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MARSCHNER REVIEW

Root anatomy and soil resource capture

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Abstract
Background Suboptimal water and nutrient availability are primary constraints in global agriculture. Root anatomy plays key roles in soil resource acquisition. In this article we summarize evidence that root anatomical phenotypes present opportunities for crop breeding.
Scope Root anatomical phenotypes influence soil resource acquisition by regulating the metabolic cost of soil exploration, exploitation of the rhizosphere, the penetration of hard soil domains, the axial and radial transport of water, and interactions with soil biota including mycorrhizal fungi, pathogens, insects, and the rhizosphere microbiome. For each of these topics we provide examples of anatomical phenotypes which merit attention as selection targets for crop improvement. Several cross-cutting issues are addressed including the importance of phenotypic plasticity, integrated phenotypes, C sequestration, in silico modeling, and novel methods to phenotype root anatomy including image analysis tools.
Conclusions An array of anatomical phenes have substantial importance for the acquisition of water and nutrients. Substantial phenotypic variation exists in crop germplasm. New tools and methods are making it easier to phenotype root anatomy, determine its genetic control, and understand its utility for plant fitness. Root anatomical phenotypes are underutilized yet attractive breeding targets for the development of the efficient, resilient crops urgently needed in global agriculture.

Keywords Root · Anatomy · Water · Nutrients · Transport · Insects · Pathogens · Mycorrhiza · Carbon sequestration · Modeling · Image analysis · Plasticity

Abbreviations

AM	Arbuscular Mycorrhizas
CCS	Cortical Cell Size
CCFN	Cortical Cell File Number
LCA	Living Cortical Area
MCS	Multiseriate Cortical Sclerenchyma
RCA	Root cortical aerenchyma
RCS	Root cortical senescence

Introduction

A better understanding of resource capture by plant roots is important because water and nutrient availability limit plant growth in the majority of terrestrial

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ecosystems. In natural ecosystems, improved understanding of this topic will expand our knowledge of key factors driving the productivity and function of these systems and will be useful in mitigating the increasingly severe consequences of global climate change and human encroachment. In managed ecosystems, such insight would create opportunities to sustain productivity despite environmental degradation and increasing population pressure. This is most clearly evident in the case of crop production. In rich nations, intensive fertilization and irrigation of crops is costly, damages the environment, depletes limited resources, and is unsustainable (Lynch 2007, 2019). In developing nations, low crop yields caused by drought and low soil fertility are primary constraints to food security, economic development, and political stability (Lynch 2007, 2019). These challenges are intensifying because of population growth, soil degradation, depletion of freshwater resources, and global climate change (Oldeman 1992; St. Clair and Lynch 2010; Mbow et al. 2019). We urgently need better crops and cropping systems that can sustain adequate yields with less demand for fertilizers and irrigation, while sustaining or improving soil fertility (Lynch 2007, 2019). Resource capture by plant roots is closely linked to soil exploration, and therefore carbon sequestration from the atmosphere, which is a promising avenue to mitigate global climate change (Kell 2011; 2012, Lynch and Wojciechowski 2015). Improved understanding of soil resource capture by plant roots is therefore an important component of the grand challenge of the twenty-first century: how to sustain 10B people while reversing environmental degradation.

While improved understanding of soil resource capture has manifold benefits, it would be directly useful in the breeding of more resource-efficient, stress-tolerant crops. The identification of traits improving soil resource capture is needed for the development of ideotypes for specific environments, for the deployment of specific traits in breeding programs (phenotypic selection), and when possible, for the use of marker-assisted or genotypic selection in molecular breeding (Lynch 2019). The large number of root traits affecting soil resource capture, and their genetic and mechanistic complexity, means that it is highly improbable to select optimal phenotypes on the basis of coarse metrics of plant performance such as yield under stress (Lynch 2019). While breeding programs that directly employ root phenotypes for improved water and nutrient capture are rare, they have been successful when attempted (e.g. Burridge et al. 2019).

In this perspective we provide an overview of root anatomical phenotypes that are potential targets to improve soil resource capture by crops. We do not attempt a comprehensive review of a very large topic, but rather highlight promising breeding targets based on the magnitude of potential benefits given natural phenotypic variation present in crops (rather than mutant studies for example), as evaluated in realistic environments, and discuss knowledge gaps and future prospects.

Soil exploration

Anatomy regulates the metabolic cost of soil exploration

The availability of water and nutrients in soil varies greatly in time and space and is often unpredictable (Lynch 2018). Soil exploration is therefore a primary challenge in plant biology. Since roots and their symbionts are heterotrophic, biomass allocation to roots reduces allocation to photosynthetic tissues, which can limit overall plant growth. This is especially true under conditions of edaphic stress, which in the case of the three primary soil resources—water, nitrogen (N), and phosphorus (P)—increases resource allocation to roots relative to shoots. For example, root maintenance respiration accounted for up to 72% of the growth reduction caused by suboptimal potassium (K) availability in maize plants in silico, and up to 38% of the growth reduction caused by suboptimal N or P availability (Postma and Lynch 2011b). The metabolic cost of soil exploration can be analyzed from the perspective of any limiting resource such as P, N, energy, or water, but is most often analyzed in the context of C fluxes and budgets, which are easier to measure than energy analogs and capture respiratory costs more readily than other currencies such as P, N, or water. More comprehensive microeconomic analyses can encompass opportunity costs, risk, and competition within and among organs and organisms (Lynch 2015). In the context of soil exploration, analysis of resource efficiency in terms of the costs of root elongation, root depth, or volume of soil explored is often useful. In accord with microeconomic theory, a plant that can acquire a limiting soil resource at reduced metabolic cost would benefit by being able to acquire more of the limiting resource as well as by having greater availability of internal resources to allocate to competing plant functions, such as the growth of photosynthetic tissue, resource storage, and reproduction.

Anatomy is a primary determinant of the metabolic costs of root construction and maintenance. Some tissues are more metabolically demanding than others. For example, mature xylem vessels and some sclerenchyma cells are dead, in contrast with xylem parenchyma or phloem companion cells which are highly active. Cell walls, cytoplasm, and vacuole have very different construction and maintenance costs. Living cell types have varying proportions of polysaccharides, protein, and nucleic acids. Some cells like cortical parenchyma are relatively expendable while others like phloem cells are critical for root function. Anatomical features like aerenchyma regulate oxygen availability and thereby respiration. By determining the proportion of living and dead cells, highly active vs. less active cells, cell composition and oxygen availability, root anatomy is a key determinant of the metabolic costs of soil exploration.

Reducing cortical burden increases soil exploration

Root cortical parenchyma is often ephemeral and has variable dimensions, suggesting that it may be reduced without loss of critical functions. Reduced metabolic costs of cortical tissue may be especially useful in monocots, which have a persistent cortex, unlike dicots, in which the cortex is destroyed in secondary growth (Postma and Lynch 2011a; Strock and Lynch 2020) (see "Secondary growth in dicotyledonous species" in this article). Jaramillo et al. (2013) measured this 'cortical burden' as living cortical area (LCA, *i.e.* transversal root cortical area minus aerenchyma area and intercellular air space), and showed that LCA is highly correlated with root respiration, and reduced LCA is associated with greater drought tolerance among contrasting maize phenotypes (Jaramillo et al. 2013). Several anatomical phenes ('phene' is a fundamental unit of the phenotype, as opposed to phene aggregates, sensu (York et al. 2013)) may reduce LCA, thereby reducing the metabolic cost of soil exploration, including root cortical aerenchyma (RCA), root cortical senescence (RCS), and cortical cell file number (CCFN) (Lynch 2019).

Root cortical aerenchyma (RCA) is caused by programmed cell death of cortical parenchyma, resulting in air-filled lacunae (Fig. 1). Although RCA has been primarily researched for its role in oxygenation of root tissue under hypoxia (Jackson et al. 1985), constitutive RCA formation is common in grasses. In addition to hypoxia, RCA is induced by a range of abiotic stresses (Jackson et al. 1985), including drought (Zhu et al. 2010a; Chimungu et al. 2015b), heat (Hu et al. 2014), and suboptimal availability of N (Saengwilai et al. 2014), P (Fan et al. 2007; Galindo-Castañeda et al. 2018), and S (Bouranis et al. 2003). RCA formation under edaphic stress could be adaptive by reducing the metabolic costs of soil exploration by converting living cortical parenchyma into air space, thereby reducing root nutrient content and respiration. RCA formation also reduces the radial transport of water and nutrients to the stele (Fan et al. 2007; Hu et al. 2014; Bo et al. 2014). Reduced radial transport of water may not be disadvantageous in dry soils since the majority of water uptake occurs in lateral roots (Schneider and Lynch 2018) and younger root tissue before RCA develops. Reduced radial transport offers potential benefits of parsimonious water capture under drought, which would conserve soil water for future use, and enforce more efficient use of water via reduced leaf growth and stomatal aperture. Likewise, under nutrient stress, reduced radial nutrient transport of mature root axes with RCA may not be detrimental since most nutrient capture occurs by younger root tissues and lateral roots. In silico analysis found substantial fitness benefits for RCA in maize growing in soils with suboptimal availability of N, P and K, via reduced root respiration as well as nutrient reallocation from cortical tissue (Postma and Lynch 2011a). The benefits of RCA for P capture were greater in maize than bean (Postma and Lynch 2011b), showing the importance of a persistent root cortex in monocots, as opposed to dicots, which lose their root cortex through secondary growth (Strock et al. 2018). In silico results were confirmed in empirical studies in the field and in greenhouse mesocosms under suboptimal N and P availability, where RCA

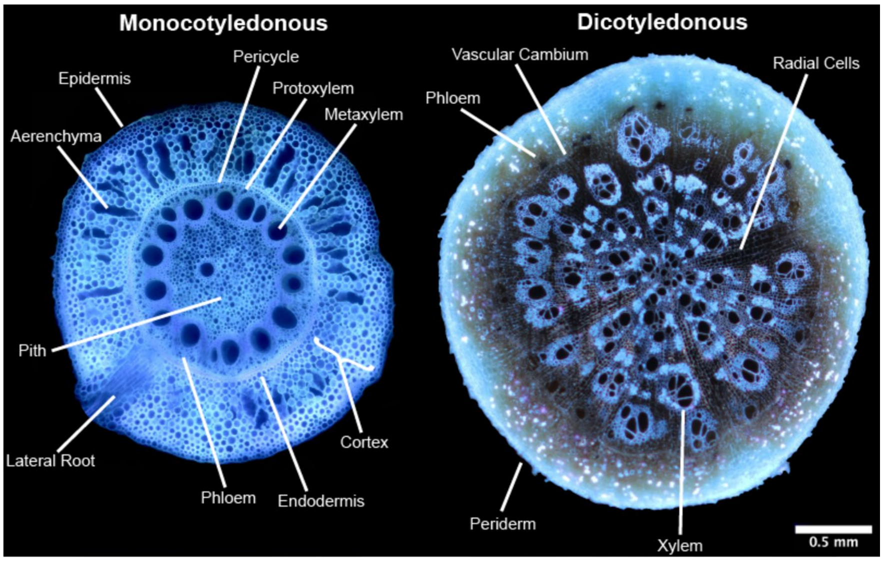

Fig. 1 Comparison of maize (*Zea mays*) and common bean (*Phaseolus vulgaris*) root cross-sections highlighting differences in the anatomical arrangement between monocotyledonous and dicotyledonous species. Dicot roots are characterized by having a vascular cambium (lateral meristem) that produces new xylem and phloem continuously through secondary growth, thereby destroying primary tissues like the epidermis, cortex, and endodermis. Contrastingly, most monocots lack a lateral meristem and have a persistent cortex that encircles a central stele containing a fixed number of metaxylem and phloem. All cross-sections are at the same scale. Scale bar = 0.5 mm

formation among contrasting maize phenotypes was associated with reduced root respiration, greater root growth, greater N and P capture, better shoot nutrient status under nutrient stress, and hence greater photosynthesis, growth, and yield (Saengwilai et al. 2014; Galindo-Castañeda et al. 2018). Similar benefits were observed for RCA formation under drought stress, where greater RCA formation among contrasting maize lines was associated with reduced root respiration, greater rooting depth, better shoot water status, leaf photosynthesis, plant growth, and yield (Fig. 2; Zhu et al. 2010a). These results were supported by analysis of maize yields under natural drought environments in Malawi, which found greater yields in high RCA landraces than in low RCA landraces (Chimungu et al. 2015b). A recent study associated greater RCA with drought adaptation in specific phenotypic clusters of diverse maize inbreds (Klein et al. 2020).

Root cortical senescence (RCS) is similar to RCA in being formed via programmed cell death but differs in having more restricted taxonomic distribution, having been reported in the *Poaceae*, including principal cereal crops such as wheat, barley, rye, and oat, and in causing entire loss of the cortex instead of the formation of discrete lacunae as in RCA (Schneider and Lynch 2018) (Fig. 3). As with RCA, loss of cortical parenchyma by RCS reduces root respiration and nutrient content as well as radial water and nutrient transport (Schneider et al. 2017b). In silico analysis showed that these effects would be beneficial for barley plants growing with suboptimal availability of N, P, and K (Schneider et al. 2017a). As with RCA, reduced radial water transport in older axes caused by RCS may not be detrimental under drought because such root segments are not normally active in water transport, although further research is needed (Schneider and Lynch 2018).

Another avenue to reduced cortical burden is simply by reducing the number of parenchyma cells in the cortex, as shown in maize, which displays substantial phenotypic variation for the number of cortical cell files in nodal roots (CCFN, Fig. 2). Among contrasting maize phenotypes under drought stress, reduced CCFN was associated with reduced root respiration, greater rooting depth, better water capture from deep soil, better shoot water status, leaf photosynthesis, growth, and yield (Lynch et al. 2014). In silico analysis suggests similar benefits under

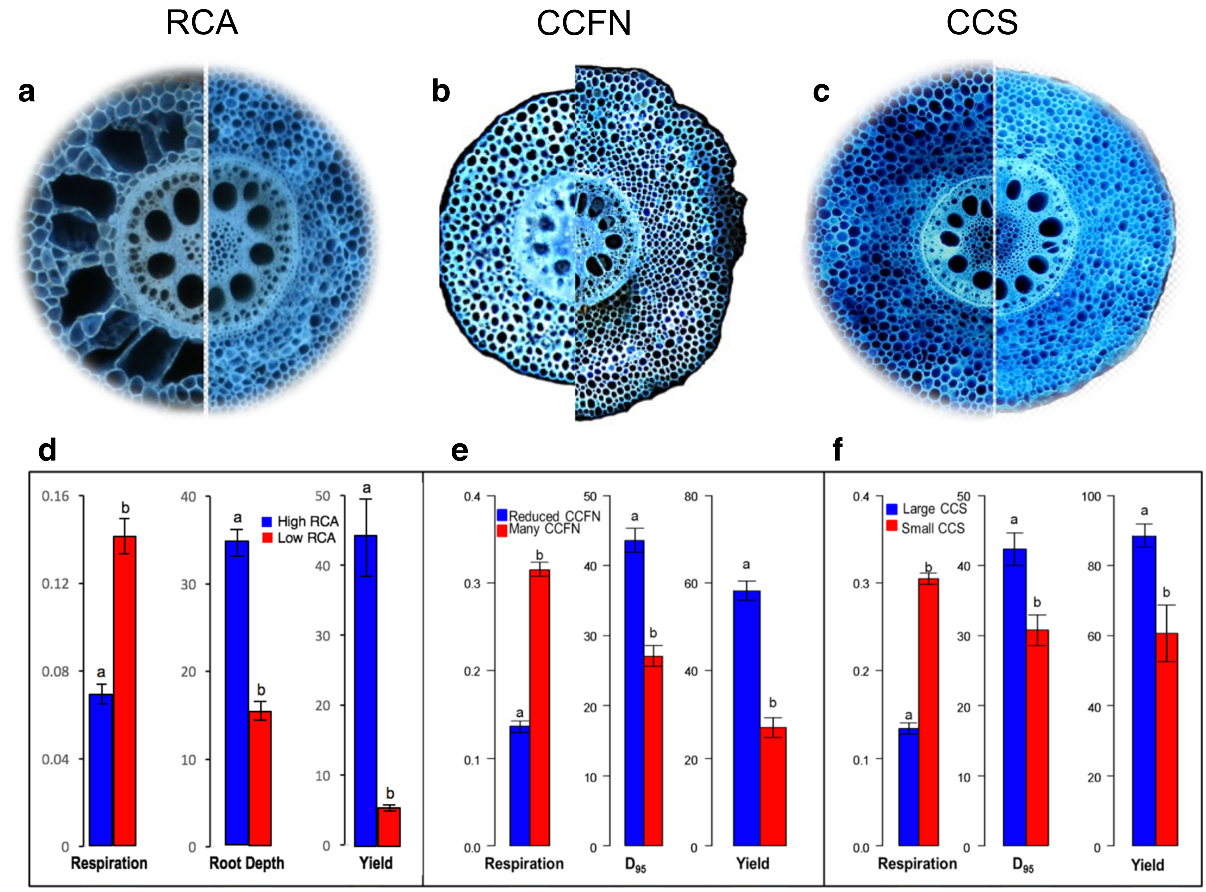

Fig. 2 Phenotypic variation in maize for root cortical aerenchyma (RCA, **a**), cortical cell file number (CCFN, **b**) and cortical cell size (CCS, **c**). (**d**) Under water stress, genotypes with greater RCA have less respiration (nmol CO_2 s^{-1} cm^{-1}), deeper rooting (a, cm roots at 40–50 cm soil depth; b, c, D_{95}, which is the depth in cm attained by the 95th percentile of roots), and greater yield (g per plant), as did genotypes with reduced CCFN (**e**) and greater CCS (**f**). Data shown are means ± SE ($n = 3$ or 4). Means with different letters are significantly different ($P \leq 0.05$). Redrawn from Lynch (2018)

suboptimal availability of N, P, and K (Yang et al. 2020).

