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# Rhizosphere Enzyme Dynamics: Spatial and Temporal Regulation by Plant Growth, Root Architecture, and Soil Microbiome Interactions

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## Abstract

The rhizosphere represents one of the biochemically active microenvironments in terrestrial ecosystems, where bidirectional fluxes of carbon compounds, ions, enzymes, and signaling molecules govern the nutritional and structural dynamics of plant-soil systems. Extracellular enzyme activities serve as integrative

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biomarkers of rhizosphere function, reflecting the combined metabolic contributions of plant roots, associated microorganisms, and their residues. This review synthesizes current knowledge on the spatial and temporal regulation of enzyme activities in the rhizosphere, with particular emphasis on the roles of root morphology, plant developmental stage, rhizodeposition chemistry, and abiotic environmental drivers. We examine how root architectural traits including root hair density, lateral root proliferation, and fine root turnover — modulate the volume and intensity of enzymatic hotspots. The roles of key hydrolase and oxidoreductase families (glucosidases, phosphatases, proteases, chitinases, and lactases) are discussed in relation to carbon, nitrogen, phosphorus, and sulfur cycling within the rhizosphere. We further address how soil physicochemical properties (pH, moisture, texture, and sorption capacity) modulate enzyme persistence and diffusion gradients from root surfaces. Advances in two-dimensional zymographic imaging have revealed previously unresolved spatial heterogeneity in enzyme distribution, yet critical gaps remain in understanding the enzyme-level links between root ontogeny and microbial community assembly. Future research priorities include integrating zymography with stable isotope probing, metatranscriptomics, and functional genomics to attribute enzymatic activity to specific biotic sources. Such integrated approaches will be essential for leveraging rhizosphere enzyme ecology in designing sustainable agricultural strategies that minimize fertilizer inputs and enhance nutrient-use efficiency.

*Keywords: Rhizosphere; extracellular enzymes; root morphology; zymography; rhizodeposition; microbial hotspots.*

## 1. Introduction

Soil functions as a heterogeneous matrix in which chemical, physical, and biological processes are tightly interlinked across spatial scales ranging from micrometers to meters. Within this matrix, the zone of soil directly influenced by living root activity the rhizosphere stands out as a region of exceptional biochemical intensity. First conceptualized by Lorenz Hiltner in the early twentieth century, the rhizosphere has since been recognized as a critical interface where plant nutrition, microbial ecology, and soil organic matter dynamics converge (Hartmann et al., 2008). Contemporary research has expanded this view considerably, revealing that the rhizosphere is not a static boundary but a continuously renegotiated zone shaped by the interplay of root growth, exudate chemistry, microbial succession, and soil physical properties.

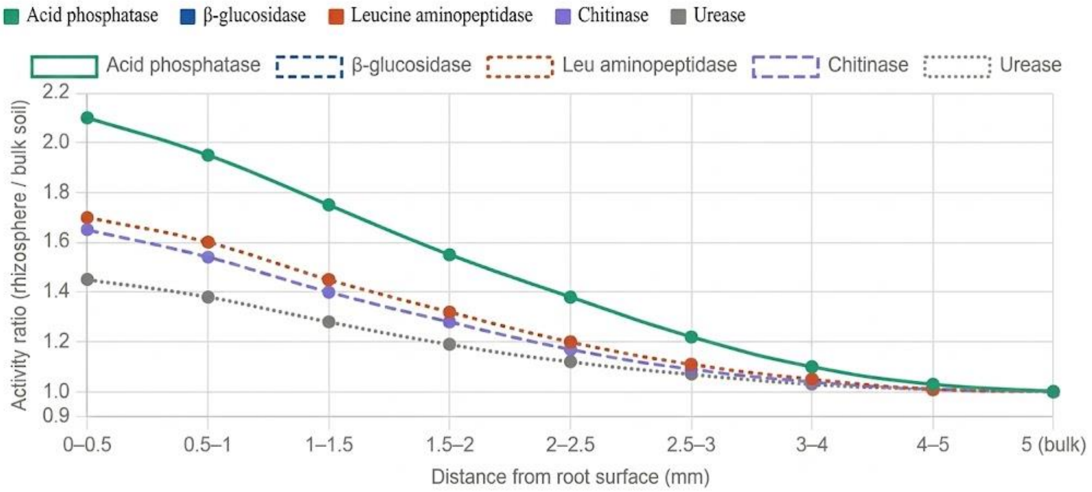

**Fig. 1. Radial enzyme activity gradients from the root surface to bulk soil for five key rhizosphere enzyme classes**

Values represent mean activity (nmol $g^{-1}$ soil $h^{-1}$) normalized to bulk soil at 5 mm. Gradient profiles are based on zymographic measurements from Zea mays and Lens culinaris (Razavi *et al.,* 2016). Shaded bands indicate ± SE. The functional rhizosphere radius (FRR, dashed vertical line) at 1–3 mm marks the zone of significant enzyme enrichment (>30% above bulk soil).

Central to rhizosphere function are extracellular enzymes biocatalysts capable of transforming complex organic polymers into bioavailable monomers. These enzymes originate from multiple sources: actively secreted by living root cells and associated microorganisms, passively released through cell lysis during root senescence, and retained in soil matrices through stabilization on mineral surfaces and organic matter (Burns *et al.*, 2013). Enzyme activities in the rhizosphere consistently exceed those of bulk soil by margins of 30 to 200 percent depending on the enzyme class and plant species under investigation, underscoring the amplifying role of rhizodeposition on belowground biological activity (Kuzyakov & Blagodatskaya, 2015).

Despite substantial progress, a mechanistic understanding of how plant developmental stage and root morphological complexity jointly regulate the spatial and temporal distribution of enzyme activities remains incomplete. Classical approaches relying on destructive soil sampling provide spatially averaged measurements that obscure the microscale heterogeneity intrinsic to the rhizosphere. The emergence of soil zymography a non-destructive, membrane-based imaging technique has transformed this landscape by enabling direct visualization of enzymatic activity patterns in intact root-soil systems (Razavi *et al.*, 2019; Ma *et al.*, 2017). However, translating zymographic observations into process-level understanding requires complementary knowledge of root physiology, exudate biochemistry, and microbial ecology.

This review aims to provide an integrative synthesis of the current understanding of rhizosphere enzyme dynamics, drawing on literature spanning plant physiology, soil biochemistry, microbial ecology, and advanced imaging science. We specifically focus on four interconnected themes: (i) The structural and functional biology of root systems as drivers of enzyme distribution; (ii) The biochemistry of rhizodeposition and its relationship to enzyme induction; (iii) The spatial and temporal patterning of individual enzyme groups; and (iv) The environmental and management factors that modulate these patterns. We conclude by identifying key knowledge gaps and outlining research priorities for advancing the field toward applied outcomes in sustainable crop production.

## 2. Root Architectural Determinants of Rhizosphere Enzyme Activity

### 2.1 Root System Complexity and Functional Zonation

Root systems are architecturally complex organs whose functional attributes extend far beyond mechanical anchorage. The spatial configuration of primary roots, lateral branches, root hairs, and mycorrhizal associations collectively determines the geometry and intensity of soil volume explored by root-derived compounds. Root volume a composite function of root length and diameter ultimately sets the upper boundary for rhizosphere extent and enzyme activity footprint (Fageria, 2005). However, it is the qualitative distribution of root functional zones, rather than total root mass alone, which most strongly predicts enzyme patterns within the rhizosphere.

Functionally, roots are organized into longitudinal zones that differ substantially in their rates of exudate release and enzyme secretion. The root tip, enclosed by the root cap and meristematic zone, is characterized by active cell division and the release of mucilage, border cells, and water-soluble organic acids. Moving apically, the elongation zone exhibits passive exudation of sugars and amino acids through plasma membrane transporters, while the mature zone behind the zone of root hair development is associated with secondary metabolite release and enzyme secretion tied to nutrient mobilization (Sasse *et al.*, 2018). Lateral root emergence zones represent particularly important hotspots, as tissue disruption during lateral primordia breakthrough transiently increases membrane permeability and localizes enzyme activity to specific nodal points along the root axis (Mathesius *et al.*, 2000).

Taxonomic and cultivar-level differences in root architecture translate into measurable differences in rhizosphere enzyme distribution. Species with higher specific root length and root hair density establish broader enzymatic gradients per unit root surface area. Conversely, roots with larger mean diameters tend to be associated with narrower activity gradients, partly because the longer diffusion path from epidermis to bulk soil allows greater enzymatic substrate-product cycling to occur within a restricted zone. These architectural considerations underline the importance of root phenotyping in rhizosphere ecology studies (Waisel & Eshel, 2002).

