

## Review

## Root cell types as an interface for biotic interactions

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**Root responses to environmental stresses show a high level of cell type and developmental stage specificity. Interactions with beneficial and pathogenic organisms – including microbes and parasites – elicit a set of transcriptional responses unique to each root cell type, often dependent on their differentiation state. Localized changes to the cell wall and to the integrity of root cell types can serve as a physical barrier for a range of pests. Conversely, certain microorganisms weaken existing barriers within root cell types. Interactions with microorganisms vary between roots of different developmental origins and cellular architectures. Here we provide an overview of the molecular, architectural, and structural properties of root cell types crucial to both maintaining beneficial interactions and protecting from pathogens.**

**Root cell-type-specific interactions with the environment**

Hidden below the ground, roots play an important role in plant life. They anchor the plant in the ground and facilitate nutrient and water acquisition. The root is also the first organ to sense adverse conditions such as soil salinization, nutrient shortages, or flooding. Their life underground exposes roots to a myriad organisms, including bacteria, oomycetes, fungi, nematodes, and other plants. These interactions can be pathogenic and result in plant disease, or non-pathogenic; the latter includes symbiosis, where the relationship is beneficial for both organisms involved, and commensalism, where plant function is neither harmed nor improved. Bacteria and fungi participate in all three types of interactions, while nematodes or parasitic plants are pathogens. Thus, roots are an important center for plants' responses to abiotic and biotic signals.

While the root system of the mature plant is a complex network of higher-order root types, the arrangement of individual cell types (known as cellular architecture) displays an elegant simplicity [1]. Root cells form a developmental gradient along the root's longitudinal axis. Closest to the root tip, the stem cell niche (quiescent center and initial or stem cells) and proliferating cells constitute the meristematic zone, followed in a shootward direction by the elongation zone, and further by differentiating cells within the maturation zone. Radially, the majority of root cell types are organized in concentric cylinders surrounding the root stele, which consists of the pericycle (from which lateral roots originate) and vascular cells, including the xylem, phloem, and (pro)cambial cells (Figure 1). Soil-borne organisms penetrate root cell types radially, beginning with the epidermis and followed by the exodermis (if present), the (inner) cortex, and the endodermis, to reach the vasculature, which allows them to spread to other plant organs. Parasitic plants establish a connection between their vasculature and that of their host plant, to obtain water, nutrients, and in some cases sugars [2,3]. Each root cell type presents a unique set of molecular signatures, and root responses to environmental signals show a high level of cell-type specificity [4,5]. While root cell-type-specific responses to a myriad abiotic stresses have been studied thoroughly in the past 10 years, the impact of biotic stresses on individual root cell types has only recently received more attention [6–11]. Cell-type-specific transcriptional reprogramming can result in functional

**Highlights**

Individual root cell types perceive and respond to biotic factors in unique and distinct manners dependent on their developmental stage.

Plants discriminate soil-borne pathogens from commensal microorganisms via spatially restricted immune responses and cell-type-specific transcriptome reprogramming.

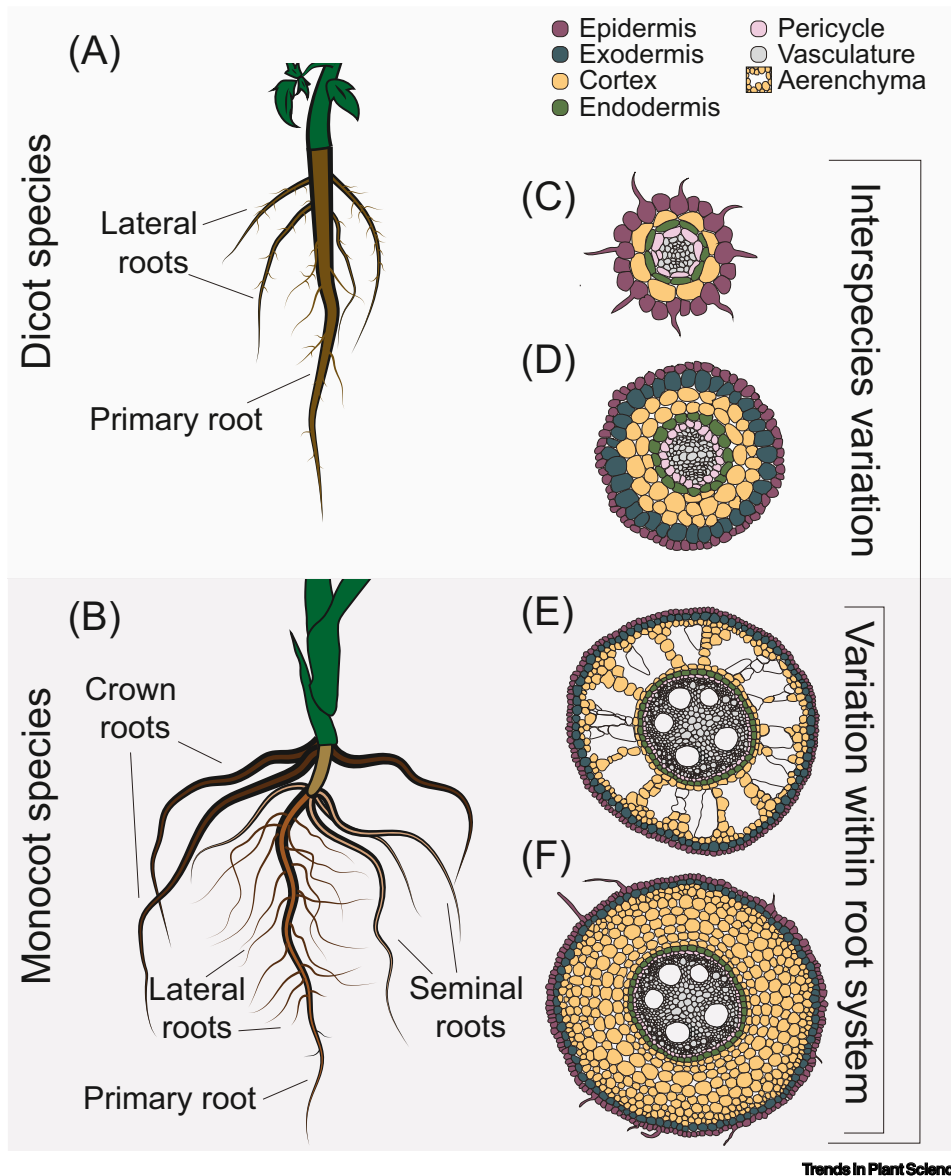
The integrity of root endodermal diffusion barriers influences root interactions with soil-borne microbes and vice versa.

