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Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes

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Plants employ immunological and ecological strategies to resist biotic stress. Recent evidence suggests that plants adapt to biotic stress by changing their root exudation chemistry to assemble health-promoting microbiomes. This so-called 'cry-for-help' hypothesis provides a mechanistic explanation for previously characterized soil feedback responses to plant disease, such as the development of disease-suppressing soils upon successive cultivations of take all-infected wheat. Here, we divide the hypothesis into individual stages and evaluate the evidence for each component. We review how plant immune responses modify root exudation chemistry, as well as what impact this has on microbial activities, and the subsequent plant responses to these activities. Finally, we review the ecological relevance of the interaction, along with its translational potential for future crop protection strategies.

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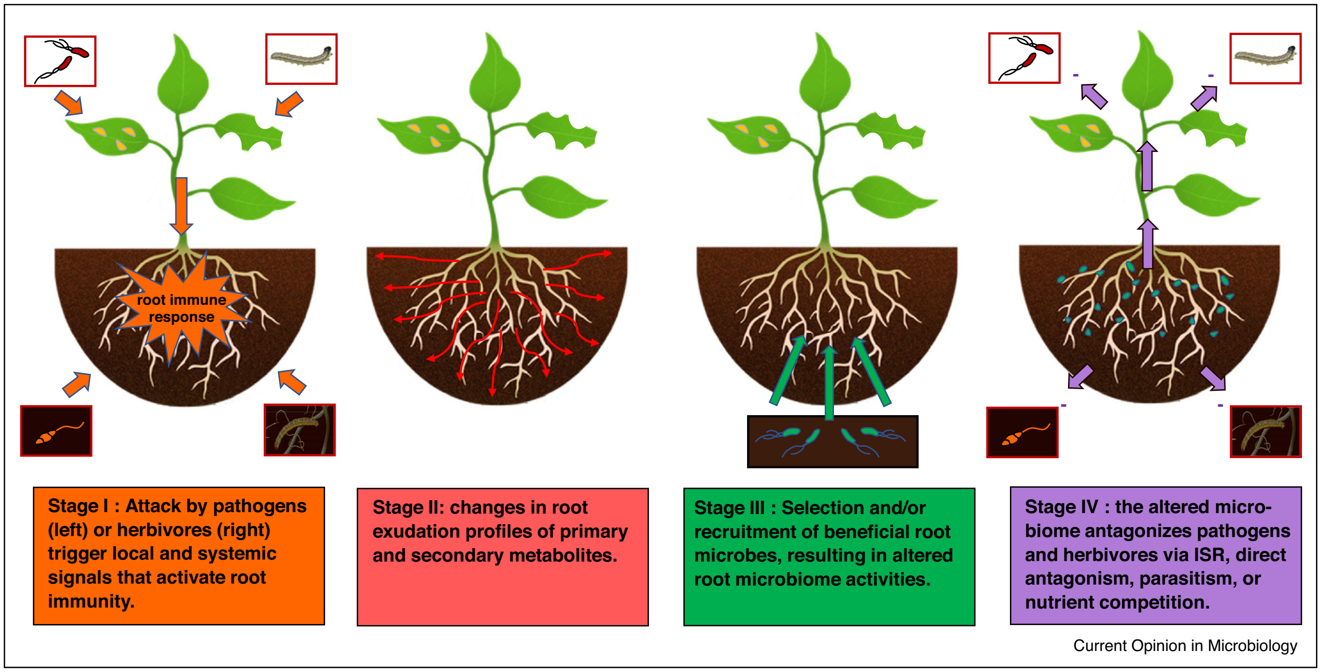
Introduction

