranging from studying specific genes in specific contexts that are difficult to generalise, to broad-scale ecological dynamics and concepts that have difficulties in explaining particular cases. However, relatively few studies integrate both worlds while trying to understand invasions as an ecological and mechanistic complex phenomenon. Ensuring bioinoculant benefits on plant fitness, either directly or indirectly, demands working in harmony with ecological principles and the molecular mechanisms that depend on, and give rise to, those principles. In this sense, future efforts should focus on defining the precise microhabitats where the bioinoculant can provide its most benefits, and study how its invasion depends on its own physiology under the conditions therein, and how interactions with the local community and the host determine its engraftment and its effects in the field.

Data availability

No data were used for the research described in the article.

Conflict of interest statement

The authors declare no conflict of interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Salas-González I, Reyt G, Flis P, Custódio V, Gopaulchan D, Bakhoum N, Dew TP, Suresh K, Franke RB, Dangl JL, *et al.*: **Coordination between microbiota and root endodermis supports plant mineral nutrient homeostasis**. *Science* 2021, **371**:eabd0695.

2. He D, Singh SK, Peng L, Kaushal R, Vílchez JI, Shao C, Wu X, Zheng S, Morcillo RJL, Paré PW, *et al.*: **Flavonoid-attracted *Aeromonas* sp. from the *Arabidopsis* root microbiome enhances plant dehydration resistance**. *ISME J* 2022, **16**:2622-2632.

3. Li H, La S, Zhang X, Gao L, Tian Y: **Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress**. *ISME J* 2021, **15**:2865-2882.

4. Gu Y, Banerjee S, Dini-Andreote F, Xu Y, Shen Q, Jousset A, Wei Z:
•• **Small changes in rhizosphere microbiome composition predict disease outcomes earlier than pathogen density variations**. *ISME J* 2022, **16**:2448-2456.
In nature, some plants remain unaffected even though the pathogen is present. In this study, the authors show how this phenomenon is dependent on the early stages of microbiome assembly, when initial microbiome configurations from the same homogeneous soil community diverge into different assembly states that differentially affect pathogen success.

5. Xu S, Liu Y-X, Cernava T, Wang H, Zhou Y, Xia T, Cao S, Berg G, Shen X-X, Wen Z, *et al.*: **Fusarium fruiting body microbiome member *Pantoea agglomerans* inhibits fungal pathogenesis by targeting lipid rafts**. *Nat Microbiol* 2022, **7**:831-843.

6. Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, DiLeo MV: **From the lab to the farm: an industrial perspective of plant beneficial microorganisms**. *Front Plant Sci* 2016, **7**:1110.

7. Wang NR, Wiesmann CL, Melnyk RA, Hossain SS, Chi M-H,
• Martens K, Craven K, Haney CH: **Commensal *Pseudomonas fluorescens* strains protect *Arabidopsis* from closely related *Pseudomonas* pathogens in a colonization-dependent manner**. *mBio* 2022, **13**:e02892-21.
This study shows how a set of commensal pseudomonads confers protection against a closely-related opportunistic pathogen. In co-inoculation assays of commensals and the pathogen, plant biomass correlated with colonisation success of the phylogenetically-related commensal strains. This result suggests that plant protection can be achieved by using commensal inoculants whose niche overlap with the niche of a pathogen.

8. Kurkjian HM, Akbari MJ, Momeni B: **The impact of interactions on
• invasion and colonization resistance in microbial communities**. *PLoS Comput Biol* 2021, **17**:e1008643.
The authors of this paper explore the main variables affecting colonisation resistance in mathematical simulations. In these simulations, invasion success is maximised with bioinoculant densities of at least 10% of the total microbial density, with high bioinoculant growth rate, and in communities that show low internal cooperation but that contain facilitators for the bioinoculant.

9. Qian JJ, Akçay E: **The balance of interaction types determines the assembly and stability of ecological communities**. *Nat Ecol Evol* 2020, **4**:356-365.