**Table 1. Extracellular enzyme classes commonly characterised in rhizosphere studies, with substrate specificities, associated nutrient cycles, EC numbers, typical fold-enrichment relative to bulk soil, and primary biological sources. Enrichment values represent ranges compiled from zymographic and bulk assay studies across multiple crop species**

| Enzyme Class | Substrate Specificity | Nutrient Cycle | EC Number | Rhizosphere Enrichment (fold) | Primary Source |
|---|---|---|---|---|---|
| β-Glucosidase | Cellobiose, glucosides | Carbon | 3.2.1.21 | 1.5 – 2.8 | Root exudate + bacteria |
| Acid Phosphatase | Organic P monoesters | Phosphorus | 3.1.3.2 | 1.8 – 3.5 | Root cells + AM fungi |
| Alkaline Phosphatase | Organic P monoesters | Phosphorus | 3.1.3.1 | 1.3 – 2.4 | Bacteria + fungi |
| Leucine Aminopeptidase | N-terminal leucine peptides | Nitrogen | 3.4.11.1 | 1.4 – 2.2 | Bacteria |
| Urease | Urea hydrolysis | Nitrogen | 3.5.1.5 | 1.2 – 2.0 | Bacteria + archaea |
| Chitinase | Chitin (N-acetylglucosamine) | Nitrogen / Carbon | 3.2.1.14 | 1.6 – 2.6 | Fungi + bacteria |
| Arylsulfatase | Aryl sulfate esters | Sulfur | 3.1.6.1 | 1.3 – 2.1 | Bacteria |
| Laccase | Polyphenols, lignin | Carbon | 1.10.3.2 | 1.1 – 1.9 | Fungi |
| Phytase | Phytic acid (inositol hexaphosphate) | Phosphorus | 3.1.3.26 | 1.7 – 3.2 | Bacteria + roots |
| Cellulase (Endoglucanase) | β-1,4-glucan chains | Carbon | 3.2.1.4 | 1.2 – 2.0 | Fungi + bacteria |

*AM = arbuscular mycorrhizal fungi*

## 2.2 Lateral Roots as Enzymatic Amplifiers

Among the different root types comprising a typical root system, lateral roots exert a disproportionate influence on rhizosphere enzyme dynamics. Compared with taproots or primary axes, lateral roots exhibit substantially higher rates of carbon exudation per unit root surface area, generating enzyme activity that may exceed that of the primary root by two to three fold when expressed on an area-normalized basis. This disparity reflects both a higher surface-to-volume ratio in fine lateral roots and their greater metabolic activity per unit dry weight during active growth phases.

**Table 2. Spatial characteristics of rhizosphere enzyme activity gradients in selected crop and grain legume species. Activity ratio indicates the ratio of enzyme activity measured at the root surface relative to bulk soil. Functional rhizosphere radius (FRR) defines the zone in which enzyme activity exceeds bulk soil values by ≥30%**

| Plant Species | Enzyme Class | Activity Ratio (Rhizosphere / Bulk Soil) | Max Enrichment (fold) | Functional Rhizosphere Radius (mm) | Reference | Method |
|---|---|---|---|---|---|---|
| Zea mays (maize) | Acid phosphatase | 1.8–2.5 | 2.9–3.4 | 2–4 | (Razavi et al., 2016) | Zymography |
| Zea mays (maize) | β-Glucosidase | 1.5–2.1 | 2.4–3.0 | 1–3 | (Razavi et al., 2016) | Zymography |
| Lens culinaris (lentil) | Acid phosphatase | 2.1–3.2 | 3.1–4.2 | 1–3 | (Razavi et al., 2016) | Zymography |
| Lens culinaris (lentil) | Leucine aminopeptidase | 1.4–1.9 | 1.8–2.4 | 1–2 | (Razavi et al., 2016) | Zymography |
| Oryza sativa (rice) | Acid phosphatase | 1.7–2.8 | 2.5–3.3 | 2–3 | (Ge et al., 2017) | Bulk assay + model |

| Plant Species | Enzyme Class | Activity Ratio (Rhizosphere / Bulk Soil) | Max Enrichment (fold) | Functional Rhizosphere Radius (mm) | Reference | Method |
|---|---|---|---|---|---|---|
| Oryza sativa (rice) | β-Glucosidase | 1.4–2.0 | 2.0–2.6 | 1–3 | (Ge et al., 2017) | Bulk assay |
| Triticum aestivum (wheat) | Urease | 1.2–1.8 | 1.6–2.2 | 1–2 | (Liu et al., 2017) | Zymography |
| Hordeum vulgare (barley) | Acid phosphatase | 1.9–2.7 | 2.7–3.5 | 2–4 | (Holz et al., 2018) | Zymography |
| Hordeum vulgare (barley)* | Acid phosphatase | 1.2–1.8 | 1.8–2.5 | 1–2 | (Holz et al., 2018) | Zymography |
| Glycine max (soybean) | Leucine aminopeptidase | 1.5–2.1 | 2.0–2.8 | 1–3 | (Ma et al., 2017) | Zymography |

**Root hair-deficient mutant of Hordeum vulgare. DAP = days after planting*

The transition between taproot and lateral root zones corresponds to shifts in the community composition of rhizosphere microorganisms, which in turn feedback on enzyme production patterns. Lateral root emergence temporarily disrupts the soil-root interface, creating physical disturbances that transiently expose intercellular space to soil solution and alter local gradients of oxygen, pH, and dissolved organic carbon. These perturbations select for specific microbial taxa capable of rapid exploitation of labile substrates, leading to localized bursts of hydrolytic enzyme activity that can be captured by zymographic imaging (Razavi *et al*., 2016). The cumulative contribution of lateral root-associated enzyme activity to total rhizosphere enzymatic output represents a major, and often underappreciated, component of belowground carbon cycling.

### 2.3 Root Hairs and Microscale Enzyme Distribution

Root hairs, the tubular extensions of epidermal cells, magnify root surface area by an order of magnitude in many plant species. Beyond their well-established role in water and phosphorus uptake, root hairs contribute significantly to the spatial architecture of enzyme activity in the immediate perirhizosphere. Comparisons between wild-type barley genotypes and root hair-deficient mutants have demonstrated that root hairs substantially expand the radial extent of exudate deposition, shifting the zone of maximal enzyme activity outward from the root surface and broadening the gradient profile (Holz *et al*., 2018).

The mechanism underlying this effect is primarily diffusional: root hairs position the source of exudate release further into the soil matrix, extending the distance over which exudates can diffuse before microbial consumption attenuates their concentration. In soils with high microbial biomass, this extension may be modest (1–2 mm), but in lower-activity soils or under conditions of suboptimal moisture, root hairs can double the effective rhizosphere radius for certain enzyme classes. This architectural amplification is particularly significant for phosphatases and phytases, enzymes directly involved in mobilizing organic phosphorus pools that are often concentrated in the outermost millimeters of the rhizosphere.

## 3. Rhizodeposition Chemistry and Enzyme Induction Mechanisms

### 3.1 Diversity and Dynamics of Root-Derived Carbon Inputs

Rhizodeposition encompasses the full spectrum of organic materials delivered to soil by living root systems, including passively released soluble exudates, mucilage secretions from border cells, sloughed epidermal and cortical cells, volatile organic compounds, and gases including carbon dioxide and ethylene. On a global scale, plants allocate an estimated 20 percent of photosynthetically fixed carbon to belowground pathways, a proportion that varies substantially with plant species, nutrient status, developmental stage, and abiotic stress (Fischer *et al*., 2010; Nguyen, 2003). This continuous carbon subsidy creates energetic conditions that sustain microbial populations in the rhizosphere at densities one to two orders of magnitude above those found in bulk soil.

The composition of root exudates directly determines which enzyme systems are induced in the rhizosphere microbial community. Low molecular weight compounds including organic acids (citrate, malate, oxalate, fumarate), simple sugars (glucose, fructose, sucrose), and amino acids are rapidly consumed by bacteria within minutes of release, providing energy and carbon skeletons that sustain enzyme production. High molecular weight secretions such as polygalacturonans, mucilage polysaccharides, and phenolic glycosides, by contrast, require prior enzymatic depolymerization before microbial assimilation can proceed, creating a co-regulatory loop in which enzyme production governs substrate availability and substrate availability governs enzyme production (Carminati *et al*., 2017; Kalbitz *et al*., 2005).

Spatial and temporal heterogeneity in exudate composition drives corresponding heterogeneity in enzyme distribution along the root axis. Sucrose efflux is concentrated in apical root regions and mediates the induction of glucosidases and sucrases in the immediate vicinity of root tips. Amino acid exudation is associated with emerging lateral root zones, and stimulates protease and aminopeptidase activities in the lateral rhizosphere. Organic acid secretion, regulated in part by ALMT and MATE transporter families at the plasma membrane, is most pronounced under phosphorus- and iron-limited conditions and drives localized increases in acid phosphatase activity in the rhizosphere of cluster roots and phosphorus-efficient genotypes (Mora-Macías *et al*., 2017; Dinkeloo *et al*., 2018).