Soil is a crucial resource for agricultural crop production. While agri-technological progress has made spectacular progress over recent decades, most innovations are based on agrochemicals and crop breeding technologies. By comparison, soil as a target for crop improvement has been largely overlooked, despite ample evidence for its plant protective activities [1]. The ability of soil to suppress plant diseases is a common characteristic of soil health and is determined by the soil-associated and root-associated microbiome [2•,3]. While crop rotation, conservation tillage and soil organic amendments improve soil health [1], these practices are not always financially feasible for farmers. However, disease-suppressive soil activity can also develop in high-intensity production systems that rely on successive crop monocultures. The classic example is take-all decline, during which continued wheat cultivation in soil infested with the pathogenic take-all fungus *Gaeumannomyces graminis* pv. *tritici* initially leads to increased disease, followed by a progressive decline in disease [4]. There are ample other examples whereby prolonged disease exposure leads to disease-suppressing soil activity [2•]. These observations have led to the hypothesis that disease-exposed and herbivore-exposed plants employ a strategy that involves active selection and/or recruitment of disease-suppressing soil microbiomes. This adaptive strategy not only benefits the plant that is under attack, but also subsequent plant generations, which is why these feedback responses are sometimes referred to as 'legacy' or 'soil memory' effects [5–8]. Analogous to aboveground multitrophic interactions between plants and arthropods [9], the mechanisms initiating this long-term adaptation are encompassed by the 'cry-for-help' hypothesis (Figure 1). This concept gained significant traction in the rhizosphere research community after a pioneering study that identified shifts in the microbial community structure of a disease-suppressive soil following prolonged cultivation of *Rhizoctonia solani*-infected sugar beet [10••]. In subsequent years, various other studies have confirmed enrichment of disease-suppressing microbes in disease-suppressive soils [2•]. In addition, there is an impressive body of evidence to support that root exudation chemistry is crucial for the assembly of plant health-promoting microbiomes [11]. However, there remain knowledge gaps in the successive stages predicted by the belowground cry-for-help model. In this review, we evaluate the evidence for each stage of the process, after which we will discuss the ecological relevance and translational opportunities of this long-term plant adaptation strategy.

Stage I: root immune responses to belowground and aboveground attackers

Of all plant tissues, roots are exposed to the highest microbial density and diversity [12]. In that regard, it is unsurprising that immune responses by roots differ from those by aboveground tissues [13]. Detailed studies of root responses to microbe-associated molecular patterns

Figure 1



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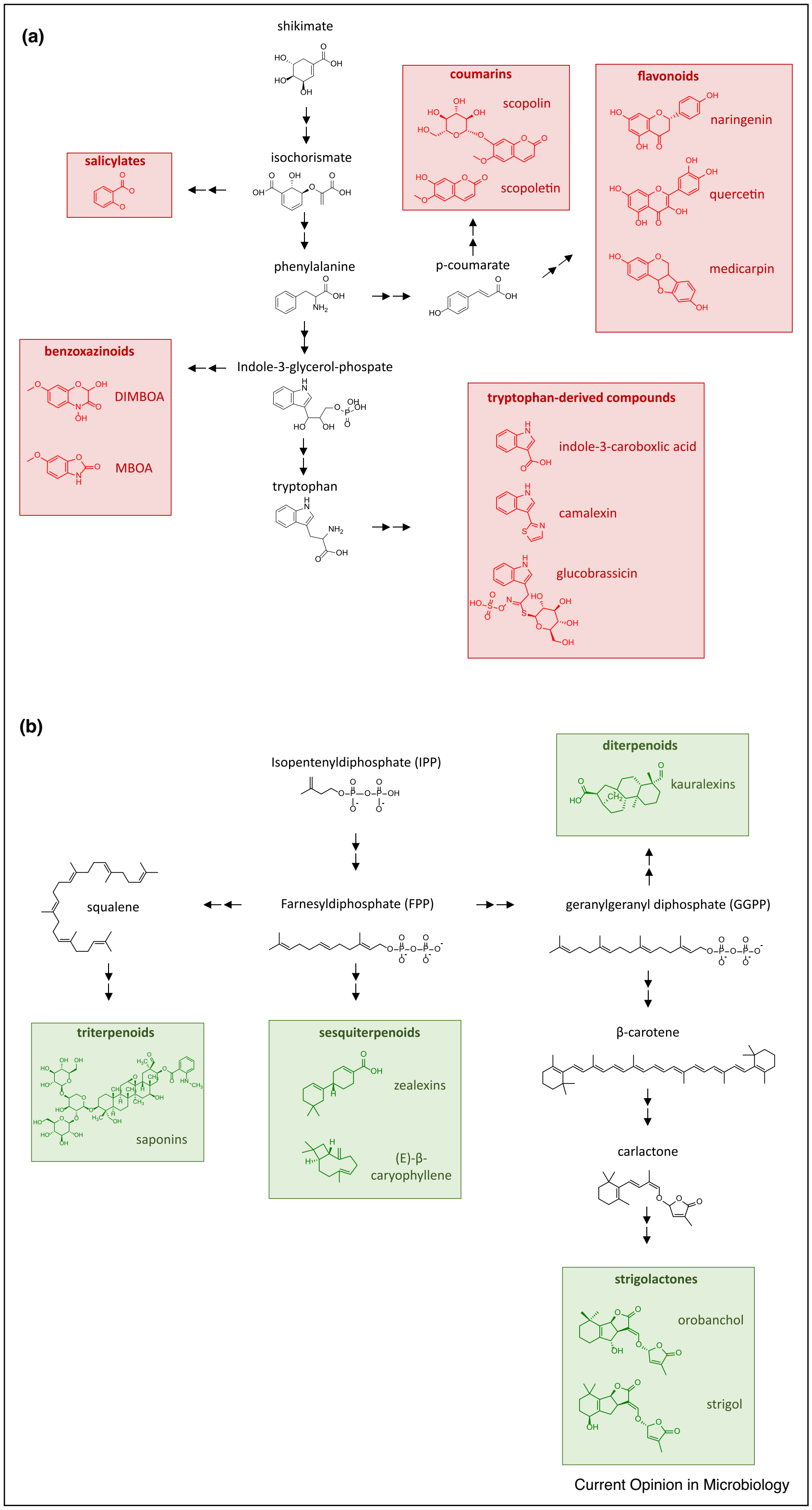
Model of the successive stages of the 'cry-for-help' hypothesis. Local and systemic signals elicited by pathogens or herbivores activate root immune responses (stage 1), which alter root exudation profiles of primary and secondary metabolites with biocidal and/or semiochemical activities (stage 2). Altered root exudation profiles influence the microbiome by recruiting and selecting specific microbiota and inducing microbial activities (stage 3). Some of these activities involve direct and indirect mechanisms that antagonize plant attackers, such as antibiosis, nutrient competition and induced systemic resistance (ISR; stage 4).