RCA, RCS, and CCFN all affect living cortical area- the symplastic area of the cortex as observed in cross sections. An alternative avenue to reduced cortical burden without concomitant reduction of root diameter is by enlarging cortical cell size (CCS, Fig. 2), since larger cells have proportionately greater vacuole/cytoplasm volume, given that the cytoplasm is much more metabolically active than the vacuole. Among contrasting maize phenotypes under drought stress, greater CCS was associated with reduced root respiration, greater rooting depth, better water capture from deep soil, better shoot water status, leaf photosynthesis, growth, and yield (Chimungu et al. 2014a). In silico analysis suggests comparable benefits under suboptimal availability of N, P, and K (Yang et al. 2020). Larger CCS in wheat grown in compacted soils reduced root respiration by ~ 50% and improved penetration of hard soils (Colombi et al. 2019).

Taken as whole, the fact that RCA, RCS, CCFN, and CCS all improve the capture of soil resources supports the proposal that anatomical phenotypes that reduce the metabolic costs of soil exploration, by reducing nutrient content and respiration, promote greater soil exploration and therefore are beneficial under drought and low soil fertility. It is noteworthy that comparable benefits were observed in maize for greater RCA, reduced CCFN and larger CCS under drought despite the fact that these three

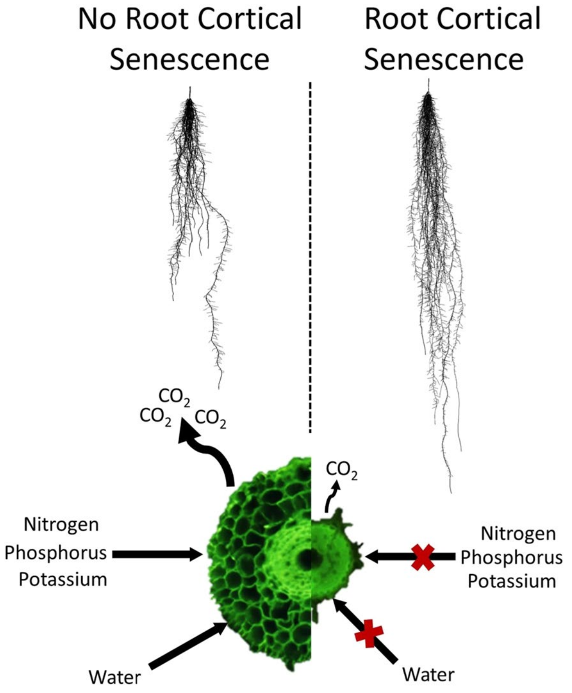

Fig. 3 Schematic of RCS and soil resource capture. In edaphic stress, plants with RCS have greater root length, reduced root respiration, and reduced radial water and nutrient uptake. Greater root growth in plants with RCS is driven by savings in metabolic costs of root tissue. Reduced radial water and nutrient transport of axial root tissue after RCS has small effects on total plant nutrient uptake, as lateral roots do not form RCS and perform the majority of root nutrient and water uptake. From Schneider and Lynch (2018)

phenes are under distinct genetic and developmental regulation (Chimungu et al. 2014a, b, 2015a, b; Lynch et al. 2014) (Fig. 2). Benefits of RCA for N (a mobile resource) and P (an immobile resource) capture in low fertility soil are congruent with benefits for water capture under drought. Comparable benefits were observed in the field, in greenhouse mesocosms with simplified soil biota, and in silico, which is a highly simplified environment that serves to test the adequacy of a logic model.

Secondary growth in dicotyledonous species

In dicotyledonous species, secondary growth causes thickening of roots as they age and develop (Fig. 4). Radial expansion is a result of cellular divisions that occur within the vascular cambium and cork cambium (or phellogen) (Fig. 1; Evert and Eichhorn 2006). The deposition of secondary tissues produced by these cambia ultimately cause the destruction of the primary root tissues (epidermis, cortex, and endodermis) (Dickison 2008). The formation of these secondary tissues includes metaxylem vessels that are produced centripetally (inside) and phloem centrifugally (outside) to the vascular cambium, while a protective tissue called phellem is produced centrifugally to the cork cambium (Sanio 1873; Larson 1994; Evert and Eichhorn 2006; Smetana et al. 2019). In annual dicotyledonous crops like *Phaseolus* species, most of the root thickening from the production of these secondary tissues is driven by the production of metaxylem elements and parenchyma centripetally to the vascular cambium (Strock and Lynch 2020). These dramatic anatomical changes that occur via secondary development effectively shift the functional role of a root from soil resource capture to axial transport of water and nutrients (Fig. 4; McCully 1999a; Steudle 2000; Strock et al. 2018). Specifically, the destruction of the primary tissues and deposition of heavily lignified and suberized secondary tissues decrease the absorptive capacity of roots as they age while the production of metaxylem increases capacity for axial conductance (Bouma et al. 2001; Volder et al. 2005;

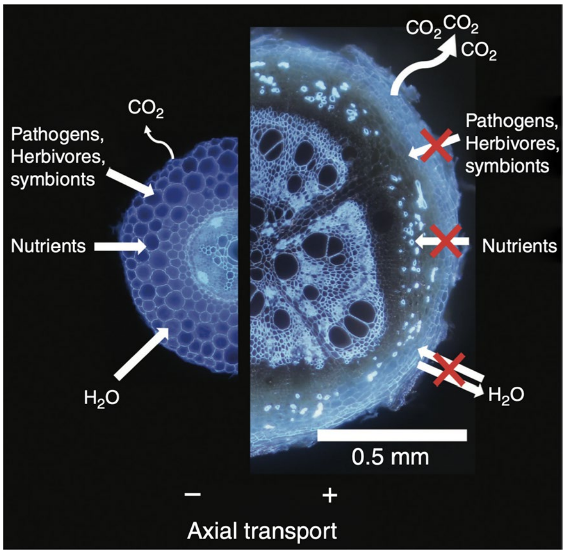

Fig. 4 Diagram summarizing the relationships between secondary growth of roots and soil resource acquisition, metabolic costs, and interactions with soil organisms. Images of cross-sections from roots of common bean (*Phaseolus vulgaris*). All cross-sections are at the same scale. Scale bar = 0.5 mm. Modified from Strock and Lynch (2020)

Guo et al. 2008; Rewald et al. 2011). From a functional perspective, this restriction of radial transport may serve an important role in preserving the integrity of the vasculature under drought by hydraulically isolating older root segments from drying surface horizons and preventing leakage and air seeding through pit membranes (Sperry and Saliendra 1994; Hacke and Sperry 2001; Zwieniecki et al. 2002; Cuneo et al. 2016). Additionally, the production of these secondary tissues serves to provide mechanical support for the growing shoot and increase resistance to edaphic herbivores and pathogens (Eissenstat 1992; Valenzuela-Estrada et al. 2008; Gerrienne and Gomez 2011; Hoffman and Tomescu 2013).

As the heavily lignified and suberized periderm is developed during secondary growth, radial transport of water and nutrients restricted, and the periderm also plays a significant role in protecting the root from biotic stress (Eissenstat 1992; Guo et al. 2008; Valenzuela-Estrada et al. 2008; Rewald et al. 2011). Deposition of suberin in this secondary tissue is a key component in inhibiting penetration by soil pathogens such as *Phytophthora* (Lulai and Corsini 1998; Valenzuela-Estrada et al. 2011; Machado et al. 2013), and in *Malus domestica* the intensity of pathogen colonization in roots was closely linked to the senescence and loss of the cortex (Emmett et al. 2014). Secondary growth also impairs the formation of mycorrhizae. In *Vitis* ssp., root age significantly affects formation of arbuscular mycorrhizae, where younger roots have more arbuscules and older roots have more vesicles and/ or spores (Vukicevich et al. 2019). Under phosphorus deficit, secondary growth of *P. vulgaris* roots is suppressed, thereby prolonging AM (Strock et al. 2018). Valenzuela-Estrada et al. (2008) similarly observed in *Vaccinium* ssp. that roots with greater radial growth had less mycorrhizal colonization.

In most plants, activity of the vascular cambium is indeterminate, suggesting that the process of secondary growth is regulated by homeostatic mechanisms (Tomescu and Groover 2019). While the constitutive nature of secondary growth in dicot roots suggests that this process increases fitness in most environments, a significant level of plasticity in the meristematic activity of the vascular cambium does afford the capacity to respond to the environment (Brewer et al. 2013; Strock and Lynch 2020). In some environments, root secondary development can hinder overall plant growth as radial expansion increases root metabolic and construction costs. Specifically, as secondary growth progresses, the metabolic (i.e. carbon, nutrient and energy) costs of producing and maintaining root length increase, and it has been proposed that suppression of secondary growth in some environments may be an adaptive strategy to improve the metabolic efficiency of soil exploration (Lynch 1995, 2007; Lynch and Brown 2008; De la Riva and Lynch 2010; Strock et al. 2018; Strock and Lynch 2020). Allocation of resources to greater total root length rather than radial thickening facilitates greater exploration of soil domains where resources are more abundant. Even small changes in the density of root tissue can have significant effects on the soil volume explored per unit of carbon invested (Ma et al. 2018). For example, under P deficit, suppression of secondary growth is associated with greater root elongation, increased soil exploration and greater phosphorus acquisition (Strock et al. 2018).

Reallocation of resources within a plant is a hallmark adaptive response to nutrient stress (Fohse et al. 1988), and further investigation into the effect of nutrient and water limitation on secondary growth of roots is warranted. While the influence of radial thickening of roots on metabolic and construction costs is obvious, because secondary growth affects multiple aspects of root function, the utility of suppressing this developmental process could be limited to specific environments. Thin roots with a low tissue density are more metabolically efficient in soil exploration, but have less hydraulic conductance, root longevity, and ability to penetrate strong soils (Bengough et al. 2006; Strock and Lynch 2020). Targeted studies exploring how abiotic and biotic factors affect this process, as well as the relevance of secondary growth in roots to fitness of annual dicotyledonous crop species under stress, are needed. Interactions between root secondary growth, edaphic conditions and soil resource acquisition may have significant effects on plant fitness. Specifically, the implications of secondary growth for resource capture, axial transport, metabolic costs and interactions with soil organisms in roots of annual crops are important research foci that warrant investigation (Strock and Lynch 2020).

Thicker cortical cell walls/apoplastic phenes

The primary cell wall is constructed during cell division and is extended during cell expansion (Cosgrove 1999, 2005; Höfte and Voxeur 2017; McQueen-Mason and Cosgrove 1995; Zhong et al. 2019). In roots, secondary cell walls are deposited in mature cells depending on the cell type, *e.g.* xylem tracheids have thicker secondary cell walls as compared to cortical parenchyma (Zhong et al. 2019) and secondary walls occupy most of the apoplastic volume in mature roots. Secondary cell walls are mainly composed of cellulose, hemicellulose, lignin, and pectin (Somssich et al. 2016; Zhong et al. 2019). Dicots have higher pectin percentage while in monocots the role of pectin is replaced by glucorono-arabinoxylan (Carpita 1996; Carpita and Gibeaut 1993; Jarvis et al. 1988; Riboulet et al. 2008). Because cell walls are rich in carbohydrate, they require significant initial carbon and energy investment, but because they have low metabolic activity, are not costly to maintain. We propose that the maintenance cost of the cell walls would be much less than the cost of maintaining a similar volume of living cell lumen. Therefore, cell wall volume and composition may influence the metabolic costs of soil exploration, by analogy with root cortical phenotypes (see "Reducing cortical burden increases soil exploration").

Root hairs

Root hairs are subcellular outgrowths of root epidermal cells that are present in nearly all land plants (Fig. 5). Their role in nutrient acquisition, soil adhesion, and microbe interaction has been postulated for nearly a century (Comber 1922). Early reports indicate that a single rye plant could have as many as 4 billion root hairs, leading to an increase in surface area of 400 m^2 (Dittmer 1937). Root hairs have long been known to play an important role in nutrient uptake, especially phosphate, as found through modeling and empirical studies (Bouldin 1961; Nye 1966; Barley and Rovira 1970; Bhat and Nye 1974; Itoh and Barber 1983). As phosphate is immobile in soil and the soluble phosphate pool is slowly replenished, a root must access new pools of phosphate by coming into contact with soil which has not been recently depleted of phosphate (Barber 1995). New root growth into these areas can be costly, but root hair growth has low carbon costs and can allow for an increase in the size of the depletion zone at little cost to the plant (Bates and Lynch 2000). Potassium

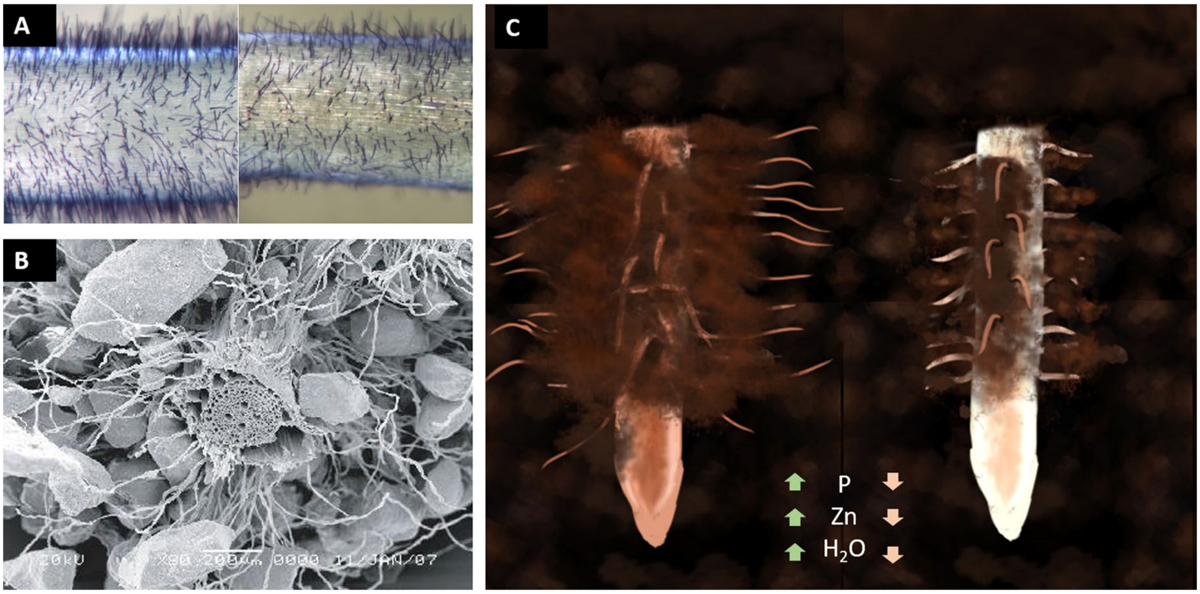

Fig. 5 Importance of root hairs and rhizosheaths in improving nutrient uptake and plant performance. **A**) Scanning electron micrograph of root hair less mutant (Rht-1). **B**) Scanning electron micrograph of wild type – B73, compare the density and length of root hairs with Rht-1. **C**) None to bare minimum rhizosheath formation on the Rht-1 root and **D**) Root hairs promoting rhizosheath formation in wild type B73. Increased rhizosheath formation improves nutrient uptake e.g. P, Zn and improves stress tolerance e.g. drought

is also immobile in soil, though to a lesser extent than phosphate, and root hairs contribute to uptake in a similar fashion (Jungk 2001). Similarly, uptake of zinc in barley was inhibited in a hairless mutant (Genc et al. 2007).

