

The Use of Synthetic Microbial Communities to Improve Plant Health

Samuel J. Martins,^{1,†} Josephine Pasche,¹ Hiago Antonio O. Silva,^{1,2} Gijs Selten,³ Noah Savastano,⁴ Lucas Magalhães Abreu,² Harsh P. Bais,⁴ Karen A. Garrett,¹ Nattapol Kraitsitudomsook,¹ Corné M. J. Pieterse,³ and Tomislav Cernava^{5,6}

¹ Department of Plant Pathology, University of Florida, Gainesville, FL, 32611, U.S.A.

² Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil

³ Plant–Microbe Interactions, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

⁴ Department of Plant and Soil Sciences, 311 AP Biopharma, University of Delaware, Newark, DE 19713, U.S.A.

⁵ Institute of Environmental Biotechnology, Graz University of Technology, Graz, 8020, Austria

⁶ School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, SO17 1BJ, U.K.

Accepted for publication 28 February 2023.

Abstract

Despite the numerous benefits plants receive from probiotics, maintaining consistent results across applications is still a challenge. Cultivation-independent methods associated with reduced sequencing costs have considerably improved the overall understanding of microbial ecology in the plant environment. As a result, now, it is possible to engineer a consortium of microbes aiming for improved plant health. Such synthetic microbial communities (SynComs) contain carefully chosen microbial species to produce the desired microbiome function. Microbial biofilm formation, production of secondary metabolites, and ability to induce plant resistance are some of the microbial traits to consider when designing SynComs. Plant-associated microbial communities are not assembled randomly. Ecological theories suggest that these communities have a defined phylogenetic organization structured by general community assembly rules. Using machine learning, we can study these rules and target microbial functions that generate desired plant phenotypes. Well-structured assemblages are more likely to lead to a stable SynCom that thrives under environmental stressors as compared with the classical selection of single microbial activities or taxonomy. However, ensuring microbial colonization and long-term plant phenotype stability is still one of the challenges to overcome with SynComs, as the synthetic community may change over time with microbial horizontal gene transfer and retained mutations. Here, we explored the advances made in SynCom research regarding plant health, focusing on bacteria, as they are the most dominant microbial form compared with other members of the microbiome and the most commonly found in SynCom studies.

Keywords: biofilm, dysbiosis, eubiosis, food security, induced systemic resistance (ISR), inoculants, microbial volatile organic compounds (mVOCs), microbiome, phytobiome, plant growth promoting (PGP), plant–bacteria interaction

As our global population and the number of diseases it must contend with both increase steadily, microbes beneficial to human health have gained more attention as an alternative treatment. Some of these beneficial microbes, often termed probiotics, have been studied with positive outcomes through fecal microbiome trans-

plantation from healthy donors to sick patients to treat infectious diseases and to overcome resistance to powerful immunotherapies (Erdmann 2022). These probiotics act by antagonizing specific pathogens and/or inducing host immunity (Sanders et al. 2019). Similar to the human gut, the plant rhizosphere, defined as the narrow area of soil around the root under direct influence of root exudates, is colonized by diverse microbes. These microbes play a crucial role in plant physiological processes, in addition to being the first line of defense against invading pathogens/parasites (Berg et al. 2020; Trivedi et al. 2020). When isolated and applied to the plant, these microbes can act as probiotics and increase plant nutrient uptake, control diseases, alleviate environmental stress, and promote growth (Martins et al. 2015, 2022; Poudel et al. 2021). Despite the numerous benefits of these probiotics to the plant, maintaining consistent results between applications is a challenge, especially under field conditions, where there are other native microbes, and plants experience a wide range of abiotic stressors. For over 100 years, the use of beneficial microbes to support plant health has had the

†Corresponding author: S. J. Martins; sj.martins@ufl.edu

Funding: Support was provided by the U.S. Department of Agriculture-National Institute of Food and Agriculture Hatch Project 1024881, the Novo Nordisk Foundation (project InRoot), NWO Gravitation Programme MiCRop “Harnessing the second genome of plants” (grant 024.004.014), and the Foundation for Food and Agriculture Research (grant FF-NIA19-000000050).

e-Xtra: Supplementary material is available online.

The author(s) declare no conflict of interest.

potential to become a reliable, sustainable approach in agriculture, but there are still persistent challenges in realizing this potential. To enhance probiotic performance and reliability, we need a better understanding of microbial interactions as a community and the factors that contribute to successes and failures of probiotics in different environments.

In recent years, cultivation-independent methods based on profiling marker genes or shotgun metagenome sequencing, associated with reduced sequencing costs, have considerably improved the overall understanding of microbial ecology in the plant environment. As a result, we can now engineer small consortia of microbes that can mimic the observed function and structure of the microbiome in natural conditions (De Souza et al. 2020). Such consortia are known as synthetic microbial communities (SynComs), and each species in these communities has to be carefully chosen to mimic the function of the microbiome and preserve the symbiotic interactions within the plant (Shayanthan et al. 2022). To effectively manipulate and engineer SynComs, integrated with the approximately 10 billion microorganisms present in 1 g of soil, has been a major challenge in microbial ecology. In this review, we explored the advances made with SynComs to support plant health. We focused on bacteria, as they are the most dominant microbial form, and most SynComs use bacteria.

From Single Microbes to SynComs

To feed our growing population, it is imperative to increase agricultural productivity 70% by the year 2050 (Singh et al. 2020). To meet this goal, a possible sustainable solution involves alterations of the microbial communities in the rhizosphere, where the majority of plant microbes are found. The beneficial microbes associated with suppressive soils were termed plant growth-promoting rhizobacteria (PGPR) by Kloepper and Schroth (1978), who also showed that there are bacteria in the rhizosphere that specifically influence plant growth. These realizations sparked a world of research opportunities, as the influence of these suppressive soil microbes could have more beneficial effects than previously known. The question of disease suppression was addressed by Broadbent et al. (1971), who determined that some strains of *Bacillus* and *Streptomyces* had suppressive qualities against damping-off, caused by *Rhizoctonia solani*. Later, Schroth and Hancock (1982) determined that inoculating soil with *Pseudomonas* spp. could produce a soil suppressive to deleterious rhizobacteria (including *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Flavobacterium*, *Achromobacter*, and *Anthrobacter*) in sugar beet (*Beta vulgaris* subsp. *vulgaris*). Although single-strain inoculation showed promise, the importance of the community of microbes became increasingly clear, as microbial communities and their interactions are beneficial to plants and increase their resistance to diseases (Qu et al. 2020). In the same train of thought as fecal microbiome transfer from healthy to sick patients, the idea of transferring a percentage of the soil is being explored to transfer the beneficial microbiome and turn conducive soils into suppressive soils (Jiang et al. 2022).

The transfer of rhizosphere microbes from one community into another has been addressed by several methods, each with its own set of strategies and challenges. The most classic of these approaches is soil transfer, which involves a donor and recipient soil. The donor soil is disease-suppressive, whereas the recipient is conducive to the disease of interest (Jiang et al. 2022). Mendes et al. (2011) sought to understand the possibility of using this soil-transfer method to control disease. They specifically focused on a soil that was previously identified as suppressive to *R. solani* infections. The team transferred a portion of the suppressive soil into a conducive soil in a 1:9 (wt/wt) ratio. The authors concluded that the transfer of soil partially conferred disease suppression of *R. solani* infections in sugar beet (*Beta vulgaris* subsp. *vulgaris*) as the result of microbes in the soil. Another approach to microbiome transfer is extraction

of soil microbes. The rhizosphere microbiome transplant approach eliminates the abiotic factors of soil, and the microbes from the suppressive rhizosphere are extracted for transplant into the recipient soil (Jiang et al. 2022). The strategy begins very similarly to the soil-transfer approach, as a disease-suppressive soil is identified, but the microbiome will be prepared into a slurry that will be added to the conducive soil, as described by Elhady et al. (2018), Silva et al. (2022), and Zhou et al. (2019). There are slight variations between these protocols, but the methods begin with a donor soil from the rhizosphere being collected for extraction. Soil is extracted in a 1:10 ratio of soil to salt buffer, and the solution is then shaken or blended (Zhou et al. 2019). After vigorous mixing, the supernatant is passed through a sieve and centrifuged (Silva et al. 2022). The remaining microbiome slurry is resuspended in water to then transplant into the recipient soil (Elhady et al. 2018). Another benefit of both soil-transfer and rhizosphere microbiome transplant methods is the delivery of microbes in the exact proportion that was found in the suppressive soil.

Another approach to microbial transfer that has been explored recently is the isolation of key microbial taxa and their assembly into SynComs. The number of microbes present in a SynCom for crop health typically varies from 2 to 23, and more than half of SynCom studies used between 2 and 5 microbes (Fig. 1A; Supplementary Table S1).

Although the average number of microbes used in SynComs was seven (with the average influenced by the outliers with bigger values), the mode and probably the most meaningful value was four (Fig. 1A). The top five bacterial genera used in a SynCom are *Bacillus*, *Pseudomonas*, *Enterobacter*, *Streptomyces*, and *Pantoea* (Fig. 1B). Despite beneficial organisms being identified, plant colonization and maintaining the long-term stability of a SynCom are still challenges to be overcome, as the SynCom may change over time with horizontal gene transfer among microbes and retained mutations (Jiang et al. 2022; Shayanthan et al. 2022).

In contrast to selecting microbes based on single microbial *in vitro* activities or taxonomy, as in the classic method, in SynCom development, multiple attributes should be considered. These attributes include microbes associated with desirable plant phenotypes and microbial traits that will give the microbes fitness to persist in different environments and to colonize plants. These microbial traits include the production of exometabolites, such as secondary metabolites and volatile organic compounds (VOCs), robust biofilm formation, and the ability to chemically trigger plant defense mechanisms.

Network Analysis to Understand Community Interactions

Although SynComs with greater numbers of species have the potential to be more resilient, there are increasing challenges for characterizing the systems, as the number of interactions among species also increases. Greater resilience could result if species produce microenvironments that support the SynCom, suppress natural enemies of the SynCom in the environment to which the SynCom is introduced, and/or compensate for each other's functions when environmental fluctuations favor one species over another. Ecological concepts include a wide range of potential benefits of diversity, including the idea that SynComs with greater numbers may be more likely to include a particularly effective species by chance (Tilman et al. 2014). Challenges arise in understanding each type of interaction among species and how those interactions may change across abiotic environments and in the presence of other background microbes. Evaluating the microbiome network, the network of associations among microbial taxa, and potentially associations with plant phenotypic traits can help to identify candidate assemblages for a SynCom (Fig. 2).

