CellPress

Special Issue: Unraveling the Secrets of the Rhizosphere

Review

New Methods To Unravel Rhizosphere Processes

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Root-triggered processes (growth, uptake and release of solutes) vary in space and time, and interact with heterogeneous soil microenvironments that provide habitats for (micro)biota on various scales. Despite tremendous progress in method development in the past decades, finding a suitable experimental set-up to investigate processes occurring at the dynamic conjunction of biosphere, hydrosphere, and pedosphere in the close vicinity of active plant roots still represents a major challenge. We discuss recent methodological developments in rhizosphere research with a focus on imaging techniques. We further review established concepts that have been updated with novel techniques, highlighting the need for combinatorial approaches to disentangle rhizosphere processes on relevant scales.

Trends

Visualization of 2D and 3D rhizosphere processes via chemical imaging, microbial imaging, and non-invasive imaging has significantly enhanced our knowledge of rhizosphere processes across a range of scales from cm to sub-µm.

The use of stable isotopes in combination with complementary techniques such as mass spectrometry, high-throughput sequencing, nanoscale secondary ion mass spectrometry, and neutron radiography has great potential to unravel mechanisms of uptake, storage, and translocation of carbon and nutrients at plant–microbe–soil interfaces.

The importance of rhizodeposits in shaping the rhizosphere has been increasingly put forward, leading to the development of novel experimental approaches that enable root exudation sampling from soil-grown plants.

Investigating the Rhizosphere

The conceptual term 'rhizosphere' is commonly used to describe the volume of soil influenced by roots but may also be extended to describe root–soil interfaces that can be separated into ecto-rhizosphere (rhizosphere soil), rhizoplane (root surface), and endo-rhizosphere (inner root). Rhizospheres are complex and highly-dynamic environments where an immense number of interactions between roots, minerals, organic compounds, solutes, gases, and (micro)organisms drive the biogeochemical cycling of elements [1]. The interlinked multicomponent complexity of the rhizosphere requires experimental designs and measuring techniques capable of resolving processes on a wide range of scales (cm to sub-µm). Given the spatial resolution needed to investigate rhizosphere dynamics, and the unpredictability of field conditions, most studies seek to simulate field conditions in semi-artificial experimental set-ups. Instrumental limitations (e.g., immobility) also frequently constrain experiments to the laboratory. However, these can be at least partially overcome by analyzing *in situ* collected plant and soil samples (e.g., undisturbed soil columns of various size). We aim here to take a closer look at recently developed laboratory applications including destructive and non-destructive imaging, isotope and 'metaomic' techniques, to briefly summarize their main advantages, problems, and applicability in the field and to undisturbed field samples (Table 1), and highlight promising combinatorial approaches (Table 2). Developments in root phenotyping and mathematical modeling are not within the scope of this review. Elaborate reviews and methodological summaries of phenotyping and mathematical modeling as well as of well-established experimental set-ups in rhizosphere research can be found elsewhere [2–6].

Accessing the Rhizosphere and Rhizosphere Traits

Sampling Rhizosphere Soil – Growth Designs

Three major designs of growth containers can be distinguished that enable a wide range of investigations and are frequently combined with new applications. Pot/compartment systems (i) [7–9] provide the simplest differentiation between bulk soil and rhizosphere samples (soil and

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roots). Horizontal [10] or vertical [11,12] root-mat rhizoboxes (ii) offer the possibility of rhizosphere sampling in well-defined distance from the root mat (e.g., using thin sectioning [13]) to investigate biogeochemical gradients. Rhizotrons of various sizes (iii) positioned at a 30–45° angle during plant growth [2] are used for investigations of single roots along the root axis and are usually chosen when 2D imaging techniques are applied (see below). While taking rhizosphere samples enables us to analyze biogeochemical changes induced by roots and (micro)biota *ex situ*, increasing focus in the past years has been on visualizing rhizosphere processes *in situ* as well as on investigating rhizodepositions that trigger the latter.

Sampling Root Exudates

Significant advances in the analytical sector allow us to move from targeted compound analysis to root exudate fingerprinting, thereby providing novel in-depth information on the compound composition in its entire complexity that triggers many rhizosphere processes (for more details see the review paper on metabolomics by Bouwmeester and van Dam in this issue). However, the impact of high-end analytical approaches is determined by our ability to identify relevant compounds as well as by our exudate sampling techniques. To avoid alterations in the exudation profile as a result of sorption to the soil matrix and microbial decomposition, the majority of studies on root exudates have been and still are carried out in hydroponic systems. While the altering effect of non-sterile conditions on the exudation profile is well investigated [14], the few comparisons between soil and nutrient solution culture as a growth medium showed a significant, soil-dependent difference in compound concentrations released by roots [9,15]. Although hydroponic set-ups allow (relatively) simple collection of root exudates, the relevance of obtained results remains questionable with respect to field conditions.

Soil-based exudate sampling approaches targeting the entire root system to date include (i) the 'quick and dirty' approach where plants are grown in soil-filled pots followed by careful root washing (soil removal) and hydroponic exudate sampling [9,15]; (ii) rhizobox growth and hydroponic sampling [12]; (iii) a novel method using rhizoboxes in combination with a root exudate-collecting (REC) tool [9,15] where unaltered exudates are repeatedly and non-destructively collected. In addition, (iv) single roots or root segments from plants grown in rhizotrons can be sampled by applying agar sheets, specialized resins, or filter papers (this allows only qualitative and semi-quantitative comparison of compounds [16–18]) or (v) excavation/cuvette-hydroponic-sampling can be applied [19] (see also Table 1). To date a thorough methodological comparison of exudate sampling techniques to evaluate the effect of different experimental set-ups on the quantity and quality of rhizodepositions in the laboratory/greenhouse as well as in the field is still missing.

Sampling Rhizosphere Soil Solution

Micro-suction cups (MSC) are a powerful tool to investigate biogeochemical changes in rhizosphere soil solution at a high spatial resolution (µl/mm) [15,20,21]. However, they are not suited for the collection of unaltered root exudates or exudation rates because immediate interactions with the soil matrix (sorption) as well as microbial activity (the decomposition and release of microbial metabolites) will change compound composition. In a novel approach, microcapillaries coupled with capillary electrophoresis/inductively coupled plasma mass spectrometry (ICP-MS) were successfully used to monitor the effect of organic acids on depleted uranium in the rhizosphere of white lupin on a nl/µm scale (single-cell sampling and analysis, SiCSA) [22]. Recently, microdialysis has been introduced as a novel method to sample diffusive fluxes of metals [23,24] and nitrogen (N) species [25,26] from soil. While the miniature design enables installation with minimal disturbance and highly localized sampling suitable for rhizosphere dimensions, accurate calibration and deduction of external soil solution concentrations is problematic due to the inhomogeneous physical characteristics of the porous soil matrix [26] (Table 1).