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### Authors

Ahkami, Amir H

Qafoku, Odeta

Roose, Tiina

et al.

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## Emerging sensing, imaging, and computational technologies to scale nano-to macroscale rhizosphere dynamics – Review and research perspectives

Amir H. Ahkami<sup>a,\*</sup>, Odeta Qafoku<sup>a,\*</sup>, Tiina Roose<sup>b</sup>, Quanbing Mou<sup>c</sup>, Yi Lu<sup>c</sup>, Zoe G. Cardon<sup>d</sup>, Yuxin Wu<sup>e</sup>, Chunwei Chou<sup>e</sup>, Joshua B. Fisher<sup>f</sup>, Tamas Varga<sup>a</sup>, Pubudu Handakumbura<sup>a</sup>, Jayde A. Aufrecht<sup>a</sup>, Arunima Bhattacharjee<sup>a</sup>, James J. Moran<sup>a,g</sup>

<sup>a</sup>. Environmental Molecular Sciences Laboratory (EMSL), Pacific Northwest National Laboratory (PNNL), Richland, WA, 99454, USA

<sup>b</sup>. Bioengineering Sciences Research Group, Faculty of Engineering and Environment, University of Southampton, University Road, Southampton, England, SO17 1BJ

<sup>c</sup>. Department of Chemistry, The University of Texas at Austin, 105 East 24<sup>th</sup> Street, Austin, TX 78712, USA

<sup>d</sup>. Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA, 02543, USA

<sup>e</sup>. Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720 USA

<sup>f</sup>. Schmid College of Science and Technology, Chapman University, 1 University Drive, Orange, CA, 92866, USA

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\*Corresponding authors: Amir H. Ahkami [amir.ahkami@pnnl.gov](mailto:amir.ahkami@pnnl.gov), Odeta Qafoku [Odeta.qafoku@pnnl.gov](mailto:Odeta.qafoku@pnnl.gov).

Author's contribution:

Amir H. Ahkami: Conceptualized the work, wrote the Abstract, Introduction and section 5.1 of Biosensors for monitoring nutrient and chemical exchanges in the rhizosphere, contributed to developing Figures 1,2, and 7, reviewed, and edited the manuscript.

Odeta Qafoku: Conceptualized the work; wrote Introduction and section 2; Integrated imaging and biochemical methodologies to resolve rhizosphere processes in space and time; contributed to developing Figures 1,2, and 7, reviewed, and edited the manuscript.

Tamas Varga: Wrote section 4.1–4.2 of Image-based modeling of plant-soil interaction: rhizosphere multiscale measurement and modeling; contributed to developing Figures 1 and 7.

Tiina Roose: Wrote section 4, Image-based modeling of plant-soil interaction: rhizosphere multiscale measurement and modeling; contributed to developing Figure 7.

Pubudu Handakumbura: Wrote section 3.2 of Constructing environments for laboratory to field investigations of rhizosphere processes; contributed to developing Figure 2.

Jayde A. Aufrecht: Wrote section 3.1 of Constructing environments for laboratory to field investigations of rhizosphere processes; contributed to developing Figure 2.

Arunima Bhattacharjee: Reviewed and edited section 3.2

Yi Lu: Wrote section 5.2, Biosensors for monitoring nutrient and chemical exchanges in the rhizosphere; developed Figure 3.

Quanbing Mou: Wrote section 5.2, Biosensors for monitoring nutrient and chemical exchanges in the rhizosphere; developed Figure 3.

Zoe Cardon: Wrote section 6, Measurement of the distribution and dynamics of rhizosphere chemical gradients in the field; developed Figure 4.

Yuxin Wu: Wrote section 7, Detection of rhizosphere interactions across scales: challenges in upscaling in complex systems; wrote section 8.2 of Fate of the Terrestrial Biosphere: Scaling plant-soil-microbe interactions to the landscape and the world; developed Figure 5.

Joshua B. Fisher: Wrote section 8, Fate of the Terrestrial Biosphere: Scaling plant-soil-microbe interactions to the landscape and the world; developed Figure 6.

James J. Moran, involved in planning the work and assisted in organizing and reviewing the manuscript, wrote the Introduction and section 2, Integrated imaging and biochemical methodologies to resolve rhizosphere processes in space and time, contributed to developing Figures 2 and 7.

<sup>9</sup> Michigan State University, Department of Integrative Biology and Department of Plant, Soil, and Microbial Sciences, East Lansing, MI, 48824, USA

## Abstract

The soil region influenced by plant roots, i.e., the rhizosphere, is one of the most complex biological habitats on Earth and significantly impacts global carbon flow and transformation. Understanding the structure and function of the rhizosphere is critically important for maintaining sustainable plant ecosystem services, designing engineered ecosystems for long-term soil carbon storage, and mitigating the effects of climate change. However, studying the biological and ecological processes and interactions in the rhizosphere requires advanced integrated technologies capable of decoding such a complex system at different scales. Here, we review how emerging approaches in sensing, imaging, and computational modeling can advance our understanding of the complex rhizosphere system. Particularly, we provide our perspectives and discuss future directions in developing *in situ* rhizosphere sensing technologies that could potentially correlate local-scale interactions to ecosystem scale impacts. We first review integrated multimodal imaging techniques for tracking inorganic elements and organic carbon flow at nano- to microscale in the rhizosphere, followed by a discussion on the use of synthetic soil and plant habitats that bridge laboratory-to-field studies on the rhizosphere processes. We then describe applications of genetically encoded biosensors in monitoring nutrient and chemical exchanges in the rhizosphere, and the novel nanotechnology-mediated delivery approaches for introducing biosensors into the root tissues. Next, we review the recent progress and express our vision on field-deployable sensing technologies such as planar optodes for quantifying the distribution of chemical and analyte gradients in the rhizosphere under field conditions. Moreover, we provide perspectives on the challenges of linking complex rhizosphere interactions to ecosystem sensing for detecting biological traits across scales, which arguably requires using the best-available model predictions including the model-experiment and image-based modeling approaches. Experimental platforms relevant to field conditions like SMART (Sensors at Mesoscales with Advanced Remote Telemetry) soils testbed, coupled with ecosystem sensing and predictive models, can be effective tools to explore coupled ecosystem behavior and responses to environmental perturbations. Finally, we envision that with the advent of novel high-resolution imaging capabilities at nano- to macroscale, and remote biosensing technologies, combined with advanced computational models, future studies will lead to detection and upscaling of rhizosphere processes toward ecosystem and global predictions.

## Keywords

Rhizodeposition; carbon flow; nutrients gradients; biosensors; image-based modeling; ModEx

## 1. Introduction

The rhizosphere, defined as the soil region influenced by plant roots, is one of the most dynamic soil domains on Earth that facilitates intra- and interkingdom interactions between plants and microorganisms (Hinsinger et al., 2005, Vetterlein et al., 2021). Plant roots play a significant role in shaping the rhizosphere microbial community by supplying photosynthetically fixed carbon and other metabolites, while microorganisms growing in

close association with plant roots further affect the plant productivity and ecosystem resilience (Balasubramanian et al., 2021; Saeed et al., 2021; Ling et al., 2022).

Rhizosphere constitutes a dynamic environment where interplay and connectivity among its components (i.e., plant root, microbial community, and soil) and flow of nutrients and metabolomic exchanges dictate system behavior and functionality. A number of integrated abiotic and biotic processes and stimuli continuously occur in the dynamic rhizosphere region that drives many biogeochemical and physicochemical reactions, which regulate the supply of inorganic and organic nutrients (Ahkami et al., 2017). A persistent past and current challenge in rhizosphere science involves understanding the individual abiotic and biotic reactions and their impact on the larger, integrative rhizosphere behavior, especially given the dynamic responses to seasonality, climate perturbations, and land-use changes (Philippot et al., 2009). Embedded within this challenge is the ability to quantify the individual and cumulative contributions of different rhizosphere components to larger scale nutrient cycling, soil organic matter formation, greenhouse gas priming, ecosystem response to climate change, and carbon sequestration (Jones et al., 2009; Kell 2012; Jia et al., 2023).

Critical rhizosphere processes are often scale dependent with emergence of new processes and behaviors across scales. Mechanistic understanding of behaviors and key processes of such complex systems needs to consider the spatial gradients and interaction of plant root–microbial–mineral interfaces and the driving factors that influence correlations between these components. Addressing such great challenges in regard to rhizosphere function necessitates the use of the most advanced technologies available (Védère et al., 2022) and development of novel tools suitable for application within the rhizosphere region including in situ techniques, high-resolution imaging, biochemical sensing, and computational technologies. For example, addressing specific rhizosphere-related hypotheses can benefit from application of less complex controlled and managed systems such as synthetic soil/plant habitats (Gao et al., 2018) and rhizogrids (Maskova and Klimes, 2020, Handakumbura et al., 2021), which are compatible with multimodal imaging technologies and able to incorporate tight control of environmental variables. Other approaches include application of isotope labeling integrated with chemical and mass spectrometry imaging techniques that can be used to spatially track and quantify carbon fluxes and transformations in the rhizosphere to improve understanding of controls on these reactions. In addition, genetically encoded fluorescent biosensors coupled with nanotechnology-mediated deliveries can enable tracking of chemical exchanges in natural systems, a promising direction particularly for in situ monitoring of rhizosphere components. Such sensing technologies are critical for real-time monitoring of hydrobiogeochemical processes and chemical gradients (Neelam and Tabassum 2023), for in situ detection of key analytes (e.g., O<sub>2</sub> and NO<sub>3</sub>) (Capstaff et al., 2021), and for expanding our knowledge from controlled and managed environments to natural field conditions.

Understanding the rhizosphere processes in both managed and natural systems relies not only on development of advanced sensing capabilities to capture critical spatiotemporal dynamics in situ but also on developing a predictive understanding of rhizosphere parameters that drive ecosystem behavior at larger scales. This requires holistic characterization of high-throughput observational datasets that capture the system behavior

and, even more importantly, ecosystem response under simulated scenarios. Singular, isolated measurements often provide only a “sliced” or biased view of the system, and correlations derived from such observations are often noncausal and difficult to scale beyond observation locality. Furthermore, the volume of high-throughput, field-deployable data attainable with various sensor platforms, combined with the richness of biogeochemical transformations contributing to the observed activity, require advanced computational and machine learning (ML) techniques to extract relationships leading to the larger scale processes (Zhu et al., 2023). Many of these modeling techniques remain to be developed and will need to evolve constantly as sensor capabilities are improved for deployment in rhizosphere studies. Lastly, the rhizosphere typically constitutes hotspots of biogeochemical transformations across terrestrial landscapes with tremendous impacts on nutrient cycles (Pathan et al., 2020). However, the small spatial confinement of the rhizosphere makes accurate incorporation of this system into land-scale Earth system models tenuous at best. Improved inclusion of rhizosphere components in these models will aid in the prediction of rhizosphere processes under future climate perturbations and under different mitigation scenarios.

Although recent review articles have greatly advanced our knowledge on rhizosphere interactions with other biogeochemical interfaces (Blagodatskaya et al., 2021), the roles of rhizodeposition on promoting plant growth (Ravelo-Ortega et al., 2023) as well as on reducing nitrogen emissions and promoting low-carbon agriculture (Lu et al., 2023), we still lack an understanding of the global effects of the rhizosphere on soil microbial communities, carbon cycling, and climate change in terrestrial ecosystems (Lv et al., 2023). This requires gaining a deeper insight on the current and emerging technologies that bridge the nano-to macro-scales rhizosphere studies. Therefore, here, we present a review and provide our perspective views on several key sensing, imaging, and modeling technologies, spanning from lab to field to ecosystem scales, for addressing the great challenges in understanding rhizosphere functions and the role of the rhizosphere on larger ecosystem responses to climate change and soil carbon sequestration.

## **2. Integrated imaging and biochemical methodologies to resolve rhizosphere processes in space and time**

In situ advanced methodologies that are based on seismic reflection, acoustic, optical, and nuclear magnetic resonance have been successfully deployed to monitor geophysical and geochemical properties and processes from belowground to deep subsurface (Holbrook et al., 2019). Applications of these technologies have provided an in-depth understanding of how physical and chemical factors influence abiotic processes, such as rock conversion and transformation to weathered bedrock and to soil at the critical zone (Holbrook et al., 2019). On the contrary, in situ technologies that continuously and comprehensively, in space and time, monitor the biogeochemical processes, mechanisms, and fluxes of organic carbon, (micro)nutrients, metabolites, and enzymes in the spatially confined rhizosphere region are less developed. One of the main reasons that the in situ technology development is moving at a very slow pace, despite the decades of laboratory and field-scale studies, is that many components of the rhizosphere coexist in an overlapping space (Lee et al., 2022). The

nearby environment is a function of many interconnected reactions and processes, making it challenging to disentangle and to comprehensively interrogate. Processes that contribute to this complexity, include root exudation and cell release, which increase organic matter content and alter the soil's chemical composition including pH (Toal et al., 2000, Kim et al., 1999; Ma et al., 2022). This enhancement in organic matter furthermore stimulates soil microbial activity, resulting in organic matter decomposition, mineralization, and nutrient cycling (Paterson, 2003). The combined influence of root exudates and microbial activity significantly impacts soil mineral transformation through weathering processes and in addition promotes organic matter stabilization or priming, soil particle aggregation, and consequently changes the soil structure (van Breemen et al, 2000; Landeweert et al., 2001; Hoffland, 2004; Mikutta et al., 2006, Keiluweit et al., 2015, Uroz et al., 2015; Totsche, et al, 2018; Rillig et al., 2017, Tisdall and Oades, 1982). Understanding the function of each individual rhizosphere component is a challenging task, as interactions between plant roots, soil microbes, and soil minerals are further impacted by mutual mycorrhizal associations (Leake, 2008; Bonfante and Genre, 2010). Studying these complex processes requires advanced imaging approaches to monitor key rhizosphere components including C and inorganic nutrients at different scales.

