Review

The rhizosphere microbiome and plant health

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The diversity of microbes associated with plant roots is enormous, in the order of tens of thousands of species. This complex plant-associated microbial community, also referred to as the second genome of the plant, is crucial for plant health. Recent advances in plant–microbe interactions research revealed that plants are able to shape their rhizosphere microbiome, as evidenced by the fact that different plant species host specific microbial communities when grown on the same soil. In this review, we discuss evidence that upon pathogen or insect attack, plants are able to recruit protective microorganisms, and enhance microbial activity to suppress pathogens in the rhizosphere. A comprehensive understanding of the mechanisms that govern selection and activity of microbial communities by plant roots will provide new opportunities to increase crop production.

The rhizosphere microbiome

Pathogens can have a severe impact on plant health. The interactions between plants and pathogens are regularly simplified as trench warfare between the two parties, ignoring the importance of additional parties that can significantly affect the infection process. Plants live in close association with the microbes that inhabit the soil in which plants grow. Soil microbial communities represent the greatest reservoir of biological diversity known in the world so far [1–4]. The rhizosphere, which is the narrow zone of soil that is influenced by root secretions, can contain up to 10^{11} microbial cells per gram root [5] and more than 30,000 prokaryotic species [6]. The collective genome of this microbial community is much larger than that of the plant and is also referred to as the plant's second genome. In humans, the effects of intestinal microbial communities on health are becoming increasingly apparent [7]. Similar functions can be ascribed to microbial communities in the human gut and plant rhizosphere (Table 1). An increasing body of evidence also signifies the importance of this root microbiome, which consists of the entire complex of rhizosphere-associated microbes, their genetic elements and their interactions, in determining plant health. Here, we discuss how rhizosphere microbial communities, with an emphasis on bacteria, affect the plant and vice versa.

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The root microbiome: effects on plant health

Disease-suppressive soils

The impact of the root microbiome on plant health is evidenced most clearly in disease-suppressive soils (Figure 1). The microflora of most soils is starved. As a result, there is a fierce battle in the rhizosphere between the microorganisms that compete for plant-derived nutrients [8]. Most soil-borne pathogens need to grow saprophytically in the rhizosphere to reach their host or to achieve sufficient numbers on their host before they can infect host tissue and effectively escape the rhizosphere battle zone. The success of a pathogen is influenced by the microbial community of the soil in which the infection takes place. Every natural soil has the ability to suppress a pathogen to a certain extent. This can be deduced from the disease severity following pathogen inoculation in pasteurized soils compared with non-pasteurized soils. This phenomenon is known as general disease suppression and is attributed to the total microbial activity. Organic amendments can stimulate the activity of microbial populations in a conducive soil, resulting in enhanced general disease suppressiveness [9]. 'Specific suppression' occurs when specific microorganisms cause soils to be suppressive to a disease [8,10,11]. Specific disease suppressiveness is superimposed on the general disease suppressiveness of soils and is more effective. The biotic nature of specific disease suppressiveness is also demonstrated by the removal of suppressiveness through pasteurization of the soil, but is distinguished from general suppressiveness because specific suppressiveness can be transferred to disease conducive soil by adding 0.1–10% of the suppressive soil [6,8,10,11].

Build-up of disease suppressiveness

A further differentiation is made among specific disease-suppressive soils; some soils retain their disease suppressiveness for prolonged periods and persist even when soils are left bare, whereas other soils develop suppressiveness only after monoculture of a crop for several years. Induction of suppressiveness by itself is remarkable, because for most plant species, successive monocultures will lead to a build-up of specialized plant pathogens [12]. Nonetheless, development of disease suppressiveness in soils has been reported for various diseases, including potato scab disease caused by *Streptomyces* species, Fusarium wilt disease of several plant species, Rhizoctonia damping-off disease of sugar beet, and take-all disease of wheat (*Triticum aestivum*) caused by *Gaeumannomyces graminis* var. *tritici* [6,8,11]. Microorganisms that can confer suppressiveness

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Table 1. Similarities of the microbiomes of the human gut and plant roots