10. da Costa PB, van Elsas JD, Mallon C, dos Anjos Borges LG, Pereira, Passaglia LM: **Efficiency of probiotic traits in plant inoculation is determined by environmental constrains**. *Soil Biol Biochem* 2020, **148**:107893.

11. Wippel K, Tao K, Niu Y, Zgadzaj R, Kiel N, Guan R, Dahms E, Zhang
•• P, Jensen DB, Logemann E, *et al.*: **Host preference and invasiveness of commensal bacteria in the *Lotus* and *Arabidopsis* root microbiota**. *Nat Microbiol* 2021, **6**:1150-1162.
Plants recruit a specific set of microbes that is distinguishable even when hosts are grown in the same habitat. The authors demonstrate that microbes show a host preference when grown in a community context. Priority effects were evident during root microbiome assembly, and microbes were better able to invade communities of their cognate hosts, even after communities were already established.

12. Birt HWG, Tharp CL, Custer GF, Dini-Andreote F: **Root phenotypes as modulators of microbial microhabitats**. *Front Plant Sci* 2022, **13**.

13. Verbon EH, Liberman LM, Zhou J, Yin J, Pieterse CMJ, Benfey PN, Stringlis IA, de Jonge R: **Cell-type specific transcriptomics reveals roles for root hairs and endodermal barriers in interaction with beneficial rhizobacterium**. *bioRxiv* 2022, https://doi.org/10.1101/2022.05.09.491085

14. Dressler MD, Conde J, Eldakar OT, Smith RP: **Timing between successive introduction events determines establishment success in bacteria with an Allee effect**. *Proc R Soc B Biol Sci* 2019, **286**:20190598.

15. Purtschert-Montenegro G, Cárcamo-Oyarce G, Pinto-Carbó M, Agnoli K, Bailly A, Eberl L: ***Pseudomonas putida* mediates bacterial killing, biofilm invasion and biocontrol with a type IVB secretion system**. *Nat Microbiol* 2022, **7**:1547-1557.

16. O'Callaghan M, Ballard RA, Wright D: **Soil microbial inoculants for sustainable agriculture: limitations and opportunities**. *Soil Use Manag* 2022, **38**:1340-1369.

17. Dal Co A, van Vliet S, Kiviet DJ, Schlegel S, Ackermann M: **Short-range interactions govern the dynamics and functions of microbial communities**. *Nat Ecol Evol* 2020, **4**:366-375.

18. Vliet S, van, Hauert C, Fridberg K, Ackermann M, Co AD: **Global dynamics of microbial communities emerge from local interaction rules**. *PLoS Comput Biol* 2022, **18**:e1009877.

19. Passarelli-Araujo H, Franco GR, Venancio TM: **Network analysis of ten thousand genomes shed light on *Pseudomonas* diversity and classification**. *Microbiol Res* 2022, **254**:126919.

20. Rieusset L, Rey M, Muller D, Vacheron J, Gerin F, Dubost A, Comte G, Prigent-Combaret C: **Secondary metabolites from plant-associated *Pseudomonas* are overproduced in biofilm**. *Microb Biotechnol* 2020, **13**:1562-1580.

21. Hansen ML, Wibowo M, Jarmusch SA, Larsen TO, Jelsbak L:
• **Sequential interspecies interactions affect production of antimicrobial secondary metabolites in *Pseudomonas protegens* DTU9.1**. *ISME J* 2022, https://doi.org/10.1038/s41396-022-01322-8.
Pyoluteorin and DAPG are biocontrol traits in *Pseudomonas protegens*. Diffusible signals from a nearby *Pseudomonas capeferrum* can trigger production of pyoluteorin in *P. protegens*, killing *P. capeferrum*. Antibiosis results in the release of intracellular signals that further alter *P. protegens* production of biocontrol traits, in this case DAPG. This study shows how interactions among pseudomonads drive expression of relevant bioinoculant traits.

