Co-evolution within the plant holobiont drives host performance

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Abstract

Plants interact with a diversity of microorganisms that influence their growth and resilience, and they can therefore be considered as ecological entities, namely "plant holobionts," rather than as singular organisms. In a plant holobiont, the assembly of above- and belowground microbiota is ruled by host, microbial, and environmental factors. Upon microorganism perception, plants activate immune signaling resulting in the secretion of factors that modulate microbiota composition. Additionally, metabolic interdependencies and antagonism between microbes are driving forces for community assemblies. We argue that complex plant–microbe and intermicrobial interactions have been selected for during evolution and may promote the survival and fitness of plants and their associated microorganisms as holobionts. As part of this process, plants evolved metabolite-mediated strategies to selectively recruit beneficial microorganisms in their microbiota. Some of these microbiota members show host-adaptation, from which mutualism may rapidly arise. In the holobiont, microbiota members also co-evolved antagonistic activities that restrict proliferation of microbes with high pathogenic potential and can therefore prevent disease development. Co-evolution within holobionts thus ultimately drives plant performance.

Introduction

In nature, a wide diversity of microorganisms inhabit above- and belowground plant tissues (Müller *et al*, 2016; Gross, 2022). These communities are referred to as "plant microbiota" and comprise prokaryotes (i.e., bacteria and archaea) and eukaryotes (e.g., fungi, protists including oomycetes, algae, and even animals such as nematodes and small insects) that mostly rely on plant-derived carbon compounds for energy production. By promoting plant growth, health, and resilience, microbiota generally are beneficial for their hosts (Durán *et al*, 2018; Gross, 2022). Accordingly, plants growing in the absence of microbiota are particularly vulnerable to disease and are unlikely to survive in natural settings (preprint: Paasch *et al*, 2023).

While a large fraction of the plant microbiota is horizontally acquired from the environment, some taxa are stably associated with their host, independently from soil and environmental conditions (Compant *et al*, 2019). A subset of these core microbiota members can be vertically transmitted through seeds, but also during clonal reproduction of the plant (Vannier *et al*, 2018; Abdelfattah *et al*, 2023). As they engage in stable associations, microbiota are likely to co-evolve with their hosts. Accordingly, the phylogenetic relatedness of two plants typically correlates with the similarities of their microbial communities (Bouffaud *et al*, 2014; Kembel *et al*, 2014; Abdelfattah *et al*, 2022).

Since its inception, plant evolution is believed to be driven by interactions with microbes (Ramanan *et al*, 2016; Delaux & Schornack, 2021). Symbiotic associations with fungi are ancient and have been instrumental for land plant evolution, by facilitating the uptake of nutrients from soils and possibly even contributing to early root development (Kenrick & Strullu-Derrien, 2014; Martin *et al*, 2017). Moreover, the chloroplasts and mitochondria that have become responsible for photosynthesis and aerobic respiration, respectively, are derived from ancient bacterial endosymbionts (Archibald, 2015). Furthermore, plant immune systems evolved under the continuous threat of pathogenic microbes (Cook *et al*, 2015; Delaux & Schornack, 2021).

Given their long-lasting and continuous association with co-evolving microbiota, plants may be considered as ecological entities, generally termed "holobionts," rather than as singular organisms. Contrary to the definition of "meta-organism" that implies specificity of time and place and a function for each member, a holobiont represents a eukaryotic host with all external and internal associates, regardless of their stability and functionality (Jaspers *et al*, 2019). A contrasting and more specific definition of the "plant holobiont" implies evolutionary selection between plants and microorganisms, contributing to an overall stability of the system (Trivedi *et al*, 2020; Lyu *et al*, 2021; Berg *et al*, 2023). Since stable associations concern only a fraction of the interactions in the plant holobiont, we adopt the former holobiont definition and consider that

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co-evolution occurs within plant holobionts, although it may not involve all members. Arguably, plant evolution in the holobiont favors associations with beneficial partners and counter-selects for detrimental interactions.

Experimental dissection of root- and leaf-associated microbial communities revealed that the effects of microbiota members on host performance are diverse (Durán et al, 2018; Eitzen et al, 2021; Mesny et al, 2021). In addition to mutualists and commensals, both opportunistic and obligate pathogens may occur in holobionts (Box 1). Co-evolution between plants and various types of pathogens with diverse lifestyles has been well-documented and is often

referred to as an "arms-race" in which the plant evolves mechanisms to detect and intercept individual pathogens, while pathogens evolve to evade or suppress this interception to continue host colonization (Cook et al, 2015; Möller & Stukenbrock, 2017; Han, 2019). However, little is known about the evolution of nonpathogenic microbes in plant-associated environments.

Complex plant–microbe and intermicrobial interactions, together with environmental cues, influence microbiota structure and composition (Müller et al, 2016; Hassani et al, 2018). Likely, many mechanisms behind these interactions result from co-evolution within the holobiont. In this review, we assemble evidence that supports co-adaptation within the holobiont as a driver for plant performance.

Box 1. Pathogenic potential in plant microbiota

Microbiota of healthy plants generally comprise microbes with disease-causing potential (Cregger et al, 2018; Nelson & Shaw, 2019; Thiergart et al, 2020; Rudgers et al, 2022), which is evident from the so-called "recolonization experiments" where isolates negatively affect plant health when individually inoculated on otherwise sterile plants (Kia et al, 2017; Nelson et al, 2018; Mesny et al, 2021). In holobionts, disease is mitigated by the combined action of plant immune systems and the collective microbiota composition that keeps microbes with pathogenic potential in check (Wolinska et al, 2021). In agreement with the "disease triangle" concept, stating that disease develops from the interplay of host genetics, pathogen genetics, and environmental factors, which can be understood to comprise microbiota composition besides other biotic and abiotic cues (McNew, 1960), disease development may be promoted by external stimuli that increase stochasticity in plant microbiota assemblies and therefore affect mitigative forces (Arnault et al, 2023).

Given that microbes with disease-causing potential frequently occur in healthy holobionts, we should consider how to treat pathogens. However, rather than classifying microorganisms as commensals, pathogens, or mutualists with strictly separated lifestyles, we need to realize that this classification occurs as a continuum. Accordingly, the ability to cause disease (i.e., pathogenicity) should be appreciated as a continuous variable (i.e., the "pathogenic potential") incorporating the occurrence of host damage, time, and virulence factors (Casadevall, 2017, 2022). Microorganisms with low pathogenic potential may not (or rarely) cause disease, presently often referred to as opportunistic pathogens, while the ones with the highest pathogenic potential are virulent, obligate pathogens. The development of disease may be determined by the extent of microbial colonization, which depends on the holobiont composition, which in turn is modulated by environmental stimuli. Thus, importantly, pathogens are part of holobionts too, irrespective of their infection strategy. This concept agrees with the endophytic continuum theory, stating that the interaction of fungal endophytes with their hosts is never neutral (Schulz & Boyle, 2005). A particular degree of "virulence" is required to enable host colonization of plant tissues, and disease establishment is eventually repressed by plant immunity and environmental factors.

When immunity is disrupted, or environmental factors are permissive, plants may develop disease depending on the pathogenic potential of their microbiota members (Plett et al, 2014; Lahrmann et al, 2015; Hiruma et al, 2016; Chen et al, 2020a; Wolinska et al, 2021). Accordingly, defense responses targeting all plant-colonizing microorganisms, including those with performance-promoting effects, are essential for plant survival and fitness. The common conception that plants evolved immune systems to defend against particular "pathogens" is thus incongruous, and immunity should be considered as an organism's capacity to withstand any microbial invader that may cause damage, which can be any member of their microbiota.

Plant immune systems shape beneficial microbiota composition

While plants evolved continuously surrounded by a wealth of microorganisms, they established diverse symbiotic associations ranging from detrimental to beneficial (Han, 2019). Simultaneously, they evolved a complex innate immune system. Pathogen-induced biotic stresses affecting fitness have long been considered the sole drivers of plant immune system evolution. However, numerous studies have demonstrated that also nonpathogenic microbes are recipients of plant immune responses, and they likely contributed to shaping them (Teixeira et al, 2019; Yu et al, 2019a; Box 1). An increasingly accepted view proposes that immune systems act as microbial management systems to control microbiota assembly and host–microbe homeostasis (Cook et al, 2015; Hacquard et al, 2017; Teixeira et al, 2019). After introducing how plant immune systems operate, we will show that plant microbiota are shaped by immune responses and argue that plants evolved mechanisms to mediate beneficial microbiota assembly.

Plant immune systems are integrated surveillance systems

While recognized as integrated surveillance systems to detect microbial invasion (Thomma et al, 2011; Cook et al, 2015), plant immune systems have been molecularly portrayed as composed of two layers (Chisholm et al, 2006; Jones & Dangl, 2006), recently realized to be mechanistically strongly interconnected (Pruitt et al, 2021; Parker et al, 2022; Feehan et al, 2023). As a first layer, plasma membrane-localized receptors known as pattern recognition receptors (PRRs) perceive conserved structural molecules, such as bacterial lipopolysaccharides and fungal chitin, referred to as microbe-associated molecular patterns (MAMPs). These receptors are known as pattern recognition receptors (PRRs), and they include receptor-like kinases (RLKs) and receptor-like proteins (RLPs). Recognition of MAMPs leads to the activation of immune signaling to establish MAMP-triggered immunity (MTI). Major immune signaling pathways are mediated by reactive oxygen species (ROS) and by the phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) (Peng et al, 2018). Local concentrations in these compounds change upon MAMP perception and mediate activation of plant defenses. Analysis of transcriptomic reprogramming upon perception of different MAMPs in *Arabidopsis thaliana* identified a congruent set of rapidly activated responses that are also induced when plants face abiotic stresses, suggesting that early transcriptional responses to

MAMPs overlap with general stress responses (Bjornson et al, 2021).

To overcome this first defense layer, microbes secrete small proteins, typically referred to as "effectors," supporting host colonization by repressing or evading MTI, leading to "effector-triggered susceptibility" (ETS) (Chisholm et al, 2006; Jones & Dangl, 2006). However, to counteract this breach of MTI, plants re-activate immunity upon sensing the presence or activity of microbial effectors. This so-called "effector-triggered immunity" (ETI) was proposed to rely on intracellularly located receptors from the nucleotide-binding domain leucine-rich repeat containing (NLR) family. Like microbial effector catalogs, NLR gene repertoires are highly plastic and diversify rapidly, driven by duplication, translocation, deletion, and promiscuous gene exchange events (Han, 2019; van de Weyer et al, 2019). A recent genomic analysis showed that NLRs display higher variability than PRRs in A. thaliana (Pruitt et al, 2021). The perpetual adaptation of NLR sequences to rapidly evolving effector proteins, and of PRRs to MAMPs, is generally referred to as an "arms race" in which plants and microorganisms aim to detect and overcome detection, respectively. While ETI may culminate in host localized cell death, it also reinstates and amplifies MTI-related defenses that were perturbed through microbial effector activities (Parker et al, 2022).

Plant immune systems shape microbiota assemblies

Plant tissues are colonized by phylogenetically diverse microorganisms that collectively evolved a broad variety of MAMPs and effectors and may therefore differentially interact with plant immune systems. The impact of MTI responses on microbiota structure and composition is increasingly recognized. When treated with the bacterial flagellin-derived MAMP epitope flg22, A. thaliana plants overexpressing the PRR-encoding gene FLS2 revealed an effect of MTI responses on root microbiota composition (Ma et al, 2021). Consistently, the A. thaliana WRKY33 transcription factor, which positively regulates expression of immunity genes upon flg22 treatment, shapes root-associated microbiota (Wolinska et al, 2021). In A. thaliana, a PRR complex that includes the receptor kinase FERONIA restricts rhizosphere colonization by Pseudomonas bacteria (Song et al, 2021). Upon phosphate starvation, PRR complex formation is repressed, leading to enrichment in the root microbiota of several bacterial genera that alleviate low-phosphate stress (Tang et al, 2022). Deletion of three A. thaliana PRR genes (namely FLS2, EFR, and CERK1) together with a gene involved in vesicle-trafficking (MIN7) resulted in an endophytic phyllosphere community shift and microbial overproliferation under high humidity, causing detrimental effects on plant performance (Chen et al, 2020a). Interestingly, deletion of the A. thaliana NADPH oxidase RBOHD, responsible for respiratory bursts that limit microbial colonization, has a greater impact on the leaf microbiota than deletion of PRR genes (Pfeilmeier et al, 2021). Taken together, these findings highlight the importance of MTI for microbiota modulation and the restriction of microbial proliferation in plant tissues. While the impact of ETI on microbiota assembly remains to be demonstrated, recent studies linking natural genetic variation in barley and sorghum to their rhizosphere microbiota revealed correlations between community composition and the presence of particular NLR genes (Deng et al, 2021; Escudero-Martinez et al, 2022).

Although immune systems shape whole microbiota, mechanisms accommodating beneficial microbes while keeping pathogenic invaders at bay have been positively selected during plant evolution. This implies that plants can distinguish beneficial from detrimental microbes. This is the case in arbuscular mycorrhizal symbioses, in which plant RLKs perceive fungal short-chain chitooligosaccharides and nonsulfated lipochitooligosaccharides, commonly referred to as "Myc factors," resulting in the activation of symbiosis signaling and in the suppression of immunity to accommodate the mycorrhizal fungus (Feng et al, 2019). Similarly, to induce root nodule symbioses, rhizobia synthesize lipochitooligosaccharides after sensing the presence of legume-secreted flavonoids. Upon perception of these lipochitooligosaccharides by host cell surface receptors, symbiotic programs are induced and immune signaling is repressed (Ghantasala & Roy Choudhury, 2022). Opportunities for mutualist associations to develop arise from the rapid evolution and high variability of MTI. Accordingly, intraspecific variation of immune responses upon MAMP perception occurs in A. thaliana (Vetter et al, 2016) and in tomato (Veluchamy et al, 2014; Roberts et al, 2019), while microorganisms may exhibit considerable variation in their MAMPs (Teixeira et al, 2019).