**Table 3. Temporal dynamics of key rhizosphere enzyme activities across principal plant developmental stages. Activity levels (Low / Moderate / High / Peak / Declining) reflect relative enrichment compared to bulk soil and are expressed as fold-enrichment ranges where quantitative data are available**

| Developmental Stage | Enzyme Class | Activity Level | Enrichment vs. Bulk Soil (fold) | Driving Mechanism | Key Reference |
|---|---|---|---|---|---|
| Early seedling (0–14 DAP) | β-Glucosidase | Moderate | 1.3 – 1.7 | Sucrose exudation, root elongation | (Ge et al., 2017) |
| Early seedling (0–14 DAP) | Leucine aminopeptidase | Moderate | 1.2 – 1.5 | Amino acid exudation at tips | (Ge et al., 2017) |
| Vegetative (15–45 DAP) | Acid phosphatase | Increasing | 1.6 – 2.4 | Lateral root proliferation, P demand | (Razavi et al., 2016) |
| Vegetative (15–45 DAP) | β-Glucosidase | Moderate–High | 1.5 – 2.1 | Biomass expansion, exudate flux increase | (Ge et al., 2017) |
| Reproductive / Flowering | Acid phosphatase | Peak | 2.5 – 3.5 | Maximal P demand for seed development | (Ge et al., 2017) |
| Reproductive / Flowering | Leucine aminopeptidase | Peak | 2.0 – 2.8 | N demand for protein biosynthesis | (Ren et al., 2016) |
| Reproductive / Flowering | Urease | High | 1.7 – 2.3 | N recycling from senescent tissue | (Liu et al., 2017) |
| Grain fill / Post-anthesis | Acid phosphatase | Declining | 1.8 – 2.5 | Partial redirection of C to seeds | (Ma et al., 2017) |
| Senescence | Chitinase | Increasing | 1.5 – 2.2 | Fungal biomass decomposition | (Burns et al., 2013) |
| Senescence | β-Glucosidase | Low | 1.1 – 1.5 | Root cell lysis, low active secretion | (Ge et al., 2017) |

*DAP = days after planting. L-AP = leucine aminopeptidase*

## 3.2 Membrane Transporters and the Regulation of Exudate Release

For several decades, root exudation was conceptualized primarily as a passive process driven by concentration gradients and membrane permeability. Contemporary molecular evidence has substantially revised this view, demonstrating that many exudate components are actively transported across root plasma membranes by specific protein families whose expression is subject to transcriptional regulation by nutrient availability and developmental cues. This active control mechanism confers on plants a capacity to modulate the composition and spatial distribution of exudates in response to soil conditions, with direct consequences for rhizosphere enzyme induction (Sasse *et al*., 2018).

Key transporter families mediating exudate efflux include the SWEET family for sugars, UMAMIT and CAT transporters for amino acids, and ALMT (aluminum-activated malate transporter) and MATE (multidrug and toxin extrusion) families for organic acid secretion. The ABC (ATP-binding cassette) transporters constitute a distinct category of active efflux systems directly coupled to ATP hydrolysis, enabling energy-dependent export of flavonoids and secondary metabolites implicated in microbiome assembly and nodulation signaling (Dinkeloo *et al*., 2018; Yang & Hinner, 2015). Crucially, the spatial expression patterns of these transporters along the root axis determine which zones release which compounds, thereby establishing the blueprint for enzyme induction patterns in the surrounding soil.

### 3.3 Priming Effects and Co-metabolic Enzyme Synthesis

The introduction of labile carbon substrates into the rhizosphere triggers a cascade of microbial responses collectively termed the rhizosphere priming effect (RPE). When easily mineralizable compounds such as sugars and amino acids increase in soil solution, microbial communities accelerate their metabolic activity, increasing both the synthesis of intracellular enzymes and the secretion of extracellular hydrolases into the surrounding soil matrix. This enhanced enzymatic capacity facilitates the co-metabolic decomposition of recalcitrant soil organic matter (SOM) components — humic acids, lignocellulose, and microbial necromass — that would otherwise turn over slowly in the absence of rhizosphere priming (Kuzyakov *et al*., 2000; Haichar *et al*., 2014).

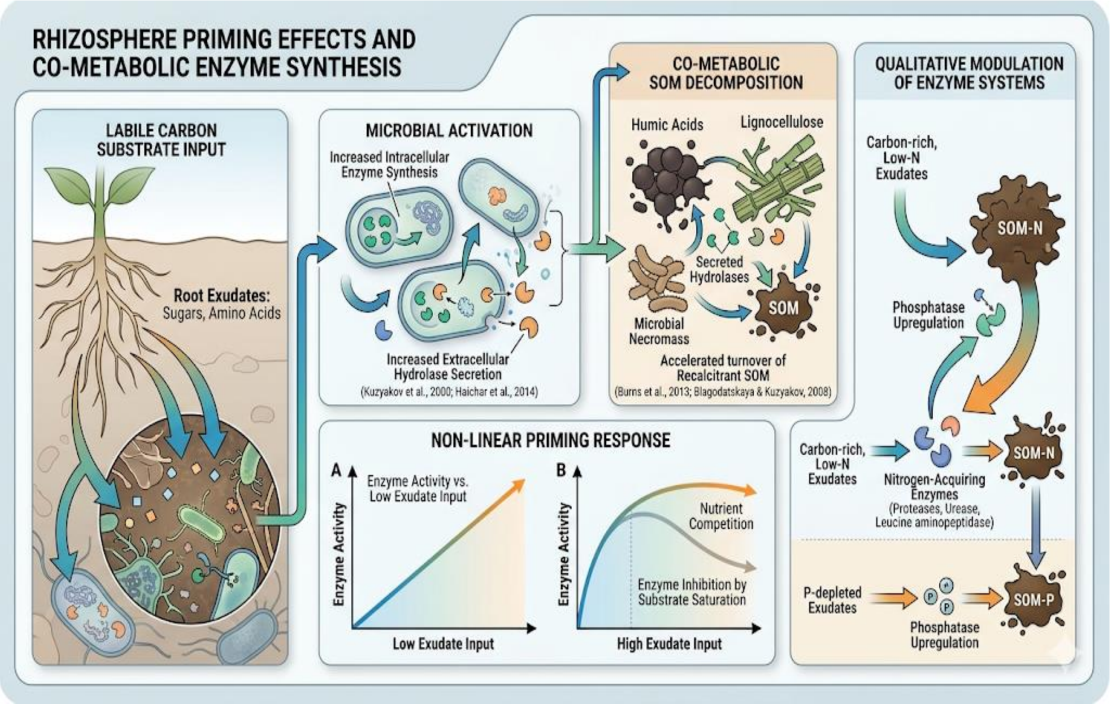

**Fig. 2. Rhizosphere priming effects and co-metabolic enzymes synthesis**

The relationship between priming magnitude and enzyme production is not strictly linear. Under low exudate input conditions, enzyme activity may increase proportionally with substrate availability. At higher inputs, however, microbial nutrient competition and enzyme inhibition by substrate saturation introduce non-linearities that complicate prediction of enzyme responses from exudate flux measurements alone. Furthermore, the qualitative composition of exudates modulates which enzyme systems are preferentially induced: carbon-rich exudates with low nitrogen content tend to stimulate nitrogen-acquiring enzymes (proteases, leucine aminopeptidase, urease), while phosphorus-depleted exudate conditions drive phosphatase upregulation in root-associated microbial communities (Burns *et al*., 2013; Blagodatskaya & Kuzyakov, 2008).

## 4. Spatial and Temporal Patterns of Rhizosphere Enzyme Activity

### 4.1 Radial Gradients from Root Surface to Bulk Soil

Enzyme activity in the rhizosphere is fundamentally a gradient phenomenon, declining continuously from the root surface outward toward bulk soil in a pattern that integrates the competing influences of enzyme source strength, diffusional transport, microbial consumption, and soil sorption. For most enzyme classes that have been characterized, the transition from rhizosphere to bulk soil enzymatic activity follows a sigmoidal curve when plotted against distance from the root surface. Activity at the root surface typically exceeds bulk soil values by a factor of 1.3 to 2.0 for enzymes such as leucine aminopeptidase, phosphatase, glucosidase, and chitinase, with the precise magnitude depending on plant species and soil properties (Razavi *et al.*, 2016).

