Shaping 3D root system architecture

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Summary

Plants are sessile organisms rooted in one place. The soil resources plants require are often distributed in a highly heterogeneous pattern. To aid foraging, plants have evolved roots whose growth and development is highly responsive to soil signals. As a result, 3D root architecture is shaped by myriad environmental signals to ensure resource capture is optimised and unfavourable environments are avoided. The first signals sensed by newly germinating seed, gravity and light, direct root growth into the soil to aid seedling establishment. Heterogeneous soil resources such as water, nitrogen and phosphate also act as signals that shape 3D root growth to optimise uptake. Root architecture is also modified through biotic interactions that include soil fungi and neighbouring plants. This developmental plasticity results in a 'custom made' 3D root system best adapted to forage for resources in each soil environment a plant colonises.

Introduction

Land plants have evolved root systems with complex 3D shapes. The volume of soil explored by a root system is largely determined by its architecture and hence modifications to its 3D shape can significantly impact a plant's efficiency at acquiring resources. Root system architecture (RSA) is essentially determined by four major shape parameters- growth, branching, surface area and angle. Roots regulate these shape parameters in response to signals in their local soil environment such as water and nutrient availability, in addition to genetically determined developmental programmes. This highly adaptable behaviour, termed developmental plasticity, has been a major determinant for the success of land plants [1, 2].

In order to understand the need for root systems to be developmentally plastic, it is necessary to consider that soil is spatially and temporally highly heterogeneous (Figure 1). For example, soil structure varies between types of soil, but also as a result of different tillage practices [3] (Figure 1A). This results in huge variations in the size and connectivity of pores (Figure 1A-C). Soil pores are the gaps in between and within particles and aggregated material. They are either gas or water filled. Heterogeneity of nutrient availability can arise due to patchy organic input and microbial decomposition [4]. This is further compounded by movement of nutrient ions in soil varying widely. For example, nitrate is highly soluble in water, whereas phosphate quickly forms insoluble complexes greatly reducing its mobility [5]. Water heterogeneity also occurs at a macro-scale, as the top soil dries before deeper profiles, and on a micro-scale within pores that vary in their capacity to hold water (Figure 1D) (See supplementary video 1) [2, 6, 7]. Broadly speaking the water in micropores (< 30 µm) is held at very high suctions typically above what a plant is able to extract, water in

mesopores (30-1000 µm) is regularly accessible to plants and water in macropores (>1000µm) is free drainage and usually passes through the upper portion of the root zone before a plant can extract it [8]. Soil structure in particular is a dynamic property, changing significantly over time (from minutes to years) in response to changes in weather and the associated variations in water content, temperature, vegetative growth, soil organism activity and anthropogenic management [9-11] . The developmental plasticity of RSA is therefore not surprising, given the complexity of soil structure and fluid dynamics that cross many scales.

Studying RSA in soil poses major practical challenges. The development of shovelomics has enabled high throughput root phenotyping of field grown crops [12, 13]. However, destructive sampling often results in finer scale root architectural features being lost (e.g. lateral roots) and only a snapshot of development being measured [13]. Non-destructive imaging techniques enable temporal changes in architecture to be observed throughout root development. Classical non-destructive techniques such as agar plates, rhizotron, paper-based and hydro/aeroponic systems have been integral in gaining a better understanding of root development (Figure 2A) [14-18]. Nevertheless, these techniques essentially force roots to develop in two dimensions.

Integrating the third, and fourth (time), dimension enables researchers to better observe how roots forage and compete for resources. Non-destructive analysis of 3D root growth has been made possible using transparent gels (Figure 2B) [19, 20]. Growing roots in non-soil based systems helps decrease experimental variability by reducing, for example, heterogeneity of resource distribution or the impact of microbial populations. However, by removing these factors the conditions faced by the plant are artificial and results may be

difficult to extrapolate to growth in the field. To non-invasively study 3D growth in soil, more sophisticated imaging approaches are needed. For example, Magnetic Resonance Imaging (MRI; Figure 2C) and X-ray Computer Tomography (CT; Figure 2D) have been used successfully to observe 3D root systems in the soil [21-25]. However, these techniques are expensive to employ and are low throughput. Until such techniques overcome these issues, 2D imaging will remain important for root research.

This review explores the diverse architectures, adaptive responses and molecular mechanisms employed by root systems to optimise resource capture and adapt to abiotic and biotic stresses.

Root system architecture: myriad variations on common themes

At the root system level, different plant species appear to display myriad shape variations, yet common types of root distributions can be discerned. For example, in dicotyledonous plants such as *Arabidopsis thaliana* L. (thale cress) and *Raphanus sativus* L. (tillage radish), the embryo derived primary root remains active throughout the plants life cycle. Several orders of lateral roots then develop from the primary root to form the mature root system (Figure 3A & 4B). In contrast, seedlings in monocotyledonous species like *Triticum aestivum* L. (wheat) form several seminal roots (Figure 3B). When visualised from above (Figure 3C), seminal roots serve to multiply the volume of topsoil a seedling's roots are able to initially colonise to obtain nutrients and anchorage. At later stages of development, cereal root systems are dominated by a new root class termed adventitious crown or brace roots [26]. This class originate post-embryonically from shoot tissues like grain forming tillers and provide greater anchorage and resource acquisition capacity [27]. The striking differences in

the 3D structures of dicot and monocot roots result in them being classified as tap root and fibrous types of architectures, respectively.

At the local root level, almost every class of root (irrespective of species) branches to aid efficient exploration of the adjacent soil volume (Figure 3). A top down CT image of a wheat root segmentation reveals how branches emerge at different angles relative to the root circumference (Figure 3D). The term rhizotaxis has been used to describe the spacing and pattern of lateral root emergence akin to phyllotaxis in leaves [28, 29]. As in leaves, altering the angle of lateral root emergence relative to the previous and next branch will serve to minimise competition between a plant's own roots. Despite its obvious importance, almost nothing is known about how roots select where branches emerge radially [30]. In all flowering plant species, lateral roots originate from small groups of pericycle-derived stem cells (Figure 4A). Depending on the plant species, lateral root stem cells are either positioned between or opposite phloem and/or xylem vessels [31]. In Arabidopsis lateral roots emerge from opposing xylem poles in an alternating pattern as two laterals will not develop at one cross section of the root (Figure 4B) [32, 33]. In cereals like wheat, lateral roots emerge from phloem poles (of which 10 or more may be present) enabling branches to simultaneously emerge at many different radial positions in one cross section (Figure 3D) [34, 35]. The net benefit is the capacity to form a much denser root system when conditions favour, such as encountering a localised source of nutrients (Figure 5A & 5B).