Root cortex cellular architecture impacts root interactions with fungi.

The endodermis is a likely barrier for a broad range of pathogens across kingdoms.

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**Figure 1. Variation in the root system and cellular architecture between dicot and monocot species.** (A) A dicot species root system consists of the primary and lateral roots. (B) In monocot species, the root system is initially established by the primary root and seminal roots, and elaborated by shoot-derived crown roots. (C–F) Variation in root cellular anatomy includes differences in cortex cell layer number, the presence/absence of the exodermis, and aerenchyma production. This diversity can be observed between species (C–F) and well as between different root types within an individual root system (E,F).

consequences to root cell morphology, including modifications to the primary or secondary walls of some root cell types which form physical barriers to certain pathogens. Recent reports suggest that root diffusion barriers in the endodermis are also reshaped by non-pathogenic soil-borne bacteria [12]. Plant roots face an incredible abundance of biotic organisms in the soil. Recently, cellular and cell-type-resolution approaches have determined some mechanisms by which roots can distinguish innocuous, beneficial, and pathogenic organisms from each other simultaneously and spatiotemporally [7]. Understanding the role of individual root cell types in these

biotic interactions will facilitate the improvement of plant resistance to root pathogens and will further enable the use of beneficial microbes to enhance plant performance and stress resilience.

In this review, we highlight commonalities in how roots respond to different types of biotic agents. Specific examples are provided with respect to the barriers produced in specific cell types to mount responses to multiple types of pathogens. We further indicate how these interactions differ depending on whether the microbes are commensal organisms, and finally provide a perspective on how the properties of individual root cell types enable a simultaneous balance of interactions with both pests and beneficial microbes. This review is organized according to the order of events during an interaction between a plant root and another organism. We begin with the way in which the root cell types perceive a biotic agent, and how differentiation features of root cell types (both spatial and temporal) influence progression of these agents through the root; we further demonstrate how these interactions can be bidirectional. We finally focus on the optimal features of root cell types to provide beneficial interactions with microbes while protecting from pathogens and minimizing tradeoffs.

### Cell type resolution sensing and transcriptome reprogramming during biotic interactions

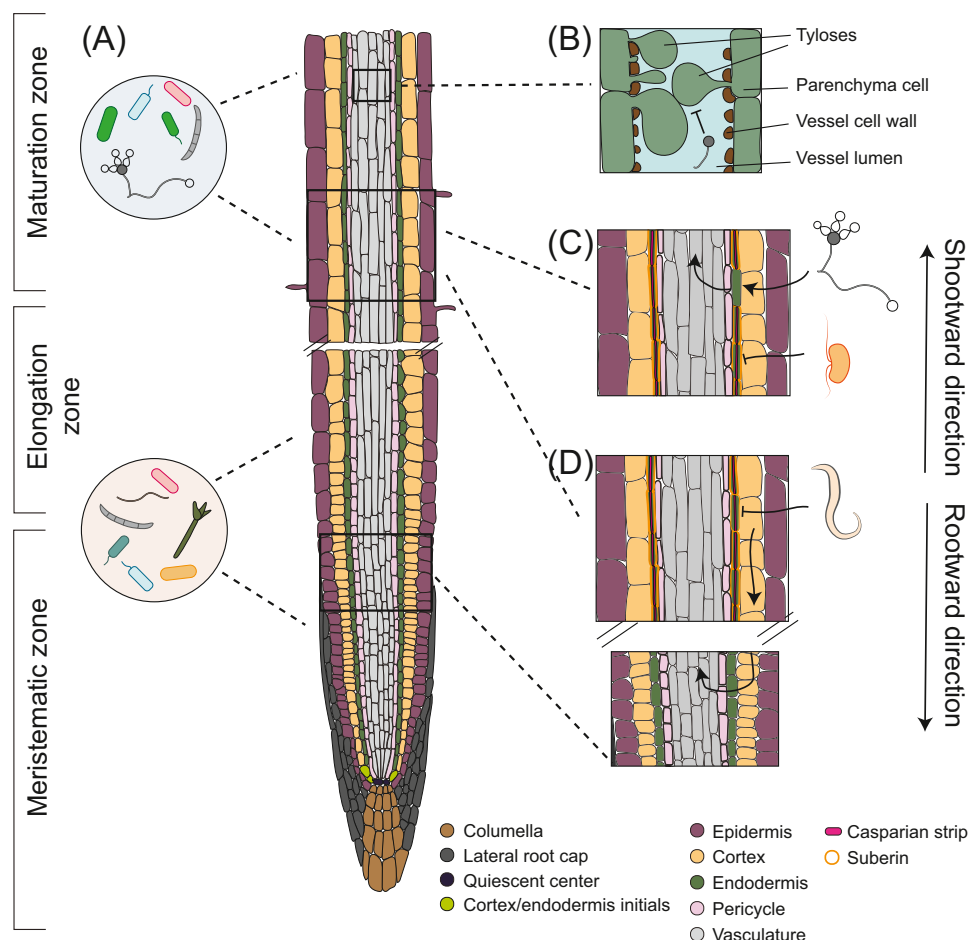
The first step for a plant to establish an interaction with any soil-inhabiting organism – whether beneficial, pathogenic, or commensal – is an exchange of molecular signals leading to recognition of its presence. Our understanding of how plants sense microorganisms is substantial, with certain similarities found for the perception of nematodes or parasitic plants. Detection of neighboring organisms by a plant relies on the molecular signatures of microbe-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs) [13]. MAMPs are exogenous molecules produced by bacteria, fungi, oomycetes, and nematodes, and have recently been found in parasitic plants, while DAMPs are endogenous compounds released from plant tissues upon wounding that occurs during infection [13–16]. Both MAMPs and DAMPs are perceived by plant pattern recognition receptors (PRRs) localized in the plasma membrane; these initiate a signaling cascade called pattern-triggered immunity (PTI) [17,18].

