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Root Growth and Development in "Real Life": Advances and Challenges in Studying Root–Environment Interactions

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Abstract

Plant roots play myriad roles that include foraging for resources in complex soil environments. Within this highly dynamic soil environment roots must sense, interact with, and acclimate to factors such as water availability, microbiota, and heterogeneous distribution of nutrients. To aid their acclimation, roots alter their growth and development to optimize their architecture and actively regulate the physical, chemical, and biological properties of their rhizosphere. Understanding the complex interactions between roots and rhizosphere is critical for designing future crops with improved root traits better adapted to diverse and challenging soil conditions. However, studying roots and their interactions with soil under real-world conditions presents significant challenges. Addressing these challenges demands developing realistic laboratory-based model systems and innovative field-based root imaging techniques. Our review surveys the current knowledge and recent advances in understanding root–environment interactions while proposing future solutions to study roots under more "real-life" soil conditions.

Contents

INTRODUCTION

Roots are fundamental to the success of modern terrestrial plants. The earliest land plants, colonizing dry land ~450 million years ago, initially relied on simple root hairlike structures termed rhizoids for limited anchorage and fungal symbionts to enhance resource capture (31). Plants later developed root systems ~400 million years ago to meet the challenges of terrestrial life. For example, the evolution of true roots and the greater anchorage they provided allowed for greatly increased plant sizes. Early vascular plants such as lycophytes shaped their root architecture by undergoing dichotomous branching, which involves splitting the root tip into two new meristems. Over time, root branching shifted away from apical meristem to root elongation zone. Vascular plants initiated priming lateral root (LR) stem cells in the basal meristem at the boundary with the elongation zone (89). This shift has contributed to higher branching densities, environmental responsiveness, and plasticity.

The survival and success of plant species hinges on their ability to sense and acclimate to environmental signals and stresses. Roots are particularly adept at adapting to heterogeneous conditions in their soil environments, as demonstrated by the remarkable levels of plasticity in root architecture and anatomy. For example, roots branch in response to soil moisture availability to optimize water foraging (83). Similarly, roots form suberized and lignified barriers to prevent the diffusion of root oxygen into hypoxic soil (33). These interactions between plant roots and their surroundings, or rhizosphere, reciprocally lead to changes in their soil environment, through modifying their physical, chemical, and biological properties. For example, the secretion of root exudates alters the hydraulic properties of soil, thereby influencing water uptake (16). Such complex interactions and feedback highlight the importance of studying root–environment interactions to fully understand plant life and the ecosystems they support.

Studying roots noninvasively in soil is often challenging. Many classical and advanced imaging techniques, such as rhizotrons, X-ray computed tomography (CT), and magnetic resonance imaging (MRI), provide scientists with the means to observe root growth and interactions within a soil environment (as discussed in the section titled Challenges and Solutions to Studying

Rhizoid: a root hairlike structure emanating from nonvascular plants that helps anchor plants and absorbs moisture from soil

Rhizotron: a 2D root architecture phenotyping chamber that allows monitoring of root architecture responses visible on the surface of a thin soil layer

X-ray computed tomography (CT): X-ray–based phenotyping system that enables noninvasive imaging of 3D and 4D root–soil interactions at up to a submicron-scale resolution

Roots Under Real-World Conditions and summarized in **Table 1**) (88, 106, 123). Nevertheless, developing highly innovative and disruptive research methodologies and technologies is essential for deeper understanding of root–environment interactions, particularly in natural ecosystems and field-structured soil settings. Our review addresses root growth and development in "real life," discussing recent advances and challenges in studying root–soil environment interactions.

Magnetic resonance imaging (MRI): noninvasive root imaging technology that visualizes 3D and 4D images of roots and transport of abundant proton-containing molecules like water

Rhizodeposit: an extracellular compound released by roots into soil

Mycorrhizosphere: zone of interaction among mycorrhizal fungi, roots, and soil

DYNAMIC INTERACTIONS AT THE ROOT–SOIL INTERFACE

The Rhizosphere: A Matter of Exchange

The rhizosphere, which describes the volume of soil and associated organisms influenced by the activity of living roots, is shaped by the interactions between plant physiological and soil physical, chemical, and biological processes (69, 90, 136, 142) (**Figure 1*a*,*b***). Despite the pivotal role of the rhizosphere for the functioning of terrestrial ecosystems (69, 90), and thus agricultural productivity (139, 149), the definition of the boundaries of the rhizosphere varies widely (142). This is due to the spatial impact of different plant physiological processes such as root growth, rhizodeposition, or water and nutrient uptake on rhizosphere formation and dynamics (136, 142). For example, distinct spatial concentration gradients of plant nutrients have been reported around roots (75). Similarly, spatial concentration and activity gradients differ between root exudates and exoenzymes, respectively (69), while contrasting water uptake rates can also exist between root classes (1).

The rhizosphere always includes roots that either (*a*) add matter to the soil in the form of root biomass, various rhizodeposits (e.g., mucilage, sugars, organic and amino acids, H^+, exoenzymes), and metabolic byproducts such as CO_2 or (*b*) extract matter, predominately water and plant nutrients, from the soil (90, 142) (**Figure 1*b*,*c***). Hence, the size and shape of the rhizosphere is essentially determined by this dynamic exchange between plants and soil. It should be noted that carbon, nutrient, and water transfer through hyphal networks of mycorrhizal fungi can substantially increase the volume of soil that is indirectly affected by root activity, termed the mycorrhizosphere (139, 142).

Soil Structure Development Is Key to Rhizosphere Formation and Processes

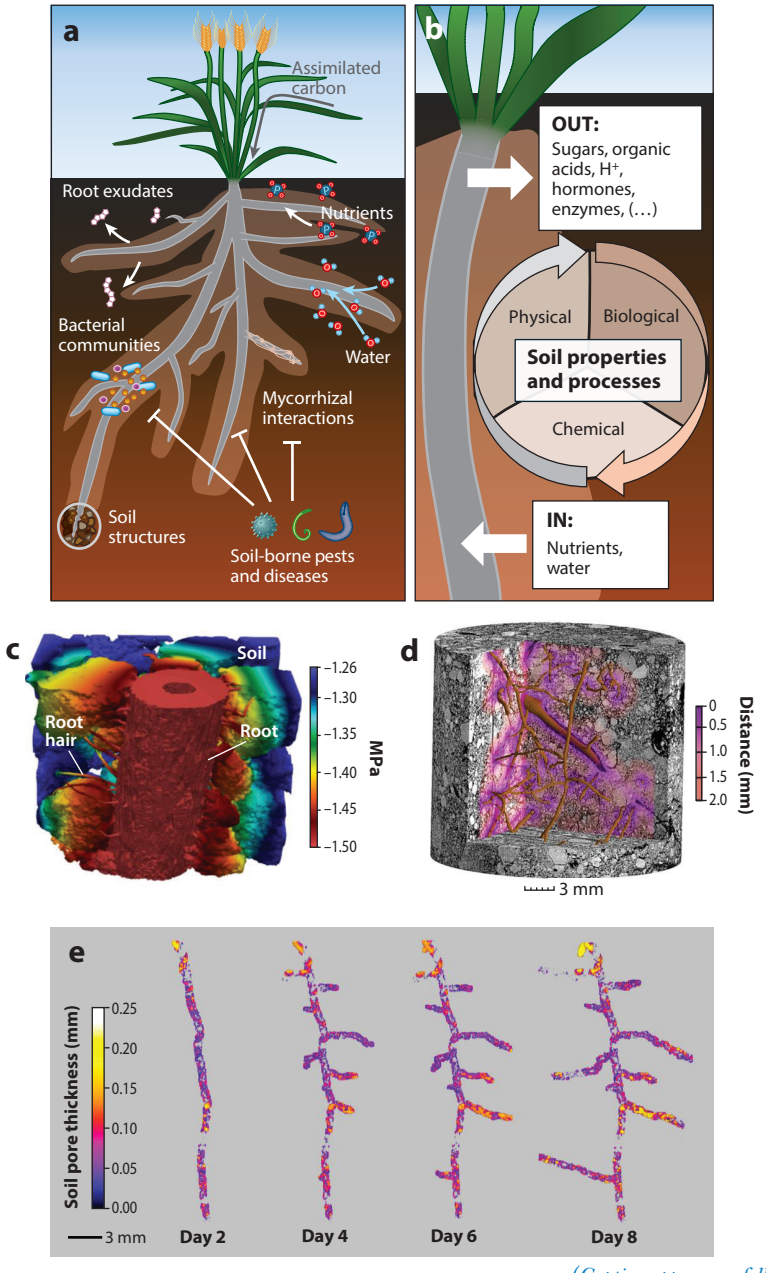
Rhizosphere formation is initiated by the effects of living roots on soil structure, i.e., the spatial arrangement of solids and pores (90) (**Figure 1*b***). Depending on the architecture of the preexisting pore system, the displacement of soil particles by growing roots can increase or decrease soil porosity in the vicinity of roots (77, 133) (**Figure 1*d***). Subsequently, the pore system adjacent to roots can be further modified by root hairs (67). Moreover, root hairs entangle soil particles (90) and increase the root surface area and thus rhizodeposition (69), which support soil aggregation (90). Studies show that root water uptake is substantially influenced by the increased root–soil contact facilitated by root hairs (**Figure 1*c***). However, soil drying causes shrinkage of root hairs, reducing their effectiveness in water uptake (32). Root water uptake leads to localized soil drying, which in many soils results in soil shrinkage and subsequent crack formation (22). These changes in soil structure alter gas and water transport characteristics in the vicinity of roots, which directly impacts soil exploration and resource acquisition. For example, low soil porosity impedes ethylene, O_2, and CO_2 exchange between roots, the rhizosphere, and bulk soil, leading to decreased root growth (90, 98). Conversely, greater soil density around roots helps to maintain hydraulic conductivity and thus water uptake in drying soil (2).