3D root architecture is shaped by environmental signals throughout plant development

The first environmental signals to shape root architecture immediately after seed germination are gravity and light. This was strikingly demonstrated by Ma & Hasenstein

(2006) who observed that the primary root of flax seedlings develop the ability to sense the direction of gravity 8 hours after seed imbibition (rehydration), which was 11 hours prior to root emergence from the seed coat [36]. The onset of this gravity-sensing ability is correlated with the formation of starch-filled plastids termed statoliths. These specialised plastids function as gravity sensors that sediment to the bottom of columella cells (root cap) and move in response to seedling re-orientation [37-39]. Statolith movement triggers the formation of a lateral gradient of the hormone auxin within minutes after a gravity stimulus [40]. Unequal distribution of the hormone between the upper and lower sides of the root in the elongation zone results in differential cell elongation, leading to root curvature [39, 41, 42]. A seedling's ability to orient the growth of the primary root in the direction of gravity (termed positive gravitropism) will greatly enhance its chances of establishment. A time series of CT images charting the development of an Arabidopsis seedling root system (Figure 4B) (See supplementary video 2), reveals the critical role that the primary root performs during the first 12 days after germination, prior to lateral root emergence. Up to this point, the seedling is entirely reliant on the primary root for its establishment, providing anchorage and foraging for critical resources such as nutrients and water.

Like gravity, light is amongst the very first environmental factors to regulate root growth, directing it to penetrate the soil surface [43]. Plant roots are negatively phototropic meaning they will grow away from the direction of light [44]. Light is able to penetrate the topsoil and provide a directional signal for root growth that is perceived immediately after germination. Plant roots express phytochrome, phototropin and cryptochrome classes of light receptors [45-48]. Polar localisation of auxin efflux transporters PIN2 and PIN3 has been proposed to result in an asymmetric distribution of auxin, causing the root to bend away from light and into the soil [49-52]. Interestingly recent research has demonstrated

that light received by the shoot can activate the photoreceptor phyB in roots which has a role in controlling organ growth. This suggests that roots are able to integrate light signals from both the stem and the root to control growth [53].

Gravity continues to play a crucial role controlling 3D RSA following seedling establishment. The angles at which roots grow varies widely between the different classes of roots within a single plant (Figure 3A & 3B) [54, 55]. For example, lateral roots emerging from primary and seminal roots often grow horizontally in a manner termed diagravitropic behaviour (Figure 3A, 3B & 4B) [55, 56]. Furthermore, higher orders of lateral roots (originating from first order laterals) exhibit highly variable angles termed plagiotropic behaviour [57]. Seminal roots grow at angles intermediate to the vertical and horizontal, distinct to the vertical (positive gravitropic behaviour) of the primary root (Figure 3B). Such differences in angle between these distinct root classes are likely to reduce self-competition, whilst serving to maximise the volume of soil being explored.

Exactly how roots grow at angles that deviate from vertical is currently unclear as the majority of research to date has focused on studying the primary root and its positive gravitropic response [58, 59]. This non-vertical pattern of growth is termed the gravitropic set-point angle (GSA) [56, 60]. GSA is an important trait of RSA determining whether a plant develops a steep or shallow root system, which in turn has an impact on water and nutrient uptake [61]. GSA may change in the course of development and can be modified by environmental factors such as nutrient availability [62].

A single root shape is not optimal for capturing heterogeneously spread nutrients in soil

A key root system function for the plant is acquisition of mineral and organic nutrients. There are seventeen nutrient elements that are widely classified as essential for plant

growth and development [63]. Of these nutrients two elements, nitrogen and phosphorus, are required in high quantities in agriculture yet are deficient in many soils. As the availability of nutrients are spatially and temporally heterogeneous in the soil, roots need to forage for such resources. Root architecture thus has a profound effect on the uptake of nutrients and consequently the yield potential of a crop. Root adaptive responses to nutrient supply have been widely studied. Despite the diversity across the plant kingdom, several nutrient-related root responses are common in most land plants. For example, roots that respond to the local availability of a nutrient often proliferate in this zone (Figure 5A & 5B) [64-67]. Therefore, 3D RSA is shaped by the availability of these nutrients in order to optimise resource capture.

Phosphorus is largely unavailable for uptake as it is insoluble in the soil solution [68]. Phosphate (P) is an inorganic form of phosphorus that is highly immobile and is concentrated in the topsoil due to plant bioaccumulation and deposition [69, 70]. Adaptations to RSA for increased topsoil foraging has been shown to enhance P acquisition. Architectural changes to RSA in response to low P availability include: increased numbers and lengths of roots in patches of high P availability (Figure 5A) [67, 71], shallower root angle [69, 72], increased numbers and lengths of root hairs [73, 74] and cluster root formation [75, 76]. Cluster roots represent dense arrays of branched roots. Their high numbers help to increase the surface area of the root system in order to access a greater amount of P [75, 77]. These adaptive responses highlight the importance of root architecture in acquiring P compared with other nutrients, as a result of its low mobility in soil. Plants also appear to manipulate their associated root microbiome in response to phosphate stress to enhance their performance [78]. Nevertheless, once an area of soil has been depleted of P the root must expand its surface area to forage further. It is evident that

root architecture must be plastic to respond spatially and temporally to nutrient heterogeneity for efficient uptake.