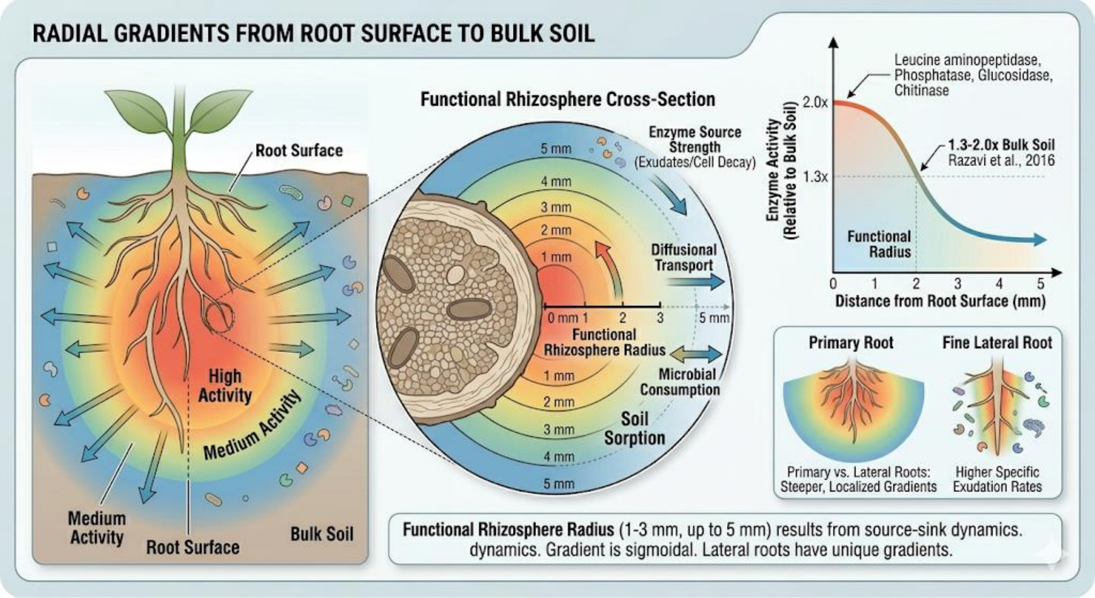

**Fig. 3. Radial gradients from root surface to bulk soil**

The radial extent of significant enzyme enrichment above bulk soil values — the functional rhizosphere radius averages between 1 and 3 mm for most commonly studied enzyme groups, though this range can extend to 4 or 5 mm under conditions of high soil moisture, elevated temperature, or intense exudation during reproductive growth stages. Fine lateral roots exhibit steeper and more localized gradients than primary roots due to their smaller diameter and higher specific exudation rates, a finding with important implications for modeling rhizosphere enzyme contributions to whole-soil biogeochemical processes.

### 4.2 Axial Variation along the Root Axis

In addition to radial gradients, enzyme activity displays pronounced variation along the longitudinal axis of individual roots. Maximum activity is consistently observed in the zone immediately proximal to the root tip, encompassing the root cap, meristematic zone, and early elongation zone. Glucosidase and phosphatase activities at the root tip commonly exceed activities measured 6 to 8 centimeters from the tip by a factor of two to four, reflecting the concentration of metabolically active cells and the intensity of exudate release in this region (Razavi *et al.*, 2016; Ge *et al.,* 2017).

Moving toward the root base, enzyme activity generally declines, though secondary peaks are frequently observed at sites of lateral root emergence, root hair zones, and regions of active nutrient uptake. These axial hotspots correspond to anatomically distinct zones where membrane transport activity, oxygen consumption, and carbon release rates differ markedly from surrounding tissue. The temporal persistence of these hotspots changes with root age: young root segments maintain high activity associated with active metabolism, while older suberized root sections shift toward lower enzyme activity driven primarily by microbial communities colonizing senescent root tissue rather than by direct root secretion.

**Table 4. Environmental and soil physicochemical factors regulating rhizosphere enzyme activity, with documented activity responses and underlying mechanisms**

| Environmental Factor | Condition / Level | Affected Enzyme(s) | Activity Response | Mechanism | Key Reference |
|---|---|---|---|---|---|
| Soil pH | 4.5 – 5.5 (acid) | Acid phosphatase | Increase (+40–90%) | Proton release under NH4+ nutrition acidifies rhizosphere | (Hinsinger et al., 2009) |
| Soil pH | 4.5 – 5.5 (acid) | Alkaline phosphatase | Decrease (–20–50%) | Conformational inactivation at low pH | (Marschner et al., 1982) |
| Soil pH | 6.5 – 7.5 (neutral–alkaline) | Laccase / Phenol oxidase | Increase (+30–60%) | Optimal conformation at near-neutral pH | (Burns et al., 2013) |
| Soil moisture (θ) | 0.3 – 0.5 $m^3 m^{-3}$ | All hydrolases | Maximum activity | Optimal diffusion-reaction balance | (Olesen et al., 2000) |
| Soil moisture (θ) | < 0.15 $m^3 m^{-3}$ | Phosphatase | Compressed gradient, concentrated | Mucilage hydrogels preserve microsites | (Holz et al., 2018) |
| Temperature | 25 – 35 °C | All enzyme classes | Optimal (100%) | Michaelis–Menten kinetics peak | (Ge et al., 2017) |
| Temperature | > 40 °C | All enzyme classes | Sharp decline (–30–70%) | Denaturation + microbial community shift | (Allison & Treseder, 2008) |
| Elevated CO2 (> 550 ppm) | Increased photosynthate flux | Proteases, L-aminopeptidase | Increase (+20–45%) | C enrichment drives N-mining enzymes | (Burns et al., 2013) |
| Clay content (> 30%) | High adsorption capacity | All classes | Stabilized, narrow gradient | Enzyme immobilization on mineral surfaces | (Kalbitz et al., 2005) |
| SOM content (> 5%) | High stabilization potential | Glucosidase, phosphatase | Elevated background, prolonged persistence | Organo-mineral enzyme complexes | (Burns et al., 2013) |

*SOM = soil organic matter. FRR = functional rhizosphere radius. θ = volumetric soil water content. All percentage changes are relative to activity at optimal or reference conditions unless otherwise stated*

### 4.3 Temporal Dynamics across Plant Developmental Stages

Plant development imposes a strong temporal structure on rhizosphere enzyme activity, with distinct patterns associated with vegetative growth, reproductive development, and post-reproductive senescence. During early vegetative stages, rapidly elongating root systems establish new soil contacts and release primarily simple sugars and amino acids, inducing corresponding increases in glucosidases and peptidases. Root growth rate during this phase is the dominant driver of enzyme activity expansion, as new soil volumes are colonized and primed by exudate deposition.

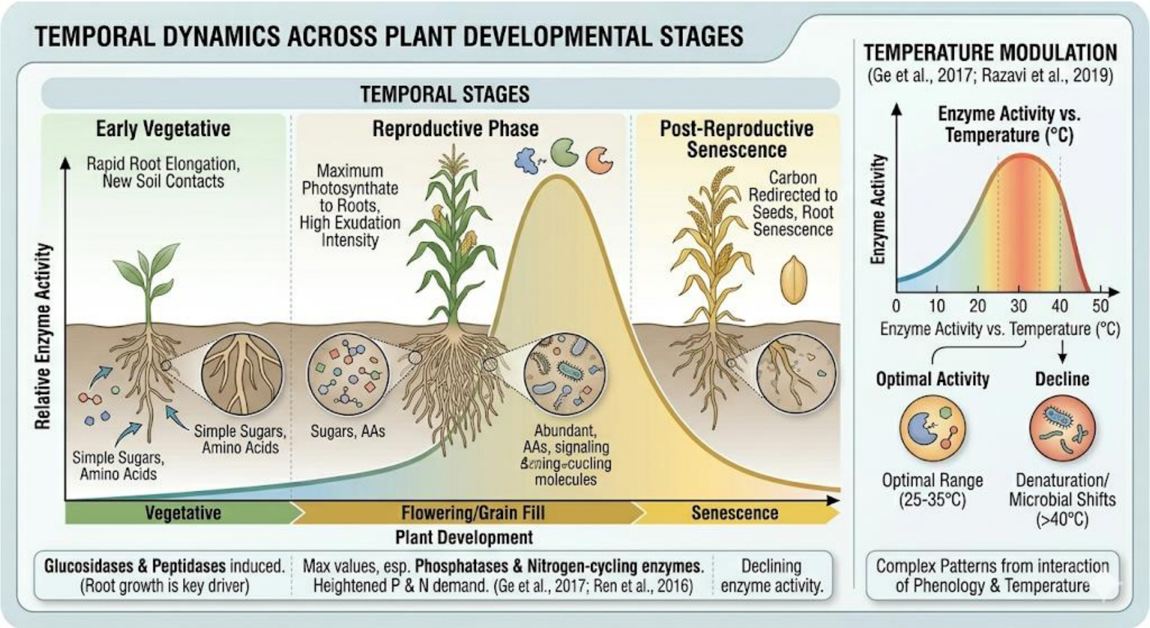

**Fig. 4. Thermal dynamics across plant development stages**

Enzyme activity reaches maximum values during the reproductive phase, when photosynthate allocation to belowground systems peaks and root exudation intensity is at its highest. Studies on maize, rice, and various legume species consistently report that rhizosphere enzyme activities during flowering and grain fill exceed those at vegetative stages, and this elevation is most pronounced for phosphatases and nitrogen-cycling enzymes, reflecting the heightened demand for phosphorus and nitrogen during reproductive organ development (Ge *et al.,* 2017; Ren *et al.,* 2016). Following the reproductive peak, enzyme activity declines progressively as carbon investment in root exudation is redirected toward seed filling and root senescence accelerates.

