

## Review

## Plant Microbiome Engineering: Expected Benefits for Improved Crop Growth and Resilience

Inessa Arif,<sup>1,2</sup> Maria Batool,<sup>1,2</sup> and Peer M. Schenk<sup>1,\*</sup>

**Plant-associated microbiomes can boost plant growth or control pathogens. Altering the microbiome by inoculation with a consortium of plant growth-promoting rhizobacteria (PGPR) can enhance plant development and mitigate against pathogens as well as abiotic stresses. Manipulating the plant holobiont by microbiome engineering is an emerging biotechnological strategy to improve crop yields and resilience. Indirect approaches to microbiome engineering include the use of soil amendments or selective substrates, and direct approaches include inoculation with specific probiotic microbes, artificial microbial consortia, and microbiome breeding and transplantation. We highlight why and how microbiome services could be incorporated into traditional agricultural practices and the gaps in knowledge that must be answered before these approaches can be commercialized in field applications.**

**Constraints in Agriculture and Customized Solutions**

The global demand for agricultural crops is expected to rise by at least 60% by 2050; however, in the 20 years between 1985 and 2005 the global crop output only increased by 28% [1]. In addition, there is an increasing demand for biofuel, fiber, and biomaterials from plants that is further exacerbated by a growing global meat and dairy industry that requires fodder. The green revolution brought about a significant increase in agricultural productivity through genetically improved crop varieties, chemical pesticides, and fertilizers, but this success is tainted by many unsustainable practices such as the degradation of soil health. Several studies have shown that yields are stagnating or decreasing for major crop plants, such as maize, rice, and wheat, which together form ~57% of global agricultural calorific intake [1], accompanied by increasing incidences of environmental constraints including drought, heavy metal contamination, soil salinity, and heat stress [2]. Apart from abiotic stresses, biotic stresses from pests and pathogens also limit crop productivity, leading to US\$ 40 billion annual losses globally [3].

Chemical fertilizer applications currently form core agricultural practices, but ultimately lead to soil degradation, nutrient pollution, greenhouse gas emissions, and eutrophication, and, for phosphorous (P), also present a finite resource, and nitrogen (N) fertilizer is energy-intensive to produce. Furthermore, one study reported that only 0.1% of chemical pesticides reach their target, and the remaining amount is leached into surrounding soil and water, polluting the environment [4]. Although genetically modified (GM) plants remain a viable option as a low-input, sustainable agricultural practice, the research and regulation necessary to develop new varieties are expensive and can take decades to gain consumer and regulatory approval [3]. Sustainable agricultural practices aim to ensure high yields by focusing on increasing the uptake of readily available nutrients, while not depleting nutrient levels in the soil [5]. However, some reports suggest that 48% of farms have experienced soil nutrient deficiencies that cannot be easily mitigated by

**Highlights**

Symbiotic bacteria can boost plant growth, control pathogens, or alleviate abiotic stress.

Microbiome engineering incorporated into traditional agricultural practices can improve microbial ecosystem services for crop yield and resilience.

New agricultural practices may include microbiome breeding, transplantation, and targeted microbiome engineering, for example by strategic soil amendments in which selective addition of plant exudates attracts and maintains beneficial microbes, or by directly applying microbial consortia as probiotics.

Customized microbiome engineering will be necessary to cope with the many variables, including soil type, environmental/climatic conditions, growth stage, and genotype of the plant, to influence the microbiome in a purposeful and effective manner.

Breeding 'microbe-friendly' crops can complement microbiome engineering to better attract and maintain beneficial microbiomes.

<sup>1</sup>Plant–Microbe Interactions Laboratory, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, QLD 4072, Australia

<sup>2</sup>These authors contributed equally to this work.

\*Correspondence: [p.schenk@uq.edu.au](mailto:p.schenk@uq.edu.au) (P.M. Schenk).

the addition of mineral fertilizers [5]. These often coincide and are linked to low soil organic carbon contents and reduced soil microbial biodiversity [6].

Plant-associated microbes and microbiomes provide important 'ecosystem services' by promoting plant growth, controlling pathogens, and alleviating abiotic stresses [3,5,6]. This review explores various practical ways by which beneficial plant–microbe interactions can be manipulated to improve crop production in an agricultural context. It focuses on how **microbiome engineering** can be incorporated into traditional and emerging agricultural practices, and on emerging methods that build on these microbial ecosystem services to further improve crop yields and resilience.

## Traditional Microbiome Engineering

### Soil Amendments

Soil amendments comprise organic and inorganic additions to the soil or agricultural practices that farmers use to optimize productivity and that influence and manipulate plant–microbiome interactions [5,7]. The following sections provide some examples.

Inorganic amendments include lime, vermiculite, perlite, and sand that have been used to reduce the impact of soil acidity and salinity on crop plants [8]. They can also lead to increased plant biomass triggered by an increase in root exudates, as well as a larger, more active microbiome, even in N-deficient soils [7]. Inorganic amendments used in tandem with organic amendments showed a 30% crop yield increase, compared with only 8% with organic fertilizers alone [7].

Organic amendments encompass the application of a variety of additives to the soil which act as an energy source for heterotrophic bacteria, fungi, and invertebrates that are beneficial for plants [5]. They have the dual advantage of supplementing depleted soil fertility and reducing farming waste [5]. Long-term application of traditional organic fertilizers leads to increased microbial biomass and diversity, whose composition depends on the amendment type [6]. For example, manure has been used for several centuries because it is rich in N, P, and organic matter that can alter the soil microbiome and the physical and chemical properties to increase agricultural yields [9]. Addition of sheep manure enhanced microbial growth and lowered metal toxicity by decreasing lead, cadmium, and zinc levels in alfalfa plants, resulting in significantly higher crop yields [10]. Bones have been used as a renewable source of P since the mid 19th century. It is well known that soil microorganisms, such as *Bacillus megaterium* var. *phosphaticum* and *Bacillus mucilaginosus*, can solubilize the P present in bone to make it available for plant uptake by releasing organic acids [11].

The large amounts of organic carbon and other nutrient-rich waste in crop residues can be valorized by composting or vermicomposting to generate biostimulants, leading to improved soil aggregation [6]. Vermicompost end-products are both environmentally friendly and rich in nutrients, and can be used as soil conditioning agents to promote plant growth [12]. Such biostimulants are expected to increase the mineralization of nutrients that rely on changing the composition and biomass of the soil microbiome [13]. Compost soil amendment is a popular alternative to chemical fertilizers and also reduces farming waste. Biocomposting is a microbiological oxidative process that uses various recyclable organic substances, and is often used in organic farming for plant protection and growth promotion [14]. Many studies have named compost and agriwaste as rich sources of biocontrol soil microbiomes, and green compost was also a management tool to control soil-borne plant pathogens [14]. The mechanisms and processes resembling naturally suppressive soils were also exploited in greenhouse-based horticultural soil-less systems. Compost is considered to be a reliable option by farmers who do not have many options for

## Glossary

### 1-Aminocyclopropane-1-carboxylic acid (ACC):

an ethylene precursor whose concentration is elevated in plants subjected to biotic and abiotic stresses. It is an important root exudate that plants can release into the rhizosphere to attract plant growth-promoting rhizobacteria (PGPR). ACC deaminase is the enzyme responsible for cleaving the plant ethylene precursor, ACC, into ammonia and  $\alpha$ -ketobutyrate.

