Chapter IV : The Rhizosphere : The Critical Interface

1. Root Exudates : The Role of Signaling Molecules and Organic Acids in Nutrient Solubilization

1.1. Plant Root Architecture and Anatomy

Understanding the functions of plant roots and their interactions with the soil environment requires knowledge of their structure and anatomy.

The root cap, located at the root tip, protects the delicate meristematic tissues and facilitates root penetration through the soil. The apical meristem, situated immediately above the root cap, consists of actively dividing cells responsible for root growth and the formation of new tissues. These cells undergo division, elongation, and differentiation to produce the primary tissues of the root, including the epidermis, cortex, endodermis, pericycle, and vascular tissues

The epidermis constitutes the outermost layer of the root and plays a fundamental role in the absorption of water and mineral nutrients. Specialized epidermal cells form root hairs, which increase the root surface area in contact with the soil and enhance resource acquisition. The cortex, composed mainly of parenchymatous cells, contributes to the storage of carbohydrates and other reserves and facilitates the movement of water and dissolved mineral nutrients toward the central vascular cylinder.

The endodermis regulates the movement of water and dissolved ions into the vascular cylinder, thereby acting as a selective barrier between the cortex and the internal conducting tissues. Its Casparian strips restrict uncontrolled apoplastic transport and contribute to the selective uptake of substances. The pericycle, located immediately inside the endodermis, retains the capacity to initiate lateral root formation, contributing to root branching and the exploration of a greater volume of soil. The vascular tissues comprise the xylem and phloem: the xylem transports water and mineral nutrients from the roots to the aerial organs, whereas the phloem distributes sugars and other organic compounds throughout the plant.

Plant root architecture and anatomy vary according to plant species, developmental stage, and environmental conditions. Variations in root length, branching patterns, diameter, and growth orientation influence the capacity of roots to explore the soil and acquire water and mineral nutrients. Soil properties, nutrient availability, moisture conditions, and interactions with soil microorganisms can further influence root development and distribution. These structural and functional adaptations are essential for understanding plant–soil interactions, resource acquisition, and the establishment of the rhizosphere as a dynamic zone of interaction between plant roots and soil microorganisms.