Phytohormone signaling modulates plant microbiota composition

Downstream of MAMP and effector perception, the phytohormones salicylic acid (SA), and jasmonic acid (JA) mediate immune signaling as part of MTI and ETI (Venugopal et al, 2009; Liu et al, 2016; Cui et al, 2017). These two phytohormones were the first immunity components proven to directly impact microbiota diversity and composition. Induction of SA-mediated defenses reduced endophytic bacterial community diversity in A. thaliana leaves, whereas plants deficient in JA-mediated defenses revealed greater epiphytic diversity (Kniskern et al, 2007). In roots, both SA and JA influence bacterial community composition in the rhizosphere and in the endosphere (Lebeis et al, 2015). A third phytohormone of importance for plant immunity is ethylene (ET), a volatile compound produced at early MTI stages that transcriptionally activates FLS2 production in A. thaliana (Mersmann et al, 2010; Jones et al, 2019). Both the phyllosphere and the rhizosphere of ET-insensitive ein2 mutants host bacterial communities that significantly differ from wild-type plants (Doornbos et al, 2011; Bodenhausen et al, 2014). Finally, brassinosteroids, phytohormones that act at the growth-defense interface, indirectly shape root microbiota composition. In A. thaliana, these hormones bind to a molecular complex that includes the transmembrane receptor kinase BRI1. While this interaction activates growth, it also represses defense-gene expression, callose deposition, ROS accumulation, and spontaneous cell death (He et al, 2007). Bacterial and fungal community profiling of BRI1 knock-out plants revealed major differences when compared with wild-type plants (Hou et al, 2021; Wolinska et al, 2021).

Fine modulation of phytohormone-signaling mediates the accommodation of beneficial microbes in a context-dependent manner. Treatment of bulk soil with methyl-JA lowered the variability of rhizosphere microbial communities (Carvalhais et al, 2013). The JA-induced community shift was associated with an enrichment of bacterial taxa known to suppress plant pathogens and herbivore attack, and with a depletion of growth-promoting taxa. In the ectomycorrhizal association of Laccaria bicolor with Populus trichocarpa, JA signaling prevents intercellular fungal overcolonization to sustain a long-term symbiotic association (Plett et al, 2014). Interestingly, foliar application of methyl-JA on Lotus japonicus plants inhibited

root nodulation, pointing to a potential role of JA signaling in the repression of rhizobial recruitment (Nakagawa & Kawaguchi, 2006). Therefore, plants may modulate JA signaling to remodel their microbiota in a context-dependent manner, leading to the recruitment of microbes that can alleviate stress. Signaling mediated by other phytohormones also contributes to recruitment of beneficial microbiota members. For instance, ET is enriched in peanut roots grown in a cyanide-rich soil. Cyanide triggered increased belowground production of ET, resulting in altered root microbiota composition and enrichment of *Catenulispora* bacteria (Chen *et al*, 2020b). The reshaped root microbiota correlated with increased soil ammonium, nitrogen, and available phosphorus concentrations, pointing to a role of ET in the assembly of beneficial microbiota to alleviate mineral stress. Thus, phytohormones are key mediators of plant microbiota composition.

Immune-related metabolites shape beneficial plant microbiota

Phytohormone signaling results in the production and secretion of compounds with direct effects on plant-associated microorganisms. For instance, application of exogenous JA to *A. thaliana* roots alters root exudate profiles, correlating with changes in bacterial and archaeal communities in the rhizosphere (Carvalhais *et al*, 2015). While such exudates include hydrocarbon compounds that can be used by microbes as energy source, others, especially nucleotides, can act as chemotaxis signals to recruit microbes into the microbiota (Yang *et al*, 2015; Jones *et al*, 2019). Tryptophan-derived metabolites, secreted as part of plant immune responses, act in shaping plant microbiota composition. *Arabidopsis thaliana* cyp79b2-cyp79b3 mutants do not convert tryptophan into indole-3-acetaldoxime, a precursor of indole glucosinolates, but also of camalexin and indole-3-carboxylic acids, and they host highly different root-associated bacterial communities from wild-type plants (Wolinska *et al*, 2021). These mutants are adversely affected by a synthetic microbial community that represents a native root microbiota, and by the beneficial fungi *Colletotrichum tofieldiae, Serendipita vermifera,* and *Serendipita indica* due to uncontrolled fungal growth in root endophytic compartments (Nongbri *et al*, 2012; Lahrmann *et al*, 2015; Hiruma *et al*, 2016; Wolinska *et al*, 2021). In other plants, tryptophan-derived metabolites impact root-associated microbial communities as well. Maize mutants that lack benzoxazinoids combine different root metabolomic profiles with altered bacterial community composition (Cotton *et al*, 2019; Cadot *et al*, 2021). Furthermore, root-secreted antimicrobial triterpenes shape bacterial community composition in *A. thaliana* and melon (Huang *et al*, 2019; Zhong *et al*, 2022).

Plant metabolites and exudates are also used to actively recruit beneficial microbiota members through chemotaxis or growth stimulation (Sasse *et al*, 2018). For instance, *Pseudomonas putida* KT2440, a beneficial bacterium in the maize rhizosphere, is chemotaxically attracted to a benzoxazinoid in maize root exudates (Neal *et al*, 2012). Triterpenes act in the active selection of microbes through plant defense signaling and antimicrobial activities. *Arabidopsis thaliana* triterpene biosynthesis affects root microbiota composition, and purified triterpene cocktails differentially affect microbial growth (Huang *et al*, 2019). Consistently, cucurbitacins (bitter triterpenes) modulate rhizosphere composition of cucurbit plants, recruiting *Enterobacter* and *Bacillus* bacteria that protect against the soil-borne fungal pathogen *Fusarium oxysporum* (Zhong *et al*, 2022). When colonized

by the pathogen *Fusarium culmorum*, *Carex arenaria* roots emit a specific blend of volatile organic compounds (VOCs) that attract bacteria with antifungal properties (Schulz-Bohm *et al*, 2018). Involved in iron nutrition, coumarins are semiochemical phenolic secondary metabolites that shape *A. thaliana* root microbiota (Stringlis *et al*, 2018a; Stringlis *et al*, 2019; Voges *et al*, 2019). Under iron limitation, *A. thaliana* reshapes its root microbiota by secreting coumarins to recruit beneficial microbes that alleviate iron stress (Harbort *et al*, 2020). Taken together, these findings illustrate that immune-related metabolites shape plant microbiota and help plants to overcome various stresses.

Microbiota evolved to persist without adverse effects on host plants

As plants evolved immune systems to face biotic stresses and shape their microbiota, microorganisms evidently adapted to plant-associated environments. Microorganisms have evolved a plethora

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Box 2. Mechanisms of plant growth promotion by microbiota members

Microorganisms evolved a plethora of mechanisms to promote plant host performance. First, they provide nutrition. Root nodule-forming rhizobia catalyze the reduction and ionization of atmospheric dinitrogen into ammonium to improve nitrogen acquisition by legumes (Singh *et al*, 2022). Other microbiota members mediate nutrient uptake from soil. The hyphae of mycorrhizal fungi take up mineral phosphate, ammonium, nitrate, sulphate, potassium, and water, and they provide them to the plant in exchange for carbohydrates and lipids (Martin *et al*, 2016; Liu *et al*, 2020; Berger & Gutjahr, 2021; Kakouridis *et al*, 2022). Rhizosphere microbiota likely act in host nutrition by processing nutrients to permit plant uptake. Many microbes can solubilize inorganic phosphate (Hiruma *et al*, 2016; Rawat *et al*, 2021). Moreover, microbes can relieve iron starvation by reducing ferric into ferrous ions that can be taken up by plant roots (Harbort *et al*, 2020). Likewise, other essential soil macro- and micronutrients can be solubilized, including potassium, zinc, and manganese (Singh *et al*, 2022).

Plant growth can also be promoted by microbes through interference with host metabolism, especially through modulation of phytohormone signaling. Microbial auxin production is a major plant growth-promoting trait, since the most abundant auxin, indole-3-acetic acid, is synthesized by a broad variety of microbes (Bulgarelli *et al*, 2013). By activating root proliferation, auxin may increase absorption of nutrients and water. In contrast, ethylene inhibits root growth and, consequently, plant growth. Thus, some microorganisms secrete 1-aminocyclopropane-1-carboxylate deaminase to convert an ethylene precursor to α-ketobutyrate and ammonia, to prevent ethylene accumulation. Microbes may also promote growth through emission of volatile organic compounds, some of which modulate phytohormone signaling, while others promote photosynthesis (Tyagi *et al*, 2018).

In addition to these direct effects on plant growth, microbes may promote plant performance under abiotic and biotic stresses. Some protect hosts from environmental stressors that include light deprivation, salt stress, warming, heavy metal and cyanide pollution (Chen *et al*, 2020b; Hou *et al*, 2021; Carrell *et al*, 2022; Haque *et al*, 2022; King *et al*, 2022). Other microbes may antagonize potentially pathogenic microbes or elicit defense responses (Vogel *et al*, 2021) to prevent disease development in the holobiont (see subsection "Plant microbiota mediate disease suppression").

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of mechanisms that promote host performance (Box 2). We will first detail known evolutionary trajectories of microbiota members. Then, we will detail how microbes adapt to plant immune responses. Next, we will argue that assimilation or deconstruction of plant carbohydrates is a major driver for microbial adaptation to plants. Finally, we will show that microbes can rapidly evolve to engage in mutualistic plant associations.

Microbiota members evolved from ancestors with diverse lifestyles

Microorganisms have interacted with land plants for 450 million years from their evolutionary origin onwards, and presently engage in a broad range of associations spanning the mutualism-to-parasitism continuum (Delaux & Schornack, 2021). However, the evolutionary trajectory of microbiota members remains poorly understood. Likely, plant-adapted microbes originate from ancestors with diverse lifestyles. The bacterial capacity to fix nitrogen evolved several times independently, in association with plants that are genetically predisposed to form root nodules (Martin *et al*, 2017). Given the differences in root colonization strategies, nitrogen-fixing bacteria likely derive from ancestors with different lifestyles. The adaptation of mycorrhizal fungi to plant roots results from long-term genome evolution, involving extensive gene losses. Glomeromycotina arbuscular mycorrhizal fungi possibly evolved from ancestors forming endosymbioses with cyanobacteria, and the emergence of mycorrhiza was concomitant with substantial gene losses in thiamine sugar and fatty acid metabolism (Mahdi *et al*, 2021). Ectomycorrhizal fungi from the Agaricomycetes class are derived from saprotrophic ancestors by major losses of plant cell wall-degrading enzymes (Kohler *et al*, 2015; Miyauchi *et al*, 2020). Some ancestral saprotrophs of the Agaricomycetes class evolved into endophytic fungi (Garnica *et al*, 2016; Mesny *et al*, 2021). However, most fungal endophytes in the *A. thaliana* root microbiota belong to the Sordariomycetes and Dothideomycetes, and they likely evolved from pathogenic ancestors (Mesny *et al*, 2021). It has been proposed that some endophytic fungi are "in the wait" for co-evolution with their host and development of mycorrhizal symbiosis (Selosse *et al*, 2009, 2022). However, microbiota members can also evolve into virulent pathogens (Bhunjun *et al*, 2023). In both prokaryotes and eukaryotes, only few genes or transcriptomic changes can mediate the differentiation between beneficial and detrimental strains (Mohr *et al*, 2008; Hacquard *et al*, 2016; Melnyk *et al*, 2019; preprint: Hiruma *et al*, 2022; preprint: Thoms *et al*, 2023). Thus, gains and losses of pathogenicity frequently occur.

In advanced stages of their evolutionary history, some microbiota members adapted to particular host plants only. Endophytic shoot microbiota composition was correlated with host evolutionary distance in a study of 11 *Malus* species (Abdelfattah *et al*, 2022). The microbiota of domesticated apple tree (*Malus domestica*) is an admixture of its wild progenitors, suggesting introgression of microbial communities and supporting host-microbiota co-evolution during plant domestication. After bacterial isolation from the roots of *A. thaliana* and *L. japonicus*, reciprocal inoculations of sterile host plants with synthetic communities revealed a competitive advantage of native strains when colonizing roots of their cognate host, but not its rhizosphere (Wippel *et al*, 2021). As this host preference could neither be linked to plant immunity nor to root exudates, its genetic and evolutionary basis remains poorly understood. Host and

nonhost communities did not show significant impact on plant performance but may help their host resist biotic and abiotic stresses in nature. Adaptation to specific plant species was also described for eukaryotic microbiota members, such as for the beneficial fungal endophyte *Epichloë typhina* (Schirrmann & Leuchtmann, 2015), which is reflected in their effector catalogs (Schirrmann *et al*, 2018). Evidence that microbiota are adapted to their cognate hosts further supports co-evolutionary history with potential implication for holobiont health, although the genetic and molecular basis determining this specialization remains mostly unknown.