### Anaerobic soil disinfestation (ASD):

a process by which anaerobic soil conditions are created to disinfest the soil by incorporating soil amendments which are easily degradable, covering the treated area with polyethylene mulch, and saturating the soil by irrigation to provide a 2–6 week treatment period to the soil.

**Benzoxazinoids:** naturally occurring organic plant defense metabolites that are produced mainly by the Poaceae plant family, and that have antimicrobial activity against various microbiological threats to plants.

### Biological soil crust (BSC):

comprises groups of microorganisms living in the upper layer of soils; these are typically fungi, cyanobacteria, lichens, mosses, liverworts, and microalgae that play an important role in stabilizing and protecting the soil from erosion and are a rich source of fixed carbon in sparsely vegetative areas.

**Coumarins:** organic chemical compounds that are found naturally in many plants and that have medicinal uses in the pharmaceutical industry. These are also used as a defense against herbivore infestation because they are bitter in taste

### Microbe-associated molecular

**patterns (MAMPs):** molecular signatures from small molecules that are present in groups of microbes but are not present in the host, for example flagellin for bacteria, chitin for fungi.

### Microbial biofertilizers and

**biopesticides:** microorganisms that assist in plant growth and biocontrol of plant pests or pathogens, respectively.

**Microbiome engineering:** the purposeful manipulation of microbial communities.

### Operational taxonomic units

**(OTUs):** taxa of closely related microbes that are classified according to a similar specific taxonomic marker gene in their DNA sequence. These are commonly

disease control in the absence of soil fumigants and fungicides [14]. **Anaerobic soil disinfestation** (ASD) with wheat bran or ethanol soil amendments as carbon sources not only reduced root rot disease severity on tomato caused by *Pyrenochaeta lycopersici* but also resulted in modification soil microbiomes, increasing the abundance of Firmicutes [15].

used when analyzing microbiomes using 16S or 18S rDNA (prokaryotes or eukaryotes) marker gene sequences.

Agricultural practices such as crop rotation, tillage, and intercropping can also have a strong impact on soil microbiome conditioning and variation. For example, almost 10% modification of wheat root and soil bacterial communities was observed when wheat root and soil microbial communities were investigated in a cropping system with traditional and organic soil amendments, and microbial population diversity was sensitive to cropping and tillage practices [16]. Yield decline in sugarcane cropping can be reversed by legume crop rotation that is linked to a restoration of soil microbial biomass [17].

#### Less-Traditional Organic Addendums

A scaling law described the uppermost layer of soil to harbor >1.0 trillion microbial species, including fungi, lichens, bacteria, actinomycetes, microalgae, and cyanobacteria, that are closely associated with each other [18]. This **biological soil crust** (BSC) is believed to play a primary role in promoting agricultural yield and soil fertility. Many microalgae, including N-fixing cyanobacteria, assist in the decomposition of organic wastes, biocontrol of phytopathogens, detoxification of harmful chemicals, recycling of nutrients, and the production of metabolites such as enzymes, hormones, and vitamins that influence soil nutrient composition and plant growth [19]. Exploiting the relationship between soil algae and microbes to generate biofertilizers is a burgeoning area of research. Algae/microbe fertilizers could maximize N, P, and potassium uptake, clean pollutants in the soil [20], and inhibit plant nematode parasitic activity in association with other microorganisms belonging to Proteobacteria and Bacteroidetes [21].

Biochar is a C-rich product formed from the partial or complete pyrolysis of organic matter such as manure, bones, crop residues, and slaughterhouse wastes that generally increase soil nutrient availability and water-holding capacity [22]. Biochar provides a habitat for soil microbes [8] and has a positive effect on crop yield (with some exceptions) [23]. Biochar can also suppress various diseases caused by pathogens such as *Botrytis cinerea*, *Fusarium oxysporum*, *Podosphaera aphanis*, *Pythium aphanidermatum*, *Phytophthora cactorum*, and *Rhizoctonia solani* [24], the nematode *Pratylenchus penetrans* [25], and the parasitic weed *Phelipanche aegyptiaca* [26]. Disease suppression on lettuce by *Fusarium oxysporum* f.sp. *lactucae* or *Sclerotinia sclerotiorum* was observed with wood biochar and alfalfa leaf litter application that was linked to soil microbiome modifications [27].

Microbial soil inoculants include **microbial biofertilizers and biopesticides** of usually individual or a limited number of known microbial strains that have beneficial attributes for boosting plant productivity (e.g., *Rhizobium* spp.) and protecting plants against pathogens [3]. Table 1 provides some examples of benefits gained from microbial inoculation of plants.

Because N is the most essential nutrient, the global biofertilizer market is dominated by products containing N-fixing microorganisms. Currently, the most commonly used N-fixing biofertilizer microorganisms include *Actinorhizobium* spp., *Azospirillum* spp., *Azotobacter* spp., and *Rhizobium* spp. [28]. Most are used for legumes, but some also find uses as free-living N-fixing bacteria with other crops such as sugarcane and rice [28]. Once activated by specific legume root exudates (flavonoids), rhizobia induce a physiological change in root hairs, resulting in curling and bulging to form nodules that rhizobacteria can colonize [29]. Many soil bacteria can also solubilize inorganic P and make it available for plant absorption [11]. Souza and colleagues identified 101

Table 1. Examples of Benefits Gained from Microbial Inoculations of Plants

| Plant               | Beneficial microbe                                      | Benefit to plants   | Refs. |
|---------------------|---|---|-------|
| Tomato seeds        | <i>Azospirillum brasilense</i>                          | The bacteria increased both, shoot and root length  | [74]  |
| Cucumber and tomato | <i>Bacillus</i> strain QST713                           | Increased P content by 40% and thereby overall plant growth   | [2]   |
| Common bean         | <i>Pseudomonas putida</i> UW4+<br><i>Rhizobium</i> PGPR | Significantly enhanced nodule formation in plants under P stress and supplemented growth promotion by <i>Rhizobium</i>                        | [75]  |
| Tomato              | <i>Burkholderia unamae</i>                              | Plants inoculated with wild <i>B. unamae</i> strains showed better growth than <i>B. unamae</i> mutant strains without ACC deaminase activity | [30]  |
| Rice                | <i>Azospirillum amazonense</i>                          | Significantly improved grain yield in rice by increasing panicle number and N content at maturity   | [76]  |

bacterial strains such as *Burkholderia*, *Enterobacter*, and *Pseudomonas* spp. that are associated with rice plant roots and that could solubilize tricalcium phosphate [30].

Advances in genome sequencing, metagenomics, 16S or 18S rDNA, and internal transcribed spacers (ITS) amplicon community sequencing, and qPCR and non-PCR-based methods, such as T-RFLP, FISH, and DNA arrays, have helped researchers to gain a better understanding of the composition and function of microbial communities [31]. This has led to the development of multispecies ‘designer’ microbial biofertilizers with strains that complement each other or strains that can tolerate different environmental conditions [31]. The use of microbiomes to boost plant productivity and resilience is further reviewed later.

