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Minireview

Back to our roots: exploring the role of root morphology as a mediator of beneficial plant–microbe interactions

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Summary

Plant breeding for belowground traits that have a positive impact on the rhizosphere microbiome is a promising strategy to sustainably improve crop yields. Root architecture and morphology are understudied plant breeding targets despite their potential to significantly shape microbial community structure and function in the rhizosphere. In this review, we explore the relationship between various root architectural and morphological traits and rhizosphere interactions, focusing on the potential of root diameter to impact the rhizosphere microbiome structure and function while discussing the potential biological and ecological mechanisms underpinning this process. In addition, we propose three future research avenues to drive this research area in an effort to unravel the effect of belowground traits on rhizosphere microbiology. This knowledge will pave the way for new plant breeding strategies that can be exploited for sustainable and high-yielding crop cultivars.

Introduction

By the year 2050, the global agricultural output will need to increase in order to feed 10 billion people, but without increasing the amount of land used for cultivation (Tian *et al*., 2021). Traditional strategies, namely, fertilizer and pesticide applications, are not long-term solutions to increased crop productivity due to their harmful effects on the environment. Plant breeding technologies have been successfully used to breed for high yielding crops (Yu *et al*., 2021). However, despite the progress made in plant breeding, high-performing cultivars still struggle in the face of challenging climatic and stress scenarios (Shew *et al*., 2020). This may be attributed to the negative belowground effects of crop domestication and breeding for, i.e. aboveground traits on the plant root microbiome and plant–microbe interactions in the rhizosphere (Szoboszlay *et al*., 2015; Pérez-Jaramillo *et al*., 2018; Kavamura *et al*., 2020). The *rhizosphere* is a dynamic environment governed by a highly complex network of interactions between plants and diverse soil organisms. Each organism inhabiting the rhizosphere plays a role in ecological processes, e.g. nutrient cycling, competition, symbiosis and even microscopic predation. The microorganisms in the soil, notably bacteria and fungi, are also capable of providing benefits to the plant host such as preventing fungal and bacterial infections (Cheng *et al*., 2019), increasing drought and salinity tolerance (Etesami and Beattie, 2018; De Vries *et al*., 2020), and improving plant nutrient acquisition (Pii *et al*., 2015). These beneficial effects of belowground plant-associated rhizobacteria on crop health and productivity have been well-documented since the 1970s (Vessey, 2003).

Hence, harnessing the rhizosphere microbiome through the promotion of its beneficial effects on crop health and productivity is a promising strategy to fortify our future food supply without damaging the environment (Bakker *et al*., 2012). While the use of microbial inoculants has been substantially researched and recently reviewed in Basu *et al*. (2021), the approach of engineering the microbiome by plant breeding is only just now coming into focus. Specifically, microbiome-based approaches for sustainable agriculture should bolster plant-beneficial rhizosphere microbiome traits through plant breeding strategies that take into account the effect

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on the rhizosphere, not just yield (Fig. 1). Breeding for pathogen resistance in bean was shown to improve antifungal functions in the root microbiome (Mendes *et al*., 2018, 2019), highlighting that breeding for increased crop health and productivity via the rhizosphere microbiome is a propitious avenue for sustainable agriculture.

Only recently has work been done to link specific plant genes to the rhizosphere microbiome through genome-wide association studies (Bergelson *et al*., 2019; Deng *et al*., 2021). As this work continues, plant breeding can target these plant genes for improved rhizobacterial function for enhanced plant performance. However, many of these genes have wide-ranging functions such as cell-wall integrity and systemic immunity (Bergelson *et al*., 2019), and thus may not be good targets for plant breeding due to off-target effects. Alternatively, since the genetic loci for root phenotypic traits continue to be unravelled with higher resolution (Rufo *et al*., 2020), plant breeding can shift towards manipulating root traits that improve microbial colonization and functioning in the rhizosphere.

