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OPINION PAPER

New methods for new questions about rhizosphere/plant root interactions

Peter J. Gregory · Timothy S. George ·
Eric Paterson

Abstract In this opinion paper we review recent methodological developments underpinning the study of roots, the rhizosphere and interactions affecting soil functions, and explore new understanding resulting from these advances. We focus on methods that have improved our understanding of rhizodeposition, rhizosphere enzymatic processes and root growth, water and nutrient acquisition at several levels. Finally, we suggest that the future will require new methods that continue to overcome the difficulties posed by the opacity of soil, can scale results spatially and temporally, and integrate multiple aspects of rhizosphere processes simultaneously.

Keywords Enzymology · Rhizosphere ·
Rhizodeposition · Root methods

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Introduction

The root/soil interface is highly complex and dynamic, and its study is bedevilled by the fact that soil is opaque and any disturbance alters the environment leading, in turn, to modifications to plant growth, microbial composition and rhizospheric processes. During the 30 years that Hans Lambers has been Editor in Chief of Plant and Soil, methods for studying roots and the rhizosphere have advanced substantially. Initially, methodological advances tended to focus on either the study of roots and their responses to soil properties or the composition and activity of microbes and other organisms in proximity to roots. More recently, attention has increasingly focussed on methods that allow characterisation of the multiple processes occurring at the rhizosphere/root interface. Ideally, such methods need to encompass the whole plant and soil system, be non-invasive and non-destructive at levels that are realistic, resolvable and meaningful for the processes being studied, and deliver information in three dimensions and in real-time to resolve spatial and temporal dynamics (for examples see reviews by Oburger and Schmidt (2016), York et al. (2016) and Vetterlein et al. (2020)).

New methods generally arise as a consequence of two interacting processes. First, scientific curiosity and specific questions drive the quest for a means of answering them. For example, determining how much of a crop's photosynthate supports root growth

and the acquisition of nutrients and water drove the early development of soil coring and root washing methods to measure root dry matter (Mengel and Barber 1974; Gregory et al. 1978). Similarly, questions about the distribution of roots in soil led to the adoption of trench profiling, radioactive tracer techniques, minirhizotron and core break technologies with a focus on root length (e.g. Majdi and Persson 1997). Second, advances in physics and biology provide new technologies that facilitate better means of answering old questions and stimulate the study of previously unanswerable questions. For example, ground penetrating radar and x-ray computed tomography both allow insights into the distribution of roots in soil, but the latter also allows study of root interactions with pores and particles (Schmidt et al. 2012). Similarly, the emergence of new questions about nutrient cycling for sustainable crop production and sequestration of soil carbon to mitigate climate change, the concept of soil as a holobiont (Finlay et al. 2020) and the role of roots as a key element of soil formation and functioning (Gregory 2022) have been spurred by the development and application of synchrotron techniques, high-throughput genomic pipelines and metabolomic technologies, and stable isotope methods.

The aims of this opinion paper are to: i) review recent methodological developments underpinning the study of roots, the rhizosphere and interactions affecting soil functions; ii) explore new understanding resulting from these methodological advances; and iii) suggest remaining issues for which new approaches are urgently required and the technologies that might facilitate answers.

Recent methodological developments

Root detection

Cabal et al. (2021) characterised techniques to identify roots of specific plants growing in stands or mixed communities as being either extraction, observation or inference detection methods. The current state and recent advances in these methods are outlined in Table 1.

In addition to these techniques, rhizoboxes are widely used in laboratory and glasshouse studies to give measures of roots and their activities (e.g.

George et al. 2002). This method is still widely used to assess root activities such as enzyme reactions and biochemical changes, but has the disadvantage that only a planar surface is viewed and root growth against a surface or mesh may introduce artefacts. In the laboratory, root observation has been transformed by the development of magnetic resonance imaging and x-ray computed tomography (Mooney et al. 2012) which have also imaged root/soil processes including root/soil contact, gap formation and the development of pores (Schmidt et al. 2012; Carminati et al. 2013; Tracy et al. 2015; Helliwell et al. 2017; Perelman et al. 2020). Radiation sources and detectors now allow μm resolution and new software permits root tracing through soil volumes (Phalempin et al. 2021).

Characterisation of rhizodeposition and impacts on microbial communities

A key driver of plant-microbe interactions is the release of organic compounds from living roots (rhizodeposition), delivering diverse substrate sources to the rhizosphere, impacting microbial species composition and the magnitude of biogeochemical cycling (Kuzyakov and Blagodatskaya 2015). More recently, there has been rapid progress in understanding of functional consequences of these interactions, driven particularly in the context of research priorities to achieve sustainable food production and climate change mitigation/ adaptation (Jones and Hinsinger 2008; Philippot et al. 2013). These advances have been facilitated by rapid development of powerful approaches to characterise microbial communities in association with roots (high-throughput and lower costs of sequencing technologies for metagenomics, Schlaeppi and Bulgarelli 2015), functional assays of microbial processes (isotopic tracing and transcriptomics, Nkongolo and Narendrula-Kotha 2020) and the increasing resolution of analytical methods to provide comprehensive quantification of plant and microbial metabolites (metabolomics, Kellogg and Kang 2020).

Observing the root-soil interface

Use of high-powered X-ray beams has allowed in-situ measurement of the intimate relationship between roots and soil including interactions between root

Table 1 Field methods for dectecting plant roots

Means of detection	Method	Current state	Issues to be resolved	References
Extraction	Trenching and washed soil cores	Few advances in last two decades, but better drilling machines, washing techniques and optical detection and software have made data easier to collect. Such techniques have been adopted as one of the only means of studying deep roots	Disadvantages of the methods are widely understood	Smit et al. 2000 for summaries. Maeght et al. 2013 for deep roots
Observation	Minirhizotrons	Used with digital cameras these have advanced the study of fine roots especially root longevity and turnover.	Roots growing against a solid surface can produce artefacts; sequential coring and ingrowth cores are used to complement this method.	Rewald and Ephrath 2013; Arnaud et al. 2019.
	Tomography using electromagnetic waves	Ground-penetrating radar (GPR) has been successfully used to map tree roots non-invasively, to estimate root biomass and to characterise phenotypic variation in root traits	Accuracy is affected by root diameter, proximity to other roots and rock fragments, soil depth, root and soil water content and organic litter meaning that it requires careful interpretation	Butnor et al. 2003; Wasson et al. 2020; Lombardi et al. 2021; Cabal et al. 2021; Tanikawa et al. 2021
Inference	Electrical methods	Electrical capacitance has the longest history of use and has been widely used in hydroponics and pot experiments with wet soils	Determination of root traits and architecture is still problematic with species- and soil moisture-specific calibration required	Wu et al. 2017; Cabal et al. 2021; Cseresnyés et al. 2020; Gu et al. 2021

hairs and rhizosphere microporosity and soil water (Fig. 1; Keyes et al. 2013; Koebernick et al. 2017).