Nitrogen (N) is an essential macronutrient for plant growth and development that is primarily assimilated in the form of nitrate (NO_3^-) and ammonium (NH_4^+). In soil NH_4^+ is immobile because it rapidly forms insoluble complexes with cations whereas NO_3^- is transported with soil water [63]. NO_3^- therefore is a particular challenge for root capture as it is mobile in the soil water solution and leaches into the deeper soil layers. When soil is uniformly low in N, the following root responses have been reported: steeper root angle in brace and crown roots [79], elongation of lateral and seminal roots [80-82], reduced root length density near the soil surface and reduced numbers of axial roots [83]. This increases the foraging capacity of the root system with exploration of deeper soil layers where N is more abundant.

Root adaptive responses are important even when N requirements are sufficient. An increased concentration of NH_4^+ inhibits primary root elongation whereas an excess of NO_3^- represses lateral root elongation and branching [84, 85]. Such suppression of root growth in response to increased N allows reallocation of carbon and metabolites to the shoots of the plant [81].

Modifications in RSA in response to N level are sensitive to whether the supply is global or local. Root growth is inhibited in soils with globally high N but the opposite response is observed when a root growing in N poor soil encounters a locally enriched nitrogen patch (Figure 5B) [66]. Uneven distribution and breakdown of organic matter in soil cause localised nutrient rich patches. Root strategies for exploiting localised N include stimulation of lateral root initiation and lateral root elongation in these zones [86-89].

Root architectural plasticity is a beneficial adaptation particularly in natural ecosystems when resources are scarce and there is competition with other plants. In agricultural systems however plasticity can be considered a maladaptive trait. For example, it may be advantageous for a plant to not proliferate in response to local nutrient pockets but to grow deeper irrespective of nutrient availability in anticipation of leaching N fertiliser later in the year [83, 90, 91]. By repressing adaptive responses to heterogeneous nutrients, a plant could prioritise growing a deep root system with increased distribution of lateral roots at depth for better access to resources [92].

It is clear that nutrient heterogeneity has a major impact on RSA as a result of changes in branching, elongation and angle of root growth. With crop productivity gains not increasing at the required rate for food security [93], and flat lining for key crops and some regions [94], increased focus on root research could provide further gains by increasing nutrient efficiency. Development of an ideotypic root system for each root type per environment and management practice system could be a more realistic aim. The biggest challenge ahead is to comprehend the complexity of nutrient spatial and temporal heterogeneity in soil in respect to the diverse varieties of crops we grow.

Symbiotic mycorrhiza act as a surrogate root system that extends the range of foraging

In both wild and agricultural soil habitats roots do not grow in isolation, but interact with the soil microbiome, often forming symbiotic relationships with bacteria like rhizobium and frankia that trigger root nodule formation to fix nitrogen [95, 96]. Moreover, more than 90% of land plants form symbioses with mycorrhizal fungi [97, 98]. Indeed, early land plants heavily relied on mycorrhizal fungi interactions to provide a surrogate root system, prior to

the evolution of roots [99].The most common type is the arbuscular mycorrhizas (AM) which colonise 80% of terrestrial plant species [98]. The Glomeromycota fungus and the infected plant both benefit from this symbiotic relationship. Extraradical mycelia can extend far beyond the root depletion zone to capture P which is then passed to the plant through arbuscules, highly branched intraradical hyphae, that form in the roots cortical cells. In return the plant provides photosynthate to support the fungi's obligate biotrophic lifestyle [100, 101].

As P is extremely immobile this extension of the root system increases the plants ability to access P and it is known that mycorrhizal colonisation can increase plant P level and plant growth [101]. The level of mycorrhizal colonisation responds to P availability in the soil [102]. When P availability is low in the soil intraradical development of the fungus can occur in over 80% of the root length, preferentially in lateral roots [103, 104]. On the other hand the ability of mycorrhiza to improve N plant content is less well understood [97]. Nevertheless, it can be argued that plants have enlisted the help of mycorrhiza to act as a surrogate root system in order to explore a greater soil volume and improve P uptake.

Mycorrhizal colonisation leads to changes in 3D root architecture due to an increase in lateral root development [104, 105]. Research has shown that mycorrhiza can cause this response in root architecture both before and during colonisation. AM fungal spores produce a diffusible factor that stimulates lateral root formation before colonisation occurs [106]. Furthermore, this response was seen clearly during colonisation when AM fungi colonisation was able to rescue lateral root branching in the *Zea mays* L. (maize) mutant *lateral rootless1* (*lrt1*), normally lacking embryonic laterals [107]. It has been argued that the increase in density is an adaptive strategy to create more suitable sites for further

colonisation [103]. However, the response may not be specific to mycorrhiza and may simply be caused by the locally high P or sugar levels at sites of colonisation [108].

Roots growing in water deficit respond by increasing their angle to reach deeper soil

One of the key functions of the root system is to source water. As the topsoil commonly dries first there is a vertical gradient of water availability, ranging from lower water availability in shallow soil to more abundant water availability in the deep soil. The steep root architecture in *Pennisetum glaucum* L. (pearl millet) is hypothesised to be an adaptation to the low water availability in the topsoil where it was domesticated [109]. In *Oryza sativa* L. (rice) a shallow rooting cultivar had improved yield under drought stress conditions when its seminal root angle was made steeper by overexpression of the DEEPER ROOTING1 (DRO1) gene [110]. This novel sequence is an early auxin responsive gene suggesting the water deficit signal is integrated into the auxin-regulated gravitropic response pathway. Recent research in Arabidopsis has observed that when a root system is exposed to a soil water deficit in the upper soil profile, it responds by increasing the gravitropic angle of its lateral roots in order to explore deeper levels (Figure 5C) [111]. This novel adaptive root response appears to be distinct from another water-related root response termed hydrotropism [112], since hydrotropic mutant *miz1* does not disrupt lateral root re-orientation, leading this response to be termed xerotropism [111, 113].