Structural features of the rhizosphere such as porosity gradients have been linked to the emergence of increased chemical interactions and biological activity around living roots (48, 133)

Table 1 Examples of phenomics techniques for plant root imaging in field and laboratory scenarios

Technique	Visualization	Scale (m)	Time/throughput (per day)	Study setting	Roots	Soil	Process/function	Example/ reference(s)
X-ray computed tomography (CT)	Noninvasive	Root architecture (10^{-3}–10^{1})	10 samples/day	Laboratory	Yes	Yes	Structural	74, 123, 137
Magnetic resonance imaging (MRI)	Noninvasive	Root architecture (10^{-3}–10^{-1})	10 samples/day	Laboratory	Yes	No	Water	85
Positron emission tomography (PET)	Noninvasive	Root architecture (10^{-3}–10^{-1})	10 samples/day	Laboratory	Yes	No	C/N isotope tracing	86, 145
Rhizotrons	Noninvasive	Root architecture/ single root ($\sim10^{-3}$–10^{-1})	900 rhizotrons	Greenhouse/CE (controlled environment)	Yes	Yes	RSA (root system architecture), shoots via images	91
Neutron computed tomography (CT)/radiology	Noninvasive	RSA/water (1×10^{-3})	10 s/tomogram	Laboratory	Yes	No	D_2O imaging	47, 128
Rhizotubes	Noninvasive	Multiple roots ($\sim10^{-3}$–10^{-2})	Single timepoint sampling	Field	Yes	No	Root images	57
Shovelomics	Destructive	Root architecture ($\sim10^{-2}$–10^{-1})	10 min/plant	Field	Yes	No	Root crown architecture	135
Electrical resistivity tomography (ERT)	Noninvasive	Root biomass/water ($\sim10^{-1}$–10^{2})	Continuous recoding at 2 s/sample	Field	Yes	Yes	Water	105
Electrical impedance tomography (EIT)	Noninvasive	Root biomass ($\sim10^{-1}$–10^{2})	~10 pots	Greenhouse/CE	Yes	Yes	Conductivity mapping	24
Thermoacoustic tomography (TT)	Noninvasive	Biomass (1×10^{-2})	Continuous imaging	Pots/field	Yes	No	Ultrasound maps from microwave illumination	120
Electrical current source density (ECSD)	Noninvasive	Water/biomass (5×10^{-2})	Single plant chamber	Rhizotrons	Yes	Yes	Current density map	101
Laser ablation tomography (LAT)	Destructive	Tissue-scale (micron-level)	~250 samples/day	Laboratory	Yes	No	Laser ablation	116, 122

(**Figure 1***e*). Plants release myriad organic compounds into soil in the form of mucilage, sloughed off root cap cells, and exudates, which fuel the activity of soil microorganisms in the rhizosphere and thus turnover of soil organic matter (SOM) (69, 139). Depending on the composition of SOM, e.g., stoichiometric ratios between carbon and plant nutrients, microbial activity can lead to mobilization as well as immobilization of plant nutrients (69, 102). Exudation of exoenzymes further contributes to SOM breakdown (69), while rhizosphere acidification via release of H^+ and organic



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Dynamic interactions at the root–soil interface. (*a*) Illustration highlighting key processes involved in the formation of the rhizosphere, i.e., the volume of soil and associated organisms affected by living roots (*light brown*, immediately surrounding the roots). Soil structural changes caused by the activity of living roots (e.g., growth, exudation, resource uptake) initiate rhizosphere formation, leading to the emergence of spatial gradients in soil physical, chemical, and biological properties and processes around living roots. In turn, these heterogeneities characterizing the rhizosphere shape the overall environment of the roots, leading to feedback with root growth and architecture. (*b*) Roots modify the physical, biological, and chemical properties of the rhizosphere by releasing rhizodeposits (e.g., mucilage, sugars, organic acids, ions, hormones, and exoenzymes) and extracting resources such as nutrients and water from soil. (*c*) Distribution of resources in the rhizosphere, such as water, is highly heterogeneous, and soil stresses, such as soil drying, exacerbate this variability. Root structures, such as root hairs, enhance root–soil contact and influence the uptake of unevenly distributed water resources. Synchrotron-based X-ray computed microtomography and image-based modeling demonstrate the gradients in water potential within the root–soil continuum under dry soil conditions. The color scale represents gradients in soil matric potential (MPa). Panel *c* adapted from Reference 32 (CC BY 4.0). (*d–e*) Growing roots alter soil structural properties, such as porosity, in the vicinity of roots, depending on the architecture of the preexisting pore system. For instance, (*d*) Lucas et al. (77) performed 3D visualization of root–soil contact, with a distance map (*gradient color*) showing the distance from every pixel to the nearest biopore. The study revealed that roots compact the rhizosphere when the soil structure does not have enough well-connected large pores. Panel *d* adapted from Reference 77 (CC BY 4.0). Similarly, (*e*) high-resolution X-ray computed tomography images reveal how plant roots impact the structural development of the rhizosphere by causing changes in soil pore thickness over time. Panel *e* adapted from Reference 48 (CC BY 4.0).

acids alters solubility of nutrients bound to soil particles (133, 149). Moreover, mucilage allows greater water retention in the rhizosphere and the formation of liquid bridges connecting soil particles, which are critical to water transport in the rhizosphere and plant water uptake in dry conditions (15).

Living and Dead Roots Shape Spatiotemporal Heterogeneities That Define Soil Environments

Root activities including root growth and respiration, rhizodeposition, as well as water and nutrient uptake lead to the development of distinct spatial gradients of rhizosphere processes and properties (136). For example, root exudate concentration and microbial and enzyme activity decrease with the distance from the root surface, while the opposite pattern occurs for plant nutrient concentration and microbial diversity (69, 136). Certain gradients emerge almost immediately (e.g., soil porosity), while others can take several days (e.g., nutrient and enzyme concentrations) to weeks and even months (e.g., microbial community structure) (69). Mounting evidence suggests these spatiotemporal gradients are the foundation for a functioning rhizosphere (136, 149) and that root acclimation and adaptation to rhizosphere heterogeneity are key to plant growth (23, 139).