MAMPs are highly conserved molecules produced by both pathogenic and non-pathogenic microorganisms [19]. Beneficial and commensal bacteria use several strategies to either evade MAMP recognition or to actively suppress the plant immune response [19]. Soil is composed of an extraordinary microbial diversity [20,21]; thus, the capacity to ward off pathogens without compromising beneficial interactions is especially important for roots. In differentiated *Arabidopsis* (*Arabidopsis thaliana*) root cells, the transcriptional response to flg22 (a bacterial MAMP) is low, which curtails defense and permits colonization of non-pathogenic bacteria [6]. Moreover, individual root cell types exhibit quantitative differences in the transcriptional output of flg22, suggesting a range of the potential to mount response to recognition by the FLS2 receptor, from the epidermis (extremely capable of responding) to the vasculature (almost non-responsive) [7]. Cell-type specificity was also observed in some species responses to fungal MAMPs, where only atrichoblasts (epidermal cells that do not produce a hair) and not trichoblasts (epidermal cells that develop a hair) initiate the calcium oscillations required for the establishment of symbiosis [22]. In the presence of pathogenic bacteria, defense responses are initiated only when MAMP detection is accompanied by likely mechanical damage, each occurring in different cell types [6]. For example, cell death induced by pathogenic bacteria in differentiated epidermis activates transcriptional responses to MAMPs in neighboring cortex cells [6]. Together, these highly localized immune responses, restricted to individual cell types, allow the root to accommodate innocuous bacteria and to prevent exhaustion of root growth within non-differentiated cells from excessive defense programs.

Cell-type identity – rather than the type of the immune-eliciting molecule – is the primary factor regulating transcriptional changes in roots exposed to specific MAMP and DAMP molecules [11]. Furthermore, cell-type identity regulators are robustly unchanged to perturbations by biotic stress-associated signals; this is similar to observations with abiotic signals [4,23]. This exemplifies another cell-type-specific mechanism restricting the defense responses to the functional capacity of root developmental programs.

Microbial progression through an Arabidopsis root is accompanied by transcriptional changes within individual root cell types, without a change in cell identity. In their biotrophic stage, the pathogenic fungus *Verticillium longisporum* colonizes Arabidopsis root vasculature, while another root pathogen, the oomycete *Phytophthora parasitica*, colonizes only the epidermis and cortex but cannot cross the endodermis. Endodermis differentiation includes first the synthesis and deposition of a lignified Casparian strip that forms a centralized ring around endodermal cells. This barrier seals endodermal intercellular space and blocks apoplastic transport. The second differentiation feature is the uniform deposition of suberin lamellae in its secondary cell wall, which restricts the transcellular transport pathway [24,25]. Upon infection with *V. longisporum*, genes associated with suberin biosynthesis and Casparian strip formation in the endodermis are downregulated, which corresponds with decreased suberin deposition. This resulting weakening of the diffusion barrier grants the fungus entry to the vasculature (Figure 2) [8]. In contrast, *P. parasitica* does not elicit such changes and cannot pass through the endodermis [8]. It remains to be addressed how durable this restriction is, especially as in other plant species *P. parasitica* has been shown to enter endodermal cells at later oomycete life cycle stages [26]. These pathogens can also mount distinct responses in different cell types. *V. longisporum* induces transcription of antimicrobial aliphatic glucosinolate biosynthetic genes in the cortex, while infection with *P. parasitica* represses transcription of the same gene sets in vasculature [8]. *V. longisporum* is also able to induce changes in the host root by changing the identity of non-xylem cells into xylem cells (a process known as transdifferentiation), thus reprogramming the cell's transcriptome [27]. Such a long-distance influence on cell type processes is also observed with a symbiotic microbes. In the early penetration phase of colonization by arbuscular mycorrhizal fungi, there is activation of cell cycle transcriptional machinery and cell divisions in inner cortex cells at a distance from the colonizing hyphae (reviewed in [28]). This suggests that microbes can influence the transcriptomes of the cells they do not physically reach. The cell-type-resolution transcriptional data (Table 1), along with those describing transcriptional responses to flg22 exposure, clearly demonstrates that microbes can act in a local (in the cell type they reside) and distal (in the cell type they are absent from) manner and elicit cell-autonomous and noncell-autonomous responses in individual cell types [7].