22. Andrić S, Rigolet A, Argüelles Arias A, Steels S, Hoff G, Balleux G,
•• Ongena L, Höfte M, Meyer T, Ongena M: **Plant-associated *Bacillus* mobilizes its secondary metabolites upon perception of the siderophore pyochelin produced by a *Pseudomonas* competitor**. *ISME J* 2023, **17**:263-275.
This study, together with Hansen et al. 2022, highlights the relevance of interactions of bioinoculants with resident microbes. Here, the authors describe how *Bacillus velezensis* senses pyochelin produced by competitor pseudomonads and deploys an array of competitive traits in response, including broad-range antimicrobial compounds and motility traits.

23. Andrić S, Meyer T, Rigolet A, Prigent-Combaret C, Höfte M, Balleux G, Steels S, Hoff G, De Mot R, McCann A, *et al.*: **Lipopeptide**

interplay mediates molecular interactions between soil *Bacilli* and Pseudomonads. *Microbiol Spectr* 2021, **9**:e02038-21.

24. Qi SS, Bogdanov A, Cnockaert M, Acar T, Ranty-Roby S, Coenye T, Vandamme P, König GM, Crüsemann M, Carlier A: **Induction of antibiotic specialized metabolism by co-culturing in a collection of phyllosphere bacteria**. *Environ Microbiol* 2021, **23**:2132-2151.

25. Tyc O, van den Berg M, Gerards S, van Veen JA, Raaijmakers JM, de Boer W, Garbeva P: **Impact of interspecific interactions on antimicrobial activity among soil bacteria**. *Front Microbiol* 2014, **5**:567.

26. Seyedsayamdost MR: **High-throughput platform for the discovery of elicitors of silent bacterial gene clusters**. *Proc Natl Acad Sci USA* 2014, **111**:7266-7271.

27. Kehe J, Ortiz A, Kulesa A, Gore J, Blainey PC, Friedman J: **Positive interactions are common among culturable bacteria**. *Sci Adv* 2021, **7**:eabi7159.

28. Shalev O, Karasov TL, Lundberg DS, Ashkenazy H, Pramoj Na
• Ayutthaya P, Weigel D: **Commensal *Pseudomonas* strains facilitate protective response against pathogens in the host plant**. *Nat Ecol Evol* 2022, **6**:383-396.
In this study, six natural *Arabidopsis thaliana* genotypes collected around Tübingen (Germany) were inoculated with pathogenic or commensal *Pseudomonas* synthetic communities, or with a mixed community of pathogens and commensals. Interestingly, the authors found that commensal communities could protect *A. thaliana* from pathogens, and that the extent of protection depends on the host genotype.

29. Heiman CM, Maurhofer M, Calderon S, Dupasquier M, Marquis J, Keel C, Vacheron J: **Pivotal role of O-antigenic polysaccharide display in the sensitivity against phage tail-like particles in environmental *Pseudomonas* kin competition**. *ISME J* 2022, **16**:1683-1693.

30. Kraigher B, Butolen M, Stefanic P, Mandic Mulec I: **Kin discrimination drives territorial exclusion during *Bacillus subtilis* swarming and restrains exploitation of surfactin**. *ISME J* 2022, **16**:833-841.

31. Kobayashi K, Diverse LXG: **toxin and antitoxin systems specifically mediate intraspecies competition in *Bacillus subtilis* biofilms**. *PLoS Genet* 2021, **17**:e1009682.

32. Eckshtain-Levi N, Harris SL, Roscios RQ, Shank EA: **Bacterial community members increase *Bacillus subtilis* maintenance on the roots of *Arabidopsis thaliana***. *Phytobiomes J* 2020, **4**:303-313.

33. Sun X, Xu Z, Xie J, Hesselberg-Thomsen V, Tan T, Zheng D, Strube
• ML, Dragoš A, Shen Q, Zhang R, *et al*.: ***Bacillus velezensis* stimulates resident rhizosphere *Pseudomonas stutzeri* for plant health through metabolic interactions**. *ISME J* 2022, **16**:774-787.
These authors show that the integrity of the biofilm is essential for a positive interaction between two bioinoculants that together stimulate plant growth. Both species occupied distinct niches within the biofilm, in which *Pseudomonas stutzeri* resides at the bottom, nutrient-rich, oxygen-limited section, while Bacillus velezensis could be found at the top which is oxygen-rich, yet nutrient-limited.