Root architecture and morphology are essential for plant resource acquisition, and different root ideotypes have been suggested as breeding targets for climate-resilient genotypes (Ober *et al*., 2021). However, lacking attention is the untapped potential of root architecture and morphology as a plant breeding target for an improved rhizosphere microbiome. *Root architecture* describes the spatial configuration of the entire root system, and includes traits such as root system length, root density, root branching and branching intensity, root angle, and biomass (Bardgett *et al*., 2014). *Root morphology* describes the physical traits of the individual root such as diameter, surface area, cell wall structure, root hairs and specific root length (Bardgett *et al*., 2014). The focus of this review is therefore to draw attention to the overlooked importance of root architecture and morphology, especially root diameter, in mediating beneficial rhizosphere interactions, thereby proposing a paradigm shift in plant breeding towards below ground root traits to improve rhizosphere microbiome functioning. We also propose root diameter in particular as an important plant breeding target for a robust rhizosphere microbiome, and hypothesize the potential mechanisms by which root diameter can mediate beneficial rhizosphere interactions.

Root-driven bacterial assembly and function on the root

One of the most important drivers of rhizosphere development is the plant itself (Bulgarelli *et al*., 2013). Plant species identity is a strong determinant of bacterial community structure in the rhizosphere (Schmid *et al*., 2019), and plant roots in particular are capable of selecting and filtering soil bacteria to uniquely shape the microbiome in each section of the root (i.e. rhizosphere, rhizoplane and endosphere; Edwards *et al*., 2015). This is due to the many plant root traits which impact bacterial colonization

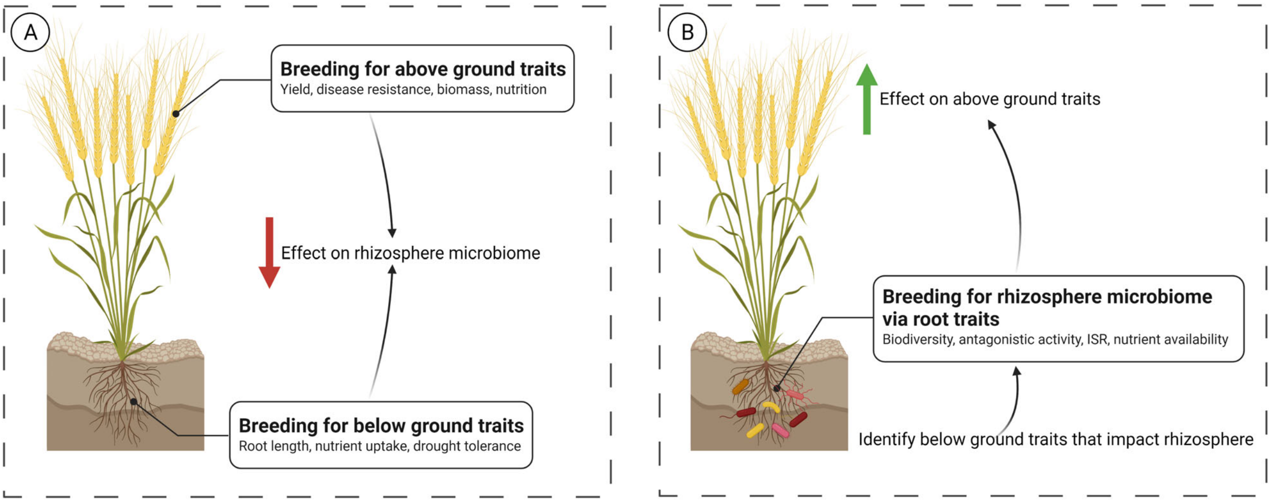

Fig. 1. A. Traditional plant breeding strategies focus on above ground (most common) or below ground (rarely) traits with no concern for effect on plant microbiome. This may result in negative impacts on holobiont functioning through off-target effects on the root microbiome and reduces overall plant performance despite the intended breeding outcome.
B. A bottom-up plant breeding approach should be used in combination with breeding for yield. The proposed strategy first identifies the root traits showing an effect on the rhizosphere. It then breeds for the root traits, which promote a healthy rhizosphere function (diversity, antagonism, abiotic stress fortification, etc.). This has a positive impact on the holobiont and holistically improves plant performance with long-term success. ISR, induced systemic resistance (Created with Biorender.com).

and function, namely, root exudation as well as root architecture and morphology.