### Microbiome Services

Box 1 and Figure 1 provide overviews of plant microbiomes and the services they offer to plants. The microbiome is now known as the ‘second genome’ of an organism that strongly influences its health and general well-being. As a result, future diagnostics and therapy in plants, animals, and humans will be heavily dependent on understanding and manipulating microbiome functions and its services [32]. It is worthwhile to explore parallels between the plant and human microbiomes. At least 105 diseases have been associated with changes in the human microbiota [33]. A better understanding of the human microbiome is expected to contribute to the use of ‘personalized medicine’ which switches from the traditional ‘disease-specific’ to patient-specific therapy [32]. Probiotics (beneficial microbes) and prebiotics (compounds in food that induce the growth or activity of beneficial microbes) are increasingly gaining traction in the market as a therapy for gut disturbances and related ailments [34]. It can be suggested that the equivalents for plants of the gut are the roots – probiotics for plant roots are PGPR, and prebiotics for plants are substrates or additives that alter the composition of plant microbiomes, such as soil amendments (see earlier sections). Plant microbiomes (similarly to gut microbiomes) strongly influence nutrient availability as well as host organism growth and development [35,36] (e.g., increased root and shoot growth in plants vs metabolic syndrome in humans). It has been shown conclusively that plants actively recruit and ‘engineer’ their microbiome through exudates [37,38], and further research may reveal equivalent mechanisms for various microbiome-colonized human organs. The following sections review the growing evidence of positive interactions between plants and their microbiome, and examine examples of purposeful plant microbiome engineering.

### Targeted Plant Microbiome Engineering

The root microbiome is the primary determinant for the development and growth of a plant by assisting in protection against biotic stresses, nutrient uptake, and abiotic stress tolerance [39], and manipulation of this microbiome has the potential to further enhance these features. Various microbes can interact with each other in the rhizosphere in the form of functional consortia. For

## Box 1. The Plant Microbiome

## The Plant Holobiont

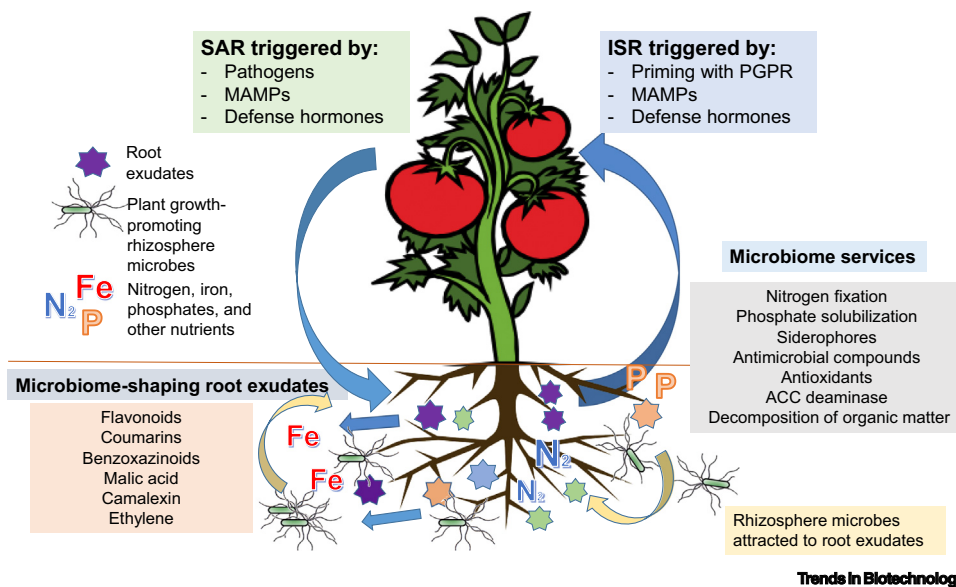
Microbial colonies can exist inside plants (endosphere), externally on roots (rhizosphere), and on leaves (phyllosphere) [31]. For below-ground bacteria, exudates from the root, soil pH, salinity, soil organic matter, and moisture determine the composition and function of the bacteria that are present in and around the roots. The root microbiota, although highly diverse, generally include *Acidobacteria*, *Bacteroidetes*, *Proteobacteria*, *Planctomycetes*, and *Actinobacteria* [77]. In addition to the host, environmental factors play a significant role in shaping the plant microbiome – that under ideal conditions provides biological control agents, assists plant growth, and promotes overall adaptability to abiotic stress [30]. Axenic plants (plants without microbes) grow significantly slower [35], and there is mounting evidence that plants recruit, degrade, and consume symbiotic microbes according to their function (e.g., assistance with growth vs defense) on an as-needed basis [31].

## Plant Microbiome Composition

The constant communication between the plant and its microbiota can be used to manipulate the abundance and type of microorganisms and alter the development of the plant and/or defense against pests and diseases, which can improve overall crop productivity. The diversity of the microbiota species is dependent on members of the same genera (horizontal transfer via the soil environment) but can also be vertically transferred from the seeds across generations [31]. Xia and colleagues [78] investigated the effects of isolated bacterial endophytes across 32 species, including corn, tomato, melon, and pepper. Of the isolates tested, 61% were found to boost growth in tomato, and 50–64% were shown to improve biomass accumulation.

## Beneficial Attributes of PGPR

There are various direct and indirect mechanisms by which plant growth-promoting bacteria contribute towards plant growth promotion and development [31]. Table 1 lists some examples. Some PGPR modulate plant endogenous hormone levels by producing phytohormones such as auxins, gibberellins, and cytokinins. Several PGPR, including *Pseudomonas* spp., *Arthrobacter* spp. and *Bacillus* spp., secrete ACC deaminase, which degrades ACC, a precursor of the plant stress hormone ethylene, leading to reduced stress levels in plants under suboptimal conditions (e.g., salinity or drought) [40,41]. A wide range of PGPR, including *Pantoea* spp., *Pseudomonas* spp. and *Paraburkholderia* spp., have shown plant growth-promoting activities in wheat and soybean, including N fixation, phosphate solubilization, ACC deaminase and auxin (indole acetic acid) production, and various other mechanisms associated with stress tolerance and improved nutrient uptake by plants [79].