Detritusphere: volume of soil affected by dead and decaying plant residues that impact soil structure dynamics and microbial processes

While the rhizosphere needs living roots by definition (69, 90, 136, 142), the effects and ultimately the legacy of plant physiological processes on the soil environment are not constrained to living root tissues. Dead root tissue constitutes a major input of the detritusphere—i.e., the volume of soil affected by dead root tissue, a hotspot for biological activity, organic matter turnover and nutrient cycling, and soil structure dynamics (90). Moreover, macropores formed by roots improve water infiltration and can be exploited by subsequent plants as preferential growth paths to gain access to water stored in deeper soil layers (141). Thus, short- and long-term effects of the complex interactions at the root–soil interface collectively shape the heterogeneous yet organized environment of plant roots and thereby crop yield.

ROOT ADAPTATIONS TO ENVIRONMENTAL SIGNALS AND STRESSES

Plant roots are able to sense and acclimate to a diverse range of soil structures and environmental signals and stresses, as demonstrated by the remarkable levels of plasticity in their architecture (**Figure 2**). The following section reviews a selection of these environmental signals and the chemical, biological, and physical stresses including water, nutrients, oxygen, microbiome, and mechanical impedance. For details on other root environmental stresses such as salt, pH, or heavy metals, we recommend several excellent reviews (50, 59, 132).

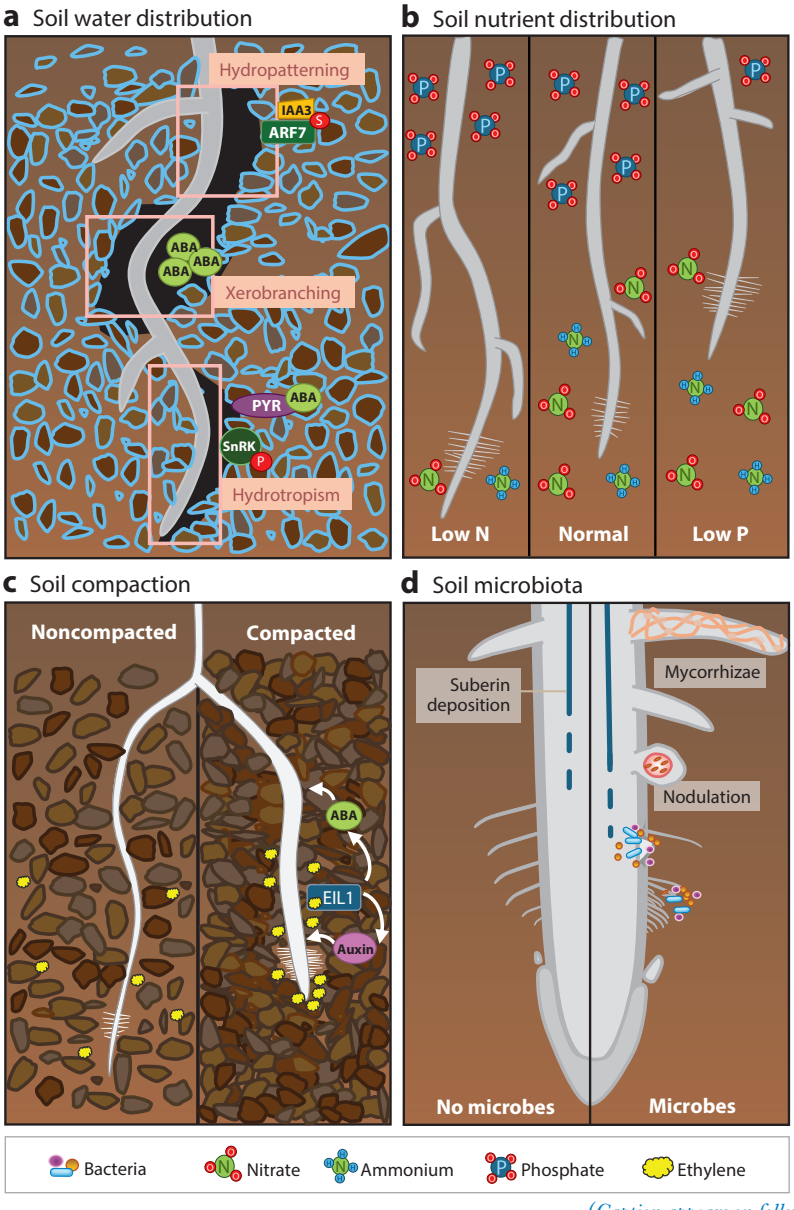


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Figure 2 (*Figure appears on preceding page*)

Root adaptations in response to soil environmental signals. (*a*) Schematic illustration of mechanisms of root adaptations to heterogeneous soil water availability. Roots show hydropatterning to position their lateral roots toward the side in contact with soil water (*blue*) (96). When roots temporarily lose contact with water in soil pore spaces (*black*), they exhibit xerobranching to pause lateral root formation. Root tips navigate toward areas with high water potential by directing their tip growth via hydrotropism. Blue boundaries surrounding soil particles indicate areas with available water. (*b*) Roots employ adaptive strategies to cope with nutrient limitations in soil. Under low nitrogen availability, roots enhance lateral root formation in deeper soil layers to access nitrogen sources that may be more concentrated at depth. Conversely, in phosphorus-deficient soils, roots enhance lateral root growth in the topsoil where phosphate is more abundant. Additionally, roots elongate their root hairs to expand their surface area, thereby increasing their capacity for nutrient uptake. (*c*) Soil physical properties such as soil bulk density influence root growth. Higher bulk density or compacted soil conditions restrict the diffusion of ethylene, resulting in inhibited root growth and triggering a thickening response in roots (73, 97). (*d*) Roots establish symbiotic associations with mycorrhizal fungi and nitrogen-fixing bacteria, significantly enhancing a plant's ability to obtain essential nutrients. Root-inhabiting microbiota also regulate the plasticity of root branching and deposition of the endodermal diffusion barriers such as the Casparian strip and suberin lamellae, crucial for nutrient homeostasis in plant. Abbreviations: ABA, abscisic acid; ARF7, AUXIN RESPONSE FACTOR 7; IAA3, INDOLE-3-ACETIC ACID INDUCIBLE 3; P, phosphate; S, SUMO protein.

Divining for Water in Soil

Plants continuously seek out water through their root systems. Reduced access to soil water due to poor rooting has been identified as a key factor contributing to lower crop yield improvements in recent decades (140). For wheat, it is estimated that each additional millimeter of water extracted by deep-growing roots can increase yield by up to 55 kg per hectare, highlighting the importance of water availability (81). The availability of water to roots is influenced by the heterogeneous distribution of moisture in soil. Factors such as soil type, depth, variability in precipitation, and availability of irrigation impact how water is distributed. Additionally, root distribution in soil and the dynamics of root water uptake further contribute to this variability. To adapt to these heterogeneous soil conditions, roots perceive gradients of soil moisture availability and employ various acclimative responses to optimize water uptake (**Figure 2*a***).

