Trends in Plant Science

CellPress

Review

Cell walls and their role in the plant root microbiome

Vicky J. Waymouth [1,#], Amelia C.W. Keynton [1,#], Douglas R. Brumley [2], Berit Ebert [1,3], and Michelle Watt [1,*]

Plant roots form a microbiome that interacts at the cell wall extracellular matrix before entering the cell. The root primary and accessory walls present a dynamic, cell-type-dependent scaffold that microbes must navigate, using shared cellulose or contrasting chitin motifs and influencing plant gene responses that encode enzymes for cell wall biosynthesis and degradation. We propose that an interface evolves as microbes reach the root tip and interact with host polymers, potentially driving concurrent degradation of root and microbial cells. Knowledge gaps span diffusion, fluid flow, nutrient exchange, and the physics of microbial motion within the wall boundary. Advances in *in situ* imaging and mathematical modelling can help understand the dynamics of cell walls to design root microbiomes to function in agroecosystems.

Highlights

Plant roots have a microbiome of bacteria, fungi, archaea, and protists that influence plant nutrition, water status and health, and soil organic matter and porosity.

Roots and microorganisms interact first at their extracellular walls, which provide signals, attachment sites, transport pathways, and barriers.

Plants actively adjust their root cell walls based on microorganism encounters, where wall synthesising and degrading enzymes help shape the microbiome.

Root microbiomes develop over time in a soil volume, such that the root-microbiome wall is dynamic, with highly complex biochemistry and currently unpredictable functions in soil and for the plant.

New *in situ* analytical methods using fabricated ecosystems and microfluidics that track, in space and time, defined microorganism communities around plant genotypes will allow the discovery of the walls of the root–microbiome interface.

Cell walls: scaffolding for the root microbiome

Plant roots host microorganisms from their growing environment, usually soil, to form the **root microbiome** (see Glossary) [1]. The diversity and function of the root microbiome impact plant production in agriculture and natural ecosystems, soil nutrients, water and carbon cycles, and provide alternatives to synthetic pesticide use [2]. Research on microbiome colonisation has focused extensively on the role of root **exudates**, notably the exudates that diffuse away from the root into the soil or solution that is sampled separately from the root ([3–5] and references therein). Here, we turn attention to the **cell walls** of the roots (Box 1) and associated microorganisms, and the molecular changes that influence the root microbiome, especially bacteria and fungi, which are extensively studied (Figure 1). For the purposes of this review, the space occupied by the root microbiome combines spaces traditionally treated and sampled separately, including the endosphere, the rhizoplane, and the rhizosphere.

Root cell walls are the first and final plant surfaces hosting the root microbiome, from the arrival of a root tip to years after the host plant has died [16,17]. Bacterial and fungal populations bound to the root are significantly different in diversity, succession rates, and function compared with the microbiome colonising the rhizosphere outside the root and in the bulk soil [18,19]. Root cell walls provide cues to root microbiome diversity [20,21], attraction and attachment points [22,23], niches [24–26], protection of living cell cytoplasm [27,28], intercellular apoplastic barriers [29,30], and a large source of energy to soil microorganisms [31]. Indirectly, the chemical composition and thickness of root cell walls likely influence the biophysical pathways for the exchange of signals and for the transport of nutrients, water, gases, and metabolites [29].

In this review, we cover the molecular aspects of the root cell walls and their associated microorganisms and the spatial and temporal arrangement of root and microorganism walls as the root microbiome develops. We propose that an **interface** of plant and microorganism walls arises

[1]School of Biosciences, The University of Melbourne, Biosciences 2, Royal Parade, Parkville, Victoria 3010, Australia
[2]School of Mathematics and Statistics, The University of Melbourne, Parkville, Victoria 3010, Australia
[3]Faculty of Biology and Biotechnology, Ruhr University Bochum, Universitätsstraße 150, Bochum 44801, Germany
[#]These authors contributed equally

*Correspondence:
watt.m@unimelb.edu.au (M. Watt).

Box 1. Root cell types and their cell walls

If one cuts a transverse section through a young plant root, cell types are arranged in layers from the outer epidermis in towards the centre (Figure I). Each cell type can differentiate different cell walls and apoplastic barriers (see Section below, 'Molecular structure of cell walls'). All root cells develop a primary wall and many retain that wall only and are called parenchyma cells. Some parenchyma cells retain an ability to divide throughout their life, including the pericycle cells that divide to form lateral roots; cambial cells that develop new xylem and phloem and outer layers in dicotyledonous species [6]. Some cortical cells develop walls thickened with primary wall material but remain living (collenchyma cells [6,7], while others thicken with secondary wall material and die (sclerenchyma cells) [6]. In exceptional cases including *Brassica napus* (Figure I), inner cortical cells have a radial scaffolding of lignified secondary wall called phi thickening [8]. Invariably across all species examined, walls of the endodermal cells become modified with Casparian bands and suberized lamella [6,9]. The Casparian strip is a localised impregnation of the primary cell wall that surrounds the endodermal cells as a belt in the longitudinal direction that is made mainly of lignin [9]. Polymerized suberin is deposited to form the lamellar structure between the plasma membrane and the cell wall [10]. Many species (an exception is *Arabidopsis thaliana*) develop an exodermis that shares genes and a Casparian strip and suberized lamella with the endodermis [11]. Changes in cell wall chemistry towards greater autofluorescence in the epidermis occurs frequently, especially in roots grown in soil, indicative of the deposition of lipophilic compounds [12]. The outer epidermal walls of root hairs and nonhair cells can be extremely resistant to breakdown in the abiotic and biotic conditions of the field [13].

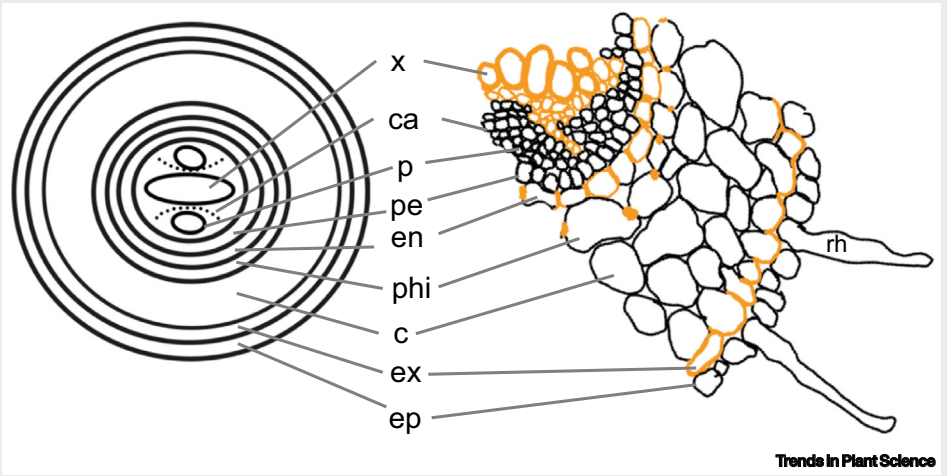

Figure I. An example of variation in cell walls across a root. Cell layers (left) 2 cm from the base of a tap root of the dicotyledon *Brassica napus* (canola) 12 days after planting from seed in soil. Walls of cells (right, pie section of left) traced from the cross section viewed with brightfield optics to observe primary walls (black) and autofluorescence to observe lignin and suberin in secondary walls and barriers (orange). From the outer layer: epidermis (ep) with occasional root hair (rh) single cell projections; exodermis (ex) which has developed a Casparian strip and suberized lamella, typical of certain species; cortical (c) cells; Phi secondary wall thickening (phi) found uniquely in cortical cells of Brassicaceae and few other genera; endodermis with Casparian bands (deposited in radial walls) and suberized lamella (deposited outside the plasma membrane of some of the cells); pericycle (pe); phloem (p); vascular cambium (ca); xylem (x). Note that that root cell layers can vary among plant genotypes, age, and environment.