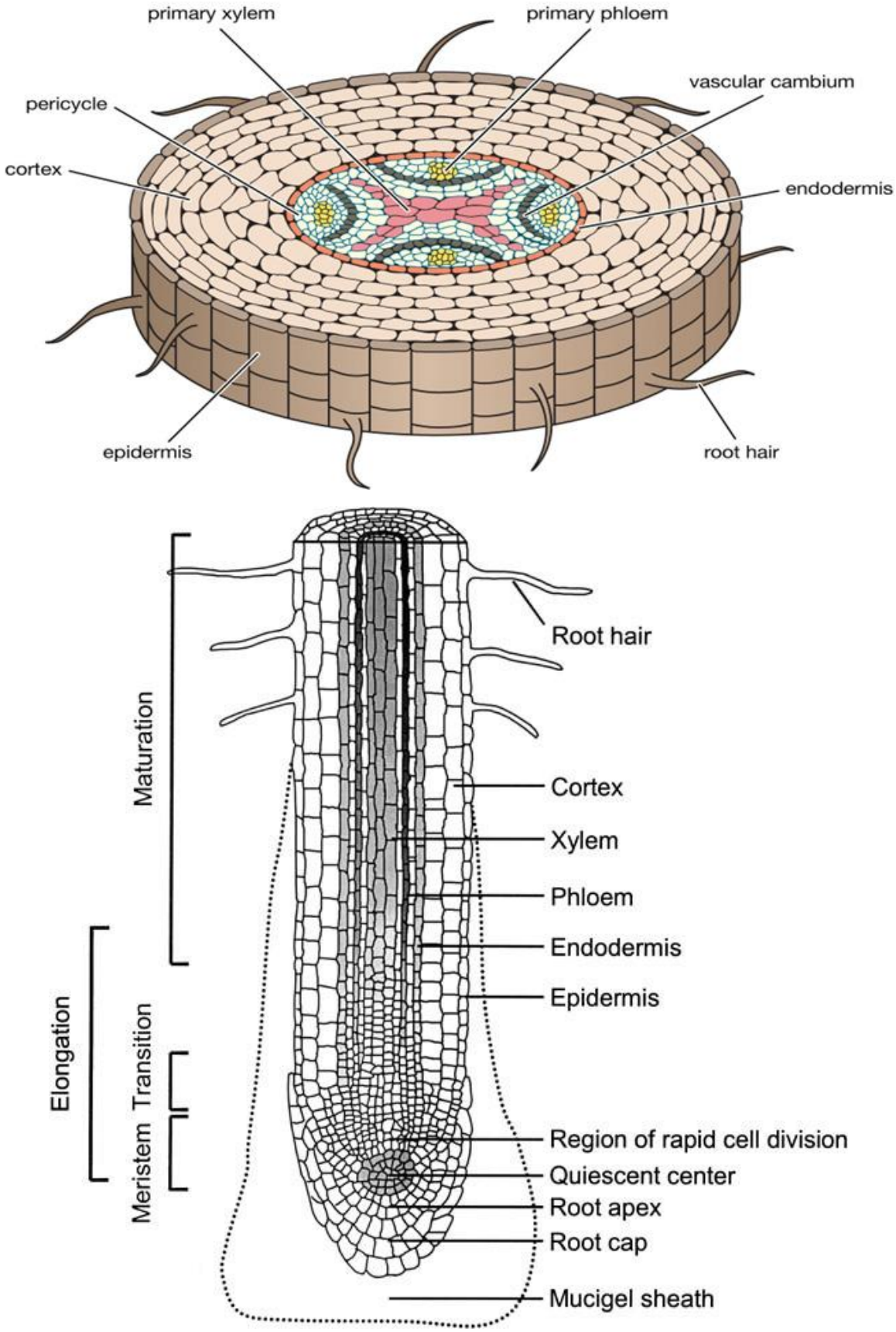

Figure 1 : Anatomy of plant root.

1.2. The rhizosphere

The rhizosphere typically represents approximately 2–3% of the total soil volume and constitutes a hotspot of biological activity. It extends approximately 1–2 mm from the root surface, although its influence may reach up to 20 mm, depending on root characteristics and activity. Compared with bulk soil, the rhizosphere exhibits distinct physical, chemical, and biological properties. The high density, diversity, and activity of microbial communities make this zone particularly important for ecological processes and agricultural productivity.

The rhizosphere effect refers to the stimulation of microbial growth and activity by root-derived compounds, particularly root exudates. Consequently, microbial populations in the rhizosphere may be 10–50 times more abundant, and decomposition rates may be two to three times higher than those observed in bulk soil.

Rhizosphere processes are influenced by several interconnected factors :

- Plant factors : Plant species, cultivar, age, developmental stage, and health influence the composition and quantity of root exudates. For example, legumes release flavonoids that stimulate chemical signaling and attract compatible nitrogen-fixing bacteria of the genus *Rhizobium*.

- Soil and environmental factors : Soil texture, pH, organic matter content, nutrient availability, temperature, moisture, light intensity, and CO_2 concentration affect root exudation and microbial activity. For instance, iron deficiency can stimulate the release of phenolic compounds and organic acids, enhancing iron mobilization and influencing the availability of other minerals.

- Microbial interactions : Interactions between plant roots and microorganisms, including bacteria and fungi, can modify root exudation and influence the composition and activity of rhizosphere microbial communities. These interactions also affect carbon cycling, nutrient availability, and microbial community structure.

- Agricultural practices : Fertilization, irrigation, tillage, and crop rotation influence root exudation, rhizodeposition, and the composition of rhizosphere microbial communities. These practices can consequently affect nutrient availability, soil biological activity, and plant growth.

The rhizosphere can be divided into three main zones :

-Ectorhizosphere : The soil surrounding the roots, enriched with root exudates that support microbial growth and activity. This zone is characterized by gradients in nutrient availability, pH, and redox potential.

-Rhizoplane : The root surface, where microorganisms attach directly to root tissues and interact with plant cells. These interactions contribute to nutrient exchange, microbial colonization, and chemical signaling.

-Endorhizosphere : The internal root tissues colonized by endophytic microorganisms, including bacteria and fungi. These microorganisms may contribute to nutrient acquisition, plant growth, and defense against pathogens.