34. Wang Z, Chen Z, Kowalchuk GA, Xu Z, Fu X, Kuramae EE: **Succession of the resident soil microbial community in response to periodic inoculations**. *Appl Environ Microbiol* 2021, **87**:e00046-21.

35. Hu J, Yang T, Friman V-P, Kowalchuk GA, Hautier Y, Li M, Wei Z, Xu Y, Shen Q, Jousset A: **Introduction of probiotic bacterial consortia promotes plant growth via impacts on the resident rhizosphere microbiome**. *Proc R Soc B Biol Sci* 2021, **288**:20211396.

36. Emami S, Alikhani HA, Pourbabaee AA, Etesami H, Motasharezadeh B, Sarmadian F: **Consortium of endophyte and rhizosphere phosphate solubilizing bacteria improves phosphorous use efficiency in wheat cultivars in phosphorus deficient soils**. *Rhizosphere* 2020, **14**:100196.

37. Berendsen RL, Vismans G, Yu K, Song Y, de Jonge R, Burgman WP, Burmølle M, Herschend J, Bakker PAHM, Pieterse CMJ: **Disease-induced assemblage of a plant-beneficial bacterial consortium**. *ISME J* 2018, **12**:1496-1507.

38. Gu S, Wei Z, Shao Z, Friman V-P, Cao K, Yang T, Kramer J, Wang
•• X, Li M, Mei X, *et al*.: **Competition for iron drives phytopathogen control by natural rhizosphere microbiomes**. *Nat Microbiol* 2020, **5**:1002-1010.
In some cases, the rhizosphere microbiome resists the invasion of the pathogen *Ralstonia solanacearum*, consequently protecting the host plant. In others, though, it can strongly facilitate pathogen engraftment and promote disease. This variability is attributed to the ability of the pathogen to uptake the siderophores produced by residential microbes. In communities that produce siderophores for which the pathogen has receptors, invasion is facilitated. Otherwise, the community resists to invasion. This article is a great work on setting the molecular basis of resistance to invasion at the community level.

39. Deng X, Zhang N, Li Y, Zhu C, Qu B, Liu H, Li R, Bai Y, Shen Q, Falcao Salles J: **Bio-organic soil amendment promotes the suppression of *Ralstonia solanacearum* by inducing changes in the functionality and composition of rhizosphere bacterial communities**. *New Phytol* 2022, **235**:1558-1574.

40. Li M, Wei Z, Wang J, Jousset A, Friman V-P, Xu Y, Shen Q, Pommier T: **Facilitation promotes invasions in plant-associated microbial communities**. *Ecol Lett* 2019, **22**:149-158.

41. Li M, Pommier T, Yin Y, Wang J, Gu S, Jousset A, Keuskamp J, Wang H, Wei Z, Xu Y, *et al*.: **Indirect reduction of *Ralstonia solanacearum* via pathogen helper inhibition**. *ISME J* 2022, **16**:868-875.

42. Hu J, Wei Z, Kowalchuk GA, Xu Y, Shen Q, Jousset A: **Rhizosphere microbiome functional diversity and pathogen invasion resistance build up during plant development**. *Environ Microbiol* 2020, **22**:5005-5018.

43. Hu J, Wei Z, Friman V-P, Gu S, Wang X, Eisenhauer N, Yang T, Ma J, Shen Q, Xu Y, *et al*.: **Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression**. *mBio* 2016, **7**:e01790-16.

44. Piccardi P, Alberti G, Alexander JM, Mitri S: **Microbial invasion of a toxic medium is facilitated by a resident community but inhibited as the community co-evolves**. *ISME J* 2022, **16**:2644-2652.