upon plant and microorganism colonisation, and that the biochemistry of the interface wall depends on the abundance and function of enzymes and other nonenzymatic proteins, such as expansins, in roots and the associated microbiome. Many of the myriads of cell wall enzymes expressed upon interaction are common to host roots and colonising organisms, offering common targets for regulating the microbiome in agriculture and natural systems. Although complex, a predictable sequence of events takes place during root microbiome succession and the evolution of wall biochemistry. The **dynamics** could be quantified using live imaging in microfluidic microcosms, analysed with **biophysical models** that can be coupled to crop and land use models that inform the development of commercial plant and microorganism products and policy for agronomic practices [32].

Glossary

Biophysical models: mathematical prescriptions or numerical simulations, which represent key features of a biological system, for the purpose of studying underlying physical processes and properties. For root microbiomes, biophysical models help simulate dynamic interactions to (i) design experiments, (ii) extract quantitative information from images (e.g., image processing or cell tracking), and (iii) calculate rates and fluxes (e.g., cell colonisation rates, fluid flow, and nutrient transport along and through wall interfaces) [32].
Cell wall: mechanically strong yet dynamic extracellular matrix deposited by a cell outside its plasma membrane. It is in plants, bacteria, algae, and fungi but not protozoa and most animal cells.
Dynamics: changes in space and time and the forces underlying those changes (referenced for plant roots [178]).
EcoFABs: fabricated ecosystems for multilaboratory testing of repeatable plant–microorganism interactions [179].
Exudates: low and high molecular weight molecules (e.g., sugars, organic acids, polysaccharides, and proteins) released from the inside to the outside of cells by a transport process across a membrane or detachment from shedding or dying cells [180]. Exudates are considered to act on microorganisms outside the root, often distances away from their point of release (e.g [3]).
Fluid flows: the movement of fluid (i.e., water) in response to external forces. Driven, for example, by evaporation, transpiration, and groundwater, fluid flows redistribute nutrients, gases, and microorganisms and modify chemical gradients used by microorganisms to navigate. Velocity gradients—i.e., spatial variations in the fluid velocity—are expected to be low but alter the motility of microorganisms so that they cannot swim in a straight line [181], and this can result in strong accumulation of bacteria in particular locations.
Interface: used here in this review to describe where cell wall polymers of root and microbial origin interact and evolve, regulated by the activity of cell wall enzymes, the availability of precursors, and chemical conditions such as pH.
Microfluidic devices: confine and control dynamic processes at the scale of individual microorganisms, to allow

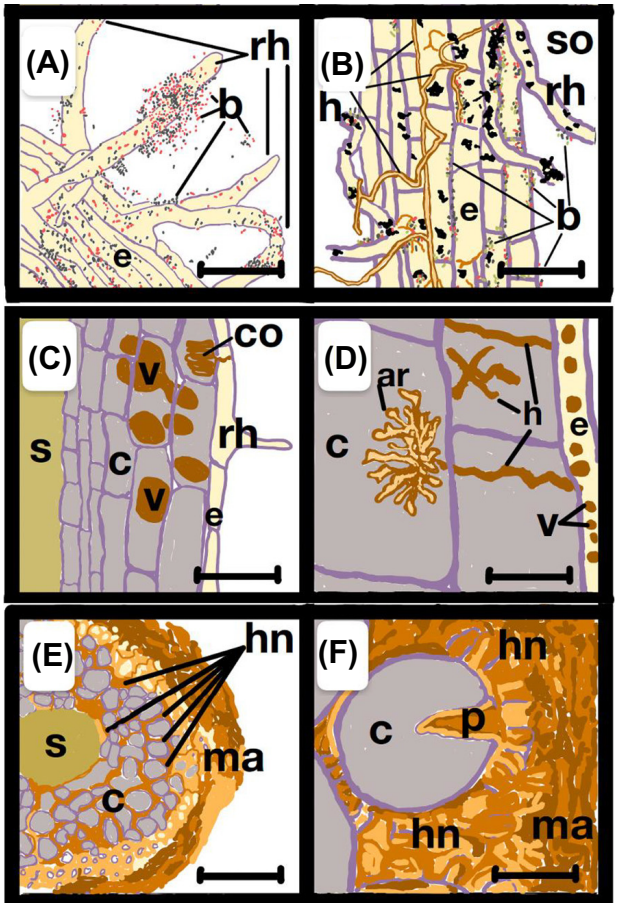

Figure 1. Examples of bacterial and fungal microbiome interactions with roots and cell wall surfaces. (A) Bacteria (b, red and blue points) attached to epidermal (e) cells and root hair (rh) of wheat root grown without soil (Watt, unpublished; root processed using fluorescence in situ hybridisation (FISH) as per [14]). (B) Epidermal (e) surface of a wheat root harvested from the field in soil (so) with fungal hyphae (h) and bacteria (b) colonising the root epidermis (e) and root hairs (rh) (from [15]). (C) and (D) Colonization of root cortical cells (c) by arbuscular mycorrhizal fungi vesicles (v), coiled hypha (co), hypha (h), and arbuscules (ar). (unpublished images from hand sectioned fresh root material of herbs; vesicles (v) that appear to go across two cells may be within single cells above or below the focal plane). (E) Cross section of tree root cortex (c) colonized with ectomycorrhizal fungus Hartig net (hn) (brown pigment in spaces between cell walls) and surrounded by melanin (ma) of hypha (sketches were based on images from [15]). (F) Fungal peg (p) in a cortical cell (c) and surfaces of Hartig net (hn) and melanin (ma) appressing root cell wall surfaces (sketches were based on images from [15]). Bar = 20 μm. Figures traced from microscopy images from referenced sources. Purple = root cell wall; Cream = epidermal cell (e); Grey = root cortical cell (c); Gold = root stele (s); Browns = fungal structures; Red, blue = bacteria (b).

visualisation [182]. Devices are typically fabricated through soft lithography using elastomers such as polydimethylsiloxane, and microfluidic devices enable the precise control of physical and chemical microenvironments, while facilitating live imaging through video-microscopy.

Middle lamella: thin (50–100 nm) pectic-rich macro-molecular layer between plant cells that is biochemically distinct and external to the much thicker (ca 5000 nm) primary cell walls. Potential functions include enabling cell-to-cell contact for tissue and organ cohesion of plants [183].

Root microbiome: microbial community in and around the root habitat that has distinct and dynamic physico-chemical properties to the growth medium [1]. Organisms in root microbiomes can include unicellular organisms from the Bacteria and Archaea domains and Fungi and Protists from the Eukarya domain. For the purposes of this review, we consider the root microbiome colonising cell walls of endosphere, rhizoplane, and rhizosphere spaces.

SynCOM: combination of two or more microorganism species selected based on ecological or metabolic principles [184] that are isolated, cultured, and combined to be applied repeatedly in plant-microbe experiments [185].