(MAMPs) have revealed that defence-related gene expression is spatially restricted to specific cell types, which vary according to the applied MAMP [14••,15•]. The immunological differences between roots and shoots may result from the lack photosynthesising chloroplasts in the roots, which generate high concentrations of defence-enhancing reactive oxygen and nitrogen species [16]. Furthermore, although pathogen-infected roots are capable of accumulating salicylic acid (SA) [17], the initial biosynthetic steps occur in chloroplasts, indicating phloem-mediated transport of SA and/or derivatives from shoot to root tissues [18,19]. Indirect evidence that jasmonic acid (JA)-dependent and SA-dependent immune reactions in roots generate rhizosphere-active signals is based on rRNA amplicon sequencing experiments, showing that exogenous hormone treatments or mutations in these pathways influence the root-associated microbiome [20•,21•]. In addition, systemic immune responses to aboveground pests and defence elicitors have been reported to alter root interactions with belowground microbes in a SA-dependent manner [22,23]. In the following section, we will review how root immune responses lead to exudation and accumulation of rhizosphere-active metabolites and derivatives thereof.

Stage II: stress-induced changes in root exudation of antimicrobials and semiochemicals

Roots release primary metabolites, such as carbohydrates, amino acids, organic acids and membrane lipids, which provide energy and nutrients to the soil microbiome [24]. The concentration and composition of these compounds in root exudates changes upon exposure to biotic stress and can have specific signalling effects in the rhizosphere. For instance, foliar infection of Arabidopsis by *Pseudomonas syringae* increases L-malic acid exudation, leading to increased root colonisation by resistance-inducing *Bacillus subtilis* [25•]. In cucumber, local root infection by pathogenic *Fusarium oxysporum* alters the concentrations of 89 mostly primary metabolites in exudates from distal roots, of which increased tryptophan and reduced raffinose correlated with root colonisation by beneficial *Bacillus amyloliquefaciens* [26••]. However, it seems unlikely that primary metabolites alone are responsible for the assembly of disease-suppressive root/soil microbiomes. Secondary root metabolites seem equally, if not more important, since they are often inducible by biotic stress, are less quickly metabolized by microbes, and typically have antimicrobial and/or signalling activities. In accordance with previous studies reviewed by [27–29], Figure 2 provides an overview of the