The advantage of increased root hair length and density for phosphate acquisition has been shown in a number of species, through both the use of mutant studies and analysis of natural phenotypic variation (Gahoonia and Nielsen 1997, 2004; Gahoonia et al. 2001; Bates and Lynch 2001; Zhu et al. 2005; Nestler and Wissuwa 2016). The longevity of an individual root hair, generally thought to be active for less than 48 h, is a trait that has been studied less, and its contribution to nutrient acquisition is not as well understood but could be beneficial in specific soil conditions (Brown et al. 2013). In addition to being beneficial for nutrient acquisition, root hairs are also highly responsive to their surrounding environment. Root hairs of multiple species, including Arabidopsis and maize, become longer and denser under P stress (Foehse and Jungk 1983; Bates and Lynch 2000; Ma et al. 2001a, b; Zhu et al. 2010a, b; Vejchasarn et al. 2016; Giri et al. 2018). In silico analysis showed that increased root hair length and density (and in Arabidopsis, the position and geometry of their initiation) are synergistic for P capture, *i.e.* their benefits for P capture are much greater in combination than in isolation (Ma et al. 2001b). Other nutrient stresses, including iron (Müller and Schmidt 2004), manganese (Wei Yang et al. 2008), ammonium (Liu and von Wirén 2017), and nitrate (Canales et al. 2017) alter root hair length and/or density. The root hair growth response is rapid in response to low P (Bhosale et al. 2018; Janes et al. 2018). The majority of our understanding of root hair growth and P responses derives from problematic artificial growth systems (Nestler et al. 2016; Hanlon et al. 2018) using Arabidopsis, which has a unique root hair formation pattern and is non-mycorrhizal (Pemberton et al. 2001; Dolan 2017).

Root hairs have also been implicated in the acquisition of water and mobile soil resources. Recent work has shown that root hairs reduce the drop in matric potential at the root surface, leading to increased water uptake (Carminati et al. 2017). Increased root hair length and density enhanced water acquisition in barley in droughted field conditions, conferring a yield advantage (Marin et al. 2020). The specifics by which root hairs impact water acquisition are confounded by multiple factors, including differences in water uptake at different times of day (Carminati et al. 2017), differences in osmotic potential at the root surface due to nutrient concentration (Carminati et al. 2017), and differences in evaporative demand (Dodd and Diatloff 2016). The role of root hairs in water uptake is directly related to rhizosheath formation (Rhizosheaths). Further understanding of the interplay of root hairs and rhizosheaths is necessary to understand how water uptake and soil contact occurs at the root surface. Root hair length and density are both increased under low nitrogen availability in maize (Gaudin et al. 2011). In Arabidopsis, different accessions have differential root hair length and density responses to nitrogen conditions dependent on the form (ammonia versus nitrate) (Vatter et al. 2015). However, no work has yet shown a direct link between root hair traits and nitrogen acquisition. Investigation of root hair traits in leguminous species such as soybean, alfalfa, pea or bean may be further complicated by rhizobial symbiosis, as the root hair is the starting point for these interactions (Ibáñez et al. 2017).

In addition to their direct benefits for soil resource capture, root hairs contribute to the formation of rhizosheaths (see "Rhizosheaths") and have been proposed as being important for the penetration of root axes into hard soil (see "Anatomical phenes that improve adaptation to soil mechanical impedance").

Many studies investigating the functional role of root hairs have been completed using root hairless mutants, such as *brb* in barley and *rth1*-*rth6* in maize. Though these allow for targeted experimental design, they fail to consider pleiotropic effects of the genetic mutation and the complex roles of root hairs in maintaining a normal plant-soil interface. In maize, *rth1* has short root hairs, but a dwarf phenotype (Wen and Schnable 1994). The *rth3* mutant in maize lacks root hairs, has no obvious aberrant shoot phenotypes, but has greatly reduced yield (Hochholdinger et al. 2008). The *rth3* mutant has altered root system architecture, forming many more fine roots (Klamer et al. 2019). The *brb* mutant lacks apoplastic barriers (Burke et al. 2020), which could greatly alter patterns of hydraulic flow and nutrient uptake as compared to its wildtype counterpart, and has altered root architecture (Dodd and Diatloff 2016). The *brb* mutant can effectively replace root hairs with mycorrhizal interaction (Jakobsen et al. 2005), though the complexities of this relationship across species and in naturally varying populations is less well understood (Maherali 2014). A different set of mutants in barley that alter root hair formation and growth have been isolated, though these have not been studied extensively in terms of nutrient responses or acquisition (Chmielewska et al. 2014). One of the isolated mutants, *rhs1.a,* had pleiotropic growth effects, similar to what had been seen with *rth1* in maize (Chmielewska et al. 2014). Root hairs, mycorrhizae, and rhizosphere development may all have overlapping roles in nutrient acquisition at the root surface, but our standard methods by which we study these relationships may currently be lacking. Using the hairless *rth3* mutant of maize, Kumar et al. (2019) found plants had increased mycorrhizal colonization, presumably to increase P acquisition in the absence of hairs. The *rth3* plants also had more fine lateral roots, indicating that these mutations can impact the entire root architectural system and are not solely isolated to root hairs. As root hairs are only one mechanism by which plants obtain nutrients from soil, they must be considered within this larger context. Exploiting natural variation in crop plants, including bean, maize, and rice may allow for targeted selection and improvement in the field, as well as a more nuanced understanding of how natural variation for root hair phenotypes contributes to resource capture (Brown et al. 2013; Lynch 2019; Burridge et al. 2019).

Rhizosheaths

Rhizosheaths are sheaths of small soil aggregates adhered to an immature root surface (Fig. 5; McCully 1999b). Rhizosheaths form up to 5 cm basipetal to the root tip (depending on the root elongation rate) and this zone can be 15–30 cm long (McCully 1999b; St. Aubin et al. 1986; Watt et al. 1994). They are generally identified by carefully excavating roots from soil and then shaking the loose soil, with remaining soil adhering to roots being considered rhizosheath (McCully 1999b). Rhizosheaths were first reported in desert taxa (Wullstein and Pratt 1981) but now have been reported in many mesophytic grass taxa (Bailey and Scholes 1997; Duell and Peacock 1985) including principal crops like wheat (Basirat et al. 2019; James et al. 2016), barley (Haling et al. 2014), rice (Zhang et al. 2020), maize (McCully 1999b; St. Aubin et al. 1986), foxtail millet (Liu et al. 2018) and sorghum (Duell and Peacock 1985).

Mucilage released by epidermal cells and border cells along with root hairs play major roles in the formation of rhizosheaths (McCully 1999b). Mucilage released by the border cells is believed to first act as a lubricant for tip growth and assists rhizosheath development (Watt et al. 1994). Alternate wetting and drying of mucilage give rise to its important adhesive properties (Watt et al. 1993, 1994), which leads to adherence of soil aggregates to roots as well to each other (Fig. 5B). Selecting for mucilage phenes is challenging because not much is known about the genetics of mucilage secretion that can be directly applied in breeding (Vermeer and McCully 1982).

Root hair length is related to rhizosheath size to different degrees in different species; however, root hair presence is required for the maximum expression of the rhizosheath (Haling et al. 2014; George et al. 2014; Delhaize et al. 2015; Brown et al. 2017) although root hair length may not improve rhizosheath mass (Brown et al. 2017). In contrast, other studies in wheat (Haling et al. 2010) and barley (Brown et al. 2012) showed that root hair length improves rhizosheath mass. The link between root hair length and rhizosheath formation deserves further investigation. Increasing the size of the rhizosheath may contribute to increased water uptake and resistance to soil drying (Choi and Cho 2019; Basirat et al. 2019). Root hairs and rhizosheaths improve plant performance under low phosphorus conditions (Brown et al. 2013; Nielsen et al. 1998; Zhu et al. 2010a, b), aluminum stress (Delhaize et al. 2015, 2012), combined low P and drought stress (Brown et al. 2012), and assist in associative nitrogen fixation (Bergmann et al. 2009). Root hairs release exudates, including phosphatases, that mineralize phosphate and promote interactions with soil microbes (Holz et al. 2017, 2020; McKay Fletcher et al. 2020). The extent to which a plant can capture P from surrounding soil is greater than the individual contributions of either root hairs or rhizosphere formation, indicating a synergistic relationship between the two components (Holz et al. 2020). This highlights the importance of root hairs for rhizosheath formation and function, reinforcing their potential value as selection targets for crop improvement (Lynch et al. 2014).

Other major functions of the rhizosheath include providing a habitat for microorganisms (Vermeer and McCully 1982) and improved acquisition of nutrients like zinc (Nambiar 1976). It is also known to help plants better tolerate acidic soils (Delhaize et al. 2015, 2012), and mechanical stress (Haling et al. 2014, 2013). Most of these functions are attributed to the rhizosheath's property of maintaining contact between the soil and roots (Fig. 5C). The exact mechanism by which the rhizosheath can improve plant performance is poorly understood. For example, the mucilage in the rhizosheath improves water holding capacity of this zone (Watt et al. 1993; Young 1995) and reduces the risk of an air gap between soil and roots (McCully 1995; Walker et al. 2003), suggesting a role of the rhizosheath in improving plant performance under drought stress, first proposed over a century ago (Price 1911). Recent research has shown that wheat cultivars with greater rhizosheath formation had sustained transpiration rates under drought stress as compared to genotypes with less rhizosheath formation (Basirat et al. 2019). The observation that xylem vessels in the rhizosheath zone are still alive and are not conductive (McCully 1999b) suggests that one benefit may be the maintenance of hydration of the growing root tip and surrounding soil. It has been proposed that greater length of the immature vessel zone may increase the length of rhizosheath and thereby affect plant performance under abiotic stress (Lynch et al. 2014). This merits investigation.

Anatomical phenes that improve adaptation to soil mechanical impedance

Soil mechanical impedance limits root growth and elongation, soil quality, and crop production globally (Whalley et al. 2005; Lipiec et al. 2012; Bengough et al. 2011). Root phenotypes that are able to overcome impedance have an advantage in water and nutrient capture at depth, improving plant performance in edaphic stress (Barraclough and Weir 1988; Ishaq et al. 2001; Lynch 2018). Physical soil properties influence mechanical impedance including soil water content, texture, bulk density, and overburden pressure due to depth (Gao et al. 2015; Vanhees et al. 2020). In most soils, soil strength and therefore mechanical impedance increases with decreasing water content (Whalley et al. 2005), and is therefore synergistic with water deficit stress (Bengough et al. 2011). Soil compaction causes impedance by increasing soil bulk density while reducing porosity and pore connectivity (Chen et al. 2014a) and therefore increases the risk of hypoxia by reducing hydraulic conductivity and air permeability (Chen et al. 2014a). In addition, in soils with limited well-connected macropores, root growth may further compact the rhizosphere (Lucas et al. 2019a). Root phenes that place roots in deeper soil strata may also aid in the avoidance of soil hardening in drying topsoil (Lynch 2013). Roots have innate characteristics that enable them to penetrate hard soils. Roots that are less likely to buckle, provide root tips better anchorage, or better locate biopores and cracks in the soil may be advantageous in environments with mechanical impedance (Whiteley et al. 1982). In hard soils, maize increased the proportion of coarse roots (Vanhees et al. 2021b) and roots were larger in diameter (Lucas et al. 2019a). In maize, deeper-rooting plants in hard soils were associated with increased cortical cell file number, greater mid-cortical cell area at node three and greater crown root cortical aerenchyma formation at node four (Vanhees et al. 2020), and the formation of multiseriate cortical sclerenchyma (Schneider et al. 2021). Intraspecific variation in root penetration ability has been observed in maize (Bushamuka and Zobel 1998; Chimungu et al. 2015a), lupin (Chen et al. 2014b), rice (Yu et al. 1995; Price et al. 2000), cotton (Taylor and Kasperbauer, 1999), wheat (Kubo et al. 2006; Botwright Acuña et al. 2007), soybean (Bushamuka and Zobel 1998), and common bean (Rivera et al. 2019). Genetic diversity for penetration of hard soils gives potential for trait discovery.

Root diameter

Root anatomical phenes influence penetration ability in impeded soils (Vanhees et al. 2020). For example, cortical cell thickness, cortical cell count, cortical cell wall area, and stele diameter are all associated with increased root penetration and bend strength (Fig. 6; Chimungu et al. 2015a). Generally, thicker roots have greater penetration ability in hard soils as they are more resistant to buckling and deflection (Whiteley et al. 1982; Jin et al. 2013). Root thickening in response to impedance involves both changes in the underlying tissues and cellular structures in both the cortex and stele. In response to compaction, the formation of coarse roots, addition of cell layers (Colombi et al. 2019) or the expansion of cortical cells (Atwell and Russell 1989; Colombi and Walter 2017; Colombi et al. 2019; Vanhees et al. 2021a, b) have been observed. In wheat, root

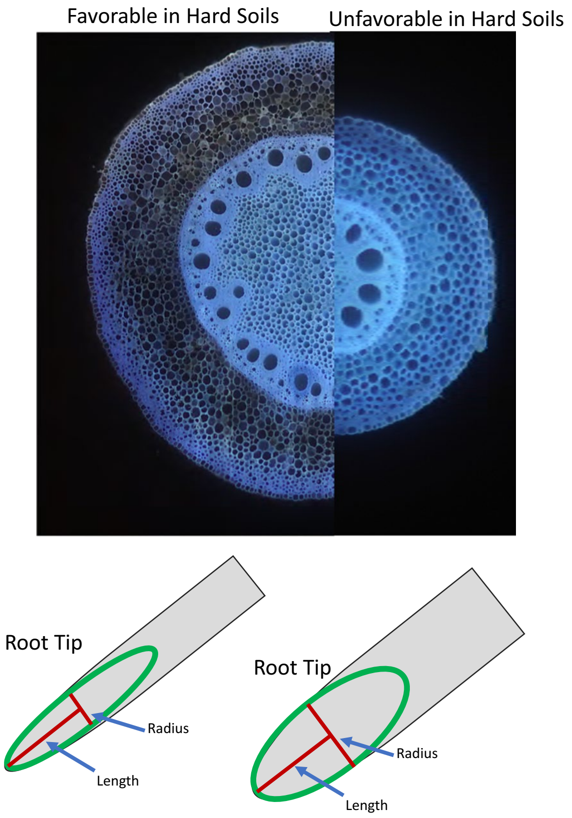

Fig. 6 Roots with a greater root diameter, greater stele area, many cortical cell files, small cortical cells in the outer cortex, and a small root tip radius to length ratio enhance root penetration in hard soils

cross-sectional area, stele and cortical area, root cortical aerenchyma, and cortical cell size and file number were plastic in response to impedance, and these responses were genotype and root class specific (Colombi and Walter 2017). Classically, radial expansion in response to mechanical impedance has been assumed to enhance soil penetration. However, recent studies have demonstrated that root thickening is not associated with rooting depth in compacted soils (Vanhees et al. 2020). Ethylene may signal the root to radially thicken in response to compaction and may act as a stop signal for axial root growth (Vanhees et al. 2021a). Compacted soil reduces ethylene diffusion and therefore increases the concentration of ethylene near root tissues. A signalling cascade triggered by the accumulation of ethylene may impede root growth through hard soil (Pandey et al. 2021).