Biochemistry of cell walls and surfaces of the root microbiome

Plant roots, like all plant organs, are made up of cells with varying cell wall chemistries and morphology, separated by a thin **middle lamella** (Box 1). All cells develop a primary wall (Figure 2A), and specialised cells eventually develop a secondary cell wall (Figure 2B), enriched in lignin, suberin, or callose, depending on cell type and age (Figure 3) and abiotic and biotic growth conditions (Figure 4).

Plant cell walls are an interwoven network of cellulose microfibrils and hemicelluloses embedded in a matrix of highly diverse pectins and hydroxyproline-rich glycoproteins, predominantly extensins and arabinogalactan proteins (AGPs) [49] (Figure 2A,B). Polysaccharide structures and linkages are heterogeneous, and this heterogeneity is implicated in the differential colonisation of root cells by microorganisms [29,50]. For pathogenic interactions, molecular changes in walls have long been studied. The degree and pattern of pectin methyl esterification regulate pathogen infections [51]. *Arabidopsis* mutants with defects in cellulose, xylan, glucuronoxylan, pectin, xyloglucans, or lignin biosynthesis have altered susceptibility to diverse pathogens [52]. Cell wall-derived oligosaccharides can act as damage-associated molecular patterns (DAMPs) to elicit pattern-triggered immunity (cell wall-associated immunity is reviewed in [53]). Cell wall molecules are also emerging as key determinants of the microbiome

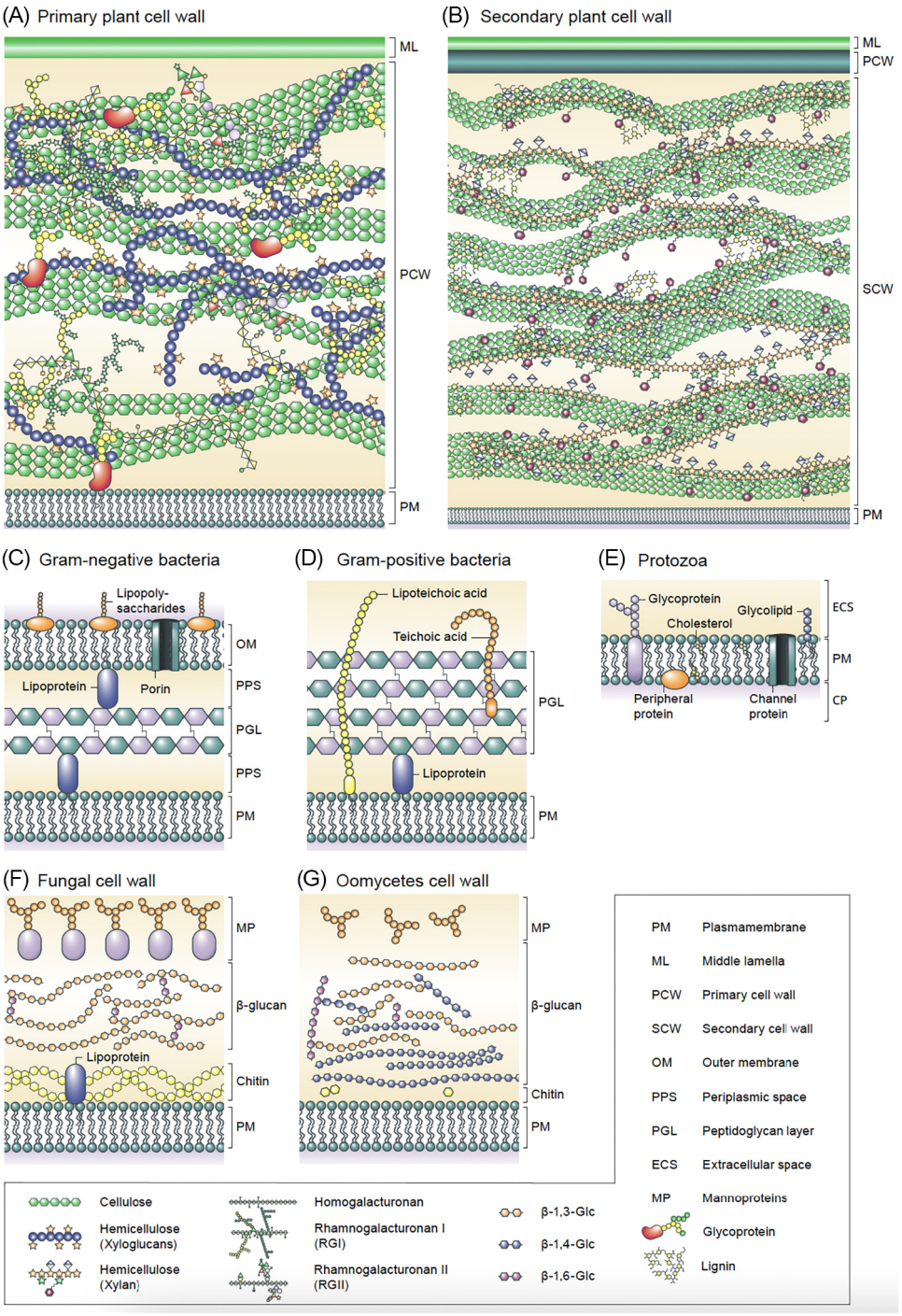

Figure 2. Schematic representation of cell surrounding structures of the root microbiome. (A) All root cells first develop a primary wall. Type I primary cell wall of plants illustrating the main components: cellulose (15–40%), hemicellulose (predominately xyloglucans, 20–30%), and pectins (homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II, 30–50%). The specific hemicelluloses and pectins, as well as the proportions of each component vary across species, development, and cell type. Glycoproteins (such as extensins and arabinogalactan proteins) are also

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community, including commensal and beneficial organisms, for example, in *Arabidopsis* [20] and the barley microbiome [21].

Microorganism invasion, in general, leads to thicker root cell walls, which serve as reinforcements and assist in wound sealing, particularly at penetration sites [54]. This thickening of the existing cell wall is caused by the deposition of phenolic compounds such as lignin or glucans such as cellulose, hemicellulose, and callose and is further reinforced by the deposition of a secondary matrix of suberin (Figure 4A,F) [55–59]. Suberin (like lignin) is also a complex hydrophobic polymer that contains fatty acids and their oxidised derivatives, glycerol, and ferulic acid, linked by ester bonds to form a largely poly-aliphatic polyester [58]. During normal plant growth, suberin can be diffusely deposited into pre-existing cell walls and as a distinct secondary suberised wall, typically in root endodermal and exodermal cells after the development of the Casparian strip, but it is also produced in response to wounding by root pathogens (Box 1, Figure 4A,F) [60]. These barriers can also affect the composition of the rhizosphere, with *Arabidopsis* mutants affected in Casparian strip and suberin endodermal barrier formation influencing the composition of a natural microbiota community [61]. Furthermore, studies in Sorghum as well as *Arabidopsis* have demonstrated the ability of individual bacterial isolates to control the pattern of suberin deposition. Isolates were able to reduce the region of continuous activation of a suberisation reporter by increasing the root zone with a patchy pattern of reporter activation in Arabidopsis, while an increase in the suberisation of endodermis and exodermis was seen with inoculated Sorghum [61,62].

Callose (β-1,3-glucan with β-1,6 branches) is a glucan polymer involved in multiple developmental processes, for example, cell-to-cell signalling in the root meristem [63]. It reinforces the cell wall in response to damage caused by pathogen penetration [64] by forming transient depositions in plasmodesmata to regulate permeability upon pathogen attack [65]. In the *Arabidopsis sid2* mutant, the loss of callose deposition permitted *Trichoderma harzianum* to enter the vascular system, from which it is normally excluded [66].