### 2.1. Tracking inorganic nutrients in the rhizosphere

Currently, advances have been made using a number of novel laboratory technologies, from nano- to microscale, that are able to interrogate interfaces of key components of rhizosphere (root, minerals, microbes) and reveal evidence of biotically driven processes and reactions (Weaver et al., 2021, Lybrand et al., 2019, Lybrand et al., 2022, Christophe et al., 2013, Hoffland et al., 2004, Landeweert et al., 2001). These advanced high-resolution methodologies are often combined or cross-correlated with each other to address limitations that individual techniques frequently present when addressing complex and challenging materials. For example, measurements based on scanning electron microscopy (SEM) (van Scholl et al., 2008), transmission electron microscopy, and energy dispersive X-ray spectroscopy (TEM/EDX) combined with nanoscale atom probe tomography, (APT) (Lybrand et al., 2022) reveal in detail biogenic processes that occur in the rhizosphere at nano- to micro-scale (Figure 1). The evidence provided by these methods demonstrates how exudates from microbes cement soil particles and may contribute toward mineral stabilization, which promotes soil micro- and macro-aggregates formation and long-term soil carbon sequestration (Rillig et al., 2017, Ma et al., 2022).

Indirect evidence of redox and ligand-promoted biotic reactions, driven by microbial pursuit for resource acquisition in the rhizosphere, can also be provided by interrogating microbe–mineral interfaces (Leake et al., 2008, van Breemen et al., 2000). The evidence of interfacial mineral bioweathering by fungi, for example, reveals mineral dissolution and macro- to micronutrient (i.e., Ca, P, Fe) solubilization by acidification or chelation. More evidence on bioweathering can be gathered from three-dimensional (3D) analysis at atomic scale, which shows at unprecedented high resolution the elemental distribution between mineral and microbe interfaces (Figure 1, adapted from (Lybrand et al., 2022). The 3D characterization further demonstrates that biotically driven mineral alterations in field settings occur at regions with exposed bioavailable nutrients such as Ca/P enrichments

or with exposed energy sources, such as Fe mineral inclusions. The regions that contain energy or nutrients are opportunistically sought after by soil microbes and fungi in their search for vital mineral-bound nutrients. Other cross-correlation techniques (e.g., atomic force microscopy coupled with high-resolution TEM) can also assess mineral dissolution and provide additional evidence of alteration zones such as channel formation induced from mineral interactions with microorganisms (Li et al., 2022). The combination of optical microscopy with nanoscale topography by vertical scanning interferometry further provides a framework for in situ quantification of biotic mineral dissolution rates in natural settings (Wild et al., 2021). Other examples of multimodal approaches include coupling structural information from X-ray computed tomography (XCT) with chemical information obtained by synchrotron X-ray fluorescence spectroscopy (XRF) and X-ray absorption near-edge structure (XANES) to show how root induced soil deformation influences the presence and uptake of Fe, S, and P in the rhizosphere (Schlüter et al., 2019; van Veelen et al., 2020; Keyes et al., 2022; more refs). Moreover, when high-resolution methods such as electron microscopy are coupled with gene expression analysis, it provides critical insights on biotic mineral weathering processes and nutrient uptake. For instance, a combination of SEM with gene expression analysis clarifies microorganism adaptation and metabolic responses to nutrient stress and deficiencies (Pinzari et al., 2022). Understanding how specific genes contribute to microbially-derived processes with soil minerals, such as oxidation/reduction of Fe containing minerals (Shi et al., 2016a, Yadav et al., 2006; Yu et al., 2019), can lead to unraveling underlying mechanisms that guide such processes in the rhizosphere.

Current and future works should further investigate the selective interactions between plant roots and microbial communities with soil minerals to address open questions on nutrients dissolution, transformation, and release into the rhizosphere. The outcomes of these studies have the potential to contribute significantly towards the identification of target nutrients and other compounds for development and deployment of in situ sensors in field settings (see sections 5 and 6 below) for understanding rhizosphere processes across scales.

## 2.2. Monitoring carbon flux in the rhizosphere

In addition to tracking inorganic nutrients, a key component for elucidating microbial and geochemical processes in the rhizosphere is accounting for and tracking organic carbon flow (Figure 2, left panel). From a microbial perspective, soil is frequently limiting in (bioavailable) carbon and a defining distinction between rhizosphere and bulk soil is the deposition of plant-derived carbon into the rhizosphere, which can spatially relieve this nutrient stress and stimulate microbial populations (Kuzyakov and Blagodatskaya, 2015). Carbon can be supplied to the rhizosphere in many forms (root necromass, root exudates, mucilage, etc.), but is often collectively referred to as rhizodeposition, and multiple approaches are available for collecting, quantifying, and identifying constituents of this material (Jones et al., 2009). The amount, chemical composition, and spatial localization of the emplacement of plant-derived organics into the rhizosphere can vary in response to root and plant lifecycle, environmental conditions, or the rhizosphere microbial community (Metlen et al., 2009, Zhahnina et al., 2018, Keller et al., 2021). An important frontier, therefore, in developing a mechanistic understanding of rhizosphere processes is gaining insights into how the plasticity of rhizodeposits is influenced by and exerts control

over other rhizosphere processes; understanding the adaptive interplay between plants, the local environment, and associated microbial populations. A fundamental requirement for unraveling these interactions is the ability to measure and track rhizodeposition and there are a variety of techniques dedicated to this goal (Oburger and Schmidt, 2016). A wide array of analytical tools for resolving the chemical composition of root exudate with various mass spectrometry and spectroscopy approaches provide detailed information on these typically complex samples (see review in Casas and Matamoros, 2021). However, complexity of the rhizosphere gives rise to a highly dynamic system that greatly complicates isolation of pristine root exudate samples without incurring various forms of collection or handling bias (Oburger and Jones, 2018). In short, sampling for root exudates encounters a constant tradeoff between factors such as maintaining ecologically relevant conditions, avoiding traumatic impacts on plant roots (with associated release of injury associated compounds), securing root exudates before microbial or reuptake processes alter their chemical composition, etc. Thus, typical approaches such as hydroponics, direct sampling of rhizosphere washed off of plant roots, and rhizon-based sampling of soil moisture all provide viable samples, but interpretation of the resulting data should reflect potential modifications and bias to the sample during and after collection as well as any analytical artifacts associated with the chosen analysis method(s).

While traditional root exudate analysis offers insightful information on the chemical composition and related shifts in response to external factors, these methods also tend to be spatially agnostic and reflect average composition blended over a sampling region. This may be an analytical requisite in many cases, but rhizosphere is nearly synonymous with spatial heterogeneity and new, emergent techniques are making progress toward better linking organic composition with specific locations and conditions in the rhizosphere to better reflect spatiotemporal organization (Vetterlein et al., 2020). For instance, mass spectrometry imaging (Figure 2, left panel) has been used for identification of key organics in rhizodeposition within various forms of constructed systems and can perform high spatial specificity analysis of organic speciation in root tissues (Velickovic and Anderton, 2017); yet it is challenging to directly apply this imaging to the natural soil matrix due to multiple interferants and analytical requirements. To help overcome this limitation, recent progress demonstrated spatial extraction of organics onto a membrane followed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry for organic speciation mapping (Velickovic et al., 2020). While effective, there are biases in the method linked to the surface chemistry of the membrane used, so the results are not comprehensive. Further, it is often challenging to specifically identify the organic compounds made concurrently within an experiment versus legacy materials present in the system before a test occurs.

Various isotope approaches have been used to help circumvent issues related to distinguishing recent from more relict organic material, as when using isotope tracers, only the new (i.e., more recent than the application of the tracer) materials contain the added tracer. In some cases, application of these tracers is measured in specific organic compounds. Commonly, an isotope tracer is used to perform a more integrated quantification (e.g., of total rhizodeposition, respiration of root exudates, etc.). Choices of isotope systems and associated detectors can be driven by specific scientific questions. For instance, application of a radiocarbon ( $^{14}\text{C}$ ) tracer can be linked to spatial imaging of the



distribution of rhizodeposition in plant systems (Pausch and Kuzyakov, 2011). Similarly,  $^{11}\text{C}$  radiocarbon tracers can provide more sensitivity due to their rapid decay rates and, when imaged using a positron emission tomography system, provide high spatial resolution mapping of root exudates (Kiser et al., 2008). The very short half-life of this isotope (only ~20.4 minutes) requires the source of  $^{11}\text{C}$  to be in close proximity to the experimental system and necessitates calibration for loss of signal over time while also making the imaging potentially infeasible in larger plants (where transport time for photosynthate from leaves to roots to rhizosphere is in a timeframe similar to the radioactive decay half-life due to loss of signal). However, this short half-life enables the use of multiple pulses of the tracer at different timepoints during a plant's growth since decay effectively removes a previous pulse and is not destructive to the plant or rhizosphere (Oburger and Schmidt, 2016). In other cases, the ease of applying stable isotope tracers makes this the isotope of choice for tracing and quantifying various carbon processes. For instance,  $^{13}\text{CO}_2$  can be added to a plant system and used to quantify distribution of photosynthates between plant, rhizosphere, and soil components. Depending on the amount of  $^{13}\text{C}$  applied, the signal can be tracked into specific microbial groups to identify and help quantify the level of metabolic coupling between rhizosphere microorganisms and plant roots (Starr et al., 2021). It is also feasible to spatially map the localization of the  $^{13}\text{C}$  signal into the rhizosphere to estimate rates of root exudation. Various mass spectrometry and spectroscopy (including infra-red spectroscopy) approaches coupled to laser ablation sampling can provide spatial resolution in the tens of  $\mu\text{m}$  range (Denis et al., 2019, Rodionov et al., 2019, Moran et al., 2022). In cases where more spatial resolution is required, NanoSIMS analysis can reveal  $^{13}\text{C}$  incorporation at the 10s nm range through plant tissues and into associated fungal and microbial biomass (Kaiser et al., 2015, Vidal et al., 2018). NanoSIMS can also be linked to the use of taxon-specific tags to enable combined spatial, taxon-specific, and stable isotope measurements (Dekas et al., 2019; Schmidt et al., 2023), although this approach is time consuming, best applied to address targeted (versus screening) questions and is rarely applied to rhizosphere studies.

Spatially resolved assessments of carbon introduction into soil typically reveal focused areas of organic deposition that align with hotspots of microbial activity. These hotspots are associated with increased biogeochemical activity and encompass significantly higher rates of nutrient mobilization and exchange than their spatial areas would suggest. Yet, the small spatial scales of these hotspots combined with their ephemeral nature make them challenging to interrogate and major questions remain regarding recruitment of microbial activities and the role these processes play in directing the overall fate of organic carbon and associated impacts on plant performance. While the requisite analytical techniques for enabling spatially specific co-analysis of nutrients and associated biological processes remain somewhat limited, emerging capabilities are enabling better observation of linkages between these phenomena than ever before. For instance, zymography enables targeted mapping of specific enzymatic processes within living rhizosphere systems (Spohn et al., 2013, Ghaderi et al., 2022). This approach leverages a surrogate substrate for an enzyme that has been modified to produce a fluorescent signal upon enzymatic reaction. Zymography is adaptable to a wide range of different hydrolytic and oxidative enzymatic processes (Spohn and Kuzyakov, 2013, Razavi et al., 2019; Kravchenko et al., 2019; Khosrozadeh et al., 2022). Emerging micro-zymography approaches employed modified techniques to

apply a labeled substrate are being shown to reduce some limitations and artifacts of traditional zymography to enable enhanced the spatial resolution of the technique (Ghaderi et al., 2022). Importantly, zymography can also be coupled to additional analysis. For example, Kravchenko et al. (2019) linked  $\mu$ CT and zymography to elucidate correlation between carbon abundance, pore spaces, and localization of enzymatic activity. Finally, amino-mapping is an emerging technique reminiscent of zymography that uses abiotic reactions to map amino-derived nitrogen in soil and rhizosphere (Khosrozadeh et al., 2023). This opens up the possibility of spatially tracking microbial activity in rhizosphere and correlating it to changes in carbon and nitrogen nutrient status.