Water deficit in the upper soil profile has also been observed to suppress lateral root and crown root growth in different plant species [111, 114]. In the cereal *Setaria viridis* L. (green foxtail) crown root growth is completely repressed during water deficit whilst primary root growth is promoted causing significant changes to the overall root architecture [114]. Water

deficit is sensed locally at the crown and results in an accumulation of arrested roots. This repression of branching is an adaptive response to save metabolic resources [115, 116]. The few but long lateral ideotype of root architecture is suggested to be the most efficient shape during water stress as metabolic resources are saved to be used to extend the root system into deeper soil profiles. Indeed, when grown under water stress maize lines with few but long root architecture had 144% greater yield than lines with many but short architecture [115].

Root systems also respond to micro-scale heterogeneity in water availability

Historically research into the effect of water on root architecture has focused on water deficit and drought [6]. However, water availability in soil is heterogeneous on a much smaller scale than has been previously considered. At a microscale soil consists of aggregates and pores of different sizes [117]. The size of the pore alters the matric potential imposed on water and hence how strongly water adheres to the soil matrix. Therefore, some pores will be filled with water whilst other pores will only contain a layer of water adhered to the soil and the majority of the pore will contain air. This creates heterogeneity of water availability as the water in a filled pore is much easier to access for a root than water adhered to the soil matrix or water contained in humid air [113, 118].

Recent research has highlighted how root architecture responds to microscale water heterogeneity by altering the pattern of branching [18, 30]. It has been observed that when the primary root is exposed to a difference in water availability across its circumferential axis lateral roots initiate preferentially on the side with greater water availability (Figure 5D) [30]. This novel adaptive response, called hydropatterning, has been observed in

Arabidopsis and several cereals when grown on agar plates and in soil [30]. Therefore, branching is patterned to best place root growth in areas where water (and hence soluble nutrients) are present. Similarly, when primary and seminal cereal roots are exposed to a localised water deficit, lateral root initiation is repressed specifically in the segment of root exposed to the stress [18]. Both responses appear very similar, with the only difference being one represses lateral root growth in a radial pattern whilst the other represses growth in a longitudinal pattern. Although the molecular mechanisms controlling each response have not been fully elucidated, initial research suggests that hydropatterning is regulated by the hormone auxin [30]. These patterning responses are sensitive to microscale differences in water highlighting that 3D root architecture can respond to the soil environment at a spatial scale much lower than previously thought.

Branching is not the only way root architecture can be altered by water heterogeneity. Roots are able to grow towards areas with higher water availability using hydrotropism. This response has been demonstrated in soil grown Arabidopsis exposed to a directional source of water [119]. Hydrotropism is distinct from gravitropism, involving the hormone abscisic acid (ABA) [120] and a different set of tissues to sense and respond to micro-scale gradients of water availability located in the elongation zone [112]. Roots are also able to grow away from soil areas with high salt using halotropism, which (like gravitropism) is mediated by auxin redistribution [121]. Both responses ensure that root architecture is shaped to optimise root growth in areas of high water availability. The patterning of branching and tropisms into favourable growth environments mean that root systems are not at the whim of the environment but actively grow into more conducive soil environments.

Finding the path of least resistance in deeper soil

Whilst roots colonising deeper soil profiles is favourable due to its higher water content, this zone is also much more challenging to penetrate due to its higher bulk density created by overburden pressure. As a soils bulk soil density increases, the pore connectivity usually decreases. This results in increased mechanical impedance for roots and reduced air permeability [122-124]. Higher soil bulk density has been shown to impede root growth by decreasing the spread of lateral roots and total root length [125]. *Solanum lycopersicum* L. (tomato) roots respond to soil compaction by increasing their diameter and lateral root number in an attempt to overcome the reduction in root surface area [125]. However, wheat roots growing 90 cm below the ground are only found in macropores suggesting that their roots only grow by soil deformation in shallow un-compacted soil [126]. Furthermore, root tips of *Glycine max* L. (Soybean) die if they do not meet a macropore before a soil depth of 30 to 45 cm [127]. Therefore, once roots reach deep compacted soil they rely on the presence of macropores for a pathway of least resistance, hence these pores shape the 3D root system.

Macropores, often in the shape of cracks or tubular passages, are most commonly associated with the bioturbation of previous roots, the burrowing activity of macro organisms (like worms) and shrinkage of soil due to dehydration [126]. Interestingly maize and soybean roots preferentially grow towards macropores using a process termed trematotropism [128]. Macropore availability is a key determinate of plant growth in compacted soil, with dry shoot biomass increasing by 27-67% when artificial pores were created in a compacted field [128]. Despite the potential importance of this response, the mechanisms behind it remain unclear. Instead it has been assumed that the ability to

deform strong soil is the most important trait for deep rooting [129]. However, the ability to locate and grow through macropores may be more important for conferring a deep rooting architecture to reach water supplies.

Root systems architecture is shaped by interactions with neighbouring plants

In natural and agricultural environments plants grow alongside neighbours. Roots from different trees are able to graft together to form a common root system [130]. However, in many plant species, roots compete for the same resources in overlapping soil volumes [131]. Many different responses to below ground competition have been documented [132]. It has been observed in multiple studies that plants respond by over proliferating their root system to compete for resources [133-135]. Over proliferation has been deemed a "tragedy of the commons" as both plants increase the size of the root system in an attempt to take up more resources than the other. However, multiple studies that observed a tragedy of the commons response failed to control soil volume meaning it is difficult to conclude whether the plant responded to the neighbouring plant or the extra soil volume [136, 137].

Research by Nord et al., (2011) showed that root responses of *Phaseolus vulgaris* L. (common bean) to neighbouring plants was caused by resource depletion by the neighbour rather than the direct presence of a neighbour [138]. In this study over proliferation of roots was not observed but RSA was altered to reduce the distribution of roots in the soil volume occupied by the neighbour. This response to spatially segregate root systems and avoid resource competition has also been observed in Arabidopsis and rice [139, 140]. Transcriptomic analysis of Arabidopsis indicates plants sense neighbouring roots before

resource depletion occurs suggesting that resource depletion is not the only signal for segregation [139].