In addition to radial thickening rendering roots more resistant to buckling, it has also been speculated that thickening may facilitate root anchorage to enable root penetration in strong soils. The anchorage of roots may be accomplished by friction of soil particles and maturing tissues through the production of root hairs and lateral roots behind the zone of elongation. Root anchorage may support the maximum growth pressure (i.e. growth force exerted by the root per unit cross-sectional area), which is largely driven by turgor pressure in the expanding cells at the root elongation zone, to force the root tip further into strong soils (Bengough et al. 2011; Lynch and Wojciechowski 2015). Radial thickening of the root through an increase in the number of cell files, cell size, or cell wall thickening is speculated to relieve stress from the root tip by deforming the soil near the root tip allowing penetration into compacted soil (Atwell 1993; Smith 2007). When a root encounters impeded soil, radial thickening occurs within the elongation zone and the elongation zone becomes shorter (Bengough et al. 2006). In response to mechanical impedance, the cell walls in the axial direction of the elongation zone stiffen and increase tension (Bengough et al. 2006). The elongation zone shortens upon mechanical impedance presumably due to decreased cell wall extensibility in the axial direction while the local growth rate is maintained at the apical end of the elongation zone (Bengough et al. 2006; Spollen and Sharp 1991). Upon exposure to mechanical impedance, root diameter may increase up to twofold due to radial expansion of cortical cells caused by microfibril reorientation in the primary cell wall (Veen 1982). This smaller elongation zone under mechanical impedance will produce shorter cells with a larger diameter (Bengough et al. 2006; Colombi et al. 2019). Increased root diameter in response to impedance (Atwell 1993; Pfeifer et al. 2014; Colombi and Walter 2015) may be accompanied by decreases in cell division (Clark et al. 2003) and therefore root elongation (Atwell 1993; Smith 2007). Therefore, increasing penetration resistance results in decreased root elongation (Colombi et al. 2019; Vanhees et al. 2020). Root thickening is often considered to be adaptive, however, increased root diameters are associated with greater energy costs for root elongation and growth and may be node-dependent (Yang et al. 2019; Vanhees et al. 2020; Colombia et al. 2019). Root thickening has been primarily observed on small, seedling roots. However, in maize, thinner (seedling) roots may have a greater propensity to thicken when compared to younger, thicker roots of older plants. As roots from younger nodes are innately thicker, these roots may experience less impedance stress than thinner, older roots. Root thickening is node-specific, dependent on root anatomy, and may be obscured by allometric effects. In future studies it will be important to distinguish between the utility of innately thicker roots that have the capability to grow under mechanical impedance and roots that thicken as a reaction to mechanical impedance.

Studies of mechanically impeded soils in controlled growth conditions often involve repacking soil in pots, which not only changes the soil bulk density but also significantly changes the soil structure including pore size and continuity. Altering soil structure, and therefore pore characteristics and air permeability can have significant effects on root growth (Lipiec and Hatano 2003). In dense repacked soils, roots have a limited ability to locate existing macropores and soil aeration was reduced (Lucas et al. 2019a). Soils with a high bulk density (> 1.60 g cm^{-3}) did not limit root growth in the field, but limited root growth in repacked soils (Lucas et al. 2019b). In the field highly connected pore systems enable roots to bypass zones of high mechanical impedance (Colombi et al. 2017b; Stirzaker et al. 1996). Soil structure including pore size and connectivity should be considered as an important mechanism for deep rooting in hard soils. Soil structure disruption in repacked soils should also be considered when interpreting results from controlled experiments.

Root tip

For a root to penetrate soil, it requires pressure to expand the cavity in the soil and to overcome the frictional resistance between the root and the soil. The cavity expansion pressure is primarily determined by the shape of the root tip, its rate of penetration, and soil mechanical properties. Generally, more narrowly pointed root tips are more efficient in cylindrical deformation of soil, whereas more blunt root tips have less efficient spherical-like soil deformation (Bengough et al. 1997). In hard soils, a smaller root tip radius to length ratio was associated with greater root elongation rates by reducing root penetration stress (Fig. 6). However, root tips do not become more acute in response to mechanical impedance (Colombi et al. 2017a). Changes in the mechanical properties around the root tip including hydraulic or water release properties of the rhizosphere may occur through root mucilage or other rhizodeposits (Read et al. 2003; Whalley et al. 2005; Carminati et al. 2010). In addition, rhizodeposits may also interact with charged clay particles at the root tip to alter viscosity (Barré and Hallett 2009). A better understanding of mucilage and exudate interaction with the soil at the root tip will be important to understand root penetration in impeded soils. In addition, the frictional resistance between the root and the soil may account for up to 80% of the total penetration resistance (Bengough et al. 1997). However, the frictional resistance between the root and soil may be reduced through lubrication by border cells and mucilage production (Bengough et al. 2011) which largely explains why penetrometer resistance is typically five times greater than root penetration resistance (Bengough and Mullins 1990). Under increasing mechanical impedance roots produce more border cells and mucilage to better facilitation root penetration (Barber and Gunn 1974; Iijima et al. 2000).

Multiseriate cortical sclerenchyma

Sclerenchyma are complexes of thick, lignified cell walls that enable plant organs to withstand stretching, bending, and pressure strains (Evert and Eichhorn 2006). The size and composition of peripheral cortical cell files have been shown to be important for soil penetration (Lux et al. 2010; Chimungu et al. 2015a). Outer cortical tissues in roots may consist of a hypodermis in several cortical cell files and centripetally developed internal sclerenchyma layers (Lux et al. 2010). Multiseriate Cortical Sclerenchyma (MCS) is characterized by small cells in the outer cortex that have thick cell walls impregnated with lignin, and has utility in environments with greater mechanical impedance (Schneider et al. 2021). MCS is genetically controlled and displays variation in several *Poaceae* species including maize, wheat, barley, and sorghum. In maize and wheat, MCS enhanced cortical tensile strength and bending force of the root tip which increased penetration in mechanically impeded soils (Fig. 7). Greater penetration ability in strong soils enabled greater root depth distribution and greater shoot biomass in compacted soils.

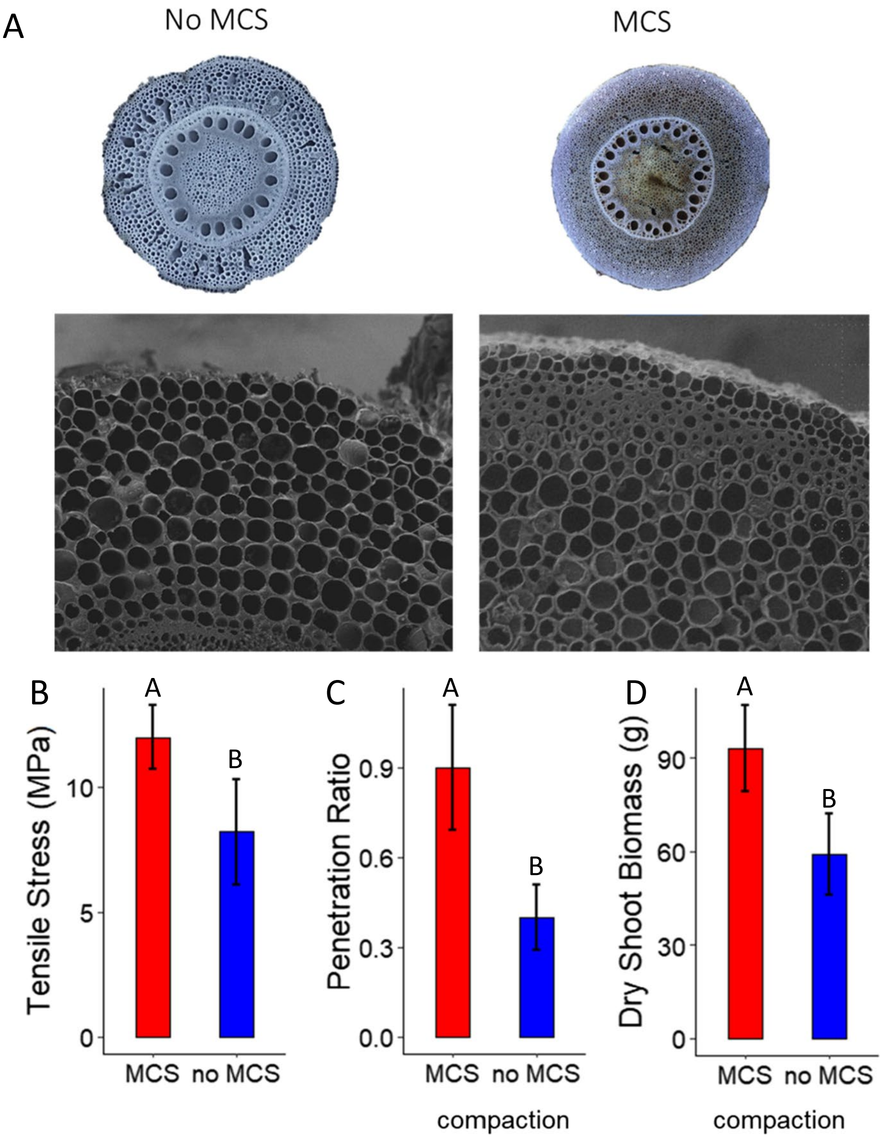

Fig. 7 **A**) Multiseriate cortical sclerenchyma (MCS) is characterized by small cells with thick cell walls in outer cortical cells. Genotypes with MCS have a **B**) greater root tensile stress, **C**) penetration ability in compacted soils, and therefore a **D**) greater plant performance in compacted soils when compared to genotypes with no MCS. Penetration ratio is measured as the ratio of the number of roots penetrating the hard layer to the number of roots reaching the hard layer per plant. Data is summarized from Schneider et al. (2021)

Roots with MCS have greater lignin concentration. Lignification is associated with mechanical support, water transport through xylem, and defense against pests and microorganisms (Boudet 2000). Lignin is primarily deposited in secondarily thickened cell walls rendering them rigid and impervious and improving the mechanical strength of the cell wall (Janssen 2000). Lignin concentrations are elevated in several root tissues including the endodermis, exodermis, and hypodermis. In barley, compacted soils increased root lignin concentrations (Scippa et al. 2008; Bingham et al. 2010; Bingham and Bengough 2003). Increases in lignin concentration are associated with increased root tensile strength and therefore penetration of hard soils (Zhang et al. 2014; Chimungu et al. 2015a).

It will be important to understand phenotypes that are capable of penetration under dynamic stresses, for example water deficit in compacted soils. A strategy for water capture in compacted soils may be to develop MCS for greater penetration ability and subsequently greater root depth or exploit paths with reduced resistance including cracks and biopores.

Anatomical phenes for transport

Metaxylem

Xylem vessels are responsible for the bulk of axial transport of water and nutrients through the root system. Xylem vessels are located within the root stele, which is enclosed by the pericycle with a single layer of endodermis and the Casparian band that restricts the radial movement of water and nutrients (Evert and Eichhorn 2006). Throughout root development, two types of xylem vessels are formed: the narrow protoxylem vessels, which are first differentiated in immature root tissues, and the larger metaxylem vessels that provide greater capacity for axial transport as the root matures (Evert and Eichhorn 2006). In monocotyledonous species lacking secondary growth, the axial transport capacity of the root is fixed following the maturation of metaxylem beyond the elongation zone. These metaxylem vessels provide the majority of axial transport capacity in the root system with smaller contributions from the protoxylem (Evert and Eichhorn 2006; Carlquist 2012). In contrast, through secondary growth, roots of dicotyledonous species have the capacity for continued production of xylem, thereby augmenting axial transport capacity throughout their phenology (Fig. 1; Evert and Eichhorn 2006; Strock and Lynch 2020).

The capacity for axial transport through the root is largely determined by the number and size of xylem vessels, and substantial intra- and inter-specific variation for root vasculature phenotypes has been reported in several agronomically important species (Fig. 8A-D; Purushothaman et al. 2013; Kadam et al. 2015; Oyiga et al. 2020; Klein et al. 2020; Strock et al. 2021). Genetic variation for xylem vessel size and number is probably linked to other physiological components of water transport, and in turn, can have significant implications on water use strategies (Fig. 8E-G; Sperry and Saliendra 1994; Gallardo et al. 1996; Purushothaman et al. 2013). This concept is reflected in the geographic distribution of many species, where plants with a larger transport capacity (i.e. many, large xylem vessels) tend to be endemic to environments with wet, humid conditions while plants with a smaller transport capacity (e.g. fewer, narrower xylem vessels) are distributed in more xeric environments (Tyree et al. 1994; Purushothaman et al. 2013).

The Hagen-Poiseuille formula demonstrates that the axial flow rate of water through a conduit is proportional to the fourth power of its radius, thus small increases in metaxylem radii translate to substantial increases in axial conductance capacity (Lewis and Boose 1995; Hacke et al. 2017). Roots with few, narrow xylem vessels have reduced capacity for axial transport and therefore require a larger water potential gradient between the soil and atmosphere to transport an equivalent volume of water as roots with many, wider xylem vessels. Several studies have linked improved drought tolerance to root phenotypes where metaxylem are narrower (Richards and Passioura 1989; Abd Allah et al. 2010; Purushothaman et al. 2013; Klein et al. 2020) and more abundant (de Souza et al. 2013; Oyiga et al. 2020; Klein et al. 2020). This may be because narrow vessels that restrict axial transport capacity improve water use efficiency by simultaneously reducing the overall shoot size and limiting transpiration by reducing stomatal conductance (Lynch et al. 2014; Vadez et al. 2014). Narrow root metaxylem that restrict net water use may be beneficial under terminal drought by limiting water use early in the growing season, thereby increasing the availability of water during reproductive growth (Zaman-Allah et al. 2011; Feng et al. 2016). Metering water transport in environments with limited water availability may also help moderate desiccation of root tips and the surrounding rhizosphere, thereby allowing continued soil exploration later in the season (Richards and Passioura 1989; Vadez 2014; Lynch et al. 2014; Strock et al. 2021).

In *Phaseolus* species with shallow root length distribution, fewer, narrower root metaxylem vessels were associated with superior plant water status under terminal drought, suggesting adaptation to drought through a strategy of conserving soil water (Fig. 8A, B, E–G; Strock et al. 2021). Computer modeling of maize root systems also revealed that roots with reduced hydraulic conductance were associated with water saving behavior while roots with greater conductance enabled a high initial transpiration rate that decreased rapidly as soil moisture was depleted (Leitner et al. 2014). However, severely diminished flow rates of small vessels may risk limiting maximum yield potential or relative growth rate in non-stress conditions (Wahl and Ryser 2000). Nevertheless, in Australia where wheat is grown on stored soil water, a narrowing in the metaxylem of seminal roots was