To complement spatial quantification of enzymatic activity, proteomics targeting the rhizosphere can provide additional information on biochemical pathways or taxonomy of microorganisms linked to consumption of plant-derived carbon containing an isotopic tracer (Pett-Ridge and Firestone, 2017). An initial study even suggests the ability to perform spatially resolved proteomics targeting microbial hotspots (White et al., 2021).

Taken together, the introduction of organic carbon by plants into the subsurface is the crucial step initiating rhizosphere development. The spatiotemporal variability of the rhizosphere challenges traditional efforts to elucidate key determinants of biogeochemical processes within these spatially constrained environments. Emerging novel technologies to elucidate carbon introduction to the rhizosphere and the microbial activities associated with this carbon provide new insights and better understanding of controls on associated processes. However, it is important to note that there is not a single analytical technique capable of capturing the full intricacy of rhizospheric carbon processing but that each of the techniques mentioned specializes in elucidating a limited set of the overall complexity (e.g., enzymatic transformation, quantification of total carbon flux, chemical speciation of carbon exudates, spatial localization of carbon, etc.). In many, but not all, cases a multi-capability approach is feasible, but the choice of the targeted techniques needs to be made carefully to avoid limiting subsequent analysis. For instance, regulatory concerns can prevent sequential use of positron emission tomography from being used in conjunction with other techniques due to fears of radioactive contamination of mass spectrometers or other lab equipment. In other cases, sample preparation may limit subsequent analyses where, for example, application of a sputter coat to prevent sample charging during NanoSIMS analysis could negatively impact the use of the sample for other techniques. In these cases, it may be feasible to adjust the order of technique application to a sample to enable subsequent analyses (e.g., perform zymography evaluation prior to NanoSIMS sample preparation). Ultimately, however, the cumulative effects of the destructive nature of some sample analyses, artifacts associated with various forms of sample preparation, and cost considerations typically limit the suite of techniques that can be brought to bear on a particular sample. Still, where advantageous, combinations of these emerging efforts may propel advances towards gaining a mechanistic understanding on rhizosphere-driven activities central to organic matter preservation, plant nutrition, and responses to environmental perturbations.

### 3. Constructing environments for laboratory-to-field investigations of rhizosphere processes

**3.1. Synthetic soil micromodel habitats**—Elucidating rhizosphere processes at the microscale in field settings is an ongoing challenge. However, some of these processes can be replicated and understood through developing a controlled and managed laboratory environment by using model rhizosphere (soil) habitat micromodels. These synthetic soil habitats are known by different names and types such as micromodels or TerraForms (Pacific Northwest National Laboratory) or EcoFABs (Lawrence Berkeley National Laboratory). Microfabrication (i.e., microfluidics, 3D printing) is a popular approach to creating model rhizospheres and typically employs a transparent, bio-inert polymer to replicate a customizable habitat design (Figure 2, right panel). Synthetic rhizosphere habitats offer distinct advantages over a native rhizosphere, including higher control over environmental conditions and soil physical parameters, as well as increased spatial resolution to sample down to the scale of an individual microbial hotspot. Moreover, the transparent material (Liu et al., 2021) used to fabricate these habitats makes them amenable to optical imaging, affording the ability to track plant root architecture development and interaction with microbes, which is a central challenge in soils due to its opacity.

Microfabricated habitats have enabled high-resolution imaging of microorganisms that are typically found underground, including bacteria, fungi, and nematodes (Arellano-Caicedo et al., 2021, Mafla-Endara et al., 2021). These model systems are also able to accommodate several plant species during the early stages of seedling development, including small model plants (*Arabidopsis thaliana*), fast-growing grass species (*Brachypodium distachyon*, *Oryza sativa*), and even some tree species (*Populus* spp.) (Gao et al., 2018, Yanagisawa et al., 2021, Sasse et al., 2019). Although the small size of these model systems limits most plant studies to the early and seedling developmental stages, this can be a critical time for characterizing the establishment of a rhizosphere microbiome. Increasingly, recent studies have focused on imaging interkingdom interactions within microfabricated habitats. These studies have described the colonization dynamics of microbes on plant roots and the spatial interaction between microbial species in the presence of a plant (Massalha et al., 2017, Aufrecht et al., 2018, Noirot-Gros et al., 2020). The optical images produced from these interaction studies offer an exciting perspective of never-before-seen rhizosphere dynamics which can lead to the development of specific ecological hypotheses.

Along with offering enhanced spatial resolution, microfabricated habitats also offer defined environmental parameters and high experimental control. These reduced complexity platforms can be used as a complement to field studies to test causal relationships between specific soil parameters and associated ecological responses. Several research groups have used microfabrication techniques to mimic the rhizosphere environment in reduced complexity habitats and, depending on the target science question, these approaches generally aim to recreate physical or chemical parameters within a rhizosphere ecosystem (Stanley et al., 2016).

Synthetic micromodel habitats that mimic the structure of the soil environment can range in design complexity from simple repeated structures (i.e., homogeneous) to complex,

interconnected porous networks (i.e., heterogenous) (Alekklett et al., 2018, Aufrecht et al., 2019). The structure of the soil is an important parameter to emulate since porous media can influence nutrient transport, organism movement and development, and even the interactions between species (Borer et al., 2018). These synthetic soil platforms have been successfully used to characterize microbial processes in porous media such as chemotaxis, biofilm and hyphal network development, and bacterial transport in flow (de Anna et al., 2021, Zhang et al., 2010). Recently, these synthetic soil habitats have been extended to the culture of whole plants and have been used to show that the physical structure of the habitat influences the distribution of root exudates (Aufrecht et al., 2022, Walton et al., 2022). Although microfabricated habitat structures are often created using a bio-inert polymer and rely on dissolved components of a liquid media to create any chemical gradients or treatments, some recent work has been focused on adding solid-phase compounds to recreate the chemical complexity of the soil environment (Bhattacharjee et al., 2022).

Future development of microfabricated rhizosphere habitats should focus on increasing chemical complexity and pairing with chemical imaging techniques in order to characterize biogeochemical transformations and molecular interactions between organisms in the rhizosphere. These types of studies are expected to help illuminate many ecological interactions in the rhizosphere, including mechanisms for plant-growth promoting microbial interactions and organic acid solubilization of mineral-bound micronutrients. Although microfabricated rhizosphere habitats are extremely simplified compared to a complex natural rhizosphere, these platforms can be used as a complement to field studies to target specific rhizosphere processes. Future work should aim to quantify molecular transformations within these habitats (i.e., carbon use efficiency, mineral weathering) so that results from these platforms can more easily be scaled to larger studies such as greenhouse or field studies.

### 3.2. Microscopic to ecosystem scale synthetic habitats

In addition to soil habitats, other platforms and approaches are currently used to bridge the ecosystem to microscopic scale, and to study the root influenced rhizosphere. Ecotrons, also known as macrocosms, are one of the largest controlled growth units available for whole ecosystem scale investigations (Roy et al., 2021) (Fig. 2, Right panel). These units harbor soil monoliths and allow a wide range of natural environment manipulations while measuring complex traits such as flux and greenhouse gas emission and tracking flow of matter with the aid of isotopic labels. Deep soil ecotron is a similar research capability currently being constructed at the University of Idaho that will enable both abiotic and biotic factor manipulations of subsurface soils to understand their impact on whole ecosystems. More specifically, the deep soil ecotron can be used to study the dynamic processes of soil microbes beyond the typically investigated depth of ten centimeters (Marx 2023). Microcosm and EcoPODs (Yee et al., 2021) have similar controls and manipulation capacities to ecotrons but are much smaller in footprint. These are considered pilot-scale ecosystems, furthering lab-scale experiments to mimic the environment and field-scale experiments that cannot be carefully controlled. Ecotrons and EcoPODs present great opportunities to refine ecosystem scale models and allow the study of intact soil systems.

On a smaller scale, rhizoboxes, a flat-by-design growth container, allows root visualization and can vary in shapes and sizes (Maskova and Klimes, 2020). One defining characteristic of rhizoboxes is their root growth angle which are inclined, forcing the roots to grow along one of the two flat planes of the rhizobox. Therefore, instead of the 3D growth space found in pots and in the field, the root system is forced to grow in essentially two dimensions within a rhizobox (Mašková and Klimeš, 2020). This reduction of dimensionality facilitates full tracking of root system growth and allows for a more comprehensive visualization of the root system. Moreover, the roots grown against the flat plane allow direct access for root and rhizosphere sampling. Over the years, rhizoboxes have been customized to have removable panels for ease of sampling and integration of planar optodes for measuring CO<sub>2</sub>, O<sub>2</sub>, and pH gradients (Lenzewski et al., 2018). Recently, the conventional pot-based controlled growth chamber experiments are further augmented by RhizoGrids (Handakumbura et al., 2021).

RhizoGrid root cartography an innovative, integrated imaging and spatial multi-omics platform, maps metabolomics and metagenomics measurements to root structures. RhizoGrids are 3D printed and assembled modular units that are customizable based on the study root system size. They act as a scaffold for holding the growing root system and the soil within the 3D printed pots with minimal interference to the growing root system allowing the root to grow normally. This approach preserves the 3D architecture of a plant root system mimicking a root system observed in a field setting. A variety of growth media, including sand and different types of soils, can be accommodated in these pots, and therefore RhizoGrid - guided root cartography workflow enables investigating root rhizosphere interactions in a near-native environment. One main challenge of whole plant system scale and the existing molecular imaging technologies is the lack of spatiotemporal resolutions. A root cartography workflow coupled with RhizoGrid indexing is built and optimized to include non-invasive imaging of the root system in the 3D space using XCT coupled with harvesting and processing of the roots and the associated rhizosphere for metabolite and microbial profiling. Based on the position of the RhizoGrid location each excised root segment is given a unique coordinate to mark its spatial location in the 3D space within the pot. Using the RhizoGrid guided indexing spatially resolved omics data is projected onto the reconstructed 3D root images derived from XCT data (Handakumbura et al., 2021). By combining the molecular-level measurements linked to specific locations within a 3D root system, we can begin to understand the specific microenvironment effects within the root rhizosphere system. This RhizoGrid system has also been tested with isotopically labeled CO<sub>2</sub> to monitor the partitioning of recent photosynthate within the 3D root system and how this partitioning is affected by microbes and environmental perturbations. Other possibilities include coupling fluorescence labeled microbes and cell sorting within RhizoGrid -grown root systems for 3D visualization of microbial recruitment, colonization, and competition within the rhizosphere.

## 4. Image-based modeling of plant–soil interaction: rhizosphere multiscale measurement and modeling

In the previous sections we emphasized the extraordinarily challenging task of finding a suitable experimental setup to investigate rhizosphere processes occurring at the dynamic conjunction of biosphere, hydrosphere, and pedosphere in close vicinity of active plant roots (Oburger and Schmidt, 2016). Nevertheless, during the past decades we have witnessed tremendous advances in the imaging technology (discussed below) that have increasingly made possible imaging of plant–soil interaction (Bandara et al. 2021). Yet the imaging techniques come with tradeoffs between sample throughput, sample size, phase contrast, image resolution, and information gained; therefore, application of modeling tools are quite important to extract information and make predictions. However, like in imaging, there are several computational challenges that need to be overcome when embarking on an image-based modeling endeavor. Here, we show the general workstream of image-based modeling with special emphasis on the computational aspects and discuss steps to give the reader a basic understanding of the technical skills needed to overcome computational challenges.

### 4.1. Image acquisition, reconstruction, and methods of image analysis

In the recent decades, 3D-imaging techniques have become widely available, including laboratory X-ray microtomography and synchrotron X-ray micro- and nanotomography as well as fluorescence microtomography. A more recent method, neutron imaging for the rhizosphere, has enabled complementary experiments where X-rays and neutrons interact with heavier and lighter chemical elements differently. Other 3D-imaging techniques include electrical resistivity tomography, electrical impedance tomography, ground penetrating radar, and magnetic resonance imaging (Anderson and Hopmans, 2013). In addition, correlation of 3D imaging with 2D microscopy and spectroscopy techniques offer options for chemical mapping, that is spatially resolved chemical information. Sampling, imaging steps, and challenges must be considered for these techniques, which are described below as examples for future applications.

**4.1.1. Question of sampling**—The size and nature of the sample (pot or core size used for root/soil imaging, probing pots vs. aggregates/microaggregates, use of fabricated devices to grow plants under controlled conditions, etc.) depends on the scale and purpose of the investigation. Sample format and preparation may be different for a rhizosphere study than for a field-scale investigation. The process to be studied, maybe a measurement of nutrient uptake, soil organic matter analysis, tracking root growth or root exudates, as well as the modeling goal (Vereecken et al., 2016), will determine the sampling approach. As an example, XCT imaging in the laboratory has enabled the nondestructive exploration of root growth processes by imaging whole roots in pot-size samples, while synchrotron-based X-ray microtomography was used on millimeter-size samples to image plant–soil interactions at fine root hairs to enhance models of nutrient uptake by roots (Downie et al., 2015, Keyes et al., 2013). We note that sample preparation can range from none or close to none for tomographic imaging, to quite extensive for multimodal measurements involving imaging (e.g., spectroscopy of carefully prepared slices of a sample). Targeted sampling of the

rhizosphere coupled with correlative imaging with chemical information, and performing these to gather in situ information, is where the future of rhizosphere imaging lies.