**Figure 1. Plant Microbiome Services Provided by Beneficial Plant–Microbe Interactions.** Systemic acquired resistance (SAR) and induced systemic resistance (ISR) against plant pathogens can be achieved by **microbe-associated molecular patterns** (MAMPs) from either plant pathogens or beneficial plant growth-promoting rhizobacteria (PGPR). Microbiome services assist plants with growth, disease resistance, and stress tolerance, leading to up to fourfold higher biomass production [70].

example, arbuscular mycorrhizal (AM) fungi and PGPR in a model grassland complemented each other with regard to various limiting nutrients and ecosystem functions [38]. The rhizosphere microbiome can be manipulated by soil conditioning (including traditional soil amendments) or by the addition/activation of signal molecules or substrates (including root exudates) to induce or attract the desired microbiota. In this regard, several studies have been carried out to encourage the use of specific exudates and substrates in field trials [40–42]. Soil can also be inoculated with key microbial strains to change the structure of microbial communities [43]. For example, consortium inoculation of the plant-beneficial bacterium *Pseudomonas libanensis* TR1 and the AM fungus *Claroideoglomus claroideum* BEG210 into the sunflower rhizosphere, aiming to harness the potential of plant growth-promoting microbiota to elevate stress tolerance, resulted in improved plant growth under metal and salinity stress alone or in combination [44]. Treating roots of growing chili plants with bacterial cultures of *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Acinetobacter* sp. UQ202 promoted plant growth and concomitantly the ability to withstand soil-borne *Phytophthora capsici* infection [45]. Using *Agrobacterium* sp. 10C2 on bean increased overall nodule number and plant biomass as well as flavonoids, antioxidants, and P in the harvested pods [46]. At 15 days after inoculation, the bacterial community in the soil around the plant changed to harbor several species of PGPR such as *Actinomyces* spp. and *Brevibacterium* spp.

The seed microbiome has also been shown to improve germination, plant survival, and performance, and can be inherited over generations [47]. Coinoculating the rhizosphere of tomato seedlings with *Pseudomonas stutzeri* and *Stenotrophomonas maltophilia* boosted plant growth, and both emitted diffusible compounds (e.g., dimethyl disulphide) that are active against the leaf pathogen *Botrytis cinerea* [37]. The bacteria integrated as endophytes could proliferate and colonize the next generation. In the same way as root exudates enrich for beneficial microbes in the rhizosphere [37,38,48], roots act as important gatekeepers to select beneficial microbes that may enter the plant as endophytes [25]. Interestingly, plants also expel bacteria into the rhizosphere [49], but the mechanisms by which microorganisms (pathogenic or otherwise) enter and exit the plant holobiont require further investigation. In addition, roots can consume associated microbes directly and use them as an N source [50], suggesting that the presence of microbial biomass in the rhizosphere also contributes to plant growth in an unspecific manner, although further studies will be necessary to determine whether specific microbes are preferred by plants for consumption.

Recent efforts in plant breeding aim towards crops that are more resilient and able to cope with climate variability and pest/pathogen attack. However, plants are typically unable to defend themselves against both biotic and abiotic stress simultaneously because the respective pathways are usually antagonistic [51]. [Box 2](#) provides an overview of how plant-associated microbes can alleviate biotic and abiotic stress.

### Emerging Areas in Microbiome Engineering

Several promising emerging areas are presented later (also [Figure 2](#) and [Box 3](#)), where microbiome engineering can have further impact to produce higher-yielding and more resilient crops. These include soil additives (including new organic soil amendments and root exudates), artificial microbial consortia, microbiome breeding and transplantation, the breeding and use of 'microbe-friendly' crop cultivars (host-mediated microbiome engineering), and combinations of these.

#### Using Organic Soil Amendments and Root Exudates To Attract and Maintain Beneficial Microbiomes

Organic soil amendments include applications of crop residues, organic wastes, compost, peat, and biochar, but also involve biofumigation, for example for disease suppression through soil

Box 2. Roles of Microbiomes in Alleviating Plant Stress

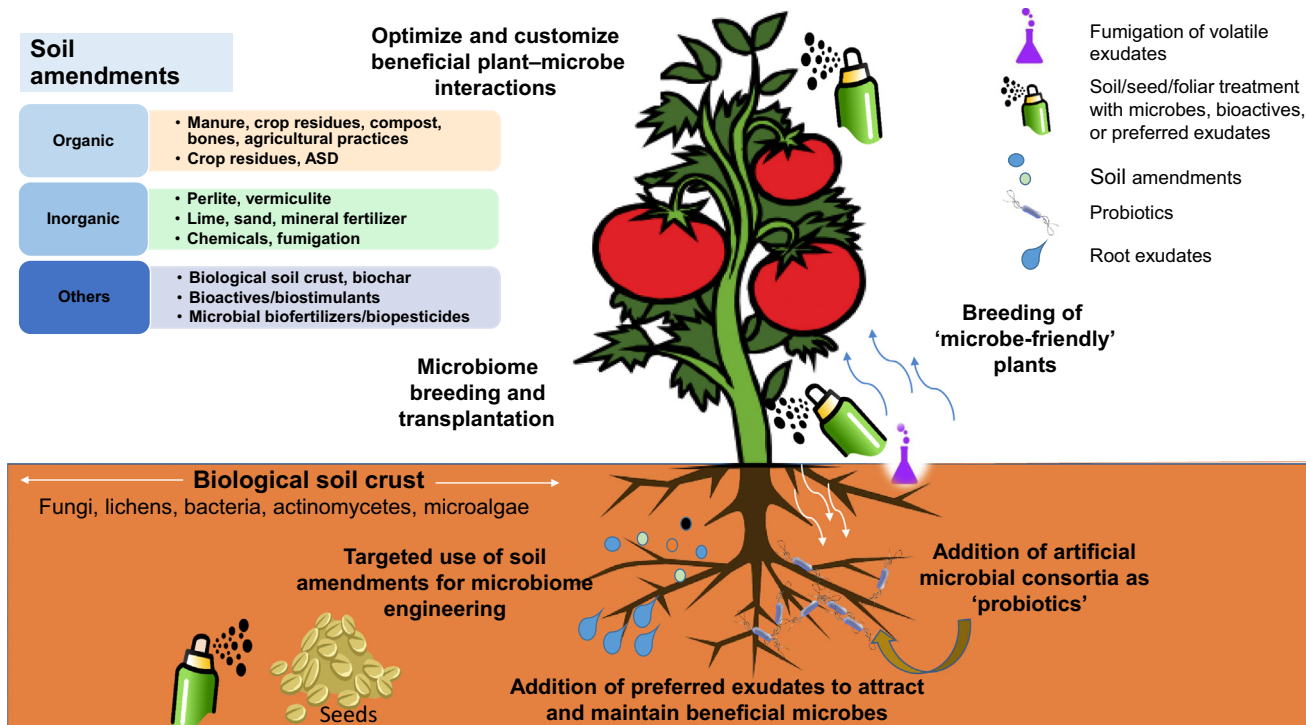
Abiotic Stress Tolerance Mechanisms

Microbiomes play important roles in abiotic stress alleviation in plants by increasing the bioavailability of nutrients and water uptake, reducing oxidative stress, decreasing metal toxicity, producing plant hormones, and regulating various signaling pathways (including degradation of ACC, a precursor of the stress hormone ethylene) [29,35,40,41]. Nearly all abiotic stresses lead to the production of reactive oxygen species (ROS) [80], but PGPR release catalase, peroxidase, and other enzymes to reduce oxidative stress and hence damage to the plant [2]. Plants without microbes can suffer from iron deficiency [38], and bacterial siderophores provide chelator agents to capture iron that is usually unavailable for plants to absorb. Similarly, PGPR can help with iron homeostasis in anaerobic and saline soils where iron toxicity is common. The composition of the fungal microbiome also plays a major role in abiotic stresses. For example, plants with mycorrhizal fungi display significantly improved water and nutrient uptake, whereas many endophytic fungal pathogens (e.g., *Fusarium* spp.) obstruct water transport through the vascular system, leading to wilting.