Currently, the most well-studied trait in rhizosphere microbiome development is root exudation. Root exudates are molecules, e.g. sugars, amino acids and organic acids, secreted by the plant, responsible for attracting bacteria from the soil to the root system. They create a hospitable environment for microorganisms by serving as a carbon source and providing necessary nutrients. The unique fingerprint of the root exudation profile of a plant contributes heavily to the recruitment, colonization and function of specific rhizobacteria (Sasse *et al*., 2018). For example, the content of the exudates is reflected in the presence of rhizobacterial genes that degrade those specific exudates (Song *et al*., 2020). Similarly, rhizobacteria that flourish on plant roots have higher metabolic potential in regards to root exudates. e.g. organic acids compared to bulk soil microorganisms (Zhalnina *et al*., 2018). Specific root exudates can even be linked to the recruitment of certain bacterial taxa (Neal *et al*., 2012; Vieira *et al*., 2020). The production of bacterial secondary metabolites such as siderophores, cyclic lipopeptides and antibiotics is an important indicator of overall rhizosphere microbiome functioning and can also be dictated by plant root exudates. For example, the regulation of cyclic lipopeptide synthesis is influenced by various plant exudates, e.g. arbutin and phenyl-β-D-glucopyranoside (Christiansen *et al*., 2020). Thus, the plant is capable of shaping its rhizosphere by attracting bacteria that are well-suited to grow in the presence of its root exudates. Yet, the balance between an established plant microbiome and one that the plant continuously builds in response to various stressors, i.e. the cry-for-help theory (Liu *et al*., 2021), is still under debate.

Root architecture and root morphology are root traits that also impact rhizosphere microbiome assembly and function, yet have not been as thoroughly studied in connection to microbial assembly and function as root exudation. Thus, we are emphasizing the overlooked importance of root architecture and morphology in mediating beneficial plant–microbe interactions.

Root architecture traits have the potential to affect the rhizosphere microbiome through its impact on the root system as a whole. Microbial diversity in the rhizosphere can be linked to various root architecture traits such as root system length, biomass and branching intensity (Eisenhauer *et al*., 2017; Wang *et al*., 2017; Iannucci *et al*., 2021; King *et al*., 2021). Many root architecture traits are related to root system size, and thus, a positive correlation between rhizobacterial colonization levels and root architecture traits are not unexpected.

Root morphological traits impact the rhizosphere microbiome by altering specifically the interface of the plant–microbe interactions. At a cellular level, bacterial colonization is favoured near root cell borders due to grooves that offer accumulation of nutrients and physical protection from predation (Schmidt *et al*., 2018). Molecularly, the root cell surface contains many important components for bacterial cell attachment, biofilm formation and growth (Wheatley and Poole, 2018). For example, lipids in the plant plasma membrane are increasingly recognized for their importance as signalling molecules in plant–microbe interactions (Macabuhay *et al*., 2021). On a larger scale, the presence of root hairs is important in stimulating rhizosphere microbiome diversity and complexity (Robertson-Albertyn *et al*., 2017) as well as selecting for plant-beneficial genes, e.g. ACC deaminase (Gebauer *et al*., 2021). Root morphology is also capable of shaping the rhizosphere by affecting soil organic matter and nutrient availability, either by increasing or decreasing resource availability. For example, high specific root length reduces the amount of available nitrogen in the rhizosphere and subsequently decreases microbial biomass (Pérez-Jaramillo *et al*., 2017; Wan *et al*., 2021).