**4.1.2. Choice of imaging method**—The choice of imaging technique depends on the characterization goal. For structural/morphological characterization of root systems and the rhizosphere, nondestructive imaging tools such as tomography (X-ray, neutron, optical coherence, electrical resistivity, etc.), magnetic resonance imaging in 3D (Mooney et al., 2012, Kumi et al., 2015), and other imaging methods such as photography using a transparent soil medium in 2D are excellent. For in vivo, time-resolved studies of plant/root growth or rhizosphere processes, researchers turn to techniques that allow for rapid image acquisition such as synchrotron-based XCT (Keyes et al., 2016). However, these imaging techniques do not provide chemical information. In order to complement the morphology with chemical or phenological information, one needs to apply multimodal methods, described in Section 2. To provide with a holistic view of soil microenvironments, it is imperative to combine the correlative imaging studies that are based on image registration of 3D microstructures obtained by XCT with biogeochemical microscopic data from various modalities and scales such as light microscopy, fluorescence microscopy, electron microscopy, and secondary ion mass spectrometry (Schlüter et al., 2019, van Veelen et al., 2020; Keyes et al., 2022; Lippold et al., 2023).

#### 4.2. Image correlation/registration

Employing multimodal and multiscale approaches to combine 3D structural information with 2D chemical information brings about the issue of image correlation, also known as image registration or co-registration. Images obtained by different techniques, using different imaging windows (fields of view) or scales, present a challenge when researchers try to align chemical maps with, for instance, tomography-based 3D images of the same sample. Solutions to this challenge are being developed. From the medical imaging field, an automated approach to match 2D histological images to 3D computed tomography data has been reported (Chicherova et al., 2014). This landmark-based approach in combination with a density-driven RANSAC (RANDOM Sampling and Consensus) plane fitting allows the localization of 2D images within the 3D data with relative accuracy. A statistical method-based approach to align 2D chemical maps with 3D XCT images of soils describes an efficient way to locate a 2D surface in the 3D data, and offers an automated, statistical technique to correct, a posteriori, the geometric distortions generally associated with 2D chemical maps obtained on cross-sections through soil samples (Hapca et al., 2011). Their method uses Pearson correlation coefficient (PCC) as a measure of image similarity to compare two 2D images of the same dimensions. In a multi-image-based study of plant–fertilizer interaction, each microscopy thin-section was compared to the XCT of the sample before resin perfusion and thin-sectioning. This was achieved by finding the XCT slice that corresponds to the thin-section plane and measuring similarity using the statistical measure of PCC (Fletcher et al., 2019). In some cases, elemental maps are overlaid via visual inspection to the corresponding data of the same sample to correlate the chemical and structural information (Keyes et al., 2022). This approach works well when the number of chemical maps is relatively low. Image co-registration between XCT and fluorescence microscopy to look at bacterial distributions has been demonstrated (Schlüter et al., 2019,

Juyal et al., 2019). Light and near-infrared microscopy images were co-registered with XCT images in studies of the spatial distribution of organic matter (Kravchenko et al., 2019, Lucas et al., 2020). While spatial resolution of microscopy techniques is comparable to that of XCT, with techniques such as NanoSIMS or laser ablation mass spectrometry, a dimensional or scale difference poses additional challenges. In these cases, a two-step registration approach may be necessary, where an imaging technique bridging the two scales is used. For image registration to be successful, the structural integrity of the samples is critical, as is the use of appropriate markers for sample orientation and co-registration between images (Lippold et al., 2023). Some of the freely available image registration tools include the medical image registration tool Elastix (Klein et al., 2010) and the ImageJ plugin Correlia (Rohde et al., 2020).

### 4.3. Model equation

Once geometry has been determined from the images, the next question for modeling is: what are the right equations to use? The answer needs to reflect the complexity of the science issue considered. The equations should be as complex as necessary, but as simple as possible. The systematic way of doing this step involves writing down the transport and conservation equations based on all the physics and known biology. The problem then for modeling is to determine if all the physical and biological processes that were deemed to be important actually are important on the time and space scales under consideration as the number of equations needing to be solved increases the computational resource, time, and computer memory needed. Thus, it is often useful to simplify or reduce the equations to the set that is minimally dominant. The process of simplification itself can often learn new science even prior to solving the model equations numerically. Simplification of equations involves two steps: model parameterization and model analytic analysis for simplification. Parameterization is an obvious step and involves determining the model input parameters based on previous published literature. The model simplification process involves finding the dimensionless groupings of parameters by the process of nondimensionalization, which is a formal mathematical process of comparing different physical phenomena present in the model equations (Fowler, 1997). Historically the most popular dimensionless numbers, such as Reynolds number and Peclet number, came about equation free by comparing inertial forces to viscous forces and convective movement to diffusive movement, respectively. In the case of very complex biological phenomena with multiple different processes like the rhizosphere system, a formal nondimensionalization approach is shown to be more productive and systematic. This process involves scaling all state and independent variables with problem-based dimensional quantities so that all variables in the resulting equations are dimensionless (Schnepf et al., 2022). The size of these dimensionless quantities then enables the simplification using perturbation and matched asymptotic analysis techniques that formalize the importance of including and discounting different terms in a quantitative manner. As a result, a new minimal but necessary set of equations needing to be solved numerically is derived.

### 4.4. Solving the models

Which solution method or computational package to use depends on a number of system properties. The balance between learning and implementing the method versus



computational demand/time is often not an easy decision to make. For example, if the model equations are well established and we need to run the simulations on a large number of images, then a fast, efficient parallel solution algorithm is needed. However, while the actual computational solution time for this is low and efficient, the setup time for the computational campaign can be as long as the fast algorithms/packages are often not presented in a very user-friendly manner and hence require more staff time to learn the package and to set up the high-throughput computational campaign. For example, the computational campaign could be OpenFOAM, which is a free, open-source computational fluid dynamics (CFD) software that can be used to solve complex fluid flows involving chemical reactions, turbulence and heat transfer, acoustics, solid mechanics, and electromagnetics (Jasak 2009). For rhizosphere-related works, OpenFoam can be used to model how pore-scale geometry of the surrounding soil affects nutrient dynamics, for instance. On the other hand, when the set of model equations is speculative in terms of science and there is no need to run the simulations on large set of images, a more user-friendly, but less computationally efficient, approach is probably more time efficient as packages like Comsol Multiphysics and Abacus are now becoming very user friendly, with excellent online knowledge bases, without requiring much time investment to learn to use them. So, any time lost in terms of computation time increasing is time saved for the implementation stage. At the end of any computational campaign, the research is often in the stage of visualization and, again, some of the best ways to present it is to use the image analysis software mentioned earlier, as fundamentally the computational step outputs a new image that needs to be analyzed and presented as part of the research project reporting.

Ultimately, any endeavor in modeling can be judged successful even if it fails, e.g., if it comes to the point where new science questions have been generated to plan new experimental and modeling campaigns to advance our learning. Thus, model failure can be an essential ingredient of modeling campaign success as we learn new science. However, having done the modeling, this next step of experiment planning can now be aided by the model being run in the predictive mode to make informed decisions about, for instance resolution needed for spatial and time-resolved sampling, to enable our learning of new science.

Collectively, under sections 2–4 we highlighted the use of different imaging methods, synthetic soil habitats, and image-based modeling as critical capabilities and approaches for monitoring root-soil-microbe interface and specifically for tracking inorganic nutrients and Carbon flux in the rhizosphere. However, we need advanced in situ and real-time rhizosphere tracking systems for better understanding and upscaling small-scale rhizosphere properties and further linking rhizosphere processes across scales. This is a significant challenge that requires a robust response from the research community for development and engineering of efficient, low-cost, in situ sensing systems, able to be deployed at the rhizosphere for continuous monitoring and data transmission of key physiochemical and biological variables. In the following sections, we provide our insights on current and emerging sensing technologies and their potential integrations with advanced predictive models for studying rhizosphere across scales. 5. Biosensors for monitoring nutrient and chemical exchanges in the rhizosphere

## 5.1. Genetically encoded biosensors

As stated earlier, rhizosphere is strongly influenced by plant metabolism through the release of photoassimilates as root exudates, i.e., plant-derived primary and secondary metabolites of both low molecular weight (MW) that is <1,000 Da (e.g., sugars, organic acids, phenolics, vitamins) and high MW compounds that are >1000 Da (e.g., enzymes, mucilage) (Oburger and Jones, 2018). As communicating molecules, root exudates provoke interactions between the soil microbiome and plant roots through modifying the chemical and physical properties of the soil and the soil microbial community (Ahkami et al., 2017).

Several methods have been reported for tracking root exudates, root-microbe interactions, and chemical exchanges including reporter genes, immunological reactions, and nucleic acids (Rilling et al., 2019). Released exudates in the rhizosphere could impact the nutrient cycling and, in some cases, facilitate the uptake of certain nutrients from the environment. Specific rhizobacteria benefit crops through nutrient solubilization or biological nitrogen fixation. Several techniques have been used to measure or image nutrient levels in roots and rhizosphere region like X-ray spectrometry or use of radioisotopes (Kanno et al., 2016). However, none of these techniques allow in vivo monitoring of nutrient and chemical profiles with cellular resolution in real time. In contrast, genetically encoded fluorescent biosensors provide an opportunity to report the levels of the compound of interest as measurable fluorescent signals.

Genetically encoded biosensors directly interact with a molecule of interest through the action of fusion proteins that serve as fluorescent indicators. This technology has been deployed to improve our real-time spatiotemporal understanding of different molecules like calcium, sugars, and phytohormones, whose dynamics have significant influence on plant physiology and development under environmental changes. Fluorescence resonance energy transfer (FRET) technology, as a genetically encoded biosensor, measures the levels of the target compound within individual cells in living organisms and in real time. Sensor proteins are produced with two properties: they can bind to the target compound in a reversible manner, and they contain two 'tags' that fluoresce at different wavelengths (Waadt et al., 2014). Essentially, FRET magnifies microscopic conformational changes by emitting light, which can be captured by a sensor, providing a unique tool to study biological processes well below the resolution of standard optical microscopy (Zadran et al., 2012).

FRET-based biosensors have been widely used to measure sugars like glucose (Zhu et al., 2017), phytohormones including ABA (Jones et al., 2014) and auxin (Herud-Sikimic et al., 2021), and ions such as  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  (Zhang et al., 2022). These biosensors provide novel insights on plant interaction with the environment, such as the discovery of salt stress-induced  $\text{Ca}^{2+}$  waves in *Arabidopsis* roots (Choi et al., 2014) or identification of a new sensing mechanism of nitrate in soil (Ho et al., 2009). Focusing on root-microbe interactions, a FRET-based biosensor reveals spatiotemporal variation in cellular phosphate content in *Brachypodium distachyon* mycorrhizal roots (Zhang et al., 2022).

The ideal sensor for the visualization of root system dynamics and functions, and specifically to track root exudates as an important component of the rhizosphere region, should have two key attributes: first, physical interaction of the sensor with the compound

of interest should elicit a fluorescent signal in a reversible manner, so that changes in molecular concentration can be monitored; second, the sensitivity of the sensor should be sufficiently high to image the dynamic distribution of the compound of interest over time (Herud-Sikimic et al., 2021).

Major challenges in developing and applying genetically encoded root biosensors in future rhizosphere research include: (1) large-scale identification of key compounds (root exudates) with important functions in rhizosphere dynamics and root-microbe interactions under different environmental conditions as candidates for biosensor development; (2) relatively low throughput of the number of biosensors and the ability of screening a limited number of compounds under a given time and condition; and (3) deploying genetically engineered crops in the field so the true validation of detected target compounds can be determined. Although obtaining permission from government agencies for such field trials requires significant effort, scientific communities should collaborate more effectively to take advantage of the existing expertise and available field locations worldwide.

## 5.2. Nanotechnology-mediated delivery of biosensors into plant tissues

In the effort to unravel rhizosphere biogeochemical complexity and dynamics, development of sensor technology plays a critical role as sensors can be used for identifying and quantifying different targets in the rhizosphere. Consequently, a variety of sensors have been developed based on small organic molecules, nucleic acids, and proteins. Because these sensors are designed to image different targets in the rhizosphere, they need to be delivered into different parts for plant imaging, genetic engineering, and gene function study. With the development of nanotechnology, a new frontier of nanomaterial-based delivery methodologies emerged. Here, we focus the discussion on the recently developed method of nanomaterial-based delivery in intact plants and how this technology can be applied in root and rhizosphere research.

Compared to classic delivery methodologies, including biolistic and *Agrobacterium* transformation, nanomaterial-based delivery does not have narrow host range limitation and plant extensive damage issues. Moreover, based on their effective delivery systems, nanomaterials exhibit great potential to be used for delivering sensors in the plant research field. Therefore, a variety of nanomaterials, including mesoporous silica nanoparticle, carbon nanotube, clay nanosheet, quantum dot, DNA nanostructure, and metal-oxide based nanomaterials, are currently being used for delivering sensors in leaves or in roots.