Conventionally, the selection of microbes for plant health has generally followed the *in vitro* screening approach, targeting the selection of well-known microbial taxa for the control of a specific pathogen or pest, or aiming at improving some plant phenotypic traits, such as plant growth, nitrogen fixation, and phytohormone production (De Souza et al. 2020; Glick 2012). In practice, these inoculants often fail to have the intended effect and may simply be unable to establish in agricultural environments (Besset-Manzoni et al. 2019; Zimmer et al. 2016). As an alternative, there can be a greater chance of success if we identify relevant microbes with key traits in each system. Plant-associated microbial communities are not assembled randomly. Instead, these communities have a defined phylogenetic organization structured by general community assembly rules (Carlström et al. 2019; Trivedi et al. 2020). Machine learning for identifying potential SynCom composition is a potentially powerful approach. The phytobiome is defined as a network of interactions between plants, their environment, and their associated micro- and macroorganisms (American Phytopathological Society 2016). Methods such as machine learning and network analysis can integrate analysis of microbes and plant phenotypes as a system for understanding how phytobiome structure influences crop health.

The position and identity of microbes in microbiome networks can indicate their importance within the microbial community, where their role in determining plant phenotypes can be either positive or negative (Fig. 2). For instance, highly interactive members of the core microbiome, known as “hub” microbes, may strongly shape the phytobiome communities (Muller et al. 2018; Trivedi et al. 2020) by promoting or suppressing the growth and diversity of other microbes within and across kingdoms (Agler et al. 2016). Hub microbes may play significant roles in plant health by suppressing pathogenic microbes and/or promoting the expression of disease-resistant genes (Poudel et al. 2016). Identifying these hub microbes in each plant species could be key to creating successful and effective SynComs (Toju et al. 2020). Identification of hub microbes, and other taxa with key effects, must involve multiple steps. First, associations across samples should be evaluated to see which taxa co-occur more or less often than would be expected by chance or are associated with desired plant phenotypes. Microbial associations may simply be due to shared or opposing preferences

for particular abiotic environmental conditions; however, additional tests are needed to determine whether there are actual interactions between species rather than simply different niches (Poudel et al. 2016). Likewise, taxa may be associated with abiotic conditions that favor plant health rather than having a causal relationship with plant health. Thus, an additional step is needed to understand causal relationships, testing plant phenotypes with and without combinations of specific microbial taxa. Successfully integrating studies of associations and confirmation of causal effects can usher in a new era of plant health enhanced by SynComs.

SynComs and Plant Biotic Stress Improvement

The potential of microbial VOCs in SynComs for improving plant health

Microorganisms generally occur in mixed-species communities in which biological diversity is accompanied by chemical diversity. The latter is partially composed of secondary metabolites produced by microorganisms. One specific type of such metabolites are VOCs that are often secreted as mediators for molecular communication or as antimicrobial compounds (Cernava et al. 2015; Weisskopf et al. 2021) (Fig. 2).

Microbe-produced VOCs comprise a broad spectrum of small molecules that can spread in the atmosphere at ambient temperatures (Schmidt et al. 2015). One of their key characteristics is that they can reach long distances not only in the air, but also in soil (Schulz-Bohm et al. 2018; Terra et al. 2018). This makes them highly efficient as mediators of intra- as well as interspecific communication that can even extend between kingdoms, such as bacterial–fungal or bacterial–plant interactions via volatiles (Martins et al. 2019; Schmidt et al. 2016; Weisskopf et al. 2021). An even more complex interplay was deciphered when tomato plants were inoculated with a specific *Bacillus amyloliquefaciens* strain (Kong et al. 2021). Plants that were inoculated with this bacterium released a specific VOC that was received by neighboring plants, resulting in a modulation of their rhizosphere microbiome. Other VOCs that exhibit strong antimicrobial effects on certain pathogens have become the focus of research due to their potential for biotechnological applications (Wiltshi et al. 2020). Pathogen suppressiveness in certain soils can be partially attributable to VOCs that are

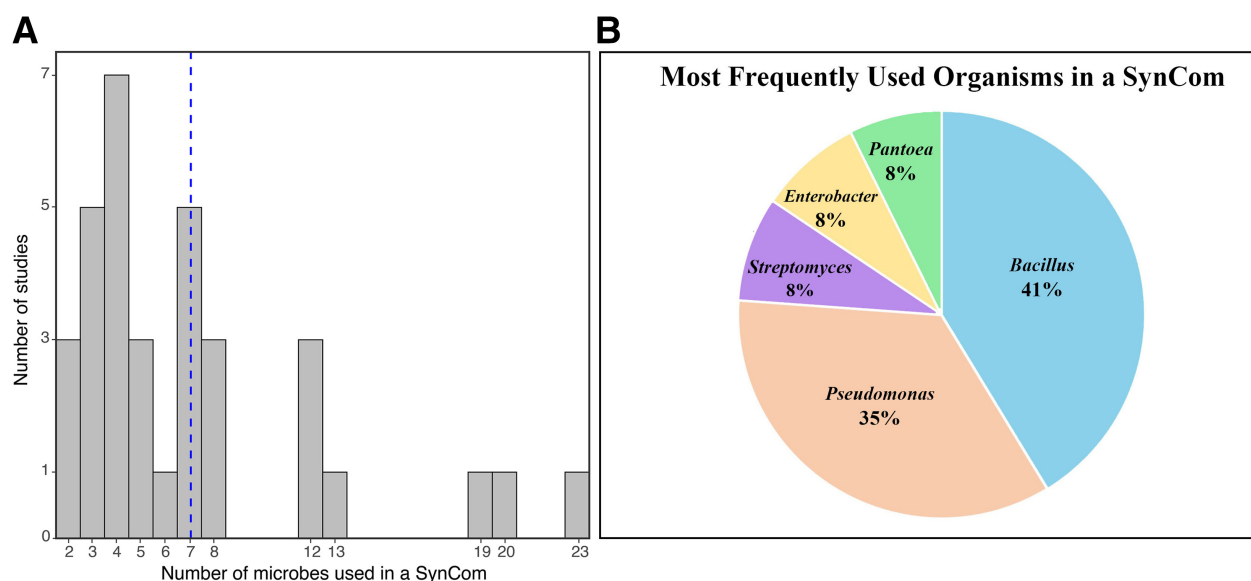


FIGURE 1

Number of microbes used in synthetic microbial communities (SynComs) when the authors used *in vivo* experiments ($n = 28$), excluding model plants. **A**, The blue dashed line represents the mean value. **B**, Top five bacterial genera used in an *in vivo* SynCom study, excluding model plants. The five most frequently used genera in 34 SynComs across 28 studies.

likely of microbial origin (Ossowicki et al. 2020). Unambiguous results were obtained in binary systems where specific pathogens were suppressed by the VOCs of isolated bacteria (Cernava et al. 2015; Mülner et al. 2019). In fact, many bacteria known for their high potential in plant protection, such as *Bacillus* spp. and *Pseudomonas* spp., were shown to produce bioactive VOCs that inhibit pathogen growth without direct contact (Asari et al. 2016; Mülner et al. 2019). When isolated and grown under laboratory conditions, these bacteria usually produce distinct mixtures of VOCs that are detectable by sampling the gas phase and subsequent analysis via gas chromatography-mass spectrometry (Cernava et al. 2015). A study by Mülner et al. (2019) demonstrated that specific mixtures of VOCs have a significantly stronger inhibitory effect on phytopathogenic fungi than the individual VOCs. What is even more interesting is that microorganisms will alter their VOC emission when exposed to volatiles of other bacteria or fungi. Rybakova et al. (2022) showed that VOC production in the beneficial bacterium *Serratia plymuthica* specifically responds to the presence of three different pathogenic fungi. This knowledge opens up new possibilities for the design of SynComs where the integrated strains mutually steer each other's VOC production in a certain direction. Such SynComs might not only be applicable for plant protection against pathogens and pests but also to improve plant growth (Türksoy et al. 2022). For the design of such SynComs, it is important not only to select

compatible strains but also to ensure that they are present in appropriate concentrations; otherwise, they may cause undesirable effects (Cordovez et al. 2018). It was previously observed that certain microbial VOCs positively affect plant growth at low concentrations but become detrimental after a certain threshold is reached (Cordovez et al. 2018; Song et al. 2022). The design of SynComs might also benefit from VOC producers with dual functions (i.e., that can inhibit the growth of pathogens while simultaneously promoting plant growth) (Asari et al. 2016). Overall, microbial VOCs can elicit various desirable effects in plant production, and their targeted activation in SynComs will be an important milestone.

Nonvolatile secondary metabolites

Microbial plant symbionts produce and secrete a large number of secondary metabolites (SMs) of diverse biosynthetic origins that play pivotal roles in interspecies interactions, from communication to direct antagonism. Knowledge about the diversity and functions of SMs produced by members of the plant microbiome has traditionally come from studies conducted with single strains isolated from specific niches, such as PGPR selected as antagonists of plant pathogens and used as biological control agents (Paulsen et al. 2005). In this context, the production and excretion of a few major groups of SMs by biological control agents inoculated and maintained at high cell densities result in direct pathogen suppression