Characteristic	The human gut microbiome	Refs	The rhizosphere microbiome	Refs
Important for nutrient uptake	Microbiota assist in the breakdown of dietary products and produce essential nutrients, such as vitamins B and D. In return, the microbiome is provided with a steady supply of carbon in the form of mucins	[85,86]	Mycorrhiza and rhizobia assist plants with the uptake of phosphorus and nitrogen, respectively. Furthermore, the microbiome assists in the weathering of minerals and the degradation of recalcitrant organic matter. In return, the microbiome is provided with carbon in root exudates and other rhizodeposits	[38,87]
Prevent colonization by pathogens	Mechanisms include competition for nutrients or for adhesion receptors, production of inhibitory metabolites and modulation of toxin production or action	[86]	Mechanisms include competition for (micro)nutrients, production of antibiotic compounds or lytic enzymes and consumption of pathogen stimulatory compounds	[13,14]
Modulate host immunity	Host innate immune systems adapt to colonization by microflora and shift to a primed state that not only affects the intestinal mucosa, but can also regulate immune response in respiratory mucosa	[7,86,88]	Many beneficial soil-borne microorganisms have been found to boost systemically the defensive capacity of the plant. This ISR is a state in which the plant immune system is primed for accelerated activation of defense	[27]
	Furthermore, development of the gut microflora in the first year of life is of crucial importance in the development of the immune system and for susceptibility to development of disease later in life			
Distinguish friend from foe	As symbionts and pathogens express similar molecular patterns that are recognized by the innate immune system, it is not entirely known how the innate immune system distinguishes friend from foe. Multiple mechanisms are present to avoid aberrant activation of the immune system (e.g. physical barrier provided by mucus, induced desensitization of epithelial cells to bacterial lipopolysaccharide, and low levels of pathogen receptors on apical surface of epithelial cells)	[89,90]	Symbionts and pathogens express similar molecular patterns that are recognized by the innate immune system, and it is largely unknown how plants distinguish friend from foe	[15]
	Many commensal microbes are potentially pathogenic but are controlled by the host immune system; commensals can become pathogenic in immunity-impaired mice		Both pathogens and beneficials are also known to suppress plant immune response to promote their own colonization through secretion of effector molecules	
	The adaptive immune system is trained to be tolerant of commensals. Regulatory T-cells not only suppress immune response to self, but are also educated to suppress immune responses to the commensal gut microbiota			
Microbiome density and diversity	Although microbial density is high, with typically 10^{11}–10^{12} microbial cells per ml of intestinal fluid, the phylogenetic diversity is relatively low. Only seven of the 55 described bacterial phyla are found in the human gut, which is dominated by Firmicutes and Bacteroidetes. It is estimated that some 500–1000 species of bacteria exist in the human gut	[91,92]	In the rhizosphere, the microbial density is typically higher than in bulk soil and ranges from 10^8 to 10^9 bacteria per gram. However, soil microbial communities are considered to hold the most diverse microbial communities in the world, with up to 10^4 bacterial species per gram	[62,93]
	There is an indication that intestinal microbial variation between individuals is stratified rather than continuous, and that there is a limited amount of classifiable communities that can exist in the gut, coined 'enterotypes'		Root microbiomes of plants grown in the same soil differ between plant species and between genotypes within a species. However, the existence of classifiable 'rhizotypes' has not yet been reported	

to otherwise conducive soils have been isolated from many suppressive soils. Take-all, an important root disease of wheat caused by *G. graminis* var. *tritici* spontaneously decreases after several years of monoculture of wheat and a severe outbreak of the disease (Figure 1). This phenomenon is known as 'take-all decline'. It is observed worldwide and has been associated with the build-up of antagonistic fluorescent *Pseudomonas* spp. that produce the antifungal compound 2,4-diacetylphloroglucinol (DAPG) [11]. Other microorganisms that can confer suppressiveness have been found among the Proteobacteria and Firmicutes and for fungi among the Ascomycota (reviewed in [8]). Mechanisms through which rhizosphere microorganisms can affect a soil-borne pathogen have been identified and include production of antibiotic compounds, consumption of pathogen stimulatory compounds, competition for (micro)nutrients and production of lytic enzymes [13,14].

Modulation of the host immune system by beneficial microbes in the rhizosphere

In addition to direct effects on deleterious microbes in the rhizosphere, many beneficial soil-borne microorganisms have been found to boost the defensive capacity in aboveground parts of the plant [15]. This induced systemic resistance (ISR) is a state in which the immune system of the plant is primed for accelerated activation of defense