On the other hand, the reciprocal relationship also exists. Data from single-strain inoculation studies have highlighted the capability of rhizosphere bacteria to modify root architecture and morphology through the secretion of various phytohormones, notably auxins and cytokinins (Grover *et al*., 2021). Two recent experiments have demonstrated the ability of *Bacillus* inoculations to increase root length, volume and branching of wheat and soybean (Cataldi *et al*., 2020; Araujo *et al*., 2021), and this effect is not limited to only *Bacillus* (Verma *et al*., 2018). Indeed, over 70 bacterial strains encompassing a wide range of phyla have been identified as capable of altering plant root growth (Grover *et al*., 2021). However, the effect of bacterial inoculations on certain root morphological traits is less clear, as inoculation of various strains in rice, wheat, or soybean increased, decreased, or had no effect on root diameter respectively (Rêgo *et al*., 2014; Liu *et al*., 2019; Araujo *et al*., 2021). Thus, while inoculations are a well-examined route for sustainable agriculture, there remains a gap in knowledge on the relationship between root traits, especially root diameter, and the microbiome that can be applied to plant breeding strategies.

Potential of root diameter to impact the rhizosphere microbiome

Root diameter determines the plant's nutrition acquisition strategies, water transportation, ion uptake, soil penetration and other root morphological traits (Popova *et al*., 2016; Ma *et al*., 2018a). *Root diameter* can be measured against a thin versus thick threshold, which is defined on a species-by-species basis (Wang *et al*., 2020; Zai *et al*., 2021), as the average root diameter of one cultivar compared to another (Szoboszlay *et al*., 2015; Li *et al*., 2018), or by the branching order (Wang *et al*., 2017; Pervaiz *et al*., 2020). In general, *thin roots* is a relative term that describes the thinnest roots of the system, excluding the root hairs. Previous studies have documented that root diameter can influence rhizobacterial diversity. Different bacterial compositions can be found between thick- and thin-rooted lettuce and wheat (Schreiter *et al*., 2014; Kavamura *et al*., 2020). There is also growing evidence that a thinner root diameter recruits a more diverse rhizosphere community in, e.g. a natural shrubland, black locust trees, peach trees, poplar trees, but also in the agriculturally relevant crops maize and wheat (Szoboszlay *et al*., 2015; Wang *et al*., 2017, 2020; Pervaiz *et al*., 2020; Zai *et al*., 2021; C. H. Herms, unpublished).

Microbial diversity in the rhizosphere is critical for plant health; biodiversity increases interference and competition with plant pathogens (Hu *et al*., 2017) and is directly linked to the prevention of pathogen attack (Jousset *et al*., 2011; van Elsas *et al*., 2012; Yang *et al*., 2017). Since increased microbial diversity often has a positive impact on crop health and productivity (Berg *et al*., 2017; Mendes *et al*., 2018; Saleem *et al*., 2019), root diameter would be important in promoting plant-beneficial microbial interactions if it can reliably improve rhizosphere biodiversity. Thus, it is imperative to unravel how root diameter influences rhizosphere development, i.e. plant-growth-promoting (PGP) bacterial colonization and activity, so that it can be utilized via plant breeding to promote crop performance. Thin root traits, i.e. limited root surface, improved root exudation, and enriched nutrient cycling (Fig. 2), may promote bacterial diversity and activity in the rhizosphere. We can also consider how increased nutrient availability via root exudation and carbon cycling concentrated in a reduced physical space work in combination to drive bacterial diversity.

Reduced root diameter means that for an individual root, there is less surface area available for bacterial colonization. Space as a limiting resource is a well-documented phenomenon (Grams and Lüttge, 2010) and can also be applied to colonization of the root surface (Martinuz *et al*., 2012; Essarioui *et al*., 2017). Competitive exclusion is already an important rhizosphere trait for combating plant pathogens (Bakker *et al*., 2012). Limited physical space of the thin root may increase microbial competition; due to this competition for space, bacteria which can produce plant-beneficial antibiotics may outcompete other microbes attempting to colonize the root