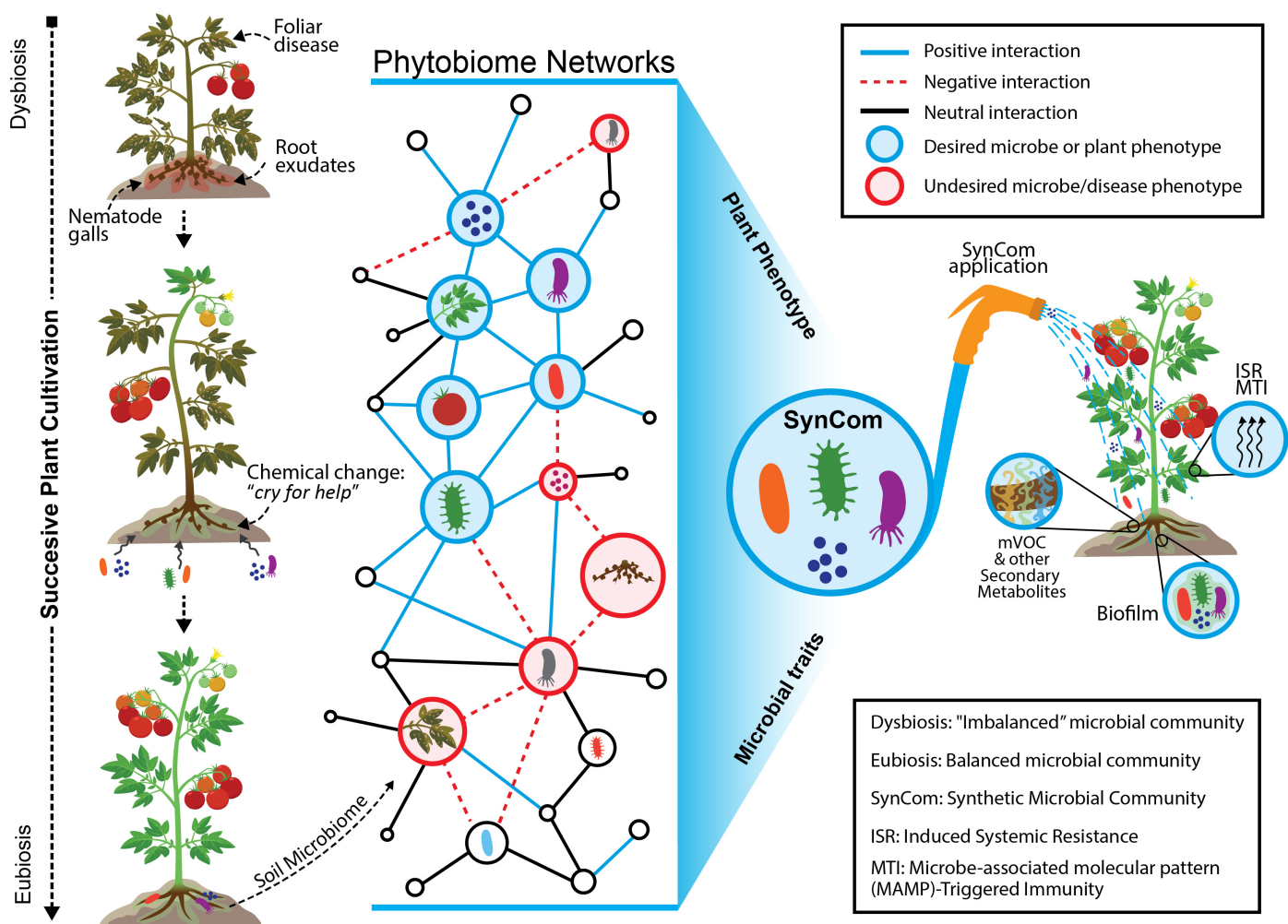


FIGURE 2

Roadmap to design a synthetic microbial community (SynCom) involving plant phenotypes and microbial traits. Characterizing plants across a range of levels of infection by different pathogens provides information about the network of microbial associations with each other and with specific plant phenotypes. Microbes with positive associations with desirable plant phenotypes, or microbes with negative associations with undesirable microbes or positive associations with desirable microbes, can be tested for potential inclusion in SynComs.

(antibiosis) or the activation of defense mechanisms in the host plant (induced systemic resistance) (Ongena et al. 2007; Raaijmakers et al. 2002).

Several species of *Bacillus* and members of the *Pseudomonas fluorescens* clade are dominant PGPR taxa that produce complex mixtures of SMs with a wide range of bioactivities toward prokaryotes and eukaryotes (Raaijmakers et al. 2010). Lipopeptides are amphiphilic molecules with surfactant properties that interact with lipid bilayers and cause damage to the cell membranes of susceptible organisms (Balleza et al. 2019). Plant-associated *Bacillus* produce antifungal and antibacterial cyclic lipopeptides of the iturin, fengycin, surfactin, and kurstatin biosynthetic families; these SMs are active against many plant pathogens and may also induce systemic disease resistance in some plants (Ongena and Jacques 2008). Fluorescent pseudomonads are also prolific producers of many linear and cyclic lipopeptides with antimicrobial activities (Zhao et al. 2019). Interestingly, lipopeptides produced by plant-pathogenic *Pseudomonas* can act as phytotoxins (Götze and Stallforth 2020). Phloroglucinols and phenazines are other well-known bioactive SM produced by fluorescent pseudomonads in the rhizosphere of wheat and other crops cultivated in soils that are suppressive to soilborne pathogens (Raaijmakers et al. 2002). A third group of prominent PGPR is composed of Actinobacteria, especially those of the genus *Streptomyces* that produce thousands of bioactive metabolites in their secondary metabolism, a feature that has traditionally attracted the attention of researchers looking for new pharmaceuticals (Bérdy 2012).

In natural microbial communities, complex interspecies interactions govern the biosynthesis and excretion of SMs through quorum-sensing control mechanisms (Chodkowski and Shade 2017). In this context, most SMs are present at subinhibitory concentrations in the medium, where they may participate in formation of biofilms, mediate cell mobility, or function as signaling molecules affecting quorum-sensing-dependent phenotypes, including the production of other SMs by different microbial species (Chevrette et al. 2022; Raaijmakers and Mazzola 2012). On the other hand, the accumulation of antifungal molecules such as 2,4 diacetylphloroglucinol and phenazines, produced by fluorescent pseudomonads, and several cyclic lipopeptides of *Bacillus* and *Pseudomonas* spp. in the rhizosphere of crops planted in naturally suppressive or artificially inoculated nonsterile soils provides evidence that major SMs produced by dominant PGPR can reach inhibitory concentrations in nature (Kinsella et al. 2009; Mavrodi et al. 2012; Nielsen and Sørensen 2003; Raaijmakers et al. 1999).

Recent microbiome-based studies have used culture-independent multi-omic approaches combined with dedicated databases and analysis pipelines to characterize the biosynthetic gene clusters (BGCs) responsible for biosynthesis of microbial SMs. These studies have uncovered a large number of microbial BGCs responsible for the biosynthesis of polyketides, nonribosomal peptides, terpenes, aryl polyenes, ribosomally synthesized and post-translationally modified peptides, and metabolites of mixed origin in the metagenomes of plant-associated microbes (Carrión et al. 2019; Dror et al. 2020; Tracanna et al. 2021). In one study involving the protection of sugar beet seedlings against damping-off caused by *R. solani* in a suppressive soil, metagenomic analyses of the root endosphere showed an expressive enrichment of bacteria-derived BGCs in the endophytic compartments of plants subjected to pathogen inoculation in the suppressive soil. Interestingly, only 12 out of 117 enriched BGCs could be linked to known bioactive SMs produced by PGPR (Carrión et al. 2019). In another study, partial sequencing of the adenylation domain of nonribosomal peptide synthetases and functional metagenomics were used to study the diversity of nonribosomal peptides in the rhizosphere microbiome of wheat planted in soils classified as suppressive or conducive to root rot caused by *Fusarium culmorum*. More than 50,000 unique domains were detected, and functional annotations showed that siderophores

and cyclic lipopeptides are enriched in the rhizosphere of plants in suppressive soils (Tracanna et al. 2021).

Collections of culturable bacteria representing the dominant taxa in the plant microbiome can be used to test the insights gained from culture-independent studies. Helfrich et al. (2018) conducted thousands of in vitro pairwise confrontations with dominant members of the phyllosphere microbiome of *Arabidopsis thaliana* and found that the most potent inhibitors belonged to the orders *Bacillales* and *Pseudomonadales*. A single strain of *Brevibacillus* was the top inhibitor in dual confrontations, and MALDI imaging mass spectrometry showed the accumulation of several SMs in the inhibition zones induced by this strain. The mixture of antibacterial SMs produced by this strain contained the cyclopeptides, streptocidins and marthiapeptide A, and the novel metabolites macrobrevin and phosphobrevin (Helfrich et al. 2018). In another study, the microbiome-informed isolation of fluorescent pseudomonads from the rhizosphere of sugarbeet cultivated in a suppressive soil inoculated with *R. solani* led to the discovery of a strain capable of protecting the plants against damping-off through the production of a chlorinated cyclic lipopeptide, later identified as a new metabolite, thanamycin (Mendes et al. 2011).

Synthetic communities containing key taxa selected from the plant microbiome can be used for studying more complex roles of SMs in interspecies interactions. A three-member SynCom was composed of *Bacillus cereus*, *Flavobacterium johnsoniae*, and *Pseudomonas koreensis*, three taxa that coexist in the rhizosphere of soybean and interact in distinct ways in vitro. In dual cultures, *P. koreensis* partially inhibits the growth of *F. johnsoniae*, but in tripartite interactions, *B. cereus* protects the latter (Lozano et al. 2019). The novel alkaloids koreenceines are produced by *P. koreensis* in co-cultures with the other members of the SynCom and induce extensive reprogramming of gene expression in both bacterial partners while also having direct antimicrobial effects on *F. johnsoniae* (Hurley et al. 2022). Meta-metabolomic analysis showed that several SMs are only produced in tripartite interactions, such as a biotransformation derivative of the cyclic lipopeptide lokisin produced by *P. koreensis*, whereas the relative concentration of several SMs of *B. cereus*, including siderophores and the antibiotic kanosamine, varied in response to the presence or absence of koreenceines in the medium (Chevrette et al. 2022).

The use of SynComs for studying the function of microbial SMs in pathogen suppression in situ was shown in the work of Carrión et al. (2019). Metagenome data provided evidence for the key role of BGCs from *Flavobacterium* and chitinases from *Chitinophaga* in the suppression of *R. solani* by the endophytic root microbiome of sugarbeet. Accordingly, a minimal two-strain SynCom composed of *Flavobacterium* and *Chitinophaga* isolates representing dominant haplotypes found in the root endosphere protected sugarbeet seedlings against damping-off. Gene expression analyses and site-directed mutagenesis confirmed the production of chitinases by *Chitinophaga* and showed that a specific BGC from *Flavobacterium*, responsible for the biosynthesis of a yet unknown nonribosomal peptide-polyketide hybrid, was crucial for maintaining the level of plant protection provided by SynComs.

The more in-depth understanding of the function of SMs produced by key members of the microbiome provided by (meta)genomic studies can directly inform the selection of microbial taxa for the composition of SynComs having plant protecting roles. Selection can favor strains that secrete antimicrobial SMs in sufficient amounts for pathogen inhibition while causing no major disruptions in the stability of the assembled communities. Attention should also be given to producers of SMs having more subtle roles in interspecies interactions taking place in the SynCom, including attenuated antagonism toward more competitive members and protection of less aggressive ones and induction of desirable community-level phenotypic expressions by SMs having quorum-sensing signaling roles.

Biofilms

Biofilms are microbial communities within an extracellular matrix, adhering to a fixed surface (Lakshmanan et al. 2012; Vlamakis et al. 2013). Bacterial biofilms can be found on plant surfaces, specifically on colonizing seeds through the release of seed exudates (Martins et al. 2018), on leaves (Beattie and Lindow 1999), and on roots (Lakshmanan et al. 2012). During biofilm formation, bacteria grow to form microbial colonies composed of one (homogenous) or multiple (heterogeneous) bacterial species. In heterogeneous microbial colony formation, different bacterial species can migrate or be enveloped into the microbial colony. This heterogeneity can enhance the survival and growth of the biofilm (Beattie and Lindow 1999). In the root, biofilm formation starts in the rhizosphere, where the bacteria feed on these root exudates, increasing their growth (Lakshmanan et al. 2012). Signaling from the plant triggers bacterial cells to express matrix genes and form a biofilm, adhering to the root surface via an extracellular matrix (Vlamakis et al. 2013). Compounds, such as surfactin, activate kinase C and kinase D, which, in return, both influence the activity of the *Spo0A-P* pathway that controls multiple genes involved in biofilm formation (Vlamakis et al. 2013). Other kinases and external signals influencing biofilm formation can be produced by plants and other soil microorganisms (Vlamakis et al. 2013). Signaling between microbes of different species in a singular biofilm may have enhanced benefits. For example, microbe-microbe enhancements include increased biofilm formation, colonization, and synergism (Niu et al. 2020). *Bacillus* species present in soil positively influenced biofilm formation and growth of *Bacillus subtilis* by activating kinase D (Shank et al. 2011). The exact mechanisms of microbe-microbe enhancement within biofilms are still unknown (Niu et al. 2020).