Hydrotropism. Roots can modify their direction of growth toward zones with a higher water potential using hydrotropism (29) (**Figure 2*a***). This tropic response has served as a model for water-driven root adaptive responses since the classical studies of Darwin and Sachs (26, 111). However, designing experimental setups that closely replicate natural soil moisture distribution patterns has proved challenging. Typically, researchers resort to using high concentrations of osmolytes, which induce osmotic shock and fail to mimic the gradual water stress roots experience under field conditions (43). The widely used split-agar-based method illustrates this approach, where 1% agar with or without 400 mM sorbitol are poured side by side. Seedling root tips are positioned close to the agar–sorbitol interface. Takahashi and coauthors (125) demonstrated that this system can establish a water potential gradient ranging from −0.2 MPa (plain agar) to −1.0 MPa (400 mM sorbitol) across the agar–sorbitol front. At the agar–sorbitol interface, the water potential gradient was measured at −0.33 MPa. Interestingly, these water potential values closely resemble the varying water potentials of soils used to study plant water stress responses, such as Ψsoil −0.1 MPa (well-watered), Ψsoil −0.8 MPa (moderate stress), and Ψsoil −1.4 MPa (severe water stress conditions). However, it is important to note that the water potential gradient in the split-agar-based method diminishes gradually over 24 h due to diffusion. Nevertheless, the split-agar-based method has proven instrumental in uncovering key mechanisms underlying hydrotropism.

Hydrotropism: acclimative plant response involving roots growing toward volumes of soil with higher water potential

The split-agar-based system helped identify that abscisic acid (ABA) is a key signal controlling hydrotropism. Mutants defective in ABA biosynthesis such as *aba1-1* (*ABA-deficient 1*), or in ABA signaling like *abi2-1* (*ABA-insensitive 2*) (see **Supplemental Table 1**), exhibit reduced hydrotropic bending (125). Cell type–specific complementation assays of the ABA signaling mutant *snrk2.2/2.3* (*sucrose nonfermenting 1-related protein kinase 2.2/2.3*) (**Supplemental Table 1**) and mathematical modeling revealed that asymmetric ABA response in the elongation zones of hydrostimulated roots leads to enhanced cortical cell expansion on the convex side of roots facing low water potential (30). Nevertheless, it remains unclear whether this asymmetric ABA response is preceded by a moisture-driven lateral ABA gradient in the elongation zone.

Supplemental Material >

Xerotropism: acclimative plant response triggered by topsoil drying, causing roots to grow at a steeper downward angle to access water in deeper soil layers

The asymmetric distribution of other signals such as Ca^{2+} have also been shown to regulate hydrotropism (119). Like the ABA response, Ca^{2+} becomes asymmetrically distributed in the elongation zone with a stronger accumulation on the convex side of the root. Treatments with a calcium chelator (BAPTA-AM) inhibited hydrotropism, whereas treatment with the Ca^{2+} ionophore (Br-A23187) enhanced hydrotropic response (119). The important regulator of root hydrotropism, *MIZ1* [*MIZU-KUSSEI 1*; meaning hydro (Mizu, 水) tropism (Kussei, 屈性) in Japanese; 66], regulates levels of cytosolic Ca^{2+} upon hydrostimulation. *MIZ1* encodes a protein of unknown function localized on the surface of the endoplasmic reticulum (ER), where it inhibits the ER-localized calcium-ATPase pump (ECA1). Inhibition of ECA1 enhances cytosolic Ca^{2+} concentration, which is critical for root hydrotropic bending. Mutations in *miz1* lead to impaired Ca^{2+} accumulation, specifically in columella cells (119). These findings are intriguing, as MIZ1 primarily functions in the root cortex, prompting the question of how root cap calcium dynamics are linked to MIZ1 activity. Contrary to these findings, Dietrich et al. (30) reported that the elongation zone, and specifically the cortex layer, acts as the primary site of hydrotropic perception. Laser ablation or microdissection of the entire *Arabidopsis* root cap disrupts gravitropism but does not inhibit hydrotropism. This finding suggests that different root tissues play distinct roles in perceiving hydrotropic versus gravitropic stimuli.

Xerotropism. When topsoil dries out, roots often respond by growing deeper and steeper in search of water. This phenomenon, where roots exhibit enhanced gravitropism in response to a water deficit, is referred to as xerotropism (34). Xerotropism involves LRs changing their growth direction from shallow to steeper angles. Interestingly, this tropic response is independent of hydrotropism, since mutants like *miz1* still exhibit xerotropism. Instead, auxin has been implicated since auxin receptor mutants such as *tir1* (*transporter inhibitor response 1*) fail to reorient LRs in response to water deficit (105). In rice, the expression of auxin-regulated *DRO1* (*deeper rooting 1*) has been associated with steeper root growth. *DRO1* is negatively regulated by auxin on the lower side of gravistimulated roots, leading to reduced cell elongation compared to the upper side. This asymmetric growth results in root curvature toward gravity (131). *DRO1* alleles improve drought tolerance in rice by promoting deeper rooting. Recent studies in maize identified ABA response factor binding elements in the promoter of *ZmDRO1* (*Zea mays deeper rooting 1*) required for ABA induction. Expression of *ZmDRO1* using a synthetic ABA-inducible promoter also conferred increased yield under water-deficit conditions, further emphasizing the role of ABA in shaping root architecture in response to soil water availability (35).

Since the discovery of *OsDRO1* in rice, three homologs have been identified in *Arabidopsis: AtDRO1/AtLAZY4*, *AtDRO2/AtLAZY3*, and *AtDRO3/AtLAZY2* (45). Similar to rice, mutants of *AtDRO1/AtLAZY4* exhibit LRs with wider root angle (45). The cellular mechanism of LAZY protein action on gravitropism was recently elucidated by several groups. Studies have shown that LAZY proteins rapidly change their polarity in response to gravistimulation. Gravistimulation triggers phosphorylation of LAZY proteins, enhancing their interaction with amyloplast

surfaces in columella cells. Sedimentation of amyloplasts causes AtLAZY proteins to relocate from the amyloplast surface to the adjacent plasma membrane, initiating signaling pathways that drive asymmetric auxin distribution and differential root growth along the gravity vector (18, 93). Despite significant efforts to understand the role of *DRO1*-like genes in *Arabidopsis*, it remains uncertain whether these proposed mechanisms are universally conserved across crop species, particularly in regulating root growth angle under xerotropism.

Xerobranching: acclimative response where lateral root initiation is suppressed when root tips temporarily lose contact with moisture (e.g., in soil air gaps)

Hydropatterning: acclimative response where roots exposed to an asymmetric water source (e.g., in soil macropore) preferentially branch toward high water availability

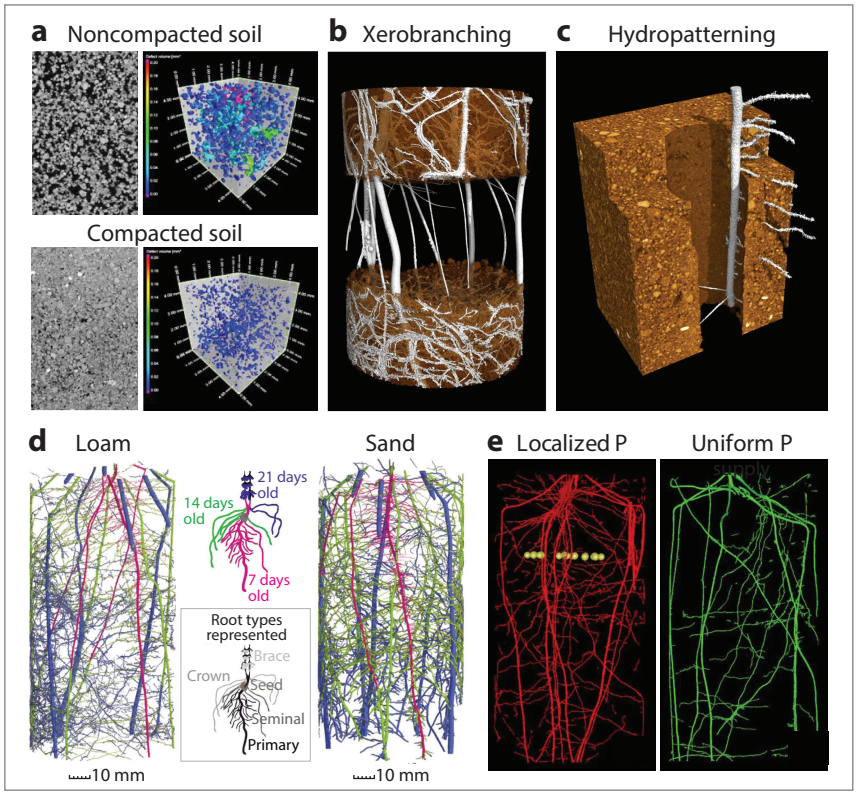
Xerobranching. Apart from promoting deeper and steeper root growth to enhance water foraging during drought conditions, ABA also regulates LR plasticity in response to microscale differences in soil moisture. For instance, when growing root tips temporarily lose contact with water in soil pore spaces, ABA suppresses root branching until roots reconnect with moist soil. This suppression of root branching in dry soil pore spaces is termed xerobranching (84, 95) (**Figures 2*a*** and **3*b***). Studies on ABA-deficient maize and tomato mutants, which continue to branch in soil air gaps, validate the role of ABA in xerobranching (84). Similar results were observed in *Arabidopsis* ABA signaling and biosynthetic mutants using an agar plate–based air-gap assay, which replicates xerobranching in soil.