The chemical structures of the walls of roots and microorganisms have similarities and differences [67] (Figure 2). Bacteria are broadly divided into two classes, Gram-negative or Gram-positive (Figure 2C,D), based on their appearance in a Gram stain, which is a direct reflection of their wall structure. The Gram stain is not retained by Gram-negative bacteria as they have an additional outer membrane that Gram-positive bacteria lack (Figure 2C,D). The outer membrane of Gram-negative bacteria is primarily composed of lipopolysaccharides (LPSs) and contains

present in the primary cell wall. The commelinid clade of monocots have a type II primary cell wall (not shown), which is enriched with mixed linkage glucans and glucuronoarabinoxylan rather than pectins [33,34]. (B) Some cells develop a secondary cell wall (Box 1 for root cell types with secondary cell walls), which mainly contains cellulose and the hemicelluloses xylan and glucomannan [35] (Figure 2B). Thick secondary cell walls of plants comprise rigid cellulose (40–80%), the hemicellulose xylan (10–40%), and are enriched in lignin (5–25%). (C) Gram-negative bacteria have two membranes with lipopolysaccharides on the outer membrane. Lipopolysaccharides (LPS) have three regions: lipid A; the core oligosaccharide; and the O-antigen, which is composed of hydrophilic species-specific polysaccharide chains of repeating monosaccharides, such as galactose, rhamnose, and mannose [36]. Between the cytoplasm and outer membrane is an approximately 5nm thick layer of peptidoglycan, repeating units of *N*-acetyl-glucosamine linked to *N*-acetylmuramic acid cross-linked by diverse pentapeptide side chains [37–39]. (D) Gram-positive bacteria have a single membrane and a thicker peptidoglycan layer (20–50 nm) than Gram-negative bacteria. Highly negatively charged teichoic acids and lipoteichoic acids, polyol phosphate polymers, are major components of the Gram-positive bacteria cell wall and are embedded in the plasma membrane or covalently attached to the peptidoglycan layer, respectively, on the periplasmic side of the cytoplasmic membrane as an outer protective barrier [40]. (E) An example of a protozoan cell surface (no cell wall) is shown. (F) Fungal cell walls are composed primarily of chitin (repeating units of β-(1,4)-*N*-acetyl-D-glucosamine), β-1,3 glucans, and mannoproteins [41]. (G) The cell wall of oomycetes consists mainly of cellulose, β-1,3 and β-1,6 glucans, mannose, and in some species, small amounts of chitin. (C), (D), and (F) were reproduced from [42] with permission.

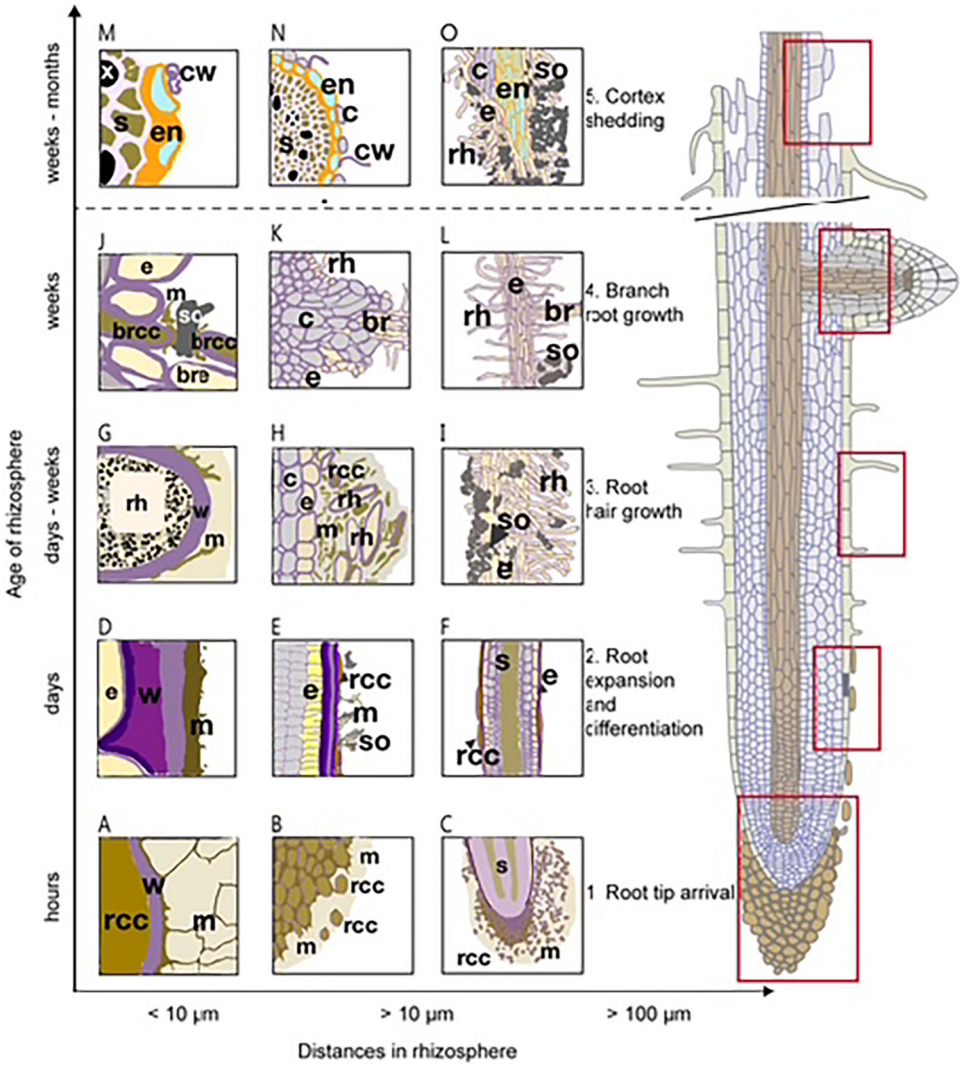

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Figure 3. Evolution of the plant root and attachment sites for microorganism interactions, presented from high to lower magnification (left to right) and through time (bottom to top). **1.** The root microbiome starts with the arrival of a root tip (A–C), which is surrounded by root cap cells (rcc) (B) that secrete a mucilage (m) that can be seen outside the wall of root cap cells (w). Underlying the root cap, a dividing root meristem makes root tissue cells including the stele (s). **2.** Within days, the root is expanding and differentiating in the zone that hosted the root cap (D–F) and root cap cells (rcc), mucilage (m), and soil (so) can be seen attached to the epidermal (e) root cells. (D) In this expansion zone, the wall (w) of epidermal (e) cells is overlayed with mucilage (m). **3.** Days to weeks after arrival of the tip, root hairs (rh) extend from some epidermal (e) cells (G–I) and the surface of the root can be a mixture of root cap cells, mucilage, and soil particles (so) overlying the epidermal (e) cell wall (w). **4.** Within weeks, branch roots (br), also termed lateral roots, emerge (J–L) into the rhizosphere. New root and root cap meristem emerges from the parent root such that the root surface is renewed with younger cells including the lateral root epidermis (bre) and lateral root cap cells (brcc) and mucilage (m) that mix with the parent root epidermis (e), root hairs (rh), cortical cells (c), and soil (so). (A–C) reproduced with permission from [43] Figs 6, 2, and 7, respectively. (D) reproduced from [44] Fig 5 with permission. (E) reproduced from [25] Fig 16 upon permission from publisher. (F) reproduced from Synopsis image from [45] with permission of the author. (G) reproduced with permission from the publisher from [46] Fig 13. (H) reproduced from [25] Fig. 20 with permission from the publisher. (N) reproduced from [47] with permission from the publisher.