Microbiota members adapt to plant immune responses

Microbial adaptation to plants involves immunity avoidance and repression. The rapid evolution of genes encoding MAMPs may prevent perception by PRRs and may therefore promote microbial survival (Teixeira *et al*, 2019). Nitrogen-fixing bacteria encode flg22 variants that do not elicit defenses in *L. japonicus*, pointing to mutations to evade immune responses (Lopez-Gomez *et al*, 2012). Similarly, the *A. thaliana* root microbiota comprises a majority of bacteria that produce nonimmunogenic variants of flg22, shown to be under strong evolutionary pressure due to FLS2 receptor activity (Colaianni *et al*, 2021; Parys *et al*, 2021). An alternative approach to evade flg22-triggered immunity evolved in *Pseudomonas* bacteria that repress the synthesis of flagellae (Pfeilmeier *et al*, 2016). Finally, fungi produce proteins capable of binding their MAMPs with high affinity to prevent recognition by PRRs. Typically, lysin-motif (LysM) effectors bind cell wall chitin to prevent perception (Kombrink & Thomma, 2013). While the first LysM effector was characterized in the context of plant pathogenicity (de Jonge *et al*, 2010), such effectors are also produced by nonpathogenic fungi, such as the mycorrhizal symbiont *Rhizophagus irregularis* (Zeng *et al*, 2020). The beneficial endophyte *S. indica* furthermore produces a β-glucan-binding lectin, which sequesters this fungal MAMP but also induces cell wall remodeling to prevent recognition (Wawra *et al*, 2016). In root-associated Betaproteobacteria and Sordariomycetes of *A. thaliana* (Fig 1A and B; Appendix), many genes encoding LysM proteins (CAZyme family CBM50) and other fungal cell wall-active enzymes are under positive selection (Fig 1C–G; Datasets EV1 and EV2). While LysMs can also bind bacterial peptidoglycan and may contribute to a diversity of functions, it is likely that fungal cell wall-binding proteins are under accelerated evolution, so fungi can avoid host detection and survive in plant microbiota. Although some microbes evolved mechanisms to avoid MAMP perception by plants, they may still elicit MTI responses. However, some commensal microbiota members evolved strategies to restrain or suppress these responses. An *A. thaliana* root-associated community of commensal bacteria was recently shown to comprise taxonomically diverse MTI suppressor strains with efficient root colonization abilities (Teixeira *et al*, 2021). Studies of individual microbiota members revealed mechanisms behind such immunity suppression. The beneficial root-colonizer *Pseudomonas simiae* WCS417 actively suppresses more than half of the MAMP-triggered transcriptional responses by modulating plant auxin signaling (Stringlis *et al*, 2018b), whereas *Pseudomonas capeferrum* WCS358 produces organic acids to lower the extracellular pH, thereby suppressing the flg22-mediated oxidative burst and transcriptional reprogramming (Yu *et al*, 2019b). In the fungal kingdom, a common MTI-suppression strategy involves the secretion of effector proteins with diverse functions. *In planta*,
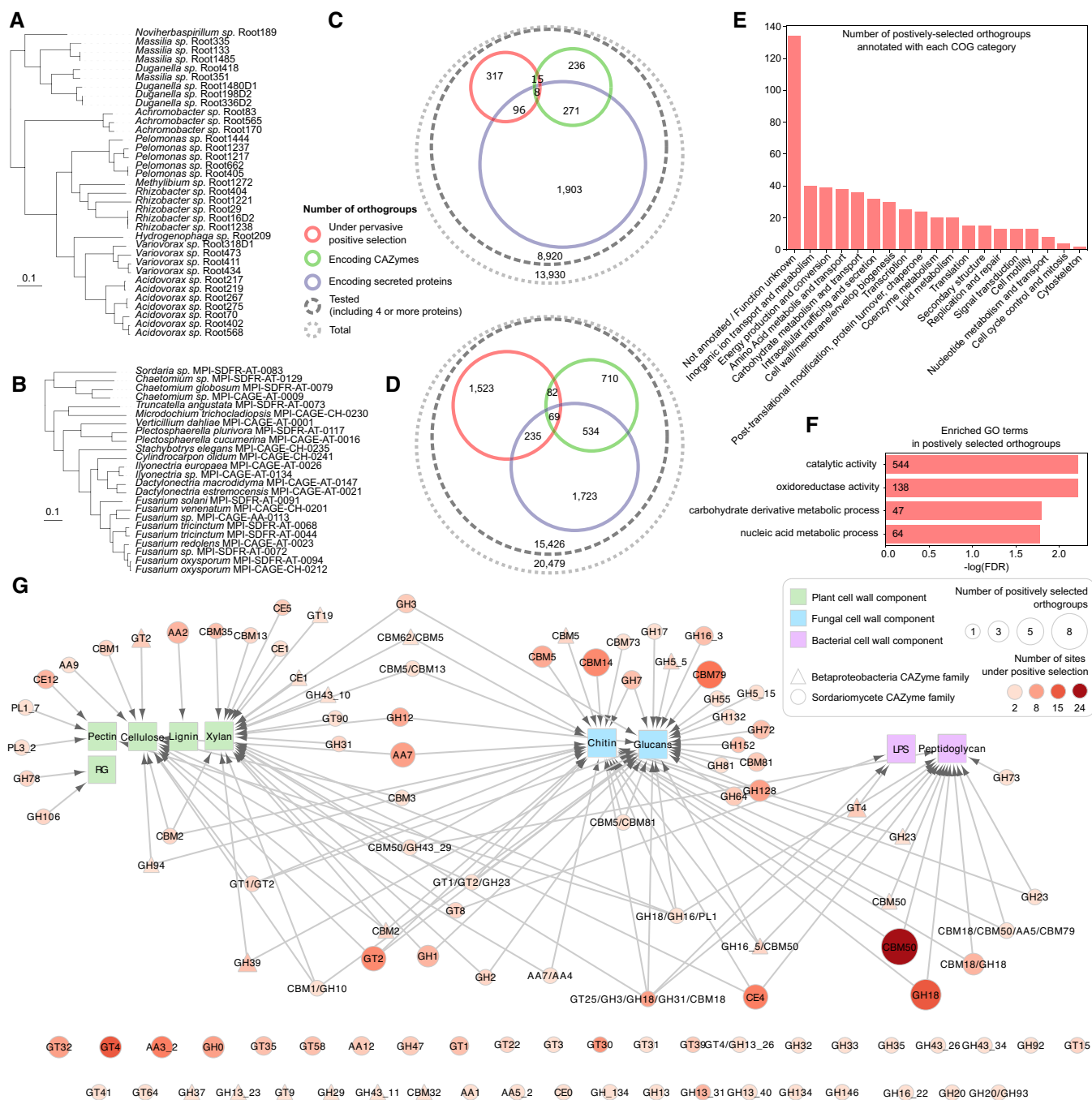
Figure 1. Gene families under positive selection in the *Arabidopsis thaliana* root-associated microbiota.

› We performed dN/dS analyses to identify gene families under pervasive positive selection in the *A. thaliana* root microbiota (Appendix). Phylogenomic trees presenting microbiota members included in the analysis are shown on panels (A) (Betaproteobacteria) and (B) (Sordariomycetes). Counts of gene families under positive selection, encoding carbohydrate-active enzymes (CAZymes) and encoding secreted proteins are depicted on panels (C and D), for Betaproteobacteria and Sordariomycetes respectively (Dataset EV1). Since Betaproteobacteria gene families under positive selection do not show any significant enrichment in Gene Ontology (GO) terms, panel (E) presents the number of families in each Cluster of Orthologous Groups (COG) functional category. Panel (F) shows GO terms significantly enriched in the set of Sordariomycete gene families under positive selection. On panel G, each CAZyme family under positive selection is linked to its putative substrate in plant, microbial and/or fungal cell walls (RG—Rhamnogalacturonan, LPS—Lipopolysaccharide; Dataset EV2).

expression of candidate effector genes is not limited to pathogens but is common for many fungal endophytes, suggesting that effectors are important for their successful endophytic colonization (Lahrmann *et al*, 2015; Hiruma *et al*, 2016; Mesny *et al*, 2021). Moreover, the MiSSP7 effector secreted by the mycorrhizal fungus *L. bicolor* prevents JA-mediated transcriptional activation of immunity-related

genes, thereby promoting the ectomycorrhizal symbiosis (Plett *et al*, 2014). The phylogenetically distant arbuscular mycorrhizal fungus *R. irregularis* produces SP7 effectors that interact with the rice transcription factor ERF19 to attenuate ethylene-mediated immune responses (Kloppholz *et al*, 2011). Similar to pathogens (Wu & Derevnina, 2023), microbiota members may have also evolved adaptive strategies to disrupt plant ETI. Some secrete proteins with domains similar in sequence to some NLR motifs, possibly interfering *in planta* with key protein interactions in the effector-triggered immune signaling (Levy *et al*, 2017). Together, these findings show that the ability to avoid or repress immune responses constitutes an essential trait of plant-adapted microbes.

Importantly, some microbiota members even evolved molecular mechanisms that depend on plant immune responses. While SA-dependent defenses contribute to shaping microbiota composition, several bacterial strains benefit from SA, using it either as growth signal or as carbon source (Lebeis *et al*, 2015). A similar observation was made for root endophytic fungi of horseradish that adapted to defensive glucosinolates (Szűcs *et al*, 2018). Furthermore, oak-adapted leaf endophytic fungi show increased tolerance when compared with generalists to host antifungal polyphenolic compounds and can even utilize some of them as carbon source (Nickerson *et al*, 2023). Root-associated *Bacillus velezensis* bacteria are also adapted to MTI, as ROS produced during *A. thaliana* colonization stimulate bacterial auxin production, promoting efficient root colonization, but also activating plant defense against fungal pathogens (Tzipilevich *et al*, 2021). Finally, studies of individual growth-promoting fungi and bacteria revealed that uncompromised plant immune systems are necessary for their beneficial effects. Accordingly, the fungal endophytes *S. indica* and *C. tofieldiae* are unable to promote growth of immunity-compromised *A. thaliana* mutant lines (Lahrmann *et al*, 2015; Hiruma *et al*, 2016). Similarly, the beneficial root-associated bacteria *Pseudomonas* sp. CH267 requires the *A. thaliana*-specific tryptophan-derived phytoalexin camalexin to promote plant growth (Koprivova *et al*, 2019). Thus, collectively these findings show that the ability to avoid or repress immune responses is key to persist in plant-associated microbiota. Additionally, plant-adapted microorganisms evolved molecular mechanisms that rely on immunity outputs.

Efficient catabolism of plant-derived carbohydrates underlies microbial adaptation to plants

Arguably, plant microbiota members are metabolically adapted to utilize plant-derived carbon compounds, including cell wall components (Müller *et al*, 2016). Different plants have different cell wall composition, and cell walls of monocots and dicots are structurally clearly distinct (Vogel, 2008). Dicot cell walls are pectin-rich (dicots: 35%, maize: 10%), whereas grass cell walls contain more hemicellulose (grasses: 60%, dicots: 30%) and phenolics (up to 5%). Major differences between the microbiota of sugarcane (monocot), *A. thaliana* (dicot), and lignin-rich poplar trees (dicot) (Getzke *et al*, 2019) may reflect differential microbial abilities to utilize different plant cell wall components. Large-scale comparative genomics revealed that the genomes of plant-associated bacteria are enriched in genes involved in carbohydrate metabolism (Levy *et al*, 2017), possibly acquired from plant hosts through horizontal gene transfer (preprint: Haimlich *et al*, 2022). When investigating the functions of genes under positive selection in *A. thaliana* root-associated Betaproteobacteria and Sordariomycetes (Fig 1A–D; Appendix; Dataset EV1), enrichment in enzyme-encoding genes and in genes involved in the metabolism of carbohydrate derivatives was found in Sordariomycetes (Fig 1F). Since microbiota members utilize plant-derived carbohydrates as main energy source, optimization of carbohydrate catabolism is important for microbial adaptation to plant-associated environments. The CAZyme-encoding gene families under positive selection in both Sordariomycetes and Betaproteobacteria comprise plant cell wall-active enzymes acting on xylan, cellulose, and pectin (Fig 1G; Dataset EV2), the main constituents of *A. thaliana* cell walls (Bacic, 2006). Moreover, a positively selected fungal pectin-degrading enzyme constitutes a key genetic determinant underlying efficient colonization of the *A. thaliana* root endosphere (Mesny *et al*, 2021). Cellulose catabolism has also been suggested to rule microbiota assembly, as genetic modification of rice plants revealed that accumulation of cellulose in leaves caused a shift in community composition (preprint: Roman-Reyna *et al*, 2019). Interestingly, the evolution of arbuscular mycorrhizal symbiosis correlated with substantial losses of plant cell wall-degrading enzymes (Malar C *et al*, 2021), and fungal nutrition relies on plant-encoded molecular mechanisms, as plant invertases convert sucrose into glucose and fructose before transfer to the fungal symbiont via monosaccharide transporters (Salvioli di Fossalunga & Novero, 2019). This points to a co-evolutionary model where plants modulate carbon allocation to "reward," and thus select, the symbiont (Kiers *et al*, 2011). Taken together, these findings suggest that evolution of plant cell wall-degradation ability, and more generally carbohydrate metabolism, likely drives plant-microbiota co-evolution.