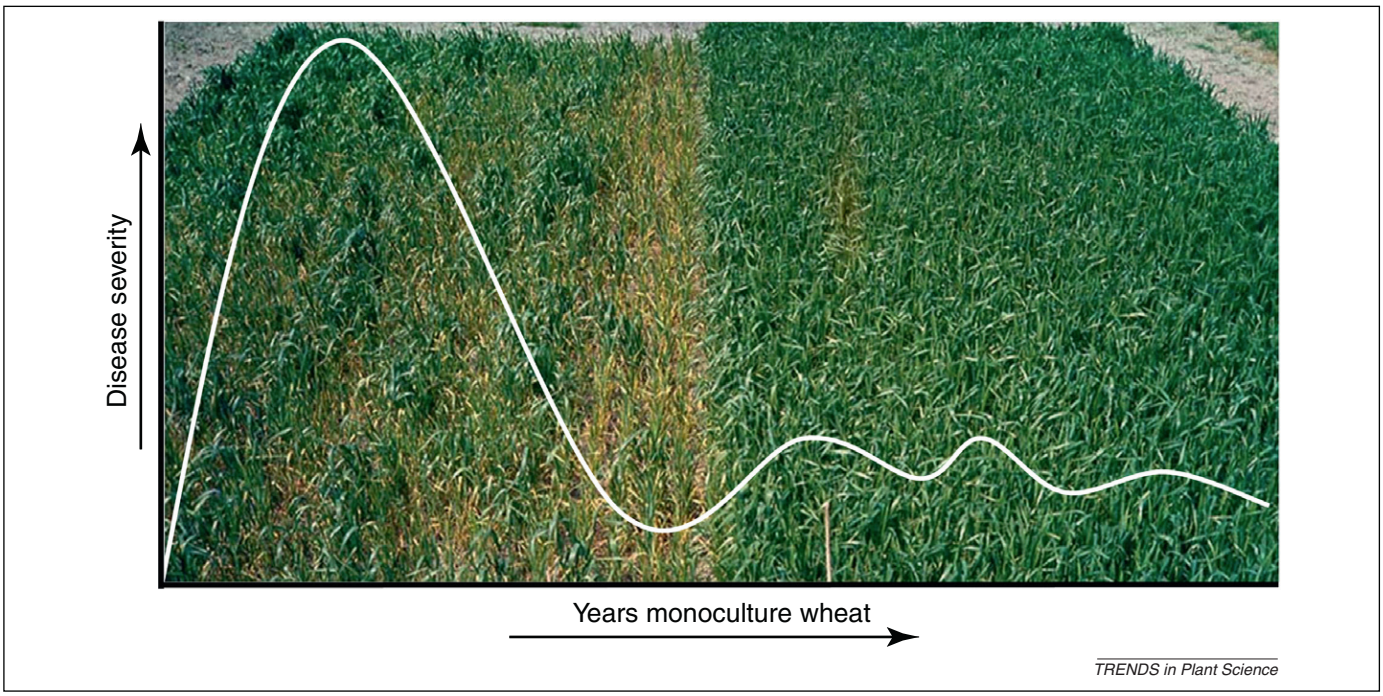

Figure 1. Disease-suppressive soils. For many soil-borne plant diseases, it has been found that the incidence of disease in some soils is lower than in surrounding soils, even though a virulent pathogen is present. Such disease-suppressive soils are especially well studied for take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*. During monoculture of wheat, take-all disease usually develops as depicted. An initial increase of disease severity as pathogen inoculum builds up with recurring presence of a susceptible host is followed by a decline of disease severity. This is typically associated with an increase of *Pseudomonas* spp. that produce the antifungal 2,4-diacetylphloroglucinol. The picture in the background shows an experimental field (Flevoland, the Netherlands) in which wheat had been grown in rotation with other crops (left) or in monoculture (right). Following inoculation with *G. graminis* var. *tritici*, less disease developed in the wheat monoculture plot. Adapted from [11].

[16–20]. Induction of ISR in *Arabidopsis* (*Arabidopsis thaliana*) by the plant growth-promoting rhizobacterium *Pseudomonas fluorescens* WCS417 is well studied. Locally in roots, WCS417 is able to suppress flagellin-triggered immune responses via apoplastic secretion of one or more low-molecular-weight molecules [21]. Although locally plant immunity is suppressed, an immune signaling cascade is initiated systemically that confers resistance against a broad spectrum of pathogens and even insects [22–24]. Initiation of this ISR in the roots of *Arabidopsis* is regulated by the root-specific transcription factor MYB72, which acts locally in the generation or translocation of a so far unknown systemic signal [25]. The ISR response is often not associated with direct defense activation, but instead with priming for accelerated defense-related gene expression and increased deposition of callose at the site of pathogen entry [23]. Establishment of WCS417-ISR in systemic leaf tissues depends on the hormones jasmonic acid and ethylene and requires the transcriptional (co)-activators NPR1 and MYC2 ([26,27]. In addition to plant growth-promoting rhizobacteria, beneficial fungi such as mycorrhizal fungi [18], *Trichoderma* spp. [28] and other fungal biocontrol agents [29] have also been found to induce ISR. As well as inducing systemic resistance, mycorrhizal fungi can also form a connecting network between plants that can convey a resistance-inducing signal to neighboring plants [30].

Microbiome complexity

The identification of specific microorganisms responsible for disease suppressiveness has relied mainly on cultivation-dependent techniques. Recently, bacterial communities have been characterized [6] in the rhizosphere of sugar beet grown in *Rhizoctonia solani*-suppressive soils using a high-density 16S ribosomal DNA oligonucleotide microarray referred to as PhyloChip [31]. More than 33,000 bacterial and archaeal operational taxonomic units were present in the rhizospheres of plants grown in either suppressive or conducive soil. However, neither the number nor the exclusive presence of microbial taxa could be related to disease suppressiveness. Instead, the relative abundance of several taxa was found to correspond to disease suppressiveness. The culture-independent approach identified the Gammaproteobacteria, Betaproteobacteria and Firmicutes as groups of bacteria that are important in disease suppressiveness. It was concluded that disease suppressiveness in the *R. solani* suppressive soil could not be attributed to a single taxon, but that it was brought about by a consortium of microorganisms.