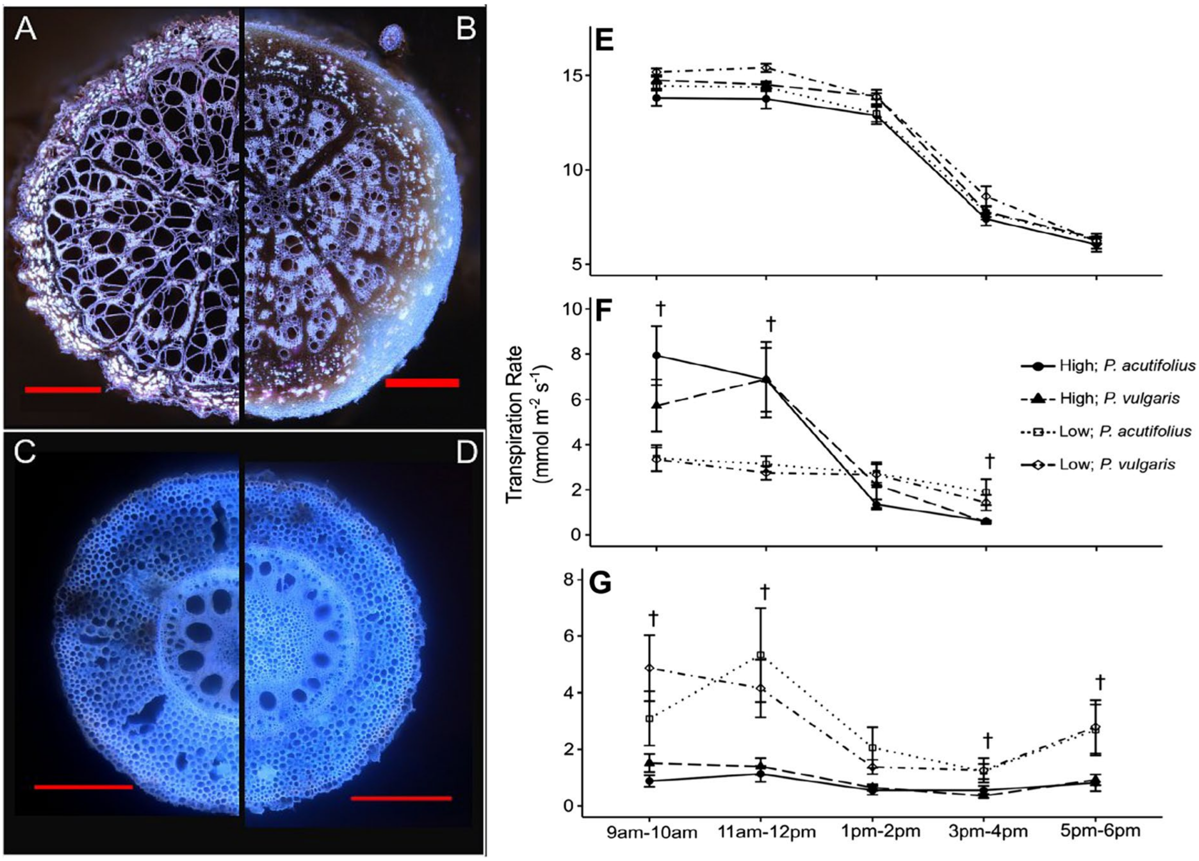

Fig. 8 Comparison of common bean (*Phaseolus vulgaris*) (**A**, **B**) and maize (*Zea mays*) (**C**, **D**) root cross-sections highlighting genetic variation within each of these species for the number and size of xylem vessels. All cross-sections are at the same scale. Scale bar = 0.5 mm. (**E**–**G**) This variation in xylem number and size affects axial hydraulic conductance capacity of the root system and can relate to differences in net transpiration rate (mmol m^{-2} s $-^{1}$) of common bean (*Phaseolus vulgaris*) and tepary bean (*Phaseolus acutifolius*) accessions with contrasting conductance under terminal drought stress at 13 (**E**), 27 (**F**), and 41 days after planting (**G**). Values shown are mean ± SE. Comparisons are made using ANOVA and Tukey's HSD across species and phenotypic groups within each timepoint. * indicates significant species differences and † indicates significant phenotypic differences at $\alpha \leq 0.05$. Modified from Strock et al. (2021)

related to greater wheat yields under drought with no yield penalty under optimal watering (Richards and Passioura 1989), possibly because of compensating conductance by nodal roots.

In addition to improving water use efficiency, narrow root metaxylem are also more resistant to hydraulic dysfunction from cavitation (Hacke and Sperry 2001; Sperry et al. 2006), a common occurrence in dry soils where there is greater tension of xylem sap. High rates of drought-induced cavitation can greatly impair biomass accumulation (Williams et al. 1997; Gitz et al. 2015) and is a direct cause of mortality in trees (Barigah et al. 2013). Though cavitation has been observed in the roots of herbaceous annuals (McCully et al. 1998) and has been shown to weaken the overall hydraulic system of non-resilient species (Hacke et al. 2001), it is unclear whether cavitation significantly hinders productivity of annual crops. Because drought-induced cavitation results from air-seeding at the interconduit pits, the pit membrane anatomy, such as the pit pore size or total pit area, may play a more direct role in vulnerability to cavitation than xylem vessel diameter (Wheeler et al. 2005; Hacke et al. 2006; Sperry et al. 2006).

While narrower metaxylem vessels that meter water use may be adaptive under water-limited conditions, restricted axial transport can also have ancillary effects on acquisition of other soil resources.

Suppressed extraction of water from the rhizosphere may reduce nutrients delivered via transpiration-driven mass flow (Chapman et al. 2012; Lynch 2019). Therefore, it may be advantageous to couple the restrictive root metaxylem phenotype with augmented ion uptake kinetics (Kelly and Barber 1991; York et al. 2016) and root architectural phenotypes that optimize root foraging in resource-rich soil domains. The 'steep, cheap and deep' ideotype would improve N capture by facilitating deeper root growth (Lynch 2013) whereas Mg and Ca acquisition would be aided by root architectures optimized for topsoil foraging (Lynch 2019). In contrast, acquisition of nutrients bound to soil constituents and taken up by diffusion, like P and K, is less affected by restricting water extraction. Their uptake is more dependent on root architectural phenotypes that maximize exploration in the topsoil where these resources are more bioavailable (Lynch and Brown 2001; Ho et al. 2005; Lynch 2011, 2019).

Axial transport phenes may affect hydraulic lift, the phenomenon in which root redistribute water from deep stores to the drier shallow layers overnight to be reabsorbed by shallow roots during the day (Caldwell and Richards 1989; Dawson 1993). Hydraulic lift has been observed in multiple species as a mechanism that facilitates favorable edaphic conditions for sustained mineral nutrient availability, fine root longevity, and microbial processes in the rhizosphere, particularly under water-limited conditions (Caldwell et al. 1998). While root abundance, root diameter, and root volume were positively correlated with increased water availability spurred by hydraulic lift, and in turn improved drought tolerance, in greenhouse-grown maize (Wan et al. 2000), the contributions of root anatomy have not yet been shown. Theoretically, root transport phenes that accommodate high axial hydraulic conductance and greater efflux from the roots (*e.g.* numerous or wide metaxylem vessels) may encourage hydraulic lift to occur but at a potential cost to daytime drought tolerance. Genotypic variation in hydraulic lift prevalence has been observed (Wan et al. 2000), which suggests that hydraulic lift may offer opportunities for crop improvement.

Radial transport

Cell wall modifications and anatomical phenes can restrict the radial movement of water, nutrients, and gases into the root. The exodermis, hypodermis, and endodermis are often embedded with suberin and/or lignin which regulate the movement of water and ions, primarily though reducing apoplastic transport (Steudle 2000; Colmer 2003; Enstone et al. 2003; Schreiber et al. 2005). The amount of suberin in roots typically is negatively correlated with water uptake in maize (Zimmermann et al. 2000), water and Ca, Mn, and Zn in Arabidopsis (Baxter et al. 2009), and radial oxygen loss in rice (Kotula et al. 2009). Edaphic stress can induce cell wall modifications. For example, water-stressed rice roots had enhanced endodermal suberization and most drought-tolerant genotypes had greater endodermal suberization (Henry et al. 2012), and increased lignification (Hazman and Brown 2018). Intraspecific variation in the degree of suberization and lignification in roots of many species suggest a potential for breeding for stress-tolerant crops. However, we need to better understand the environmental and genetic factors that influence the development of apoplastic barriers.

Other root anatomical phenes can also influence radial water transport and the development of apoplastic barriers. For example, increased cortical width caused by a greater number of cortical cell files and/or an increase in cortical cell size reduces radial conductance (Heymans et al. 2020b). Root cortical senescence is a type of programmed cell death in the cortex of several temperate grain species. Senescence of cortical tissue is accompanied by increased suberization of the endodermis and a reduction in nutrient and water transport (Schneider et al. 2017b, 2020a, b, c). Similarly, the formation of root cortical aerenchyma may have similar functions in reducing radial and water transport. Root cortical aerenchyma formation in maize reduced radial nutrient transport (Hu et al. 2014; Heymans et al. 2020b) and root hydraulic conductivity (Fan et al. 2007).

In water-limited environments, reduced radial hydraulic conductivity may be advantageous by conserving soil water throughout the growth season, a strategy known as "water banking" (Feng et al. 2016). Under water deficit, a reduced hydraulic conductivity may prevent desiccation of mature roots and the root tip and surrounding soil for continued root elongation. Root phenes like root cortical senescence and root cortical aerenchyma, may promote water banking by reducing water flow from the root to soil and enhanced suberization by reduce radial water loss from older root tissue. Root phenotypes that prevent desiccation of the root tip and surrounding soil may also be beneficial in strong soils, such as delayed xylem maturation, increased suberization of the endodermis, or hydraulic isolation of rhizosheaths (Lynch and Brown 2012; Lynch 2018).

Root anatomy and biotic interactions

Mycorrhizas

Arbuscular mycorrhizae (AM) are wide-spread symbioses between Glomeromycota fungi and plant roots (Smith and Read 2008). By creating a hyphal network outside of the root tissue, AM can expand the soil volume explored by a plant increasing nutrient uptake for at least 15 cm beyond the root surface (Jansa et al. 2003). AM trigger the expression of aquaporins in root tissue under water-limiting conditions, thereby increasing water uptake under drought (Bárzana et al. 2014). Plant defense response is also triggered during the symbiosis formation (Pozo and Azcon-Aguilar 2007). Furthermore, AM help maintain soil aggregate stability (Rillig and Mummey 2006). Thus, the promotion of the symbiosis is considered a beneficial practice in agriculture (Smith and Smith 2011) with overall positive growth response when inoculated plants are compared to non-inoculated controls in greenhouse (Gerlach et al. 2015) and field (Hijri 2016) conditions. Nevertheless, the effects of inoculation with spores or infective mycelia of AM on plant growth range from positive to negative (Kaeppler et al. 2000; Sawers et al. 2017) and are accentuated under abiotic stress. Mycorrhizal inoculum is abundant in normal soil. Therefore, practical applications of AM in intensive agriculture have been questioned (Ryan and Graham 2018). Mycorrhizal symbiosis has potential to promote plant growth but the multifactorial nature of this interaction makes it challenging to design AM-based applications to promote plant growth under contrasting agronomic conditions (Smith and Smith 2011). Hence, more research is needed to find possible factors that may consistently affect colonization rates and benefits from the symbiosis.

It is well established that fine, young roots have increased active AM colonization compared to lignified, coarse and older roots within the same plant (Brundrett et al. 1996; Guo et al. 2008; Smith and Read 2008). Such changes are linked to the anatomy of each root class (Guo et al. 2008). However, the effect of anatomical variation within the same root class between related plant species on AM has been in general overlooked. Root cortical aerenchyma and a continuous sclerenchymatic ring in the outer cortex was associated with decreased AM colonization in related palm species (Dreyer et al. 2010). A multispecies study in which three root anatomical traits, namely proportion of root length with a suberized hypodermis, distribution of hypodermal passage cells, and the proportion of root volume that is intercellular air space were quantified in parallel with AM under low phosphorus, revealed that none of these traits were significantly correlated to AM colonization, suggesting that this relation might be species-specific (Sharda and Koide 2010). Mycorrhizal colonization was absent in pioneer roots of trees (Acer, Juglans, Liriodendron, and Populus), which in comparison with fibrous roots with AM, have more layers of hypodermis, a smaller percentage of putative passage cells and more protoxylem clusters than fibrous roots of similar age in the same plants (Zadworny and Eissenstat 2011).

Few studies address the associations of intraspecific variation for root anatomy and the AM symbiosis. Reduced living tissue and increased root cortical aerenchyma were correlated with AM colonization in inbred maize lines grown in the greenhouse and field, but the opposite pattern and a correlation between AM and root diameter were found in field-grown hybrids (Fig. 9; Galindo-Castañeda et al. 2019). When AM colonization in common beans was compared under high and low P conditions, colonization was greater in basal root segments vs. apical root segments under low P, and the opposite was found for high P (Strock et al. 2018). A better understanding of possible AM-tradeoffs of root anatomical phenotypes would be facilitated by comparative analyses of AM in plants from the same species with contrasting levels of aerenchyma, root hair length and density, lignification of cortical layers, cortical cell size, etc. Moreover, the relationship between axial and lateral root anatomy would be crucial for the understanding of AM associations given that lateral roots host the most active part of the symbiosis. From a physiological perspective, the relative contribution of the symbiosis versus

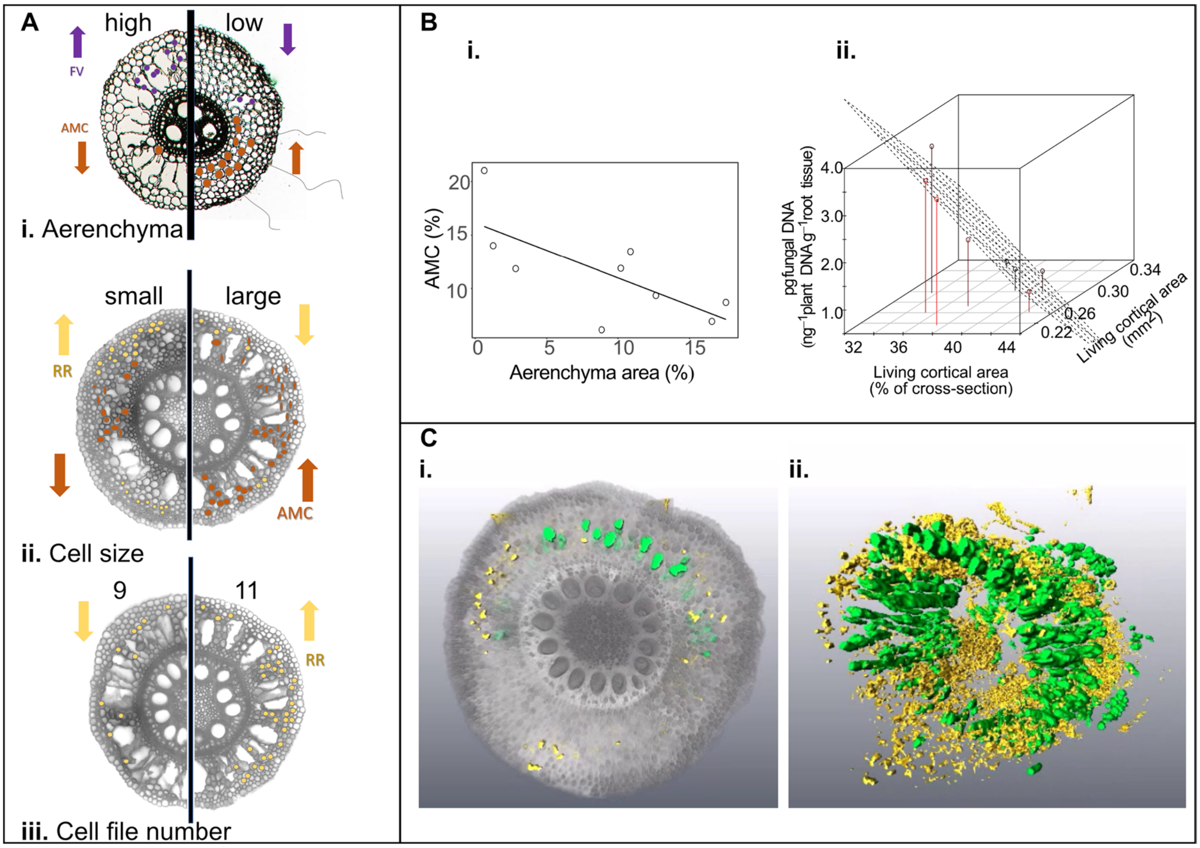

Fig. 9 Relationships between maize root anatomy and fungal colonization as found by Galindo-Castañeda et al. (2019). **A. i.** Root cortical aerenchyma was negatively correlated with mycorrhizal colonization (AMC) and inversely correlated with *F. verticilliodes* colonization in greenhouse-grown inbred maize lines. **ii.** Cell size was directly correlated with AMC, and inversely correlated with root rots in field-grown maize hybrids. **iii.** Plants with reduced cortical cell file numbers had reduced root rots in field-grown maize hybrids. **B. i.** Relationship of AMC and percent aerenchyma area ($P=0.030$, $R^2=0.443$, $n=4$) in greenhouse-grown inbred maize lines under suboptimal P conditions. **ii.** Three-dimensional scatterplot of *F. verticillioides* colonization with the root anatomical phene aggregates living cortical area (LCA) and percent of the cortex that is living cortical area (perXSisLCA) in greenhouse-grown inbred maize lines. The plane is the linear fit of the model *F. verticillioides* concentration = LCA + perXSisLCA ($P=0.002$, $R^2=0.881$, $n=4$). Red intensity relates to the LCA coordinates, with red in the lowest values and black in the highest values. **C.** Tridimensional reconstructions of a root segments colonized by AM, highlighting arbuscules (yellow) and aerenchyma lacunae (green). Roots collected in 4-weeks old maize plants growing in the greenhouse. **i.** Front of the segment before rotation showing the cross-section. **ii.** Front and side view of the reconstruction in which the root tissue was faded and the aerenchyma channels and arbuscular networks are shown (Photos by Tania Galindo Castañeda)

the root phenotype itself for soil resource capture would reveal the real importance of one or the other mechanism to improve plant growth under stress. Experimental systems where AM colonization can be controlled, and where both the measurement of root anatomy and AM colonization is measured in parallel with soil resource capture will facilitate further research efforts focused on untangling the relative contributions of root traits and AM. Such a system needs to be further developed, but mesocosm systems in combination with microscopy and laser ablation tomography are promising approaches to study this bipartite phenomenon. Mutants that do not form AM are useful but may display other phenotypic differences from wildtype plants because of pleiotropy. Complementation with gene expression and metabolic profiling would help in understanding the hierarchy of events that take place when roots use both symbionts and root phene states to cope with abiotic stress.