How do hormone signals like ABA control xerobranching? Hydrodynamic modeling revealed that when external water uptake is transiently blocked as a root tip transits an air gap, phloem-derived water is required to flow outward to sustain root growth. This reversal in water flux comobilizes ABA from its phloem companion cell source toward outer root tissues. Using a high-resolution FRET-based hormone biosensor, nlsABACUS2 (109), it was observed that ABA levels reach approximately 300 nM in outer root tissues following a xerobranching stimulus. Elevated ABA levels reversibly close plasmodesmata in these outer root tissues, which temporarily blocks radial inward symplastic auxin movement from the epidermis to the LR stem cells in the pericycle, thereby suppressing branch formation. Strikingly, this transient ABA response is attenuated as soon as the root tip reconnects with moist soil, allowing LR formation to resume. This dynamic hormone flux illustrates a hydrosignaling mechanism involving the redistribution of signals such as auxin and ABA in response to changing water availability (84). By integrating hydraulic fluxes with the redistribution of these hydrosignals (ABA and auxin), roots can adapt their branching according to soil water availability.

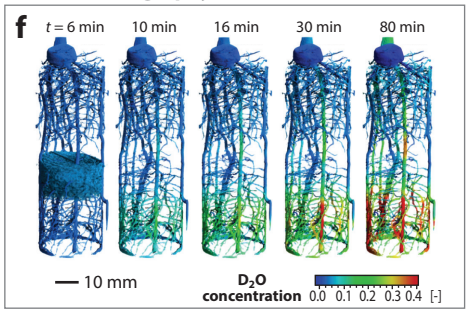
Hydropatterning. Soil water distribution also influences the radial patterning of LRs. For example, roots in contact with soil water on only one side preferentially position branches toward that side, a response known as hydropatterning (5) (**Figures 2*a*** and **3*c***). Both hydropatterning and xerobranching demonstrate how local, microscale differences in water availability continuously optimize root architecture and foraging for soil resources. Despite their superficial similarities, hydropatterning and xerobranching are regulated by distinct hormone signals. While auxin signaling regulates hydropatterning, ABA serves as the primary regulator of xerobranching (5, 84). This reveals that spatial differences in water availability activate distinct signaling mechanisms: A radial water potential gradient experienced by one side of a root triggers auxin-mediated hydropatterning, while the absence of an external water source by a root tip transiting a soil air space induces an ABA-mediated xerobranching response.

Hydropatterning requires biosynthesis and transport of auxin on the water-exposed side of the root compared to the air-exposed side, mediated by TAA1 (TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1) and PIN3 (PIN-FORMED 3) (5). This response is also regulated by the posttranslational modification of LR regulator ARF7 (AUXIN RESPONSE FACTOR 7) (96). On the air-exposed side of the root, ARF7 undergoes SUMOylation, which leads to the preferential recruitment of the auxin signaling repressor IAA3 (INDOLE-3-ACETIC ACID INDUCIBLE 3) via its SUMO interaction motif (SIM). This recruitment

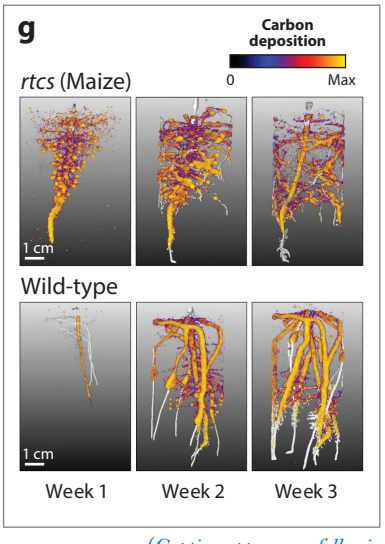
X-ray CT



Neutron tomography



MRI–PET



LAT

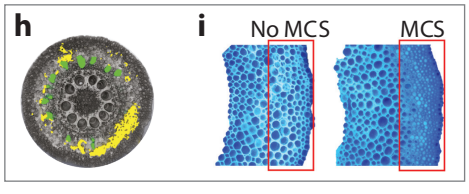


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Figure 3 (*Figure appears on preceding page*)

Phenotyping methods to visualize root–environment interactions. (*a*) X-ray computed tomography (CT) images compare noncompacted and compacted soil cores, offering 3D visualizations of air-filled soil pores (98). Soil compaction reduces soil pore volume, leading to significant alterations to soil structure. Panel *a* adapted from Reference 98. (*b*) X-ray CT image of maize roots illustrating xerobranching response, where roots suppress branching when they lose contact with soil moisture in air gaps. (*c*) X-ray CT image demonstrates hydropatterning, where maize roots grown through a macropore position lateral roots toward the contact side with high water availability. (*d*) Time-resolved X-ray CT scanning reveals the impact of varying soil textures (sand and loam) on maize root growth. Superimposed sequential CT scans at 7, 14, and 21 days show detailed temporal root development, including root diameters and root types (primary, seminal, and lateral roots). Panel *d* adapted from Reference 74 (CC BY 4.0). (*e*) X-ray CT imaging compares maize root responses to localized phosphate (P, *yellow*) versus uniform phosphate supply. Roots proliferate more in the localized phosphate patch compared to the uniform phosphate supply. Panel *e* adapted from Reference 39 with permission from SNCSC (CC BY-NC-ND 4.0). (*f*) Time-resolved neutron tomography of maize roots showing water uptake and axial transport by individual roots using deuterated water (D_2O). Panel *f* adapted from Reference 128 (CC BY 4.0). (*g*) Time-resolved magnetic resonance imaging and positron emission tomography (MRI–PET) reveal that the maize seminal rootless mutant *rtcs* produces more lateral roots and allocates more carbon to lateral root development compared to wild-type maize, which has a higher number of seminal roots. Panel *g* adapted from Reference 145 with permission from SNCSC (CC BY-NC-ND 4.0). (*h*) Laser ablation tomography (LAT) showing the colonization and distribution of arbuscular mycorrhizal fungi in the cortex of maize roots (*yellow*) and the presence of root cortical aerenchyma (*green*). Panel *h* reproduced from Reference 122 with permission from Oxford University Press. (*i*) LAT images of maize roots with and without multiseriate cortical sclerenchyma (MCS). Roots with MCS exhibit greater tensile strength, enhancing root penetration in compacted soil conditions (116). Panel *i* adapted with permission from Hannah Schneider/The Pennsylvania State University.

blocks ARF7-dependent expression of the LBD16 (LATERAL ORGAN BOUNDARIES-DOMAIN 16) transcription factor, which is required to trigger LR initiation. In contrast, on the water-exposed root side, non-SUMOylated ARF7 continues to trigger LR initiation via *LBD16*. *Arabidopsis* mutants defective in ARF7 SUMOylation disrupt hydropatterning. This example reveals the critical role of posttranslational modifications in the rapid regulation of root adaptive responses within heterogeneous soil environments.