Rapid microbial adaptation may result in mutualism

Experimental evolution suggests that microbes may rapidly evolve towards mutualism *in planta*. Detrimental *Pseudomonas protegens* evolved beneficially in the rhizosphere of *A. thaliana* within six plant growth cycles (Li *et al*, 2021). Interestingly, after co-cultivating the yeast *Saccharomyces cerevisiae* with the alga *Chlamydomonas reinhardtii*, many yeast adaptive mutations were identified, ranging from competitive to mutualistic (Venkataram *et al*, 2023). While the presence of the alga did not determine which mutations were adaptive, it favored yeast mutants promoting both fungal and algal yields, resulting in stronger mutualism. To decipher the emergence of root nodule symbioses, pathogenic *Ralstonia solanacearum* bacteria transformed with rhizobial nitrogen-fixation and nodulation genes were inoculated onto *Mimosa pudica* legume plants (Marchetti *et al*, 2010). While this did not induce nodule formation upon bacterial infection, rapid transition to mutualism occasionally occurred due to adaptive mutations that inactivated type-III secretion. Finally, after repeated inoculations onto five *Medicago truncatula* accessions, the nitrogen-fixing bacteria *Ensifer meliloti* rapidly became more beneficial toward the host (Batstone *et al*, 2020), confirming that microbial adaptation can be highly host-specific (Schirrmann & Leuchtmann, 2015; Wippel *et al*, 2021). Thus, microorganisms can evolve into mutualistic symbionts over short evolutionary time frames, demonstrating their capacity to rapidly adapt to plant-associated environments, with implications for host health. By evolving plant-beneficial properties, microbiota members can sustain the health of a host, which likely supports their fitness in turn.

To persist in nature, microbes must also evolve ways to survive upon host death. A recent study of the interaction between *Sulfitobacter* D7 and microalga *Emiliania huxleyi* revealed that by sensing algal metabolites, *Sulfitobacter* can switch from coexistence to pathogenicity by upregulating flagellar motility and diverse transport systems, presumably to maximize assimilation of metabolites released upon algal cell death (Noa Barak-Gavish et al, 2023). Also, some fungal microbiota members switch lifestyle to persist in soil after host death. Most oak-associated leaf endophytes show saprophytic capacity to survive in soil litter (Davis et al, 2023). In *Serendipita indica*, transition to a saprotrophic lifestyle is inhibited by host signals (Lahrmann et al, 2013). Mutualism can therefore rapidly arise from plant-microbe co-evolution, but microbial fitness is also dependent on the ability to switch lifestyle and survive upon environmental changes.

Intermicrobial competition evolved to protect the holobiont

While plant cell walls and immune responses constitute major forces driving microbial evolution in holobionts, microbiota members are connected in complex networks of positive and negative interactions. We will first show that plant microbiota are shaped by these interactions, particularly by highly interactive keystone microbes. We will then present how microbiota are structured by intermetabolic dependencies and by microbial antagonism. Finally, we argue that antagonistic intermicrobial interactions can protect holobionts against disease.

Keystone microbes shape microbiota structures

As for any microbial community, interactions between microorganisms structure plant microbiota and drive their assemblies (Hunter et al, 2010; Kemen, 2014; Hassani et al, 2018). In theory, two microorganisms in each community can interact positively, negatively, or not at all. Microbes showing the highest number of interactions are referred to as "hubs" and are thought to be keystones of community structures. If hub microbial presence is affected, major community shifts are typically observed. For instance, in the *A. thaliana* phyllosphere, environmental conditions and host genotypic factors directly impact the presence of two eukaryotic hub microbes, the oomycete *Albugo laibachii* and the fungus *Dioszegia* sp., with cascading consequences on bacterial colonization capabilities and community composition (Agler et al, 2016). Fungi generally represent hubs in plant microbiota, influencing bacterial community composition (Hassani et al, 2018). Mycorrhizal fungi influence bacterial assemblages on grass roots (Singh et al, 2008), and distinct arbuscular mycorrhizal fungi recruit different bacterial communities to the same plant species (Zhou et al, 2020). Importantly, the presence and identity of nonfungal symbionts, especially nitrogen-fixing bacteria, impact root-associated microbial communities (Uroz et al, 2019). These findings prove that intermicrobial interactions structure plant-associated communities and demonstrate the importance of hub microbes for microbiota assemblies.

Metabolic interdependencies shape microbiota composition

While interaction processes favoring microbial coexistence within plant holobionts remain poorly understood (Hassani et al, 2018),

metabolic interdependencies have been described in synthetic microbial communities (Pontrelli et al, 2022). When artificially feeding a community with a single carbohydrate polymer (e.g., a plant cell wall component), it is possible to distinguish degraders that digest the polymer to acquire nutrients from exploiters that feed on the resulting digestion products and are therefore dependent on the presence of the initial degraders, and from scavengers that rely exclusively on metabolites produced by degraders and exploiters and thus depend on their presence for growth (Fig 2). Importantly, microbes can change between trophic levels during their life cycle and scavengers eventually implement positive feedback loops supporting the growth of exploiters and degraders that provide their preferred by-products (Sulheim & Mitri, 2023). However, such simplified tri-trophic hierarchical community structure illustrates that microbial interdependency consists essentially of cross-feeding, generally termed "syntrophy," and relies on different metabolic capabilities. A single limiting carbon compound was shown to rule the assembly of highly diverse communities of leaf-isolated bacteria *in vitro* (Goldford et al, 2018; Murillo-Roos et al, 2022). These communities subsist through cross-feeding owing to differential metabolic capabilities. Bacteria without the ability to utilize the available carbon source can survive by utilizing only secreted or leaked metabolites from other community members (Murillo-Roos et al, 2022). Arguably, metabolic interdependencies evolve through resource-saving gene losses, occurring when the synthesis of a compound becomes dispensable, as provided by community co-inhabitants (Morris et al, 2012). Importantly, by nutritional selective pressure on bacterial interactions, plants shape intermetabolic dependencies (Mataigne et al, 2022). Evolutionary selection will favor the assembled community if beneficial for the plant. In turn, bacterial production of specific metabolites will be selected if cross-feeding results in a reward for the producer, for example, through nutrients from the plant.

Since the ability to degrade plant cell wall components is one of their key traits, fungi act as essential degraders in plant microbiota, further affirming their hub status (Nagy et al, 2017). While feeding on complex cell wall components, fungal microbiota members produce metabolites that maintain communities of bacteria along their hyphae, referred to as the "hyphosphere" (Wang et al, 2022). For instance, arbuscular mycorrhizal fungi carry bacteria along their extraradical hyphae that are fed by hyphal exudates and enhance mineralization of organic phosphorus (Jiang et al, 2021). To reach the legume rhizosphere and trigger nodulation, rhizobia use mycelia as dispersal networks, likely benefiting from fungal exudates (Zhang et al, 2020). In fact, diverse soil bacteria were shown to form biofilms on the hyphae of ectomycorrhizal fungi that are modulated by plant- and fungi-encoded mechanisms (Guennoc et al, 2018). Thus, metabolic interdependencies constitute key forces underlying microbiota assembly.

Intermicrobial competition and antagonism shape microbiota composition

While microbes coexist in communities through cross-feeding and benefit from each other, negative interactions similarly shape microbiota assemblies (Fig 2). A survey of antagonistic interactions among rhizosphere soil, root, and leaf bacteria of the medicinal plant *Echinacea purpurea* revealed that microbial antagonistic abilities and sensitivities differ based on taxonomy, but also ecological
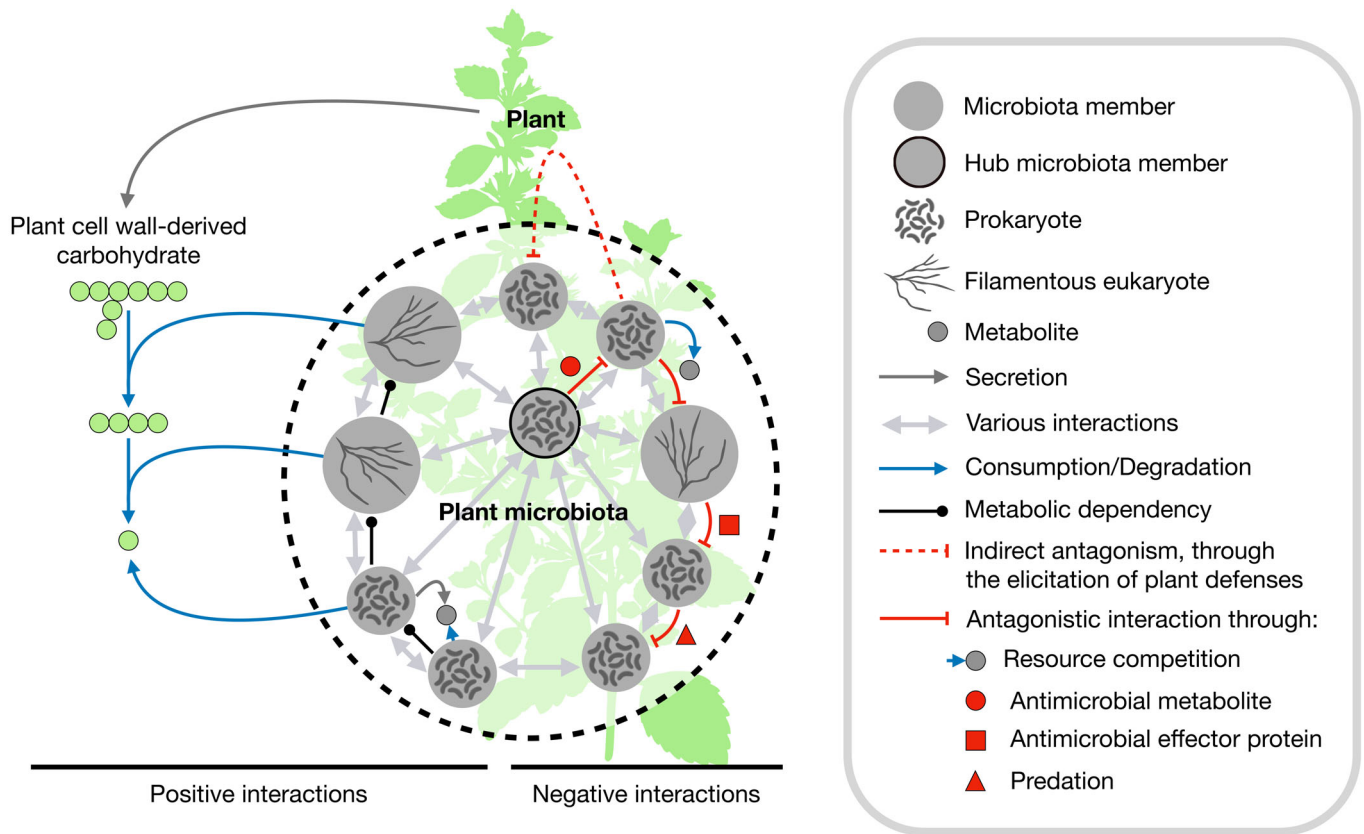
**Figure 2. Intermicrobe interactions drive community structure.**

(Left) Positive interactions between microbiota members mostly consist in cross-feeding and can be observed as organized hierarchically. (Right) Antagonism between microbiota members can be either direct or indirect and relies on a diversity of mechanisms.

niche (Maida et al, 2016). Bacteria isolated from aboveground tissues were much more sensitive to antagonism than bacteria from underground compartments. A high-throughput screening of binary interactions among 224 bacteria isolated from A. thaliana leaves linked antagonistic activities to biosynthetic gene clusters and showed that Brevibacillus sp. is a potent antagonist of microbiota co-inhabitants through the synthesis of antimicrobials such as streptocidins, phosphobrevin, macrobrevin, and marthiapeptide (Helfrich et al, 2018). A similar analysis of binary interactions among 198 A. thaliana root-isolated bacteria identified Pseudomonas brassicacearum as a potent antagonist that relies on the combined action of two exometabolites, an antimicrobial and an iron chelator, that suppress competitors, and thereby promote its root colonization (Getzke et al, 2023).

Resource competition represents an important mechanism for indirect microbial antagonism (Hassani et al, 2018). The ability to rapidly utilize a limited resource can be detrimental for a less competitive microbe. Additionally, microbes can sequester resources, preventing utilization by other community members. Siderophores chelate soil iron for microbial uptake and are differentially exploitable to prevent other microbes to obtain essential iron resources (Joshi et al, 2006). For instance, pyoverdine is an iron-chelator produced by some A. thaliana root-associated bacteria contributing to their competitiveness (Getzke et al, 2023).

Microorganisms also directly suppress growth of microbial opponents. Some Pseudomonas bacteria produce 2,4-diacetylphloroglucinol, a secondary metabolite with both antibacterial and antifungal properties, affecting root-associated bacteria and fungi of A. thaliana and wheat (Bakker et al, 2002; Getzke et al, 2023). Additionally, antimicrobial volatile organic compounds may be produced, for instance by potato-isolated Pseudomonas strains to antagonize oomycete growth (De Vrieze et al, 2015). Endophytic fungi are well-known to produce antibiotic metabolites (Martinez-Klimova et al, 2017). For instance, Fusarium and Alternaria spp., frequently found in A. thaliana root microbiota (Thiergart et al, 2020), secrete metabolites that restrain the growth of other microbes (Martinez-Klimova et al, 2017).