45. Molina-Santiago C, Vela-Corcía D, Petras D, Díaz-Martínez L, Pérez-Lorente AI, Sopeña-Torres S, Pearson J, Caraballo-Rodríguez AM, Dorrestein PC, de Vicente A, *et al*.: **Chemical interplay and complementary adaptative strategies toggle bacterial antagonism and co-existence**. *Cell Rep* 2021, **36**:109449.

46. Mawarda PC, Lakke SL, Dirk van Elsas J, Salles JF: **Temporal dynamics of the soil bacterial community following *Bacillus* invasion**. *iScience* 2022, **25**:104185.

47. Mallon CA, Le Roux X, van Doorn GS, Dini-Andreote F, Poly F, Salles JF: **The impact of failure: unsuccessful bacterial invasions steer the soil microbial community away from the invader's niche**. *ISME J* 2018, **12**:728-741.

48. Kiesewalter HT, Lozano-Andrade CN, Strube ML, Kovács ÁT: **Secondary metabolites of *Bacillus subtilis* impact the assembly of soil-derived semisynthetic bacterial communities**. *Beilstein J Org Chem* 2020, **16**:2983-2998.

49. Niu B, Paulson JN, Zheng X, Kolter R: **Simplified and representative bacterial community of maize roots**. *Proc Natl Acad Sci USA* 2017, **114**:E2450-E2459.

50. Chen C, Wang M, Zhu J, Tang Y, Zhang H, Zhao Q, Jing M, Chen Y,
• Xu X, Jiang J, *et al*.: **Long-term effect of epigenetic modification in plant–microbe interactions: modification of DNA methylation induced by plant growth-promoting bacteria mediates promotion process**. *Microbiome* 2022, **10**:36.
This study is a great example of using bioinoculants to promote plant health without focusing on colonisation levels of the beneficial microbe. The authors show that bioinoculant-induced DNA methylation in the host plant promotes growth, even after the bioinoculant is eliminated from the community.

51. Case TJ: **Invasion resistance arises in strongly interacting species-rich model competition communities**. *Proc Natl Acad Sci USA* 1990, **87**:9610-9614.

52. Mallon CA, Elsas JD, van, Salles JF: **Microbial invasions: the process, patterns, and mechanisms**. *Trends Microbiol* 2015, **23**:719-729.

53. Tilman D: **Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly**. *Proc Natl Acad Sci USA* 2004, **101**:10854-10861.

54. Herren CM: **Disruption of cross-feeding interactions by invading taxa can cause invasional meltdown in microbial communities**. *Proc R Soc B Biol Sci* 2020, **287**:20192945.

55. Dini-Andreote F, Raaijmakers JM: **Embracing community ecology in plant microbiome research**. *Trends Plant Sci* 2018, **23**:467-469.

56. Nuccio EE, Starr E, Karaoz U, Brodie EL, Zhou J, Tringe SG, Malmstrom RR, Woyke T, Banfield JF, Firestone MK, *et al.*: **Niche differentiation is spatially and temporally regulated in the rhizosphere**. *ISME J* 2020, **14**:999-1014.

57. Lee S-M, Kong HG, Song GC, Ryu C-M: **Disruption of Firmicutes and Actinobacteria abundance in tomato rhizosphere causes the incidence of bacterial wilt disease**. *ISME J* 2021, **15**:330-347.

58. Wei Z, Gu Y, Friman V-P, Kowalchuk GA, Xu Y, Shen Q, Jousset A: **Initial soil microbiome composition and functioning predetermine future plant health**. *Sci Adv* 2019, **5**:eaaw0759.

59. Ferreira DA, da Silva TF, Pylro VS, Salles JF, Andreote FD, Dini-Andreote F: **Soil microbial diversity affects the plant-root colonization by arbuscular mycorrhizal fungi**. *Microb Ecol* 2021, **82**:100-103.