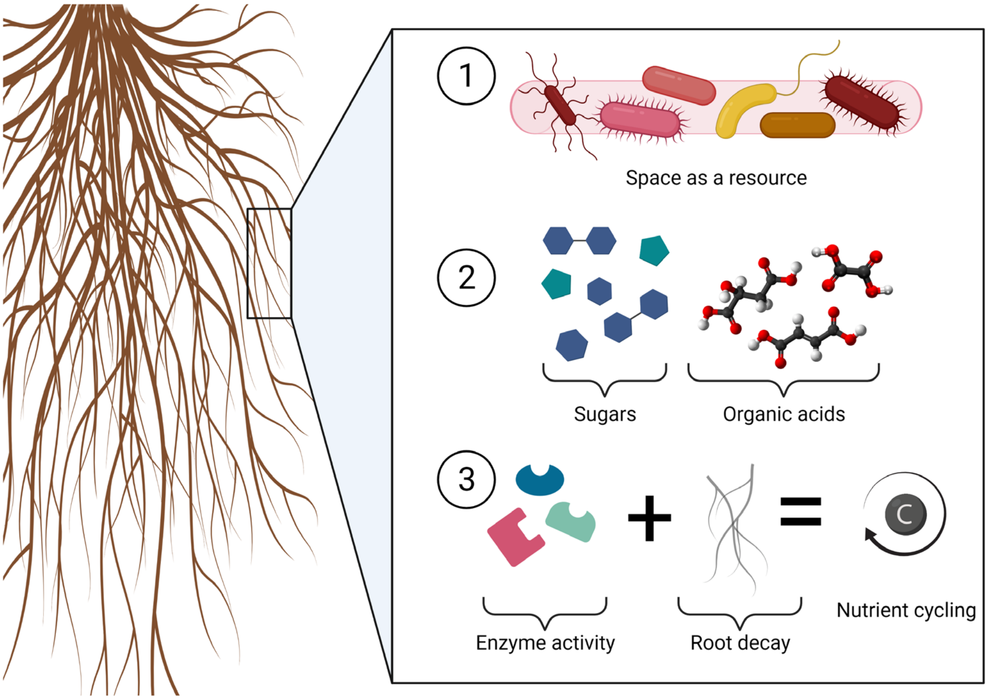

Fig. 2. Thin root have traits which may have an effect on the rhizosphere and microbial interactions on the root. (1) Reduced space on the thinner root promotes contact between diverse microorganisms colonizing the root. This may function to increase competition and antagonistic activity of rhizobacteria through the secretion of plant-beneficial antimicrobial metabolites. (2) Thin roots secrete increased levels of root exudates, especially sugars and organic acids. These exudates are involved in the recruitment of plant-beneficial microorganisms to the root. (3) Thin roots increase nutrient cycling via increased enzymatic activity and root turnover. Increased carbon availability may be capable of supporting a more metabolically active rhizosphere (Created with Biorender.com).

surface, including plant pathogens that carry toxins only affecting the plant. Indeed, there is evidence that increased microbial competition in the rhizosphere prevents pathogen invasion (Wei *et al*., 2015). This may be attributed to the competition-induced production of antimicrobials that are well documented *in vitro* (Jousset *et al*., 2014; Netzker *et al*., 2018). It is still to be determined if increased rhizosphere competition can promote other aspects of crop health such as stress tolerance. Still, the production of antagonistic compounds by rhizobacteria under space-related stress may serve the dual purpose of carving out a niche in a competitive rhizosphere and protecting the crop against pathogens, demonstrating the benefit of space limitation on rhizosphere functioning.