Intriguingly, it was recently uncovered that fungi exploit effectors with antibacterial and antifungal activities that modulate the composition of plant-associated communities (Snelders et al, 2020, 2021, 2023). This extends the range of previously considered effector activities, typically thought-out to mainly act in manipulation of host physiology to support host colonization, frequently through modulation of host immune signaling. While effectors with antimicrobial activity were initially described in the context of plant infection by the pathogenic soil-borne and broad host-range pathogen Verticillium dahliae, other fungal pathogens may produce such effectors too. More recently, it was reported that the soil-borne
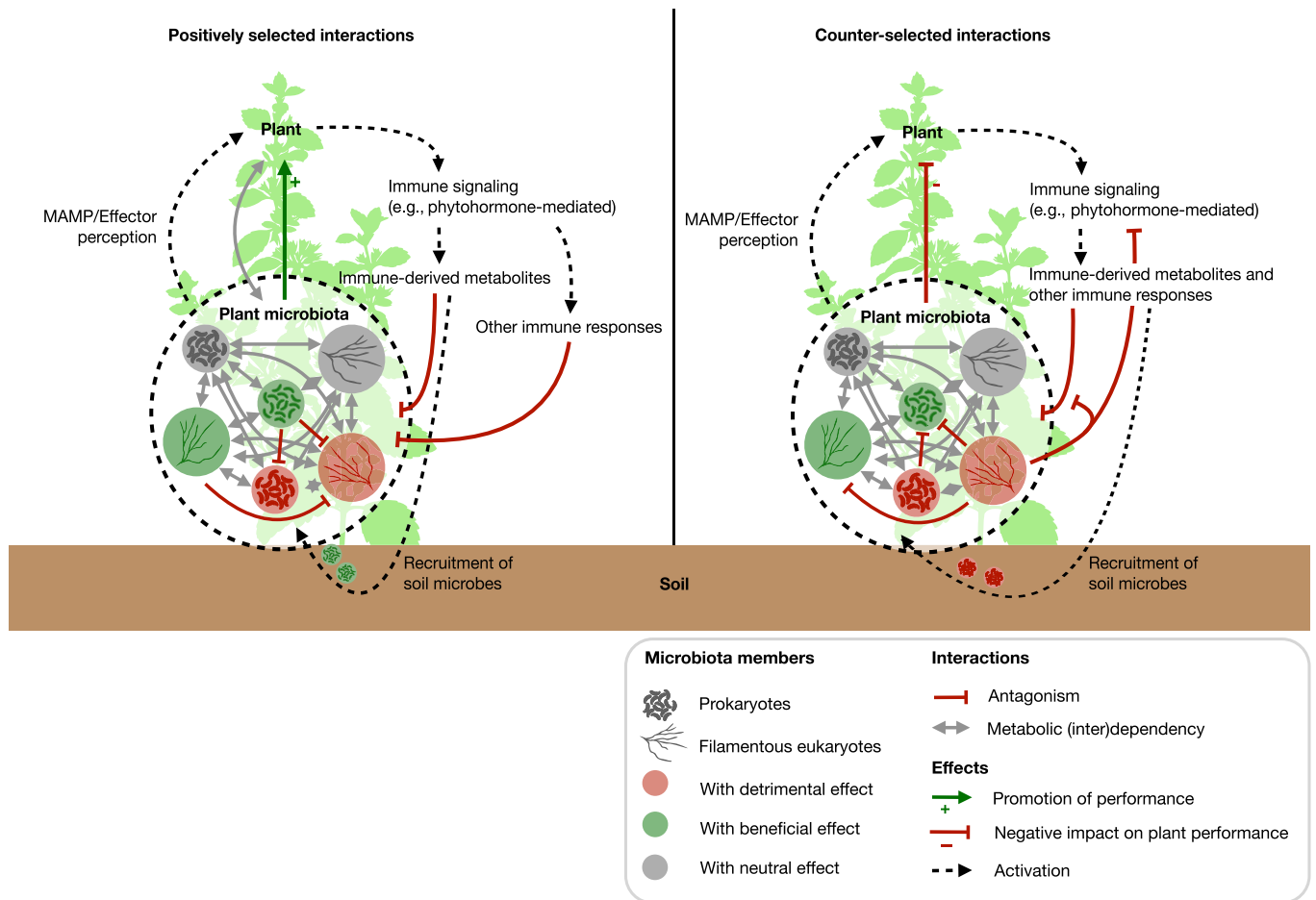
Figure 3. **Positively selected interactions within the holobiont ultimately promote plant performance.**
(Left) Graphical summary of the interactions reviewed in this article, that are positively selected by the plant in the holobiont and together, ultimately contribute to promoting plant performance. (Right) Graphical representation of interactions that are likely counter-selected for through plant-mediated mechanisms, according to the holobiont hypothesis, since they would ultimately result in negative impact on plant performance.

white root rot pathogen *Rosellinia necatrix* expresses antimicrobial effector proteins during host colonization, while smut fungi express a conserved extracellular ribonuclease with broad-spectrum cytotoxic activity to compete with host-associated bacteria on the leaves of host plants (preprint: Chavarro-Carrero *et al*, 2023; preprint: Ökmen *et al*, 2023). Pathogenic oomycetes seem to rely on a similar strategy, since *Albugo candida* secretes antimicrobials into the apoplast of *A. thaliana* leaves to repress the growth of keystone bacteria (Gómez-Pérez *et al*, 2023). The expected occurrence of these effectors across the fungal kingdom, and beyond, suggests that such effectors are likely produced by fungi with diverse lifestyles, including commensal fungi in plant microbiota (Snelders *et al*, 2022). Although several such effectors may result from inter-microbial co-evolution (Snelders *et al*, 2022), antimicrobial effectors of plant-associated filamentous eukaryotes broadly evolved to support fungal accommodation in the holobiont, since some are multifunctional and target plant processes.

Finally, a third intermicrobial antagonism mechanism that contributes to plant microbiota assembly is predation. Mycophagous bacteria that grow at the expense of living fungal hyphae occur in plant microbiota (Rudnick *et al*, 2015; Hassani *et al*, 2018). Furthermore, the bacterial prey range of the predator *Bdellovibrio* spp. isolated from roots of common bean suggests adaptation to this environment, as most targets are root-associated bacteria (Jurkevitch *et al*, 2000).

Plant microbiota mediate disease suppression

By hosting antagonistic microorganisms, plants extend their capacity to prevent disease development. This microbiota function is essential for host survival and persistence, and thus a driving force for plant-microbiota co-evolution. The "cry for help" theory proposes exudate-mediated recruitment of disease-suppressive microbiota by plants facing pathogen attack, but it remains to be validated (Rizaludin *et al*, 2021). Roots of *A. thaliana* naturally host fungi with high pathogenic potential, especially *Fusarium*, *Plectosphaerella* and *Ilyonectria* (Mesny *et al*, 2021). Detrimental overcolonization by these fungi is prevented by bacterial root commensals, including Pseudomonadaceae and Comamonadaceae, that display particularly high antifungal activities in binary interaction assays (Durán *et al*, 2018). In the *A. thaliana* phyllosphere, the oomycete hub *Albugo laibachii* affects plant health unless

controlled by a lysozyme secreted by the yeast *Moesziomyces bullatus* (Eitzen *et al*, 2021). Similarly, the bentgrass leaf endophyte *Epichloë festucae* secretes an antifungal protein that antagonizes the dollar spot pathogen *Clarireedia jacksonii* (Fardella *et al*, 2022). Upon sugar beet infection by *Rhizoctonia solani*, root endophytic bacteria prevent disease development by production of antifungal secondary metabolites (Carrión *et al*, 2019). Protective microbiota members also rely on the emission of volatile organic compounds to antagonize pathogens. Maize rhizosphere *Pseudomonas* release volatiles that significantly affect mycelial growth of *Fusarium proliferatum* (Cordero *et al*, 2014). Plant protection can additionally occur via production of microbial physical barriers, as endophytic *Enterobacter* form biofilm-mediated microcolonies and assemble multilayer root-hair endophyte stacks to constitute a physical barrier that traps *Fusarium graminearum* (Mousa *et al*, 2016). Tomato root endophytic *Rahnella aquatilis* bacteria counteract *F. oxysporum*-induced rhizosphere alkalinization through secretion of gluconic acid while moving through hyphae to reach and colonize plant roots, thereby preventing fungal infection (Palmieri *et al*, 2020). Interestingly, the poplar phyllosphere includes diverse fungal endophytes that increase or decrease *Melampsora* leaf rust disease (Busby *et al*, 2016). In a community context, the combined effect of individual microbial antagonistic activities can result in plant protection. Consistently, synergistically with root-associated bacteria the fungal root endophyte *Serendipita vermifera* protects plants against the soil-borne fungal pathogen *Bipolaris sorokiniana* (Mahdi *et al*, 2021). Finally, rhizosphere bacteria of *Mikania micrantha* preventing harm from soil-resident *Fusarium* fungi and *Ralstonia* bacteria were associated with plant invasiveness, demonstrating the contribution of disease-protective microbiota members to fitness (Yin *et al*, 2020). Therefore, plant protection from pathogens represents a key function of the microbiota, mostly through antagonistic capabilities. Similar to plant-microbe co-evolution, evolutionary arms races likely rule microbial evolution within holobionts. As previously mentioned, the wilt pathogen *V. dahliae* evolved antimicrobial effector proteins targeting antagonists in the plant microbiota to successfully colonize the plant and cause disease (Snelders *et al*, 2020, 2021, 2022, 2023). Under the selection pressure of the antimicrobials secreted by *V. dahliae*, protective strains insensitive to these effectors will be selected. Consistent with this hypothesis, plant-pathogenic *R. solanacearum* adapts to growth-inhibiting *Bacillus amyloliquefaciens* VOCs, although this increased tolerance negatively affected *R. solanacearum* virulence (Wang *et al*, 2023).

Importantly, disease suppression also occurs through microbiota-induced elicitation of plant immunity (Fig 2). The physiological state of enhanced immunity induced by microbiota members is commonly referred to as "induced systemic resistance" (Trivedi *et al*, 2020). For instance, the protective ability of leaf-isolated bacteria against the bacterial pathogen *Pseudomonas syringae* pv. tomato is reduced in MTI-compromised *A. thaliana* (Vogel *et al*, 2021). Axenic *A. thaliana* are hypersusceptible to this foliar pathogen, whereas plants recolonized with synthetic and soil-derived communities show substantially restored immunocompetence (preprint: Paasch *et al*, 2023). Further studies are needed to better characterize the relative contributions of intermicrobial antagonism and induced systemic resistance in disease suppression.

> ### Box 3. In need of answers
>
> - How are holobionts shaped, and are plant-microbe interactions more important drivers than inter-microbial ones? Are the co-evolutionary dynamics of a given plant-microbe association affected by the presence of microbiota co-inhabitants?
> - Do holobiont subcommunities exhibit different dynamics? How persistent are selected interactions across time and upon environmental changes?
> - What plant factors govern holobiont composition? Can we confirm the key structuring roles of plant cell wall composition and exudates? Besides MTI pathways, do NLR catalogs play a determining role?
> - How convergent is the evolution of phylogenetically distant microbes that adapt to the same host? Is a core set of microbial functions required?
> - Is the invasiveness of a microbe correlated with its pathogenic potential? To what extent have microbes with a high pathogenic potential evolved to manipulate the microbiota of the plant holobiont in order to colonize the host?

Conclusion and outlook

Complex plant–microbe and intermicrobial interactions in plant holobionts can result in beneficial outcomes for the plant (Fig 3). We argue that most of these interactions evolved within plant holobionts and have been selected to maintain an environment promoting the survival and fitness of the whole holobiont. On one side, plants evolved mechanisms that shape their microbiota, such as immune responses and secreted metabolites. This arsenal is key to protect plants from disease, as it fights pathogens and prevents dysbiosis. Moreover, plants evolved the capacity to actively recruit beneficial microorganisms that contribute to their performance and their resistance to biotic and abiotic stresses. On the microbial side, genomes of microbiota members carry signatures of adaptation to plant-associated environments, resulting in gene repertoire modifications for carbohydrate metabolism and allowing efficient utilization of plant carbon compounds. Recent findings based on experimental evolution suggest that mutualism can rapidly arise from evolution of microorganisms in close association with plant hosts. Importantly, plant-adapted microbes overcome plant immune outputs, and sometimes benefit from them. Therefore, microbial adaptation results in an improved capacity to persist in plant-associated environments. Finally, within plant microbiota, microorganisms are tightly interconnected, through metabolic interdependencies and antagonistic relationships. While metabolic interdependencies rule microbiota assemblies, in combination with the plant immune system, antagonism mitigates detrimental activities of microbiota co-inhabitants with high pathogenic potential. This microbial defense layer contributes to disease protection, and thereby supports holobiont persistence. Thus, because of their direct impact on plant performance, interactions within holobionts are under strong selection pressure. However, their evolutionary dynamics remain poorly understood (Box 3).

Expanded View for this article is available online.

Author contributions

Fantin Mesny: Conceptualization; funding acquisition; writing – original draft; writing – review and editing. **Stéphane Hacquard:** Supervision; funding acquisition; writing – review and editing. **Bart PHJ Thomma:** Conceptualization; supervision; funding acquisition; writing – review and editing.

Disclosure and competing interests statement

The authors declare that they have no conflict of interest.