60. Humphrey PT, Satterlee TT, Whiteman NK: **Competitive hierarchies, antibiosis, and the distribution of bacterial life history traits in a microbiome**. *bioRxiv* 2020, https://doi.org/10.1101/2020.06.15.152272

61. López JL, Pappas N, Poppeliers SWM, Sanchez-Gil JJ, Fourie-Fouche A, de Jonge R, Dutilh BE: **Copiotrophs dominate rhizosphere microbiomes and growth rate potential is a major factor explaining the rhizosphere effect**. *bioRxiv* 2022, https://doi.org/10.1101/2020.06.15.152272

62. Liu X, Le Roux X, Salles JF: **The legacy of microbial inoculants in agroecosystems and potential for tackling climate change challenges**. *iScience* 2022, **25**:103821.

63. Mawarda PC, Le Roux X, Dirk van Elsas J, Salles JF: **Deliberate introduction of invisible invaders: a critical appraisal of the impact of microbial inoculants on soil microbial communities**. *Soil Biol Biochem* 2020, **148**:107874.

64. Albright MBN, Louca S, Winkler DE, Feeser KL, Haig S-J, Whiteson KL, Emerson JB, Dunbar J: **Solutions in microbiome engineering: prioritizing barriers to organism establishment**. *ISME J* 2022, **16**:331-338.

65. Escudero-Martinez C, Coulter M, Alegria Terrazas R, Foito A, Kapadia R, Pietrangelo L, Maver M, Sharma R, Aprile A, Morris J, *et al.*: **Identifying plant genes shaping microbiota composition in the barley rhizosphere**. *Nat Commun* 2022, **13**:3443.

66. Oyserman BO, Flores SS, Griffioen T, Pan X, van der Wijk E, Pronk L, Lokhorst W, Nurfikari A, Paulson JN, Movassagh M, *et al.*: **Disentangling the genetic basis of rhizosphere microbiome assembly in tomato**. *Nat Commun* 2022, **13**:3228.

67. Sharp C, Foster KR: **Host control and the evolution of cooperation in host microbiomes**. *Nat Commun* 2022, **13**:3567.

68. Blake C, Nordgaard M, Maróti G, Kovács ÁT: **Diversification of *Bacillus subtilis* during experimental evolution on *Arabidopsis thaliana* and the complementarity in root colonization of evolved subpopulations**. *Environ Microbiol* 2021, **23**:6122-6136.

69. Li E, de Jonge R, Liu C, Jiang H, Friman V-P, Pieterse CMJ, Bakker PAHM, Jousset A: **Rapid evolution of bacterial mutualism in the plant rhizosphere**. *Nat Commun* 2021, **12**:3829.

70. Li E, Zhang H, Jiang H, Pieterse CMJ, Jousset A, Bakker PAHM, de Jonge R: **Experimental-evolution-driven identification of *Arabidopsis* rhizosphere competence genes in *Pseudomonas protegens***. *mBio* 2021, **12**:e00927-21.

71. Nordgaard M, Blake C, Maróti G, Hu G, Wang Y, Strube ML, Kovács ÁT: **Experimental evolution of *Bacillus subtilis* on *Arabidopsis thaliana* roots reveals fast adaptation and improved root colonization**. *iScience* 2022, **25**:104406.

72. Wiesmann CL, Zhang Y, Alford M, Hamilton CD, Dosanjh M, Thoms D, Dostert M, Wilson A, Pletzer D, Hancock REW, *et al.*: **The ColR/S two-component system is a conserved determinant of host association across *Pseudomonas* species**. *ISME J* 2023, **17**:286-296.

73. Tzipilevich E, Russ D, Dangl JL, Benfey PN: **Plant immune system activation is necessary for efficient root colonization by auxin-secreting beneficial bacteria**. *Cell Host Microbe* 2021, **29**:1507-1520.e4.

74. Song Y, Wilson AJ, Zhang X-C, Thoms D, Sohrabi R, Song S, Geissmann Q, Liu Y, Walgren L, He SY, *et al.*: **FERONIA restricts *Pseudomonas* in the rhizosphere microbiome via regulation of reactive oxygen species**. *Nat Plants* 2021, **7**:644-654.