Interestingly the changes in RSA documented in root: root interaction studies appear to be dependent on the relationship of the plants in competition [140-142]. The perennial grass *Buchloe dactyloides* (buffalo grass) produced fewer and shorter roots when grown in the presence of roots from the same physiological individual compared to a stranger [141]. Identity recognition can occur on multiple levels from species specific recognition to kin vs stranger and self vs non-self recognition [139-142]. The ability to identify the relationship of a neighbouring plant may be advantageous in order to prevent competition for soil resources with kin. However, how plants determine these relationships is not well understood. Proposed mechanisms include volatile cues, electric signals, hormonal rhythms, root exudates and associated microorganisms [132].

In conclusion, it is not yet clear if a plants RSA is altered directly or indirectly by its neighbours. The difficulty in observing these interactions has slowed research into this fascinating, yet under-studied topic. However, new image analysis techniques are being developed to observe 3D multi-root interactions in soil which promise to revolutionise our understanding (Figure 2D) [23, 140].

Conclusion

Roots perceive and interpret multiple, often overlapping, abiotic and biotic signals from soil. The key components of 3D RSA (root growth, branching, surface area and angle), are continuously being modified by these signals to optimise resource capture in highly

heterogeneous soil environments. The extraordinary plasticity exhibited during root development underpins the success of their foraging strategies. This review has described several major advances in our understanding of 3D root architecture gained through the use of novel imaging systems (Figure 2 & 5). However, a much greater understanding about the molecular mechanisms regulating how plants integrate these signals to control root developmental plasticity, and ultimately 3D RSA, is needed. Being able to non-invasively study gene expression throughout development in soil grown roots, using the GLO-roots imaging system, represents a major step forward to gaining a mechanistic understanding of RSA [111]. Similarly, models of 3D RSA are set to provide new knowledge about complex, non-linear processes, such as how multiple root architectural phenes interact to facilitate adaptation to a single (or multiple) abiotic stress(es) [90]. Such multi-scale mechanistic insights will underpin efforts to develop crops with improved root systems [91] and help address the urgent need for future crops better adapted to the challenge of climate change.

Supplementary Information

Supplemental information includes two videos.

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Figure legends

Figure 1. Heterogeneous soil structure, pore and water availability create a complex environment for root growth

(A-C) X-ray CT images of soil in tilled (i.e. after ploughing) and zero till (in this case after 15 years without ploughing) conditions [3]. (A) 3D rendered grey scale X-ray attenuation map of soil cores with a virtual 'cut-out' to reveal soil structure. In this case the darker the material, the lower the attenuation, hence the pore space appears as blacker regions. (B) Images highlighting 'solid' soil matrix in brown and 'pore' space in white. In this case there is no discrimination between water and air-filled pores so all pores appear in white though clear differences in size and shape can be seen (C) Images visualise the pore space only. This helps to appreciate the level of connectivity in the pores (shown to a high degree in the tilled soil and to a much lesser extent in the zero tilled soil). Scale bar is 10 mm (D) X-ray CT image of sandy loam soil (sieved < 2mm) where the soil is saturated i.e. almost all the pores are water filled. The water distribution is shown in blue, soil in grey and some air-filled pores in black. The size of the visualised region is approx. 2.5 x 2.5 x 7.5 mm. Scan resolution is 3 microns. See supplementary video 1.

Figure 2. Imaging solutions available to study root architecture

(A) *Triticum aestivum* root system imaged after 9 days of growth using a germination paper hydroponic phenotyping system [17]. (B) 3D root reconstruction of *Oryza sativa* created from 2D rotational image sequences of roots grown in a transparent gellan gum system [20]. (C) 3D surface renderings of MRI time lapse images of *Zea mays* growth at 6, 9, 12 and 15 days after sowing (DAS) show 3D root architecture but not soil structure. Maize was grown in pots with an 81 mm internal diameter and 300 mm tall [22] . (D) Interaction between two *Triticum aestivum* root systems grown in soil and imaged using X-ray CT. Root system information was recovered using the software tool RooTrak - for multiple interacting root systems (version 0.3.10). The column diameter was 5 cm [23].

Figure 3. Tap root and fibrous root systems

(A) X-ray CT rendered image of the tap root system of *Raphanus sativus* grown in a sandy loam arable soil for 58 days. (B) X-ray CT image of a 10-day-old *Triticum aestivum* root system grown in soil (column dimensions 7.5 cm [w] × 17 cm [h]). (C) Top down view of the whole *Triticum aestivum* root system shown in B. (D) Top down view of a single *Triticum aestivum* seminal root showing lateral root rhizotaxis. Applied bounding box has restricted lateral root length for image clarity.

Figure 4. Overview of lateral root development in *Arabidopsis thaliana*

(A) Time series of a developing young lateral root primordium expressing a plasmamembrane marker (UBQ10::YFP-PIP1;4) captured by confocal fluorescence microscopy. Scale bar = 50 µm. The lower panel depicts a sketch of cell contours seen in the

upper panel. Upon stage VII the cellular organization resembles the one seen in primary root tips, indicated by colours. 3D Illustration of a stage IV and emerged lateral root. (B) X-ray CT time series of a seedling growing in soil (column 1 cm [w] x 4 cm [h], imaged section 1 cm [w] x 1.8 cm [h]) over a period of 21 DAS. Day 3, 7, 10, 12, 14, 17, 19 and 21 are shown, see supplementary video 2.

Figure 5. Root development is altered in response to nutrients and water in the local soil environment in order to optimise root architecture for resource capture

(A) X-ray CT image of *Triticum aestivum* grown for 28 days with (left) and without (right) a high phosphate band. Increased root density is seen in the high phosphate section [67]. (B) *Hordeum vulgare* grown for 21 days in a sand culture split into three compartments. In the control samples a complete nutrient solution was supplied to all three compartments. In other samples only the middle section was supplied with the complete nutrient solution whilst the other sections were supplied with a solution deficient in nitrate or ammonium. Plants responded to the localised increase in nitrogen with an increased number and growth of laterals [66]. (C) *Arabidopsis thaliana* plants, imaged with the GloRoots imaging system, exposed to a water deficit (WD) after 13 DAS have enhanced xerotropism in the lateral roots compared with well-watered (WW) plants [111]. (D) X-ray CT image of *Zea mays* growing down an artificial air pore (left) and in a continuous volume of soil (right). The primary root exposed to both soil and air preferentially branches into the soil [30].