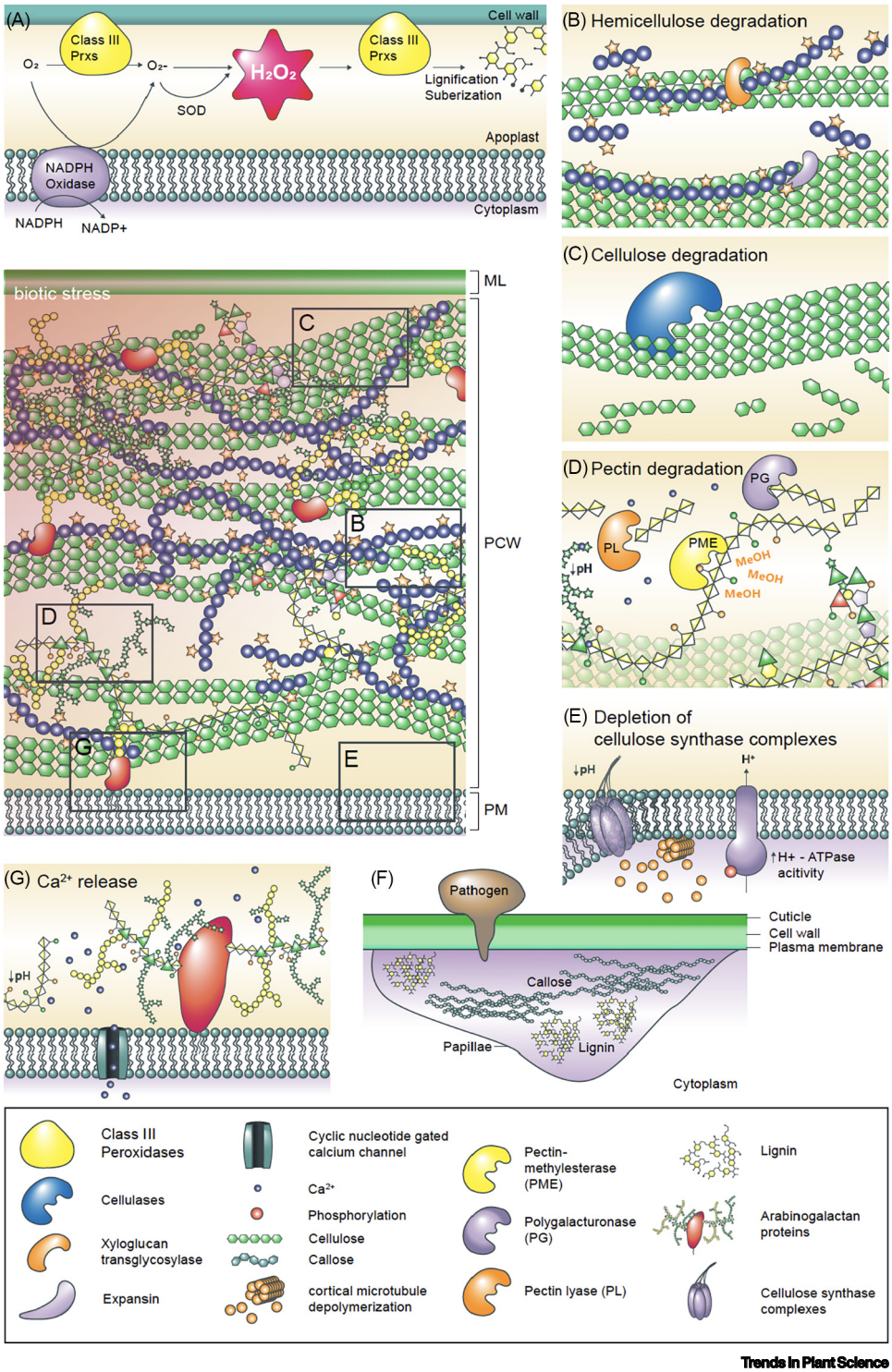

Figure 4. Schematic representation of biotic stress responses and cell wall degradation occurring at the root cell wall upon microorganism contact. (A) Cell wall class III peroxidases and plasma membrane-localised NADPH oxidases causing lignification and suberization. Cell wall degradation and loosening upon microbe contact through (B) the

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lipoproteins and β-barrel proteins [68]. LPSs form a barrier against small hydrophobic molecules [36] and have been implicated in host recognition and specificity, binding, and in the immune response [69]. Mutants with altered LPS composition have a reduced ability to colonise their host plants [70]. LPSs are considered as pathogen-associated molecular markers that can be recognised by the plant and induce cellular defence responses [69].

Compared with Gram-negative bacteria, much less is known about the wall components of Gram-positive bacteria during rhizosphere interactions. Teichoic acids and lipoteichoic acids on the outside of Gram-positive bacteria have parallels to the LPSs of Gram-negative bacteria (Figure 2C,D). Modifications of the polyol chains with D-alanine, *N*-acetyl-glucosamine, and *N*-acetyl-galactosamine form characteristic teichoic acids for certain Gram-positive bacteria and may function similarly in host-recognition to the species–specific *O*-antigens of LPSs [36]. Furthermore, both Gram-negative and Gram-positive cell wall surface molecules are negatively charged. The charge state of bacterial cell walls and root cell walls may play a role in attachment between bacteria and roots, governed by hydrophobic and electrostatic properties [70].

Fungal cell walls are thicker than bacterial cell walls and unlike plants and oomycetes, most fungal walls are devoid of cellulose (Figure 2F). The unique fungal cell wall polymer, chitin, is central to fungal root interactions, eliciting immune responses in plants for pathogenic and nonpathogenic fungi and providing signalling molecules in mutualistic interactions [71]. Fungal chitin-binding proteins can protect from plant chitinases to prevent the formation of chitin-derived microbe-associated molecular patterns that elicit pattern-triggered immunity [72].

Oomycete walls contain both small amounts of chitin and a low proportion of cellulose. Oomycete walls fall into three classes based on *N*-acetyl-glucosamine content and are further differentiated by the fine structure of their β-1,3 glucans and chitin [73] (Figure 2G).

Uniquely, compared with other rhizosphere-colonizing microorganisms, protozoa have no cell wall (Figure 2E). The phospholipid membrane contains glycoconjugates and has proteins that perform various essential functions in metabolism, transport, and signalling. In protozoan parasites, lipid-anchored molecules form protective barriers and play critical functions required for infectivity [74]. The outer membrane of protists can develop protrusions (called axopodia, cilia, and flagella) for feeding (mainly eating bacteria) and motility. These protrusions may help attach to root cell walls as part of their feeding strategy [75]. Although protozoa have long been recognised as part of the root and rhizosphere microbiome [76], it is unclear how their lack of a cell wall compared with root cells and unicellular microorganisms plays a role in their functions, particularly related to making recycled nitrogen available to plants upon the consumption and breakdown of bacteria [77].