For sensor delivery in leaves, mesoporous silica nanoparticles were first investigated for both gene and chemical delivery in an intact plant with the gene gun system (Torney et al., 2007). Subsequently, Cre recombinase protein delivery to maize cells was explored using mesoporous silica nanoparticles for DNA-free genome editing (Martin-Ortigosa et al., 2014) based on the same gene gun system, a force-dependent internalization process. Magnetic nanoparticles also have been applied in producing transgenic seeds without tissue culture regeneration, which were forced into pollen by a magnetic field with the packaged plasmid DNA (Zhao et al., 2017). However, there was only one successful application of magnetofection in plants and no evidence for transient transformation was found via pollen magnetofection in several monocot species (Vejlupkova et al., 2020). Therefore, developing

nanomaterial-based plant delivery systems with high efficiency and without the aid of external force is still needed. In 2009, single-walled carbon nanotubes (SWCNTs) were demonstrated to penetrate the cell wall and cell membrane of intact plant cells (Liu et al., 2009). SWCNTs could also enter chloroplasts of *Arabidopsis thaliana* leaves by infiltration through the leaf lamina (Giraldo et al., 2014). Since then, SWCNTs have been reported to act as a powerful tool to deliver many sensors including small molecule, nanoceria (Giraldo et al., 2014), single-stranded DNA (Liu et al., 2009, Wu et al., 2020), siRNA (Demirer et al., 2020), and plasmid DNA (Demirer et al., 2019, Kwak et al., 2019), delivery in intact plant cells. Besides, plasmid DNA has been delivered into pollen (Lew et al., 2020) by SWCNTs. Similar to SWCNTs, quantum dots have also been used for siRNA and plasmid DNA delivery in intact plant cells (Schwartz et al., 2020, Wang et al., 2020) and targeted delivery of chemicals by using a peptide recognition motif (Santana et al., 2020). With the rapid development of DNA nanotechnology, DNA nanostructures were further investigated for their internalization into plant cells and were applied to gene silencing in plants (Zhang et al., 2019). Positively charged, delaminated-layered, double-hydroxide lactate nanosheets were found to penetrate the plasma membrane via non-endocytic pathways once they formed neutral conjugates, which could be a novel gene carrier to plants (Bao et al., 2016). Recently, cell-penetrating peptide was used to deliver the actin binding peptide, Lifeact, fused to GFP11, which offers a new tool for cytosolic delivery of proteins in plants (Wang, 2022).

Similar to the development of nanomaterial-based delivery systems in leaves, systems have also been widely applied in roots. Due to the rhizodermis lateral root junctions, roots may provide easy access to nanomaterial-based delivery systems. For example, different from a force-dependent mesoporous silica delivery system in leaves, mesoporous silica nanoparticles could deliver foreign DNA into intact *Arabidopsis thaliana* roots without the aid of mechanical force. The delivery system was detected in the epidermal layer and in the more inner cortical and endodermal root tissues (Chang et al., 2013). SWCNTs could also be used to deliver plasmid DNA into tobacco root cells by taking advantage of the nanocylindrical shape (Golestanipour et al., 2018). Short arginine-rich, intracellular delivery peptide was able to deliver plasmid DNA into roots in the form of peptide/plasmid complexes (Chen et al., 2007). Positively charged, delaminated-layered, double-hydroxide lactate nanosheets can not only deliver molecules in leaves, but also can work as effective biomolecular transporters in the cytosol of epidermal cells from the root apical region (Bao et al., 2016). Moreover, dendritic polymers with positive charges were used as carriers for dsRNA, which can trigger gene silencing in plants (Jiang et al., 2014). Many metal-oxide-based nanomaterials can be uptaken by the roots, and this topic has already been systematically reviewed (Nair et al., 2010).

Due to the rapid advances of the delivery systems in both leaves and roots, some targets have been applied for chemical in situ detection and sensing in plants. For example, DNA-modified SWCNTs were delivered to leaves for NO (Giraldo et al., 2014) (Figure 3A) and H<sub>2</sub>O<sub>2</sub> (Giraldo et al., 2015, Wu et al., 2020), sensing due to the quenching in fluorescence of SWCNTs. Besides, the embedded DNA-modified SWCNTs in plant tissues have been applied to arsenic detection (Lew et al., 2020) and the SWCNTs conjugated to the peptide Bombolitin II detected nitroaromatics via infrared fluorescent emission, by using polyvinyl-alcohol functionalized SWCNTs as an invariant reference

signal (Wong et al., 2017). The microRNA expression within whole plant leaves was detected using plasmonic-active, silver-coated, gold nanostars (AuNS@Ag) functionalized with DNA strands by integrating three different and complementary techniques: surface-enhanced Raman scattering (SERS), X-ray fluorescence (XRF), and plasmonic-enhanced two-photon luminescence (TPL) (Crawford et al., 2019). This biosensor provided a dynamic visualization through a SERS map of detected microRNA targets and quantified nanoprobe concentrations using XRF and TPL (Figure 3B).

Although major progress has been made on nanomaterial-based delivery systems for plant leaves and roots, less attention has been paid to developing nanomaterial-based biosensors for environmental monitoring. Investigation on how to use and adapt state-of-the-art nanomaterial-based biosensors (currently applied for plant leaves and roots such as those shown in Figure 3A–C) for rhizosphere biosensing with appropriate reengineering is a critical need. For example, optical nanosensors can be designed for monitoring aqueous-based plant signaling molecules, and radio-frequency nanoelectronic-based wearable devices can be used to detect plant volatile compounds (Figure 3D). Smart plant sensors that communicate with electronic devices could provide detailed information on plant water, nutrients, and pesticide-specific needs. Real-time monitoring of plant health, rhizosphere activities, and root–soil–microbe interactions with nanosensors enables fine-tuning of resource inputs and has significant potential to enhance agricultural sustainability. In addition to the nanomaterial-based biosensors, some emerging technologies can be applied to plant biosensing as well. For example, recently thiol-mediated uptake has been successfully applied for nucleotides delivery in plants and a glucose aptamer FRET sensor has been used for in situ glucose detection in *Arabidopsis* (Figure 3C) (Mou et al., 2022). The advancements of biosensors and novel nanomaterial-based delivery systems in plants opens a new frontier for rhizosphere-based activity sensing that can provide insights into understanding the biogeochemical complexity and dynamics of the rhizosphere.

## 6. Measurement of the distribution and dynamics of rhizosphere chemical gradients in the field

Quantifying the distribution and dynamics of chemical gradients around roots in the field is a challenging and exciting frontier. Heterogeneity in space and time creates localized niches where diverse biogeochemistries are enabled. Investigating both their local ( $\mu\text{m}$  to  $\text{cm}$ ) scale patterns and potential aggregate effects at larger scales requires development of field methods capable of capturing dynamic rhizosphere-scale heterogeneity. Several methods have recently emerged that hold promise, including planar optodes and diffusive gradient (DGT) and diffusive equilibrium thin films (DET) or gels. Optodes are chemical sensors providing information about the presence or concentration of specific chemical targets (e.g., oxygen and  $\text{CO}_2$  concentrations, sulfide, or pH) via an optical signal (e.g., fluorescence). In planar optodes, the sensor chemistry is immobilized on a transparent sheet, enabling imaging of the 2D distribution of target chemical species. Ideally, optode signals are rapidly reversible. In early work using planar optodes, (Glud et al., 1996) demonstrated that oxygen concentrations [ $\text{O}_2$ ] could be quantified across the 2D face of a coastal sediment block at resolutions of tens of  $\mu\text{m}$ . Since then, planar optodes have provided fascinating insights into,

for example, dynamic heterogeneity in  $[O_2]$  around plant roots or in invertebrate burrows in sediment (Li et al., 2019). Most often, planar optodes have been deployed in saturated sediment in the lab (see discussion of field deployments below). Planar optode sensing systems can be custom-built from readily available components (Larsen et al., 2011) or purchased commercially (Koop-Jakobsen et al., 2021). Detailed reviews by Santner et al., (2015) and Li et al., (2019) provide the history of planar optode application, the mathematics behind various quantification strategies, matrices used, and optical indicators for diverse analytes. A number of approaches (Scholz et al., 2021) are available for detecting and quantifying chemical gradients around seagrass roots, among them using reversible planar optodes, as well as sensing analytes via irreversible reactions with immobilized binding chemicals held within thin planar DET or DGT films or gels. Such gels are amenable to simple deployment in the field, though they are not capable of providing dynamic information when irreversible reactions are used to detect analytes (Davidson, 2016). DGT and DET approaches have been used in biogeochemical studies, for example, to map the locations of accumulation of  $H_2S$  and/or iron (II) in sediment (Robertson et al., 2008). Commercial DGT gels are available for field deployment and detection of a very wide variety of metals, cations, anions, and organic molecules.

The reversibility of many planar optode reactions particularly recommends their use for field monitoring of dynamic concentrations of analytes such as  $O_2$ ,  $CO_2$ , and pH in the rhizosphere. In a recent review, Koren and Zieger, (2021) proposed avenues for advancement of the technique, and strategies for enabling optode use by a broader audience. They argued for development of reversible indicators to probe a wider variety of analytes in the environment; more sophisticated image analysis, from image noise reduction to calibration to characterization of signal drift; combinations of the application of planar optodes with other methods, including DGT/DET gels and microsensors; and deployment in the field.

From the perspective of using planar optodes to understand dynamic system-scale environmental biogeochemistry influenced by rhizosphere processes, this latter leap to field deployment is particularly important but has been challenging and rare. Glud et al. (2001) pioneered field deployment of a large, ship-based  $O_2$  planar optode system in sediment off the Danish coast. Mirrors, a camera, and an illuminator were enclosed within an inverted periscope tethered to a ship-board computer via fiber optic link. Similarly, Fan et al., (2011) built a ship-deployed system expanded to include sensing of  $O_2$ ,  $CO_2$ , and pH in coastal sediments in Long Island Sound. More recently, Turner et al. (2020) deployed an  $O_2$  planar optode system in a vegetated Alaskan thermokarst bog to examine controls over methane oxidation and production. System components were housed within a large hollow box (~1 m tall x 56 cm square) inserted into a dug hole. No oxygen pools were detected around plant roots in bog sediment in the field, though plants had aerenchyma tissue that should support diffusive transport of oxygen belowground and methane aboveground. However, the kinetics of the disappearance of oxygen from aerated bogwater injected by the investigators against the optode revealed that biological oxygen demand was higher in vegetated sediment than in unvegetated sediment, underlining an important point. Microbial communities are well known to proliferate and become more active as roots mature behind the tip zone (Cardon and Gage, 2006, Herron et al., 2013). In any planar optode study of the rhizosphere, undetectable oxygen pools along roots could be caused by no or low root oxygen release, or

by rhizosphere microbial oxygen demand outstripping root release of O<sub>2</sub>; these two causal scenarios have very different biogeochemical implications for carbon, nitrogen, and sulfur cycling in saturated systems.

To advance understanding of the influence of rhizosphere processes on local and larger scale biogeochemistry in the field, we would echo and expand on Koren and Zieger's (2021) recommendations:

1. Deployment of rhizosphere sensors in the field clearly needs to become more routine. We are developing a small, low-cost, prototype O<sub>2</sub> planar optode system (35 cm wide × 30 cm deep × 4 cm thick, (Zhang, 2021) that can be deployed in situ in the field under saturated (flooded) or unsaturated conditions, and that sends images wirelessly to a phone or laptop nearby. Figure 4 provides an example image series showing root oxygenation of sediment and tidal flushing of low oxygen water out of sediment into overlying water during tidal inundation of the salt marsh near Woods Hole, MA. The ultimate goal is deployment of many of these low-cost instruments in natural landscapes, enabling local quantification, detection of landscape-scale variation, and cross-site comparison of dynamics and heterogeneity in [O<sub>2</sub>] belowground.
2. With field images in hand, not only should image processing and analysis become more sophisticated (as recommended by Koren and Zieger, 2021), but the information provided by optodes should be incorporated into biogeochemical process models (Figure 4, e.g., the reactive transport model PFLOTRAN, (O'Meara et al., 2021)). Such models (see sections 7 and 8 and Figure 7 below) must be modified to represent spatial as well as temporal heterogeneity in belowground conditions. Optode measurements are not perfect; even in the lab, both signal drift and the physics of gas distribution when released against the optode plane affect quantification. But even imperfect measurements provide guidance for sensitivity analyses and explorations of scaling behavior for systems of nonlinear biogeochemical reactions (Ruel and Ayres, 1999, Zhou et al., 2022).
3. The co-deployment of field planar optodes with diverse field-ready DGT/DET gels and microsensors clearly is synergistic for rhizosphere mechanistic investigations and biogeochemical modeling. Deployment of multiple small-scale sensors within the footprints of eddy covariance towers (Figure 4) will provide a powerful new opportunity to examine whether, when, and how the aggregate effect of rhizosphere-scale chemical heterogeneity influences the magnitude and/or dynamics of biogeochemical process measured and modeled at larger scales.