Temperature plays a key role in modulating the temporal trajectory of enzyme activity within a growing season, independently of plant developmental stage. Most rhizosphere enzymes display optimal activity between 25 and 35 degrees Celsius, with sharp declines above 40 degrees that reflect both enzyme denaturation and shifts in microbial community composition. The interaction between phenological development and seasonal temperature variation creates complex temporal patterns that require integrated analysis of plant physiology and edaphic conditions to fully interpret (Ge *et al*., 2017; Razavi *et al.,* 2019).

## 5. Environmental and Soil Physicochemical Controls on Rhizosphere Enzymes

### 5.1 Soil pH and Enzyme Stability

Soil pH exerts pervasive control over enzyme activity in the rhizosphere through direct effects on enzyme conformation and substrate accessibility, and indirect effects on microbial community structure, nutrient speciation, and mineral surface chemistry. Root-driven pH modification — through selective release of protons or hydroxyl ions depending on the nitrogen source available — routinely generates pH gradients of 0.5 to 1.0 unit between the root surface and surrounding bulk soil, a gradient sufficient to substantially shift the activity of pH-sensitive enzymes such as phosphatases and phenol oxidases (Hinsinger *et al.,* 2009; Marschner *et al.,* 1982).

Under ammonium-dominated nitrogen nutrition, roots release excess protons to maintain electrochemical neutrality, acidifying the proximal rhizosphere and favoring acid phosphatase activity while suppressing alkaline phosphatase. Under nitrate nutrition, hydroxyl release raises rhizosphere pH, shifting the enzymatic balance toward neutral and alkaline phosphatase forms. These pH-mediated shifts in enzyme activity distribution represent an underappreciated mechanism by which nitrogen management practices influence phosphorus bioavailability in agricultural soils a connection with direct implications for fertilizer management strategies.

## 5.2 Soil Moisture and Diffusional Control

Water content in the rhizosphere governs enzyme dynamics through two primary mechanisms: the physical control of molecular diffusion, which determines how far enzyme molecules and their substrates migrate from points of release; and the biological control of microbial activity, which modulates enzyme production rates and turnover. Diffusivity in soil follows an approximately square-law relationship with volumetric water content, such that even modest reductions in moisture substantially curtail the radial extent of enzyme gradients from root surfaces (Olesen *et al*., 2000).

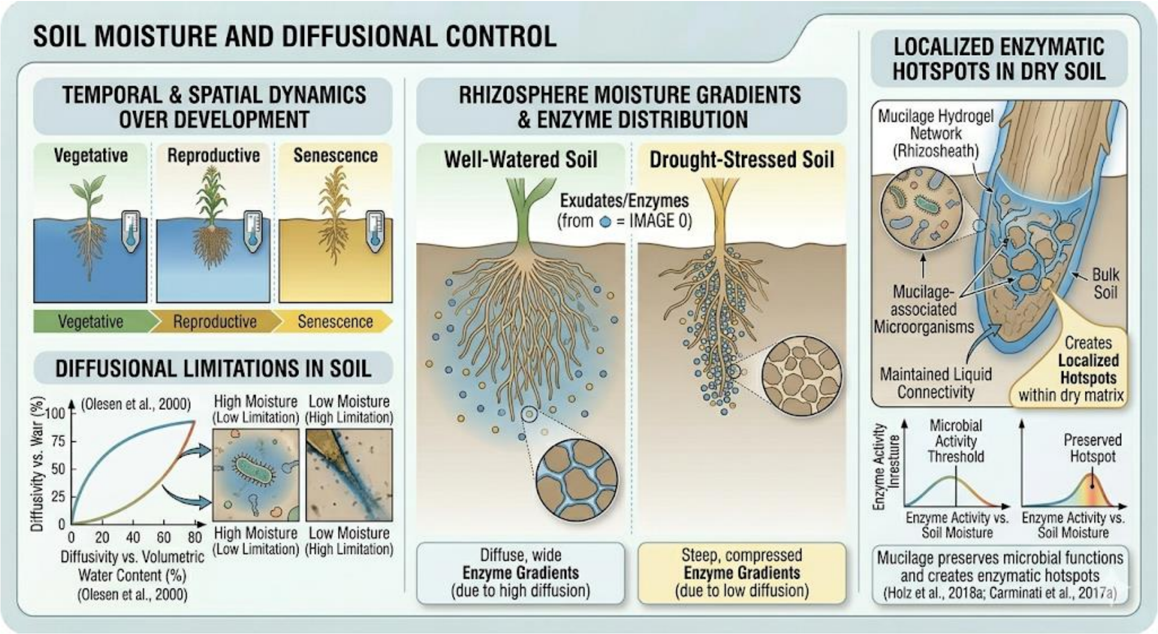

**Fig. 5. Soil Moisture and diffusion control**

Under water-limiting conditions, root-derived exudates concentrate near the root surface, creating steep but spatially compressed enzyme gradients. Mucilage released by root tips plays a disproportionately important role under drought conditions, forming hydrogel networks around roots that maintain liquid connectivity between root surface and soil particles even as bulk soil dries. The enzymatic functions of mucilage-associated microorganisms are thereby preserved in microsites where moisture content remains above microbial activity thresholds, creating localized enzymatic hotspots within an otherwise quiescent dry soil matrix (Holz *et al.,* 2018; Carminati *et al.,* 2017).

## 5.3 Soil Organic Matter, Clay Minerals, and Enzyme Stabilization

A critical, and often overlooked, dimension of rhizosphere enzyme ecology is the fate of secreted enzymes once they encounter the soil mineral-organic matrix. Enzymes released into soil solution rapidly encounter surfaces bearing variable charges clay mineral surfaces, iron and aluminum oxide coatings, and organic matter functional groups to which they adsorb with varying affinity and retention. This stabilization process simultaneously protects enzymes from protease-mediated degradation and suppresses their catalytic activity through conformational change and substrate diffusion limitation (Kalbitz *et al*., 2005).

Soils high in clay, sesquioxide content, or organic matter have proportionally greater enzyme-stabilizing capacity, which tends to narrow apparent rhizosphere extent by retaining enzyme molecules close to their point of release. Conversely, coarse-textured, low-organic-matter soils present weaker stabilization potential, allowing enzymes to diffuse further before deactivation or degradation. These textural dependencies create a soil-type dimension to rhizosphere enzyme ecology that is seldom incorporated into comparative studies but is essential for cross-site extrapolation of experimental findings (Burns *et al*., 2013).

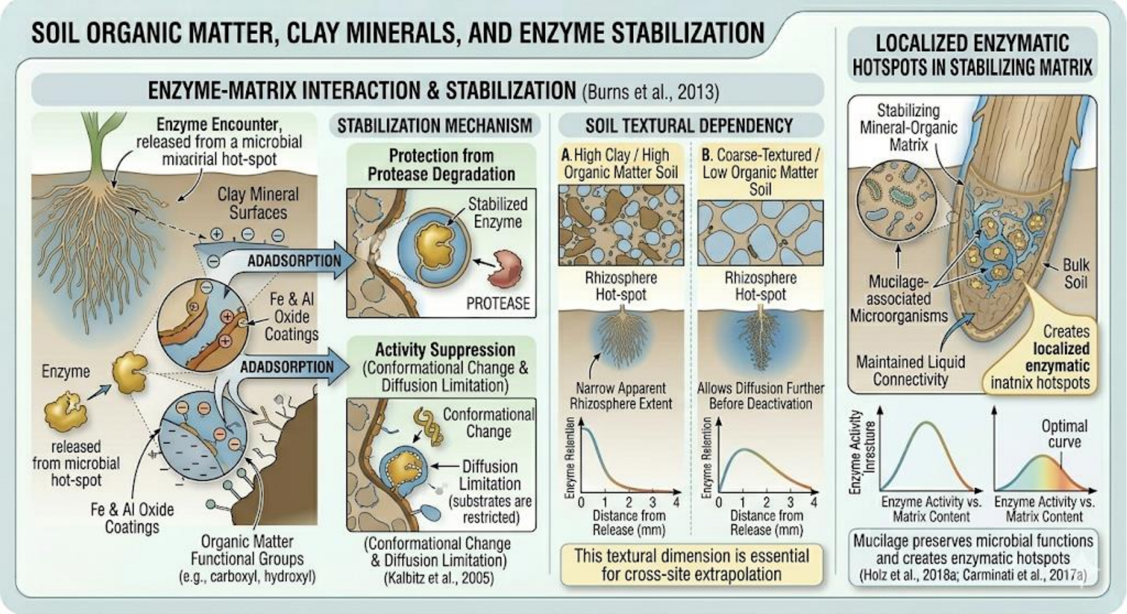

**Fig. 6. Soil Organic matter, clay minerals and enzyme stabilization**

**Table 5. Root architectural traits influencing the spatial distribution and intensity of rhizosphere enzyme activity. Gradient geometry refers to the radial extent and steepness of the enzyme activity gradient from the root surface to bulk soil**