Dynamics of the cell walls of the root microbiome

The cell wall landscape of a root is dynamic (Figure 3). Here, we present the development of the root microbiome from the reference frame of a position in space (e.g., soil) (see Figure 5A of [78]).

action of expansins and hydrolysis of hemicellulose by xyloglucan transglycosylases, (C) hydrolysis of cellulose by cellulases, and (D) hydrolysis of pectins by the combined action of polygalacturonases, pectin methylesterases, and pectin lyases. Reduced pH leads to (E) internalisation of cellulose synthase complexes from the plasma membrane and depolymerisation of stabilising cortical microtubules and (G) calcium ion release from arabinogalactan proteins and pectins. In response to invasion by pathogenic biotrophs, (F) callose and lignin production is induced to form papillae. The large central panel highlights regions where a combination of these activities occur concurrently in the cell wall and at the plasma membrane in response to biotic stress. Figure adapted from [48].

An alternative spatiotemporal reference frame would be a position on the root tip, which continues to move forward when elongating. In this study of cell wall changes of the root as the root microbiome evolves, the position is fixed at the start, and time passes within that position as the root develops. Root microbiome development starts with the arrival of the root cap in that position. From root tip arrival onwards, signalling molecules, attachment sites, barriers, and nutrient transfer develop in that position from the time that young root cap cells arrive (Figure 3A–C) to senescing cortical cells (Figure 3M–O).

Root caps are the root microbiome 'pioneers' [79] with new cells that detach (Figure 3B,C) [80], potentially living multiple centimetres past the root tip in soil-grown roots [25]. Root cap cell release requires cell wall-active enzymes, i.e., pectinases and glucanases, capable of disrupting cell wall networks [81,82] (Figure 4C,D). These cap cell walls contain pectin, cellulose [83], and in some species, heteromannan [84], producing and secreting a mucilage (Figure 2A) with AGPs, xyloglucan, and heteromannan [84,85]. Root cap mucilage significantly regulates microbiome interactions, including bacteria, fungi, and protozoa [86]. Cap cells attract and repel bacteria depending on the species [87]. AGPs [88,89] and xyloglucan [90] have both been shown to act as signalling molecules from cap cells (Figure 4G, calcium signalling). The xyloglucan secreted by cap cells acts as a soil-binding molecule [91], which alters water retention. Physically, root cap mucilage can 'entangle' the fungal hyphae of pathogens, which are subsequently sloughed off with the cap and cells [92]. Interestingly, protozoa increase the growth and production of root mucilage in the cap cell complex [93].

Underlying the root cap, the root meristem's dividing cells eventually transition to elongation (Figure 3C–F). In the specialised but widespread case of tree root tips, ectomycorrhizal colonisation abruptly causes meristems to stop dividing, vacuolate, and stop extending into the soil [94]. Wall-degrading enzymes (Figure 4) increase in colonised tips [95]. Hyphae release pectin-modifying enzymes such as fungal pectin methylesterase LbPME1 (Figure 4D), which loosens the spaces between the cells [96] (Table 1). A Hartig 'net' of highly branched fungal hyphae grow in contact with, and between, cell walls [107]. Bacteria and fungi change daily in root tips undergoing ectomycorrhizal colonisation, presumably responding to the dynamics of substrates from cells and new niches on cell and hyphal surfaces [108]. In the case of root tips in nonectomycorrhizal plants, a dividing meristem is maintained for a time defined by root type, and the duration is an important determinant of root architecture.

The 'transition zone' [109] is where meristematic cells start elongating, and the region is highly sensitive to biotic and abiotic stimuli and interference (reviewed by [110]). Normally, the cell walls and surroundings are acidic to facilitate loosening for division and subsequent expansion and immunity signalling pathways [111]. Microbial attachment can induce alkalisation, slowing growth and defence responses [111], although acidification can benefit colonisation [112]. In alkaline conditions, peroxidases generate H_2O_2 (reactive oxygen species) (Figure 4A), and cell wall-modifying enzymes become inactive, causing the wall to stiffen [113]. Specifically, the covalent crosslinking of hydroxyproline-rich glycoproteins (known as extensins) by specialised class III extensin peroxidases causes them to become insoluble, thereby hampering their extractability from the wall [114]. Proposed mechanisms by which microorganisms disrupt cell wall apoplast pH include the plasma membrane- localised proton-ATPases that regulate the root expansion zone apoplast [115] and peptide secretion that creates an alkalised environment [116].

Shorter expansion zones are associated with longer residence times of tips in soil, greater bacterial numbers [14,117] (see Figure 4 of [74]), and potentially earlier exposure of grooves between epidermal cells where bacteria tend to cluster [17]. Bacteria can attach to root cell walls via

Table 1. Plant cell wall modifying protein families

Plant cell wall modifying proteins and activity[a]	Identified in[b]	Target	Refs
Cellulose-degrading enzymes			
Cellulose 1,4-β-cellobiosidases (EC 3.2.1.91 and 3.2.1176)	B, F	Hydrolyse β-1,4-D-glucosidic linkages, releasing cellobiose	[97]
Cellulases (EC 3.2.1.4)	P, B, F	Endohydrolysis of β-1,4-D-glucosidic linkages, releasing cellodextrin	[97,98]
β-glucosidase (EC 3.2.1.21)	P, B, F	Hydrolyse terminal nonreducing β-D-glycosyl residues, releasing β-D-glucose	[97]
Hemicellulose-acting enzymes			
Xyloglucan specific endo-β-1,4-glucanases (EC 3.2.1151)	B, F	Hydrolyse β-1,4-D-glucosidic linkages in the xyloglucan backbone	[97]
Xyloglucan endotransglycosylases /hydrolases (EC 2.4.1207)	P	Cleaves and transfers a β-1,4-D-glucosidic linkages in the xyloglucan backbone	[99]
Endo-1,4-β-xylanases (EC 3.2.1.8)	P, B, F	Endohydrolysis of β-1,4-D-xylosidic linkages in xylans	[97,98]
Xylan β-xylosidases (EC 3.2.1.37 and 3.2.1.72)	P, B, F	Hydrolyse successive xylose residues from the nonreducing end of xylans	[97]
Glucuronoarabinoxylan endo-1,4-β-xylanases (EC 3.2.1136)	B	Hydrolyse xylosyl links in feruloylated arabinoxylans	
Acetylxlyan esterases (EC 3.1.1.72)	B, F	Hydrolyse acetyl groups from xylan	
β-Mannanases (EC 3.2.1.78)	P, B, F	Randomly cleave β-1,4-mannosidic linkages of the mannan backbone	[97,100]
β-Mannosidases (EC 3.2.1.25)	F, B	Hydrolyse terminal nonreducing β-D-mannose residues in β-D-mannosides	[100]
Pectin-acting enzymes			
Endo-polygalacturonases (EC 3.2.1.15)	P, B, F	Randomly cleave a-1,4-D-galactosiduronic linkages in pectate and other galacturonans	[97,98]
Galacturonan 1,4-α-galacturonidase (EC 3.2.1.67)	P, B, F	Hydrolyses the first glycosidic linkage from the nonreducing end of saturated homogalacturonan	
Pectin lyases (EC 4.2.2.10)	B, F	Eliminative cleavage of α-1,4-D-galacturonan methyl ester in pectic polysaccharides	[97]
Pectate lyases (EC 4.2.2.2)	P, B, F	Eliminative cleavage of α-1,4-D-galacturonan in pectate polysaccharides	[97,98]
Rhamnogalacturonan lyases (EC 4.2.2.23, and 4.2.2.24)	P, B, F	Cleave α-1,4-glycosidic bonds in the backbone of rhamnogalacturonan I	[97]
Pectinesterases (EC 3.1.1.11)	P, B, F	Catalyses the de-esterification of pectins	[98,101]
Arabinogalactan type II-degrading enzymes			
Galactan (1,3)-β-galactosidase (EC 3.2.1145)	P, B, F	Hydrolyse β-1,3 linked galactose residues at nonreducing ends	[97,102]
α-L-arabinofuranosidases (EC 3.2.1.55)	P, B, F	Hydrolyse α-L-arabinofuranose from side chains	[97,102]
Endo-β-(1,6)-galactanases (EC 3.2.1164)	F	Hydrolyse dearabinosylated β-1,6-D-galactose side chains	[102]
β-L-arabinopyranosidases (EC 3.2.1.88)	P, B, F	Hydrolyse β-L-arabinopyranose from the non-reducing end of side chains	[102]
β-glucuronidase (EC 3.2.1.31)	P, B, F	Hydrolyse 4-O-methyl glucuronic acid from the β-1,6 galactosyl side chains	[97,102,103]
Other cell wall modifying proteins			
Acetylesterases (AEs) (EC 3.1.1.6)	P, B, F	Removes acetyl groups from a variety of polysaccharides	[104]
Expansins (EXPs)	P, B, F	Nonhydrolytic proteins that cause cell wall loosening through an unknown mechanism	[105,106]