Supplementary video 1- 3D visualisation of the distribution of water in soil from X-ray CT data

Video showing the 3D reconstruction of an X-ray CT scan of sandy loam soil (sieved < 2mm) where the soil is saturated i.e. almost all the pores are water filled. The water distribution is shown in blue, soil in grey and some air-filled pores in black. The size of the visualised region is approx. 2.5 x 2.5 x 7.5 mm. Scan resolution is 3 microns.

Supplementary video 2- X-ray CT time series reconstruction of an Arabidopsis seedling growing in soil over a period of 21 days after sowing.

X-ray CT time series of an Arabidopsis seedling growing in soil (column 1 cm [w] x 4 cm [h], imaged section 1 cm [w] x 1.8 cm [h]) over a period of 21 days after sowing. Day 3, 5, 7, 10, 12, 14, 17, 19 and 21 are shown

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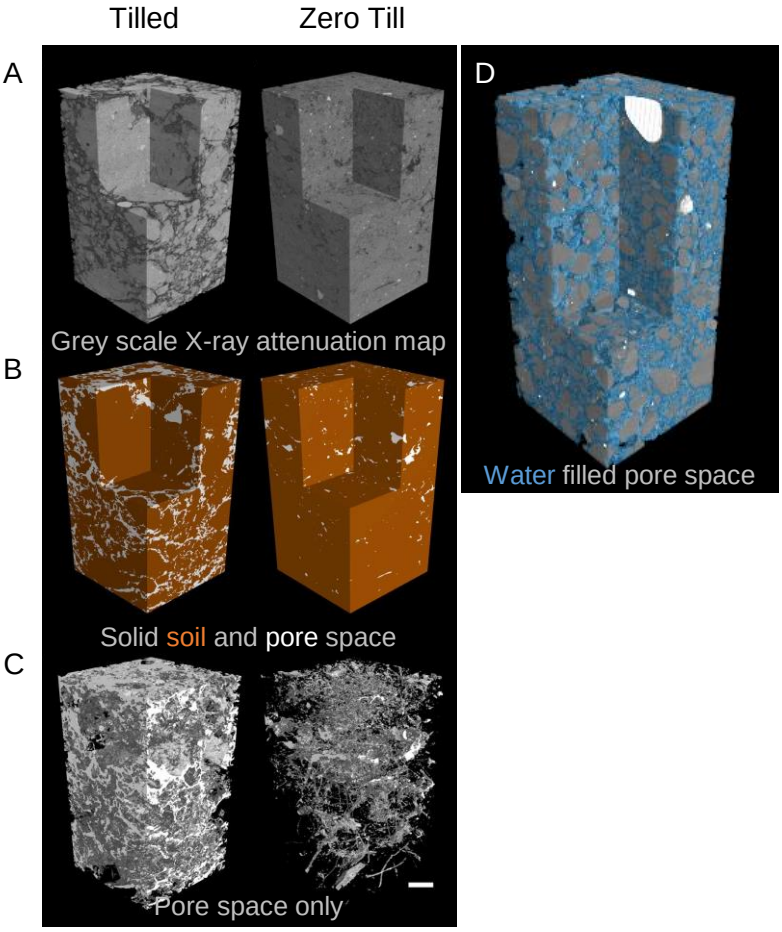
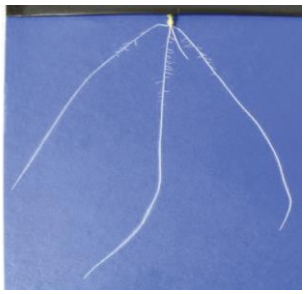

Tilled
Zero Till
A
Grey scale X-ray attenuation map
B
Solid soil and pore space
C
Pore space only
D
Water filled pore space