Pathogens

Root pathogens cause substantial yield losses in global agriculture (Stirling et al. 2016). Therefore, the study of the interactions of root anatomy with pathogen colonization is of great interest in plant breeding programs targeting root anatomical traits. The selection of specific root phenotypes linked to improved soil resource acquisition should not compromise root resistance to pathogens. Very few studies have examined associations between natural phenotypic variation for root anatomy and root pathogens. Root cortical aerenchyma has been studied as a possible factor that could affect pathogen colonization in root tissues. For example, the colonization by the fungal pathogens *Cochliobolus sativum* in spring wheat (Deacon and Lewis 1982), and *Phialophora oraminicola* in Kentucky bluegrass (Smiley and Giblin 1986) was favored in plants with increased RCA. On the contrary, safflower plants with increased RCA had reduced colonization of *Phytophthora cryptogea* (Atwell and Heritage 1994). In a recent study, fungal colonization of maize nodal roots by *Fusarium verticilliodes* was reduced in plants with increased living cortical area in greenhouse-grown inbreds, and root rots were positively correlated with cortical cell file number and inversely correlated with cortical cell size in field-grown hybrids (Fig. 9; Galindo-Castañeda et al. 2019). Possible mechanisms that may explain these observations are linked to the availability of apoplastic space where pathogens spread. In roots with a relatively greater apoplastic space (for example in cortex with smaller cell size and many cortical cell files) pathogens may encounter more avenues to spread. However, this may be modified when the apoplastic space, especially in the outer layers of the cortex, is sealed by suberization or lignification (Garrett 1981).

Pathogen susceptibility across different root classes in perennial species has been explored and could be used as reference or for examination of grasses or annual species. For example, in a study of apple rootstocks and the multi-species disease apple replant disease, roots in primary development and transitioning to secondary development had the greatest pathogen abundance (Emmett et al. 2014). It is also recognized that precocious suberization of the endodermis might prevent fungal colonization, and that tylosis could be linked to pathogen colonization resistance (Garrett 1981). Questions remain open on whether interactions in trees are also found in annual crops.

Rhizosphere microbiome

Microorganisms inhabiting the rhizosphere have the potential of promoting plant growth by increasing P and N availability (Richardson et al. 2009). Therefore, it has been proposed that the rhizosphere microbiome is a promising avenue to facilitate nutrient uptake under abiotic stress in crops (Compant et al. 2019). New approaches in plant breeding targeting root phenes should consider the effects of selecting for specific root phenotypes on the composition and functions of the rhizosphere microbiome. How root anatomy would affect the rhizosphere microbiome, and how the microbiome would affect root anatomy was until very recently generally unknown, and remains a topic to be researched in crops.

Traits that are directly linked to abiotic stress response have been recently studied in the context of microbial associations in the rhizosphere. Maize inbreds grown in the field with contrasting aerenchyma formation under nitrogen-limiting conditions were studied for their rhizosphere bacterial composition using amplicons of the 16s RNA genes. Plants with augmented aerenchyma showed significantly different bacterial communities compared to low-aerenchyma plants and the changes were nitrogen-level dependent (Galindo-Castañeda et al. 2018). Root-hair defective mutants had a distinct, more simplified procaryotic community in the rhizosphere, compared to wild-type barley lines (Robertson-Albertyn et al. 2017). Genes involved in root development (initiation and emergence of lateral roots, root hair length, root morphogenesis) were linked to microbiome traits in field-grown *Arabidopsis* (Bergelson et al. 2019). The regulatory network that controls endodermal suberization of *Arabidopsis* was linked to the composition of the root microbiome (endosphere and rhizosphere included), and microbes induced differences in the levels of endodermis suberization, which at the same time was linked to nutrient content (Salas-González et al. 2021). For the third and fourth mentioned studies, how these processes occur in agricultural-relevant species such as cereals or legumes remains to be discovered. It is noteworthy that *Arabidopsis* root anatomy and microbial associations might greatly differ from crops, and therefore, the mechanisms involved in orchestrating microbiome and root anatomy in such different soil environments need further exploration. Nevertheless, recent results indicate that the microbial composition of the rhizosphere is coordinated by both the root and the microbial community, and that interkingdom dialog and feedbacks shape the capabilities of the plant to absorb nutrients and cope with nutrient stress. Consequently, breeding efforts targeting root anatomy to improve soil resource capture, as well as the development of biostimulants, should consider microbial tradeoffs and microbial-root feedbacks that could modify or be modified by the microbe-root environment as determined by root anatomy.

Insects

Root interactions with arthropods and nematodes have significant impacts on soil resource capture, plant performance and yield. Plants are generally less tolerant of root herbivory compared to shoot herbivory since damage to roots can dramatically reduce water and nutrient uptake, open opportunities for pathogen infection, and increase susceptibility to lodging (Zvereva and Kozlov 2012). We hypothesize that resistance to these pests not only encompasses the commonly cited signaling response and plant defense biomolecules, but likely also involves physical components of root anatomy (Fig. 10). Although widely studied at the molecular and ecological scales (Meihls et al. 2012; Moore and Johnson 2017), the interactions between these soil organisms and root anatomy may have reciprocal effects but have been largely neglected in the literature (Strock et al. 2019b).

Where the interface between plants and insects has been examined at the anatomical scale, significant interactions have been reported. For example, Levin et al. (2021) found that cyst nematodes formed feeding sites adjacent to the central metaxylem in wheat (*Triticum aestivum* L.) roots, and resistance to nematodes was associated with physical separation of feeding sites and metaxylem vessels (Fig. 10A, B). Examination of root anatomy has also revealed that the allocation of defensive compounds is concentrated in specific tissues within the root (van Dam 2009). Hol et al. (2003) found that the root cortex of *S. jacobaea* had five times the concentration of pyrrolizidine alkaloids compared to the central cylinder. Similarly, McCully et al. (2008) found that glucosinolates were highly concentrated in the cell layers just beneath

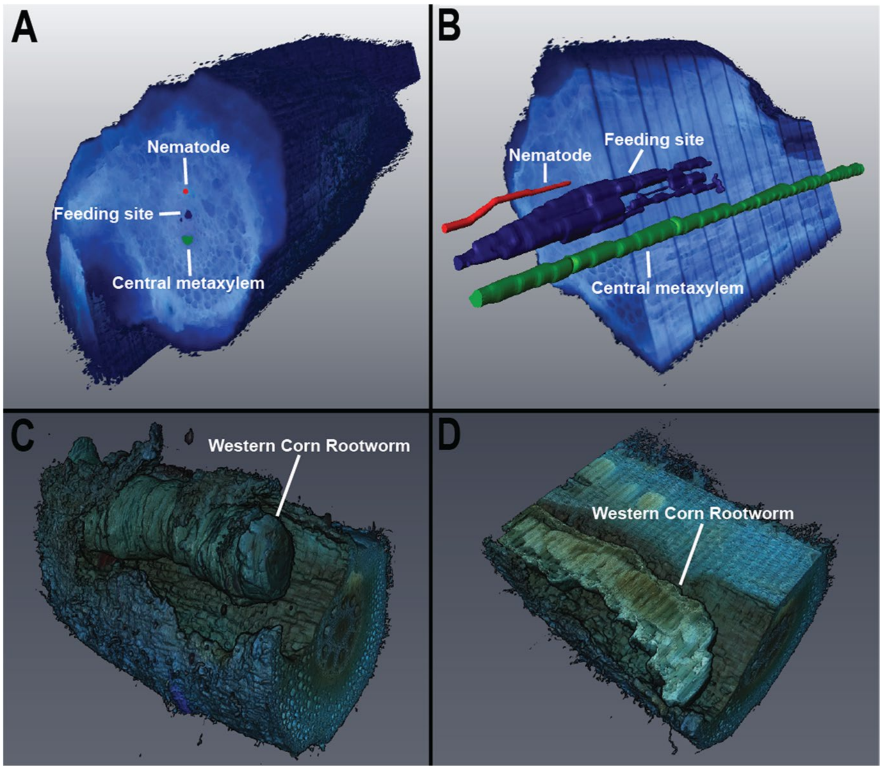

Fig. 10 (**A**, **B**) Three-dimensional reconstruction of a wheat (*Triticum aestivum* L.) root segment that contains a cereal cyst nematode (*Heterodera avenae*) and feeding site. The central metaxylem is highlighted in green, the nematode in red, and the feeding site in dark blue. (**C**, **D**) Three-dimensional reconstruction of the western corn rootworm (*Diabrotica virgifera virgifera*) feeding on a maize (*Zea mays*) crown root from a V3 stage plant, 4 d after inoculation with neonates. Modified from Strock et al. (2019a, b)

the outermost periderm layer in *Brassica napus* roots. This accumulation of defensive compounds in superficial cell layers likely serves to shield root vasculature from damage thereby preventing the disruption to axial transport of water and nutrients (van Dam 2009).

Although spatiotemporal patterns of structural compounds have not been well characterized in roots, localized incorporation of lignin, cellulose, suberin, and callose can provide physical barriers to herbivory (Meihls et al. 2012; Moore and Johnson 2017). Incorporation of mineral compounds like silica and calcium oxalate into anatomical features may also reduce the accessibility and digestibility of root tissues to these pests. For example, Riedell and Kim (1990) found that the suberized and lignified secondary cell walls of the endodermis and exodermis in maize roots act as a barrier to discourage larval feeding of western corn rootworm on maize roots (Fig. 10C, D). Similarly, Castano-Duque et al. (2017) found an association between the cutting resistance of maize roots and the intensity of western corn rootworm feeding on the cortex. Johnson et al. (2010) found that roots with greater lignin concentration and fracture toughness had delayed penetration by wireworms. In line with these observations, the deposition of lignin and suberin during secondary growth of dicotyledonous species (Strock and Lynch 2020) and multiseriate cortical sclerenchyma in monocotyledonous species (Schneider et al. 2021) would likely confer resistance to edaphic pests.

To our knowledge, targeted selection of root anatomical traits for resistance to root herbivory has yet to be utilized in breeding programs, but nevertheless, indirect selection for anatomical phenotypes conferring resistance to root herbivores may occur. For example, breeding programs for resistance to western corn rootworm consider the phenotype of reduced lodging (Castano-Duque et al. 2017). Overall, while the sparse literature on the interface between root anatomy and herbivory is likely attributed to experimental limitations, novel research techniques like laser ablation tomography have recently opened up opportunities for investigating this scale of plant–insect interactions (Strock et al. 2019b). Investigation into how root herbivores interact with root anatomy would not only provide a great contribution in our understanding of how these pests affect root health and development, but also aid in the advancement of designing integrated systems of pest management.

Cross cutting issues

Plasticity

Phenotypic plasticity is the ability of the organism to alter its phenotype in response to the environment. Plasticity of root phenes is heritable and genetically controlled and is a widespread and important phenomenon for strategic capture of nutrients and water (Schneider et al. 2020a; Schneider and Lynch 2020). However, plasticity is not necessarily a characteristic of an organism as a whole, but rather a characteristic of a specific phene and may be adaptive, maladaptive, or neutral in regard to fitness. A number of root phenes express phenotypic plasticity. For example, metaxylem number, root cortical aerenchyma, stele diameter, root angle, lateral branching density and length, and cortical cell size and file number are plastic in response to drought (Schneider et al. 2020a, b; Niones et al. 2013; Price et al. 2017). In drought, many of these adaptive plastic responses were associated with deeper rooting, yield stability, and improved plant performance (Niones et al. 2012; Wasson et al. 2012; Sandhu et al. 2016). However, the duration and the spatiotemporal conditions of phenotypic expression influence its utility. It has been speculated that plasticity is an adaptive mechanism for crop ancestors in natural ecosystems with highly variable and fluctuating environments (Schneider and Lynch 2020). However, a plastic response may be maladaptive in high-input agroecosystems with greater water and nutrient availability and reduced biotic stress (Lynch 2018). The fitness landscape of root phenotypic plasticity is complex and dependent on many variables including the environment and management practices.

Integrated anatomical phenotypes

While individual root anatomical phenes affect the metabolic costs of soil exploration, root penetration into strong soils, the radial and axial transport of soil resources, and interactions with soil organisms, efforts to conceptualize the interaction among multiple components of root anatomy can provide even greater insight into strategies of soil resource capture (Asfaw et al. 2012; York et al. 2013). The effects of root phenotypes on soil resource capture are multifaceted over space and time, and we must embrace this complexity to better define the fitness optima in which specific root phenotypes are advantageous. Investigation of the broader interactions among multiple phenes, or integrated phenotypes, that affect soil resource acquisition, transport, and utilization may reveal synergistic (*i.e.* greater than the additive effects of individual phene states) or antagonistic (*i.e.* less than additive effects on plant performance) relationships among different components of the root system (Lynch 2019). For instance, at the single phene scale, large cortical cells have been linked to improved tolerance to water deficit stress (Chimungu et al. 2014a) and low P availability (Galindo-Castañeda et al. 2018) by reducing the metabolic burden of cortical tissue maintenance (Jaramillo et al. 2013). Coupling large cortical cells with fewer cortical cell files has been suggested as a synergistic combination to facilitate deeper rooting (Lynch 2013), but may have an antagonistic effect on root penetration of hard soil. Focusing on integrated phenotypes may also reveal multiple phene combinations that result in similar fitness outcomes in a given environment (Rangarajan et al. 2018; Strock et al. 2019a; Klein et al. 2020), which presents breeders with several novel targets for crop improvement.

The sheer number of possible combinations resulting from the interactions of multiple phenes in multiple states in multiple phenotypic and environment contexts is a challenge for empirical research, but is well suited to in silico analysis. In silico approaches also permit the analysis of phenotypes that do not exist in nature. For example, use of the functional-structural plant model *SimRoot* identified substantial synergism among root hair length, density, epidermal patterning, and point of initiation for P capture in Arabidopsis (Ma et al. 2001b). Low P availability regulates these phenes in this species in a coordinated manner that increases P capture 3.7 fold greater than their additive effects, demonstrating substantial morphological synergy. *SimRoot* analysis also showed that the utility of RCA for nutrient capture depends on other root phenes and environmental factors (Postma and Lynch 2011a). On low-phosphorus soils, the utility of RCA was 2.9 times greater in plants with increased lateral branching density than in plants with normal branching. On low-nitrate soils, the utility of RCA formation was 56% greater in coarser soils with high nitrate leaching.

Though the inherent complexity of integrated phenotypes lends itself to evaluation in silico, it is possible to exploit the natural diversity that exists in some species to identify integrated phenotypes that are valuable in the field. For example, using a diverse collection of maize inbreds, Klein et al. (2020) identified three integrated phenotypes composed of root anatomical phenes that centered on likely drought survival strategies: enabling the exploration of a larger soil volume by reallocating internal resources to greater root construction (increased root cortical aerenchyma production, larger cortical cells, fewer cortical cell files), restricting uptake of water to conserve soil moisture (reduced hydraulic conductance, narrow metaxylem vessels), and improving penetrability of hard, dry soils (thick roots with a larger proportion of stele, and smaller distal cortical cells). Of the groups of root phenotypes identified in the panel, the root systems of those with improved performance contained at least one of the identified integrated phenotypes.