References

Abdelfattah A, Tack AJM, Wasserman B, Liu J, Berg G, Norelli J, Droby S, Wisniewski M (2022) Evidence for host–microbiome co-evolution in apple. *New Phytol* 234: 2088 – 2100

Abdelfattah A, Tack AJM, Lobato C, Wassermann B, Berg G (2023) From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. *Trends Microbiol* 31: 346 – 355

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol* 14: e1002352

Archibald JM (2015) Endosymbiosis and eukaryotic cell evolution. *Curr Biol* 25: R911 – R921

Arnault G, Mony C, Vandenkoornhuyse P (2023) Plant microbiota dysbiosis and the Anna Karenina principle. *Trends Plant Sci* 28: 18 – 30

Bacic A (2006) Breaking an impasse in pectin biosynthesis. *Proc Natl Acad Sci U S A* 103: 5639 – 5640

Bakker PAHM, Glandorf DCM, Viebahn M, Ouwens TWM, Smit E, Leeflang P, Wernars K, Thomashow LS, Thomas-Oates JE, Van Loon LC (2002) Effects of pseudomonas putida modified to produce phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol on the microflora of field grown wheat. *Antonie Van Leeuwenhoek* 81: 617 – 624

Barak-Gavish N, Dassa B, Kuhlisch C, Nussbaum I, Brandis A, Rosenberg G, Avraham R, Vardi A (2023) Bacterial lifestyle switch in response to algal metabolites. *Elife* 12: e84400

Batstone RT, O'Brien AM, Harrison TL, Frederickson ME (2020) Experimental evolution makes microbes more cooperative with their local host genotype. *Science* 370: 476 – 478

Berg G, Schweitzer M, Abdelfattah A, Cernava T, Wassermann B (2023) Missing symbionts – emerging pathogens? Microbiome management for sustainable agriculture. *Symbiosis* 89: 163 – 171

Berger F, Gutjahr C (2021) Factors affecting plant responsiveness to arbuscular mycorrhiza. *Curr Opin Plant Biol* 59: 101994

Bhunjun CS, Phukhamsakda C, Hyde KD, McKenzie EHC, Saxena RK, Li Q (2023) Do all fungi have ancestors with endophytic lifestyles? *Fungal Divers* 1: 1 – 26

Bjornson M, Pimprikar P, Nürnberger T, Zipfel C (2021) The transcriptional landscape of *Arabidopsis thaliana* pattern-triggered immunity. *Nat Plants* 7: 579 – 586

Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA (2014) A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genet* 10: e1004283

Bouffaud ML, Poirier MA, Muller D, Moënne-Loccoz Y (2014) Root microbiome relates to plant host evolution in maize and other Poaceae. *Environ Microbiol* 16: 2804 – 2814

Bulgarelli D, Schlaeppi K, Spaepen S, Van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. *Annu Rev Plant Biol* 64: 807 – 838

Busby PE, Peay KG, Newcombe G (2016) Common foliar fungi of *Populus trichocarpa* modify Melampsora rust disease severity. *New Phytol* 209: 1681 – 1692

Cadot S, Guan H, Bigalke M, Walser JC, Jander G, Erb M, van der Heijden MGA, Schlaeppi K (2021) Specific and conserved patterns of microbiota-structuring by maize benzoxazinoids in the field. *Microbiome* 9: 1 – 19

Carrell AA, Lawrence TJ, Cabugao KGM, Carper DL, Pelletier DA, Lee JH, Jawdy SS, Grimwood J, Schmutz J, Hanson PJ *et al* (2022) Habitat-adapted microbial communities mediate sphagnum peatmoss resilience to warming. *New Phytol* 234: 2111 – 2125

Carrión VJ, Perez-Jaramillo J, Cordovez V, Tracanna V, De Hollander M, Ruiz-Buck D, Mendes LW, van Ijcken WFJ, Gomez-Exposito R, Elsayed SS *et al* (2019) Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* 366: 606 – 612

Carvalhais LC, Dennis PG, Badri DV, Tyson GW, Vivanco JM, Schenk PM (2013) Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. *PloS One* 8: e56457

Carvalhais LC, Dennis PG, Badri DV, Kidd BN, Vivanco JM, Schenk PM (2015) Linking jasmonic acid signaling, root exudates, and rhizosphere microbiomes. *Mol Plant Microbe Interact* 28: 1049 – 1058

Casadevall A (2017) The pathogenic potential of a microbe. *mSphere* 2: e00015-17

Casadevall A (2022) Expanding the pathogenic potential concept to incorporate fulminancy, time, and virulence factors. *mSphere* 7: e0102121

Chavarro-Carrero EA, Snelders NC, Torres DE, Kraege A, López-Moral A, Petti GC, Punt W, Wieneke J, García-Velasco R, López-Herrera CJ *et al* (2023) The soil-borne white root rot pathogen Rosellinia necatrix expresses antimicrobial proteins during host colonization. *bioRxiv* https://doi.org/10.1101/2023.04.10.536216 [PREPRINT]

Chen T, Nomura K, Wang X, Sohrabi R, Xu J, Yao L, Paasch BC, Ma L, Kremer J, Cheng Y *et al* (2020a) A plant genetic network for preventing dysbiosis in the phyllosphere. *Nature* 580: 653 – 657

Chen Y, Bonkowski M, Shen Y, Griffiths BS, Jiang Y, Wang X, Sun B (2020b) Root ethylene mediates rhizosphere microbial community reconstruction when chemically detecting cyanide produced by neighbouring plants. *Microbiome* 8: 1 – 17

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124: 803 – 814

Colaianni NR, Parys K, Lee HS, Conway JM, Kim NH, Edelbacher N, Mucyn TS, Madalinski M, Law TF, Jones CD *et al* (2021) A complex immune response to flagellin epitope variation in commensal communities. *Cell Host Microbe* 29: 635 – 649

Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *J Adv Res* 19: 29 – 37

Cook DE, Mesarich CH, Thomma BPHJ (2015) Understanding plant immunity as a surveillance system to detect invasion. *Annu Rev Phytopathol* 53: 541−563

Cordero P, Príncipe A, Jofré E, Mori G, Fischer S (2014) Inhibition of the phytopathogenic fungus *Fusarium proliferatum* by volatile compounds produced by pseudomonas. *Arch Microbiol* 196: 803−809

Cotton TEA, Pétriacq P, Cameron DD, Al MM, Schwarzenbacher R, Rolfe SA, Ton J (2019) Metabolic regulation of the maize rhizobiome by benzoxazinoids. *ISME J* 13: 1647−1658

Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, Schadt CW (2018) The Populus holobiont: dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* 6: 1−14

Cui H, Gobbato E, Kracher B, Qiu J, Bautor J, Parker JE (2017) A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in *Arabidopsis* immunity. *New Phytol* 213: 1802−1817

Davis EL, Weatherhead E, Koide RT (2023) The potential saprotrophic capacity of foliar endophytic fungi from *Quercus gambelii*. *Fungal Ecol* 62: 101221

De Jonge R, Van Esse HP, Kombrink A, Shinya T, Desaki Y, Bours R, Van Der Krol S, Shibuya N, Joosten MHAJ, Thomma BPHJ (2010) Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. *Science* 329: 953−955

De Vrieze M, Pandey P, Bucheli TD, Varadarajan AR, Ahrens CH, Weisskopf L, Bailly A (2015) Volatile organic compounds from native potato-associated pseudomonas as potential anti-oomycete agents. *Front Microbiol* 6: 1295

Delaux P-M, Schornack S (2021) Plant evolution driven by interactions with symbiotic and pathogenic microbes. *Science* 371: eaba6605

Deng S, Caddell DF, Xu G, Dahlen L, Washington L, Yang J, Coleman-Derr D (2021) Genome wide association study reveals plant loci controlling heritability of the rhizosphere microbiome. *ISME J* 15: 3181−3194

Doornbos RF, Geraats BPJ, Kuramae EE, Van Loon LC, Bakker PAHM (2011) Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of *Arabidopsis thaliana*. *Mol Plant Microbe Interact* 24: 395−407

Durán P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S (2018) Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell* 175: 973−983

Eitzen K, Sengupta P, Kroll S, Kemen E, Doehlemann G (2021) A fungal member of the *Arabidopsis thaliana* phyllosphere antagonizes *Albugo laibachii* via a GH25 lysozyme. *Elife* 10: e65306

Escudero-Martinez C, Coulter M, Alegria Terrazas R, Foito A, Kapadia R, Pietrangelo L, Maver M, Sharma R, Aprile A, Morris J *et al* (2022) Identifying plant genes shaping microbiota composition in the barley rhizosphere. *Nat Commun* 13: 1−14

Fardella PA, Tian Z, Clarke BB, Belanger FC (2022) The Epichloë festucae antifungal protein Efe-AfpA protects creeping bentgrass (*Agrostis stolonifera*) from the plant pathogen *Clarireedia jacksonii*, the causal agent of dollar spot disease. *J Fungi* 8: 1097

Feehan JM, Wang J, Sun X, Choi J, Ahn H-K, Pok Man Ngou B, Parker JE, Jones JD G, Ngou M, Ahn H *et al* (2023) Oligomerization of a plant helper NLR requires cell-surface and intracellular immune receptor activation. *Proc Natl Acad Sci U S A* 120: e2210406120

Feng F, Sun J, Radhakrishnan GV, Lee T, Bozsóki Z, Fort S, Gavrin A, Gysel K, Thygesen MB, Andersen KR *et al* (2019) A combination of chitooligosaccharide and lipochitooligosaccharide recognition promotes arbuscular mycorrhizal associations in *Medicago truncatula*. *Nat Commun* 10: 5047

Garnica S, Riess K, Schön ME, Oberwinkler F, Setaro SD (2016) Divergence times and phylogenetic patterns of Sebacinales, a highly diverse and widespread fungal lineage. *PloS One* 11: e0149531

Getzke F, Thiergart T, Hacquard S (2019) Contribution of bacterial-fungal balance to plant and animal health. *Curr Opin Microbiol* 49: 66−72

Getzke F, Hassani MA, Crüsemann M, Malisic M, Zhang P, Ishigaki Y, Böhringer N, Jiménez Fernández A, Wang L, Ordon J *et al* (2023) Cofunctioning of bacterial exometabolites drives root microbiota establishment. *Proc Natl Acad Sci U S A* 120: e2221508120

Ghantasala S, Roy Choudhury S (2022) Nod factor perception: an integrative view of molecular communication during legume symbiosis. *Plant Mol Biol* 110: 485−509

Goldford JE, Lu N, Bajić D, Estrela S, Tikhonov M, Sanchez-Gorostiaga A, Segrè D, Mehta P, Sanchez A (2018) Emergent simplicity in microbial community assembly. *Science* 361: 469−474

Gómez-Pérez D, Schmid M, Chaudhry V, Hu Y, Velic A, Maček B, Ruhe J, Kemen A, Kemen E (2023) Proteins released into the plant apoplast by the obligate parasitic protist Albugo selectively repress phyllosphere-associated bacteria. *New Phytol* https://doi.org/10.1111/nph.18995

Gross M (2022) How plants grow their microbiome. *Curr Biol* 32: R97−R100

Guennoc CM, Rose C, Labbé J, Deveau A (2018) Bacterial biofilm formation on the hyphae of ectomycorrhizal fungi: a widespread ability under controls? *FEMS Microbiol Ecol* 94: 93

Hacquard S, Kracher B, Hiruma K, Münch PC, Garrido-Oter R, Thon MR, Weimann A, Damm U, Dallery JF, Hainaut M *et al* (2016) Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. *Nat Commun* 7: 1−13

Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P (2017) Interplay between innate immunity and the plant microbiota. *Annu Rev Phytopathol* 55: 565−589

Haimlich S, Fridman Y, Khandal H, Savaldi-Goldstein S, Levy A (2022) Widespread horizontal gene transfer between plants and their microbiota. *bioRxiv* https://doi.org/10.1101/2022.08.25.505314 [PREPRINT]

Han GZ (2019) Origin and evolution of the plant immune system. *New Phytol* 222: 70−83

Haque S, Srivastava N, Pal DB, Alkhanani MF, Almalki AH, Areeshi MY, Naidu R, Gupta VK (2022) Functional microbiome strategies for the bioremediation of petroleum-hydrocarbon and heavy metal contaminated soils: a review. *Sci Total Environ* 833: 155222

Harbort CJ, Hashimoto M, Inoue H, Niu Y, Guan R, Rombolà AD, Kopriva S, Voges MJEEE, Sattely ES, Garrido-Oter R *et al* (2020) Root-secreted coumarins and the microbiota interact to improve iron nutrition in *Arabidopsis*. *Cell Host Microbe* 28: 825−837

Hassani MA, Durán P, Hacquard S (2018) Microbial interactions within the plant holobiont. *Microbiome* 6: 1−17

He K, Gou X, Yuan T, Lin H, Asami T, Yoshida S, Russell SD, Li J (2007) BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. *Curr Biol* 17: 1109−1115

Helfrich EJN, Vogel CM, Ueoka R, Schäfer M, Ryffel F, Müller DB, Probst S, Kreuzer M, Piel J, Vorholt JA (2018) Bipartite interactions, antibiotic production and biosynthetic potential of the *Arabidopsis* leaf microbiome. *Nat Microbiol* 3: 909−919

Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramírez D, Bucher M, O'Connell RJ *et al* (2016) Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. *Cell* 165: 464−474

Hiruma K, Aoki S, Utami YD, Okamoto M, Kawamura N, Nakamura M, Ohmori Y, Sugita R, Tanoi K, Sato T *et al* (2022) A fungal secondary metabolism gene cluster enables mutualist-pathogen transition in root endophyte *Colletotrichum tofieldiae*. *bioRxiv* https://doi.org/10.1101/2022.07.07.499222 [PREPRINT]

Hou S, Thiergart T, Vannier N, Mesny F, Ziegler J, Pickel B, Hacquard S (2021) A microbiota–root–shoot circuit favours *Arabidopsis* growth over defence under suboptimal light. *Nat Plants* 7: 1078–1092