A Paper based screen

Triticum aestivum

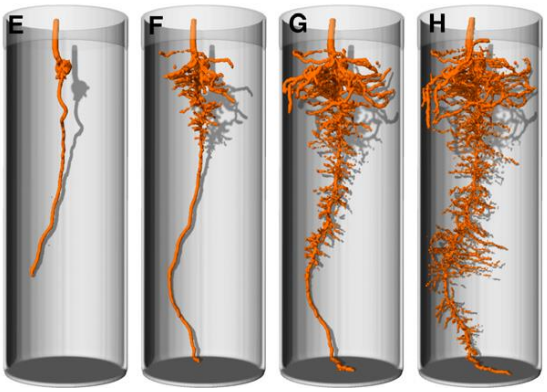


B Transparent gel system

Oryza sativa

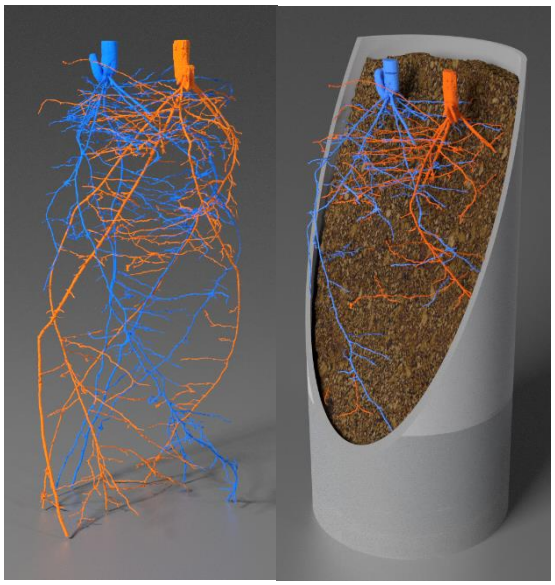
C Magnetic resonance imaging

Zea mays



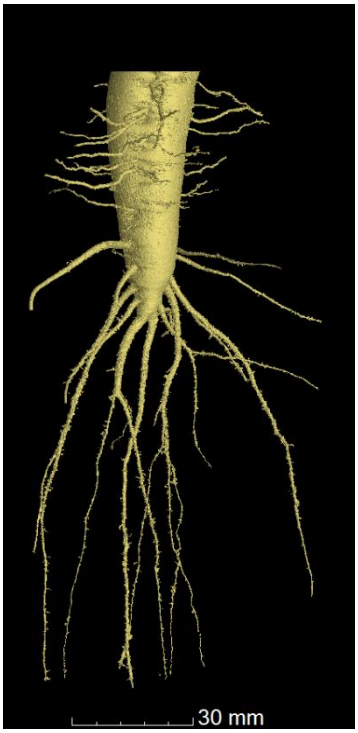
D X-ray Computed Tomography

Triticum aestivum



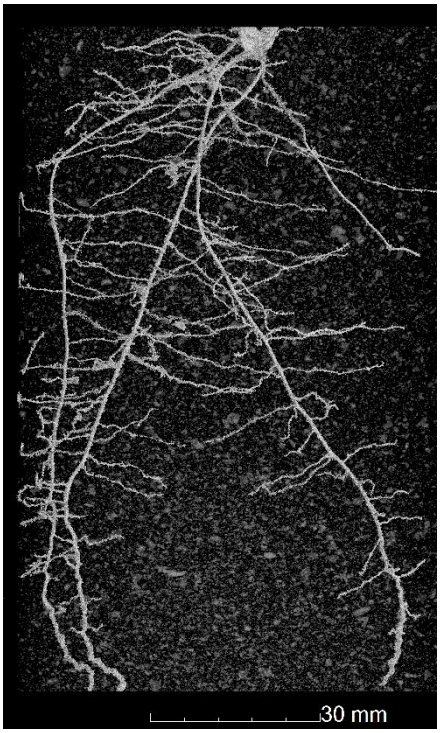
A Tap root

Raphanus sativus

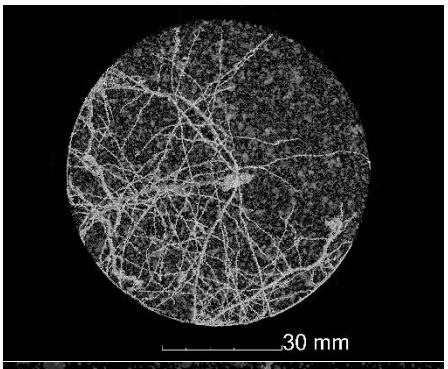


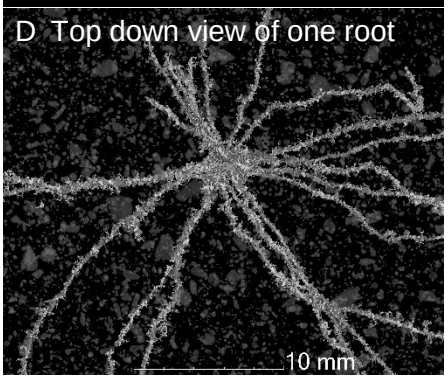
B Fibrous root

Triticum aestivum

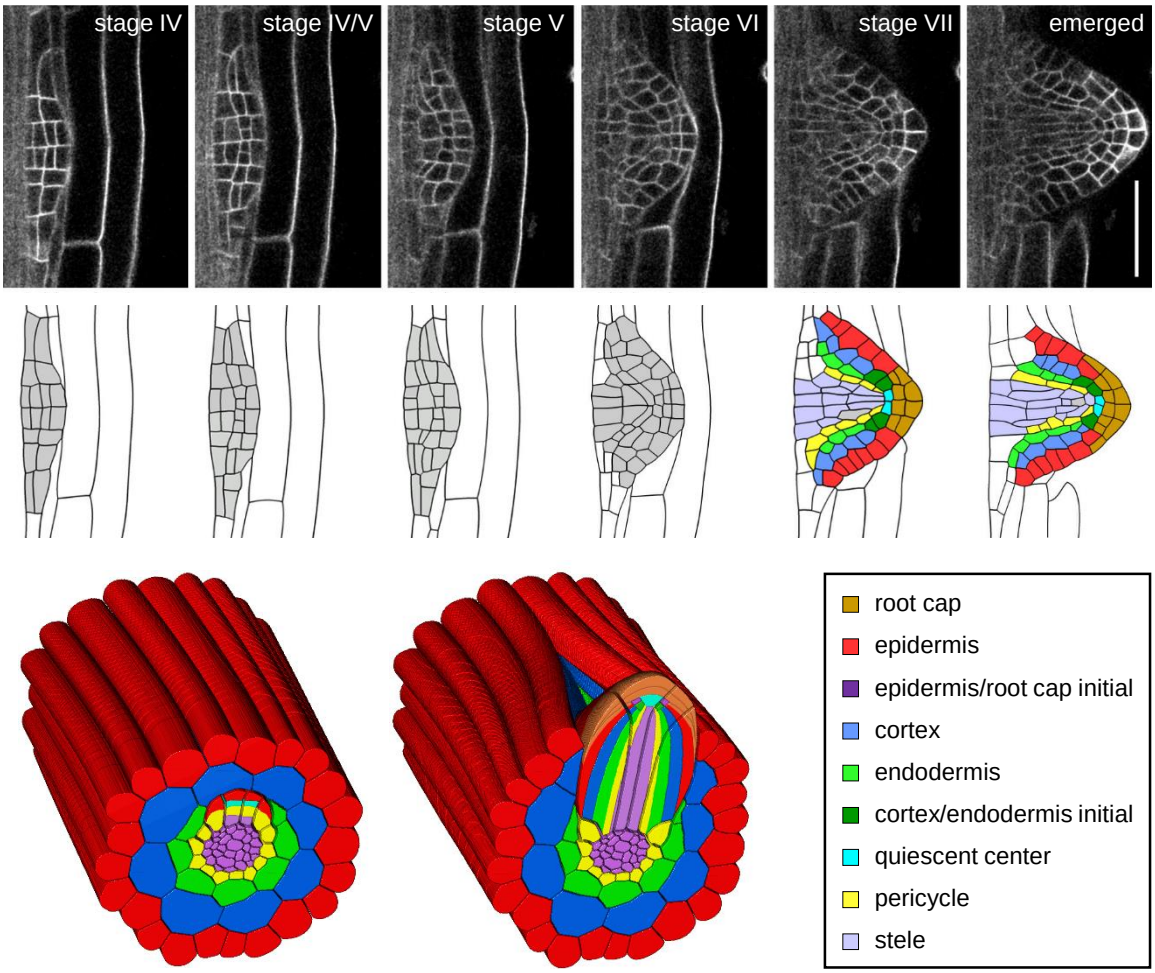


C Top down view of whole root system in panel B