Although specific microorganisms are able to protect the plant either directly or indirectly against pathogens, their efficacy is largely influenced by the rest of the microbial community. First, the pathogen-suppressing microorganisms should be present in sufficiently high numbers to have a significant effect [32,33]. Second, microorganisms that are regarded as commensals, because they neither harm nor benefit the plant directly, can compete effectively with the pathogen-suppressing biocontrol bacteria. Biocontrol bacteria may also act synergistically on each other, as seemingly non-antagonistic bacterial strains can become antagonistic when grown together with other specific strains [34]. Also, it was found that the soil-inhabiting *P. fluorescens* strain PF0-1 fine-tuned its transcriptional and metabolic responses in confrontation with different

bacterial competitors and responded differently toward different species [35]. Conversely, negative effects of pathogens on their antagonists have also been reported. For example, *Fusarium oxysporum* produces fusaric acid, which downregulates the production of the antibiotic compound DAPG, a key factor in the antagonistic activity of *P. fluorescens* CHA0 against this pathogen [36]. It can be argued that all the active microorganisms affect each other in one way or another, albeit indirectly. Hence, although specific functions can be attributed to specific microorganisms, it is the total microbiome and its interactions that affect plant health (Figure 2).

Plants actively shape their root microbiome

Species-specific microbiomes

The microflora of most soils is carbon starved [37]. Because plants secrete up to 40% of their photosynthates into the rhizosphere [38], the microbial population densities in the rhizosphere are much higher than in the surrounding bulk soil. This phenomenon is known as the 'rhizosphere effect'. In general, rhizosphere microbial communities are less diverse than those of the bulk soil [39–41]. It appears that, from the reservoir of microbial diversity that the bulk soil comprises, plant roots select for specific microorganisms to prosper in the rhizosphere. Together with the plant