However, such high resolution (μm) is accompanied by very small (mm) sample size leading to growing
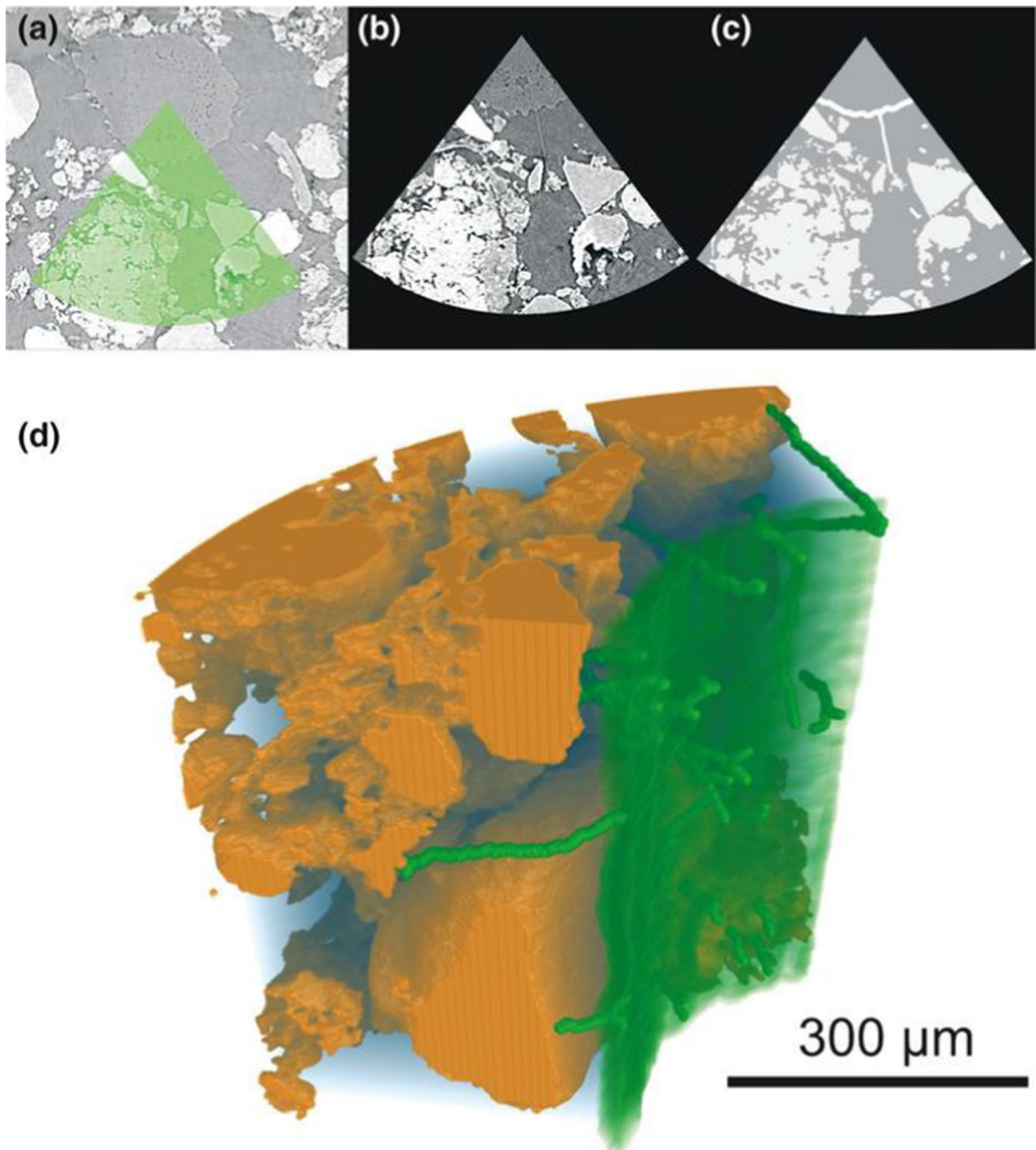

Fig. 1 High resolution synchrotron imaging of wheat root hairs growing in soil: Region selection and classification for rhizosphere simulation from synchrotron data. (**a**) A segment was defined, with centreline aligned with the centre axis of the root, (**b**) All other voxels are removed, leaving only the defined segment, (**c**) root hairs, soil, fluid and root surface regions are individually defined using different discrete grey-levels, (**d**) a volume mesh is generated, with root hairs, soil, root surface and water defined separately. (Used with permission from Keyes et al. (2013))

environments that are unrealistic of natural ecosystems. Moreover, throughput is limited and the cost of sample processing and analysis is high. Other syncotron techniques such as XANES (K-edge x-ray absorption near-edge structure spectroscopy) can observe the presence, concentration, and speciation of elements at the root soil interface providing a potentially powerful technique for understanding the dynamics of nutrients (Gillespie et al. 2009). Combined use of XANES and X-ray CT has shown the impact of soil compaction in the rhizosphere on the availability and speciation of elements (van Veelen et al. 2020). Similarly, NanoSims (nano-scale secondary ion mass spectroscopy) measures the presence of a range of elements at fine resolution, using measurement of secondary ionisation (Oburger and Schmidt 2016; Clode et al. 2009).

Elemental speciation and distributions in the rhizosphere have also been assessed using Scanning Electron Microscopy (SEM) with Energy-dispersive X-ray analysis (SEM-EDX), Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), and confocal Raman spectroscopy (μ-Raman; Bandara et al. 2021). These techniques can resolve the difference between element distribution between root and soil, within pore space, root hair zones and zones around and within active microorganisms (Oburger and Schmidt 2016; Kilburn et al. 2010). However, these techniques are destructive, and the preparation steps render samples unavailable for further analysis.

In contrast, neutron radiography, while also being used for phenotyping roots systems (Wasson et al. 2020) has been used to measure non-destructively the dynamics of water at the root soil interface (Carminati et al. 2010; Holz et al. 2018).

New understanding resulting from methodological advances

New and improved methods have enhanced our understanding of aspects of root/soil interactions, although there is still a pressing need to link mechanistically the many chemical, physical and biological processes occurring at different spatial and temporal levels (Vetterlein et al. 2020). Here we focus on advances in understanding aspects of rhizodeposition, enzymatic processes and water and nutrient acquisition.

Rhizodeposition

Quantification and chemical characterisation of rhizodeposition in natural soils has been, and remains challenging, as the release of organic compounds from roots is affected by microbial communities, and rhizodeposits are rapidly transformed by microbes in soil (Paterson et al. 2009). Labelling plant assimilate with isotopic tracers (^{13}C, ^{14}C) provides a means of differentiating root-derived C from that cycling through native SOM pools, including microbial biomass and dissolved organic carbon (DOC), and when this is done under steady-state conditions (e.g. continuous, uniform $^{13}CO_2$ labelling), this allows quantitative partitioning of plant- and SOM-derived sources to soil pools and fluxes (Paterson et al. 2009). This has provided new understanding of plant-mediated impacts on microbial communities and their functions (e.g. priming effects, Kuzyakov 2010), leading to the suggestion that plant-microbe interactions affecting soil processes may be a route to management directed to promotion of beneficial functions for crop productivity and sustainable soil health.