The approaches and data summarized here leverage our knowledge of root biotic interactions at cell-type resolution [29,30] (Table 1). While most of these reports concern pathogenic microorganisms, little is known about how these cell-type-specific transcriptomic and developmental changes translate to the properties of these cells crucial for pest resistance. In the case of beneficial bacteria, regulation of the developmental programs associated with cell wall remodeling upon inoculation with the symbiotic *Sinorhizobium meliloti* in the pericycle has been linked to its promoting effect on lateral root formation [9]. It must be noted that the majority of current transcriptome studies address microbe interactions with individual Arabidopsis root cell types. Whether the same mechanisms exist in other plant species remains unknown. An exception is cell-type-specific transcriptomes of *Rhizobium*-induced nodules and root tissue profiled in *Medicago truncatula* [10,31]. Cell-type resolution descriptions of transcriptional changes elicited by other biotic factors, such as nematodes or parasitic plants, are still missing. Furthermore, cell-type populations studied in the context of biotic interactions were derived mostly from root tips,



## Trends in Plant Science

**Figure 2. Root-cell-type developmental trajectory influences plant-biotic interactions.** (A) Distinct microbial communities assemble in the root tip (with less mature cells) relative to the root base (in the shootward direction in terms of primary, seminal, and crown roots, or in the case of lateral and secondary order roots, close to their parent root), where cells reach their mature or differentiated stages [42]. (B) Tyloses block the lumen of the xylem vessel, forming a potential barrier for vascular wilt pathogens, limiting their spread along the root longitudinal axis in the shootward direction and further progression to the shoot [49,54]. The effectiveness of tyloses for plant resistance is controversial [55,56]. (C) Endodermal cells in the maturation zone have suberized cell walls and lignified Casparian strips, creating a barrier for biotic agents. Certain fungal species reduce the suberization of endodermis, which allows them to enter the vasculature and spread through the plant [8]. Organisms unable to cross the mature endodermis reside in the cortex and/or endodermis [8]. (D) Root-knot nematodes overcome endodermal barriers by progressing through the cortex along the root longitudinal axis in the rootward direction to the elongation/meristematic zones and enter the vasculature through undifferentiated endodermis [48].

and little is known about how cells in root regions that undergo secondary growth respond to biotic agents. The advancements of single-cell transcriptome profiling technologies offer an exciting opportunity for further studies of root-biotic interactions at cellular resolution [32]. However, the changes in cell wall composition induced by the biotic stresses, which vary across cell types, might prove to be a challenge for cell nuclei/protoplast isolation, and the cellular disruption process itself might release DAMPs. Spatial transcriptomic approaches can overcome these problems as well as offer the cell-type resolution solution for species with no known cell-type markers [33].

**Table 1. Cell-type resolution profiles of root–biotic interactions**

| Species     | Biotic factor                                                                                                                            | ‘Omic technique                | Cell types profiled                                  | Major finding                                                                                                                                           | Refs |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Arabidopsis | Pathogens: fungi <i>Verticillium longisporum</i> , oomycete <i>Phytophthora parasitica</i> ; mutualists: fungi <i>Serendipita indica</i> | TRAP-seq                       | Epidermis (atrachoblasts), cortex, endodermis, stele | Pathogens colonizing vasculature downregulate suberin biosynthesis and Casparian strip formation pathways                                               | [8]  |
| Arabidopsis | MAMP flg22, DAMP Pep1                                                                                                                    | FACS, RNA-seq                  | Epidermis, cortex, pericycle                         | Set of transcription factors in epidermis and cortex connect immunity transcriptional networks with cell type identity networks                         | [11] |
| Arabidopsis | N-fixing bacterium <i>Sinorhizobium meliloti</i>                                                                                         | FACS, microarray hybridization | Pericycle, cortex                                    | Upregulation of defense responses activated in cortex, developmental changes initiated in pericycle. Responses in cortex precede those in the pericycle | [9]  |
| Medicago    | N-fixing bacterium <i>Sinorhizobium meliloti</i>                                                                                         | TRAP-seq                       | Epidermis, cortex, phloem companion cells            | Upregulation of transcripts involved in mRNA decay in epidermis and cortex promotes nodule formation                                                    | [10] |

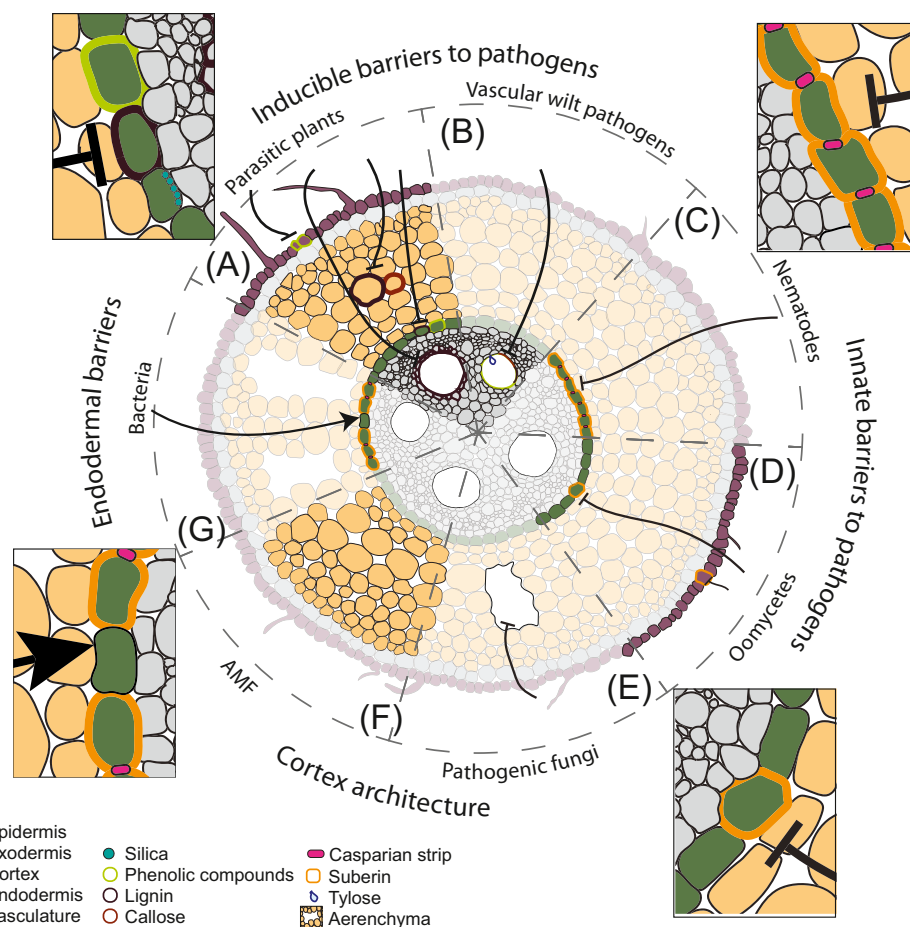
### The influence of root radial architecture and differentiation programs on biotic interactions