The concentration of exudates in the rhizosphere can also be affected by root diameter. Total root exudation rates for carbon, especially glucose, and organic acids are significantly higher in fine roots compared to thick roots across many plant species (Proctor and He, 2017; Li *et al*., 2018; Meier *et al*., 2020). However, this was examined more often in trees and shrubs with lignified coarse roots, but still the definition for thin roots in both of these studies was below 0.5 mm (Proctor and He, 2017; Meier *et al*., 2020). Nevertheless, limited research suggests that this holds true in agriculturally relevant crops (cucumber, Li *et al*., 2018). Although the relationship between root exudation and root diameter is likely complex due to many additional factors, it is important as root exudates have the potential to affect rhizosphere assembly. Organic acids in particular have been shown to promote chemotaxis, growth and root colonization of PGP rhizobacteria in *Limonium sinense* (Xiong *et al*., 2020). High levels of glucose in root exudation also promoted bacterial chemotaxis and the synthesis of antimicrobial compounds in maize (Fan *et al*., 2012). It is worth investigating how root diameter can affect the exudation of the specific root metabolites as defined by Xiong *et al*. (2020) and Fan *et al*. (2012), among others, that increase the recruitment, colonization and activity of PGP rhizobacteria.

In addition to increased root exudation rates, thin roots may impact rhizosphere development and activity by stimulating metabolic activity within the surrounding soil. It is well-documented that thin roots have shorter lifespans, have increased turnover, and are able to accelerate underground carbon cycling compared to thicker roots (Endo *et al*., 2019). Thin roots are also associated with increased plant enzyme activity regarding carbon, nitrogen and phosphorus cycles (Ma *et al*., 2018b). These mechanisms may increase the amount of metabolically relevant compounds, especially carbon, in the rhizosphere available for microbial utilization, which has the potential to alter rhizosphere development and activity. Culturing *Bacillus amyloliquefaciens* in the presence of multiple carbon sources improved both the growth efficiency and antibiotic production of the rhizobacterium, demonstrating the ability of carbon availability to promote co-expression of bacterial genes (Yang *et al*., 2019). In real soil systems, increased soil organic carbon is a key factor in promoting both soil microbial community diversity and metabolic diversity (Li *et al*., 2015). Indeed, increased carbon availability directly stimulated by thin root degradation was linked to elevated microbial metabolic activity in the rhizosphere, but without comment on plant-beneficial effects (de Graaff *et al*., 2013). Taken together, these data reveal the potential impact of thin root-driven carbon cycling on the rhizosphere microbiome.

In summary, the data from microbiology and plant science support the hypothesis that the characteristics of thin roots, namely, increased nutrient availability combined with reduced physical space, can promote biodiversity in the rhizosphere. Thus, considering root diameter in plant–microbe interactions is vitally important for developing robust future plant breeding strategies. As the genetic loci for root phenotypic traits, including root diameter, are identified (Wu *et al*., 2017; Rufo *et al*., 2020), we should explore the opportunity to breed for crop cultivars with a root diameter that improves the plant-beneficial activities of the rhizosphere microbiome (Fig. 1), but without negatively affecting water transport and soil penetration.

Future perspectives

In summary, a growing number of studies have established correlations between root architectural and morphological traits and the rhizosphere microbiome composition. However, the link between these root traits and rhizosphere microbiome function has not been made. Nevertheless, there is potential for root diameter to impact rhizobacterial activity through increased nutrient availability and space limitation. Thus, the plant root is a promising breeding target for a more robust and plant-beneficial rhizosphere microbiome. Root traits can be implemented as a selection criteria in breeding programs, but this requires that the trait is heritable and simple to screen (Richards, 2006). The trait should be observable during early plant growth and determine the root system functioning and root architecture or morphology (Manschadi *et al*., 2006). Recently, however, unique phenotyping facilities enabling studies of roots in soil have been developed, making studies on root architecture and morphology throughout the growing season possible (Svane *et al*., 2019).

Even with the aforementioned studies on the impact of root architectural and morphological traits on the rhizosphere microbiome, more work is needed before the implementation of plant breeding programs for the microbiome via root traits and root diameter can occur. First, since no prior research to our knowledge has linked root architecture and morphology to rhizosphere microbiome functioning, additional studies must be performed to further understand how these root traits affect microbial gene expression, secondary metabolite synthesis and rhizosphere interactions. Drivers of rhizosphere function are equally as, if not more, important as factors regulating rhizosphere assembly due to the high genomic diversity of closely related or clonal bacteria (Jaspers and Overmann, 2004). The integrated use of transcriptomics and proteomics in combination with novel techniques such as ion mobility in mass spectrometry to directly analyse metabolites from environmental samples (Singer *et al*., 2021) will allow us to better visualize the chemical cross-talk between plant roots and their microbiome. Reporter gene fusions to relevant bacterial promoter genes *in planta* can link plant root traits to the bacterial molecular mechanisms regulating PGP gene expression.