75. Ma K-W, Niu Y, Jia Y, Ordon J, Copeland C, Emonet A, Geldner N, Guan R, Stolze SC, Nakagami H, *et al.*: **Coordination of microbe–host homeostasis by crosstalk with plant innate immunity**. *Nat Plants* 2021, **7**:814-825.

76. Yu K, Liu Y, Tichelaar R, Savant N, Lagendijk E, van Kuijk SJL, Stringlis IA, van Dijken AJH, Pieterse CMJ, Bakker PAHM, *et al.*: **Rhizosphere-associated *Pseudomonas* suppress local root immune responses by gluconic acid-mediated lowering of environmental pH**. *Curr Biol* 2019, **29**:3913-3920.e4.

77. Teixeira PJPL, Colaianni NR, Law TF, Conway JM, Gilbert S, Li H, Salas-González I, Panda D, Del Risco NM, Finkel OM, *et al.*: **Specific modulation of the root immune system by a community of commensal bacteria**. *Proc Natl Acad Sci USA* 2021, **118**:e2100678118.

78. Vílchez JI, Yang Y, He D, Zi H, Peng L, Lv S, Kaushal R, Wang W, Huang W, Liu R, *et al.*: **DNA demethylases are required for myo-inositol-mediated mutualism between plants and beneficial rhizobacteria**. *Nat Plants* 2020, **6**:983-995.

79. Tian T, Sun B, Shi H, Gao T, He Y, Li Y, Liu Y, Li X, Zhang L, Li S, *et al.*: **Sucrose triggers a novel signaling cascade promoting *Bacillus subtilis* rhizosphere colonization**. *ISME J* 2021, **15**:2723-2737.

80. Zhang X, Dippold MA, Kuzyakov Y, Razavi BS: **Spatial pattern of enzyme activities depends on root exudate composition**. *Soil Biol Biochem* 2019, **133**:83-93.

81. Emonet A, Zhou F, Vacheron J, Heiman CM, Dénervaud Tendon V, Ma K-W, Schulze-Lefert P, Keel C, Geldner N: **Spatially restricted immune responses are required for maintaining root meristematic activity upon detection of bacteria**. *Curr Biol* 2021, **31**:1012-1028.e7.

82. Fröschel C, Komorek J, Attard A, Marsell A, Lopez-Arboleda WA, Le Berre J, Wolf E, Geldner N, Waller F, Korte A, *et al.*: **Plant roots employ cell-layer-specific programs to respond to pathogenic and beneficial microbes**. *Cell Host Microbe* 2021, **29**:299-310.e7.

83. Rich-Griffin C, Eichmann R, Reitz MU, Hermann S, Woolley-Allen K, Brown PE, Wiwatdirekkul K, Esteban E, Pasha A, Kogel K-H, *et al.*: **Regulation of cell type-specific immunity networks in *Arabidopsis* roots**. *Plant Cell* 2020, **32**:2742-2762.

84. Zhou F, Emonet A, Dénervaud Tendon V, Marhavy P, Wu D, Lahaye T, Geldner N: **Co-incidence of damage and microbial patterns controls localized immune responses in roots**. *Cell* 2020, **180**:440-453.e18.

85. Gupta R, Elkabetz D, Leibman-Markus M, Jami E, Bar M: **Cytokinin-microbiome interactions regulate developmental functions**. *Environ Micro* 2022, **17**:2.

86. Kawa D, Brady SM: **Root cell types as an interface for biotic interactions**. *Trends Plant Sci* 2022, **27**:1173-1186.

87. Engelhardt IC, Patko D, Liu Y, Mimault M, de las Heras Martinez G,
   • George TS, MacDonald M, Ptashnyk M, Sukhodub T, Stanley-Wall NR, *et al*.: **Novel form of collective movement by soil bacteria**. *ISME J* 2022, **16**:2337-2347.
   The study underlines how much we still need to learn about microbial physiology in soil. Here, the authors describe how *Bacillus subtilis* cells move collectively like flocks among polymer particles that resemble the soil matrix. This type of movement is possibly driven by chemotactic and quorum sensing mechanisms that enable *B. subtilis* cells to arrange dynamically around root tips.