## **7. Detection of Rhizosphere interactions across scales: challenges in upscaling in complex systems**

### **7.1. Integrated sensing tracking interactions among subsystems**

Modern technological advances have significantly improved our capability to measure and analyze, which brings a wide range of new possibilities for ecosystem sensing (Akyildiz et

al., 2002; Short 2008; Anderson and Gaston, 2013; Taberlet et al, 2012). While progress to improve sensing is moving forward on every front, coordinated efforts that take a holistic approach to realize next-generation ecosystem sensing have been lacking. Such a holistic approach requires a systematic view of the systems under study to decide where and how to invest efforts. The critical nature of the questions to be addressed, the spatial and temporal extent of the challenge, and the need to allocate resources efficiently require that we approach this effort with a new paradigm. Future generation ecosystem sensing networks must consist of diverse but compatible sensing modalities that can operate across a range of spatial scales with new approaches for efficient onboard or localized data processing and communication (Yick et al., 2008; Shi et al., 2016; Dizdarevic et al., 2016; Jetz et al., 2019). These sensor packages should be customized and deployed based on the type, location, and timing of data requirements, using best-available model predictions to inform these decisions, e.g., the model-experiment (ModEx) approach. Recognizing that our best-in-class models will not always represent important processes, sensing for discovery is a critical need where new sensors and new sensor combinations, in new locations, with new approaches to data interpretation and translation to knowledge are the focus (Kumar et al., 2015; Bonnet et al., 2001; Turner et al., 2003). This discovery aspect will initially require controllable systems with well-defined boundary conditions and realistic yet manageable heterogeneity, allowing multiple research groups to work together to evaluate new sensing approaches while leveraging information from well-developed sensor platforms. Manipulative laboratory platforms, such as ecotron types (Roy et al., 2021) described in Section 3, provide such an opportunity for integrated sensing system testing and optimization toward a field-deployable sensing package for both physics- and ML-based causal correlation discovery with improved scalability and predictability.

## 7.2. Examples of integrated above and belowground sensing systems

An example is given here in terms of an experimental platform to facilitate testing, prioritization, and development of the integrated ecosystem sensing system to allow sensing optimization, model benchmarking, and new scientific discovery.

Equipped with >70 streaming parameters and >20,000 distributed sensing points, the SMART (Sensors at Mesoscales with Advanced Remote Telemetry) Soils testbed is a mesoscale experimental platform with adjustable hydrological gradients and controllable biogeochemical conditions for testing key hypotheses on ecosystem behaviors and responses to perturbations (Figure 5A). In addition, the SMART Soils testbed provides a “playground” for testing sensor selection, performance, calibration, interference, and integration across scales. The sensing system is purposefully designed to mimic what is deployed in the field to test the transferability of the sensing methods, and the diverse streams of data produced from the testbed are used to benchmark mechanistic models and develop ML-based data analytical tools to improve predictability of the system behavior at field scales.

The SMART Soils testbed sits at a unique scale between tightly controlled laboratory manipulations (e.g., benchtop experimental apparatus) and field observations in that it allows selective control of some processes (e.g., hydrology), while allowing other processes to vary according to nature. It is large enough (4 m × 1 m × 1 m) to allow the inclusion of



complexity and heterogeneity at multimeter scales that are very relevant to the field, yet small enough to manage. Its proximity to the laboratory and its collected expertise facilitates rapid testing of new sensors and improved interaction and collaboration of researchers from multiple disciplines.

Experimental results during two growth seasons have proven the value of such systems in terms of testing the performance of the integrated sensing program design as well as its use for model benchmarking and new scientific discovery. Figure 5B–D shows a small fraction of the large and diverse amount of data collected from the testbed, highlighting the power of a holistic sensing system. Specifically, the 3D geophysical imaging (Binley et al., 2015) and concurrent plant phenology monitoring (Figure 5B) provide a dynamic view of soil moisture and water stress evolution and their co-variability with plant species distribution during growth cycling (Figure 5C). Together with continuous soil surface CO<sub>2</sub> flux monitoring, soil sensors, and root minirhizotron imaging (Johnson et al., 2001), the mechanistic control of soil CO<sub>2</sub> flux from hydrology and biogeochemistry is revealed (Figure 5D). While not discussed in detail, the diverse, high spatiotemporal datasets collected over multiple growth seasons under both controlled and natural conditions allows the development of datacentric ML capabilities (Kantardzic et al., 2011) to identify the main drivers and predict key soil dynamics, such as soil CO<sub>2</sub> fluxes, in such complex systems. These ML capabilities are used for data gap filling and predictions into the future. Further comparisons between the predicted behaviors and actual measurements as time progresses provide opportunities to improve the ML algorithm and guide new experimental design and data gathering to better train the ML models. Such an interactive ModEx workflow developed and demonstrated on the testbed is being evaluated for its transferability to field-scale research at multiple sites, such as the East River Watershed in Colorado.

## 8. Fate of the terrestrial biosphere: Scaling plant–soil–microbe interactions to the landscape and the world

Our paper thus far has described the importance, challenges, and technological solutions in capturing plant–soil–microbe interactions starting from the scale of nutrients and metabolomics moving upward and outward. Here, we present the next and final challenge in scaling: moving to the landscape and beyond, ultimately culminating in a global picture.

At the global scale, plant–soil–microbe interactions are critical to determine terrestrial biosphere impacts to climate change (Classen et al., 2015, Shi et al., 2019). The amount of atmospheric CO<sub>2</sub> ecosystems can sequester is a function of many environmental factors, not the least of which is nutrient limitations (Hungate et al., 2003, Fisher et al., 2012, Huntzinger et al., 2017). Progressive nutrient limitation has been hypothesized with rising CO<sub>2</sub>; plants will take up increasing amounts of CO<sub>2</sub>, but as soil nutrients gradually deplete an inflection occurs and the terrestrial carbon sink strength greatly weakens (Luo et al., 2004, Johnson, 2006). Indeed, some free air CO<sub>2</sub> enrichment (FACE) studies have shown just that (Norby et al., 2010, Norby and Zak, 2011). However, other FACE studies have curiously shown that even when soil nutrients are depleted, some trees continue to take up increasing CO<sub>2</sub> (McCarthy et al., 2010). The answer to this mystery lies in mycorrhizal

fungi: forests with ectomycorrhizal (ECM) symbioses can continue taking up rising CO<sub>2</sub> in the absence of soil nutrients, whereas forests with arbuscular mycorrhizal (AM) symbioses do not (Terrer et al., 2016).

For global-scale models to determine terrestrial carbon sink/source dynamics, we need to know where AM and ECM fungi exist throughout the landscape and globally (Braghiere et al., 2021). Because we cannot see from above the fungi below the ground, we have been unable to provide that information. We do know that most plant species associate with only one or the other mycorrhizal type (Allen et al., 1995); so if we could identify every plant species individually, then we could know which mycorrhizal type is hidden beneath. But we cannot identify the species of every tree in existence globally, so again we remain stuck.

To break through this scaling barrier, we ask a slightly different twist on the question of identifying individual plant species. Specifically, are there characteristics in common among plants of different species that associate with the same mycorrhizal type (Fisher et al., 2016)? And, if so, are those characteristics distinctly different from those of all the rest of the plant species associating with the other mycorrhizal type? If this were the case, then we could potentially see these characteristics—such as canopy spectral features—among groups of trees instead of individuals using existing remote sensing technology, allowing us to identify the underlying mycorrhizal association.

First, we test this question using airborne measurements so that we can link remote sensing measurements directly to individual trees. We used imaging spectroscopy (hyperspectral) measurements from the AVIRIS (Airborne Visible/Infrared Imaging Spectrometer) platform over 112,975 trees across six sites from Hawaii to Massachusetts (Sousa et al., 2021). Imaging spectroscopy partitions the electromagnetic spectrum into many discrete units, which enables detection of subtle reflectance differences in the spectrum due to canopy chemistry and structural differences (Green et al., 1998). These include, for example, carotenoids, xanthophyll, nitrogen, phosphorus, water, cellulose, and lignin (Sousa et al. 2021). Sure enough, these measurements revealed clearly discrete differences between the over one hundred tree species images associated with AM or ECM fungi—capturing the variability remarkably well ( $r^2 = 0.92$ ). Imaging spectroscopy was key to this differentiation, enabling a 30% improvement over simpler multispectral resolution. The next question in scaling is how does the accuracy degrade with coarsening resolution? Paradoxically, coarsening the spatial resolution to 30 m improved results likely due to a shift in signal-to-noise sensitivity and blurring of geolocation measurement uncertainty. This bodes well for scaling up further to the larger landscape.

The next step up from airborne scaling is using satellite remote sensing. Unlike the airborne remote sensing analysis, unfortunately there has been no reliable spaceborne imaging spectroscopy, although that situation is changing rapidly (Alonso et al., 2019, Green, 2020, Cawse-Nicholson et al., 2021). Still, there has been a great heritage of multispectral, high spatial resolution, remote sensing that not only facilitates global scaling, but also allows an increase in temporal resolution signals from the typically one-off flights from airborne remote sensing (Wulder et al., 2019). So, we tested this approach using 30 m Landsat data over 130,000 trees at four sites in the United States (Fisher et al., 2016). Like the

airborne results, we were able to capture variability in mycorrhizal association across these landscapes well ( $r^2 = 0.77$ ). While not better than the imaging spectroscopy results, the multispectral results benefited from seasonal differences between AM and ECM trees. The next step will be to combine the strengths of these two approaches in the coming years when we have reliable and accurate spaceborne imaging spectroscopy for multiple seasons.

In parallel and motivated by similar global-scale questions, multiple groups have constructed global maps of mycorrhizal distribution (three of which were published independently in the same year). These maps are based on site-level statistical correlations with climate and land cover, then extended globally using gridded climate and land cover datasets. Shi et al., (2016b) classified a global plant functional type map by mycorrhizal association based on literature descriptions; Sulman et al., (2019) drove a niche model with mycorrhizal data from multiple databases; Steidinger et al., (2019) synthesized forest inventory plot data with ML to generate a global map; and finally, Soudzilovskaia et al., (2019) focused on land cover and ecoregions to extrapolate site-level records. These global maps differ dramatically among each other—up to 50% disagreement for over 40% of global land area (Braghiere et al., 2021). The question that follows is: does it matter?

To understand the impact of microbial symbioses at the global scale, these dynamics need to be incorporated into global models. The first step toward this goal was the development of the Fixation and Uptake of Nutrients (FUN) model, which mathematically formalized the carbon cost to plants for nitrogen and phosphorus uptake from various sources (i.e., leaf resorption, biological nitrogen fixation, direct root uptake, mycorrhizal uptake, and passive uptake) (Fisher et al., 2010, Brzostek et al., 2014, Allen et al., 2020). FUN was originally developed for the UK land surface model, JULES (the Joint UK Land Environment Simulator), but later expanded development into Noah-MP, CLM, and eventually ELM (Cai et al., 2016, Shi et al., 2016b, Fisher et al., 2019, Lawrence et al., 2019, Braghiere et al., 2022). Finally, we gained a picture of what the global impact might be from the incorporation of these plant–soil–microbe interactions into global models (Figure 6).

In Shi et al., (2016a), mycorrhizal uptake was the dominant pathway by which nitrogen was acquired, accounting for two-thirds of the nitrogen uptake by plants. Globally, plants spent  $2.4 \text{ Pg C yr}^{-1}$  to acquire  $1.0 \text{ Pg N yr}^{-1}$ , and this loss of carbon led to a downregulation of global net primary production by 13%. Next, Braghiere et al., (2021) tested CLM with all four global maps of mycorrhizal association and found that although the differences in carbon and nitrogen cycling were ultimately small among the model runs driven by the different maps, the major impact was if they were included in the first place—again highlighting the importance of plant–soil–microbe dynamics on global carbon and nutrient cycling. Finally, Shi et al., (2019) evaluated the impact of plant–microbe symbioses on global climate. They found strong regional impacts wherein these dynamics led to an increase in temperature by up to  $1.0^\circ\text{C}$  and precipitation by up to  $9 \text{ mm yr}^{-1}$  in boreal and alpine ecosystems, for example.

Nutrient cycle dynamics have been a top priority for development among almost all global climate modeling groups in the world in recent years (Fisher et al., 2014). While model formulations and evaluations continue to evolve, it is critical that they represent plant–soil–

microbe dynamics robustly while balancing accuracy in detailed process representation versus computational load for global and centennial-scale projections. With the advent of new high spectral and high spatial resolution capabilities, in conjunction with computational power and sensing technologies at our fingertips, there is an exciting future ahead for scaling the rhizosphere to the globe (Figure 7).