The recent rapid development of molecular methods and associated bioinformatic approaches to characterise microbial community composition has facilitated recognition that both plant species and genotype can have significant influences on rhizosphere microbial community selection and development. This has led to the concept of plant-specific microbiomes, and that roots in combination with associated microbial communities should be considered as 'holobionts' affecting ecosystem processes and interactions with the environment (Vandenkoornhuyse et al. 2015). Initially, genotype-specific microbiome selection was demonstrated for *Arabidopsis thaliana*, and the generality of this has now been demonstrated for a range of species, including crop plants (Kumawat et al. 2022). Further, it is now recognised that microbiome structure varies along root axes and as a function of root type/ age, driven by differences in exudation, nutrient uptake, predation and release of signalling compounds (Bonkowski et al. 2021). However, significant challenges remain in establishing the root traits responsible for regulating specificity of microbial community selection and the functional consequences of distinct microbiomes for soil processes, such as biogeochemical cycling. In large part, uncertainty over the functional consequences of microbiome

selection is a result of the very large diversity of rhizosphere microbial communities; that understanding of the genetic bases of microbial functions is incomplete (Young 2016); and that at any point in time, a large proportion of the microbial community is inactive (Joergensen and Wichern 2018). These considerations mean that it is imperative that characterisation of plant microbiome structure is combined with measures of microbial functions to gain mechanistic understanding of plant-specific impacts on soil processes (Sokol et al. 2022).

Identification of microbial community components active in utilisation of root-derived C (interactome) can be established through $^{13}CO_2$-labelling and tracing of rhizodeposits into microbial biomarkers and nucleic acids (stable-isotope probing [SIP], Radajewski et al. 2000; Paterson et al. 2007). For example, phospholipid fatty acid (PLFA) analysis provides relatively coarse-resolution of microbial community structure but has the advantage that compound-specific isotope ratio mass spectrometry (IRMS) is a highly sensitive analytical approach for quantification of incorporation of plant-derived C (Paterson et al. 2009). In contrast, tracing ^{13}C into DNA fractions (Radajewski et al. 2000) has much higher taxonomic resolution, but is constrained by the requirement to physically separate labelled fractions via isopycnic centrifugation. An analogous approach is to target RNA rather than DNA, which has the advantage that it can be related to overall (rRNA) or function-specific (mRNA) activity of microbial populations. There is great potential in combining such SIP approaches with concurrent measurement of specific biogeochemical functions (e.g. nutrient cycling fluxes), providing opportunities to identify key components of microbial communities responsible for plant-mediated impacts on soil functions.

Advances in analytical chemistry and approaches such as metabolic flux analysis, to trace C-flow through biochemical pathways (Dijkstra et al. 2011), have greatly increased the resolution at which rhizodeposition composition and microbial C-processing in the rhizosphere can be resolved. Metabolomics, defined as the non-targeted, comprehensive analysis of metabolites in biological systems, has increased the understanding of root exudate composition, mobilisation of SOM into DOC, and microbial products in the rhizosphere, identifying molecules of low abundance that may have key

roles in communication and elicitation of functions (signalling compounds, Cotton et al. 2019). Targeted immunefluorescence techniques to image the distribution of particular polysaccharide exudates around roots (Fig. 2) have increased understanding of the role exudates play in modifying soil physical conditions. Furthermore, use of position-specific ^{13}C-labelled compounds provide opportunities to characterise microbial metabolic pathways in soil, including important community attributes such as carbon use efficiency (Apostel et al. 2013; Geyer et al. 2019). These methods have particularly strong potential to resolve the mechanistic bases of root-soil interactions when combined with metagenomic, transcriptomic, proteomic and bioinformatic pipelines in systems analysis, but requires co-development of novel statistical approaches to robustly identify significant relationships.

Enzymatic processes

Developments in zymography, where membranes impregnated with enzyme substrates are placed (with a diffusive barrier separating soil and membrane) on the soil surface at the root soil interface capture an enzyme imprint of the rhizosphere in 2D (Razavi et al. 2019). A key advantage of this techniques is that a sequence of imprints can be captured and correlated with other 2D imaging techniques to build a comprehensive picture of dynamics and functionality (Fig. 3). For example, such studies have demonstrated the importance of root hairs in the distribution of phosphatase enzymes in the rhizosphere (Giles et al. 2018). When coupled with microbial molecular (Liu et al. 2021b) and imaging approaches such as FISH (Fluorescent In-situ Hybridisation; Spohn et al. 2015), the timing and longevity of hot spots and hot moments of microbial activity in the rhizosphere can be defined (Song et al. 2019). Similarly, when zymography is combined with planar optodes (which measure the spatial distribution of pH [Ma et al. 2019, 2021]) and redox or DGT (Diffusive Gradient in Thin films) optodes (which measure depletion of nutrients from the rhizosphere [Hummel et al. 2021; Fang et al. 2021]) information about the impact of the enzymes on soil chemical properties and vice versa can be elucidated.

Fig. 2 Immunoblotting analysis of root exuded polysaccharides (glycan epitopes) at the wheat root surface: Imaging of glycan epitopes in high-molecular-weight (HMW) exudates released from large wheat roots grown hydroponically for 2 weeks and then lain on sheets of nitrocellulose for 10 min prior to probing with monoclonal antibodies (MAbs). Reproduced with permission from Galloway et al (2020)
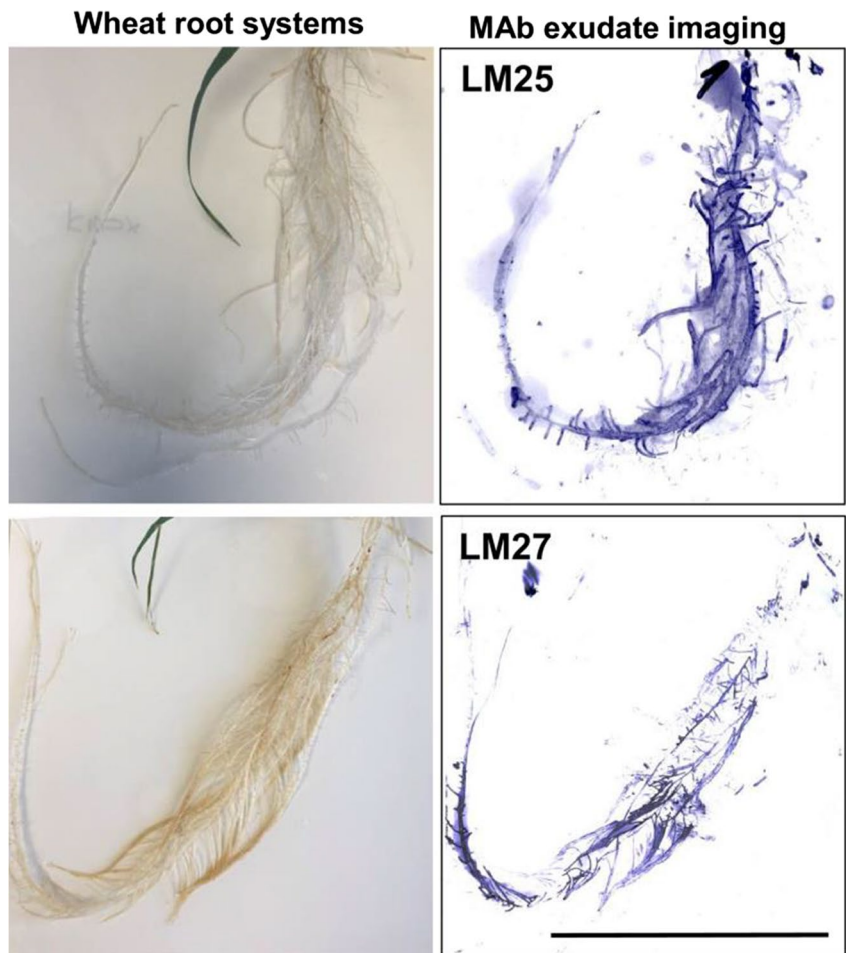
Water and nutrient acquisition