88. Gavriilidou A, Kautsar SA, Zaburannyi N, Krug D, Müller R, Medema MH, Ziemert N: **Compendium of specialized metabolite biosynthetic diversity encoded in bacterial genomes**. *Nat Microbiol* 2022, **7**:726-735.

89. Anand A, Falquet L, Abou-Mansour E, L'Haridon F, Keel C,
   • Weisskopf L: **Much more than a toxin: hydrogen cyanide is a volatile modulator of bacterial behaviour**. *bioRxiv* 2022, https://doi.org/10.1101/2021.09.29.462390.
   This study, still asa preprint, is a good example of how much there is still to learn from the physiology of current bioinoculants. Hydrogen cyanide (HCN) is a toxin produced by *Pseudomonas protegens*, and one of the main metabolites that enable biocontrol activity in this bacterium. Here, the authors describe how HCN behaves like a quorum-sensing signal that affects critical traits like motility, biofilm formation, and production of other secondary metabolites.

90. Zheng W, Zhao S, Yin Y, Zhang H, Needham DM, Evans ED, Dai CL, Lu PJ, Alm EJ, Weitz DA: **High-throughput, single-microbe genomics with strain resolution, applied to a human gut microbiome**. *Science* 2022, **376**:eabm1483.

91. Chen W, Guillaume-Gentil O, Rainer PY, Gäbelein CG, Saelens W, Gardeux V, Klaeger A, Dainese R, Zachara M, Zambelli T, *et al*.: **Live-seq enables temporal transcriptomic recording of single cells**. *Nature* 2022, **608**:733-740.

92. Pan S, Zhu C, Zhao X-M, Coelho LP: **A deep siamese neural network improves metagenome-assembled genomes in microbiome datasets across different environments**. *Nat Commun* 2022, **13**:2326.

93. Price MN, Deutschbauer AM, Arkin AP: **Filling gaps in bacterial catabolic pathways with computation and high-throughput genetics**. *PLoS Genet* 2022, **18**:e1010156.

94. Saarenpää S, Shalev O, Ashkenazy H, de Oliveira-Carlos V, Lundberg DS, Weigel D, Giacomello S: **Spatially resolved host-bacteria-fungi interactomes via spatial metatranscriptomics**. *bioRxiv* 2022, https://doi.org/10.1101/2022.07.18.496977

95. Dar D, Dar N, Cai L, Newman DK: **Spatial transcriptomics of**
   • **planktonic and sessile bacterial populations at single-cell resolution**. *Science* 2021, **373**:eabi4882.
   This is a great example of the complexity of microbial physiology. The authors of this study follow the expression *in vivo* of 105 marker genes in *Pseudomonas aeruginosa* single cells during planktonic growth and in a biofilm. They show how physiologically different subpopulations emerge, based on mutually exclusive transcriptional states.

96. Alon S, Goodwin DR, Sinha A, Wassie AT, Chen F, Daugharthy ER, Bando Y, Kajita A, Xue AG, Marrett K, *et al*.: **Expansion sequencing: spatially precise *in situ* transcriptomics in intact biological systems**. *Science* 2021, **371**:eaax2656.

97. Wang L, Zhang X, Tang C, Li P, Zhu R, Sun J, Zhang Y, Cui H, Ma J, Song X, *et al*.: **Engineering consortia by polymeric microbial swarmbots**. *Nat Commun* 2022, **13**:3879.

98. García-Cárdenas E, Ortiz-Castro R, Ruiz-Herrera LF, Valencia-Cantero E, López-Bucio J: *Bacillus* **sp. LC390B from the maize rhizosphere improves plant biomass, root elongation, and branching and requires the phytochromes PHYA and PHYB for phytostimulation**. *J Plant Growth Regul* 2022, https://doi.org/10.1007/s00344-022-10770-7