## 9. Forward looking: integrated imaging, sensing, and modeling approaches for studying rhizosphere across scales

To detect rhizosphere processes for global predictions, we propose that future works employ integrated imaging and remote sensing technologies, combined with computational models. For that, sensor technology is the key to provide in situ and real-time information on rhizosphere processes. Future generation ecosystem sensing needs to promote connectivity and interaction between managed and natural systems. It also needs to improve integrated sensing across compartments and components (soil, microbes, plant, atmosphere) and across scales (molecules to ecosystems) by using a variety of technologies across discipline boundaries such as biology, chemistry, physics, and hydrology (Figure 7). The development of future sensing capabilities needs to take a tiered approach: (1) building sensor packages to measure “critical to know everywhere” parameters for robust deployment across vast spatial scales; (2) parallel development of data processing and communications schema essential for development of the next generation of sensors for “important to know but hard to measure” parameters; and (3) testing and incorporation of future generation sensors into robust and customized packages. These could include in situ sensors to measure chemical and biological species with improved spatial resolutions as well as sensors to measure more structural and flux parameters.

Development of advanced data retrieval, transmission, and edge computing efforts for ecosystem and global sensing is also critical. This should leverage what is already occurring in the field of wireless telecommunications and the Internet of Things smart networking technologies in other fields. Developments of advanced data analytical tools need to consider the characteristics of ecosystem data and need to improve the accessibility of such tools to the broader community. Further, data analytics experts need to work closely with disciplinary scientists and modelers to ensure reliable data interpretation and build trust in each other.

The ModEX approach needs to be incorporated into sensing system design and implementation, where model inputs are key to experimental design and data collection, so that data collection is more efficient and most impactful in terms of contributing to model improvements. Such interactions from the beginning also facilitate better integration of models and measurements across different scales, critical to improving the representation of the rhizosphere small-scale mechanistic processes in large-scale models with better transferability and predictability.

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### Acronyms:

<b>SMART</b>	Sensors at Mesoscales with Advanced Remote Telemetry
<b>ML</b>	Machine Learning
<b>SEM</b>	Scanning Electron Microscopy
<b>TEM</b>	Transmission Electron Microscopy
<b>EDX</b>	Energy Dispersive X-ray Spectroscopy
<b>APT</b>	Atom Probe Tomography
<b>3D</b>	Three – Dimensional
<b>2D</b>	Two-Dimensional
<b>XCT</b>	X-ray Computed Tomography
<b>XRF</b>	X-ray Fluorescence
<b>XANES</b>	X-ray Absorption Near Edge Structure
<b>MALDI</b>	Matrix Assisted Laser/Desorption Ionization
<b>NanoSIMS</b>	Nanoscale Secondary Ion Mass Spectrometry
<b>PCC</b>	Pearson Correlation Coefficient
<b>MW</b>	Molecular Weight
<b>FRET</b>	Fluorescence Resonance Energy Transfer
<b>SWCNT</b>	Single-Walled Carbon Nanotubes
<b>SERS</b>	surface-enhanced Raman scattering
<b>TPL</b>	two-photon luminescence

<b>DGT</b>	diffusive gradient
<b>DET</b>	diffusive equilibrium thin films
<b>ModEx</b>	Model Experiment
<b>FACE</b>	free air CO <sub>2</sub> enrichment
<b>ECM</b>	ectomycorrhizal
<b>AM</b>	arbuscular mycorrhizal
<b>AVIRIS</b>	Airborne Visible/Infrared Imaging Spectrometer
<b>FUN</b>	Fixation and Uptake of Nutrients
<b>JULES</b>	Joint UK Land Environment Simulator
<b>CLM</b>	Community Land Model
<b>ELM</b>	E3SM Land Model

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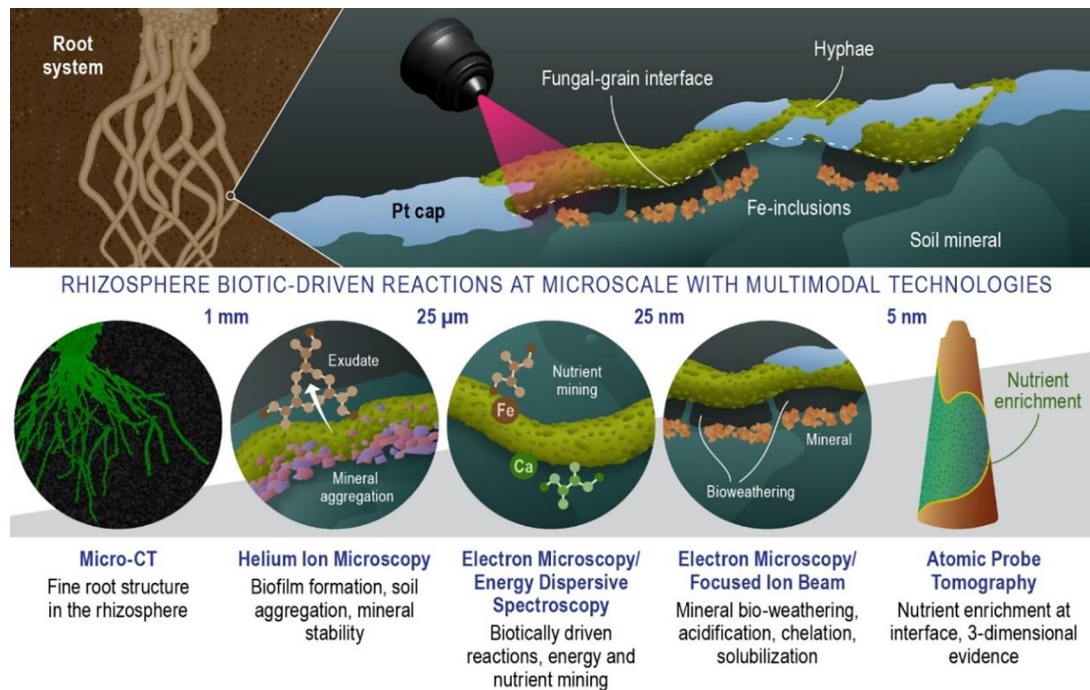
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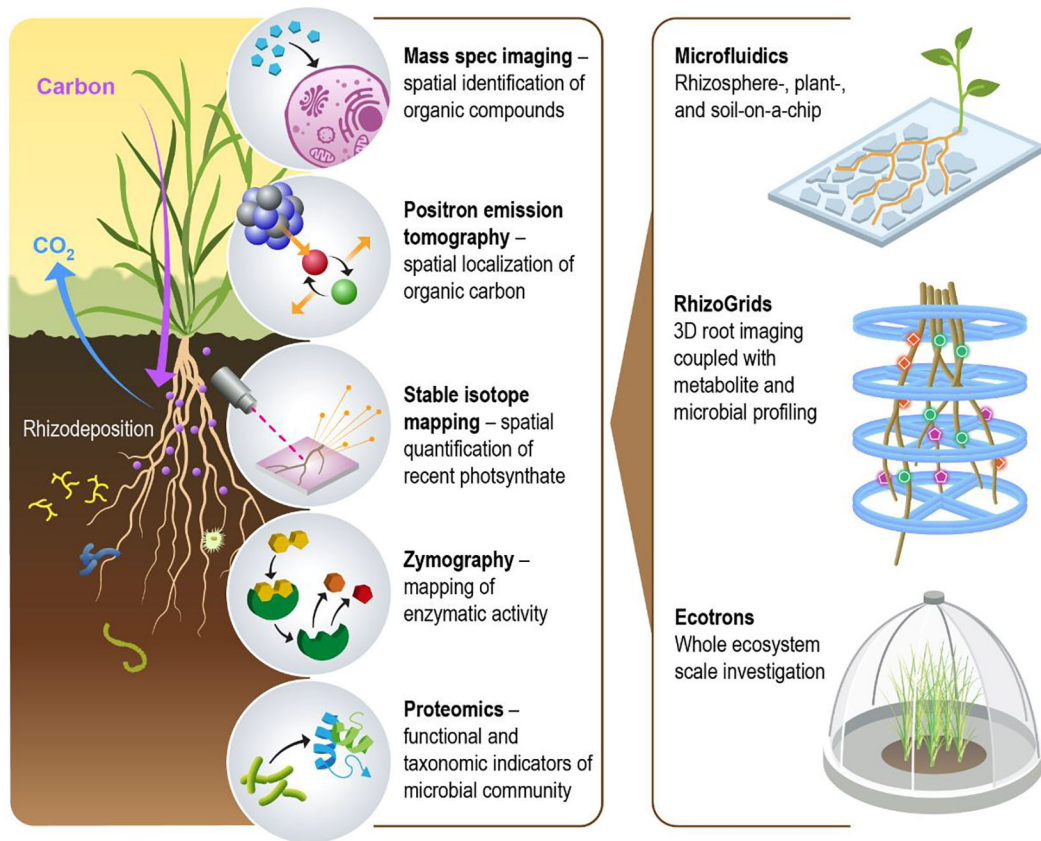


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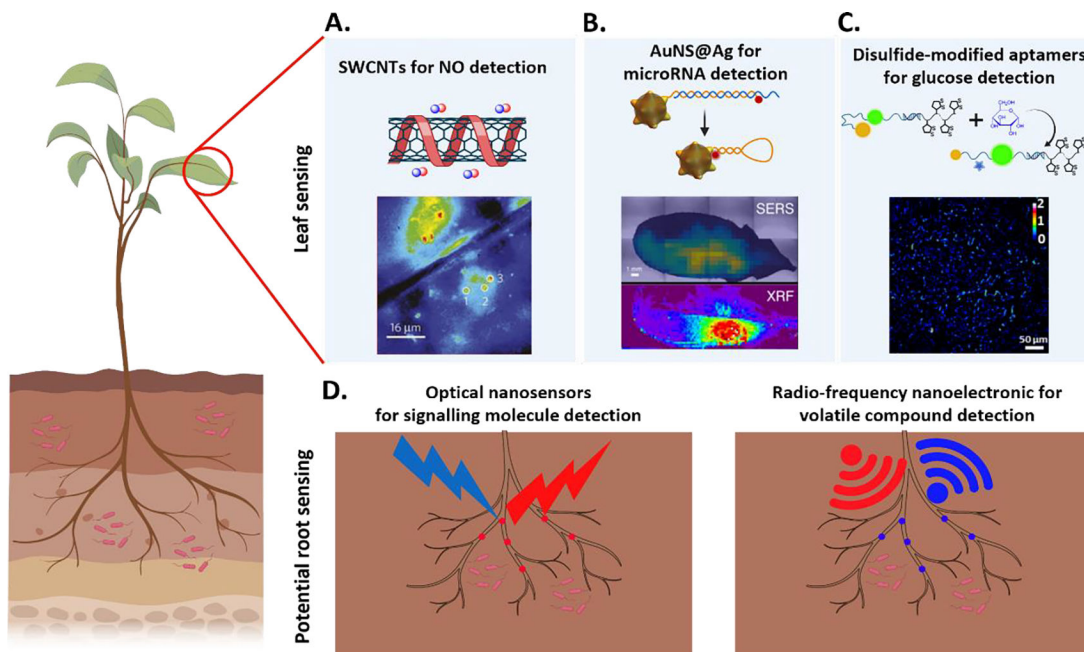


**Figure 1.** Representation of a multimodal imaging technologies for investigating and disentangling the heterogeneity and complexity of rhizosphere-integrated processes and reactions with increasing resolution, from millimeter to nanometer scale.

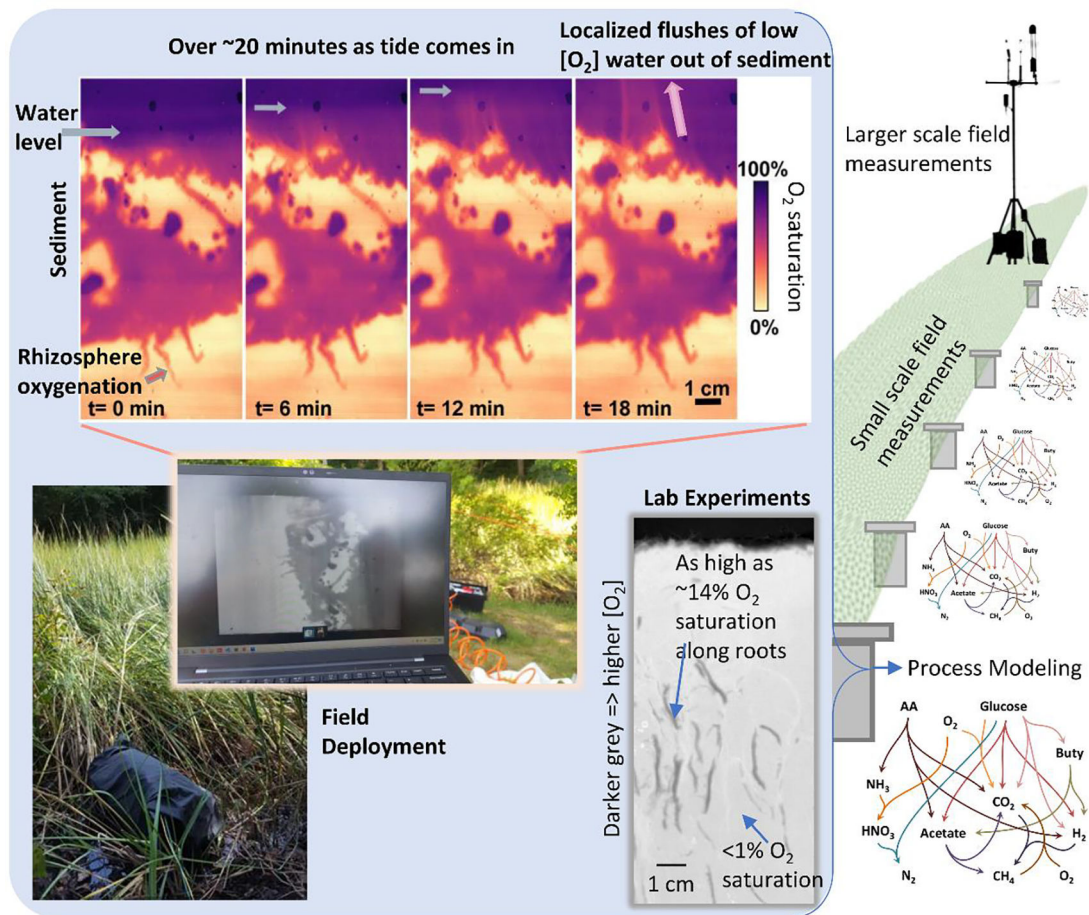


**Figure 2.**

Current cutting-edge and emerging mass spectrometry, imaging, tomography, and omics technologies (left panel) can be coupled with specific platforms like synthetic soil habit micromodels, rhizogrids, and ecotrons (right panel) to study rhizosphere processes at different scales. In many cases, multiple techniques can be applied to a single sample to enrich the resulting dataset and enable evaluation of complex questions (e.g., related to nutrient exchange, organic-inorganic interactions, or spatial organization of processes). Thus, these analytical techniques and platforms can provide critical mechanistic understanding of the rhizosphere processes and reactions associated with carbon fluxes, including rhizodeposition and root exudation.