[a]Enzyme activities are described by their KEGG EC identifier.
[b]Identified in plants (P), bacteria (B), and fungi (F).

flagella/archaella, pili, fimbriae, and surface adhesins [118]. Bacterial attachment can lead to biofilm formation, which is mediated by weak, nonspecific, and reversible binding [70]. Forces include hydrophobic and electrostatic interactions between bacterial cells and root walls,

suggesting that cell wall chemistry changes could mediate biofilm formation on roots [119,120]. Different extracellular polysaccharides from both plants and bacteria are linked during biofilm formation [121]. For example, polysaccharides exuded from the root cell wall in *Arabidopsis* trigger a signalling cascade leading to biofilm formation with *Bacillus subtilis* [122]. Exopolysaccharides exuded from bacterial cell walls are often required for biofilm formation and root colonisation. *Herbaspirillum seropedicae*, however, represses exopolysaccharide synthesis in maize roots [123]. Cellular and ultrastructural microscopy combined with antibody labelling of cell walls could help define changes that sustain microorganism colonisation along roots growing at different rates.

Root hairs increase the surface area and complexity of wall chemistry, mucilage, and soil surfaces of the epidermal layer [85] (Figure 3G–H). In an extreme example, mutation of root hairs decreased the richness and evenness of the bacterial community in barley [124]. Root hairs extend by polarized tip growth from epidermal cells [125] (Figure 3G), with associated pH changes and wall-loosening enzymes such as expansins and pectate lyases [126] (Figure 4B,D). Cellulose microfibrils are short and randomly oriented at the tip and more ordered basally [127]. Enzymatic and structural changes associated with root hair walls may promote changes in the root microbiome, such as in the symbiotic case of legume infection by rhizobia [128] (Table 1). The breakdown of the cell walls at root hair tips arises from enzymes from both plant and bacteria [129]. Root hairs are sites of bacterial biofilm clusters [17], endophytic colonisation [130], and callose depositions in response to pathogens [131]. Callose on the inside of the wall can act as a physical or chemical barrier and limit cell cytoplasm-to-cytoplasm movement of proteins and effectors through plasmodesmata [132].

The cortical cells expanding under the epidermis and root hairs begin to form fungal, endophytic bacterial, and oomycete trophic associations [29]. There is a diversity of fungal colonisation structures, occurring either between the cells (apoplastically) or intracellularly. ‘Fungal pegs’ are a common intracellular protrusion where turgor pressure from melanin-filled appressorium builds up, forcing hyphae into the plant cell [133](Figures 1F and 4F) while releasing cell wall-loosening enzymes, including peroxins (PEX proteins) [134] (Table 1). Arbuscular mycorrhizae also form intracellular structures (Figure 1C,D), where arbuscules are surrounded by the periarbuscular space, forming close connections with the plant’s endoplasmic reticulum and cytoplasm [135]. The cell wall structure of fungi is also modified during colonisation, including secretion of fungal cell wall-binding proteins, which protect from mannose-binding antifungal plant proteins, or hydrophobins, which can play a role in surface attachment [72].

Lateral root emergence [136] expands the parent root surface with young lateral root cap cells, mucilage, epidermal cells, and root hairs (Figure 3J–I). Lateral root development involves enzymes, including xyloglucan-transglycosylases [137] and pectin methylesterases (Figure 4B,D), as well as nonenzymatically functioning expansins [138] (Figure 4B, Table 1). Mutations in cell wall-modifying enzymes can alter the time at which a lateral root emerges into a position in the soil [139]. Notably, root-colonising bacteria also shorten the time that lateral roots emerge in a position in soil relative to the position of the tip of the parent root, depending on plant and microorganism genotype, as well as nutrition and temperature [140,141]. Where the lateral root is emerging is frequently an entry point for bacteria [142]. We speculate that root-associated microorganisms may alter the abundance and activity of wall-loosening enzymes and other proteins associated with lateral root development.

Roots older than weeks and months shed the cortex and the epidermis (Figure 3M–O) ([143]; recently reviewed in [144,145]), potentially profoundly altering the root microbiome. Cortical cell

colonisers such as endophytic fungi and bacteria, and bacterial biofilms on the epidermis, detach from the root and the rest of the plant. The outer cell layers of the stele, such as the endodermis or pericycle, become the outer layer of the root [143]. Endodermal wall deposits can influence plant immunity. In *Arabidopsis*, abscisic acid-mediated enhancement of suberin deposition restricted infection by *Verticillium longisporum*, a fungal root-vascular pathogen [146]. Further inside the root stele, xylem vessels have secondary cell wall depositions of lignin and other phenylpropanoid compounds that hinder the entry of some pathogens into the vascular system. In *Arabidopsis*, lignin-based barriers have been observed to spatially restrict the spread of the motile bacterial pathogen *Pseudomonas syringae*. Similiary, in jujube trees (*Zizphus jujuba*), lignin depositions increased and p-Coumaric acid decreased in the presence of *Candidatus Phytoplasma ziziphi* [147]. The fungus *V. longisporum* is an exception, as it modifies non-xylem cells into functional xylem elements [148], presumably to maintain plant viability and the ability to host the fungus. The increased thickness, lignin content, and suberin content of the mature endodermis and the underlying xylem and phloem vessel walls may slow microorganisms from entering the vasculature because of the increased energy required to break down thicker walls with stronger chemical bonding than primary cell walls (Figure 2A,B). The process of detachment is thought to be mediated by the plant through programmed cell death [149,150]. Interestingly, the timing of death of epithelial cells of the gut can be altered by microorganisms [151]. An area for future study is the role of microorganisms in cortical cell shedding, and mechanisms in animal microbiomes may provide insight into plant cell death in the root microbiome.