Root cellular architecture is diverse between plant species, including variation in the number of cell-type layers and the presence of specialized cell types, which could influence root–biotic interactions (Figure 1). While the *Arabidopsis* root has one layer of cortex cells, two to three cortex layers are present in tomato species (*Solanum lycopersicum* and *Solanum pennellii*) [34], and from eight to 15 in maize (*Zea mays*) [35,36]. In most angiosperm species, the outermost cortex layer differentiates into an exodermis, with suberized cell walls additionally reinforced with lignin in some species [37]. Non-cell-autonomous microbe-elicited immune signaling observed in *Arabidopsis* demonstrates that immune responses require signals from outer cell types [6,7]. Presumably this regulation is more complex in species with multiple cortex layers. An interesting question is how immune perception occurs in species with an exodermis. A suberized endodermal cell wall blocks flg22 perception by FLS2, while lignified Casparian strips prevent flg22 diffusion to the stele [6]. It is thus possible that an exodermis with similar properties could also affect MAMP diffusion. Suberin is known to form a protective barrier to pathogens [38–40]. Thus, the presence of suberized cell types located nearer to the outer surface of the root, as opposed to *Arabidopsis* where only endodermal cells are suberized, may influence biotic agent penetration and account for interspecies differences in susceptibility to certain pests.

Variation in root radial cellular architecture can be also found within the root system of an individual plant (Figure 1B). Monocot species possess complex root systems with a singular primary root and numerous seminal roots, both of embryonic origin, as well as two types of shoot-borne roots, crown (also referred to as nodal) roots developed from the underground internodes, and brace roots formed from aboveground stem nodes [41]. These roots have distinct cellular patterning, primarily within the cortex layers. This heterogeneity of radial patterning across a root system affects the composition of the root microbiome. Differences in the bacterial and fungal communities have been found between the crown and seminal roots of wheat (*Triticum aestivum*) and rice (*Oryza sativa*), as well as between the tip and base region of individual root types (Figure 2) [42]. In species with multiple cortex cell layers, some of these cells undergo lysis leading to the formation of air-filled spaces called aerenchyma [43]. Depending on the plant species, aerenchyma forms constitutively, to reduce the metabolic cost of root tissue, or is induced as an adaptive strategy in response to waterlogging or nutrient deficiencies [44,45]. Aerenchyma formation impacts association with arbuscular mycorrhizal fungi (AMF). AMF penetrate roots and form arbuscules in cortex cells. Roots with a high proportion of aerenchyma, and hence a low number of live cortex cells, are colonized with AMF to a lesser extent than roots with more intact cortex tissue [46,47]. Fine lateral



roots of rice are an extreme case, as they do not possess a cortical layer [35] and do not serve as a host for AMF [46]. Interestingly, a high area of aerenchyma restricts infection with pathogenic fungi [47]. This suggests that cortical architecture shapes interactions with beneficial and pathogenic fungi in a distinct manner. Most of the evidence for the influence of root radial patterning on biotic interactions are correlative, and it remains unknown whether cortex layer number and cortex cell-type specialization directly or indirectly influence fungal colonization. However, these data show the importance of using crop models to account for the heterogeneous character of root system and



**Figure 3. Radial root cellular anatomy and plant-biotic interactions.** Changes in cell wall composition form physical barriers to biotic agents. (A) Infection with a parasitic plant induces localized accumulation of phenolic compounds in the epidermis [58] and endodermis [66], lignin in the cortex [58], endodermis [66], and xylem vessels [66], callose in the cortex [60], and silica in the endodermis [64], that form a barrier for further parasite penetration or that prevent establishment of a xylem connection. (B) Vascular wilt pathogens induce coating of the xylem vessel with lignin, callose, and phenolic compounds which block these pathogens from entry into the vasculature and/or spread from vessel to vessel [50,51]. Tyloses have been suggested to form additional barriers in xylem [49,54], but their effectiveness remains controversial [55,56]. (C,D) Differentiation programs of root cells act as innate barriers for pathogens. (C) Suberized cell walls and Casparian strips in the endodermis protect the vasculature from nematodes [48]. (D) Suberin in the endodermis and epidermis prevent the radial progression of oomycetes through root tissue [39]. (E,F) Cortex architecture plays a role in root interactions with soil-borne fungi. (E) The presence of aerenchyma limits the number of cortex cells through which pathogenic fungi can enter, and thus limits infection levels [47]. (F) Intact cortex cells favor interactions with arbuscular mycorrhizal fungi (AMF) [46,47]. (G) Endodermal suberized barriers are weakened by soil-borne bacterial communities [8]. (A,C,D) The endodermis functions as a barrier to a broad spectrum of pathogens. Insets present a magnified view of the type of barriers present in endodermis.

cellular architecture. In addition, it remains unknown whether the number of cortical cell files or their specialization directly influences the assembly of the root microbiome and by which mechanism, as well as whether this gradient in microbial distribution across the root system affects the functional aspects of associated microbiota.

### Cell-type-specific barriers to pathogens

Soil-borne pathogens invade the plant through the root. These pests can traverse the root radially, and those that succeed in entering the vasculature traverse shootward (Figure 2). Properties of certain cell types and their associated differentiation features may form obstacles for disease-causing intruders both radially and along the root's longitudinal axis (Table 2, Figures 2 and 3).