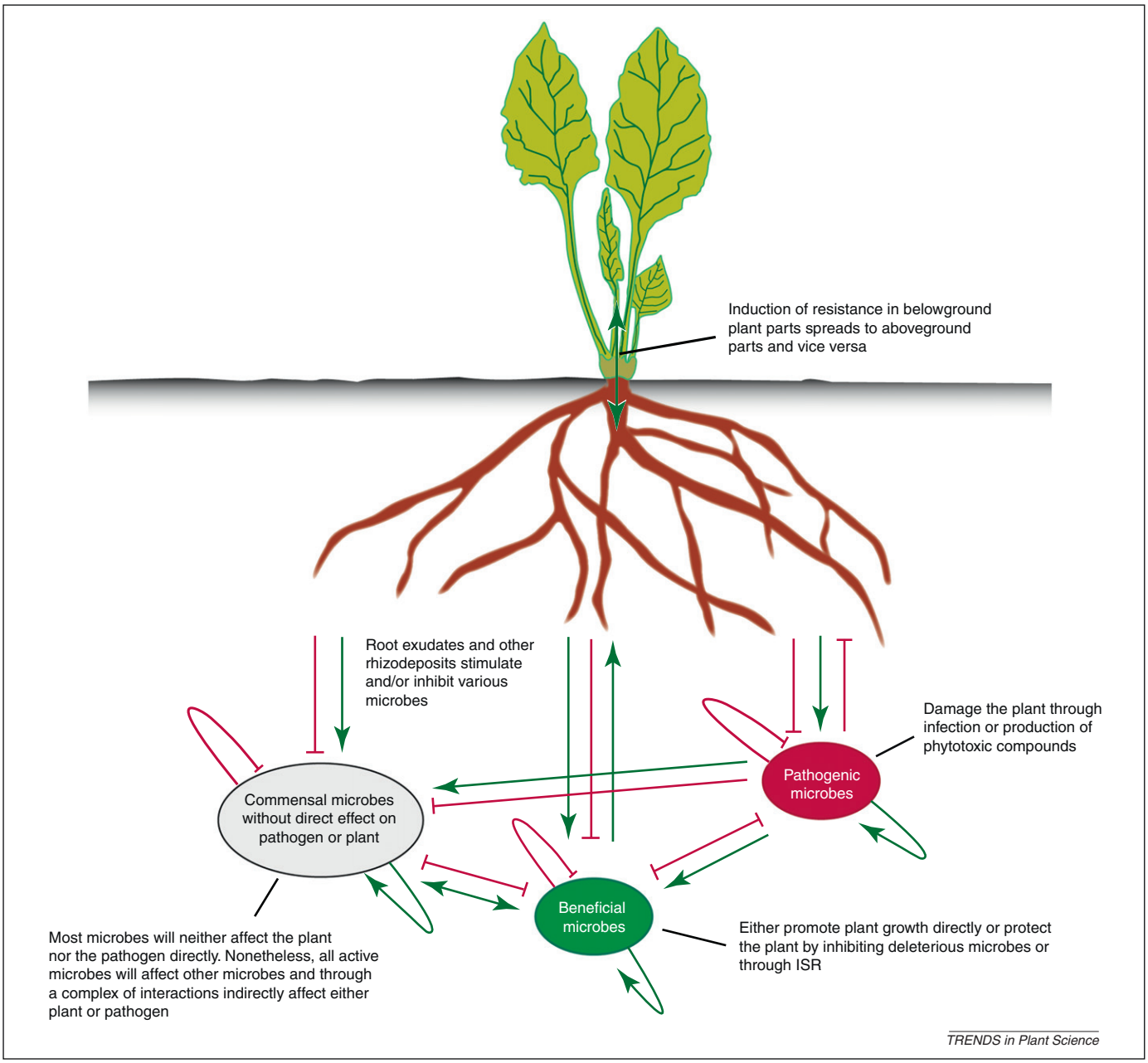

Figure 2. Interactions in the rhizosphere. Plants are able to influence the composition and activation of their rhizosphere microbiome through exudation of compounds that stimulate (green arrows) or inhibit (red blocked arrows). Vice versa, a wide range of soil-borne pathogens are able to affect plant health. Prior to infection, these deleterious microbes are in competition with many other microbes in the rhizosphere for nutrients and space. In this battle for resources, beneficial microbes will limit the success of the pathogen through production of biostatic compounds, consumption of (micro)nutrients or by stimulating the immune system of the plant. Most microbes might neither affect the plant nor the pathogen directly because they occupy different ecological niches (commensal microbes), but are likely to affect every other organism to a certain extent through a complex network of interactions. Abbreviation: ISR, induced systemic resistance.

genotype, soil type is an important driver of the microbial community composition in the rhizosphere [42,43]. However, microbial communities in the rhizospheres of different plant species growing on the same soil are also often different [41,43,44]; it has even been demonstrated that some plant species can create similar communities in different soils [45]. Even within species, different genotypes can develop distinct microbial communities in the rhizosphere [46], suggesting that plants are able to shape the composition of the microbiome in their rhizosphere.

Microbiome management by the plant

Plants can determine the composition of the root microbiome by active secretion of compounds that specifically stimulate or repress members of the microbial community [13]. In axenically collected exudates of seed, seedlings and roots of tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*) and sweet pepper (*Capsicum annuum*), organic acids were predominant, with citric acid, succinic acid and malic acid being the most common [47]. The ability of rhizobacterial strains to grow *in vitro* on citric acid as the sole carbon source appeared to correlate with their root-colonizing ability. This indicates that plant species can select bacteria through the production of specific root exudates. Stable isotope probing of plants grown under $^{13}CO_2$ revealed that bacteria assimilate root exudates [48]. Using DGGE community profiling, this study also demonstrated that exudate-consuming bacterial rhizosphere populations of four plants species were more distinct than populations that did not utilize the root exudates, emphasizing the stimulatory role of root exudates in shaping the microbiome of a plant. Plant roots can also secrete secondary metabolites that inhibit growth of specific microbes in the rhizosphere [49,50]. For instance, benzoxazinoids are exuded in relatively large quantities from cereal roots and can inhibit rhizosphere microbes. In maize (*Zea mays*), 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) is the main antimicrobial benzoxazinoid. Interestingly, the plant-beneficial rhizobacterium *Pseudomonas putida* KT2440 is not only relatively tolerant to DIMBOA, but is also chemotactically attracted by this compound [51]. Roots of DIMBOA-deficient maize mutants were significantly less colonized by KT2440 than were wild-type plants, indicating that antimicrobial DIMBOA selectively attracts this plant-beneficial bacterium.

Furthermore, plant-associated bacteria produce and utilize diffusible *N*-acyl-homoserine lactones (AHLs) to signal to each other and to regulate their gene expression [52]. Such cell-to-cell communication is known as 'quorum sensing' (QS). AHL-mediated regulation typically makes use of two proteins that resemble the LuxI and LuxR protein families. LuxI-like proteins are AHL synthases, whereas LuxR-like proteins function as receptors of AHL that can form complexes with AHL and that in turn can affect gene expression of QS-target genes [53]. Plants can produce compounds that stimulate or repress QS-regulated responses in bacteria. A study testing the effect of seedling extracts and seedling exudates of *Medicago truncatula* found 15–20 compounds that specifically stimulated or repressed responses in QS-reporter bacteria [54]. Similar compounds that affect bacterial QS have been found in pea (*Pisum sativum*), rice (*Oryza sativum*) and green algae (*Chlamydomonas reinhardtii*) [55–57]. These QS-interfering compounds enable the plant to manipulate gene expression in their bacterial communities. Some plant-associated bacteria have LuxR-like proteins that are stimulated by plant-derived signals, whereas they themselves do not produce AHLs [57]. Also, bacterially produced AHLs have been shown to affect root development of *Arabidopsis* [58] and have been suggested to elicit ISR in tomato [59], further highlighting the importance of AHLs in cross-kingdom signaling in the rhizosphere.