Huang AC, Jiang T, Liu YX, Bai YC, Reed J, Qu B, Goossens A, Nützmann HW, Bai Y, Osbourn A (2019) A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. *Science* 364: eaau6389

Hunter PJ, Hand P, Pink D, Whipps JM, Bending GD (2010) Both leaf properties and microbe-microbe interactions influence within-species variation in bacterial population diversity and structure in the lettuce (lactuca species) phyllosphere. *Appl Environ Microbiol* 76: 8117–8125

Jaspers C, Fraune S, Arnold AE, Miller DJ, Bosch TCG, Voolstra CR (2019) Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. *Fortschr Zool* 133: 81–87

Jiang F, Zhang L, Zhou J, George TS, Feng G (2021) Arbuscular mycorrhizal fungi enhance mineralisation of organic phosphorus by carrying bacteria along their extraradical hyphae. *New Phytol* 230: 304–315

Jones JDG, Dangl JL (2006) The plant immune system. *Nature* 444: 323–329

Jones P, Garcia BJ, Furches A, Tuskan GA, Jacobson D (2019) Plant host-associated mechanisms for microbial selection. *Front Plant Sci* 10: 862

Joshi F, Archana G, Desai A (2006) Siderophore cross-utilization amongst rhizospheric bacteria and the role of their differential affinities for Fe^{3+} on growth stimulation under iron-limited conditions. *Curr Microbiol* 53: 141–147

Jurkevitch E, Minz D, Ramati B, Barel G (2000) Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria. *Appl Environ Microbiol* 66: 2365–2371

Kakouridis A, Hagen JA, Kan MP, Mambelli S, Feldman LJ, Herman DJ, Weber PK, Pett-Ridge J, Firestone MK (2022) Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. *New Phytol* 236: 210–221

Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL (2014) Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proc Natl Acad Sci U S A* 111: 13715–13720

Kemen E (2014) Microbe–microbe interactions determine oomycete and fungal host colonization. *Curr Opin Plant Biol* 20: 75–81

Kenrick P, Strullu-Derrien C (2014) The origin and early evolution of roots. *Plant Physiol* 166: 570–580

Kia SH, Glynou K, Nau T, Thines M, Piepenbring M, Maciá-Vicente JG (2017) Influence of phylogenetic conservatism and trait convergence on the interactions between fungal root endophytes and plants. *ISME J* 11: 777–790

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A *et al* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333: 880–882

King WL, Kaminsky LM, Gannett M, Thompson GL, Kao-Kniffin J, Bell TH (2022) Soil salinization accelerates microbiome stabilization in iterative selections for plant performance. *New Phytol* 234: 2101–2110

Kloppholz S, Kuhn H, Requena N (2011) A secreted fungal effector of glomus intraradices promotes symbiotic biotrophy. *Curr Biol* 21: 1204–1209

Kniskern JM, Traw MB, Bergelson J (2007) Salicylic acid and jasmonic acid signaling defense pathways reduce natural bacterial diversity on *Arabidopsis thaliana*. *Mol Plant Microbe Interact* 20: 1512–1522

Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A *et al* (2015) Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat Genet* 47: 410–415

Kombrink A, Thomma BPHJ (2013) LysM effectors: secreted proteins supporting fungal life. *PLoS Pathog* 9: e1003769

Koprivova A, Schuck S, Jacoby RP, Klinkhammer I, Welter B, Leson L, Martyn A, Nauen J, Grabenhorst N, Mandelkow JF *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

Lahrmann U, Ding Y, Banhara A, Rath M, Hajirezaei MR, Döhlemann S, Von Wirén N, Parniske M, Zuccaro A (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. *Proc Natl Acad Sci U S A* 110: 13965–13970

Lahrmann U, Strehmel N, Langen G, Frerigmann H, Leson L, Ding Y, Scheel D, Herklotz S, Hilbert M, Zuccaro A (2015) Mutualistic root endophytism is not associated with the reduction of saprotrophic traits and requires a noncompromised plant innate immunity. *New Phytol* 207: 841–857

Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S, Del Rio TG, Jones CD, Tringe SG *et al* (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349: 860–864

Levy A, Salas Gonzalez I, Mittelviefhaus M, Clingenpeel S, Herrera Paredes S, Miao J, Wang K, Devescovi G, Stillman K, Monteiro F *et al* (2017) Genomic features of bacterial adaptation to plants. *Nat Genet* 50: 138–150

Li E, de Jonge R, Liu C, Jiang H, Friman VP, Pieterse CMJ, Bakker PAHM, Jousset A (2021) Rapid evolution of bacterial mutualism in the plant rhizosphere. *Nat Commun* 12: 1–13

Liu L, Sonbol FM, Huot B, Gu Y, Withers J, Mwimba M, Yao J, He SY, Dong X (2016) Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. *Nat Commun* 7: 1–10

Liu Y, Li X, Kou Y (2020) Ectomycorrhizal fungi: participation in nutrient turnover and community assembly pattern in forest ecosystems. *Forests* 11: 453

Lopez-Gomez M, Sandal N, Stougaard J, Boller T (2012) Interplay of flg22-induced defence responses and nodulation in Lotus japonicus. *J Exp Bot* 63: 393–401

Lyu D, Zajonc J, Pagé A, Tanney CAS, Shah A, Monjezi N, Msimbira LA, Antar M, Nazari M, Backer R *et al* (2021) Plant holobiont theory: the phytomicrobiome plays a central role in evolution and success. *Microorganisms* 9: 675

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

Mahdi LK, Miyauchi S, Uhlmann C, Garrido-Oter R, Langen G, Wawra S, Niu Y, Guan R, Robertson-Albertyn S, Bulgarelli D *et al* (2021) The fungal root endophyte Serendipita vermifera displays inter-kingdom synergistic beneficial effects with the microbiota in *Arabidopsis thaliana* and barley. *ISME J* 16: 876–889

Maida I, Chiellini C, Mengoni A, Bosi E, Firenzuoli F, Fondi M, Fani R (2016) Antagonistic interactions between endophytic cultivable bacterial communities isolated from the medicinal plant *Echinacea purpurea*. *Environ Microbiol* 18: 2357–2365

Malar C M, Krüger M, Krüger C, Wang Y, Stajich JE, Keller J, Chen ECH, Yildirir G, Villeneuve-Laroche M, Roux C *et al* (2021) The genome of *Geosiphon pyriformis* reveals ancestral traits linked to the emergence of the arbuscular mycorrhizal symbiosis. *Curr Biol* 31: 1570–1577

Marchetti M, Capela D, Glew M, Cruveiller S, Chane-Woon-Ming B, Gris C, Timmers T, Poinsot V, Gilbert LB, Heeb P *et al* (2010) Experimental evolution of a plant pathogen into a legume symbiont. *PLoS Biol* 8: e1000280

Martin F, Kohler A, Murat C, Veneault-Fourrey C, Hibbett DS (2016) Unearthing the roots of ectomycorrhizal symbioses. *Nat Rev Microbiol* 14: 760–773

Martin FM, Uroz S, Barker DG (2017) Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. *Science* 356: eaad4501

Martinez-Klimova E, Rodríguez-Peña K, Sánchez S (2017) Endophytes as sources of antibiotics. *Biochem Pharmacol* 134: 1–17

Mataigne V, Vannier N, Vandenkoornhuyse P, Hacquard S (2022) Multi-genome metabolic modeling predicts functional inter-dependencies in the *Arabidopsis* root microbiome. *Microbiome* 10: 1–20

McNew GL (1960) The nature, origin and evolution of parasitism. In *Plant Pathology: An Advanced Treatise*, pp 19–69. New York, NY: Academic Press

Melnyk RA, Hossain SS, Haney CH (2019) Convergent gain and loss of genomic islands drive lifestyle changes in plant-associated pseudomonas. *ISME J* 13: 1575–1588

Mersmann S, Bourdais G, Rietz S, Robatzek S (2010) Ethylene signaling regulates accumulation of the FLS2 receptor and is required for the oxidative burst contributing to plant immunity. *Plant Physiol* 154: 391–400

Mesny F, Miyauchi S, Thiergart T, Pickel B, Atanasova L, Karlsson M, Hüttel B, Barry KW, Haridas S, Chen C *et al* (2021) Genetic determinants of endophytism in the *Arabidopsis* root mycobiome. *Nat Commun* 12: 1–15

Miyauchi S, Kiss E, Kuo A, Drula E, Kohler A, Sánchez-García M, Morin E, Andreopoulos B, Barry KW, Bonito G *et al* (2020) Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nat Commun* 11: 1–17

Mohr TJ, Liu H, Yan S, Morris CE, Castillo JA, Jelenska J, Vinatzer BA (2008) Naturally occurring nonpathogenic isolates of the plant pathogen pseudomonas syringae lack a type III secretion system and effector gene orthologues. *J Bacteriol* 190: 2858–2870

Möller M, Stukenbrock EH (2017) Evolution and genome architecture in fungal plant pathogens. *Nat Rev Microbiol* 15: 756–771

Morris JJ, Lenski RE, Zinser ER (2012) The black queen hypothesis: evolution of dependencies through adaptive gene loss. *MBio* 3: e00036-12

Mousa WK, Shearer C, Limay-Rios V, Ettinger CL, Eisen JA, Raizada MN (2016) Root-hair endophyte stacking in finger millet creates a physicochemical barrier to trap the fungal pathogen *Fusarium graminearum*. *Nat Microbiol* 1: 1–12

Müller DB, Vogel C, Bai Y, Vorholt JA (2016) The plant microbiota: systems-level insights and perspectives. *Annu Rev Genet* 50: 211–234

Murillo-Roos M, Abdullah HSM, Debbar M, Ueberschaar N, Agler MT (2022) Cross-feeding niches among commensal leaf bacteria are shaped by the interaction of strain-level diversity and resource availability. *ISME J* 16: 2280–2289

Nagy LG, Tóth R, Kiss E, Slot J, Gácser A, Kovács GM (2017) Six key traits of fungi: their evolutionary origins and genetic bases. In *The Fungal Kingdom*, pp 35–56. Washington, DC: ASM Press

Nakagawa T, Kawaguchi M (2006) Shoot-applied MeJA suppresses root nodulation in Lotus japonicus. *Plant Cell Physiol* 47: 176–180

Neal AL, Ahmad S, Gordon-Weeks R, Ton J (2012) Benzoxazinoids in root exudates of maize attract pseudomonas putida to the rhizosphere. *PloS One* 7: e35498

Nelson J, Shaw AJ (2019) Exploring the natural microbiome of the model liverwort: fungal endophyte diversity in *Marchantia polymorpha* L. *Symbiosis* 78: 45–59

Nelson JM, Hauser DA, Hinson R, Shaw AJ (2018) A novel experimental system using the liverwort Marchantia polymorpha and its fungal endophytes reveals diverse and context-dependent effects. *New Phytol* 218: 1217–1232

Nickerson MN, Moore LP, U'Ren JM (2023) The impact of polyphenolic compounds on the in vitro growth of oak-associated foliar endophytic and saprotrophic fungi. *Fungal Ecol* 62: 101226

Nongbri PL, Johnson JM, Sherameti I, Glawischnig E, Halkier BA, Oelmüller R (2012) Indole-3-Acetaldoxime-Derived Compounds Restrict Root Colonization in the Beneficial Interaction Between Arabidopsis Roots and the Endophyte *Piriformospora indica*. *Mol Plant-Microbe Interact* 25: 1186–1197

Ökmen B, Katzy P, Wemhöner R, Doehlemann G (2023) A conserved extracellular ribonuclease with broad-spectrum cytotoxic activity enables smut fungi to compete with host-associated bacteria. *bioRxiv* https://doi.org/10.1101/2023.04.19.537453 [PREPRINT]

Paasch BC, Sohrabi R, Kremer JM, Nomura K, Martz J, Kvitko B, Tiedje JM, He SY (2023) A critical role of a eubiotic microbiota in gating proper immunocompetence in *Arabidopsis*. *bioRxiv* https://doi.org/10.1101/2023.03.02.527037 [PREPRINT]

Palmieri D, Vitale S, Lima G, Di Pietro A, Turrà D (2020) A bacterial endophyte exploits chemotropism of a fungal pathogen for plant colonization. *Nat Commun* 11: 1–11

Parker JE, Hessler G, Cui H (2022) A new biochemistry connecting pathogen detection to induced defense in plants. *New Phytol* 234: 819–826

Parys K, Colaianni NR, Lee HS, Hohmann U, Edelbacher N, Trgovcevic A, Blahovska Z, Lee D, Mechtler A, Muhari-Portik Z *et al* (2021) Signatures of antagonistic pleiotropy in a bacterial flagellin epitope. *Cell Host Microbe* 29: 620–634

Peng Y, van Wersch R, Zhang Y (2018) Convergent and divergent signaling in PAMP-triggered immunity and effector-triggered immunity. *Mol Plant Microbe Interact* 31: 403–409

Pfeilmeier S, Saur IML, Rathjen JP, Zipfel C, Malone JG (2016) High levels of cyclic-di-GMP in plant-associated pseudomonas correlate with evasion of plant immunity. *Mol Plant Pathol* 17: 521–531

Pfeilmeier S, Petti GC, Bortfeld-Miller M, Daniel B, Field CM, Sunagawa S, Vorholt JA (2021) The plant NADPH oxidase RBOHD is required for microbiota homeostasis in leaves. *Nat Microbiol* 6: 852–864