The proportion of a root which has completely differentiated and suberized endodermal cells can be influenced by developmental or environmental cues. Some organisms can take advantage of this aspect of developmental time along the root's longitudinal axis. Root-knot nematodes evade differentiated endodermal cells and their barriers, and enter through either the root meristem, the elongation zone where cell walls are relatively weak, or via wounded epidermal cells in the maturation/differentiation zone [48]. In the latter case, the nematode travels in a rootward direction via the cortex towards the root meristem to cross through undifferentiated endodermal cells that lack intercellular barriers (Figure 2) [48]. Plants with perturbed endodermal function, by either decreased suberization or defective Casparian strips, are more prone to nematode infection [48]. In the roots of soybean (*Glycine max*), suberin is present not only in endodermal cells but also to a lesser extent in epidermal cells. Soybean roots are infected by the oomycete *Phytophthora sojae*, but suberized lamellae between epidermal, cortical, and endodermal cells delay *P. sojae* radial progression (Figure 3) [39]. Decreased mortality in infected soybean plants is directly correlated with suberin levels, dependent on the soybean cultivar [39]. These two examples illustrate innate physical cell-type barriers to pathogens.

Table 2. Cell-type-specific barriers for root pathogens

| Root cell type | Structural modification                                   | Character        | Plant species                    | Pathogen                           | Refs       |
|----------------|-----------------------------------------------------------|------------------|----------------------------------|------------------------------------|------------|
| Epidermis      | Suberin deposition                                        | Innate           | Soybean                          | <i>Phytophthora sojae</i>          | [39,83]    |
|                | Unknown                                                   | Unknown          | <i>Phtheriospermum japonicum</i> | <i>Striga hermonthica</i>          | [58]       |
|                | Accumulation of phenolic compounds                        | Inducible        | Chickpea                         | <i>Orobanche crenata</i>           | [59]       |
| Cortex         | Lignin accumulation                                       | Inducible        | <i>Lotus japonicus</i>           | <i>Striga hermonthica</i>          | [58]       |
|                | Callose accumulation                                      | Inducible        | Pea                              | <i>Orobanche crenata</i>           | [60]       |
|                | Aerenchyma                                                | Innate           | Maize                            | <i>Fusarium verticillioides</i>    | [47]       |
| Endodermis     | Suberin deposition                                        | Innate           | Soybean                          | <i>Phytophthora sojae</i>          | [39,83]    |
|                | Suberin deposition, Casparian strips (lignin)             | Innate           | Arabidopsis                      | <i>Meloidogyne incognita</i>       | [48]       |
|                | Lignin/silica deposition, phenolic compounds accumulation | Innate/inducible | Rice, maize, sorghum             | <i>Striga hermonthica</i>          | [58,61–66] |
| Vasculature    | Lignin deposition                                         | Inducible        | Potato, tomato                   | <i>Ralstonia solanacearum</i>      | [49,50]    |
|                |                                                           | Inducible        | Pepper                           | <i>Verticillium dahliae</i>        | [51]       |
|                |                                                           | Inducible        | Rice                             | <i>Striga hermonthica</i>          | [50]       |
|                | Callose deposition                                        | Inducible        | Potato                           | <i>Ralstonia solanacearum</i>      | [84]       |
|                | Phenolic compounds accumulation                           | Inducible        | Grapevine                        | <i>Phaeomoniella chlamydospora</i> | [50]       |
|                | Tyloses                                                   | Inducible        | Potato                           | <i>Ralstonia solanacearum</i>      | [50]       |



Xylem cells (responsible for water and mineral nutrient transport from the root to the shoot) have pathogen-induced accumulation of complex secondary metabolites. These physicochemical barriers are commonly induced by vascular wilt pathogens, including the fungi *Fusarium oxysporum* and *Verticillium dahliae*, or the bacterium *Ralstonia solanacearum* [49]. Reinforcement of the xylem vessel secondary cell wall with lignin or other phenylpropanoid compounds impedes entry to the vasculature and vessel-to-vessel dissemination of these pests (Figure 3) [50,51]. Spatial restriction of *R. solanacearum* within root vasculature provides resistance to this bacterium in tomato plants [52]. Accumulation of phenolic compounds surrounding the xylem vessel infected with *F. oxysporum* restricts radial progression of the fungus in tomato root vasculature and colocalizes with the expression site of a resistance gene [53]. This suggests that the production of a physical barrier in xylem vessels might be induced by a cell-type-specific immune response. Additionally, xylem parenchyma cells are able to form tyloses (outgrowths of the cell that bulge into the vessel lumen via the pit), and these tyloses can secrete gel-like substances, together potentially blocking the vertical spread of the pathogen (Figure 2) [49,54]. While these responses have been detected, regardless of a plant's level of susceptibility to the pest, in resistant varieties they are induced earlier and only in the infected vessels [55]. However, the effectiveness of tyloses as a barrier remains in question, since the pathogen can still spread vessel-to-vessel, and compromises xylem hydraulic conductivity by blocking the vessel lumen [56].

Several types of localized intrinsic and inducible barriers contribute to resistance to root-infecting parasitic plants. *Striga hermonthica* and *Striga asiatica* infect grasses, but are incompatible with dicots [57]. These incompatible interactions are unsuccessful due to the barriers presented by root cell types or tissue layers [58]. The epidermis constitutes the first layer of incompatibility. Growth of the *Striga* radicle is redirected upon encountering the root of *Phtheriospermum japonicum* [58]. *Striga* penetration of the *Lotus japonicus* root is blocked in the outer cortex layer, with the accumulation of lignin in cells surrounding the traversing haustorium (penetration structure of the parasite) [58]. Plant species compatible with parasitic weeds are considered hosts, but certain host varieties are resistant to infection due to similar types of cellular barriers as described for incompatible species (Figure 3) [58]. Parasitic *Orobancha crenata* attaches to the surface of the roots of chickpea (*Cicer arietinum*), but accumulation of phenolic compounds at the site of the attachment serves as a localized barrier in the epidermis and prevents further development of the parasite [59]. Among pea plants (*Pisum sativum*), a host species for *O. crenata*, resistant varieties form a localized, physical barrier composed of callose within their cortex [60]. Together, inducible barriers in the epidermis and cortex prevent parasitic plants from infecting incompatible species as well as underlying resistance in host species.