Figure 2



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Scheme of the shikimate **(a)** and terpenoid **(b)** pathways, generating stress-inducible secondary metabolites in plant roots with previously reported anti-microbial and/or semiochemical activity in the soil. Coloured boxes show examples of compounds within each class.

main biochemical pathways controlling pathogen-inducible and herbivore-inducible secondary metabolites with antimicrobial and/or signalling activity. It is important to note that rhizosphere chemistry, rather than root (exudation) chemistry, is responsible for shaping root-associated and soil-associated microbiomes. Rhizosphere chemistry is the sum of root exudation chemicals, their breakdown products and microbial products of soil-derived chemicals. A recent study developed a new method for chemically profiling non-sterile rhizosphere soil, providing a powerful technique to identify semiochemicals in non-sterile rhizosphere soil and link them to rhizobiome activities [30••].

MAMP-treated Arabidopsis roots increase the expression of *CYP71A12* and *MYB51* [14••], which control biosynthesis of tryptophan-derived defence compounds, such as camalexin and indolic glucosinolates. These stress-responsive metabolites often have both antimicrobial and signalling activities [31,32], and are commonly detected in root exudates [14••,33,34••]. Their increased exudation from defence-expressing and/or damaged roots can influence the root-associated microbes, which was recently demonstrated for camalexin [34••]. Similarly, parasitic nematodes increase strigolactone (SL) biosynthesis in tomato roots [35]. Irrespective of the exact role of SLs in plant immune signalling [36], exudation of SLs can influence the root-associated microbiome by stimulating hyphal branching and infection by arbuscular mycorrhizal fungi (AMF) [37]. Stress-induced rhizodeposition of defence hormones may also play an important role in shaping the soil-associated and root-associated microbiome [38•]. In particular, SA is commonly detected in plant root exudates [39•] and can attain concentrations in soil sufficient to induce resistance in neighbouring plants [40•]. Because SA can be incorporated in iron-chelating siderophores by rhizosphere bacteria [39•], it is likely that rhizosphere accumulation of SA selects for siderophore-producing rhizobacteria, which contribute to disease suppression [41].

Like pathogens, herbivores can induce exudation of rhizosphere-active root metabolites. Infestation of maize roots by larvae of *Diabrotica vergifera* induces emission of the sesquiterpene (E)-β-caryophyllene (Eβc), which recruits soil-borne entamopathogenic nematodes [42]. Overexpression of an Eβc synthase gene in the genetic background of a Eβc-deficient variety [43] revealed that Eβc increases growth and susceptibility to the soil-borne fungal pathogen *Colletotrichum graminicola* [43,44•], suggesting that Eβc may have wider-ranging impacts on soil microbes. In cereals, herbivore infestation and wounding induce an increase in the accumulation of aglycone benzoxazinoids, such as methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) [45]. Three recent studies have used maize mutants in BX production to determine the extent by which these metabolites influence root-associated and soil-associated microbiomes [46••,47••,48•], all reporting significant effects. Hu *et al.* [46••] demonstrated that soil conditioned by BX-producing maize induces JA-dependent resistance against herbivores, which was linked to the presence and activity of 6-methoxy-benzoxazolin-2-one (MBOA). Since DIMBOA acts as a within-plant defence signal [49], Cotton *et al.* [47••] investigated whether *BX* biosynthesis genes influence the composition of the wider root metabolome. They reported that the *bx1* and *bx2* mutations have major impacts on the secondary metabolite profiles in roots, suggesting that the effects of BXs on root-associated microbes could partially be caused by BX-controlled root exudates, rather than BXs themselves. Indeed, correlation analysis between differentially abundant metabolites and bacterial taxa pointed to a dominant role of BX-controlled root metabolites, including compounds with known signalling activities in the rhizosphere, such as flavonoids [47••]. More research is needed to determine the (in)direct signalling activities of BXs in the soil. Does biotic stress increase DIMBOA exudation and MBOA accumulation in the soil? If so, does MBOA act as a stress-inducible soil-mobile signal that alters root exudation patterns in systemic roots and roots of neighbouring plants? And finally, does the belowground signalling activity of BXs extend to other plant species, such as wheat, raising the possibility that BXs could act as the regulatory signals driving take-all decline?