Plett JM, Daguerre Y, Wittulsky S, Vayssières A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C *et al* (2014) Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proc Natl Acad Sci U S A* 111: 8299–8304

Pontrelli S, Szabo R, Pollak S, Schwartzman J, Ledezma-Tejeida D, Cordero OX, Sauer U (2022) Metabolic cross-feeding structures the assembly of polysaccharide degrading communities. *Sci Adv* 8: 3076

Pruitt RN, Locci F, Wanke F, Zhang L, Saile SC, Joe A, Karelina D, Hua C, Fröhlich K, Wan WL *et al* (2021) The EDS1–PAD4–ADR1 node mediates *Arabidopsis* pattern-triggered immunity. *Nature* 598: 495–499

Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS (2016) Algae–bacteria interactions: evolution, ecology and emerging applications. *Biotechnol Adv* 34: 14–29

Rawat P, Das S, Shankhdhar D, Shankhdhar SC (2021) Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *J Soil Sci Plant Nutr* 21: 49–68

Rizaludin MS, Stopnisek N, Raaijmakers JM, Garbeva P (2021) The chemistry of stress: understanding the 'cry for help' of plant roots. *Metabolites* 11: 357

Roberts R, Mainiero S, Powell AF, Liu AE, Shi K, Hind SR, Strickler SR, Collmer A, Martin GB (2019) Natural variation for unusual host responses and flagellin-mediated immunity against pseudomonas syringae in genetically diverse tomato accessions. *New Phytol* 223: 447–461

Roman-Reyna V, Pinili D, Borja FN, Quibod IL, Groen SC, Mulyaningsih ES, Rachmat A, Slamet-Loedin IH, Alexandrov N, Mauleon R *et al* (2019) The rice leaf microbiome has a conserved community structure controlled by complex host-microbe interactions. *bioRxiv* https://doi.org/10.1101/615278 [PREPRINT]

Rudgers JA, Fox S, Porras-Alfaro A, Herrera J, Reazin C, Kent DR, Souza L, Chung YYA, Jumpponen A (2022) Biogeography of root-associated fungi in foundation grasses of north American plains. *J Biogeogr* 49: 22–37

Rudnick MB, van Veen JA, de Boer W (2015) Baiting of rhizosphere bacteria with hyphae of common soil fungi reveals a diverse group of potentially mycophagous secondary consumers. *Soil Biol Biochem* 88: 73–82

Salvioli di Fossalunga A, Novero M (2019) To trade in the field: the molecular determinants of arbuscular mycorrhiza nutrient exchange. *Chem Biol Technol Agric* 6: 1–12

Sasse J, Martinoia E, Northen T (2018) Feed your friends: do plant exudates shape the root microbiome? *Trends Plant Sci* 23: 25–41

Schirrmann MK, Leuchtmann A (2015) The role of host-specificity in the reproductive isolation of Epichloë endophytes revealed by reciprocal infections. *Fungal Ecol* 15: 29–38

Schirrmann MK, Zoller S, Croll D, Stukenbrock EH, Leuchtmann A, Fior S (2018) Genomewide signatures of selection in Epichloë reveal candidate genes for host specialization. *Mol Ecol* 27: 3070–3086

Schulz B, Boyle C (2005) The Endophytic continuum. *Mycol Res* 109: 661–686

Schulz-Bohm K, Gerards S, Hundscheid M, Melenhorst J, De Boer W, Garbeva P (2018) Calling from distance: attraction of soil bacteria by plant root volatiles. *ISME J* 12: 1252–1262

Selosse MA, Dubois MP, Alvarez N (2009) Do Sebacinales commonly associate with plant roots as endophytes? *Mycol Res* 113: 1062–1069

Selosse MA, Petrolli R, Mujica MI, Laurent L, Perez-Lamarque B, Figura T, Bourceret A, Jacquemyn H, Li T, Gao J *et al* (2022) The waiting room hypothesis revisited by orchids: were orchid mycorrhizal fungi recruited among root endophytes? *Ann Bot* 129: 259–270

Singh BK, Nunan N, Ridgway KP, McNicol J, Young JPW, Daniell TJ, Prosser JI, Millard P (2008) Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. *Environ Microbiol* 10: 534–541

Singh SK, Wu X, Shao C & Zhang H (2022) Microbial enhancement of plant nutrient acquisition. *Stress Biol* 2: 1–14

Snelders NC, Rovenich H, Petti GC, Rocafort M, van den Berg GCM, Vorholt JA, Mesters JR, Seidl MF, Nijland R, Thomma BPHJ (2020) Microbiome manipulation by a soil-borne fungal plant pathogen using effector proteins. *Nat Plants* 6: 1365–1374

Snelders NC, Petti GC, van den Berg GCM, Seidl MF, Thomma BPHJ (2021) An ancient antimicrobial protein co-opted by a fungal plant pathogen for in planta mycobiome manipulation. *Proc Natl Acad Sci U S A* 118: e2110968118

Snelders NC, Rovenich H, Thomma BPHJ (2022) Microbiota manipulation through the secretion of effector proteins is fundamental to the wealth of lifestyles in the fungal kingdom. *FEMS Microbiol Rev* 2022: 1–16

Snelders NC, Boshoven JC, Song Y, Schmitz N, Fiorin GL, Rovenich H, van den Berg GCM, Torres DE, Petti GC, Prockl Z *et al* (2023) A highly polymorphic effector protein promotes fungal virulence through suppression of plant-associated Actinobacteria. *New Phytol* 237: 944–958

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

Stringlis IA, Yu K, Feussner K, De Jonge R, Van Bentum S, Van Verk MC, Berendsen RL, Bakker PAHM, Feussner I, Pieterse CMJ (2018a) MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc Natl Acad Sci U S A* 115: E5213–E5222

Stringlis IA, Proietti S, Hickman R, Van Verk MC, Zamioudis C, Pieterse CMJ (2018b) Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. *Plant J* 93: 166–180

Stringlis IA, De Jonge R, Pieterse CMJ (2019) The age of coumarins in plant–microbe interactions. *Plant Cell Physiol* 60: 1405–1419

Sulheim S, Mitri S (2023) Breaking down microbial hierarchies. *Trends Microbiol* 31: 426–427

Szűcs Z, Plaszkó T, Cziáky Z, Kiss-Szikszai A, Emri T, Bertóti R, Sinka LT, Vasas G, Gonda S (2018) Endophytic fungi from the roots of horseradish (*Armoracia rusticana*) and their interactions with the defensive metabolites of the glucosinolate-myrosinase-isothiocyanate system. *BMC Plant Biol* 18: 1–15

Tang J, Wu D, Li X, Wang L, Xu L, Zhang Y, Xu F, Liu H, Xie Q, Dai S *et al* (2022) Plant immunity suppression via PHR1-RALF-FERONIA shapes the root microbiome to alleviate phosphate starvation. *EMBO J* 41: e109102

Teixeira PJP, Colaianni NR, Fitzpatrick CR, Dangl JL (2019) Beyond pathogens: microbiota interactions with the plant immune system. *Curr Opin Microbiol* 49: 7–17

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

Thiergart T, Durán P, Ellis T, Vannier N, Garrido-Oter R, Kemen E, Roux F, Alonso-Blanco C, Ågren J, Schulze-Lefert P *et al* (2020) Root microbiota assembly and adaptive differentiation among European *Arabidopsis* populations. *Nat Ecol Evol* 4: 122–131

Thomma BPHJ, Nürnberger T, Joosten MHAJ (2011) Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell* 23: 4–15

Thoms D, Chen MY, Liu Y, Moreira ZM, Luo Y, Song S, Wang NR, Haney CH (2023) Innate immunity can distinguish beneficial from pathogenic rhizosphere microbiota. *bioRxiv* https://doi.org/10.1101/2023.01.07.523123 [PREPRINT]

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant–microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol* 18: 607–621

Tyagi S, Mulla SI, Lee K-J, Chae J-C, Shukla P (2018) VOCs-mediated hormonal signaling and crosstalk with plant growth promoting microbes. *Crit Rev Biotechnol* 38: 1277–1296

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

Uroz S, Courty PE, Oger P (2019) Plant symbionts are engineers of the plant-associated microbiome. *Trends Plant Sci* 24: 905–916

Vannier N, Mony C, Bittebiere AK, Michon-Coudouel S, Biget M, Vandenkoornhuyse P (2018) A microorganisms' journey between plant generations. *Microbiome* 6: 79

Veluchamy S, Hind SR, Dunham DM, Martin GB, Panthee DR (2014) Natural variation for responsiveness to flg22, flgII-28, and csp22 and *Pseudomonas syringae* pv. tomato in heirloom tomatoes. *PloS One* 9: e106119

Venkataram S, Kuo H-Y, Hom EFY, Kryazhimskiy S (2023) Mutualism-enhancing mutations dominate early adaptation in a two-species microbial community. *Nat Ecol Evol* 7: 143–154

Venugopal SC, Jeong RD, Mandal MK, Zhu S, Chandra-Shekara AC, Xia Y, Hersh M, Stromberg AJ, Navarre DR, Kachroo A *et al* (2009) Enhanced disease susceptibility 1 and salicylic acid act redundantly to regulate resistance gene-mediated signaling. *PLoS Genet* 5: e1000545

Vetter M, Karasov TL, Bergelson J (2016) Differentiation between MAMP triggered defenses in *Arabidopsis thaliana*. *PLoS Genet* 12: e1006068

Vogel J (2008) Unique aspects of the grass cell wall. *Curr Opin Plant Biol* 11: 301−307

Vogel CM, Potthoff DB, Schäfer M, Barandun N, Vorholt JA (2021) Protective role of the *Arabidopsis* leaf microbiota against a bacterial pathogen. *Nat Microbiol* 6: 1537−1548

Voges MJEEE, Bai Y, Schulze-Lefert P, Sattely ES (2019) Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. *Proc Natl Acad Sci U S A* 116: 12558−12565

Wang F, Zhang L, Zhou J, Rengel Z, George TS, Feng G (2022) Exploring the secrets of hyphosphere of arbuscular mycorrhizal fungi: processes and ecological functions. *Plant and Soil* 481: 1−22

Wang J, Raza W, Jiang G, Yi Z, Fields B, Greenrod S, Friman VP, Jousset A, Shen Q, Wei Z (2023) Bacterial volatile organic compounds attenuate pathogen virulence via evolutionary trade-offs. *ISME J* 17: 1−10

Wawra S, Fesel P, Widmer H, Timm M, Seibel J, Leson L, Kesseler L, Nostadt R, Hilbert M, Langen G *et al* (2016) The fungal-specific β-glucan-binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *Nat Commun* 7: 1−11

van de Weyer AL, Monteiro F, Furzer OJ, Nishimura MT, Cevik V, Witek K, Jones JDG, Dangl JL, Weigel D, Bemm F (2019) A species-wide inventory of NLR genes and alleles in *Arabidopsis thaliana*. *Cell* 178: 1260−1272

Wippel K, Tao K, Niu Y, Zgadzaj R, Kiel N, Guan R, Dahms E, Zhang P, Jensen DB, Logemann E *et al* (2021) Host preference and invasiveness of commensal bacteria in the lotus and *Arabidopsis* root microbiota. *Nat Microbiol* 6: 1150−1162

Wolinska KW, Vannier N, Thiergart T, Pickel B, Gremmen S, Piasecka A, Piślewska-Bednarek M, Nakano RT, Belkhadir Y, Bednarek P *et al* (2021) Tryptophan metabolism and bacterial commensals prevent fungal dysbiosis in *Arabidopsis* roots. *Proc Natl Acad Sci U S A* 118: 21115−21118

Wu C, Derevnina L (2023) The battle within: how pathogen effectors suppress NLR-mediated immunity. *Curr Opin Plant Biol* 74: 102396

Yang Y, Pollard AM, Höfler C, Poschet G, Wirtz M, Hell R, Sourjik V (2015) Relation between chemotaxis and consumption of amino acids in bacteria. *Mol Microbiol* 96: 1272−1282

Yin L, Liu B, Wang H, Zhang Y, Wang S, Jiang F, Ren Y, Liu H, Liu C, Wan F *et al* (2020) The rhizosphere microbiome of Mikania micrantha provides insight into adaptation and invasion. *Front Microbiol* 11: 1462

Yu K, Pieterse CMJ, Bakker PAHM, Berendsen RL (2019a) Beneficial microbes going underground of root immunity. *Plant Cell Environ* 42: 2860−2870

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

Zeng T, Rodriguez-Moreno L, Mansurkhodzaev A, Wang P, van den Berg W, Gasciolli V, Cottaz S, Fort S, Thomma BPHJ, Bono JJ *et al* (2020) A lysin motif effector subverts chitin-triggered immunity to facilitate arbuscular mycorrhizal symbiosis. *New Phytol* 225: 448−460

Zhang W, Li XG, Sun K, Tang MJ, Xu FJ, Zhang M, Dai CC (2020) Mycelial network-mediated rhizobial dispersal enhances legume nodulation. *ISME J* 14: 1015−1029

Zhong Y, Xun W, Wang X, Tian S, Zhang Y, Li D, Zhou Y, Qin Y, Zhang B, Zhao G *et al* (2022) Root-secreted bitter triterpene modulates the rhizosphere microbiota to improve plant fitness. *Nat Plants* 8: 887−896

Zhou J, Chai X, Zhang L, George TS, Wang F, Feng G (2020) Different arbuscular mycorrhizal fungi cocolonizing on a single plant root system recruit distinct microbiomes. *mSystems* 5: e00929-20