During the formation of biofilms, small autoinducer molecules regulate quorum sensing, most commonly Acyl-homoserine lactones (AHLs) N-acyl homoserine lactones. A certain level of AHLs is produced by bacteria until the bacterial population grows to a spe-

cific threshold. Then, AHLs bind to global transcription regulators, LuxR or LuxR-like proteins, to trigger quorum-sensing-controlled gene activity, including virulence factors involved in bacterial colonization (Lakshmanan et al. 2012). However, our knowledge of root regions that trigger or deter biofilm formation in PGPR is limited. Conventionally, it is speculated that the root tip, a highly metabolically active site, may secrete the majority of exudates, leading to increased microbial association. The temporal secretion patterns within the specific region of roots are not fully understood (Fig. 3).

The method of microbial inoculation has been found to influence the site of root bacterial colonization. Bacteria concentration increases closer to the point of inoculation for plants inoculated as seeds. Generally, bacterial colonization increases in root locations where root exudates are released, including at the root elongation zone and on root hairs (Hassan et al. 2019). The factor of root surface roughness influences colonization in that more bacteria colonize roots where the root surface is rougher (Dandurand et al. 1997; Knights et al. 2021). Time is also a factor that influences bacterial distribution, as the location of root bacterial colonization changes with time (Benizri et al. 2001). Factors contributing to the location of bacterial colonization on roots should be further investigated.

Biofilm quantification. Biofilms can be harvested from soil and observed using the buried slide technique. The technique involves placing a sterile slide in soil for 1 to 3 weeks, then removing the slide and staining it to observe the soil microbial community. However, the method is not ideally representative of the microbial distributions within the soil because the microscope slide is a new substrate that alters the composition of microbial species in comparison with the soil populations (Cunningham et al. 2011). Quantification of biofilms in the rhizosphere involves marking bacteria with fluorescent proteins and quantifying the number of bacteria via flow cytometry (Knights et al. 2021). Eight fluorescent proteins ranging from blue to far-red can tag a range of bacterial species to study biofilms composed of multiple bacterial species (Schlechter et al. 2018).

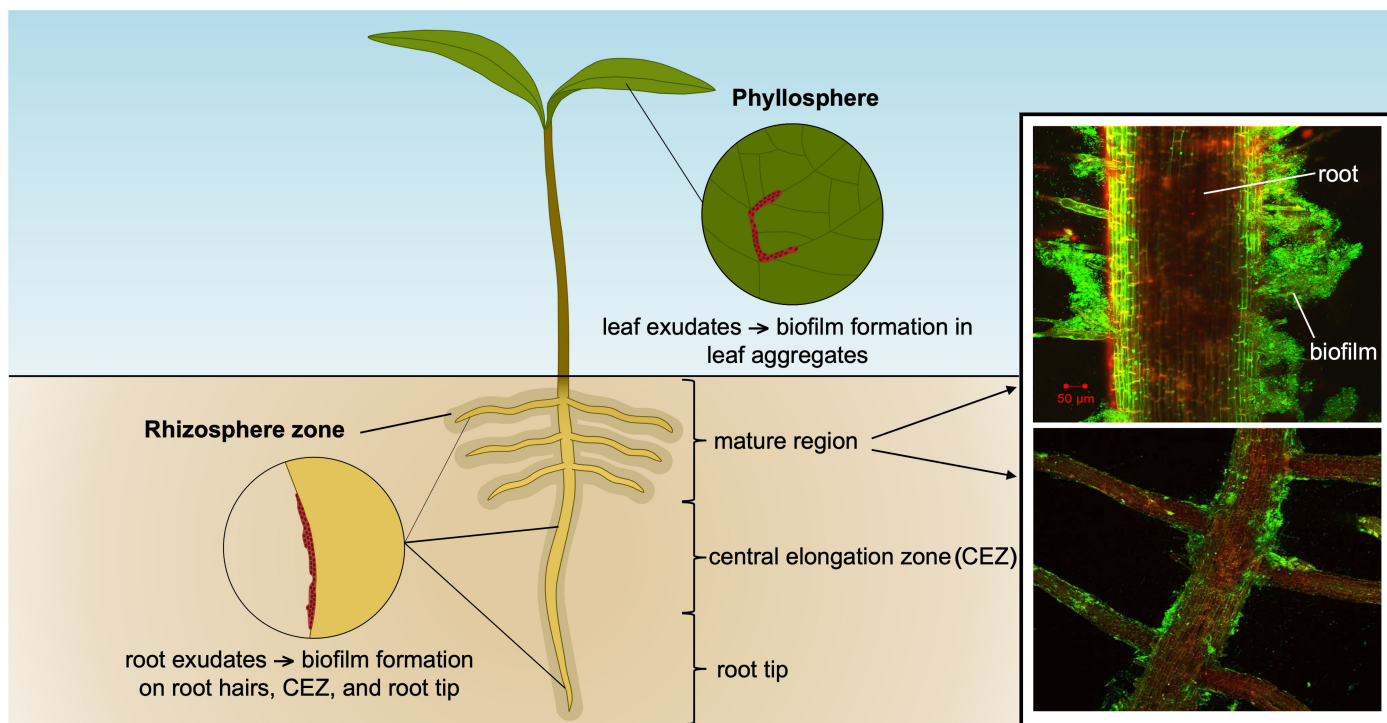


FIGURE 3

Root and leaf exudates trigger microcolonies and biofilm formation by plant growth promoting rhizobacteria (PGPR). The biofilm formation in roots is more prominent compared to the phyllosphere regions. The root regions such as tip, CEZ, and maturation zones may facilitate biofilm formation based on secretion patterns and profiles. The confocal micrographs in the inset show root colonization by PGPR (in green) in different crop species localized in the maturation root region.

Plant resistance mechanisms triggered by SynComs

To cope with pathogenic microbes, plants developed sophisticated defense mechanisms. As a first line of defense, these resistance mechanisms depend on the perception of microbe-derived compounds called microbe-associated molecular patterns (MAMPs). Examples of MAMPs include bacterial flagellin, bacterial peptidoglycan, fungal chitin, and oomycetal β -glucans (Gómez-Gómez and Boller 2000; Miya et al. 2007; Tyler 2002). These MAMPs are recognized by the plant's pattern recognition receptors, which trigger a cascade of reactions that lead to transcriptional reprogramming and activation of the plant's first line of defense, called MAMP-triggered immunity (MTI) (Boller and Felix 2009; Galletti et al. 2011; Jones and Dangl 2006; Newman et al. 2013). As the plant is putting a substantial amount of energy into its defense systems, a prolonged state of MTI can lead to growth inhibition due to growth-defense trade-offs (Gómez-Gómez et al. 1999; Zipfel et al. 2006). For that reason, it is vitally important for the plant to be selective about activating its defense systems against microbes that can activate MTI.

SynComs and the plant immune system. MAMPs are evolutionarily conserved across the bacterial, fungal, and oomycetal kingdoms. Commensal and beneficial microbes in the plant microbiome possess MAMPs similar to those of their pathogenic counterparts. It is therefore highly unlikely that SynComs consisting of commensal and/or beneficial microbes will not activate the plant's defense systems. In fact, in many SynCom studies, the bacterial strains used did not cause disease themselves. When the SynCom-treated plants were subsequently challenged with a pathogen, they actually developed fewer disease symptoms than nontreated control plants (Berendsen et al. 2018; Berg and Koskella 2018; Carrión et al. 2019; Durán et al. 2018, 2021; Gómez-Pérez et al. 2022; Hu et al. 2016; Li et al. 2021; Liu et al. 2022; Ma et al. 2021; Niu et al. 2017; Prigigallo et al. 2022; Vogel et al. 2021), either through direct competition with the pathogen or via the onset of a plant-mediated induced systemic resistance (ISR) or systemic acquired resistance (De Kesel et al. 2021; Pieterse et al. 2014). Phenotypic data from these studies revealed that in the absence of the pathogen, the SynComs either had no effect (Ma et al. 2021; Niu et al. 2017) or significantly increased plant growth (Berendsen et al. 2018; Durán et al. 2018; Liu et al. 2022), suggesting that the SynComs are able to circumvent growth-defense tradeoffs that result from the activation of plant defenses. So, how do plants accommodate plant growth-promoting microbes, such as those provided in a SynCom, while at the same time being able to ward off pathogens? To answer this question, it is important to investigate the role of different plant defense systems, such as MTI, ISR, and systemic-acquired resistance, during plant interactions with plant-beneficial SynComs.

SynComs and evasion of local host immunity. Flagellin is one of the major MAMPs in bacterial communities. Treating wildtype *Arabidopsis* plants with the immunogenic flagellin epitope flg22 of pathogenic *Pseudomonas aeruginosa* PO1 or from beneficial *Pseudomonas simiae* WCS417 leads to highly similar root defense transcriptome changes and suppression of plant growth (Stringlis et al. 2018a). This suggests that the initial response of plant roots to MAMPs of beneficial and pathogenic microbes is highly similar. However, live beneficial rhizobacteria have been shown to actively suppress this flg22-induced root MTI response (Yu et al. 2019a). Yu et al. (2019b) showed that of the tested root microbiota, 42% were able to quench local *Arabidopsis* root immune responses that are triggered by flg22. This shows that suppression of local MTI is an important function of the root microbiome, possibly to accommodate colonization by beneficial microbiota. An flg22-induced root MTI response can lead to a growth-defense tradeoff phenomenon called root growth inhibition (RGI) (Garrido-Oter et al. 2018; Gómez-Gómez et al. 1999; Huot et al. 2014). Interestingly, Teixeira et al. (2021) demonstrated in *Arabidopsis* that a 35-member SynCom was able to revert flg22-induced RGI. Additionally, a mono-association study in which 151 rhizobacterial strains were

individually inoculated on *Arabidopsis* roots indicated that roughly 40% of this selection of bacterial strains have the ability to suppress RGI (Ma et al. 2021). The mechanisms by which RGI can be suppressed can be multiple. Yu et al. (2019b) demonstrated that lowering environmental pH through the production of gluconic acid by the *Pseudomonas* rhizobacteria plays a role in the suppression of flg22-triggered MTI. Additionally, two studies found *Variovorax* and *Bradyrhizobium* species to be able to revert RGI by degrading auxin in the root compartment (Conway et al. 2022; Finkel et al. 2020). Another study found that two *Janibacter* species avoid RGI by degrading flg22 (Ma et al. 2021). In particular, bacteria from the *Variovorax* genus seem to be good RGI suppressors, as they were able to revert RGI in multiple studies (Finkel et al. 2020; Qi et al. 2022; Teixeira et al. 2021).