Extraction and observation of roots in the field has increased knowledge of the depth of rooting of native species (Canadell et al. 1996) and the role of deep roots in water and nutrient acquisition by crops. For example, White and Kirkegaard (2010) observed that while wheat roots penetrated to 1.6 m, 30-40% were clumped in pores and cracks in surface layers increasing to 85-100% below 60 cm where 44% of roots were in pores occupied by at least three other roots. These observations fed into models exploring the effects of seasonal rainfall distribution, and deeper and denser root systems on water use and demonstrated the multi-faceted nature of water use by rainfed crops (Lilley and Kirkegaard 2011). Similarly, extraction of roots coupled with soil chemical analysis has demonstrated that differences in crop rooting patterns can be

exploited to improve nitrogen use efficiency (Thorup-Kristensen 2006). Deep-rooted and 'catch' crops can recover nitrate leached during the growing season from cereal crops and raise nutrients such as potassium and phosphorus from subsoil to topsoil benefitting both yields and the wider environment (Thorup-Kristensen et al. 2020; Han et al. 2021).

X-ray CT imaging has led to insights into the processes affecting root water uptake and the influence of physical changes in the rhizosphere. Growing roots release mucilage and change the bulk density of the soil adjacent to the root thereby altering both the water retention characteristics and hydraulic conductivity of the rhizosphere (Moradi et al. 2011; Ahmed et al. 2014; Carminati et al. 2016). Several studies have shown decreased bulk density in the immediate vicinity of roots (e.g. Landl et al. 2021) but others have indicated an increase (e.g. Koebernick et al.
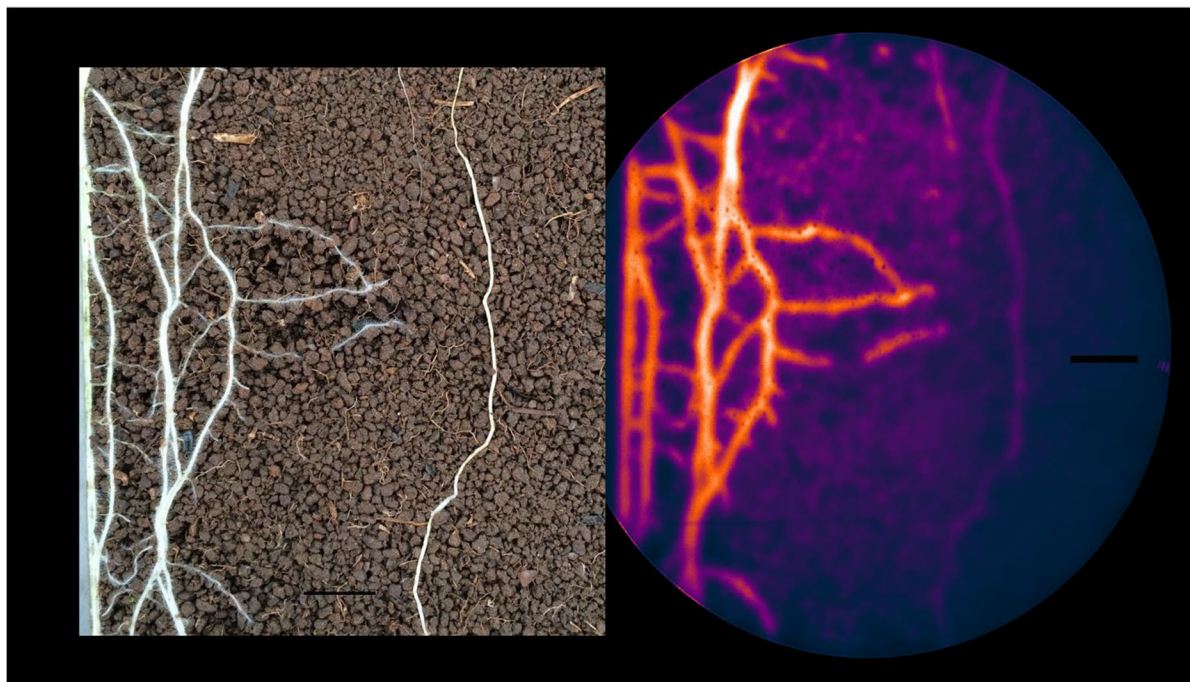
Fig. 3 Zymogrpahy image of root exuded acid phosphatase from barley and medicago roots: Comparison between a visible light image and a zymography image taken on the window of roots of barley (*Hordeum vulgare* L.) and barrel clover (*Med-icago truncatula* L.) growing on a thin layer of soil between two panes of Perspex. Image shows much greater acid phosphatase activity associated with the barley roots. Bar = 1 cm

2017). Decreased density could result from the loose packing of incompressible mineral particles displaced by the root but if particle displacement is constrained for whatever reason, then bulk density will increase because the volume occupied by the root is not matched by a decreased pore volume of the surrounding soil. When both mucilage and bulk density effects were allowed for, modelling showed that the rate of water uptake was reduced but duration was increased (Landl et al. 2021).

Spatial and temporal dynamics of element availability in the rhizosphere captures using DGT have been quantified using laser ablation ICP-MS; the laser destructively samples the imprint and transfers the material into a spectrometer for quantification (Santner et al. 2012; Fang et al. 2021; Bilyera et al. 2022). This combined technique also permits measurements of elements on, and in, live tissue such as roots and biological materials in the rhizosphere, allowing the potential production of maps of element distribution, or the rhizosphere ionome, at the soil root interface (Zaeem et al. 2021). Recently, this approach has measured the

dynamics of nano-sized fertiliser particles (Szameitat et al. 2021) and the impact of liming on nutrient availability (Smolders et al. 2020) in the rhizosphere and may assist the development of improved fertiliser practices. Such approaches when coupled with novel phenotyping tools used for screening crop genotype populations for root ion uptake and respiration (Griffiths and York 2020; Griffiths et al. 2021; Guo et al. 2021) will have profound affects on our ability to select crop genotypes that are best able to utilise the homogenously distributed resources in the rhizosphere.

What is still required

We have described methods currently used, but the future must focus on methods that provide an integrated understanding of the chemical, physical and biological changes in the rhizosphere and their consequences for plant growth. Here we focus on three issues that limit progress and need to be resolved to realise this ambition: the opacity of soil,

spatiotemporal scaling and integration of data and knowledge.

Opacity

Roots have been widely refered to as 'The Hidden Half'. X-ray CT has allowed visualisation of seedling roots in small soil samples in the laboratory, but the issue remains in studying root systems in the field. There is an urgent need for a non-destructive field technique.