The endodermis has been shown to provide a barrier to parasitic plants only in host species. In the roots of several varieties of rice [58,61,62], maize [63], or sorghum (*Sorghum bicolor*) wild accessions and landraces [64,65], *Striga* is not able to cross the endodermis. In most cases, this restriction of *Striga* penetration is associated with localized induction and accumulation of lignin [64,66], silica [64], and uncharacterized phenolic compounds [66] occurring only upon *Striga* infection. This response is likely transcriptionally controlled via upregulation of a suite of phenylpropanoid biosynthesis-associated genes, as in the roots of the *Striga*-resistant variety Nipponbare [66]. Cell-type-specific accumulation of PPRs may also be a component of the host immune response. In sunflower (*Helianthus annuus*), the HaOr7 gene – encoding a plasma membrane receptor with structural resemblance to known PPRs – confers resistance to *Orobancha cumana*, whose parasitism is blocked in the endodermis [16]. Activation of PPRs has also been shown to induce lignification [7], suggesting that PTI machinery might be involved in inducing endodermal barriers for root-parasitic plants. In several host species the role of the

endodermis as a barrier to parasitic plants, as well as to nematodes and oomycetes, suggest that the endodermis might form a protective layer to a broad range of biotic agents.

Biotic agents can also evade existing root barriers in a cell-type-dependent manner. *Phytophthora palmivora* penetrates *Medicago truncatula* and *Lotus japonicus* via epidermal cells at the root tip where it then colonizes the cortex. Successful infection depends on increased expression of the API gene within the epidermal cells, specifically in two developmental zones (the root meristem, and the elongation zone and beginning of the differentiation zone) which alters endomembrane dynamics and resulting secretion of cellulose-associated pectin and xyloglucan [67]. Thus, modification of cell-wall architecture in a specific developmental stage of epidermal cells enables *P. palmivora* to traverse the barrier constituted by the epidermal cell wall.

### **It goes both ways: the bidirectional relationship between root diffusion barriers and the soil microbiome**

The ability of a biotic agent to pass the endodermis determines its future habitat inside a plant and its potential success as a symbiont or parasite. Two types of endodermal apoplastic diffusion barriers – the lignified Casparian strip and the suberized cell wall – control the transport of water and minerals in and out of the stelar pathway [24,25]. Microorganisms capable of passing through these endodermal barriers can enter the vasculature and colonize the shoot, depending on their preferred lifestyle (Figure 2) [68]. Thus, the integrity of endodermal barriers is essential for both plant nutrient homeostasis [69] as well as interactions with members of the soil microbiome [12,70], and the two processes are interconnected.

In addition to presenting an intercellular barrier to microbes and nutrients, endodermal cells function in distinct ways necessary for microbe perception, signaling, and assembly of microbial communities. Non-differentiated endodermal cells in *Arabidopsis* are MAMP-responsive [6]; this can suppress plant defense responses to allow for colonization with commensal microbiota. In contrast, barriers in differentiated endodermal cells restrict MAMP movement and serve as a checkpoint for microbe perception and subsequent immune responses [6]. Several *Arabidopsis* mutants with impaired Casparian strip or suberin deposition have been shown to recruit distinct bacterial communities from the soil compared with the wild type [12,70]. It remains unknown whether this effect is solely a result of disruption of the endodermal barrier or the surveillance mechanism that compensates for the defective barrier. These alterations in root bacterial community profiles were further associated with the plant's ability to uptake mineral nutrients [12]. Microbial community membership is also influenced by root exudate composition. Endodermal cells with impaired diffusion barriers have distinct root exudates [70], although the precise compounds affected and their role in the recruitment of specific bacterial taxa are not yet known. Future research will also determine whether defects in endodermal barriers impact the assembly of fungal communities.

While endodermal differentiation features directly influence microbial activity, microbes can also reshape features of root endodermal differentiation, and thus the relationship between root endodermal barriers and the microbiome is bidirectional. Microbes were also found to weaken root suberized barriers in the *Arabidopsis* root endodermis [12]. The fungus *V. longisporum* showed the potential to degrade suberin [8], while several bacterial isolates can reduce the proportion of the root where endodermal cells are fully suberized as compared with roots grown axenically [12]. Inoculation of roots with a synthetic community consisting of bacterial strains reducing root suberization further allows the plant to withstand nutrient deficiencies [12]. Infection with *V. longisporum* reduced suberization of differentiated endodermal cells, suggesting the potential of this fungus to degrade suberin [8]. Modulation of suberin plasticity by bacteria is molecularly

linked to abscisic acid (ABA) signaling [12], although it remains unknown which genetic components of bacteria are essential to elicit this effect. It is also not known whether the effect of *V. longisporum* on endodermal suberization requires ABA biosynthesis or signaling. Given the downregulation of several suberin biosynthesis-related genes by the fungus, and the plethora of bacterial isolates that can reduce endodermal suberization [8,12], the existence of effector (s) that transcriptionally control suberin biosynthesis is plausible.

Root endodermal cells are not the only root cell types which form apoplastic diffusion barriers as part of their differentiation program. The exodermal layer is the cell layer underneath the epidermis; however, this layer is not found in Arabidopsis (Figure 1). Both suberized and lignified barriers are found in exodermal cells, and the pattern of lignin or suberin deposition differs between species [71]. In tomato, suberin was detected only in exodermal cell walls but was absent in the endodermis [71], further increasing the complexity with which distinct microbes can interact with root cell types. The bidirectional relationship of microbes and exodermal differentiation has not been systematically assessed.