SynComs and dysbiosis

The beneficial effects that a SynCom can have on the plant are much more evident when growth inhibition responses such as RGI are reverted by the SynCom. So, what is the role of MTI in plant interactions with complex microbial communities when it seems to stand in the way of beneficial functions of the microbiome? A SynCom study with the peptidoglycan receptor mutant *bak1 bkk1*, the flagellin-chitin receptor mutant *efrfls2 cerk1*, and the chitin receptor mutant *lyk5* indicated that a nonfunctional MTI response leads to a higher amount of bacterial and fungal cells in the rhizosphere (Wolinska et al. 2021). Similar MAMP receptor mutants, as well as the MTI response mutant *rbohD*, which is impaired in the production of reactive oxygen species (ROS), displayed a phenomenon called "dysbiosis" in the phyllosphere or rhizosphere (Chen et al. 2020; Pfeilmeier et al. 2021; Wolinska et al. 2021). Dysbiosis is a state of imbalance in the microbial community that can have adverse effects on the plant, such as the loss of disease resistance mediated by the bacterial community or the increase in opportunistic pathogens that can subsequently harm the plant (Pfeilmeier et al. 2021). Additionally, *Arabidopsis* mutants impaired in the response to the defense hormones salicylic acid, jasmonic acid, or ethylene displayed a significant shift in microbial community composition, in both a natural microbiome setting and a SynCom setting (Bodenhausen et al. 2014; Lebeis et al. 2015). Hence, besides its function in the first line of defense against pathogens, MTI and its associated defense responses also seem to be important to prevent dysbiosis in plant-associated microbial communities, as dysbiosis can possibly lead to resilient beneficial communities turning into consortia that can easily be invaded by pathogenic microbes or that can cause disease in the plants themselves.

SynComs and plant-derived metabolites. One way to keep order in the root and leaf microbiome is by the production of antimicrobial compounds such as ROS, phytoalexins, or indole glucosinolates (Favaron et al. 2009; Ferrari et al. 2007; Pascale et al. 2020; Tsuji et al. 1992; Voges et al. 2019; Wolinska et al. 2021). These compounds are known to be released to repress pathogens, although it is likely that they can also affect beneficial and commensal members of the plant microbiome and its representatives in SynComs. Expectedly, plant-associated microbial communities have developed mechanisms to cope with these antimicrobial compounds. For instance, Stringlis et al. (2018b) demonstrated that antimicrobial coumarin production in plant roots can inhibit fungal pathogens, such as *Fusarium oxysporum* f. sp. *raphani* and *Verticillium dahliae* JR2, whereas ISR-inducing rhizobacterial strains *P. simiae* WCS417 and *Pseudomonas capeferrum* WCS358 were insensitive to the antimicrobial effect of coumarins. In an in vitro screen that included *P. simiae* WCS417, Voges et al. (2019) showed that the majority of SynCom members across bacterial families were unaffected by the catecholic coumarins sideretin, fraxetin, and esculetin, which generate ROS compounds in iron-deficient conditions. Interestingly, the bacterial strains that were sensitive to these coumarins were also found in high abundance on plant roots that

were grown under iron-deficient conditions during which coumarins secretion is typically enhanced (Voges et al. 2019). A similar study focusing on the release of antimicrobial benzoxazinoids (BXs) by wheat and maize showed that Beta- and Gammaproteobacteria are largely resistant to these compounds (Schandry et al. 2021). When tested in a SynCom, these BX-resistant Beta- and Gammaproteobacteria were able to make the whole community, which also included BX-susceptible members, resistant against BXs. These findings suggest that microbes with resistance against certain antimicrobial compounds might be able to confer the whole microbial community resistance against these compounds.

SynComs and plant protection against pathogens. SynComs of well-characterized bacterial or fungal communities are used to empirically test functions of much more complex microbial communities in the root or phyllosphere microbiome (Vorholt et al. 2017). Mono-association and small SynCom studies show that for a SynCom to have a disease-resistance-enhancing effect, the level of complexity can be modest, as only one or a few strains can be enough to enhance disease resistance in a plant host against certain pathogens (Berendsen et al. 2018; Berg and Koskella 2018; Carrión et al. 2019; Li et al. 2021; Liu et al. 2022; Niu et al. 2017; Prigallo et al. 2022). How does that hold up when increasing the complexity of SynComs? Only a few studies have investigated the ability of SynCom members to provide pathogen protection in the context of increasing SynCom complexity. For instance, in a potato study, increasing SynCom complexity had stronger effects on the level of resistance against *Phytophthora infestans* (De Vrieze et al. 2018). In another study, SynComs consisting of bacterial members versus multi-kingdom members were compared in terms of circumventing growth-defense tradeoffs when the plant was grown under different light intensities (Hou et al. 2021). In this study, a multi-kingdom SynCom consisting of bacteria, fungi, and oomycetes was able to prevent the growth-defense tradeoffs that were apparent when plants under low light were grown in the absence of microbes or exposed to bacterial SynComs (Hou et al. 2021). When bacteria are left out of the multi-kingdom SynCom it can, however, lead to severe dysbiosis (Durán et al. 2018), indicating that bacterial community members are important for maintaining a healthy microbiome.

In conclusion, SynCom research has made evident that microbial communities have developed evasion strategies to avoid immune responses that the plant has evolved to cope with pathogens. The interplay between the plant's defense mechanisms and the microbial communities in its various compartments is necessary, however, as it avoids dysbiosis and allows the plant to maximally benefit from PGPR in its associated microbiome.

The potential in SynComs for improving plants' tolerance to abiotic stress

When exposed to pathogen infection, plants promote signaling in the rhizosphere to attract beneficial microbes. These microbes protect the plants against biotic stressors and improve tolerance to abiotic stressors, such as salinity, drought, and temperature stresses (Martins et al. 2014; Trivedi et al. 2020). To mitigate salinity stress in the rhizosphere, exopolysaccharides (EPS) produced by microbes for biofilm formation and adherence can bind cations such as Na⁺. EPS binds to Na⁺ and makes Na⁺ unavailable to plants, thus reducing Na⁺ plant uptake, which helps to maintain the K⁺/Na⁺ balance in plant roots under salinity stress (Gupta et al. 2017; Morcillo and Manzanera 2021). Additionally, biofilms improve salinity tolerance by forming a layer of water around bacterial cells, which improves bacterial adhesion to the plant root and mitigates salinity stress. EPS also encourages plant growth under salinity stress by improving soil aggregation. However, the influence of EPS compositional change and physical and chemical properties on plant salinity stress is still largely unknown (Morcillo and Manzanera 2021). Bacterial biofilm formation has also been found to improve drought tolerance (Martins et al. 2018). Under drought stress, bacterial EPS in the rhi-

zosphere decrease plant water loss and increase bacterial survival due to their increased water content (Roberson and Firestone 1992). This increased water content also provides plants with water in the rhizosphere, allowing more time for plants to adjust their metabolic activity to drought conditions (Morcillo and Manzanera 2021). EPS also mitigates drought stress by improving the soil structure to retain more water in the soil (Zheng et al. 2018), although the exact influence of EPS on plant physiology is not well understood (Morcillo and Manzanera 2021).

EPS-producing bacteria in the rhizosphere may also mitigate heat and cold stress, likely due to their matrix formation that also improves soil water content. EPS also produces heat shock-related proteins that protect microbes by mitigating the heat shock. However, few studies have investigated the mitigation of heat stress and heat shock by EPS-producing bacteria. More research is needed on EPS improvement of plant heat stress tolerance. Additionally, in colder temperatures, EPS binding of Na⁺ cations can improve osmotic balance in cold-stressed plants (Morcillo and Manzanera 2021).

Final considerations

In conclusion, cultivation-independent methods, more readily available due to reduced sequencing costs, have considerably improved the overall understanding of microbial ecology in the plant environment, making it possible to engineer SynComs for plant health. Using machine learning, with plant and microbial trait phenotypes as input, can generate candidate compositions for SynComs. The number of distinct microbes in the SynComs is still a practical challenge to overcome due to a lack of industrial technologies and difficulties in handling the microbes (Shayanthan et al. 2022). Moreover, although a single SynCom can provide plant resilience to multiple pathogens (Santhanam et al. 2015), it is unrealistic to think that one SynCom will act as a "one size fits all." For a scenario that is more likely to be successful, SynComs should contain microbes with multiple beneficial traits as discussed in this review (robust biofilm formation, production of desired secondary metabolites and mVOCs capable of inducing plant resistance) and possess synergistic interactions among themselves.

Acknowledgments

We thank UF/IFAS Communications and the Graphic Designer Heather Griffith for designing Figure 2.

Literature Cited

- Agler, M. T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D., and Kemen, E. M. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol.* 14:e1002352.
- American Phytopathological Society. 2016. *Phytobiomes: A roadmap for research and translation*. American Phytopathological Society, St. Paul, MN. <http://www.phytobiomes.org/Roadmap/Documents/PhytobiomesRoadmap.pdf>
- Asari, S., Matzén, S., Petersen, M. A., Bejai, S., and Meijer, J. 2016. Multiple effects of *Bacillus amyloliquefaciens* volatile compounds: Plant growth promotion and growth inhibition of phytopathogens. *FEMS Microbiol. Ecol.* 92:fiw070.
- Balleza, D., Alessandrini, A., and Beltrán García, M. J. 2019. Role of lipid composition, physicochemical interactions, and membrane mechanics in the molecular actions of microbial cyclic lipopeptides. *J. Membr. Biol.* 252:131-157.
- Beattie, G. A., and Lindow, S. E. 1999. Bacterial colonization of leaves: A spectrum of strategies. *Phytopathology* 89:353-359.
- Benizri, E., Baudoin, E., and Guckert, A. 2001. Root colonization by inoculated plant growth-promoting rhizobacteria. *Biocontrol Sci. Technol.* 11:557-574.
- Bérdy, J. 2012. Thoughts and facts about antibiotics: Where we are now and where we are heading. *J. Antibiot. (Tokyo)* 65:385-395.
- Berendsen, R. L., Vismans, G., Yu, K., Song, Y., de Jonge, R., Burgman, W. P., Burmølle, M., Herschend, J., Bakker, P. A. H. M., and Pieterse, C. M. J. 2018. Disease-induced assemblage of a plant-beneficial bacterial consortium. *ISME J.* 12:1496-1507.

- Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Coccolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ryan, M., Sarand, I., Smidt, H., Schelkle, B., Roume, H., Kiran, G. S., Selvin, J., Souza, R. S. C. D., van Overbeek, L., Singh, B. K., Wagner, M., Walsh, A., Sessitsch, A., and Schloter, M. 2020. Microbiome definition re-visited: Old concepts and new challenges. *Microbiome* 8:103.
- Berg, M., and Koskella, B. 2018. Nutrient- and dose-dependent microbiome-mediated protection against a plant pathogen. *Curr. Biol.* 28:2487.
- Bessey-Manzoni, Y., Joly, P., Brutel, A., Gerin, F., Soudiere, O., Langin, T., and Prigent-Combaret, C. 2019. Does in vitro selection of biocontrol agents guarantee success in planta? A study case of wheat protection against *Fusarium* seedling blight by soil bacteria. *PLoS One* 14:e0225655.
- Bodenhausen, N., Bortfeld-Miller, M., Ackermann, M., and Vorholt, J. A. 2014. A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genet.* 10:e1004283.
- Boller, T., and Felix, G. 2009. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60:379-406.
- Broadbent, P., Baker, K. F., and Waterworth, Y. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. *Aust. J. Biol. Sci.* 24:925-944.
- Carlström, C. I., Field, C. M., Bortfeld-Miller, M., Müller, B., Sunagawa, S., and Vorholt, J. A. 2019. Synthetic microbiota reveal priority effects and keystone strains in the *Arabidopsis* phyllosphere. *Nat. Ecol. Evol.* 3:1445-1454.
- Carrión, V. J., Perez-Jaramillo, J., Cordovez, V., Tracanna, V., de Hollander, M., Ruiz-Buck, D., Mendes, L. W., van Ijcken, W. F. J., Gomez-Exposito, R., Elsayed, S. S., Mohanraju, P., Arifah, A., van der Oost, J., Paulson, J. N., Mendes, R., van Wezel, G. P., Medema, M. H., and Raaijmakers, J. M. 2019. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* 366:606.
- Cernava, T., Aschenbrenner, I. A., Grube, M., Liebming, S., and Berg, G. 2015. A novel assay for the detection of bioactive volatiles evaluated by screening of lichen-associated bacteria. *Front. Microbiol.* 6:398.
- Chen, T., Nomura, K., Wang, X., Sohrabi, R., Xu, J., Yao, L., Paasch, B. C., Ma, L., Kremer, J., Cheng, Y., Zhang, L., Wang, N., Wang, E., Xin, X.-F., and He, S. Y. 2020. A plant genetic network for preventing dysbiosis in the phyllosphere. *Nature* 580:653.
- Chevrette, M. G., Thomas, C. S., Hurley, A., Rosario-Meléndez, N., Sankaran, K., Tu, Y., Hall, A., Magesh, S., and Handelsman, J. 2022. Microbiome composition modulates secondary metabolism in a multispecies bacterial community. *Proc. Natl. Acad. Sci.* 119:e2212930119.
- Chodkowskij, J. L., and Shade, A. 2017. A synthetic community system for probing microbial interactions driven by exometabolites. *mSystems* 2:e00129-17.
- Conway, J. M., Walton, W. G., Salas-González, I., Law, T. F., Lindberg, C. A., Crook, L. E., Kosina, S. M., Fitzpatrick, C. R., Lietzan, A. D., Northen, T. R., Jones, C. D., Finkel, O. M., Redinbo, M. R., and Dangl, J. L. 2022. Diverse MarR bacterial regulators of auxin catabolism in the plant microbiome. *Nat. Microbiol.* 7:1817.
- Cordovez, V., Schop, S., Hordijk, K., Dupré de Boulois, H., Coppens, F., Hanssen, I., Raaijmakers, J. M., and Carrión, V. J. 2018. Priming of plant growth promotion by volatiles of root-associated *Microbacterium* spp. *Appl. Environ. Microbiol.* 84:e01865-18.
- Cunningham, A. B., Lennox, J. E., and Ross, R. J. 2011. Collecting soil biofilms by the buried slide technique—instructions for students. *Biofilms: The Hypertextbook*. <http://www.hypertextbookshop.com/biofilmbook/v004/r003/>
- Dandurand, L. M., Schotzko, D. J., and Knudsen, G. R. 1997. Spatial patterns of rhizoplane populations of *Pseudomonas fluorescens*. *Appl. Environ. Microbiol.* 63:3211-3217.
- De Kesel, J., Conrath, U., Flors, V., Luna, E., Mageroy, M. H., Mauch-Mani, B., Pastor, V., Pozo, M. J., Pieterse, C. M. J., Ton, J., and Kynndt, T. 2021. The induced resistance lexicon: Do's and don'ts. *Trends Plant Sci.* 26:685-691.
- De Souza, R. S. C., Armanhi, J. S. L., and Arruda, P. 2020. From microbiome to traits: Designing synthetic microbial communities for improved crop resiliency. *Front. Plant Sci.* 11:1179.
- De Vrieze, M., Germanier, F., Vuille, N., and Weisskopf, L. 2018. Combining different potato-associated *Pseudomonas* strains for improved biocontrol of *Phytophthora infestans*. *Front. Microbiol.* 9:2573.
- Dror, B., Wang, Z., Brady, S. F., Jurkevitch, E., and Cytryn, E. 2020. Elucidating the diversity and potential function of nonribosomal peptide and polyketide biosynthetic gene clusters in the root microbiome. *mSystems* 5:e00866-20.
- Durán, P., Reinstädler, A., Rajakrut, A. L., Hashimoto, M., Garrido-Oter, R., Schulze-Lefert, P., and Panstruga, R. 2021. A fungal powdery mildew pathogen induces extensive local and marginal systemic changes in the *Arabidopsis thaliana* microbiota. *Environ. Microbiol.* 23:6292-6308.
- Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., and Hacquard, S. 2018. Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell* 175:973.
- Elhady, A., Adss, S., Hallmann, J., and Heuer, H. 2018. Rhizosphere microbiomes modulated by pre-crops assisted plants in defense against plant-parasitic nematodes. *Front. Microbiol.* 9:1133.
- Erdmann, J. 2022. How gut bacteria could boost cancer treatments. *Nature* 607:436-439.
- Favaron, F., Lucchetta, M., Odorizzi, S., Pais da Cunha, A. T., and Sella, L. 2009. The role of grape polyphenols on trans-resveratrol activity against *Botrytis cinerea* and of fungal laccase on the solubility of putative grape PR proteins. *J. Plant Pathol.* 91:579-588.
- Ferrari, S., Galletti, R., Denoux, C., de Lorenzo, G., Ausubel, F. M., and Dewdney, J. 2007. Resistance to *Botrytis cinerea* induced in *Arabidopsis* by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires *PHYTOALEXIN DEFICIENT3*. *Plant Physiol.* 144:367-379.
- Finkel, O. M., Salas-González, I., Castrillo, G., Conway, J. M., Law, T. F., Teixeira, P. J. P. L., Wilson, E. D., Fitzpatrick, C. R., Jones, C. D., and Dangl, J. L. 2020. A single bacterial genus maintains root growth in a complex microbiome. *Nature* 587:103.
- Galletti, R., Ferrari, S., and de Lorenzo, G. 2011. *Arabidopsis* MPK3 and MPK6 play different roles in basal and oligogalacturonide- or flagellin-induced resistance against *Botrytis cinerea*. *Plant Physiol.* 157:804-814.
- Garrido-Oter, R., Nakano, R. T., Dombrowski, N., Ma, K. W., McHardy, A. C., and Schulze-Lefert, P. 2018. Modular traits of the Rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host Microbe* 24:155.
- Glick, B. R. 2012. Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica* 2012:963401.
- Gómez-Gómez, L., and Boller, T. 2000. FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol. Cell.* 5:1003-1011.
- Gómez-Gómez, L., Felix, G., and Boller, T. 1999. A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. *Plant J.* 18:277-284.
- Gómez-Pérez, D., Schmid, M., Chaudhry, V., Velic, A., Maček, B., and Kemen, A. 2022. Proteins released into the plant apoplast by the obligate parasitic protist *Albugo* selectively repress phyllosphere-associated bacteria. *bioRxiv* 492175.
- Götze, S., and Stallforth, P. 2020. Structure, properties, and biological functions of nonribosomal lipopeptides from pseudomonads. *Nat. Prod. Rep.* 37:29-54.
- Gupta, G., Snehi, S. K., and Singh, V. 2017. Role of PGPR in biofilm formations and its importance in plant health. *Biofilms Plant Soil Health* 27:27-40.
- Hassan, M. K., McInroy, J. A., and Kloepper, J. W. 2019. The interactions of rhizodeposits with plant growth-promoting rhizobacteria in the rhizosphere: A review. *Agriculture* 9:142.
- Helfrich, E. J., Vogel, C. M., Ueoka, R., Schäfer, M., Ryyffel, F., Müller, D. B., Probst, S., Kreuzer, M., Piel, J., and Vorholt, J. A. 2018. Bipartite interactions, antibiotic production and biosynthetic potential of the *Arabidopsis* leaf microbiome. *Nat. Microbiol.* 3:909-919.
- Hou, S., Thiergart, T., Vannier, N., Mesny, F., Ziegler, J., Pickel, B., and Hacquard, S. 2021. A microbiota-root-shoot circuit favours *Arabidopsis* growth under defence under suboptimal light. *Nat. Plants* 7:1078.
- Hu, J., Wei, Z., Friman, V.-P., Gu, S.-H., Wang, X.-F., Eisenhauer, N., Yang, T.-J., Ma, J., Shen, Q.-R., Xu, Y.-C., and Jousset, A. 2016. Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. *mBio* 7.
- Huot, B., Yao, J., Montgomery, B. L., and He, S. Y. 2014. Growth-defense tradeoffs in plants: A balancing act to optimize fitness. *Mol. Plant.* 7:1267-1287.
- Hurley, A., Chevrette, M. G., Rosario-Meléndez, N., and Handelsman, J. 2022. THOR's hammer: The antibiotic koreneceine drives gene expression in a model microbial community. *mBio* 13.
- Jiang, G., Zhang, Y., Gan, G., Li, W., Wan, W., Jiang, Y., Yang, T., Zhang, Y., Xu, Y., Wang, Y., Shen, Q., Wei, Z., and Dini-Andreote, F. 2022. Exploring rhizo-microbiome transplants as a tool for protective plant-microbiome manipulation. *ISME Commun.* 2:1-10.
- Jones, J. D. G., and Dangl, J. L. 2006. The plant immune system. *Nature* 444:323-329.
- Kinsella, K., Schulthess, C. P., Morris, T. F., and Stuart, J. D. 2009. Rapid quantification of *Bacillus subtilis* antibiotics in the rhizosphere. *Soil Biol. Biochem.* 41:374-379.
- Kloepper, J. W., and Schroth, M. N. 1978. Plant growth-promoting rhizobacteria on radishes. Pages 879-882 in: *Proc. of the 4th Internet. Conf. on Plant Pathogenic Bacter.* Station de Pathologie Vegetale et Phytobacteriologie, INRA, Angers, France.
- Knights, H. E., Jorin, B., Haskett, T. L., and Poole, P. S. 2021. Deciphering bacterial mechanisms of root colonization. *Environ. Microbiol. Rpt.* 13:428-444.
- Kong, H. G., Song, G. C., Sim, H. J., and Ryu, C. M. 2021. Achieving similar root microbiota composition in neighbouring plants through airborne signalling. *ISME J.* 15:397-408.