Even in laboratory studies, many of the techniques (including neutron radiography) require a simplification of the growing system into two dimensions, by growing plants in thin layers of soil between plates. This requirement is generally a response to the need to get easy access to the root surface soil interface and can also be achieved in field soils using root access windows (Neumann et al. 2009). A method that usefully overcomes issues of both opacity and 2-D is the combination of light sheet microscopy with fluorescent labelled plants and microorganisms grown in transparent 'soil' (Fig. 4; Liu et al. 2021a; Jones et al. 2021). This novel development has allowed observation of the dynamics of microbial colonization of roots including dynamic waves of microbial growth at the root soil interface It has shown previously unseen extremely dynamic hot spots and hot moments associated with root growth. Of course, a limitation is that although the transparent 'soil' has been shown to behave like true soil in many respects, it is still an artificial system.

Spatiotemporal scaling

While great progress has been made in characterising the mechanistic bases of plant-soil interactions, scaling these to the level of ecosystem processes remains a significant interdisciplinary challenge (Schnepf et al. 2022). The recognised heterogeneity of soil properties and processes, over different scales of space and time, mean that quantitative translation of rates of processes at the rhizosphere scale to landscapes, or indeed globally, is highly complex (Vetterlein et al. 2020). A consequence of this is that modelling approaches, for example applied to the soil carbon cycle, have tended to greatly simplify the complexity of plant-soil interactions, favouring approaches that use environmental parameters as drivers of biologically-mediated processes in linear first-order models. However, many biological processes are characterised by non-linear functions (e.g. Michaelis-Menten enzyme kinetics), and rhizosphere research has consistently demonstrated the importance of biological diversity and context-specificity in rhizosphere functions. Theoretical advances in quantifying the consequences of non-linearity and heterogeneity of soil processes for upscaling have been made (e.g. Wilson and Gerber 2021), allowing some progress on these issues. In addition, the use of established field-to-catchment scale instrumented sites (experimental platforms) facilitate study of processes at different scales (e.g. combined use of soil flux chambers and eddy-covariance towers), while satellite remote sensing is increasingly a means to infer soil properties and plant growth at global scales. Such appoaches are also supported by the increasing availability of molecular microbial data for soils across the globe. These provide an invaluable resource with which to identify patterns of community structures across scales and are a potentially powerful means to relate biogeochemical processes with the agents that mediate them (Vereeken et al. 2016).

Integration of methods

Many of the key global challenges facing society, including climate change, agricultural sustainability, food security and the biodiversity crisis all require understanding of the dynamic zone where roots and soil intereact. They are also influenced by extremely complex systems where interactions, integration and competition between mechanisms, individuals and trophic levels are paramount. Methods and techniques are required which can capture this complexity and identify the most important interactions. We can no longer rely on reductionist approaches where the belief that understanding of one plant, one process, one microorganism or one gene will resolve these complex problems. This suggests the types of methods we need in the future. Besides dealing with opacity and spatial and temporal variation in the system, our opinion is that the prime need is to intergrate methods to study simultaneously multiple processes and their interactions. We have described several powerful methods for imaging, measuring and quantifying processes at the root soil interface. In combination, many of these techniques could become extremely powerful. Such combinations are starting to be used and have already provided insightful observations. For example, the combination of
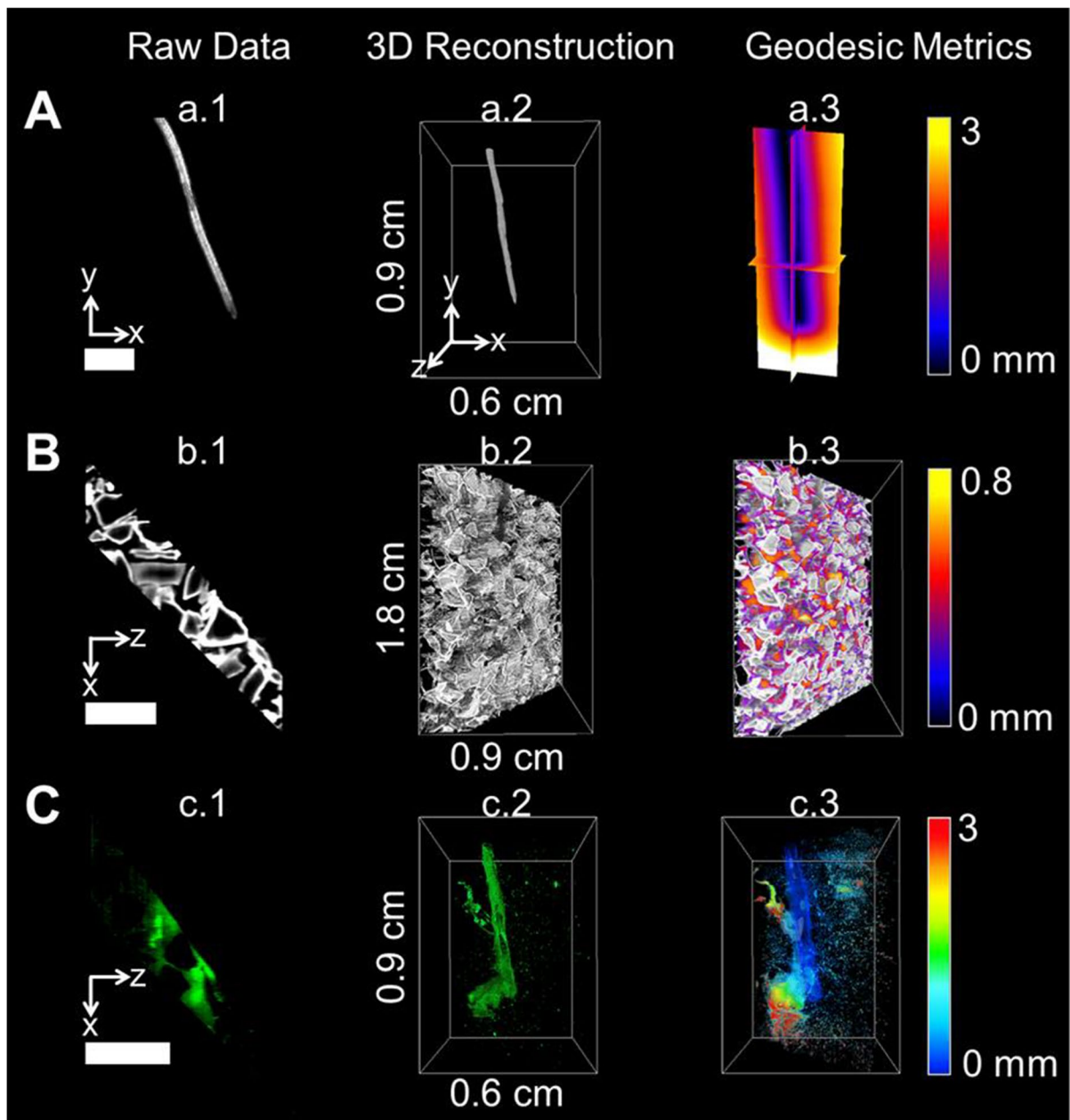
Fig. 4 Quantification of root-soil-bacteria interactions using transparent soil and light sheet microscopy. Image data from lettuce root (**A**), transparent soil particles (**B**), and GFP-labelled *Bacillus subtilis* (**C**). Processing of the data follows 3 steps. Raw data is acquired from the microscope (left). Cross sections are assembled into volume data through stitching and stacking (middle). Image processing is subsequently performed to quantify temporal and spatial patterns of biological activity in the pore space (right). The metrics obtained from the data include distance from the root surface (**A**), pore size (**B**) and bacterial cell density (**C**). The scale bar represents a distance of 2 mm. (Used with permission from Liu et al. 2021a, b)