Whether this phenomenon of reshaping endodermal differentiation is a capacity of diverse fungal taxa is unknown. *Serendipita indica* and *Colletotrichum tofieldiae*, both endophytic fungi, were tested for their progression through Arabidopsis root cell layers. Neither was able to pass suberized cells in the endodermis, although *C. tofieldiae* was able to move to the shoot [8,72]. *S. indica* has a wide range of hosts [73] – including species with a suberized exodermis such as barley [74] or tomato [71] – in which the fungus colonizes cortical cells. It has recently been suggested that passage cells, a population of non-suberized cells, present in both the endodermis and exodermis [75,76], might serve as an entry point for several mutualistic microbes [75]. Indeed, AMF hyphae enter the root via exodermal passage cells (characterized by lack of suberization) [77]. Whether other fungal classes or other microorganisms use passage cells as a gateway to the inside of the root has yet to be reported. It will be interesting to determine whether the reduction of suberization elicited by microbes is conserved between both the exodermis and the endodermis, or whether there are divergent interactions.

## Concluding remarks and future perspectives

### A balancing act: let in beneficial bugs and keep out pests

Can progress in the last years guide future directions that will lead to strengthening of root cell type interactions with beneficial microbes and prevention of interaction with pests at the same time (see Outstanding questions)? The properties of some cell types lead to contrasting effects on interactions with organisms possessing distinct lifestyles. For example, a high proportion of aerenchyma in the root cortex could restrict colonization with pathogenic fungi, but at the cost of compromising interactions with symbiotic AMF [47]. Are there beneficial microorganisms that can profit from a reduced proportion of aerenchyma? More cell-type resolution data are needed. For instance, the role of both MAMP and DAMP immunity elicitors (not just flg22) in the context of diverse host–pathogen or host–beneficial organism systems on immune responses at cell-type resolution needs to be systematically elucidated. Trade-offs need to be assessed on a more holistic scale. As an example, a response elicited in an individual root cell type may prevent interaction with a pathogenic organism but may compromise beneficial interactions or tolerance to abiotic stresses. Strengthening of lignified or suberized endodermal barriers by microbes should be localized and inducible in order to maintain mineral nutrient homeostasis and water transport. Conversely, increased suberin content as a potential physical barrier from pathogens will likely affect root exudation rates [70], and hence recruitment of beneficial microbes. Ideas for solutions to these trade-offs include the strengthening of properties of root cell types crucial for beneficial interactions and the concomitant protection from pests via other cell types. Alternatively, improving

## Outstanding questions

Is the impact of soil-borne bacteria on endodermal cell wall diffusion barriers the same in plant species possessing an exodermis?

What molecular changes lead to the establishment of localized, cell-type-specific barriers to restrict pathogen infection of the root?

Can we combine the potential of beneficial microbes to reshape specific root cell types with knowledge regarding cell-type-specific regulatory networks to enhance plant stress tolerance?

What are the genetic components of beneficial microorganisms essential for root colonization?

What is the minimal combination of genes expressed within the host root, as well as of beneficial microbes, that is sufficient to maintain their interaction?

Are the mechanisms underlying cell-type-specific responses to biotic factors unique for single microbial isolates or general for a broad range of microbial species and their molecular signatures?

Are the differences in immune perception by FLS2 between root cell types translatable to other classes of MAMPs?

Can we create an ideotype of root cellular architecture for optimal biotic interactions, strengthening beneficial interactions while minimizing pathogenic ones?

the production of physical barriers to pathogens in a highly localized and inducible manner might help to offset the potential risk of weakening interactions with beneficial microbes. This can only be achieved if we know the regulatory components that determine barrier production and deposition in individual cell types.

#### More organisms, more cell types, more problems for biotic interactions

Most crop species possess a suberized or lignified exodermis and an endodermis [37], but our knowledge of how organisms interact with roots with two barrier layers is limited. Root-cell-type resolution transcriptomes and their responses to their biotic counterparts have mostly been characterized in *Arabidopsis*, while morphological studies of interactions between root cell types and biotic factors mostly involve crop species. Thus, there is a gap in our knowledge of transcriptional regulation that reshapes root cell types to control their interactions with other organisms. Addressing this gap is an essential step to engineer optimal barriers for root pathogens.

#### Reverse engineering root cellular architecture

Can we predict an optimal root-cell-type architecture to strengthen beneficial interactions and minimize the deleterious impact of pathogens? Such ideotypes have been already proposed for acquisition of nutrients [78] and water [79]. Quantification of root radial anatomy, combined with modeling of the path by which biotic agents progress radially through the root [80,81], could pave the way to establish root ideotypes for biotic interactions. For example, modeling of the proportion of aerenchyma relative to root volume can shed light on the optimal cortex architecture for AMF recruitment balanced with protection from pathogenic fungi for improved plant growth and reproduction. The heterogeneity of root systems of crop species could help to partition optimization of the root cellular architectures into distinct root types. For example, rice fine roots that cannot establish AMF interactions would be a perfect target for optimizing their structure for protection from pests. The seedbank of parasitic plants is localized primarily in the topsoil layers [82]. Thus, strengthening intercellular barriers in the crown roots of host plants would allow for its fine, deep-soil-located roots to engage with bacterial communities that can improve nutrient uptake.

In conclusion, integrated approaches that focus on cell-type-specific perception of other organisms, transcriptional changes and reprogramming of cell identity in response to biotic interactions, together with accounting for variation in root types within individual root systems (especially in crop plant species) could bring us closer to engineering roots with cell types best suited to biotic interactions (see Outstanding questions).

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#### Declaration of interests

No interests are declared.

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