Stage III impacts of root exudates on the root-associated and soil-associated microbiome

The 'cry-for-help' hypothesis postulates that specific components of root exudates from stressed plants favour recruitment of beneficial microbes and constrain the development of pathogens. This reshaping of the rhizosphere involves multiple mechanisms. Exudates may include substrates for microbial growth, elicit chemotactic responses and facilitate root colonisation, while antimicrobial compounds limit development of susceptible microbial communities. Exudates may also interact with microbial quorum sensing systems (QSS) or be processed by community members, eliciting the release of microbially derived metabolites.

As mentioned above, Liu *et al.* showed that local infection of cucumber roots by *F. oxysporum* increases tryptophan exudation and reduces raffinose exudation [26••], resulting in increased colonisation by beneficial *B. amyloliquefaciens* SQR9 (*Ba*SQR9) and reduced pathogen colonisation, thus pushing the rhizobiome towards plant-beneficial associations. They furthermore showed that the growth-promoting activity of *Ba*SQR9 results from tryptophan-dependent auxin production, indicating further feedback loops between plant and bacteria. Other studies have implicated organic acids as important signals, acting as recruitment signals for plant growth-promoting rhizobacteria (PGPR) in cucumber, tomato, banana, watermelon and Arabidopsis. [25•,50–53]. As reviewed above, BXs are important antimicrobial metabolites

[45,49]. Neal *et al.* [54•] found that DIMBOA is chemoattractive to plant-beneficial *Pseudomonas putida* KT2440 bacteria, activating genes associated with bacterial motility, QSS and breakdown of *N*-heteroaromatic compounds. Such a selection for BX tolerance can also influence potentially hostile organisms. Sanders *et al.* [55] reported that BOA, a toxic degradation product of DIBOA, selects for BX-resistant *Fusarium* sp. in maize with the potential for grain contamination by mycotoxins.

For many microbial responses to root exudation metabolites, bacterial stress seems a recurrent theme. Exposure of PGPR to root exudates activates not only genes associated with nutrient responses and motility, but also the production of antibacterial and antifungal substances, degradation of aromatic compounds and microbial stress responses [56•,57,58•]. Thus, while many root exudates act as nutrients and recruitment factors, other root exudates induce microbial stress that lead to plant-beneficial activities. For instance, quorum sensing signals (QSS) activate transcriptional stress responses in bacteria, once a certain population density has been reached. Given the ubiquity of QSS, it is unsurprising that plants have evolved to respond to QSS molecules and manipulate QSS responses [59]. Sweet basil releases rosmarinic acid (RA) when infected by pathogenic *Pseudomonas aeruginosa* PA01 and PA14 [60]. RA not only is toxic to bacteria at high concentrations, but also binds to the response regulator RhlR triggering premature QSS responses [61••]. This QSS system is commonly found in Pseudomonads including PGPR, and may therefore also regulate PGPR responses, such as biofilm formation and antibiosis. Indeed, the protective effect of *Pseudomonas aureofaciens* strain 30–84 against take-all disease has been attributed to phenazine antibiotic production that is regulated by QSS [62,63]. Bacterial stress responses in the rhizosphere can also be an indirect consequence of microbial competition. For instance, saprotrophic fungi consume root exudates rapidly, which reduces nutrient availability to rhizobacteria that in turn triggers rhizobacterial production of antifungal compounds [64].