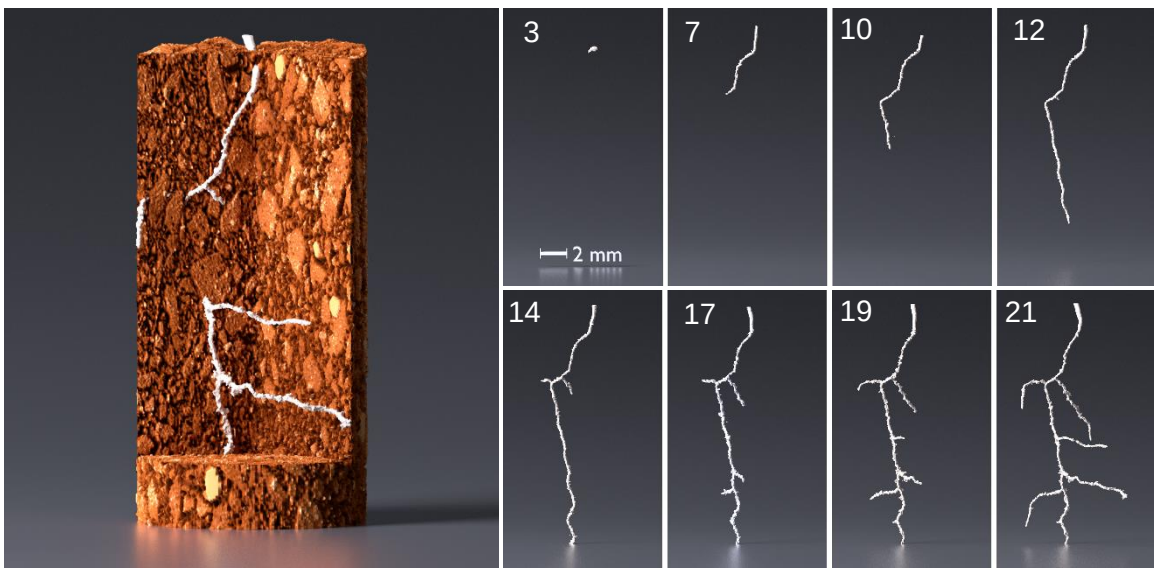




A Time series confocal imaging of a developing lateral root primordium

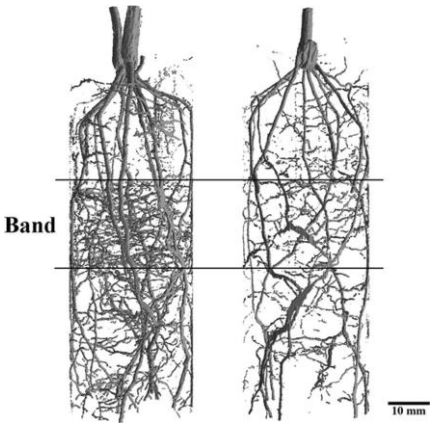


B X-ray CT time series of an *Arabidopsis thaliana* seedling growing in soil



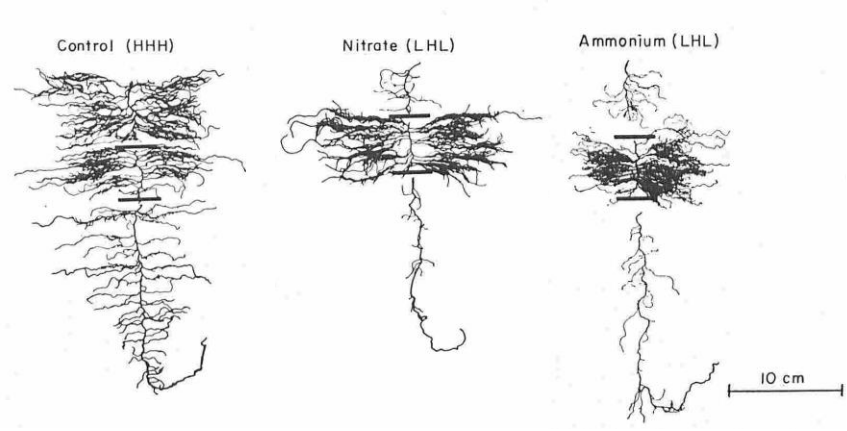
A Root proliferation in a band of high phosphate

Triticum aestivum



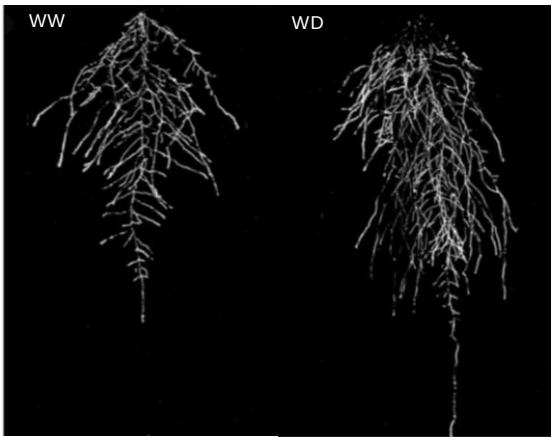
B Root proliferation in a band of high nitrate and ammonium

Hordeum vulgare



C Xerotropism

Arabidopsis thaliana



D Hydropatterning

Zea mays