- Lakshmanan, V., Kumar, A. S., and Bais, H. P. 2012. The Ecological Significance of Plant-Associated Biofilms. *Microbial Biofilms: Current Research and Applications*. Caister Academic Press, Norfolk, U.K.
- Lebeis, S. L., Paredes, S. H., Lundberg, D. S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., Glavina del Rio, T., Jones, C. D., Tringe, S. G., and Dangl, J. L. 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349:860-864.
- Li, Z. F., Bai, X. L., Jiao, S., Li, Y. M., Li, P. R., Yang, Y., Zhang, H., and Wei, G. 2021. A simplified synthetic community rescues *Astragalus mongholicus* from root rot disease by activating plant-induced systemic resistance. *Microbiome* 9.
- Liu, H., Qiu, Z., Ye, J., Verma, J. P., Li, J., and Singh, B. K. 2022. Effective colonisation by a bacterial synthetic community promotes plant growth and alters soil microbial community. *J. Sustain. Agric. Environ.* 1:30-42.
- Lozano, G. L., Bravo, J. I., Garavito Diago, M. F., Park, H. B., Hurley, A., Peterson, S. B., Stabb, E. V., Crawford, J. M., and Handelsman, J. 2019. Introducing THOR, a model microbiome for genetic dissection of community behavior. *mBio* 10:e02846-18.
- Ma, K.-W., Niu, Y., Jia, Y., Ordon, J., Copeland, C., Emonet, A., Geldner, N., Guan, R., Stolze, S. C., Nakagami, H., Garrido-Oter, R., and Schulze-Lefert, P. 2021. Coordination of microbe-host homeostasis by crosstalk with plant innate immunity. *Nat. Plants* 7:814.
- Martins, S. J., Faria, A. F., Pedrosa, M. P., Cunha, M. G., Rocha, M. R., and Medeiros, F. H. V. 2019. Microbial volatiles organic compounds control anthracnose (*Colletotrichum lindemuthianum*) in common bean (*Phaseolus vulgaris* L.). *Biol. Control* 131:36-42.
- Martins, S. J., Medeiros, F. H. V., Lakshmanan, V., and Bais, H. P. 2018. Impact of seed exudates on growth and biofilm formation of *Bacillus amyloliquefaciens* ALB629 in common bean. *Front. Microbiol.* 8:1-9.
- Martins, S. J., Medeiros, F. H. V., Souza, R. M., Faria, A. F., Cancellier, E. L., Silveira, H. R. O., Rezende, M. L. V., and Guilherme, L. R. G. 2015. Common bean growth and health promoted by rhizobacteria and the contribution of magnesium to the observed responses. *Appl. Soil Ecol.* 87:49-55.
- Martins, S. J., Medeiros, F. H. V., Souza, R. M., and Vilela, L. A. F. 2014. Is Curvobacterium wilt biocontrol temperature dependent? *Acta Scientiarum. Agronomy* 36:409.
- Martins, S. J., Taerum, S. J., Triplett, L., Emerson, J. B., Zasada, I., de Toledo, B. F., Kovac, J., Martin, K., and Bull, C. T. 2022. Soil bacteriophages and other bacterial predators for plant and human health. *Phytophymes J.* 6:184-200.
- Mavrodi, D. V., Mavrodi, O. V., Parejko, J. A., Bonsall, R. F., Kwak, Y. S., Paulitz, T. C., Thomashow, L., and Weller, D. M. 2012. Accumulation of the antibiotic phenazine-1-carboxylic acid in the rhizosphere of dryland cereals. *Appl. Environ. Microbiol.* 78:804-812.
- Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., Van Der Voort, M., Schneider, J. H., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A. H. M., and Raaijmakers, J. M. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332:1097-1100.
- Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., and Shibuya, N. 2007. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* 104:19613-19618.
- Morcillo, R. J., and Manzanera, M. 2021. The effects of plant-associated bacterial exopolysaccharides on plant abiotic stress tolerance. *Metabolites* 11:337.
- Muller, E. E. L., Faust, K., Widder, S., Herold, M., Martínez Arbas, S., and Wilmes, P. 2018. Using metabolic networks to resolve ecological properties of microbiomes. *Curr. Opin. Syst. Biol.* 8:73-80.
- Mülner, P., Bergna, A., Wagner, P., Sarajlić, D., Gstöttenmayr, B., Dietel, K., Grosch, R., Cernava, T., and Berg, G. 2019. Microbiota associated with sclerotia of soilborne fungal pathogens—a novel source of biocontrol agents producing bioactive volatiles. *Phytophymes J.* 3:125-136.
- Newman, M. A., Sundelin, T., Nielsen, J. T., and Erbs, G. 2013. MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Front. Plant Sci.* 4:139.
- Nielsen, T. H., and Sørensen, J. 2003. Production of cyclic lipopeptides by *Pseudomonas fluorescens* strains in bulk soil and in the sugar beet rhizosphere. *Appl. Environ. Microbiol.* 69:861-868.
- Niu, B., Paulson, J. N., Zheng, X. Q., and Kolter, R. 2017. Simplified and representative bacterial community of maize roots. *Proc. Natl. Acad. Sci. U.S.A.* 114:E2450-E2459.
- Niu, B., Wang, W., Yuan, Z., Sederoff, R. R., Sederoff, H., Chiang, V. L., and Borriss, R. 2020. Microbial interactions within multiple-strain biological control agents impact soil-borne plant disease. *Front. Microbiol.* 11:585-404.
- Ongena, M., and Jacques, P. 2008. Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. *Trends Microbiol.* 16:115-125.
- Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J., and Thonart, P. 2007. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ. Microbiol.* 9:1084-1090.
- Ossowicki, A., Tracanna, V., Petrus, M. L., van Wezel, G., Raaijmakers, J. M., Medema, M. H., and Garbeva, P. 2020. Microbial and volatile profiling of soils suppressive to *Fusarium culmorum* of wheat. *Proc. Royal Soc. B* 287:20192527.
- Pascale, A., Proietti, S., Pantelides, I. S., and Stringlis, I. A. 2020. Modulation of the root microbiome by plant molecules: The basis for targeted disease suppression and plant growth promotion. *Front. Plant Sci.* 10:1741.
- Paulsen, I. T., Press, C. M., Ravel, J., Kobayashi, D. Y., Myers, G. S., Mavrodi, D. V., DeBoy, R. T., Seshadri, R., Madupu, R., Dodson, R. J., Durkin, A., S., B., L., M., Daugherty, S. C., Sullivan, S. A., Rosovitz, M. J., Gwinn, M. L., Zhou, L., Schneider, D. J., Cartinhour, S. W., Nelson, W. C., Weidman, J., Watkins, K., Tran, K., Khouri, H., Pierson, E. A., Pierson, L. S., Thomashow, L. S., and Loper, J. E. 2005. Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat. Biotechnol.* 23:873-878.
- Pfeilmeier, S., Petti, G. C., Bortfeld-Miller, M., Daniel, B., Field, C. M., Sunagawa, S., and Vorholt, J. A. 2021. The plant NADPH oxidase RBOHD is required for microbiota homeostasis in leaves. *Nat. Microbiol.* 6:852.
- Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., van Wees, S. C. M., and Bakker, P. 2014. Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52:347-375.
- Poudel, M., Mendes, R., Soares, L. A., Bueno, C. G., Meng, Y., Folimonova, S. Y., Garrett, K. A., and Martins, S. J. 2021. The role of plant-associated bacteria, fungi and viruses in drought stress mitigation. *Front. Microbiol.* 12:3058.
- Poudel, R., Jumpponen, A., Schlatter, D. C., Paulitz, T. C., Gardener, B. B. M., Kinkel, L. L., and Garrett, K. A. 2016. Microbiome networks: A systems framework for identifying candidate microbial assemblages for disease management. *Phytopathology* 106:1083-1096.
- Prigallo, M. I., Cabanas, C. G. L., Mercado-Blanco, J., and Bubici, G. 2022. Designing a synthetic microbial community devoted to biological control: The case study of Fusarium wilt of banana. *Front. Microbiol.* 13:967885.
- Qi, M. S., Berry, J. C., Veley, K. W., O'Connor, L., Finkel, O. M., Salas-Gonzalez, I., Kuhs, M., Jupe, J., Holcomb, E., Glavina Del Rio, T., Creech, C., Liu, P., Tringe, S. G., Dangl, J. L., Schachtman, D. P., and Bart, R. S. 2022. Identification of beneficial and detrimental bacteria impacting Sorghum responses to drought using multi-scale and multi-system microbiome comparisons. *ISME J.* 16:1957-1969.
- Qu, Q., Zhang, Z., Peijnenburg, W. J. G. M., Liu, W., Lu, T., Hu, B., Chen, J., Chen, J., Lin, Z., and Qian, H. 2020. Rhizosphere microbiome assembly and its impact on plant growth. *J. Agric. Food Chem.* 68:5024-5038.
- Raaijmakers, J. M., Bonsall, R. F., and Weller, D. M. 1999. Effect of population density of *Pseudomonas fluorescens* on production of 2, 4-diacetylphloroglucinol in the rhizosphere of wheat. *Phytopathology* 89:470-475.
- Raaijmakers, J. M., De Bruijn, I., Nybroe, O., and Ongena, M. 2010. Natural functions of lipopeptides from Bacillus and Pseudomonas: More than surfactants and antibiotics. *FEMS Microbiol. Rev.* 34:1037-1062.
- Raaijmakers, J. M., and Mazzola, M. 2012. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annu. Rev. Phytopathol.* 50:403-424.
- Raaijmakers, J. M., Vlami, M., and De Souza, J. T. 2002. Antibiotic production by bacterial biocontrol agents. *Antonie Van Leeuwenhoek* 81:537-547.
- Roberson, E. B., and Firestone, M. K. 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Appl. Environ. Microbiol.* 58:1284-1291.
- Rybakova, D., Müller, H., Olimi, E., Schaefer, A., Cernava, T., and Berg, G. 2022. To defend or to attack? Antagonistic interactions between *Serratia plymuthica* and fungal plant pathogens, a species-specific volatile dialogue. *Front. Sust. Food Syst.* 6:1020634.
- Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., and Rastall, R. A. 2019. Probiotics and prebiotics in intestinal health and disease: From biology to the clinic. *Nat. Rev. Gastroenterol. Hepatol.* 16:605-616.
- Santhanam, R., Luu, V. T., Weinhold, A., Goldberg, J., Oh, Y., and Baldwin, I. T. 2015. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proc. Natl. Acad. Sci.* 112:E5013-E5020.
- Schandry, N., Jandrasits, K., Garrido-Oter, R., and Becker, C. 2021. Plant-derived benzoxazinoids act as antibiotics and shape bacterial communities. *bioRxiv* 425818.
- Schlechter, R. O., Jun, H., Bernach, M., Oso, S., Boyd, E., Muñoz-Lintz, D. A., Dobson, R. C., Remus, D. M., and Remus-Emsermann, M. N. 2018. Chromatic bacteria—A broad host-range plasmid and chromosomal insertion toolbox for fluorescent protein expression in bacteria. *Front. Microbiol.* 9:3052.
- Schmidt, R., Cordovez, V., De Boer, W., Raaijmakers, J., and Garbeva, P. 2015. Volatile affairs in microbial interactions. *ISME J.* 9:2329-2335.