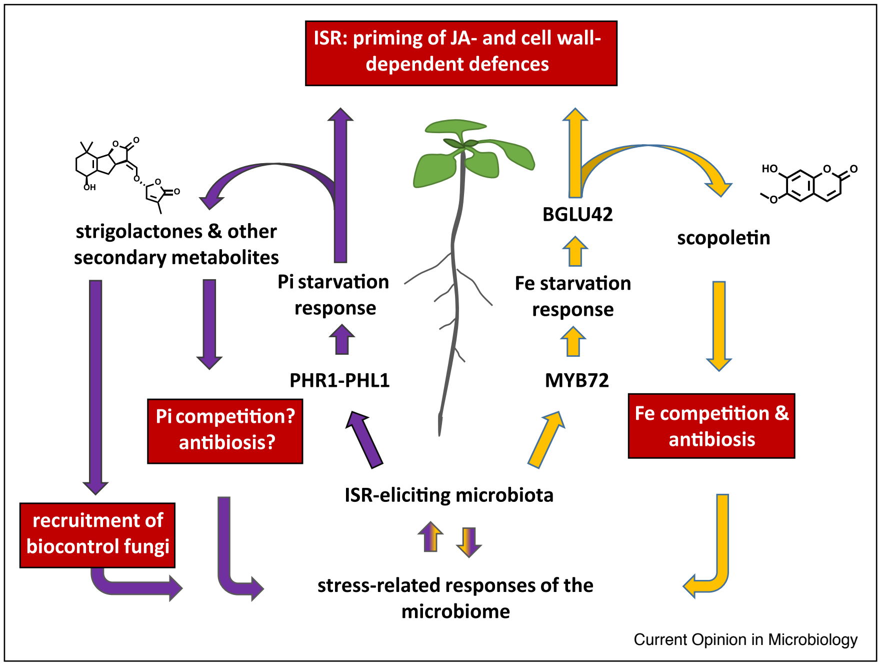
The effects of rhizosphere chemistry on the beneficial microbiome activities in the soil can be long-lived. Yuan et al. found that five generations of *Arabidopsis* plants inoculated with *P. syringae* DC3000 (*Pst*) lead to disease suppression in the sixth generation, which was associated with changes in soil microbial community [65••]. This study furthermore showed that >50 root exudation compounds changed upon infection. Soil complementation experiments with mixtures of components identified long chain organic acids as the underpinning soil signals, stimulating microbiome-mediated induced systemic resistance (ISR). Similarly, Hu *et al.* reported soil feedback responses that were linked to soil accumulation of MBOA, which induced JA-dependent resistance in maize plants of the next generation [46••]. Finally, Berendsen *et al.* [66••] isolated three community members that accumulated in soils of downy mildew-infected Arabidopsis plants, and found that this assemblage interacted to induce biofilm formation and ISR in subsequent plant generations. Notably, in all three examples, the response of the soil microbiome was crucial for the beneficial ISR response of the host plant.

Just as plants have evolved to respond to microbial signals, microbes have evolved to respond to plant signals, including plant growth regulators involved in biotic stress responses. Treatment of both plants and soil with SA, JA and ethylene (ET) induces changes in root exudates and rhizosphere communities, whereas mutations in plant JA signalling reduces root exudates that are associated with PGPR chemotaxis or that act as growth substrates for PGPR and N-fixing diazotrophs [20•,38•,67]. The emerging pattern suggests a complex network of interactions between plants and soil/plant-associated microbiomes, which are mediated by a multitude of chemicals signals that are derived from both plants and microbes. Bruto *et al.* [68] attempted to identify plant-beneficial function contributing (PBFC) genes in Proteobacterial PGPR. Interestingly, none of these genes were found in all PGPR, and many were found in non-PGPR. However, combinations of PBFC genes were only found in particular taxonomic subgroups of PGPR, indicating that specific assortments were associated with the beneficial trait. It is therefore plausible that similar, if not greater, complexity exists in PGPR responses to root exudates with combinations of signals and signalling mechanisms contributing to microbial recruitment and development.

Stage IV: mechanisms by which the root-associated and soil-associated microbiome suppress pests and diseases

The mechanisms underpinning disease-suppressing soil activity are complex [2•]. Apart from direct mechanisms, such as parasitism and the production of biocidal compounds, beneficial rhizosphere microbes suppress soil-borne attackers indirectly through competition for (micro)nutrients and elicitation of ISR. Of these, ISR provides protection against both belowground and aboveground attackers [69]. Much knowledge about the mechanisms underpinning ISR comes from the interaction between Arabidopsis and *Pseudomonas simiae* WCS417. Early studies have shown that ISR is controlled by a SA-independent signalling pathway that primes distal tissues for JA-dependent and ET-dependent defence genes and cell wall-based defences [70,71]. While there are exceptions, ISR in other plant-microbe interactions often follows a similar signalling signature [69]. This commonality could be explained by the fact that ISR-eliciting microbes trigger a general nutrient deficiency response that results in systemic upregulation of ISR-related immune pathways. Castrillo *et al.* recently demonstrated that inoculation of Arabidopsis with a synthetic rhizobacterial community