Biotic Stress Resistance Mechanisms

PGPR can outcompete or directly antagonize pathogens, or induce systemic resistance by priming plants. Several rhizospheric microorganisms directly suppress plant pathogens in soil [81]. For example, many *Bacillus* and *Pseudomonas* spp. secrete antibiotics that act as antifungal, antibacterial, anthelmintic, antiviral, and cytotoxic compounds. These competitive beneficial microbes prevent or reduce disease by competing with pathogens [82], for example by producing antimicrobial exudates [57], or they may secrete siderophores that deprive pathogens of iron to absorb [30]. There is also evidence for suppression of pathogens and disease symptoms through reconditioning of the microbiome assembly by root exudates whose excretion is induced by beneficial microbiota [48].

Prior pathogen attack can lead to systemic acquired resistance (SAR) that can protect plants from subsequent infections [83]. Many PGPR are able to 'prime' plants, leading to induced systemic resistance (ISR), a state of memory that enables the plant to respond faster and stronger to subsequent pathogen attacks [83]. There are many examples where bacteria manipulate all three major plant defense hormones. These include salicylic acid (SA) produced and secreted by bacteria, jasmonic acid (JA) through the JA-mimetic coronatine, and ethylene by degrading its precursor ACC. Pattern recognition receptors in the plant bind to microbe-associated molecular patterns (MAMPs), and respond to and differentiate between various MAMP signals emitted by non-pathogenic and pathogenic microbes. Depending on pathogenicity, they can then trigger or suppress microbial growth [53].



Trends in Biotechnology

Figure 2. New Opportunities for Applying Microbiome Engineering. The relationship between plants and microbes can be optimized to benefit plants by applying customized approaches, such as breeding 'microbe-friendly' plants, microbiome breeding and transplantation, and by the application of suitable soil amendments, such as specific microbial biofertilizers and biopesticides (including artificial consortia), or through the addition of biostimulants (e.g., root exudate compounds preferred by beneficial microbes). Abbreviation: ASD, anaerobic soil disinfestation.

### Box 3. The Future of Microbiome Engineering

Organic soil amendments could act as a 'prebiotic' that farmers can add to supplement the mechanisms by which soil microbiomes function. Further research into deciphering the relationship between the substrate and the microorganisms can lead to a targeted, plant-specific biological fertilizer. Similarly, studies that link genotypes and interactions of a host with microbial biocontrol agents will enhance our understanding of these interactions and help to develop novel microbiome-based biocontrol methods. Similar studies focusing on characterizing the holobiont and gaining a deeper understanding of the pathways within the host and its interactions with the microbiome could make 'microbe-optimized' designer plants a reality. There are now several promising methods for microbiome breeding and transplantation. However, the longevity of the microbes under field conditions will need to be considered, and the transient nature of the microbes might require ongoing soil amendments or multiple applications, including methods how to transmit and stabilize the microbial consortia over generations and various crop rotations.

Although we study plant–microbe interactions with the aim to engineer beneficial microbiomes, it is necessary to understand not only how microbiomes affect plants but also how plants manipulate microbiomes. For this, future studies can focus on devising ways to maintain beneficial microbiomes and to track how microbiome changes occur under natural conditions. There are technical issues with monitoring the complex spatial and biochemical microbiome activities and with real-time phenotypic/physiological plant characterization. A step forward could be regular DNA sampling and microbial community profiling whose changes can be correlated to plant physiological and external environmental factors. In the future, a real-time DNA monitoring probe and plant data logger may be developed.

The chemical language spoken between root exudates, attracted/repelled microbial taxa, and their benefits/detriments to the plant is poorly understood and requires extensive decoding by metabolomics and proteomics. This knowledge would then enable a customized approach to maximize beneficial plant–microbiome interactions, for example by providing specific root exudates as substrates.

Microbiome breeding, the use of 'microbe-friendly' cultivars, and customized optimization of beneficial plant–microbe interactions are emerging areas with potential to improve crop yields and resilience, and we can group core beneficial microbial consortia into categories that work for specific crop–soil–environment combinations. To best select for and breed 'microbe-friendly' crop cultivars, we can use a combination of markers to account for various benefits from microbes (e.g., MYB72 as a marker for microbe-induced priming for disease resistance).

Moreover, microbiomes vary across different plant developmental stages and continuously adapt to environmental changes. To ensure that these interactions remain positive and benefit plants in the long term, we probably can use 'prebiotics' in the form of particular soil amendments and substrates to maintain this biological stability in the plant holobiont and favor beneficial interactions.

fungistasis [27]. Organically amended soil typically harbors a potentially better operational microbiome with more positive microbial activities and more functionally interrelated species than do soils where chemical fertilizers were applied [52]. Characterizing the functional groups of microbes that are associated with particular organic amendments will be necessary to better understand soil health. Fine-tuning and optimizing the use of organic amendments for specific soil/crop combinations would establish sustainable and durable soil health and its microbial ecosystem services, resulting in equal or better yields than chemical fertilizers alone.

Signaling molecules from plants, such as salicylic acid and various root exudates, also strongly influence rhizosphere microbiome composition and dynamics [53]. This offers the possibility of using these molecules to modulate microbiomes in a purposeful manner. For example, phenolic root exudates such as **coumarins** have a role in root microbiome conditioning [54], and have benefits for disease suppression and in assisting plants to attain more bioavailable iron from the soil [55]. Various studies suggest that coumarins inhibit soil-borne pathogens while not affecting the beneficial rhizobacteria [54,55]. **Benzoxazinoids** are another class of maize root exudates that have a role in shaping the bacterial and fungal microbiome in the rhizosphere, resulting in protection against herbivore insect attacks [56]. Future studies should evaluate how exudates such as coumarins, malic acid, camalexin [57], and benzoxazinoids can contribute towards microbiome engineering.



Identifying which exudates attract which microbes is a valuable task ahead because this will enable the specific use of these compounds to attract and maintain PGPR. For example, the recruitment of a beneficial microbiome to soil that helped plants to grow under saline conditions has recently been achieved by the addition of the root exudate and ethylene precursor, **1-aminocyclopropane-1-carboxylic acid (ACC)**, before plant cultivation [40]. Plants under biotic or abiotic stress exude ACC into the soil to prevent the biosynthesis of excess ethylene that would otherwise cause additional stress to plants. By simply adding ACC to soil 2 weeks before planting, microbial consortia are attracted and proliferate that collectively facilitate ACC cleavage into ammonia and  $\alpha$ -ketobutyrate by synthesizing ACC deaminase. When plants are under stress at a later stage, the previously attracted 'ACC-hungry' microbiome quickly degrades ACC exudates, thus enabling more ACC exudation and reducing ethylene levels in plants. A long-term goal of this approach would be to analyze and validate the impacts of ACC soil amendments on the soil microbiome and yields of various crops under various stress conditions.