Plant genotype determines microbiome composition

Recent evidence suggests that differences between plant genotypes in a single gene can have a significant impact on the rhizosphere microbiome. The production of a single exogenous glucosinolate significantly altered the microbial community on the roots of transgenic *Arabidopsis* [60]. Alphaproteobacteria and fungal communities were mainly impacted, as shown by denaturing gel gradient electrophoresis of specific amplified fragments of 16S and 18S rRNA genes. Furthermore, it has been reported that an ABC transporter mutant of *Arabidopsis*, *abcg30*, had root exudates with increased phenolic compounds and decreased sugars, which also resulted in a distinct root microbiome [61].

A study of bacterial rhizosphere communities in two different soils on three cultivars of potato using the PhyloChip technology detected 2432 operational taxonomic units in the potato rhizosphere, of which 40% had a site-specific abundance [62]. Only 9% of the operational taxonomic units had a cultivar-dependent abundance in one or the other soil, whereas the abundance of 4% of the operational taxonomic units was cultivar dependent in both soils. These results underline not only the importance of the soil in determining rhizosphere communities, but also that some microbes have a particular affinity for certain plant genotypes. Interestingly, differences in abundance on the three cultivars were mainly observed for microbes belonging to the Pseudomonales, Streptomycetaceae and Micromonosporaceae, which are an order and two families of bacteria, respectively, that have been extensively studied for their ability to control plant pathogens. These results indicate that the plant genotype can affect the accumulation of microorganisms that help the plant to defend itself against pathogen attack. Indeed, differences have been found in the ability of wheat cultivars to accumulate naturally occurring DAPG-producing *Pseudomonas* spp., resulting in differences in disease suppressiveness [63,64]. In addition, the amount of antibiotics produced by specific biocontrol strains on roots has been found to differ between wheat cultivars [65]. Furthermore, specific wheat cultivars were reported to support specific biological control bacteria differentially, which further establishes that there is a degree of specificity in the interactions between plant genotype and the composition of their microbial community [63].

The root microbiome to the rescue

Microbiome changes upon defense activation

The interactions between a plant and its root microbiome might change when the plant is attacked. Recently, it was

demonstrated that infection of citrus by *Candidatus Liberibacter asiaticus*, associated with Huanglongbing, drastically altered the composition of citrus rhizosphere communities [66]. Also, *Verticillium dahliae* infections affected the microbial composition of cotton rhizospheres [67]. Changes in rhizosphere composition upon infection might be the result of the induced excretion of antimicrobial compounds by infected roots. In hairy root cultures of sweet basil (*Ocimum basilicum*), *Pythium* infection elicited the secretion of rosmarinic acid, a caffeic acid ester with antimicrobial activity [49]. Infection of barley (*Hordeum vulgare*) roots by *Fusarium graminum* induced the exudation of phenolic compounds with antifungal activity [68].

However, infection does not only lead to secretion of pathogen-deterring compounds, for example infection of water melon plants by *F. oxysporum* enhanced the stimulation of *Fusarium* spore germination by root exudates [69]. In the same study, association of the biocontrol bacterium *Paenibacillus polymyxa* SQR-21 decreased the germination-stimulatory effects of the root exudates.

Recruitment of beneficial microbes

A study on foliar phloem feeding by white fly (*Bemisia tabaci*) on sweet pepper found that it elicited resistance against the bacterial root pathogen *Ralstonia solanacearum* [70]. Intriguingly, white fly feeding also led to significant changes in the rhizosphere microbial community. Although total numbers of bacteria were unaffected, the white fly-induced plants had higher populations of Gram-positive bacteria and fungi in their rhizosphere. The authors hypothesized that plants recruit plant-beneficial microbes to their roots in response to the attack (Figure 3). In line with this, aphid (*Myzus persicae*) feeding on pepper plants increased the root populations of the plant-beneficial *Bacillus subtilis* GB03, but reduced populations of the pathogenic *R. solanacearum* [71]. Recruitment of a beneficial bacterium has also been demonstrated for *Arabidopsis* when leaves were infected with a bacterial pathogen [72]. In this study, colonization of the roots of *Arabidopsis* by the plant-beneficial soil bacterium *Bacillus subtilis* FB17 was greatly improved when aboveground plant tissues were infected by *Pseudomonas syringae* pv. *tomato*. HPLC analyses of root exudates revealed increased secretion of malic acid upon *P. syringae* pv. *tomato* infection. The *Bacillus* strain was chemotactically attracted by malic acid, whereas other rhizobacteria were not. In addition, *Atalmt1*, an *Arabidopsis* knockout mutant deficient in root malic acid secretion, could no longer recruit FB17 after infection with *P. syringae* pv. *tomato*. Also, *AtALMT1*, which encodes a malic acid transporter, was found to be upregulated upon infection of the leaves by *P. syringae* pv. *tomato*. Colonization of *Arabidopsis* roots by FB17 induced ISR and protected the aerial parts of the