Figure 3



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Emerging roles for phosphate (Pi; purple) and iron (Fe; orange) starvation responses in the orchestration of disease-suppressive mechanisms in the microbial biosphere of the plant. The model is based on recent evidence for reciprocal signalling events between nutrient starvation signalling in the host, systemic immune responses (ISR), and disease-suppressing activities by the root-associated and soil-associated microbiome [72••,77••].

induces a phosphate starvation response (PSR), which modulates systemic plant immunity and that is under control by the regulatory gene *PHR1* [72••]. Interestingly, *PHR1* has previously been reported to control ISR-related immune pathways, including JA signalling [73] and production of callose-stimulating glucosinolates [74]. If these mechanisms apply to other plant species, the PSR by ISR-eliciting microbiota could lead to increased exudation of SLs and recruitment of endophytic fungi, such as AMF, which in turn alter root-associated microbial populations [75,76]. Furthermore, a recent study of the Arabidopsis-WCS417 model system found that bacterial induction of the ISR-regulatory transcription factor MYB72 and the downstream beta-glucosidase BGLU42 result in an iron-deficiency response that is associated with increased root exudation of scopoletin [77••]. This iron-mobilizing metabolite has selective impacts on the root-associated microbiome, including biocidal activity on soil-borne pathogenic fungi. A recent study by Vogel *et al.* confirmed the importance of selected scopoletin derivatives in shaping synthetic rhizobiome communities of Arabidopsis via redox-mediated mechanisms [78••] Together, these recent studies illustrate that interactions between roots and disease-suppressing bacteria trigger a succession of signalling events, resulting in a range of disease-suppressive mechanisms, including ISR, recruitment of biocontrol fungi, (micro)nutrient competition, and antibiosis (Figure 3).

Conclusions: ecological relevance and translational opportunities

There is ample evidence to support the individual components of the cry-for-help hypothesis. However, the outcome of the response is not always adaptive and can come with ecological trade-offs. The signals used by plants to recruit plant-beneficial organisms in the soil can be hijacked by parasitic organisms. For instance, emission of Eβc increases infection by the soil-borne fungal pathogen *C. graminicola* [44•], exudation of BXs enhances damage by pathogenic fungi and the western corn rootworm [55,79], and exudation of AMF-recruiting SLs can be exploited by pathogenic nematodes and parasitic weeds to locate their host [80]. We speculate that these ecological trade-offs are determined by soil quality. Healthy soils with high microbial biodiversity are more likely to contain robust networks of beneficial rhizobacteria than poorer soils with low biodiversity [81]. Once an interaction with beneficial microbiota has been initiated, the subsequent signalling cascade leads to the establishment of a chemical and biological environment that is mutually beneficial to both partners. In situations where the soil fails to provide fast-responding beneficials, due to loss of biodiversity by overfertilization, soil compaction, or soil inversion, the cry-for-help is more likely to be hijacked by parasitic microbes and arthropods. Recent evidence that plant and microbial nutrient starvation responses control the establishment of plant health-promoting microbiomes [72••,77••] is directly antagonistic to the often excessive amounts of fertilizer applied in modern agriculture [82••]. Furthermore, human selection for aboveground yield under high fertilizer input have resulted in plant varieties with rudimentary root systems that communicate less effectively with the soil microbiome [82••]. While the importance of soil microbiomes is increasingly being recognised by farmers and the wider agri-tech sector, a better mechanistic understanding of the individual components of the cry-for-help hypothesis is necessary to reliably exploit the benefits of soil-preserving land management, biocontrol inoculations, and crop breeding programmes selecting for soil-health promoting root traits.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors have compared soil-associated and root-associated microbial communities of wheat under conventional and organic field management that varied in agrochemical input and tillage. The study discovered that 10% of the variation in microbial communities is affected by field management. The study concludes that future agricultural strategies should consider the soil microbiome to foster-specific microbiota with agricultural benefits.