#### Artificial Microbial Consortia

Artificial (or synthetic) microbial consortia (AMC) resemble synthetic biology that can reconstruct the structure and function of the plant microbiome. It is possible to compose AMC that contain multiple functions for plant growth promotion. This can potentially solve some of the drawbacks of traditional microbial biofertilizers, such as host incompatibility, ineffective competitiveness with indigenous microbes, and inadaptability to the local environment [58]. The various steps involved in designing the ideal AMC include selecting the origin of the microbes, obtaining and culturing the core microorganisms, optimizing the microbial interactions according to their compatibility, and assessing the efficacy of these consortia [59]. A bacterial consortium consisting of *Comamonas testosteroni*, *Pseudomonas putida*, *Enterobacter cloacae*, and *Citrobacter freundii* has been reported to enhance phosphate mobility and improved crop productivity by twofold [60]. Similarly, an interaction between the diazotrophic N-fixing bacterium *Azotobacter vinelandii* and the mycorrhizal fungus *Rhizophagus irregularis* has been shown to significantly improve root exploration under field conditions, leading to improved nutrient uptake in wheat [61]. AMC also play a role in stress tolerance, and a consortium containing *Pseudomonas putida* KT2440, *Sphingomonas* sp. OF178, *Azospirillum brasilense* Sp7, and *Acinetobacter* sp. EMM02 led to improved drought stress tolerance in maize [62]. Because the rhizosphere microbiome can alter plant growth by secreting particular phytohormones [63], a recent study designed two synthetic microbial communities that consisted of bacterial strains showing ACC deaminase activity [64]. Inoculation of these synthetic microbial consortia resulted in antimicrobial activity against *F. oxysporum* f. sp. *lycopersici*, reduced symptoms on tomato plants, and also enhanced growth on a poor substrate.

#### Microbiome Breeding and Transplantation

Microbiome breeding can be achieved based on the principle that the host plant exhibits traits that favor beneficial microbes through generations. It involves indirectly selecting the microbiome by allowing the host to screen which subsets of microorganisms are allowed to interact with it and which will be transmitted vertically to their offspring [65]. This method is based on propagating one phenotype of the host which is significantly affected by the microbiome. A multigenerational experimental system was recently used to select for microbes that influence late or early flowering in *Arabidopsis thaliana* [66]. Over ten generations, the four most suitable microcosms were selected based on the desired early- or late-flowering phenotype. Plants inoculated with 'late-flowering microbiomes' displayed increased inflorescence [67]. This microbiome breeding and transplantation method has potential in diverse applications, including breeding of soil microbiomes to promote growth or to withstand biotic and abiotic stresses such as soil-borne diseases, salinity, or drought. However, future research should focus on optimizing the selection

method, quantifying the generational change to track the intergenerational change within the microbiome, ensuring the stability of the selected microbiomes, developing practical microbiome transplantation methods, and assessing their functionality for improved crop production in field trials.

Microbiome transplantation can also be successful without microbiome breeding and even outside of the soil. For example, Wicaksono and colleagues [68] looked at the mechanism by which the medicinal manuka plant (*Leptospermum scoparium*) releases antimicrobial oils against the bacterial pathogen *Pseudomonas syringae* and whether this could be replicated in other models using the wound as an entry point. Indeed, transplantation of manuka plant microbiomes to kiwi fruit through wound inoculation could mimic the same antimicrobial effect and promote plant defense against *P. syringae* infection. Similarly, the role of plant-recruited rhizobacteria in disease resistance was assessed by comparing the root microbiome and metagenomes of *Ralstonia solanacearum*-resistant and -susceptible tomato varieties, and flavobacteria were found to be more abundant in the rhizosphere of the resistant plant [69]. Transplantation of the *Flavobacterium*-abundant microbiome from the rhizosphere of a resistant variety also suppressed disease symptoms in susceptible plants.

#### Host-Mediated Microbiome Engineering

It is now certain that the plant genotype plays a crucial role in the assembly and function of rhizospheric microbiomes and in benefiting from PGPR. For example, *Arabidopsis* plants inoculated with bacterial biofertilizer *Pseudomonas simiae* WCS417r produced up to fourfold more biomass in some accessions than did other genotypes where the effect was hardly noticeable [70]. This suggests that selecting and breeding 'microbe-friendly' cultivars can offer an enormous potential for improved crop yields.

At the microbiome level, different plant genotypes also attract to a variable extent beneficial and disease-suppressing microbes and restructure their microbiome assembly through variation in root exudates [47,69]. Both studies compared plant cultivars with variable resistance levels against wilt-causing diseases and reported that some bacteria were more abundant in the rhizosphere of resistant cultivars. Comparison of different bean accessions also showed a strong genotype-dependent effect on rhizosphere microbiome assembly, and only 0.7% **operational taxonomic units** (OTUs) were shared across all eight bean accessions tested [71].

Plant functional genomic studies during beneficial plant–microbe interactions has enabled the manipulation of plant genomes to attract and maintain beneficial microbiomes [72]. To this end, 'designer plants' could be genetically engineered to release hormones or exudates that attract and maintain beneficial microbiomes. Various studies have also emphasized that wild-type crop relatives can provide a new perspective into the role of wild plant genes that are associated with microbiome assembly compared with domesticated types [73]. Comparisons of wild bean accessions with modern accessions showed an increased relative abundance of Bacteroidetes in some accessions, whereas others were enriched in Actinobacteria and Proteobacteria, suggesting that the shift is associated with plant genotype and altered root morphological traits [71].

#### Concluding Remarks

Current industrial agriculture should focus on sustainable practices that minimize negative environmental impacts without compromising food security. This review has explored the complexities of the interaction between the players present in the plant holobiont ecosystem which is key to novel methods of agricultural practices. Future research should focus on customizing PGPR for various cropping systems and on delivery and maintenance methods for beneficial microbial

#### Outstanding Questions

How do microbiome changes occur under natural conditions, and how can we accurately monitor the complex plant–microbe interactions?

How can we systematically decipher the comprehensive chemical language spoken between root exudates, attracted/repelled microbial taxa, and their services to the plant?

What are useful selection technologies for breeding 'microbe-friendly' crop cultivars and 'plant-friendly' microbiomes?

Can we group core beneficial microbial consortia into categories that work better for common crop–soil–environment combinations? Would this knowledge then enable a customized approach to maximize beneficial plant–microbiome interactions?

How can we successfully inoculate plants with useful microbial consortia or transplant beneficial microbiomes, while stabilizing and maintaining their activity over time and possibly over several generations?

consortia that ensure productivity through several plant generations. Targeting seed endophytes, selecting ‘microbe-friendly’ plants or genetically engineering plant genomes to attract beneficial microbes, could ensure the continuity of the microbiome from parent to offspring. Before this technology is ready for field application, more research will be necessary to fully characterize the types of beneficial microbes for a wide variety of crop plants. The benefits provided need to be better categorized and quantified (e.g., by functional analyses using metatranscriptomics, proteomics, and metabolomics in contrasting conditions), and many new benefits are likely to be discovered. Major challenges remain for microbiome engineering (see [Outstanding Questions](#)), and [Box 3](#) provides some suggestions for how these could be addressed using emerging technologies. A combination of soil amendments, plant breeding/genetic modification, and targeted microbiome engineering could reduce the use of chemical fertilizers or pesticides and lead to higher yields and more resilient crops. The choice of which emerging technologies to use should be based on previous studies for the particular crop, soil, and environment, and on how these methods can be practically integrated to the traditional cropping system.