Enzymes and cell wall modifications during root microbiome interactions

Plant–microbe interactions involve a complex, bidirectional exchange of molecular signals that determines how the relationship develops. This communication is inherently reciprocal: microbial signals influence plant responses, while plants regulate microbial activity through both local and systemic signalling pathways. A coordinated dialogue is particularly important in beneficial associations. Enzymatic and nonenzymatic proteins produced by both plants and microbes actively modify cell walls during root microbiome interactions [50,152] (Table 1; Figure 4) with many activities shared between them. Plant cell wall-modifying enzymes include cellulases, 1,4-β-cellobiosidases, and β-glucosidases, which act on cellulose, targeting the (1→4)-β-D-glucosidic linkages to create smaller subunits (cellobiose and cellodextrin), which are then hydrolysed to release β-D-glucose (Figure 4B). Cellobiose and cellodextrin can also elicit defence-like responses in plants [153]. Xyloglucan endotransglucosylases hydrolyse the xyloglucan backbone, either to release xyloglucan or form new xyloglucan–xyloglucan links [154,155] (Figure 4C). Pectinesterases and their respective inhibitors regulate the degree of pectic homogalacturonan methylation during development [156] (Figure 4D). Demethylated homogalacturonan is more susceptible to the action of pectolytic enzymes (endo-polygalacturonases and pectin lyases), which cleave (1→4)-α-D-galacturonan methyl ester and (1→4)-α-D-galactosiduronic linkages to reduce the polymer size (Figure 4D). A suite of enzymes (β-glucuronidase, endo-β-(1,3), β-(1,4) and β-(1,6)-galactanases, β-(1,3/1,6) galactanases, α-L-arabinofuranosidases, and β-L-arabinopyranosidases) are responsible for the hydrolysis of arabinogalactan type II of AGPs (Figure 4G [102]). Acetylesterases modulate the degree of *O*-acetylation in the wall by removing acetyl groups from a variety of polysaccharides [157]. Lastly, expansins are nonhydrolytic proteins that cause cell wall loosening through an unknown mechanism [158] (Figure 4B, Table 1). The activity of expansins is frequently enhanced upon contact with microbes [159]. An expansin gene is among the most upregulated in *Medicago truncatula* mycorrhizal roots, and similar expansin/expansin-like genes are also upregulated in tomato roots [160,161].

Notably, many classes of plant cell wall-modifying enzymes are also present in the root microbiome (Table 1). Endo-1,4-β-xylanases are highly abundant in plant-pathogenic fungi

[162]. Beneficial and pathogenic fungi and bacteria can produce hydrolytic enzymes for complete depolymerisation of AGPs that plants secrete and accumulate at infection sites [102]. Often, host preference and colonisation strategy are reflected in the enzymatic activity found in the microorganism, as shown in a large-scale screening of plant-pathogenic fungi [163]. A dynamic interplay between host and coloniser enzyme activities can occur. Pectin methylesterases from external microorganisms can demethylate plant pectin to promote the action of the microorganism's polygalacturonases and pectin lyases during colonisation of the host cell [96] (Figure 4D). Plants can, in turn, utilise polygalacturonase-inhibiting proteins to reduce the hydrolytic activity of polygalacturonases, accumulate DAMPs, and elicit defence responses [164]. Plants also have pathogenesis-related proteins that target microorganism walls, including chitinases and glucanases that attack the fungal cell wall [165]. A future area for research is the extent to which microorganism walls are modified in nonpathogenic interactions.

Emerging studies on the wall interfaces of the root microbiome

Enzymatic interplay

Upon reviewing plant root and microorganism cell walls in the root microbiome, we propose that they connect and interact to form what we call an interface wall in the root microbiome (Figures 1, 3). It is unclear if cell wall changes at root–microorganism points of attachment are laid down in a complex mixture, such as that seen in the case of nematode infection [166]. Enzymatic and nonenzymatic proteins causing modifications to cell walls are excellent starting points for experimentally testing the complexity of the root microbiome interface wall (Figure 4; Table 1). Microbiome wall proteins can be manipulated using genetic transformation and tested in fabricated ecosystems **(EcoFABs)** with individual organism strains or designed synthetic microbial communities **(SynCOMS)** [167]. Live imaging of tagged proteins [23] can help reveal the biochemistry, structure, and function of the plant and microorganism walls that interact to form a new interface, including biofilms and mucilage. These experiments will be challenging on roots with multiple cell layers in the cortex and, currently, limited numbers of probes. Experiments require a space–time framework (Figure 3).

Biophysics

The biophysical principles developed for analyses of **fluid flows** in and around plant root cell walls are based on studies conducted without control of microorganisms. These can be revisited as a mixture of plant- and microorganism-derived molecules using live **microfluidic devices** and biophysical models, commonly used for the study of chemotaxis [168], biofilm growth [169], and quorum sensing [170]. Emerging applications enable simultaneous visualisation of growing plants and the root microbiome. For example, real-time imaging of *Arabidopsis* and *B. subtilis* reveals spatial distributions of bacteria [171], and imaging can be conducted from seconds to weeks in humidity-controlled devices [172]. Images can be used to identify biophysical processes using mathematical modelling [173] and harnessing tools such as TrackMate [174] or STrack [175] in ImageJ for semiautomated quantification of plant organelle and microorganism movements. In medical science specifically, brain tumour detection harnessing deep learning and artificial intelligence outperformed existing identification strategies, hinting at possible applications in plant sciences [176]. The new combined use of microfluidics and mathematical analysis enables the systematic investigation of different root–microorganism interfaces, the effects of fluid flows and wet–dry cycles in microbial recruitment, and the quantification of microbial behaviour for different plant cell wall properties.

Different domains of microorganisms

Few studies apply more than one microorganism type or strain to plant roots. Larger communities of microbes can exhibit highly complex chemical interactions. There is little data to predict mechanisms for behaviour that influence the root microbiome and the plant.

Outstanding questions

How does the cell wall composition of both microorganisms and plants influence the spatially varying and dynamic interactions between roots and the microbiome?

How does microorganism localisation differ between microbial species and among diverse plant species with distinct cell walls, and how is this related to surface chemistry and microbial behaviour?

Are cell wall-derived signals involved in a soil–root–shoot circuit signalling module, and what is the influence of microorganisms?

Which plant or microbial cell wall-related genes impact microbiota establishment on the root?

How does the physical behaviour of microorganisms—e.g., motility and chemotaxis—in structured soils with complex chemical landscapes shape the interactions with plant cell walls?

What is the influence of multikingdom microbial interactions on plant traits?

Can we tailor root cell walls to improve root microbiome interactions for better plant growth? Due to more extreme abiotic conditions, plant cell wall adaptations that attract beneficial microorganisms could be a key to future resilience.

There are broad implications for the practical functioning of the root microbiome for agriculture. For example, it is known that symbiotrophic fungi help plants acquire nutrients [177], yet there are still few studies that investigate how these relationships change in the presence of protists, bacteria, or archaea. Similarly, when metabarcoding techniques are used, these studies often only focus on bacteria, fungi, or a combination of both, rarely considering additional microorganisms (e.g., fungi, bacteria, archaea, and protists; see Outstanding questions). However, such studies could be helpful for interpreting which kingdoms of microorganisms most drive processes such as carbon cycling from cell walls. Creating a defined community root microbiome, where interactions are observed using a reductionist method at different spatial scales and times, could be one approach. In doing so, the ability to identify and visualise various members of the microbial community in real time will be essential for deciphering the complex interactions within the root microbiome and the broader rhizosphere to develop a better understanding in view of practical applications in agriculture and industry.

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Declaration of interests

The authors declare no competing interests.

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