- Schmidt, R., Etalo, D. W., De Jager, V., Gerards, S., Zweers, H., De Boer, W., and Garbeva, P. 2016. Microbial small talk: Volatiles in fungal–bacterial interactions. *Front. Microbiol.* 6:1495.
- Schroth, M. N., and Hancock, J. G. 1982. Disease-suppressive soil and root-colonizing bacteria. *Science (N.Y.)* 216:1376–1381.
- Schulz-Bohm, K., Gerards, S., Hundscheid, M., Melenhorst, J., de Boer, W., and Garbeva, P. 2018. Calling from distance: Attraction of soil bacteria by plant root volatiles. *ISME J.* 12:1252–1262.
- Shank, E. A., Klepac-Ceraj, V., Collado-Torres, L., Powers, G. E., Losick, R., and Kolter, R. 2011. Interspecies interactions that result in *Bacillus subtilis* forming biofilms are mediated mainly by members of its own genus. *Proc. Natl. Acad. Sci.* 108:E1236–E1243.
- Shayanthan, A., Ordoñez, P. A. C., and Oresnik, I. J. 2022. The role of synthetic microbial communities (SynCom) in sustainable agriculture. *Front. Agron.* 4:896307.
- Silva, J. C. P., Nunes, T. C. S., Guimarães, R. A., Pylro, V. S., Costa, L. S. A. S., Zaia, R., Campos, V. P., and Medeiros, F. H. V. 2022. Organic practices intensify the microbiome assembly and suppress root-knot nematodes. *J. Pest Sci.* 95:709–721.
- Singh, B. K., Trivedi, P., Egidi, E., Macdonald, C. A., and Delgado-Baquerizo, M. 2020. Crop microbiome and sustainable agriculture. *Nat. Rev. Microbiol.* 18:601–602.
- Song, G. C., Jeon, J. S., Sim, H. J., Lee, S., Jung, J., Kim, S. G., Moon, S. Y., and Ryu, C. M. 2022. Dual functionality of natural mixtures of bacterial volatile compounds on plant growth. *J. Exp. Bot.* 73:571–583.
- Stringlis, I. A., Proietti, S., Hickman, R., van Verk, M. C., Zamioudis, C., and Pieterse, C. M. J. 2018a. Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. *Plant J.* 93:166–180.
- Stringlis, I. A., Yu, K., Feussner, K., de Jonge, R., van Bentum, S., van Verk, M. C., Berendsen, R. L., Bakker, P. A. H. M., Fuessner, I., and Pieterse, C. M. J. 2018b. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc. Natl. Acad. Sci.* 115:E5213–E5222.
- Teixeira, P. J. P. L., Colaianni, N. R., Law, T. F., Conway, J. M., Gilbert, S., Li, H., Salas-González, I., Panda, D., Del Risco, N. M., Finkel, O. M., Castrillo, G., Mieczkowski, P., Jones, C. D., and Dangel, J. L. 2021. Specific modulation of the root immune system by a community of commensal bacteria. *Proc. Natl. Acad. Sci.* 118:e2100678118.
- Terra, W. C., Campos, V. P., Martins, S. J., Costa, L. S. A. S., da Silva, J. C. P., Barros, A. F., Lopez, L. E., Santos, T. C. N., Smant, G., and Oliveira, D. F. 2018. Volatile organic molecules from *Fusarium oxysporum* strain 21 with nematocidal activity against *Meloidogyne incognita*. *Crop Prot.* 106:125–131.
- Tilman, D., Isbell, F., and Cowles, J. M. 2014. Biodiversity and ecosystem functioning. *Annu. Rev. Ecol. Evol. Syst.* 45:471–493.
- Toju, H., Abe, M. S., Ishii, C., Hori, Y., Fujita, H., and Fukuda, S. 2020. Scoring species for synthetic community design: Network analyses of functional core microbiomes. *Front. Microbiol.* 11:1361.
- Tracanna, V., Ossowicki, A., Petrus, M. L., Overduin, S., Terlouw, B. R., Lund, G., Robinson, S. L., Warris, S., Schijlen, E. G. W. M., van Wezel, G. P., Raaijmakers, J. M., Garbeva, P., and Medema, M. H. 2021. Dissecting disease-suppressive rhizosphere microbiomes by functional amplicon sequencing and 10× metagenomics. *mSystems* 6:e01116–20.
- Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., and Singh, B. K. 2020. Plant–microbiome interactions: From community assembly to plant health. *Nat. Rev. Microbiol.* 18:607–621.
- Tsuji, J., Jackson, E. P., Gage, D. A., Hammerschmidt, R., and Somerville, S. C. 1992. Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to *Pseudomonas syringae* pv. *syringae*. *Plant Physiol.* 98:1304–1309.
- Türksoy, G. M., Carron, R., Koprivova, A., Kopriva, S., Wippel, K., and Andersen, T. G. 2022. Modulation of *Arabidopsis* growth by volatile organic compounds from a root-derived bacterial community. *bioRxiv* 488003.
- Tyler, B. M. 2002. Molecular basis of recognition between *Phytophthora* pathogens and their hosts. *Annu. Rev. Phytopathol.* 40:137–167.
- Vlamakis, H., Chai, Y., Beauregard, P., Losick, R., and Kolter, R. 2013. Sticking together: Building a biofilm the *Bacillus subtilis* way. *Nat. Rev. Microbiol.* 11:157–168.
- Vogel, C. M., Potthoff, D. B., Schafer, M., Barandun, N., and Vorholt, J. A. 2021. Protective role of the *Arabidopsis* leaf microbiota against a bacterial pathogen. *Nat. Microbiol.* 6:1537.
- Voges, M., Bai, Y., Schulze-Lefert, P., and Sattely, E. S. 2019. Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. *Proc. Natl. Acad. Sci. U.S.A.* 116:12558–12565.
- Vorholt, J. A., Vogel, C., Carlstrom, C. I., and Muller, D. B. 2017. Establishing causality: Opportunities of synthetic communities for plant microbiome research. *Cell Host Microbe* 22:142–155.
- Weisskopf, L., Schulz, S., and Garbeva, P. 2021. Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. *Nat. Rev. Microbiol.* 19:391–404.
- Wiltshi, B., Cernava, T., Dennig, A., Casas, M. G., Geier, M., Gruber, S., Haberbauer, M., Heidinger, P., Acero, E. H., Kratzer, R., Luley-Goedl, C., Müller, C. A., Pitzer, J., Ribitsch, D., Sauer, M., Schmöler, K., Schnitzhofer, W., Sensen, C. W., Soh, J., Steiner, K., Winkler, C. K., Winkler, M., and Wriessnegger, T. 2020. Enzymes revolutionize the bioproduction of value-added compounds: From enzyme discovery to special applications. *Biotechnol. Adv.* 40:107520.
- Wolinska, K. W., Vannier, N., Thiergart, T., Pickel, B., Gremmen, S., Piasecka, A., Piślewska-Bednarek, M., Nakano, R. T., Belkhadir, Y., Bednarek, P., and Hacquard, S. 2021. Tryptophan metabolism and bacterial commensals prevent fungal dysbiosis in *Arabidopsis* roots. *Proc. Natl. Acad. Sci. U.S.A.* 118:e2111521118.
- Yin, C., Hagerty, C. H., and Paulitz, T. C. 2022. Synthetic microbial consortia derived from rhizosphere soil protect wheat against a soilborne fungal pathogen. *Front. Microbiol.* 13.
- Yu, K., Pieterse, C. M. J., Bakker, P., and Berendsen, R. L. 2019a. Beneficial microbes going underground of root immunity. *Plant Cell Environ.* 42:2860–2870.
- Yu, K., Liu, Y., Tichelaar, R., Savant, N., Lagendijk, E., van Kuijk, S. J. L., Stringlis, I. A., van Dijken, A. J. H., Pieterse, C. M. J., Bakker, P. A. H. M., Haney, C. H., and Berendsen, R. L. 2019b. Rhizosphere-associated *Pseudomonas* suppress local root immune responses by gluconic acid-mediated lowering of environmental pH. *Curr. Biol.* 29:3913.
- Zhao, H., Liu, Y. P., and Zhang, L. Q. 2019. In silico and genetic analyses of cyclic lipopeptide synthetic gene clusters in *Pseudomonas* sp. 11K1. *Front. Microbiol.* 10:544.
- Zheng, W., Zeng, S., Bais, H., LaManna, J. M., Hussey, D. S., Jacobson, D. L., and Jin, Y. 2018. Plant growth-promoting rhizobacteria (PGPR) reduce evaporation and increase soil water retention. *Water Resour. Res.* 54:3673–3687.
- Zhou, D., Feng, H., Schuelke, T., de Santiago, A., Zhang, Q., Zhang, J., Luo, C., and Wei, L. 2019. Rhizosphere microbiomes from root knot nematode non-infested plants suppress nematode infection. *Microb. Ecol.* 78:470–481.
- Zimmer, S., Messmer, M., Haase, T., Piepho, H.-P., Mindermann, A., Schulz, H., Habekuß, A., Ordon, F., Wilbois, K.-P., and Heß, J. 2016. Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. *Eur. J. Agron.* 72:38–46.
- Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T., and Felix, G. 2006. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* 125:749–760.