## References

- Ray, D.K. *et al.* (2012) Recent patterns of crop yield growth and stagnation. *Nat. Commun.* 3, 1293
- Singh, V.K. (2018) Interaction of plant growth promoting bacteria with tomato under abiotic stress: a review. *Agric. Ecosyst. Environ.* 267, 129–140
- Syed Ab Rahman, S.F. *et al.* (2018) Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* 267, 102–111
- Fukamachi, K. (2019) Disease control of *Phytophthora infestans* using cyazofamid encapsulated in poly lactic-co-glycolic acid (PLGA) nanoparticles. *Colloids Surf. A Physicochem. Eng. Asp.* 577, 315–322
- Sankar Ganesh, K. *et al.* (2017) Role of organic amendments in sustainable agriculture. In *Sustainable Agriculture towards Food Security* (Dhanarajan, A., ed.), pp. 111–124, Springer, Singapore
- Saeid, A. and Chojnacka, K. (2019) Fertilizers: need for new strategies. In *Organic Farming* (Chandran, S. *et al.*, eds), pp. 91–116, Woodhead Publishing
- Wang, S. *et al.* (2015) Responses of soil microarthropods to inorganic and organic fertilizers in a poplar plantation in a coastal area of eastern China. *Appl. Soil Ecol.* 89, 69–75
- Qadir, M. *et al.* (2014) Economics of salt-induced land degradation and restoration. *Nat. Resour. Forum* 38, 282–295
- Qian, M. *et al.* (2016) Occurrence of trace elements and antibiotics in manure-based fertilizers from the Zhejiang Province of China. *Sci. Total Environ.* 559, 174–181
- Elouear, Z. *et al.* (2016) Application of sheep manure and potassium fertilizer to contaminated soil and its effect on zinc, cadmium and lead accumulation by alfalfa plants. *Sustain. Environ. Res.* 26, 131–135
- Kumar, B.L. and Gopal, D.S. (2015) Effective role of indigenous microorganisms for sustainable environment. *3 Biotech.* 5, 867–876
- Usmani, Z. *et al.* (2017) Vermicomposting of coal fly ash using epigeic and epi-endogeic earthworm species: nutrient dynamics and metal remediation. *RSC Adv.* 7, 4876
- Hellequin, E. *et al.* (2018) Specific recruitment of soil bacteria and fungi decomposers following a biostimulant application increased crop residues mineralization. *PLoS One* 13, e0209089
- De Corato, U. (2020) Disease-suppressive compost enhances natural soil suppressiveness against soil-borne plant pathogens: a critical review. *Rhizosphere* 13, 100192
- Testen, A.L. and Miller, S.A. (2018) Carbon source and soil origin shape soil microbiomes and tomato soilborne pathogen populations during anaerobic soil disinfestation. *Phytobiomes* 2, 138–150
- Hartman, K. *et al.* (2018) Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* 6, 14
- Paungfoo-Lonhienne, C. *et al.* (2017) Legume crop rotation suppressed nitrifying microbial community in a sugarcane cropping soil. *Sci. Rep.* 7, 1–7
- Locey, K.J. and Lennon, J.T. (2016) Scaling laws predict global microbial diversity. *Proc. Natl. Acad. Sci. U. S. A.* 13, 5970–5975
- Renuka, N. *et al.* (2018) Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. *Biotechnol. Adv.* 36, 1255–1273
- Marks, E.A.N. *et al.* (2017) Application of a microalgal slurry to soil stimulates heterotrophic activity and promotes bacterial growth. *Sci. Total Environ.* 605, 610–617
- Win, T.T. *et al.* (2018) Algal biofertilizers and plant growth stimulants for sustainable agriculture. *Ind. Biotechnol.* 14, 203–211
- Whitman, T. *et al.* (2016) Dynamics of microbial community composition and soil organic carbon mineralization in soil following addition of pyrogenic and fresh organic matter. *ISME J.* 10, 2918–2930
- Liu, L. *et al.* (2017) Biochar amendments increase the yield advantage of legume-based intercropping systems over monoculture. *Agric. Ecosyst. Environ.* 237, 16–23
- Eizenberg, H. *et al.* (2017) Non-chemical control of root parasitic weeds with biochar. *Front. Plant Sci.* 8, 939
- George, C. *et al.* (2016) Biochars reduce infection rates of the root-lesion nematode *Pratylenchus penetrans* and associated biomass loss in carrot. *Soil Biol. Biochem.* 95, 11–18
- Elad, Y. *et al.* (2012) The biochar effect: plant resistance to biotic stresses. *Phytopathol. Mediterr.* 50, 335–349
- Bononomi, G. *et al.* (2018) Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. *Annu. Rev. Phytopathol.* 56, 1–20
- Timmusk, S. *et al.* (2017) Perspectives and challenges of microbial application for crop improvement. *Front. Plant Sci.* 8, 49
- Miransari, M. (2014) Plant growth promoting rhizobacteria. *J. Plant Nutr.* 37, 2227–2235
- Souza, R. *et al.* (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.* 38, 401–419
- Compant, S. *et al.* (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *J. Advert. Res.* 19, 29–37
- Zmora, N. *et al.* (2016) Taking it personally: personalized utilization of the human microbiome in health and disease. *Cell Host Microbe* 19, 12–20
- Rojo, D. *et al.* (2017) Exploring the human microbiome from multiple perspectives: factors altering its composition and function. *FEMS Microbiol. Rev.* 41, 453–478
- Reardon, S. (2014) Microbiome therapy gains market traction. *Nature* 509, 269–270
- Carvalho, L.C. *et al.* (2013) Plant growth in *Arabidopsis* is assisted by compost soil-derived microbial communities. *Front. Plant Sci.* 4, 235
- Lloyd-Price, J. *et al.* (2016) The healthy human microbiome. *Genome Med.* 8, 1–11
- Rojas-Solis, D. *et al.* (2018) *Pseudomonas stutzeri* E25 and *Stenotrophomonas maltophilia* CR71 endophytes produce