How does a hydropatterning stimulus trigger a radial auxin gradient and activate its response machinery, leading to asymmetric root branching patterns? Like xerobranching, hydraulic fluxes are likely to play a key role during a hydropatterning response. Robbins & Dinneny (107) proposed a sense-by-growth theory, suggesting that the sensitivity of roots to radial water availability is highly influenced by their growth rates. Using computational modeling, the authors predicted that faster-growing root tips establish stronger water potential gradients at their tips, explaining their more pronounced hydropatterning response compared to slower-growing root tips. The authors proposed that water availability is perceived in a competence zone, which overlaps with the region where auxin primes LR formation. Mehra et al. (84) demonstrated that auxin in epidermal cells of the elongation zone moves radially inward via plasmodesmata to trigger LR stem cells. Hence, radial symplastic movement of water and auxin is coupled in this zone. In principle, root tips exposed to a radial water potential gradient would experience asymmetric water uptake. The comovement of water and auxin in the competence zone could provide a simple hydraulic mechanism for generating a radial auxin gradient that triggers asymmetric LR patterning. However, further studies are needed to validate how radial water potential gradients are perceived.

Both monocots and eudicots exhibit root adaptive responses such as xerobranching, suggesting a wide conservation of these water-sensing mechanisms. Interestingly, nonflowering vascular plants like ferns do not appear to exhibit xerobranching (83), which concurs with the hypothesis that ABA was recruited for water-stress adaptive roles in seed-forming plants (11). These observations suggest that seed plants may be better adapted to foraging in heterogeneous soil

environments. Further research is required to test this important question and also determine when water-adaptive root responses like hydropatterning and xerobranching emerged during land plant evolution.

Foraging for Soil Nutrients

In addition to water, nutrient availability in soil is critical for plant growth and development. Due to their chemical properties, solubility, and reactivity, most nutrients are heterogeneously distributed across soil profiles. For example, orthophosphate (HPO_4^{2-}, the readily available form of phosphorus) is highly immobile as it quickly reacts with positively charged chemical species (Al^{3+}, Fe^{3+}, and Ca^{2+}). HPO_4^{2-} accumulates in topsoil. In contrast, inorganic nitrogen (nitrate) can be mobilized with water into deeper soil profiles. Potassium distribution in soil is distinct from the other two major macronutrients, phosphorus and nitrogen. Despite its high abundance in soil, potassium bioavailability is limited (90–98% is present in crystalline-insoluble forms). However, there are two other forms of potassium: sparingly available (trapped between clay layers) and easily available (dissolved in soil water). This makes potassium distribution highly heterogeneous, depending on soil moisture and clay availability.

Given the complex spatial distribution of these key macronutrients (10), root nutrient sensing and adaptive responses are highly dynamic, plastic, and localized (**Figure 2*b***). Root adaptive responses in real soil environments are designed to improve foraging for a zone or a patch of nutrients. For example, since high phosphate concentrations accumulate in topsoil, rice roots use external phosphate levels to control root angle and thereby improve foraging for this macronutrient (55). Once close to a nutrient patch, plant roots often elongate their root hairs to increase the local surface area and enhance nutrient uptake. Roots employ distinct auxin-based regulatory pathways to promote hair elongation in response to local nitrogen or phosphorus availability (8, 58). Despite this localized adaptive root foraging behavior, most nutrient studies use agar-based, hydroponic, and/or aeroponic systems to study molecular and cellular responses. Such experimental conditions provide homogeneous nutrient availability, which masks the local responses that plant roots experience in heterogeneous soil conditions. In addition, researchers often use much higher levels of these macronutrients than those found in natural soils. For instance, phosphate levels in most arable soils are in the range 16–25 ppm, whereas researchers generally use 200–300 μM of H_2PO_4 in agar or hydroponic systems to study molecular and cellular responses. Typical nitrogen concentrations in soil solution range from 10 to 50 ppm, whereas most agar-based experiments use >160 ppm.

Soil is composed of more than just soil particles, water, nutrients, and microbes. The presence of SOM plays a key role in determining the availability of micronutrients (28). Besides SOM, soil pH and texture also regulate micronutrient availability. For example, boron is mostly locked up at pH levels above 7.0, especially in sandy soils. Another micronutrient, copper, is highly immobile in soil, and its availability is highly dependent on soil pH. Copper solubility increases approximately 100 times for each unit decrease in soil pH (82). Therefore, nutrient sensing and molecular responses in axenic systems often do not mimic real soil conditions, making it challenging to study the molecular, physiological, morphological, and cellular responses of roots. New methods and approaches are needed to study how plant roots sense and respond to localized nutrients in more realistic conditions which are closer to "real life."

Soil Compaction

In addition to water and nutrient availability, soil physical properties also profoundly influence root growth and development. For example, the degree to which root tips growing through a soil matrix are mechanically stimulated depends on soil density. The soil type, along with the range of

Bulk density (BD): dry weight of soil per unit volume of soil that reflects the mechanical impedance of the soil

particle and pore sizes, also influences water availability to roots. Hence, roots growing through soil versus along a vertical agar plate experience completely different growth environments. The former provides a complex 3D/4D growth matrix, while the latter offers a simple 2D surface. To illustrate the impact of soil structure on root growth, in this section we discuss compaction stress, which arises from changes in soil structure.

Soil compaction negatively affects soil properties, but not all soils are affected equally. A moderate degree of soil compaction is required to ensure good root–soil contact (143) and can increase soil moisture content or even crop yield (147). However, alternating and repeated wetting and drying cycles, as well as frequent passes of heavy machinery or cattle over the soil, increase its bulk density (BD, g cm^{-3}), which is an indication of the amount of compaction in specific agricultural land (41). Compaction stress is also influenced by properties such as soil type, SOM, soil porosity, or soil water content as they all impact the capacity of soil to deform (92). Compaction stress also reduces soil macropore volume and interconnectivity (**Figure 3*a***); with increasing BDs, most soil pore interconnections are severed, resulting in poor water drainage and gas diffusivity (76). Moreover, higher BDs increase the penetration resistance that roots need to overcome to grow in the hard soil. The topmost soil layer usually has the highest increase in penetration resistance due to soil compaction compared to deeper soil layers (147). However, long-term compaction stress can lead to higher subsoil compaction, an often-overlooked issue (62), particularly considering that deeper soil layers naturally present higher penetration resistance (3).

The combination of these detrimental soil structural effects disrupts the growth and development of root systems (97). Soil compaction reduces water infiltration and storage capacity (92), leading to an overall decline in water availability to roots, together with reduced nutrient uptake and poor soil aeration (118). The higher penetration resistance also imposes mechanical impedance, a physical stress that is one of the main factors limiting root growth (6). Moreover, root density and length of new roots (LRs, nodal roots) are reduced in several key crops like wheat and soybean (25).

Recent research has revealed monocot and eudicot roots employ a novel regulatory mechanism to detect and respond to soil compaction. As noted above, gas diffusivity is reduced in compacted soils. If gases diffuse much slower, volatile signals will accumulate in the compacted soil surrounding a root tip. Of particular interest is ethylene, a gaseous plant hormone that regulates a plethora of developmental and stress responses (9), which is released by root tips. In compacted soil, the reduction in gas diffusivity leads to the buildup of ethylene inside and around root tip tissues, triggering growth inhibition (9) (**Figure 2*c***). Interestingly, ethylene-insensitive *Arabidopsis* and rice mutants (e.g., *ein2*) remain able to grow through highly compacted soils and reach root lengths akin to those of wild-type plants growing in noncompacted soils (98). This surprising result reveals that it is actually ethylene that inhibits root growth, rather than mechanical impedance of compacted soils. Hence, roots employ ethylene as part of a novel gas diffusion–based sensing mechanisms for compacted soil (98). The compaction-induced ethylene buildup also triggers changes in the synthesis and transport of other downstream hormone signals including auxin and ABA. Higher auxin concentration in epidermal cells restricts root elongation, and greater ABA concentration causes anisotropic root swelling in compacted soil (54). Compaction triggers auxin biosynthesis at the root tip, and concomitant shootward auxin transport leads to longer root hairs, providing better anchorage and mechanical support to penetrate the compacted soil layer (68). Moreover, higher ethylene buildup in compacted soil conditions promotes crown rooting by inducing *WOX11* (*WUSCHEL-related homeobox 11*) expression, which enables better foraging in topsoil as deeper rooting is challenging due to greater mechanical impedance in compacted soil (73). Thus, ethylene orchestrates auxin, ABA, and GA (gibberellic acid) as distinct downstream signals to regulate root compaction responses such as root growth inhibition,

root hair elongation, root thickening, and crown root development in compacted soil conditions (97).