NanoSims and pulse-chase isotope labelling has helped resolve the dynamics of nutrient uptake and transfer of rhizosphere bacteria (Clode et al. 2009) and mycorrhizal fungi (Kaiser et al. 2015). This hybrid technique is now being termed NanoSIP (Pett-Ridge and Weber 2022). Similarly, the combination of several element imaging

techniques correlated with FISH have been shown to resolve the relationships between the rhizosphere microbiome and its chemical environment (Bandara et al. 2021). We earlier showed that the combined methods of DGT and ICP-MS are powerful, but further combination of these techniques with laser ablation, planar optodes, zymography and single cell transcription analysis opens the possibility for comparison of multiple chemical and biological parameters on the same sample. In the not-too-distant future, it should be possible to generate maps of the allied transcriptional response of the microbiome and root cells at the root soil interface at the same resolution as the distribution of the ionome. With such approaches, the opportunity will arise to contribute significant understanding in responding to the global grand challenges facing our society.

Declarations

References

Ahmed MA, Kroener E, Holz M, Zarebanadkouki M, Carminati A (2014) Mucilage exudation facilitates root water uptake in dry soils. Funct Plant Biol 41:1129–1137

Apostel C, Dippold M, Glaser B, Kuzyakov Y (2013) Biochemical pathways of amino acids in soil: assessment by position-specific labelling and ^{13}C-PLFA analysis. Soil Biol Biochem 67:31–40

Arnaud M, Baird AJ, Morris PJ, Haris A, Huck JJ (2019) EnRoot: a narrow-diameter, inexpensive and partially 3D-printable minirhizotron for imaging fine root production. Plant Methods 15:1–9

Bandara CD, Schmidt M, Davoudpour Y, Stryhanyuk H, Richnow HH, Musat N (2021) Microbial identification, high-resolution microscopy and spectrometry of the rhizosphere in its native spatial context. Front Plant Sci 12:668929

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Bilyera N, Hummel C, Daudin G, Santangeli M, Zhang X, Santner J, Lippold E, Schlüter S, Bertrand I, Wenzel W, Spielvogel S (2022) Co-localised phosphorus mobilization processes in the rhizosphere of field-grown maize jointly contribute to plant nutrition. Soil Biol Biochem 165:108497

Bonkowski M, Tarkka M, Razavi BS, Schmidt H, Blagodatskaya E, Koller R, Yu P, Hochholdinger F, Vetterlein D (2021) Spatiotemporal dynamics of maize (*Zea mays* L.) root growth and its potential consequences for the assembly of the rhizosphere microbiota. Front Microbiol 12:619499

Butnor JR, Doolittle JA, Johnsen KH, Samuelson L, Stokes T, Kress L (2003) Utility of ground-penetrating radar as a root biomass survey tool in forest systems. Soil Sci Soc Am J 67:1607–1615

Cabal C, De Deurwaerder HPT, Matesanz S (2021) Field methods to study the spatial root density distribution of individual plants. Plant Soil 462:25–43

Canadell J, Jackson RB, Ehleringer JR, Mooney HA, Sala OE, Schulze E-D (1996) Maximum rooting depth of vegetation types at the global scale. Oecol 108:583–595

Carminati A, Moradi AB, Vetterlein D, Vontobel P, Lehmann E, Weller U, Vogel HJ, Oswald SE (2010) Dynamics of soil water content in the rhizosphere. Plant Soil 332:163–176

Carminati A, Vetterlein D, Koebernick N, Blaser S, Weller U, Vogel HJ (2013) Do roots mind the gap? Plant Soil 367:651–661

Carminati A, Zarebanadkouki M, Kroener N, Ahmed MA, Holz M (2016) Biophysical rhizosphere processes affecting root water uptake. Ann Bot 118:561–571

Clode PL, Kilburn MR, Jones DL, Stockdale EA, Cliff JB III, Herrmann AM, Murphy DV (2009) *In situ* mapping of

nutrient uptake in the rhizosphere using nanoscale secondary ion mass spectrometry. Plant Physiol 151:1751–1757

Cotton TEA, Pétriacq P, Cameron DD, Al Meselmaui M, Schwarzenbacher R, Rolfe SA, Ton J (2019) Metablic regulation of the maize microbiome by benzoxazinoids. ISME J 13:1647–1658

Cseresnyés I, Rajkai K, Szitár K, Radimsky L, Ónodi G, Kröel-Dulay G (2020) Root capacitance measurements allow non-intrusive in-situ monitoring of the seasonal dynamics and drought response of root activity in two grassland species. Plant Soil 449:423–437

Dijkstra P, Dalder JJ, Selmants PC, Hart SC, Koch GW, Schwartz E, Hungate BA (2011) Modeling soil metabolic processes using isotopologue pairs of position-specific C-13 labeled glucose and pyruvate. Soil Biol Biochem 43:1848–1857

Fang W, Williams PN, Zhang H, Yang Y, Yin D, Liu Z, Sun H, Luo J (2021) Combining multiple high-resolution *in situ* techniques to understand phosphorus availability around rice roots. Environ Sci Technol 55:13082–13092

Finlay RD, Mahmood S, Rosenstock N, Bolou-Bi EB, Köhler SJ, Fahad Z, Rosling A, Wallander H, Belyazid S, Bishop K, Lian B (2020) Reviews and syntheses: biological weathering and its consequences at different spatial levels – from nanoscale to global scale. Biogeosci 17:1507–1533

Galloway AF, Akhtar J, Marcus SE, Fletcher N, Field K, Knox P (2020) Cereal root exudates contain highly structurally complex polysaccharides with soil-binding properties. Plant J 103:1666–1678

Galloway AF, Akhtar J, Burak E, Marcus SE, Field K, Dodd IC, Knox JP (2021) A soil-binding polysaccharide complex released from root hairs functions in rhizosheath formation. bioRxiv.