| Root Architectural Trait | Mechanism of Enzyme Influence | Effect on Gradient Geometry | Primary Enzymes Affected | Key Reference | Evidence Type |
|---|---|---|---|---|---|
| Root hair length | Extends exudate source beyond epidermal surface | Broad gradient, FRR extended 1–2 mm | Phosphatase, phytase | (Holz et al., 2018) | Barley mutant comparison |
| Root hair density | Amplifies surface-area-to-volume ratio | Higher activity per unit root mass | Phosphatase, glucosidase | (Holz et al., 2018) | Wild-type vs. mutant |
| Lateral root density | Produces enzymatic hotspots at emergence zones | Localized 2–3× peaks along root axis | All hydrolases | (Razavi et al., 2016) | 2-D zymography |
| Specific root length (SRL) | High SRL → greater soil exploration per unit mass | Broader spatial enzyme coverage | Phosphatase, aminopeptidase | (Waisel & Eshel, 2002) | Interspecific comparison |
| Root diameter | Large diameter → longer diffusion path | Narrower, steeper gradients | Glucosidase | (Razavi et al., 2016) | Maize vs. lentil |
| Cluster / proteoid roots | Mass exudation of citrate/malate under P stress | Extremely high local phosphatase activity | Acid phosphatase | (Mora-Macías et al., 2017) | White lupin, P-deficient |
| Mycorrhizal hyphal network | Extends functional reach 1–10 cm beyond root | Amplified mycorrhizosphere enzyme zone | Phosphatase, glucosidase, cellulase | (Harvey et al., 2002) | AM fungi colonized roots |
| Fine root turnover | Cell lysis releases enzymes passively | Transient enzyme flush at senescence zones | Urease, leucine aminopeptidase | (Burns et al., 2013) | 13C pulse-chase studies |

*SRL = specific root length. FRR = functional rhizosphere radius. AM = arbuscular mycorrhizal*

### 5.4 Elevated Carbon Dioxide and Climate Change Interactions

Atmospheric carbon dioxide concentration exerts upstream control over rhizosphere enzyme dynamics by modulating the photosynthetic carbon supply available for rhizodeposition. Under elevated CO2 conditions, many plant species exhibit increased rates of photosynthesis and greater allocation of assimilates to root systems, translating into higher rates of exudate release and correspondingly elevated enzyme activities in the rhizosphere. These effects are mediated in part through enhanced sucrose loading into phloem and increased root growth rates, both of which amplify the carbon flux driving microbial enzyme synthesis belowground.

However, the relationship between elevated $CO_2$ and rhizosphere enzyme activity is not uniform across enzyme classes or plant functional types. Nitrogen-acquiring enzymes, including proteases and leucine amino peptidase, may increase disproportionately under elevated $CO_2$ as plants attempt to maintain internal carbon-to-nitrogen ratios against the backdrop of carbon enrichment without commensurate nitrogen supply. This enzymatic response to $CO_2$ enrichment represents a homeostatic mechanism by which rhizosphere microbiomes buffer against stoichiometric imbalances imposed by rising atmospheric $CO_2$ levels a finding with significant implications for projecting soil nitrogen cycling under future climate scenarios.

## 6. Methodological Advances: Soil Zymography and Complementary Techniques

### 6.1 Principles and Applications of Soil Zymography

Soil zymography is a two-dimensional imaging technique that enables the spatially resolved visualization of enzyme activity in intact soil-root systems. The method involves placing fluorescent substrate-impregnated membranes in direct contact with a freshly exposed soil-root section, typically within a rhizobox or root window system, and incubating for a defined period before scanning the membrane under ultraviolet or visible excitation to detect the fluorescent hydrolysis products generated by active enzymes. The resulting images capture the spatial heterogeneity of enzyme activity at millimeter-to-submillimeter resolution, revealing hotspots, gradients, and root-associated patterns invisible to conventional bulk assay methods (Razavi *et al.,* 2019; Ma *et al.,* 2017).

The range of enzyme classes amenable to zymographic analysis has expanded considerably since the technique's initial application to soil systems. Current protocols support imaging of phosphatases (using 4-methylumbelliferyl phosphate substrates), glucosidases (using 4-methylumbelliferyl glucoside), leucine aminopeptidase (using l-leucine-7-amido-4-methylcoumarin), chitinase (using 4-methylumbelliferyl N-acetyl-beta-d-glucosaminide), and cellulase activities, among others. Multi-enzyme zymography, combining substrates with spectrally distinct fluorophores, enables simultaneous visualization of two or more enzyme activities in the same membrane, allowing functional comparisons within a single experimental unit (Liu *et al*., 2017).

Critical methodological considerations in zymography include membrane contact uniformity, substrate diffusion during incubation, and the calibration of fluorescence intensity to enzyme activity units. Imperfect membrane-soil contact leads to spatially inconsistent substrate delivery and complicates quantitative interpretation of activity maps. Recent methodological reviews have recommended optimization of incubation time, substrate concentration, membrane type, and soil moisture conditions to minimize these artifacts and maximize reproducibility across laboratories (Razavi *et al*., 2019).

### 6.2 Stable Isotope Approaches for Source Attribution

A fundamental limitation of zymography and other enzyme activity assays is their inability to distinguish the biotic sources of measured activity. Roots, bacteria, fungi, and archaea all contribute enzymes to the rhizosphere pool, yet their relative contributions vary with plant species, developmental stage, and soil conditions. Stable isotope probing approaches, including 13C- and 15N-labeling of plants followed by nanoscale secondary ion mass spectrometry (NanoSIMS) or density-gradient centrifugation of microbial DNA, offer complementary capabilities for attributing carbon flux and enzyme gene expression to specific microbial populations in defined rhizosphere compartments (Oburger & Jones, 2018).

Combining zymographic images with isotopically resolved microbial activity maps permits spatial coregistration of enzyme activity hotspots with the identity and metabolic state of the microorganisms responsible. Such integrated datasets are beginning to reveal that enzyme activity hotspots do not always coincide with zones of highest microbial biomass, suggesting that activity per cell — reflecting enzyme induction rates and secretion capacity — varies substantially across the rhizosphere microbiome. This decoupling of biomass from activity has important implications for process modeling, as it invalidates assumptions embedded in many current biogeochemical frameworks that equate microbial abundance with functional output.

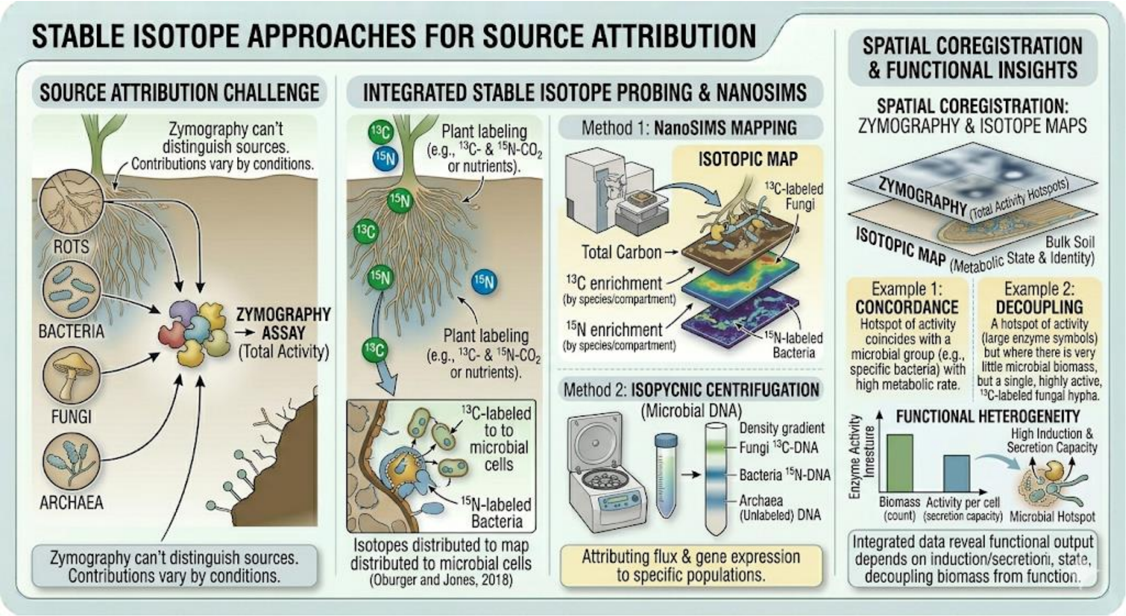

**Fig. 7. Stable isotope approaches for source attribution**

**Table 6. Comparative overview of methodologies used for characterising extracellular enzyme activities in rhizosphere research. Spatial resolution refers to the smallest discernible spatial unit of enzyme activity measurement**