Second, highly targeted community analyses are needed to discover the root traits responsible for the recruitment of specifically PGP rhizobacteria. Due to the well-conserved core root microbiome (Simonin *et al*., 2020), composition studies at a high taxonomic level may not provide significant data regarding differential rhizobacterial colonization according to differing root traits. Additionally, the high intra-genus diversity of PGP traits (e.g. *Pseudomonas*, Vacheron *et al*., 2016) demands more targeted analyses to unravel the recruitment of these plant-beneficial species and strains. Previous studies have been able to determine differential species-level colonization on different crop cultivars, which correlated with different microbial metabolite profiles (Oni *et al*., 2019). Thus, high-resolution experiments such as targeted isolations, metagenomics and fluorescence microscopy of tagged PGP bacteria would provide insight into how plants use their root architecture and morphology to promote bacteria with plant-beneficial traits. Nevertheless, these targeted population studies should align as closely as possible to natural field settings. The continued use of specific plant cultivation techniques (Song *et al*., 2020) or soil types (Simonin *et al*., 2020) leads to results that are difficult to understand in the context of real agricultural conditions. Plant experimental setups mimicking the environments of current farming systems will generate results that can be directly applied in the field.

Lastly, mechanistic understanding is vital in confirming root diameter as a driver of rhizosphere development and fully understanding the role of the plant in rhizosphere development, yet is distinctly lacking. Above, we have suggested several testable biological and ecological mechanisms by which root diameter can affect the rhizosphere. We encourage an increased focus on unravelling these through simplified consortia studies on thin versus thick roots. The use of pure culture studies for mechanistic understanding (Xiong *et al*., 2020) should be avoided if possible, as the three-way interaction between soil, the plant and its microbes is of the utmost importance to rhizosphere functioning (Tosi *et al*., 2020).

As it stands, no studies to date have attempted to unravel how and why root architecture and morphology impact the colonization and function of plant-beneficial rhizobacteria, while the effect of the microbiome on root traits via phytohormone production has been extensively researched, as reviewed by Grover *et al*. (2021). Thus, more dedicated work is urgently needed to expand the available literature of the former in order to determine how root traits can be used to improve rhizosphere microbiome functioning. Unravelling the role of root architecture and morphology as a mediator of rhizosphere interactions will be critical for breeding resistant and high yield crop cultivars.

Concluding remarks

Below-ground interactions between PGP rhizobacteria and the plant root are well recognized for their ability to improve crop performance. Breeding for a robust rhizosphere microbiome is a goal of sustainable agriculture to both reduce the reliance on harmful chemicals and fortify the food supply. A promising plant breeding target to promote rhizosphere functioning is root architecture and morphology, which influence plant performance and bacterial colonization and activity. In the present review, we have highlighted the overlooked importance of root architecture and morphology in mediating rhizosphere interactions. We suggest that the root diameter is a vitally important factor in beneficial plant–microbe interactions and the subsequent microbe–microbe interactions on the root. The impactful physical characteristics, increased root exudation and nutrient cycling of thinner roots may contribute to increased PGP rhizobacteria colonization, plant-beneficial gene expression and biodiversity in the rhizosphere. Yet, no studies to date have attempted to understand how these characteristics of root diameter influence the colonization, activity and interactions of PGP rhizobacteria with the plant and other microorganisms. The connection between root diameter and rhizobacteria raises important new questions on the role of the plant in rhizosphere development, which we see as a novel yet logical progression in the field. Even with the focus on root diameter in this review, we stress the need to unravel the full effect of root morphology and architecture traits on plant–microbe interactions in the rhizosphere.

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