George TS, Gregory PJ, Wood M, Read D, Buresh RJ (2002) Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. Soil Biol Biochem 34:1487–1494

Geyer KM, Dijkstra P, Sinsabaugh R, Frey SD (2019) Clarifying the interpretation of carbon use efficiency in soil through methods comparison. Soil Biol Biochem 128:79–88

Giles CD, Dupuy L, Boitt G, Brown LK, Condron LM, Darch T, Blackwell MS, Menezes-Blackburn D, Shand CA, Stutter MI, Lumsdon DG (2018) Root development impacts on the distribution of phosphatase activity: improvements in quantification using soil zymography. Soil Biol Biochem 116:158–166

Gillespie AW, Walley FL, Farrell RE, Leinweber P, Schlichting A, Eckhardt KU, Regier TZ, Blyth RI (2009) Profiling rhizosphere chemistry: evidence from carbon and nitrogen K-edge XANES and pyrolysis-FIMS. Soil Sci Soc Am J 73:2002–2012

Gregory PJ (2022) Are plant roots only 'in' soil or are they 'of' it? Roots, soil formation and function. Eur J Soil Sci 73:e13219

Gregory PJ, McGowan M, Biscoe PV, Hunter B (1978) Water relations of winter wheat.1. Growth of the root system. J Agric Sci (Camb) 91:91–102

Griffiths M, York LM (2020) Targeting root ion uptake kinetics to increase plant productivity and nutrient use efficiency. Plant Physiol 182:1854–1868

Griffiths M, Roy S, Guo H, Seethepalli A, Huhman D, Ge Y, Sharp RE, Fritschi FB, York LM (2021) A multiple ion-uptake phenotyping platform reveals shared mechanisms affecting nutrient uptake by roots. Plant Physiol 185:781–795

Gu H, Liu L, Butnor JR, Sun H, Zhang X, Li C, Liu X (2021) Electrical capacitance estimates crop root tritss best under dry conditions – a case study in cotton (*Gossypium hirsutum* L.). Plant Soil 467:549–567

Guo H, Ayalew H, Seethepalli A, Dhakal K, Griffiths M, Ma XF, York LM (2021) Functional phenomics and genetics of the root economics space in winter wheat using high-throughput phenotyping of respiration and architecture. New Phytol 232:98–112

Han E, Li F, Perkons U, Küpper PM, Bauke SL, Athmann M, Thorup-Kristensen K, Kautz T, Köpke U (2021) Can precrops uplift subsoil nutrients to topsoil? Plant Soil 463:329–345

Helliwell JR, Sturrock CJ, Mairhofer S, Craigon J, Ashton RW, Miller AJ, Whalley WR, Mooney SJ (2017) The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci Rep 7:1–10

Holz M, Zarebanadkouki M, Kaestner A, Kuzyakov Y, Carminati A (2018) Rhizodeposition under drought is controlled by root growth rate and rhizosphere water content. Plant Soil 423:429–442

Hummel C, Boitt G, Santner J, Lehto NJ, Condron L, Wenzel WW (2021) Co-occurring increased phosphatase activity and labile P depletion in the rhizosphere of *Lupinus angustifolius* assessed with a novel, combined 2D-imaging approach. Soil Biol Biochem 153:107963

Joegensen RG, Wichern F (2018) Alive and kicking: why dormant soil microorganisms matter. Soil Biol Biochem 116:419–430

Jones DL, Hinsinger P (2008) The rhizosphere: complex by design. Plant Soil 312:1–6

Jones CY, Engelhardt I, Patko D, Dupuy L, Holden N, Willats WG (2021) High-resolution 3D mapping of rhizosphere glycan patterning using molecular probes in a transparent soil system. The Cell Surface 7:100059

Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, Solaiman ZM, Murphy DV (2015) Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New Phytol 205:1537–1551

Kellogg J, Kang S (2020) Metabolomics, an essential tool for exploring and harnessing microbial chemical ecology. Phytobiomes J 4:195–210

Keyes SD, Daly KR, Gostling NJ, Jones DL, Talboys P, Pinzer BR, Boardman R, Sinclair I, Marchant A, Roose T (2013) High resolution synchrotron imaging of wheat root hairs growing in soil and image based modelling of phosphate uptake. New Phytol 198:1023–1029

Kilburn MR, Jones DL, Clode PL, Cliff JB, Stockdale EA, Herrmann AM, Murphy DV (2010) Application of nanoscale secondary ion mass spectrometry to plant cell research. Plant Signal Behav 5:760–762

Koebernick N, Daly KR, Keyes SD, George TS, Brown LK, Raffan A (2017) High-resolution synchrotron imaging

shows that root hairs influence rhizosphere soil structure formation. New Phytol 216:124–135

Kumawat KC, Razden N, Saharan K (2022) Rhizospheric microbiome: bio-based emerging strategies for sustainable agriculture development and future perspectives. Microbiol Res 254

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371

Kuzyakov Y, Blagodatskaya E (2015) Microbial hotspots and hot moments in soil: concept and review. Soil Biol Biochem 83:184–199

Landl M, Phalempin M, Schlüter S, Vetterlein D, Vanderborght J, Kroener E, Schnepf A (2021) Modeling the impact of rhizosphere bulk density and mucilage gradients on root-water uptake. Front Agron 3:622367

Lilley JM, Kirkegaard JA (2011) Benefits of increased soil exploration by wheat roots. Field Crop Res 122:118–130

Liu Y, Patko D, Engelhardt I, George TS, Stanley-Wall N, Ladmiral V, Ameduri B, Daniell TJ, Holden N, MacDonald MP, Dupuy LX (2021a) Plant-environment microscopy tracks interactions of *Bacillus subtilis* with plant roots across the entire rhizosphere. PNAS 118:e210917611

Liu S, Zhang X, Dungait JA, Quine TA, Razavi BS (2021b) Rare microbial taxa rather than phoD gene abundance determine hotspots of alkaline phosphomonoesterase activity in the karst rhizosphere soil. Biol Fertil Soils 57:257–268

Lombardi E, Ferrio JP, Rodríguez-Robles U, de Dios VR, Voltas J (2021) Ground-penetrating radar as phenotyping tool for characterizing intraspecific variability in root traits of a widespread conifer. Plant Soil 468:319–336

Ma X, Mason-Jones K, Liu Y, Blagodatskaya E, Kuzyakov Y, Guber A, Dippold MA, Razavi BS (2019) Coupling zymography with pH mapping reveals a shift in lupine phosphorus acquisition strategy driven by cluster roots. Soil Biol Biochem 135:420–428

Ma X, Liu Y, Shen W, Kuzyakov Y (2021) Phosphatase activity and acidification in lupine and maize rhizosphere depend on phosphorus availability and root properties: coupling zymography with planar optodes. Appl Soil Ecol 167:104029

Maeght JL, Rewald B, Pierret A (2013) How to study deep roots – and why it matters. Front Plant Sci 4:1–14

Majdi H, Persson H (1997) A study on fine-root dynamics in response to nutrient applications in a Norway spruce stand using the minirhizotron technique. Zeit Pflanzen Boden 158:429–433

Mengel DB, Barber SA (1974) Development and distribution of the corn root system under field conditions. Agron J 66:341–344

Mooney S, Pridmore T, Helliwell J, Bennett M (2012) Developing X-ray computed tomography to non-invasively image 3-D root systems architecture in soil. Plant Soil 352:1–22

Moradi AB, Carminati A, Vetterlein D, Vontobel P, Lehmann E, Weller U, Hopmans JW, Vogel H-J, Oswald SE (2011) Three-dimensional visualisation and quantification of water content in the rhizosphere. New Phytol 192:653–663

Neumann G, George TS, Plassard C (2009) Strategies and methods for studying the rhizosphere—the plant science toolbox. Plant Soil 321:431–456

Nkongolo KK, Narendrula-Kotha R (2020) Advances in monitoring soil microbial community dynamic and function. J Appl Genet 61:249–263

Oburger E, Schmidt H (2016) New methods to unravel rhizosphere processes. Trends Plant Sci 21:243–255