Beyond the integration within multiple anatomical components, integrated root phenotypes combining root anatomy with other factors affecting soil resource capture (root system architecture, shoot architecture, phenology) should be carefully considered. For example, the integrated effect of root system architecture, which affects root length distribution in the soil profile, and xylem morphology, which affects axial transport, influences water use under drought. Both synergistic and antagonistic interactions between metaxylem phenotypes and root architecture have been observed in two species of *Phaseolus* under water limitation (Strock et al. 2020). A synergistic interaction between highly conductive xylem morphology and deep root architecture in *P. acutifolius* demonstrated the utility of efficient axial transport for supporting growth when water is more available at depth. A less-conductive xylem phenotype within this species had an antagonistic interaction with deeper roots by reducing the capacity for transport of water to the growing shoot under stress. Complementarity between the low-conductance xylem phenotypes and shallow root architecture in *P. vulgaris* revealed the utility of restricting water uptake to conserve soil water usage (Strock et al. 2020). These observations indicate that xylem phenotypes integrate with root system depth to influence water use.

Interactions of root anatomy and root architecture are also important for nutrient capture. For example, long root hairs and shallow root growth angles are synergistic for P capture in common bean (Miguel et al. 2015). Compared with short-haired, deep-rooted phenotypes, long root hairs increased shoot biomass under phosphorus stress by 89%, while shallow roots increased shoot biomass by 58%. The additive benefits of these two phene states would therefore be 147%, however genotypes with both long root hairs and shallow roots had 298% greater biomass accumulation than short-haired, deep-rooted phenotypes, a synergistic combination twice as large as their additive effects. As another example, it has been proposed that RCA may have important interactions with architectural phenes increasing root depth, by alleviating hypoxia (Lynch and Wojciechowski 2015).

Trait synergisms and tradeoffs may also exist with contrasting root phenotypes originating from different root classes. For example, in soils with a high mechanical impedance, it may be beneficial for lateral roots to be highly responsive to ethylene acting as a stop signal for root growth upon reaching hard soils. This would enable more metabolic resources to be invested in axial roots, which are typically innately thicker and therefore greater ability to penetrate hard soils. The investment of metabolic resources in different root classes may interact to influence plant growth. Potential synergisms and tradeoffs between root and leaf phenotypes likely exist but presently are not well understood. While roots are directly responsible for the acquisition of soil resources, leaves have important effects on the utilization of these resources. Research attention toward understanding how these organs interact is essential for further optimizing plant fitness under limited resource availability. The optimal combination of both below- and above-ground anatomy, or potential trade-offs of acquisition and utilization strategies under varying water and N availability are poorly understood. While important synergistic effects among root phenes have been identified, the concept of aggregating multiple phenes into an integrated phenotype is not widely appreciated. However, because root and shoot development are regulated by many shared genes and signaling pathways, anatomical phenotypes in these organs may be correlated through pleiotropy or close linkage (Byrne et al. 2003; Laux et al. 2004; Chen and Lübberstedt 2010; Seago and Fernando 2013). If the anatomy of leaves has strong relationships with that of roots, collection of leaf samples may serve as an important tool in reducing labor and time required for excavating, washing, and collecting root samples in anatomical screening for below-ground traits.

Although few studies have directly addressed phene synergism and the value of integrated anatomical phenotypes, it is likely that root anatomical phenes have many important interactions with each other, with root architecture, with the shoot phenotype, and with the environment. This represents an implicit challenge to the reductionist paradigm of modern plant biology.

Carbon sequestration

In terrestrial ecosystems, soils serve as the largest reservoir of carbon, storing two times more than the atmosphere and three times more than the vegetation it supports (Post et al. 1982; Kell 2011, 2012). Despite holding such vast reserves, soils possess great capacity for further carbon retention, providing an important opportunity for combating climate change. A significant portion of plant photosynthate is deposited through roots, directly adding carbon to soil through growth, exudation, and associations with edaphic organisms (Lambers et al. 2002; Farrar et al. 2003; Jones et al. 2004). Root growth is particularly important for carbon sequestration since carbon from roots has 2.4 times longer residence time in the soil compared to carbon derived from above-ground tissues (Rasse et al. 2005). Consequently, root anatomical composition may have a significant effect on the capacity for plants to sequester atmospheric CO_2 in soil. For example, genetic variation for the deposition of lignin and suberin in roots may greatly impact plant capacity for carbon sequestration. This concept is highlighted in a meta-analysis by Poirier et al. (2018) where root suberin content was identified as one of the most influential promoters of soil organic matter stabilization. Thicker cell walls also may be a potential selection criterion for carbon sequestration. Carbon sequestration with thicker cell walls would depend on the composition; for example lignin degrades much slower as compared to hemicellulose and cellulose (Berg and McClaugherty 2003; Kögel-Knabner 2002; Yue et al. 2016). Additionally, root anatomy phenotypes that improve the metabolic efficiency of soil foraging and improve growth of above-ground biomass may, through allometry, produce greater overall root mass and augment the sequestration of carbon in soil. Since the residence time of carbon in soil is largely a function of plant allocation to roots, and their vertical distribution, root anatomical phenotypes that facilitate deeper rooting would sequester atmospheric CO_2 more effectively than plants with a shallow distribution of root length (Lynch and Wojciechowski 2015; Jobbagy and Jackson 2000; Kell 2011, 2012). Finally, root anatomical traits that modify root exudation or interactions with the rhizosphere community may further contribute to the total quantity of carbon added to the soil. Research to understand the potential that genetic variation in root anatomy has for below-ground carbon storage will only become more critical as climate change progresses.

Modelling root anatomy and resource capture

With advancement in molecular, imaging, and computational technologies, numerous mechanistic models (mathematical or computational) focusing on different aspects of plant biology have been developed, ranging from molecular and cellular biology through organs, organisms, to ecosystems, with physical structure in two or more dimensions. These models have been useful in identifying and filling knowledge gaps, quantitatively testing current paradigms, testing novel hypotheses, predicting system behavior for scenarios that are not accessible in-vivo, and designing incisive experiments to make useful discoveries and performing studies when laboratory experiments are difficult or impossible (Hodgman and Ajmera 2015; Marshall-Colon et al. 2017; Benes et al. 2020).

Evaluating the impact of variation in root anatomy on soil resource capture and identifying carbon and resource-efficient root anatomical phenotypes would be invaluable for developing improved crop varieties, as discussed in previous sections. However, such studies are extremely difficult as the number of combinations to be evaluated exceeds the capacity of empirical research. Thus, in silico evaluation becomes essential. Computational morphodynamics (Chickarmane et al. 2010) is one such approach, wherein tissue or organ scale models could either be considered as a continuum or multicellular structure, depending on the question of interest and the available computational capabilities. Under this paradigm, the functional-structural model—*SimRoot* (now, *OpenSimRoot*) (Lynch et al. 1997; Postma et al. 2017) can be classed as the only continuum model for root anatomy (Fig. 11A, Bi). Over the last three decades, *SimRoot* models have been successfully implemented for different crop species to represent various anatomical phenotypes such as root hairs, root diameter, root cortical aerenchyma, root cortical senescence, cortical cell size and cell file number, xylem size and number; and their impact on soil resource capture and plant carbon economy. These models enabled the functional evaluations of different anatomical phenotypes and their combinations under edaphic stress. It is noteworthy that these findings would have been difficult or impossible to attain with empirical approaches.

Continuum models like *SimRoot* have computational and conceptual advantages but lack precise multicellular topologies required to evaluate the influence of cellular/supracellular features (*e.g.* cell size/number/volume, cell wall, aerenchyma, casparian strip, membrane transporters, etc.) on tissue or organ scale behavior (*e.g.* resource acquisition, storage, flux, and transport) (Fig. 11B.ii). Multicellular modelling frameworks capturing root anatomy include MECHA (Couvreur et al. 2018, Fig. 11C.i) OpenAlea (Pradal et al. 2008, Fig. 11C.ii), and RootSlice (in development, Fig. 11C.iii). These frameworks use a standard programming language (e.g. java, python or C++) to couple model components and implement realistic root anatomies extracted from microscopy images (discussed in "Image analysis"). Most existing multicellular root anatomical models are focused at capturing developmental aspects (Band et al. 2012; Rutten and Ten Tusscher 2019). Only a few multicellular models of root anatomy have the capabilities to capture uptake dynamics involving water (Couvreur et al. 2018) and phosphorus (Ajmera 2016). Furthermore, to date no functional-structural model exists capturing soil resource acquisition and transport with explicit implementation of cellular and supracellular morphometry of the root. Though capabilities for capturing various features of root anatomy exist in many of the available modelling frameworks, including MECHA,

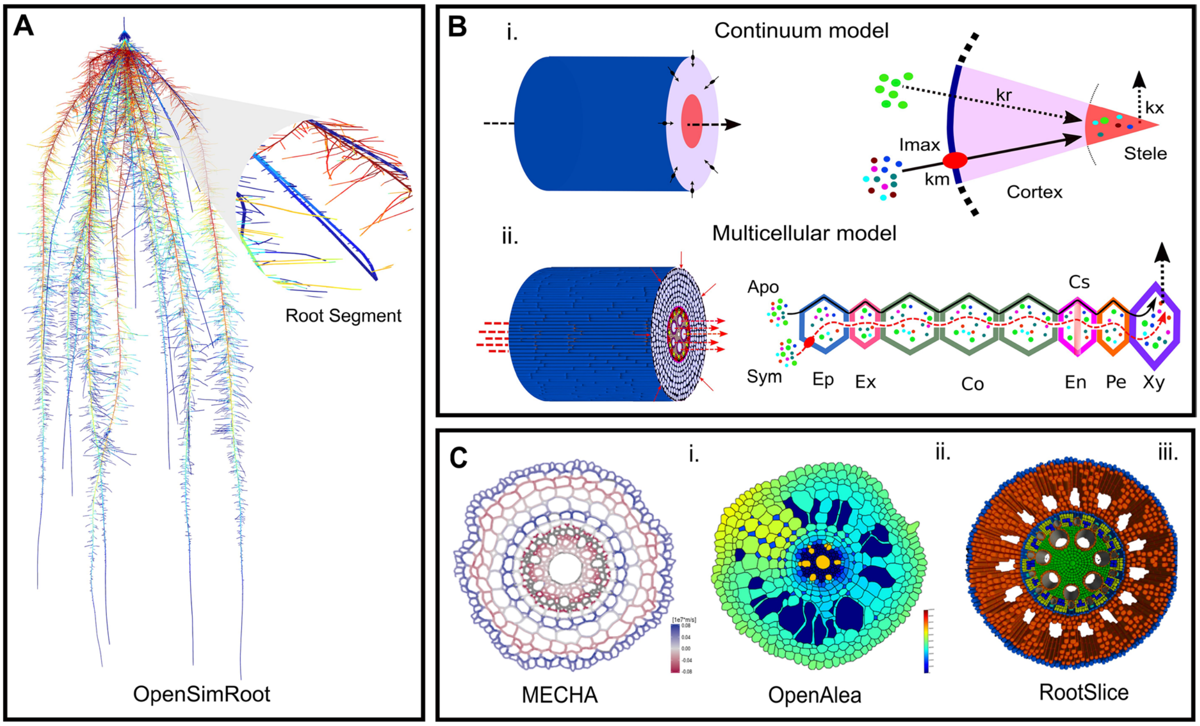

Fig. 11 *Paradigms for modeling root anatomy.* Panel **A** depicts *OpenSimRoot* simulation of maize root system at 40 days after germination. Color gradient highlights root cortical aerenchyma (RCA) formation, with red denoting the greatest proportion of RCA and dark blue denoting lack of RCA. Panel **B** depicts nutrient uptake dynamics across a root segment in continuum and multicellular models. Panel **C** highlights simulation outputs of various multicellular root anatomy models namely, *MECHA*, *OpenAlea* and *RootSlice*. Subpanel **B(i)** schematically illustrates nutrient and water uptake, flux and transport across a cylindrical root segment implemented in a continuum model like *OpenSimRoot, CRootBox* (Schnepf et al. 2018), *R-SWMS* (Javaux et al. 2008*), Root system models (*Doussan et al. 1998, 2006*)*. Water dynamics are defined by kr and kx denoting radial and axial hydraulic conductivity. Nutrient uptake kinetics are defined by the Michaelis–Menten kinetic parameters Imax and km denoting maximum nutrient uptake rate and affinity of transporter, respectively. Subpanel **B(ii)** schematically illustrates radial and axial flux of water and nutrients across a root segment in a multicellular model. The water and nutrient flow via symplastic (Sym; dash-red arrow) and apoplastic (Apo; black arrow) routes through root tissues, namely epidermis (Ep), exodermis (Ex), cortex (Co), endodermis (En), casparian strip (Cs) and pericycle (Pe), eventually entering xylem (Xy) and transported (axially) across the plant. Subpanel **C(i)** depicts *MECHA* simulation of water flux across the cell membranes in a cross section of a mature primary root of pearl millet. Blue indicates water entering a cell, red indicates water leaving a cell (image was generated via *MECHA* webtool—https://mecharoot.github.io). Subpanel **C(ii)** depicts *OpenAlea* simulation of cytoplasmic phosphate (CytoPi) distribution across an aerenchymatous rice root cross section. Red and dark blue in the scale bar depict highest and zero phosphate level, respectively. Sections of the root with aerenchyma have lower CytoPi level (light green to ocean blue) than sections with intact cortical cells (yellow). Strings of cortical cells and fluid-filled channels flanking lacunae reduce barriers to inward flow of Pi, thus increasing its uptake and lowering CytoPi levels in the outer cells. Subpanel **C(iii)** depicts a *RootSlice* simulation showing a 3D maize root cross section with RCA (white space) across the red colored cortex. Epidermis, pericycle and phloem are denoted in shades of blue, xylem, companion cells and pith are depicted in orange yellow, and green, respectively

OpenAlea, and RootSlice, their implementations are being awaited. Case studies highlighting the current capabilities of these modelling frameworks are briefly discussed below.

Root hydraulic conductivity is one of the key drivers regulating water uptake dynamics and is therefore a potential target for breeding drought tolerant crops. However, quantifying root hydraulic properties, particularly radial conductivity, and the array of factors influencing it, involves extremely complicated and error-prone procedures. To address this challenge, a two-dimensional Model of Explicit Cross-Section Hydraulic Anatomy, MECHA, was developed (Fig. 11C.i; Couvreur et al. 2018). The model can—a) estimate water flow across walls, membranes, and plasmodesmata of each individual cell; b) quantify contribution of different passages on water flow across the root, including intercellular spaces, xylem poles and passage cells, and c) predict its radial hydraulic conductivity. Certainly, MECHA offers a convenient approach to probe and cross-validate experimental root hydraulic studies.

A typical mature rice root has a distinctive cortical aerenchyma consisting of lacunae with spoke-like structures connecting the outer cell layers with the stele (Fig. 13A.iv). It has been proposed that if aerenchyma lacunae are lined or filled with aqueous solution, they would allow smooth passage for nutrients to flow across the cortex with minimal resistance (Drew and Saker 1986; van der Weele et al. 1996; Ranathunge et al. 2003; Vadez 2014). A 2D multicellular temporal OpenAlea model (Band et al. 2012) of phosphate uptake and flux across realistic rice root cross-section shows agreement with this hypothesis (Fig. 11C.ii; Ajmera 2016).

The cortex occupies a large part of the cross-sectional area of a root and its features vary across genotypes and environments. To evaluate the influence of variation in these phenotypes on its underlying rhizoeconomics, a 3D static functional-structural root anatomy modelling platform has been developed named RootSlice (unpublished, Fig. 11C.iii). In an initial study using the basal cross-sections of the maize roots, the model quantitatively showed the impact of cell wall thickness on cellular metabolic demand and apoplastic conductance. This model enables the quantification of rhizoeconomic variables for the different cylindrical root segments of varying class and age implemented in plant-scale models like *OpenSimRoot*. Such integrative compatibility opens avenues for investigating relationships between root architecture, anatomy, and soil resource availability.