Soil Oxygen

As described previously, soil compaction limits gas diffusivity. For soils such as sandy loam and sandy clay loam, moderate and high BDs progressively restrict gas diffusivity as pore size and interconnection decrease (143). Gas diffusivity is also influenced by soil water content (44), as gas diffusion in water is 10,000-fold slower than in air (99). Considering O_2 supply to soil is mainly through diffusion, O_2 from the atmosphere to a flooded soil is restricted, which affects root respiration (7) and leads to tissue hypoxia and reduced root elongation (134).

Some plant species increase root aerenchyma formation to reduce the metabolic maintenance cost of the root system (79). Continuing the waterlogging-soil compaction analogy, aerenchyma formation also enhances longitudinal O_2 diffusion from aerial parts of the root not subjected to restricted O_2 supply to the belowground parts experiencing tissue hypoxia, thus helping to sustain root respiration and growth (99). In waterlogged soils, some plant species produce suberized/lignified barriers to further maintain the O_2 diffusing from the aerial parts to the roots by preventing its diffusion to the hypoxic soil (21). In compacted soils, lignification of root tissues is also induced, to provide mechanical strength to roots so that they can penetrate harder soils (116). Additionally, it has been shown in maize that genotypes with a specific anatomical phenotype called multiseriate cortical sclerenchyma (MCS) exhibit higher concentration of lignin and better root penetration in compacted soil conditions (116). It is possible that suberization/lignification of roots induced by soil compaction could also help, in combination with aerenchyma formation, to support higher O_2 tissue levels (100).

Multiseriate cortical sclerenchyma (MCS): root anatomical trait characterized by small cells with thick walls in outer cortical cell layers

Soil Microbiota

In addition to its chemical and physical characteristics, the biological properties of soil also profoundly influence root growth and development. Over the past decades, a compelling body of scientific evidence has revealed roles for a wider range of soil microbiota beyond classical rhizobia and mycorrhizal exemplars (17, 78). Plant roots have evolved unique morphological and metabolic characteristics that have provided an attractive environment for a group of soil microbes. Roots provide a continuous supply of nutrients and a shield for the surrounding microbiota, which in turn influence root development and function, interfering with nutrient acquisition and assimilation, plant hormone homeostasis, signaling processes, or the establishment of other members of the root microbiota. To colonize and survive in the root environment, microbes use complex mechanisms related to chemotaxis toward the root, root attachment, and biofilm formation on the root surface (65, 71). Microbial mechanisms of attachment to the root surface and hairs can be influenced by soil parameters such as pH, the presence of divalent cations, and water availability (65). Likewise, bacteria–bacteria interactions can modulate the ability of the microbiota to efficiently colonize plant roots. A recent study identified the antimicrobial 2,4-diacetylphloroglucinol and the iron chelator pyoverdine as bacteria-derived exometabolites that drive competitive interactions among microbiota members with a robust effect on root microbiota composition (40). Roots control their microbiota membership via modulation of exudate composition according to the plant developmental stage (94, 114), the biosynthesis of root triterpenes (53), the colonization by specific root-associated microbes, or the activation of the immune system (70, 144). However, root-associated commensal bacteria can evade immune system activation by suppressing MAMP (microbe-associated molecular pattern) responses to promote root colonization. It is known that some peptide derivatives of flagellin, a MAMP detected by the plant immune system and present in

commensal bacteria, can evade recognition (20, 126), and effectors of the type III secretion system suppress MAMP perception (19). Recently, an additional explanation has been given to the establishment of the microbiota in the root. Nonpathogenic microbes can colonize the root, simply by avoiding damaging plant tissues. By doing this, they also avoid the strong activation of immune responses that follow cellular damage in the root and prevent microbial colonization (150). These root colonization mechanisms are modulated by the presence of endodermal diffusion barriers in the roots, since the Casparian strip and the suberin lamellae prevent the free diffusion of immune peptides derived from the microbiota, compartmentalizing the perception of colonizing microbes (150).

The root hosts bacteria primarily from the phyla Proteobacteria, Actinobacteria, and Bacteroidetes (12, 78). These populations of microbes living in close association with the roots can interfere with the root function specifically with the endogenous root responses to nutrients. Soil resident nitrogen-fixing bacteria and mycorrhizal fungi can establish symbiotic relationships with the roots (**Figure 2*d***), contributing alone or in association with other members of the root microbiota to the metabolism of nitrogen and phosphate in the root tissues (27, 138, 146). Other commensals within the plant microbiota also contribute to nutrient acquisition and use in coordination with the root responses to low nutrient levels. *Colletotrichum tofieldiae*, an endophytic fungus in natural *Arabidopsis thaliana* populations, increases root phosphorus uptake under low-phosphorus conditions. Root colonization and the benefits derived from association with this fungus are controlled by the host's phosphate starvation response in coordination with the plant's immune system (51). This crosstalk between the phosphate starvation regulatory network and plant defense elements also modulates the assembly of a beneficial plant microbiota under low-phosphorus conditions (17). Differences in nitrogen use efficiency in field-grown rice varieties are attributed to discrepancies in root microbiota composition, specifically in members of the microbiota with nitrogen metabolism functions, whose recruitment is modulated by the plant nitrate transporter NRTI1.1B (148). The adaptation of plants to iron-limited soils is based on plant beneficial interaction with the root microbiota that is mediated by coumarins exuded by the roots, the synthesis of which is regulated by the transcriptional factor MYB72 (46, 121). Therefore, the root microbiota is proposed to be an integral mediator of root function in nutrient uptake and utilization.

Root architecture and anatomy can also be modulated by its resident microbiota. Members of the bacterial genus *Variovorax* contribute to maintaining optimal root development by manipulating their hormonal levels, counteracting possible inhibitory effects on root growth caused by other bacteria (36). Furthermore, the root-inhabiting microbiota can regulate the plasticity of root branching by inducing the auxin and/or ethylene pathways that are known to control this process (42, 87). In another example, a regulatory mechanism of root endodermal differentiation driven by the root microbiota has been characterized. The deposition of the endodermal diffusion barriers (the Casparian strip and suberin lamellae) is influenced by the microbiota with consequences for mineral nutrient homeostasis. This microbial effect on endodermal function is associated with the ability of the microbiota to modulate responses to the phytohormone ABA in the root (112) (**Figure 2*d***). Furthermore, the capacity of root microbiota to induce root endodermal suberization and aerenchyma formation in sorghum plants has been associated with the inhibition of root infection by *Striga* (a genus of parasitic plants) (60).

Not only can the morphology of the root be influenced by its microbiota, but correspondingly, the morphological and anatomical characteristics of the root can also modulate the establishment of the microbiota in the root. In plant species with complex root systems like monocots, primary, seminal, crown, and brace roots with different cellular patterning accommodate distinct bacterial and fungal communities (61). In some roots, the capacity to form aerenchyma influences the level of colonization by beneficial microbes such as arbuscular mycorrhizal fungi and restricts

infection with pathogenic fungi (38). Similarly, root order (64), absence of root caps (110), and root hairs (108, 110, 124) have a strong effect on microbiota assembly as well as on the root zone in the longitudinal axis (110) and root compartments (146). The impressive progress recently being made uncovering these functional feedback and regulatory mechanisms between root tissues, zones, and soil microbiome communities is helping reveal an unprecedented level of spatial understanding into the cross-kingdom interactions occurring in the rhizosphere that underpins root–soil colonization, resource capture, and, ultimately, plant success.