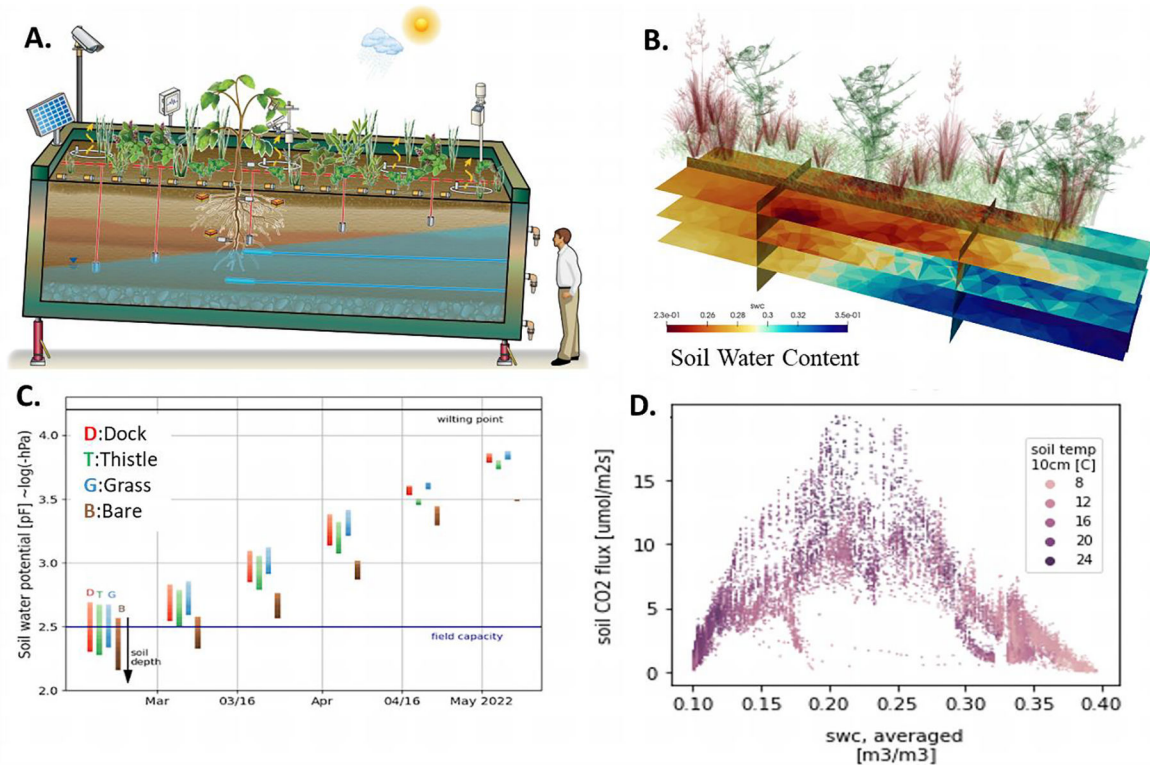


**Figure 3.** Biosensors for plant leaf and root imaging. A. Single-strand, DNA-coated, single-walled, carbon nanotube SWCNTs have been applied for NO detection in Arabidopsis leaves. B. Silver-coated gold nanostars (AuNS@Ag) have been used for microRNA detection in Arabidopsis leaves. C. Disulfide-modified aptamers have been developed for glucose detection in Arabidopsis leaves. D. Optical nanosensors and radio-frequency nanoelectronic could potentially be engineered for plant signaling molecule and volatile compound detection in roots, respectively.



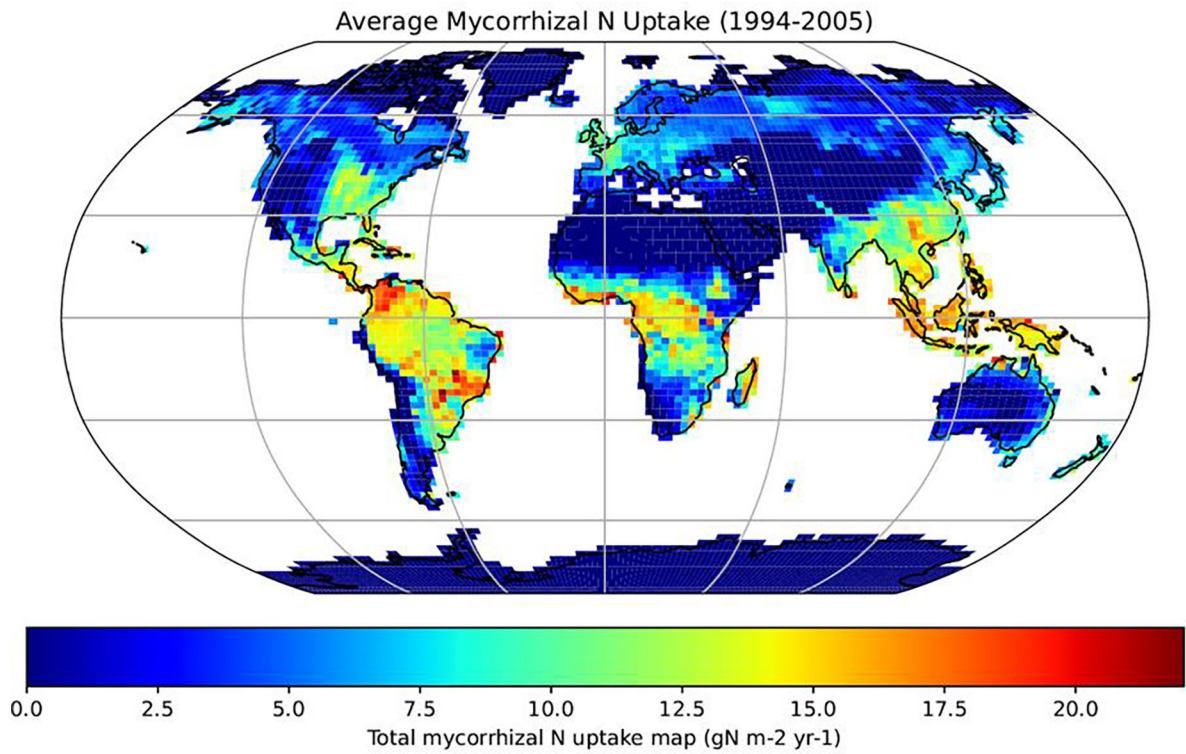
**Figure 4.**

(Left) Grey-scale laboratory and false-color field images of  $[O_2]$  in sediment vegetated with *Sporobolus alterniflorus*, a dominant salt marsh intertidal grass on Atlantic and Gulf U.S. coasts. Quantification via color ratiometric imaging following (Larsen et al., 2011), except the oxygen-sensitive fluorophore was platinum (II) meso-tetra (pentafluorophenyl) porphine (PtTFPP). Horizontal grey arrow on false-colored field images denotes the water level, (Cardon pers. comm). (Right) Coupling of replicated field planar optode measurements with small- and large-scale process modeling, in the footprint of landscape-scale eddy covariance measurements, holds great promise for determining whether and how small-scale spatial heterogeneity in sediment environmental conditions affect aggregate larger scale biogeochemical process.



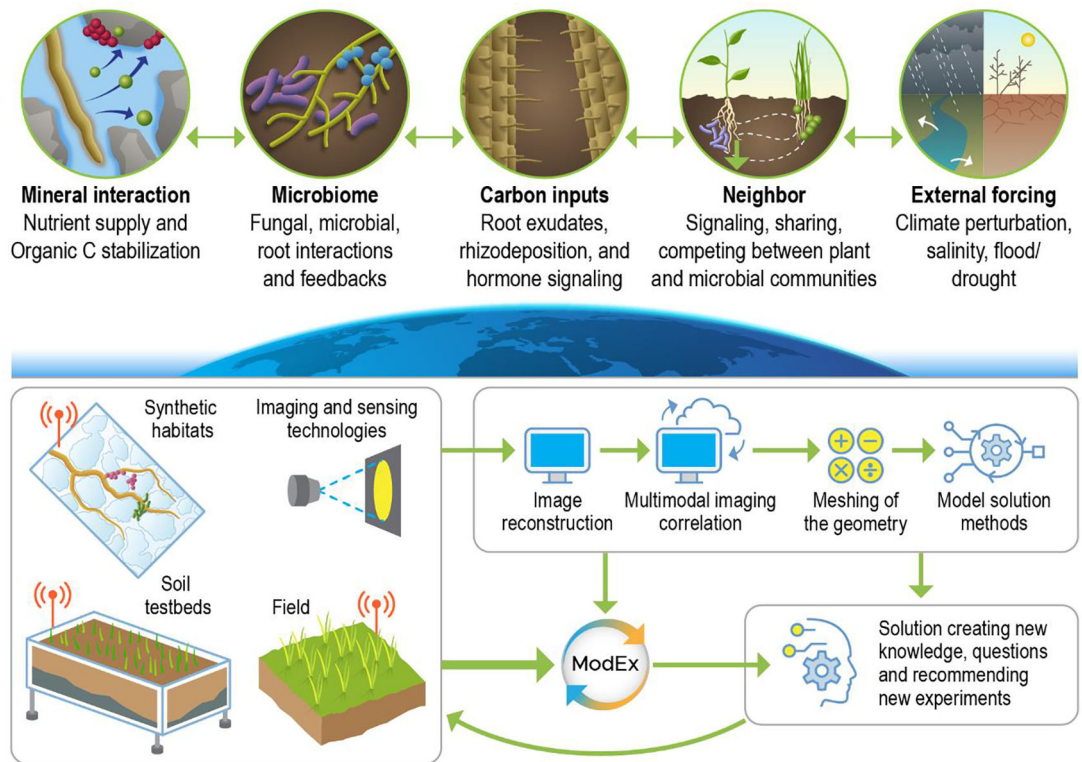
**Figure 5.**

A. Schematic setup of the SMART Soils testbed showing an arrangement of sensors deployed, including load cells, geophysical sensors, soil probes, energy, and mass flow sensors as well as micrometeorological sensors. B. A snapshot of 3D soil water content distribution and plant distribution on the SMART Soils testbed from geophysical and phenocam imaging. C. Evolution of soil water potential and the corresponding stress conditions for multiple plant species, e.g., grass, thistle, dock, or bare ground. D. Correlations between soil water content and soil CO<sub>2</sub> fluxes under different temperature conditions.



**Figure 6.** Plant nitrogen uptake from mycorrhizae at the global scale. This is an example of multiple plant–soil–microbe processes now integrated into global models such as the E3SM Land Model (ELM), including nitrogen and phosphorus uptake partitioned between AM and ECM fungi, direct root uptake of nutrients, and biological nitrogen fixation (Braghiere et al., 2022).





**Figure 7.** Envisioning the future of rhizosphere research by means of building a tiered sensing platform approach. This approach must ensure measurements of rhizosphere complex parameters and promote biosensing connectivity across components (soil, microbes, plant, atmosphere) and scales (omics to ecosystems). Development of advanced data retrieval, image-based modeling, analytical and computing tools, and integration with the ModEx approach should leverage our understanding of small-scale mechanistic processes to the large-scale field, ecosystem, and global sensing. This strategy will provide novel and important mechanistic understanding of key rhizosphere processes including nutrient cycle, root–microbe interactions, root exudation, signaling cascades, plant–plant interactions, and the global-scale impacts of climate change.