| **Methodology** | **Detection Principle** | **Sample Type** | **Spatial Resolution** | **Advantages** | **Limitations** |
|---|---|---|---|---|---|
| Conventional bulk assay | Colorimetric / fluorometric | Whole soil volume | Low (site average only) | Simple, cheap, well-validated | Cannot resolve spatial heterogeneity; artefacts from mixing |
| Soil zymography | UV fluorescence imaging | Intact root-soil section | High (mm scale) | Non-destructive, spatial maps, multi-enzyme capable | Membrane contact artefacts; semi-quantitative |
| Microplate fluorometry | MUB-substrate fluorescence | Soil slurry | Low–moderate | High throughput; simultaneous multi-enzyme | Loses spatial information; moisture artefacts |
| Stable isotope probing (SIP) | 13C / 15N labelling + NanoSIMS | Root zone to micro-site | Very high (µm scale) | Source attribution of activity to taxa | Technically complex; expensive; destructive |
| Metatranscriptomics | RNA sequencing of soil | Rhizosphere bulk | Community-level | Links gene expression to enzyme function | No direct activity measurement; RNA extraction bias |

| Methodology | Detection Principle | Sample Type | Spatial Resolution | Advantages | Limitations |
|---|---|---|---|---|---|
| Environmental proteomics | LC-MS/MS protein identification | Rhizosphere soil | Protein-level | Identifies actual secreted enzymes by species | Soil matrix inhibits extraction; low protein yield |
| Microfluidic rhizosphere chip | Micro-sensor arrays | Controlled microenvironment | Very high (µm–mm) | Real-time monitoring; replicated experimental control | Low ecological realism; not field-applicable yet |
| Laser-capture microdissection | Microscopy + RNA/protein extraction | Single root zone section | µm resolution | Zone-specific molecular profiling | Destructive; small sample mass; technically demanding |

*LC-MS/MS = liquid chromatography tandem mass spectrometry; NanoSIMS = nanoscale secondary ion mass spectrometry; PLFA = phospholipid fatty acid analysis; MUB = 4-methylumbelliferyl*

## 6.3 Emerging Omics and Functional Genomics Approaches

Advances in metatranscriptomics and environmental proteomics are enabling the direct characterization of enzyme-encoding gene expression and protein accumulation in rhizosphere samples at unprecedented taxonomic and functional resolution. Metatranscriptomics analysis of rhizosphere RNA extracts can reveal which microbial taxa are actively transcribing genes for specific enzyme families in response to root exudate gradients, providing mechanistic insight into the induction cascades linking plant carbon release to microbial enzyme synthesis. Environmental proteomic analysis of rhizosphere soil, though technically challenging due to co-extraction of inhibitory soil compounds, has successfully identified hundreds of extracellular enzyme proteins of both plant and microbial origin, enabling enzyme source attribution without the need for physical separation of soil fractions.

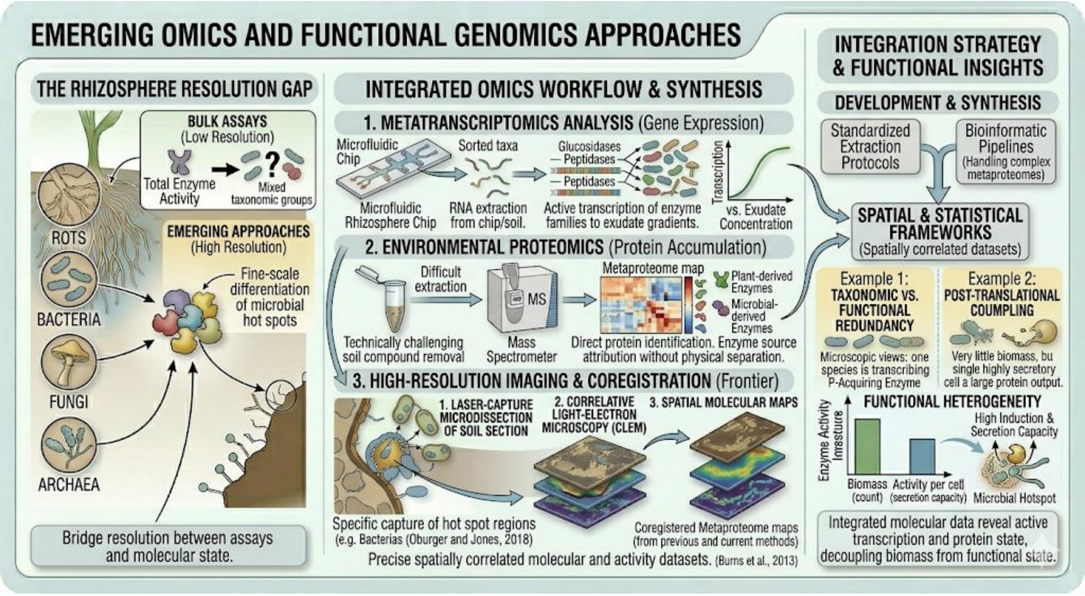

**Fig. 8. Emerging omics and functional genomics approaches**

Integration of these molecular approaches with high-resolution spatial imaging represents the frontier of rhizosphere enzyme ecology. Microfluidic rhizosphere chips, laser-capture microdissection of soil sections, and correlative light-electron microscopy are among the emerging tools that promise to bridge the resolution gap between bulk biochemical assays and molecular characterization. Successful integration of these technologies will require coordinated development of standardized extraction protocols, bioinformatic pipelines capable of handling complex soil metaproteomes, and statistical frameworks for analyzing spatially correlated molecular and activity datasets.

## 7. Plant-Microbe Interactions as Modulators of Rhizosphere Enzyme Ecology

### 7.1 Mycorrhizal Associations and Enzyme Amplification

Mycorrhizal fungi, forming symbiotic associations with the roots of approximately 80 percent of terrestrial plant species, fundamentally extend the functional reach of root systems into soil domains inaccessible to root hairs and fine roots. Arbuscular mycorrhizal (AM) hyphae, typically 2 to 7 micrometers in diameter, penetrate soil pores too small for root entry and access organic matter fractions physically protected from root-derived exudates. The secretion of phosphatases, glucosidases, and cellulases by mycorrhizal hyphae and their associated bacteria creates enzymatic activity zones extending millimeters to centimeters beyond the root surface, effectively amplifying the functional rhizosphere into what has been termed the mycorrhizosphere (Harvey *et al*., 2002; Nannipieri *et al.,* 2011).

**Table 7. Agricultural management strategies targeting rhizosphere enzyme activity enhancement for improved nutrient use efficiency**

| Management Strategy | Mechanism | Expected Enzyme / Nutrient Outcome | Target Crop System | Assessment Method | Reference |
|---|---|---|---|---|---|
| Root architecture breeding | Selection for long root hairs, high lateral root density | Broader enzyme gradients, improved organic P mobilization | Maize, barley, wheat | QTL mapping for root traits | (Holz et al., 2018) |
| Phosphate-efficient cultivar selection | High acid phosphatase exudation genotypes | 30–60% reduction in P fertilizer need | Soybean, rice | Phosphatase activity screening assays | (Nannipieri et al., 2011) |
| Bioinoculant application (PGPR) | Enzyme-producing rhizobacteria (phytase, urease) | Enhanced organic N and P mineralisation | All major crops | Inoculation trials, field-validated strains | (Siczek & Lipiec, 2016) |
| Mycorrhizal inoculation | AM fungi amplify phosphatase and glucosidase zones | Mycorrhizosphere extends enzymatic reach | Vegetable crops, cereals | PLFA / colonisation rate assessment | (Harvey et al., 2002) |
| Conservation tillage | Preserves hyphal networks, SOM stratification | Higher basal enzyme activity, lower fertiliser requirement | Wheat, maize | Enzyme assay under tillage treatments | (Spedding et al., 2004) |
| Biochar amendment | Modifies pH and sorption, stabilises enzymes | Enzyme-class-specific effects, pH-dependent | Maize, vegetables | Biochar rate × enzyme response trials | (Burns et al., 2013) |
| Compost application | Increases microbial biomass, provides enzyme substrates | General increase in all hydrolase activities | Broad spectrum | Compost rate × enzyme activity experiments | (Liu et al., 2017) |
| Nitrogen source management | NH4+ acidifies rhizosphere; NO3- raises pH | Controls balance between acid and alkaline phosphatase | Cereals, legumes | Controlled N-source pot experiments | (Hinsinger et al., 2009) |

*QTL = quantitative trait loci; PGPR = plant growth-promoting rhizobacteria; AM = arbuscular mycorrhizal; SOM = soil organic matter; PLFA = phospholipid fatty acid analysis*

The enzymatic contributions of mycorrhizal networks depend on the nutritional status of the host plant and the specificity of the fungal-plant association. Under phosphorus-limited conditions, AM fungi upregulate secreted phosphatase production in proportion to the demand signal communicated through the plant-fungus interface, creating a highly responsive enzymatic feedback mechanism. Under nitrogen limitation, ectomycorrhizal fungi in forest ecosystems produce protease complexes capable of directly mineralizing organic nitrogen from protein substrates, bypassing the conventional pathway through bulk soil nitrogen cycling. These direct nutritional pathways mediated by mycorrhizal enzymes may account for a significant fraction of annual plant nitrogen and phosphorus acquisition in low-fertility soils.