CHALLENGES AND SOLUTIONS TO STUDYING ROOTS UNDER REAL-WORLD CONDITIONS

The opaque nature of soil and its spatial and temporal heterogeneity make quantifying plant roots and rhizosphere dynamics extremely challenging. The challenge is twofold: to develop improved in agri techniques to quantify root and rhizosphere traits and to improve reproduction of real-world conditions in laboratory-based experiments. A single solution is unlikely, and a combination of modalities and scales has a greater chance of success (137). For example, by combining low- and high-resolution X-ray CT images with X-ray fluorescence microscopy, nanoscale secondary ion mass spectrometry, and laser ablation isotope ratio mass spectroscopy, carbon inputs and nitrogen uptake have been mapped in maize grown under undisturbed conditions (75). Very-deep super-resolution neural networks (63, 103) have been created to upscale images from high- to low-resolution. Cross-modal techniques (117) are now available that may enable high-resolution field-to-laboratory techniques such as X-ray CT to inform networks to upscale the lower-resolution outputs from field techniques such as electrical resistivity tomography (ERT) and electrical impedance tomography (EIT).

Phenotyping for root traits and visualization of rhizosphere components in agri generally employ either destructive techniques or less-invasive proxy measurements (**Table 1**) that measure outputs of root activity in the rhizosphere. An exception is ground-penetrating radar that has been successfully used for estimation of coarse roots (>2 mm) in soil. Direct, destructive methods include crown excavation, trenches, coring, and monolith sampling (37, 127, 130, 135). Rhizotrons allow imaging of roots that intersect a transparent window [either an access tube (minirhizotrons) or an underground chamber]. Quantification of root exudates has been performed in the field combining underground soil columns and exudate extraction methods to successfully quantify exudation ratio and composition of in-field grown roots (113). Proxy measurements include ERT (104), EIT (24), thermoacoustic tomography (TT) (120), and electrical current source density (ECSD) (101). ERT, EIT, and ECSD are indirect methods to visualize roots activity by quantifying soil water content that can be correlated with the root–soil system. However, at present, all have relatively coarse spatial resolution.

Advances in noninvasive imaging techniques have allowed investigation of rhizosphere processes in the laboratory (**Figure 3**). Tomographic approaches are the most used for direct visualization of roots in soil, in particular X-ray CT (**Figure 3*b–e***) and MRI (**Figure 3*g***) (4, 39, 74, 85, 123). X-ray CT offers higher spatial resolution than MRI, detecting even the thin roots of *Arabidopsis* (<50 μm voxel size). MRI offers a coarser spatial resolution but faster segmentation better suited for high-throughput approaches. Neutron radiography and fast neutron tomography have been used to resolve water uptake in 2D and 3D by using deuterated water and isotope tracers to indirectly measure root activity in soil to visualize the spatial distribution of water and carbon, thus quantifying root water radial and vertical lift (**Figure 3*f***) (47, 128, 129) and deposition of mucilage exudates (52). Similarly, positron emission tomography (PET) uses isotopes as tracers to visualize fluxes in carbon, water, macro- and micronutrients in plants grown in soil media (86,

Electrical resistivity tomography (ERT): imaging technique based on electrical resistivity that measures root architecture in soil

Electrical impedance tomography (EIT): noninvasive imaging technique based on electrical resistivity used to image roots and soils using surface electrodes

Thermoacoustic tomography (TT): thermoacoustic microwave illumination technology used to noninvasively image root architecture in soil

Electrical current source density (ECSD): method used to monitor soil water content by measuring extracellular electric potential at different soil depths

Positron emission tomography (PET): radioactive tracer–based functional imaging technique used to visualize dynamic processes in roots such as carbon flow

115, 145) (**Figure 3*g***). Such techniques allow visualization of rapid changes within the root–soil interphase. To phenotype root systems at high throughput, automated platforms have been developed using hydroponics, rhizotrons, and various transparent media (72). Laser ablation tomography (LAT) is another advanced imaging technology that provides 3D phenotyping of root anatomy. This innovative technique is high-throughput, provides micron-level resolution, and reveals root adaptations such as formation of root aerenchyma, MCS, and root colonization by arbuscular mycorrhizal fungi (116, 122) (**Figure 3*h*,*i***).

Improvements in controlled environment technologies now allow the precise and dynamic reproduction of climatic conditions (temperature, humidity, gas composition, and the intensity and spectral composition of illumination). Placing rhizotrons and mesocosms in such infrastructures allows the impact of predicted climate changes on rhizosphere processes to be evaluated (49). To improve replication of realistic field conditions, it is essential to reproduce the same variability in the soil where plants are grown. Temperature gradients, nutrient and water heterogeneity, microbiome composition, and temporal changes are often not reproduced in controlled experimental systems. Additionally, most plate-based laboratory experiments involve direct illumination of roots, which potentially disrupt several molecular and physiological responses, including root responses to water and nutrients and interactions with microbiota (13). These findings emphasize the significance of studying plant roots in vivo. Integrating these important parameters will allow characterization of the physiological relevance of adaptive root traits in response to more realistic, more heterogeneous, and increasingly challenging future soil conditions.

FUTURE PERSPECTIVES

Understanding complex, multiscale root–soil processes will increasingly demand greater interdisciplinary engagement (i.e., beyond agronomy and life sciences). For example, revealing how molecular mechanisms influence a root tip or system scale processes will require the integration of multiscale mathematical modeling approaches into our research (98). Similarly, performing high-throughput phenotyping of root architecture and anatomy will necessitate the use of artificial intelligence algorithms. Future root research must also consider the importance of biological time and space in soil ecosystems. Temporally, legacy effects of previous year's crops via allelochemicals highlights the importance of intergenerational timescales (80). At the other end of the biological timescale, the development of biosensors has revealed that roots experience dynamic changes in the levels of hormones and ROS (reactive oxygen species) signals within seconds to minutes after experiencing environmental stress (14, 109). Spatially, very few root–soil studies use naturally structured field soils that contain stones, biopores, or cracks, relying instead on finely sieved and repacked soil materials. This simplification is used despite the majority of crop roots being known to prefer growing via preformed biopores in the subsoil profile below the plough pan (56). Ecosystem-wise, much of the root research literature is too focused on temperate crops and their soil environments, largely ignoring other ecological settings (e.g., desert, forest). Hence, much greater consideration of time, space, scale, and ecosystem context is required to advance the field in the future.

SUMMARY POINTS

1. Soil properties vary spatially and temporally, impacting root growth differently across landscapes and seasons. This heterogeneity makes it difficult to capture root dynamics.

2. Root behavior in soils is influenced by several factors, including distribution of water, nutrient availability, soil compaction, oxygen dynamics, and interactions with soil microbiota.
3. Controlled laboratory environments often fail to replicate the complexity of field conditions, including heterogeneous nutrient gradients, water availability, gas diffusion, and microbiome interactions.
4. Advanced imaging techniques like X-ray computed tomography (CT) and magnetic resonance imaging (MRI) have proved instrumental in quantifying roots and rhizosphere dynamics in real-world conditions but require further refinement for field-scale applications.
5. Noninvasive techniques such as electrical resistivity tomography (ERT) and electrical impedance tomography (EIT) offer indirect measurements but lack fine-scale resolution.
6. Future research should focus on integrating high-resolution imaging with field-based techniques to simulate realistic soil conditions, enhancing our understanding of root responses to environmental stresses and facilitating sustainable agricultural practices.

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