Paterson E, Gebbing T, Abel C, Sim A, Telfer G (2007) Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytol 173:600–610

Paterson E, Midwood AJ, Millard P (2009) Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. New Phytol 184:19–33

Perelman A, Lazarovitch N, Vanderborght J, Pohlmeier A (2020) Quantitative imaging of sodium concentrations in soil-root systems using magnetic resonance imaging (MRI). Plant Soil 454:171–185

Pett-Ridge J, Weber PK (2022) NanoSIP: NanoSIMS applications for microbial biology. In: Microbial systems biology. Humana, New York, NY, pp 91–136

Phalempin M, Lippold E, Vetterlein D, Schlüter S (2021) An improved method for the segmentation of roots from X-ray computed tomography 3D images: Rootine v. 2. Plant Methods 17:1–19

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799

Radajewski S, Ineson P, Parekh NR, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403:646–649

Razavi BS, Zhang X, Bilyera N, Guber A, Zarebanadkouki M (2019) Soil zymography: simple and reliable? Review of current knowledge and optimization of the method. Rhizosphere 11:100161

Rewald B, Ephrath JE (2013) Minirhizotron techniques. In: Eshel A, Beeckman T (eds) Plant roots: the hidden half, 4th edn. CRC Press Boca Raton USA, pp 735–750

Santner J, Zhang H, Leitner D, Schnepf A, Prohaska T, Puschenreiter M, Wenzel WW (2012) High-resolution chemical imaging of labile phosphorus in the rhizosphere of *Brassica napus* L. cultivars. Environ Exp Bot 77:219–226

Schlaeppi K, Bulgarelli D (2015) The plant microbiome at work. Mol Plant-Microbe Interact 28:212–217

Schmidt S, Bengough AG, Gregory PJ, Grinev DV, Otten W (2012) Estimating root-soil contact from 3D X-ray microtomographs. Eur J Soil Sci 63:776–786

Schnepf A, Carminati A, Ahmed MA, Ani M, Bernard P, Bentz J, Bonkowski M, Knott M, Diehl D, Duddek P, Kroner E, Javaux M, Landl M, Lehndorff E, Lippold E, Lieu A, Mueller CW, Oburger E, Otten W et al (2022) Linking rhizosphere processes across scales: opinion. Plant Soil. https://doi.org/10.1007/s11104-022-05306-7

Smit AL, Bengough AG, Engels C (2000) In: van Noordwijk M, Pellerin S, de Geijn SC (eds) Root methods: A Handbook. Springer-Verlag, Berlin Germany

Smolders E, Wagner S, Prohaska T, Irrgeher J, Santner J (2020) Sub-millimeter distribution of labile trace element fluxes in the rhizosphere explains differential effects of soil

liming on cadmium and zinc uptake in maize. Sci Total Environ 738:140311

Sokol NW, Slessarev E, Marschmann GL, Nicolas A, Blazewicz SJ, Brodie EL, Firestone MK, Foley MM, Hestrin R, Hungate BA, Koch BJ, Stone BW, Sullivan MB, Zablocki O, Pett-Ridge J (2022) Life and death in the soil microbiome: how ecological processes influence biogeochemistry. Nat Rev Microbiol. https://doi.org/10.1038/s41579-022-00695-z

Song X, Zhang X, Zamanian K, Dippold MA, Kuzyakov Y, Razavi BS (2019) Hotspot localization but not the duration of hot moments in the rhizosphere is affected by root hair length. 21st EGU General Assembly, EGU2019, Proceedings from the conference held 7-12 April, 2019 in Vienna, Austria, id.10582

Spohn M, Treichel NS, Cormann M, Schloter M, Fischer D (2015) Distribution of phosphatase activity and various bacterial phyla in the rhizosphere of *Hordeum vulgare* L. depending on P availability. Soil Biol Biochem 89:44–51

Szameitat AE, Sharma A, Minutello F, Pinna A, Er-Rafik M, Hansen TH, Persson DP, Andersen B, Husted S (2021) Unravelling the interactions between nano-hydroxyapatite and the roots of phosphorus deficient barley plants. Environ Sci: Nano 8:444–459

Tanikawa T, Ikeno H, Yamase K, Dannoura M, Aono K, Hirano Y (2021) Can ground-penetrating radar detect adjacent roots and rock fragments in forest soil? Plant Soil 468:239–257

Thorup-Kristensen K (2006) Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. Plant Soil 288:233–248

Thorup-Kristensen K, Halberg N, Nicolaisen M, Olesen JE, Crews TE, Hinsinger P, Kirkegaard J, Pierret A, Dresbøll DB (2020) Digging deeper for agricultural resources, the value of deep rooting. Trends Plant Sci 25:406–417

Tracy SR, Daly KR, Sturrock CJ, Crout NMJ, Mooney SJ, Roose T (2015) Three-dimensional quantification of soil hydraulic properties using X-ray computed tomography and image-base modelling. Water Resour Res 51:1006–1022

Van Veelen A, Koebernick N, Scotson CS, McKay-Fletcher D, Huthwelker T, Borca CN, Mosselmans JF, Roose T (2020) Root-induced soil deformation influences Fe, S and P: rhizosphere chemistry investigated using synchrotron XRF and XANES. New Phytol 225:1476–1490

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A (2015) The importance of the microbiome of the plant holobiont. New Phytol 206:1196–1206

Vereeken H, Pachepsky Y, Simmer C, Rihani J, Kunoth A, Korres W, Graf A, Hendricks-Franssen HJ, Thiele-Eich I, Shao Y (2016) On the role of patterns in understanding the functioning of soil-vegetation-atmosphere systems. J Hydrol 542:63–86

Vetterlein D, Carminati A, Kögel-Knabner I, Bienert GP, Smalla K, Oburger E, Schnepf A, Banitz T, Tarlla MT, Schlüter S (2020) Rhizosphere spatiotemporal organisation – a key to rhizosphere functions. Front Agron 2:8

Wasson AP, Nagel KA, Tracy S, Watt M (2020) Beyond digging: noninvasive root and rhizosphere phenotyping. Trends Plant Sci 25:119–120

White RG, Kirkegaard JA (2010) The distribution and abundance of wheat roots in a dense, structured subsoil – implications for water uptake. Plant Cell Environ 33:133–148

Wilson CH, Gerber S (2021) Theoretical insights from upscaling Michaelis-Menten microbial dynamics in biogeochemical models: a dimensionless approach. Biogeosci 18:5669–5679

Wu W, Duncan RW, Ma BL (2017) Quantification of canola root morphological traits under heat and drought stresses with electrical measurements. Plant Soil 415:229–244

York LM, Carminati A, Mooney SJ, Ritz K, Bennett M (2016) The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. J Exp Bot 67:3629–3643

Young JPW (2016) Bacteria are smartphones and mobile genes are apps. Trends Plant Sci 17:478–486

Zaeem M, Nadeem M, Pham TH, Ashiq W, Ali W, Gillani SS, Moise ER, Leier H, Kavanagh V, Galagedara L, Cheema M (2021) Development of a hyperspectral imaging technique using LA-ICP-MS to show the spatial distribution of elements in soil cores. Geoderma 385:114831