### 7.2 Rhizobacterial Communities and Enzyme Co-production

Plant growth-promoting rhizobacteria (PGPR) occupy a privileged position in the rhizosphere, colonizing root surfaces and intercellular spaces where exudate concentrations are highest and enzyme induction signals are strongest. Many PGPR taxa produce extracellular enzymes that directly benefit plant nutrition — including phytases, which hydrolyze phytate-bound organic phosphorus; siderophore biosynthetic enzymes, which mobilize iron; and nitrogenases, which fix atmospheric nitrogen into bioavailable forms. The enzyme activities of PGPR communities are substantially shaped by the composition of root exudates they encounter, creating a functional selectivity mechanism whereby specific exudate profiles recruit bacterial communities with enzyme profiles matched to local nutrient constraints (Hinsinger *et al*., 2009; Siczek & Lipiec, 2016).

Quorum sensing, a cell-density-dependent signaling mechanism mediated by N-acylhomoserine lactone compounds in gram-negative bacteria and related signals in gram-positive taxa, coordinates enzyme production at the community level in the rhizosphere. At low bacterial densities near newly colonized root surfaces, quorum sensing signals are insufficient to trigger cooperative enzyme secretion, conserving cellular resources. As population density rises in response to exudate enrichment, quorum sensing threshold concentrations are exceeded and coordinated enzyme release is activated, generating the sharp spatial transitions in enzyme activity observed at the root surface in zymographic images. Understanding quorum sensing dynamics in the rhizosphere thus provides a mechanistic explanation for the spatial sharpness of enzymatic hotspots and their temporal coincidence with stages of active root colonization.

## 8. Implications for Sustainable Agriculture and Nutrient Management

### 8.1 Engineering Rhizosphere Enzyme Activity for Nutrient Use Efficiency

The rhizosphere enzyme system represents a largely untapped lever for improving nutrient use efficiency in agricultural crops. Conventional fertilizer management supplies nutrients in inorganic forms that bypass the rhizosphere biological machinery entirely, while substantial reserves of organically bound phosphorus, nitrogen, and sulfur remain unavailable to crops due to insufficient enzymatic depolymerization rates. Strategies targeting the enhancement of rhizosphere enzyme activity — through genetic improvement of crop root exudation profiles, inoculation with enzyme-producing bioinoculants, or management of soil conditions that favor enzyme persistence — offer pathways to reducing fertilizer inputs without sacrificing yield.

Root architecture breeding offers a particularly promising avenue, as cultivars with greater lateral root density, longer root hairs, and enhanced exudate secretion capacity consistently exhibit higher rhizosphere enzyme activities and superior performance on low-phosphorus soils. Quantitative trait loci (QTL) associated with root hair length, lateral root number, and organic acid exudation have been identified in multiple crop species, providing molecular targets for marker-assisted selection of rhizosphere-efficient genotypes. Integration of these root-trait QTL with enzyme activity phenotyping under field conditions will be essential to translate laboratory findings into agronomically relevant germplasm improvements.

### 8.2 Soil Management Effects on Rhizosphere Enzyme Activity

Soil management practices profoundly influence the biological and physical substrate within which rhizosphere enzyme dynamics operate. Conservation tillage systems, which preserve soil aggregation, fungal hyphal networks, and organic matter stratification, generally maintain higher basal enzyme activities than intensive tillage systems that disrupt soil structure and expose protected organic matter to mineralization. Crop rotation diversity, by introducing roots with varied exudate chemistry and architecture, maintains a functionally diverse rhizosphere enzyme pool less prone to the imbalances associated with monoculture systems (Spedding *et al*., 2004).

Amendments including biochar, compost, and organic mulches influence enzyme activity through modifications of soil pH, cation exchange capacity, microbial habitat quality, and enzyme stabilization capacity. Biochar in particular represents a complex amendment whose effects on rhizosphere enzymes depend strongly on feedstock type, pyrolysis temperature, and application rate. High-temperature biochars with alkaline pH and extensive aromatic carbon networks may stabilize certain enzyme classes while inhibiting others, necessitating a nuanced, enzyme-specific assessment of amendment suitability that current research is only beginning to address. Improved understanding of management effects on rhizosphere enzyme ecology will support the design of cropping systems that harness biological nutrient mobilization as a complement to, or partial substitute for, mineral fertilization.

## 9. Knowledge Gaps and Future Research Priorities

Despite substantial progress in characterizing rhizosphere enzyme dynamics, several fundamental questions remain unresolved. First, the quantitative partitioning of total rhizosphere enzyme activity between root-derived, mycorrhizal, and free-living microbial sources remains poorly constrained for most enzyme classes and plant species combinations. Methodological advances in isotope tracing and molecular source attribution are addressing this gap, but field-applicable methods suitable for tracking enzyme sources under complex natural conditions are still lacking.

Second, the mechanisms governing enzyme persistence and deactivation in the rhizosphere require more detailed investigation. Current models assume relatively simple adsorption-desorption equilibria between enzyme molecules and soil mineral surfaces, but recent evidence suggests that enzyme-mineral interactions are kinetically complex and influenced by the presence of competing organic molecules, pH dynamics, and ionic strength fluctuations at root surfaces. A mechanistic understanding of enzyme stabilization and deactivation kinetics in the rhizosphere would substantially improve predictions of enzyme residence time and the temporal integration of enzyme activity.

Third, the signaling networks linking plant nutritional status, exudate composition, and rhizosphere microbial enzyme production remain incompletely characterized at the molecular level. While individual exudate components have been shown to induce specific enzyme genes in model bacterial taxa, the network-level responses of complex rhizosphere microbiomes to realistic, multicomponent exudate mixtures are not predictable from single-compound studies. Systems-level approaches integrating metabolomics of exudates, metatranscriptomics of rhizosphere microbiomes, and high-resolution enzyme activity imaging are needed to bridge this mechanistic gap.

Fourth, the integration of rhizosphere enzyme ecology with global biogeochemical models represents an underdeveloped frontier. Current large-scale models of soil carbon and nitrogen cycling do not incorporate rhizosphere-specific enzyme kinetics, treating soil as a homogeneous medium with uniform turnover rates. The documented two- to three-fold enhancement of enzyme activity in the rhizosphere relative to bulk soil, and the spatial concentration of this activity in a zone comprising only a small fraction of total soil volume, create strong non-linearities in nutrient cycling rates that are structurally absent from most modeling frameworks. Parameterizing rhizosphere enzyme dynamics for inclusion in next-generation Earth system models will require coordinated experimental-modeling efforts spanning scales from root to ecosystem.

## 10. Conclusions

The rhizosphere enzyme system acts as a multi-scale interface where plant physiology, soil microbiology, and biogeochemical cycling intersect. Rhizosphere activities are governed by a complex hierarchy of controls, including molecular exudate transport, root architectural geometry, temporal plant development, and environmental soil-mineral stability. Root morphological traits—such as lateral root density, root hair length, and specific root lengthserve as strong predictors of enzyme distribution and present strategic targets for genetic and agronomic manipulation to enhance nutrient mobilization. While spatial imaging via soil zymography effectively captures localized heterogeneity, fully unlocking its potential requires integration with advanced molecular workflows like stable isotope probing, metatranscriptomics, and proteomics to resolve complex source attribution challenges. Ultimately, translating these highly spatial and mechanistic insights into targeted crop improvement, bioinoculant development, and evidence-based soil management remains a critical pathway for advancing sustainable agricultural productivity.

## Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## Competing Interests

Authors have declared that no competing interests exist.

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