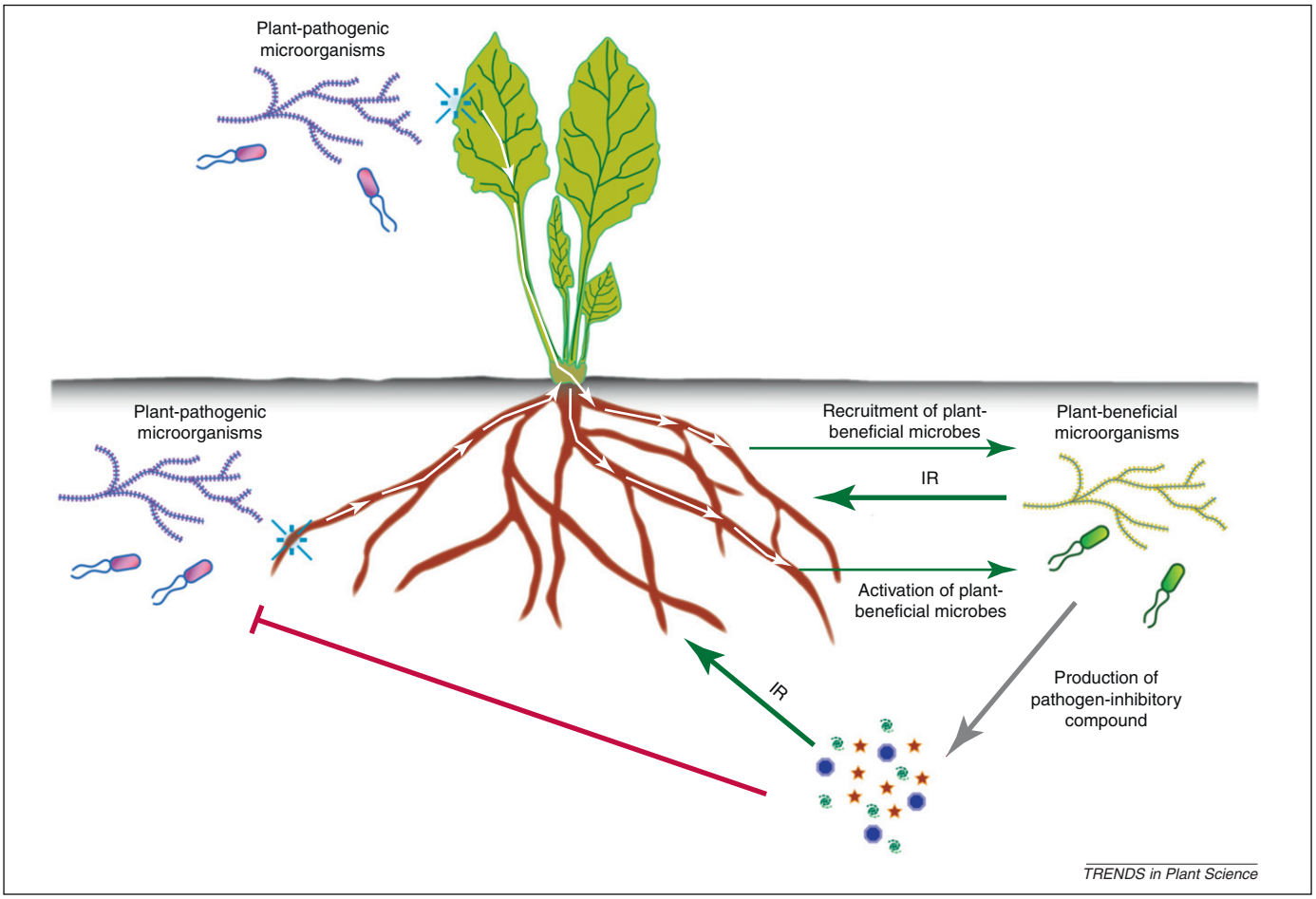

Figure 3. Microbiome to the rescue. Model of recruitment and activation of beneficials by the plant upon attack. Infected plants perceive pathogen invasion in roots or shoot and subsequently increase the secretion of microbe-stimulatory compounds in non-infected roots. These stimulants can recruit and activate plant-beneficial microorganisms. Beneficial microorganisms can induce resistance (IR) directly or produce pathogen-inhibitory compounds. Some pathogen-inhibitory compounds are known to induce resistance themselves [94].

plants against *P. syringae* pv. *tomato* infection. These data indicate a mutually beneficial relationship between *Arabidopsis* and FB17, whereby FB17 is recruited to aid in defense of the plant, and the plant provides the bacterium with malic acid.