- antifungal volatile organic compounds and exhibit additive plant growth-promoting effects. *Biocat. Agric. Biotech.* 13, 46–52
38. Vyas, P. *et al.* (2018) Screening and characterization of *Achromobacter xylosoxidans* isolated from rhizosphere of *Jatropha curcas* L. (energy crop) for plant-growth-promoting traits. *J. Adv. Res. Biotechnol.* 3, 1–8
  39. Dubey, A. *et al.* (2019) Soil microbiome: a key player for conservation of soil health under changing climate. *Biodivers. Conserv.* 28, 2405–2429
  40. Liu, H. *et al.* (2019) Soil amendments with ethylene precursor alleviate negative impacts of salinity on soil microbial properties and productivity. *Sci. Rep.* 9, 6892
  41. Danish, S. and Zafar-ul-Hye, M. (2019) Co-application of ACC-deaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress. *Sci. Rep.* 9, 5999
  42. Pascale, A. *et al.* (2020) Modulation of the root microbiome by plant molecules: the basis for targeted disease suppression and plant growth promotion. *Front. Plant Sci.* 10, 1741
  43. Orozco-Mosqueda, M.D.C. *et al.* (2018) Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. *Microbiol. Res.* 208, 25–31
  44. Ma, Y. *et al.* (2019) Potential of plant beneficial bacteria and arbuscular mycorrhizal fungi in phytoremediation of metal-contaminated saline soils. *J. Hazard. Mater.* 379, 120813
  45. Syed-Ab-Rahman, S.F. *et al.* (2019) Suppression of *Phytophthora capsici* infection and promotion of tomato growth by soil bacteria. *Rhizosphere* 9, 72–75
  46. Chihaoui, S.-A. *et al.* (2015) Inoculation of *Phaseolus vulgaris* with the nodule-endophyte *Agrobacterium* sp. 10C2 affects richness and structure of rhizosphere bacterial communities and enhances nodulation and growth. *Arch. Microbiol.* 197, 805–813
  47. Mitter, B. *et al.* (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Front. Microbiol.* 8, 11
  48. Mendes, L.W. *et al.* (2018) Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. *ISME J.* 12, 212–224
  49. Johnston-Monje, D. and Raizada, M.N. (2011) Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One* 6, e20396
  50. Paungfoo-Lonhienne, C. *et al.* (2010) Turning the table: plants consume microbes as a source of nutrients. *PLoS One* 5, e11915
  51. Anderson, J.P. *et al.* (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. *Plant Cell* 16, 3460–3479
  52. Ling, N. *et al.* (2016) Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. *Soil Biol. Biochem.* 99, 137–149
  53. Finkel, O.M. *et al.* (2017) Understanding and exploiting plant beneficial microbes. *Curr. Opin. Plant Biol.* 38, 155–163
  54. Voges, M. *et al.* (2019) Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. *Proc. Natl. Acad. Sci. U. S. A.* 116, 12558–12565
  55. Stringlis, I.A. *et al.* (2019) The age of coumarins in plant-microbe interactions. *Plant Cell Physiol.* 60, 1405–1419
  56. Cotton, T.E.A. *et al.* (2019) Metabolic regulation of the maize rhizobiome by benzoxazinoids. *ISME J.* 13, 1647–1658
  57. Koprivova, A. *et al.* (2019) Root-specific camalexin biosynthesis controls the plant growth promoting effects of multiple bacterial strains. *Proc. Natl. Acad. Sci. U. S. A.* 116, 15735–15744
  58. Hart, M. *et al.* (2018) Fungal inoculants in the field: is the reward greater than the risk? *Funct. Ecol.* 32, 126–135
  59. Kong, Z. *et al.* (2018) Paving the way from the lab to the field: using synthetic microbial consortia to produce high-quality crops. *Front. Plant Sci.* 9, 1467
  60. Baas, P. *et al.* (2016) Phosphorus mobilizing consortium Mammoth P (TM) enhances plant growth. *Peer J.* 4, e2121
  61. Dal Cortivo, C. *et al.* (2018) Effects of field inoculation with VAM and bacteria consortia on root growth and nutrients uptake in common wheat. *Sustainability* 10, 3286
  62. Molina-Romero, D. *et al.* (2017) Compatible bacterial mixture, tolerant to desiccation, improves maize plant growth. *PLoS One* 12, 1–21
  63. Stringlis, I.A. *et al.* (2018) MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc. Natl. Acad. Sci. U. S. A.* 115, E5213–E5222
  64. Tsolakidou, M.D. *et al.* (2019) Rhizosphere-enriched microbes as a pool to design synthetic communities for reproducible beneficial outputs. *FEMS Microbiol. Ecol.* 95, 10
  65. Mueller, U.G. and Sachs, J.L. (2015) Engineering microbiomes to improve plant and animal health. *Trends Microbiol.* 23, 606–617
  66. Panke-Buisse, K. *et al.* (2017) Cultivated sub-populations of soil microbiomes retain early flowering plant trait. *Microb. Ecol.* 73, 394–403
  67. Panke-Buisse, K. *et al.* (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J.* 9, 980–989
  68. Wicaksono, W.A. *et al.* (2017) Biological control of *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, using endophytic bacteria recovered from a medicinal plant. *Biol. Control* 116, 103–112
  69. Kwak, M.J. *et al.* (2018) Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nat. Biotechnol.* 36, 1100–1109
  70. Wintermans, P.C. *et al.* (2016) Natural genetic variation in *Arabidopsis* for responsiveness to plant growth-promoting rhizobacteria. *Plant Mol. Biol.* 90, 623–634
  71. Pérez-Jaramillo, J.E. *et al.* (2019) Deciphering rhizosphere microbiome assembly of wild and modern common bean (*Phaseolus vulgaris*) in native and agricultural soils from Colombia. *Microbiome* 7, 114
  72. Stringlis, I.A. *et al.* (2018) Microbial small molecules – weapons of plant subversion. *Nat. Prod. Rep.* 35, 410–433
  73. Pérez-Jaramillo, J.E. *et al.* (2018) The wild side of plant microbiomes. *Microbiome* 6, 143
  74. Ribaudo, C.M. *et al.* (2006) *Azospirillum* sp. promotes root hair development in tomato plants through a mechanism that involves ethylene. *J. Plant Growth Regul.* 25, 175–185
  75. Remans, R. *et al.* (2007) Effects of plant growth-promoting rhizobacteria on nodulation of *Phaseolus vulgaris* L. are dependent on plant P nutrition. *Eur. J. Plant Pathol.* 119, 341–351
  76. Rodrigues, E.P. *et al.* (2008) *Azospirillum amazonense* inoculation: effects on growth, yield and N<sub>2</sub> fixation of rice (*Oryza sativa* L.). *Plant Soil* 302, 249–261
  77. Fierer, N. (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15, 579–590
  78. Xia, Y. *et al.* (2015) Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. *Front. Plant Sci.* 6, 490
  79. Rascovan, N. *et al.* (2016) Integrated analysis of root microbiomes of soybean and wheat from agricultural fields. *Sci. Rep.* 6, 28084
  80. Sewelam, N. *et al.* (2016) Global plant stress signaling: reactive oxygen species at the cross-road. *Front. Plant Sci.* 7, 187
  81. Cha, J.Y. *et al.* (2016) Microbial and biochemical basis of a *Fusarium* wilt-suppressive soil. *ISME J.* 10, 119–129
  82. Zelezniak, A. *et al.* (2015) Metabolic dependencies drive species co-occurrence in diverse microbial communities. *Proc. Natl. Acad. Sci. U. S. A.* 112, 6449–6454
  83. Pieterse, C.M.J. *et al.* (2014) Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52, 347–375