Methods for studying root anatomy

One of the major limitations in exploring and utilizing root anatomical traits is the challenge of high-throughput phenotyping. Traditional methods like preparing a root cross section by hand and visualizing it with light microscopy are relatively inexpensive and a good starting point, but are limited because they may alter anatomical phenotypes and also because these methods are relatively slow and thus discourage exploration of root anatomical traits on a larger scale such as diversity panels, multi-species groups, mapping populations and breeding material, etc.. High-throughput phenotyping is needed for direct phenotypic selection, which is accessible to breeding programs without access to sophisticated genotyping capabilities, and for the discovery of genetic markers for marker- assisted breeding, which is especially important for quantitative traits like most root phenes. Therefore, high-throughput phenotyping of root anatomy progress from basic research to applied breeding. Several methods have recently become available that may be suitable for high throughput phenotyping of root anatomy.

LAT (Laser ablation tomography)

LAT (Laser Ablation Tomography) is a novel imaging technique that permits high-throughput, three-dimensional quantitative and qualitative analysis of root anatomy (Fig. 1; Chimungu et al. 2015a, b; Hall et al. 2019; Saengwilai et al. 2014; Strock et al. 2019b, 2018). The original LAT system utilizes a pulsed UV laser coupled with a galvanometer that forms a laser ablation plane by scanning the beam rapidly along a line of a defined width. The sample is mounted on a software-controlled stage that slowly moves it into the ablation plane while being imaged using a high-resolution camera equipped with a macro lens. Full color or even multi-spectrum scans of root samples at 0.1 mm to 1 cm spatial scale and μm level resolution can be acquired. LAT 2.0 is a newer version of LAT 1.0 and is equipped with superior laser physics permitting athermal ablation, hyper spectral imaging, and robotic sample processing. LAT 2.0 offers finer control over the laser power, faster sample processing speed and semi-automated handling of samples. Sample processing and imaging requires 1–2 min. The application of LAT for studies of root anatomy provides both volumetric quantification and qualitative tissue differentiation based on composition-specific autofluorescence. For three-dimensional visualization of specific features in samples, LAT scans are assembled into three dimensional stacks and compiled into three dimensional reconstructions. These three-dimensional reconstructions can be then used for volumetric measurements of different features *e.g.* three dimensional quantification of percent aerenchyma in maize (Galindo-Castañeda et al. 2018), xylem anatomy in beans (Strock et al. 2021), mycorrhizae colonization of maize and bean roots (Strock et al.

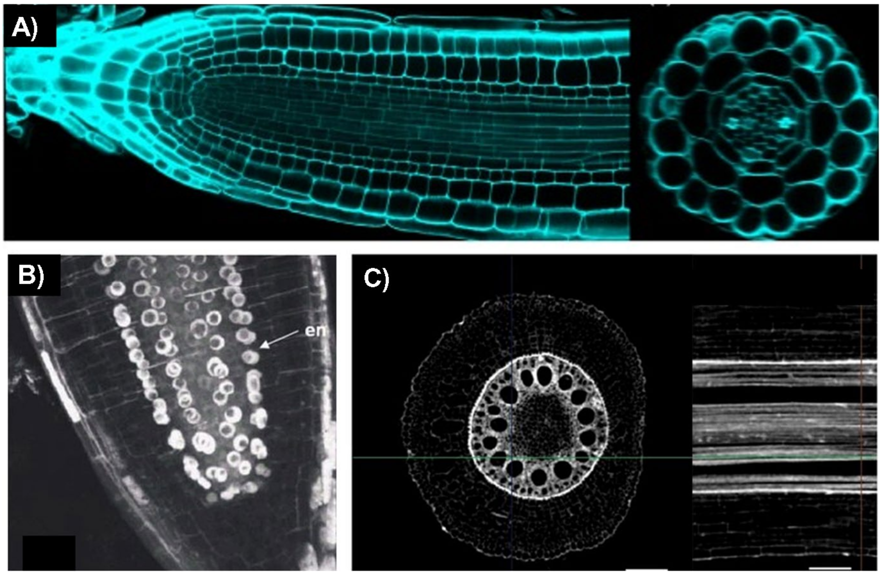

Fig. 12 Root anatomy phenotyping using **A**) confocal microscope, tissue—*Arabidopsis* root stained with propidium idodide (Bassel and Smith 2016), **B**) two photon microscope, tissue—*Arabidopsis* root (Feijó and Moreno 2002), and **C**) X-ray micro-CT, tissue – maize roots (Pan et al. 2017)

2018; Galindo-Castañeda et al. 2019), and root infection by nematodes in barley (Strock et al. 2019b; Levin et al. 2021). LAT can also be used for differentiating tissue features differing in their composition based on their autofluorescence spectra after UV excitation (Strock et al. 2019b). Hyperspectral imaging on the LAT 2.0 system can potentially help discern different tissue compounds such as lignin, xylan, and suberin. More details about LAT can be found at https://plantscience.psu.edu/research/labs/roots/methods/laser-ablation-tomography. Other

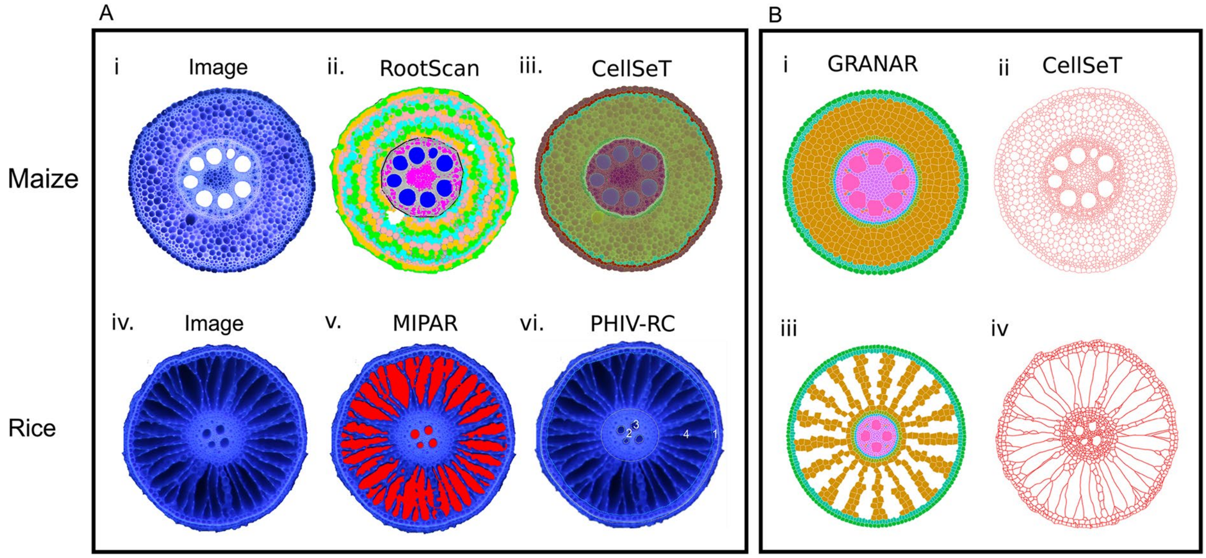

Fig. 13 *Visualization of maize and rice root cross sections processed with various image analysis tools.* Panel **A** (**i**, **iv**) highlights raw cross section images of mature maize and rice roots from laser ablation tomography. Panel **A** (**ii**, **iii**, **v**, **vi**) depicts anatomical features in the raw images quantified by different semi-automated image analysis tools such RootScan, CellSeT, MIPAR and PHIV-RC (RootCell). Panel **B** (**i**, **iii**) depicts digitalized anatomical network for mature maize and rice roots generated by GRANAR. Panel **B** (**ii**, **iv**) features structured network of cells in matured maize and rice roots extracted using CellSeT from raw image in Panel **A** (**i**, **iv**)

techniques like vibratomes coupled by imaging with light microscopy are also an alternative to hand sectioning (Verhertbruggen et al. 2017).

Phenotyping root hairs

Phenotyping for root hair length and density can easily done using a light or stereoscopic microscope, inexpensive LED pocket microscopes, or even the unaided eye (Vieira et al. 2008; Zhu et al. 2010b; Miguel et al. 2015). Root hairs can be phenotyped using seedlings germinated on germination paper or, for best representation of actual conditions, in roots extracted from soil. Root samples from soil should be harvested with care so that root hair phenotypes are not altered by the sampling method. After harvesting and washing root hairs can be stained with dyes like Toluidine blue and imaged using a light microscope. Given the intraspecific variation for root hair phenotypes and their importance for soil resource capture, they should be phenotyped routinely in crop improvement programs.

Non-destructive imaging

Certain anatomical features can be better phenotyped with non-destructive methods such as confocal microscopy, two-photon microscopy, and micro-CT etc. (Fig. 12). Confocal microscopy has been widely used to image root anatomical features of model organisms such as *Arabidopsis* and *Brachypodium* (Fig. 12A) (Hepler and Gunning 1998; Pollastri et al. 2012). Due to the small size of roots of these species, phenes like cortical cell size, cortical cell file number, cortical cell length, aerenchyma area etc. can be phenotyped non-destructively. But for phenotyping thicker root segments (> 200 um) *e.g.* in maize, simple confocal microscopes can only provide data about the epidermal cells or a few of the outer cortical cells depending on the cell diameter. Two-photon or multiphoton microscopes can image up to 1 mm into the tissue without compromising resolution (Mizuta et al. 2015), but this would not work for tissues thicker than 1 mm (Fig. 12B). Another benefit of using confocal or multiphoton microscopy is that organelles can be imaged by using different organelle specific dyes or probes (Spence and Johnson 2010; Giovanni et al. 2017). In-vivo imaging in growing medium of some root anatomical features such as root diameter etc. is possible using micro-CT (Fig. 12C; Wang et al. 2017; Teramoto et al. 2020). It is worth mentioning that all these methods, within their limits, can be used to faithfully construct root anatomical features but it most cases the time required to image samples, and the cost and complexity of these instruments, limits their application in crop breeding. We also acknowledge that there are other less common techniques for phenotyping root anatomy such as light sheet fluorescence microscopy, but we have discussed only the most used ones in this review.

Image analysis

Image processing and analysis are a major bottleneck for plant phenotyping. Broadly, it involves visualization, quantification, and management of the generated image dataset. Fundamentally, analyzing a micro-scale tissue image involves segmentation or delineation of cell walls against the background to extract the corresponding cellular morphometry. Accuracy and automation are the key attributes for the advancement of image analysis tools. For micro-scale plant phenotyping and non-invasive imaging, automation becomes vital as it requires quantification of subcellular and cellular morphometry over different spatiotemporal scales, often in 3D, from images with varying scales and depths. For more details and perspective on plant image analysis, readers are directed to several excellent reviews (*e.g.* Dhondt et al. 2013; Minervini et al. 2015; Bassel and Smith 2016; Lobet 2017; Legland et al. 2018; Zhao et al. 2019).

In recent years, various image analysis approaches and computational tools have been developed to evaluate microscopic root images from hand sections, paraffin or vibratome slices, laser ablation tomography (Fig. 13A.i, iv), fluorescence imaging and μ-CT. These tools can be classed as manual, semi-automated, and automated. Manual tools involved subjective evaluation of images and are most useful for low-throughput applications. Traditionally, ImageJ—an open-source platform (Schindelin et al. 2012; Rueden et al. 2017; Rasband 2015; http://imagej.net/), has been extensively used for manual and batch automation routines for image analysis via native macros or developing new plugins (Arena et al. 2017). On the other hand, semi-automated tools necessitate user feedback for individual images for accurate results. RootScan (Fig. 13A.ii; Burton et al. 2012), CellSeT (Fig. 13A.iii; Pound et al. 2012), PHIV-RootCell (Fig. 13A.vi; Lartaud et al. 2015) are semi-automated tools to evaluate root anatomy. An automated image analysis tool is expected to assess images and get accurate results with just a 'single-click'. RootAnalyzer (Chopin et al. 2015), DL-RootAnatomy (Wang et al. 2020) and RootScan3D (unpublished) are advanced fully automated tools for extracting anatomical metrics from root section images. DL-RootAnatomy and RootScan3D implement machine learning approaches to recognize and quantify the different anatomical root features. In machine learning approaches, initial image inputs and supervision are required for training the algorithms. Furthermore, open-source tools such as GRANAR (Heymans et al. 2020a) and CellSeT (Pound et al. 2012) allow digitalization of root anatomical networks (Fig. 13B), which can then be used for the development of multi-cellular models of root anatomy (Fig. 11C). However, there are significant differences in the processing time required by these tools, such that GRANAR (Fig. 13B.i, iii) requires seconds while CellSeT (Fig. 13B.ii, iv) could require hours to process root cross-section images with a thousand cells.

Various other plant image analysis tools, including for micro-scale, can be found in an online database (http://plant-image-analysis.org) (Lobet et al. 2013). This manually curated database enables the users to find suitable tools for their image datasets. In addition, commercial tools such as Avizo (VSG Inc., Burlington, MA, USA) and MIPAR™ (Sosa et al. 2014; Fig. 13A.v) are also being used for processing root section images and accurate quantification of different anatomical phenes (Hazman and Brown 2018; Strock et al. 2019b; Klein et al. 2020; Schneider et al. 2020a, b, c; Levin et al. 2021). There is significant progress being made in the arena of plant image processing and analysis at multiple scales. Given the challenges, AI based approaches are the way forward for the advancement of crop phenotyping.

Future prospects

As we show here, an array of anatomical phenes have substantial importance for the acquisition of water and nutrients from the soil, and natural genotypic variation for these phenotypes in crop plants provides attractive breeding targets for the development of more efficient, resilient crops. High-throughput phenotyping platforms, such as LAT accompanied by AI-assisted image analysis, are increasingly available, and in many cases are suitable for plants grown in soil and in the field, which is important since many anatomical phenes respond to environmental conditions. The expression and utility of many of these phenotypes may be restricted to specific taxa. For example, reduced secondary growth as a mechanism to expand soil exploration and nutrient capture is not relevant to monocots, which lack secondary growth. In contrast, root cortical senescence has only been observed in cereal crops. The fact that not all plant taxa display all anatomical phenotypes of interest represents a constraint for the relevance of model organisms that dominate elite plant biology research. The genetic control of many if not most of these phenotypes is likely to be highly quantitative. This poses a challenge for the gene-centric paradigm of elite plant biology. Crop breeding programs still rely heavily on phenotypic selection however. This is especially true in developing nations, which have urgent need of more resource-efficient crops but may lack the resources needed for molecular breeding approaches such as genomic selection, although such tools are increasingly powerful and inexpensive. Phenotypic selection for anatomical traits has been facilitated by the advent by inexpensive portable microscopes accessible to even the most rudimentary breeding programs.

A more challenging bottleneck is our incomplete understanding of the fitness landscape of root anatomical phenotypes. In some cases, such as that of long, dense root hairs, a phenotype appears to afford multiple fitness advantages with no obvious tradeoffs. In other cases, such as metaxylem vessel diameter, the fitness landscape is highly dependent on the environment and the background phenotype. The number of potentially interesting scenarios, given multiple states of multiple phenes in multiple environments, exceeds the capacity of empirical research. Furthermore, very few research efforts or even research communities encompass the range of relevant factors, including soil chemistry, physics, and microbiology, biotic and abiotic stressors, weather variability, crop management, etc. As we argue here, this complexity calls for greater emphasis on in silico approaches capable of spanning multiple spatiotemporal scales and multiple plant and environmental factors. However, the current organization of elite plant research, which supports and rewards success in narrow subfields, is not well suited to address this challenge. As an example that is relevant in the context of this article, very few elite plant biologists have expertise in soil science, and many do not employ natural soils at all, or if they do use real soil, make no effort to generalize their results across contrasting soil taxa. Few research efforts focused on drought also consider P stress, although these constraints commonly occur together in global agriculture. For that matter, few studies of plant response to abiotic stress also consider biotic stress factors. Such efforts are difficult to fund, conduct, and publish in modern plant science. They are what is needed to address global challenges. As a research community we need to reach beyond narrow disciplinary boundaries, dogma, and tribalism, and be willing to support and publish transdisciplinary, integrative research addressing the complexity of real world systems, and we need to train young scientists how to do this, if we are to fully exploit the remarkable potential of root anatomical phenotypes to address the two primary challenges of the twenty-first century: how to sustain 10B people in a degraded environment, and how to sustain and improve the natural resource base for future generations.

Dedication We dedicate this article to Kathleen M Brown on the occasion of her retirement. In a career spanning over 40 years she has been a model of research excellence, integrity and dedication. Her spirit of selfless service, quest for the truth, and personal kindness has inspired generations of students and scholars.

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