In early studies, it was postulated that development of disease suppressiveness requires a severe outbreak of disease, after which plants signal for help in their rhizosphere [73]. Indeed, the above-described studies [71,72] indicate that plants respond to pathogen attack by recruiting specific beneficial microorganisms. It was recently reported that irrigated wheat fields in the pacific Northwest have high populations of *Pseudomonas* spp. that produce DAPG, whereas non-irrigated fields are dominated by *Pseudomonas* spp. that produce phenazines [74] In the irrigated fields, the DAPG-sensitive *G. graminis* var. *tritici* is the main root pathogen, whereas in the dry fields *Rhizoctonia*, which is sensitive to phenazine, is more problematic. This field study suggests specific recruitment of beneficial pseudomonads that are effective against a particular pathogen.

A study on shifts in the rhizosphere microflora of barley grown for five consecutive cycles in the same soil observed that barley induced shifts in the composition of the microbial communities [75]. However, the authors found no differences in microbiome composition between microcosms inoculated with the take-all fungus *G. graminis* var. *tritici* and non-inoculated microcosms. Nonetheless, take-all decline developed only in the inoculated microcosms. It was proposed that the major shifts in composition of microflora were a result of plant-specific selection of microbes, whereas the suppressiveness was a result of pathogen-induced changes in activity of the microbes present.

Activation of beneficials

The association of the pathogenic fungus *G. graminis* var. *tritici* with wheat roots strongly altered gene expression of the biocontrol bacterium *P. fluorescens* Pf29Arp [76]. Moreover, inoculation of strawberry plants with *V. dahliae* stimulated the expression of cyanide biosynthetic genes in the biocontrol bacterium *Pseudomonas* sp. LBUM300 in addition to stimulating root colonization by the bacterium [77]. These changes in gene expression could be a result of nutrients leaking from damaged roots. In a study of barley plants in a split root system, the roots on one side of the system were inoculated with *Pythium ultimum*, whereas the other side was inoculated with the biocontrol bacterium *P. fluorescens* CHA0 [78]. Using reporter strains, it was demonstrated that the expression of the DAPG biosynthesis gene *phlA* in CHA0 was induced upon infection by *Pythium* and that root exudation of vanillic acid, fumaric acid and *p*-coumaric acid increased concurrently. Very low concentrations of these organic acids can induce DAPG production in CHA0 *in vitro*. These results imply that, upon pathogen attack, the plant launches a systemic response that can stimulate the antifungal activity of the rhizosphere microflora (Figure 3).

Effects of defense signaling on the root microbiome

Several studies have investigated the effects of defense signaling on the commensal microflora. Differences have been observed in the microbial communities both in the phyllosphere [79] and in the rhizosphere [40,80] of wild-type *Arabidopsis* plants and mutants impaired in defense signaling. In addition, defense-related plant hormones, such as jasmonic acid and salicylic acid, can mediate changes in the composition of root exudates [81]. This suggests that changes in the defense-related hormone signature, as observed during pathogen and insect attack of foliar tissues [82], potentially influence the composition of the root exudates and, hence, the composition of the microbiome in the rhizosphere. However, chemical activation of defense by foliar treatment with salicylic acid and jasmonic acid did not significantly affect the resident soil microflora [80]. Nonetheless, recruitment of beneficials upon defense activation probably involves specific interactions with only a small part of the microbiome and subtle changes in the composition of the microflora. Hence, development of specific and sensitive profiling techniques is crucial to detect such dynamic changes in the microbiome of a plant.

Concluding remarks and prospects

Current understanding of the complex plant–microbe interactions that take place in the rhizosphere is still in its infancy [83]. Experimental evidence underlines the importance of the root microbiome in plant health and it is becoming increasingly clear that the plant is able to control the composition of its microbiome. It stands to reason that those plants that manage their microbiome in a way that is beneficial to their reproductive success will be favored during evolutionary selection. It appears that such selective pressure has brought about many specific interactions between plants and microbes, and evidence is accumulating that plants call for microbial help in time of need. It is expected that the near future will bring many new insights into the selective forces that shape the microbiome of the root and how it affects the plant, because next-generation sequencing techniques will undoubtedly provide new opportunities to study the interplay between the plant and its associated microflora [84]. Metagenomic studies of the root microbiome have until now focused on phylogenetic composition, resulting in limited information on the presence of specific operational taxonomic units. Development of functional metagenomics and transcriptomics will deliver insight into the activities and functions of the microbiome. Ultimately, unraveling the mechanisms through which plants control their microbiome and through which the microbiome controls plant health will open new avenues to increase crop quality and productivity.

Acknowledgments

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