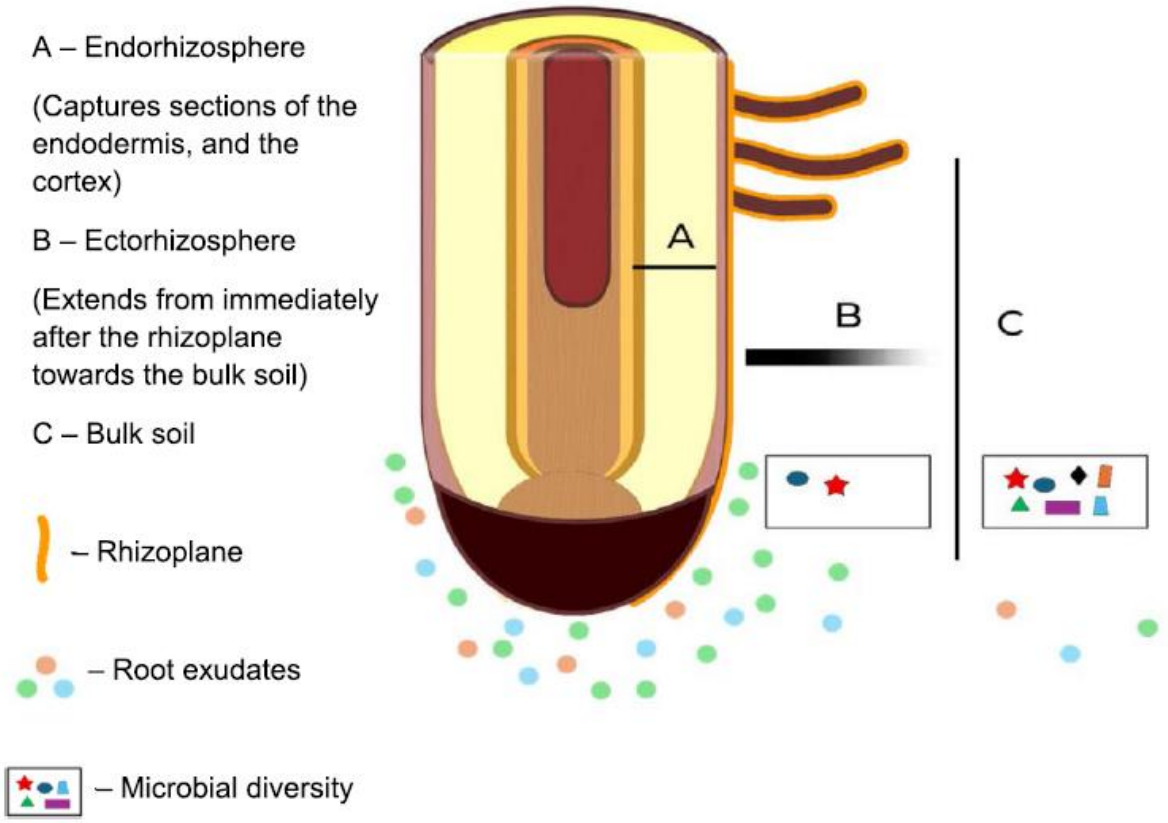

Figure 2 : Root exudate-mediated shaping of microbial diversity across the rhizosphere. Microbial diversity generally increases with increasing distance from the root surface, reflecting the selective influence of root exudates on microbial communities. The root diagram illustrates three distinct rhizosphere compartments : (A) endorhizosphere, (B) ectorhizosphere, and (C) bulk soil.

1.3. Rhizodeposition

- **Definition and Categories of Rhizodeposits**

Rhizodeposition and root-microbe interactions are fundamental components of rhizosphere biology that shape the structure and functioning of the plant-soil interface. Rhizodeposition refers to the release of organic materials from plant roots into the surrounding soil. These

materials known as rhizodeposits, include root exudates, mucilage, sloughed-off root cells, and cellular lysates, which collectively contribute to the supply of organic matter in the rhizosphere. Rhizodeposits represent an important source of carbon and energy for soil microorganisms, supporting their growth, metabolism, and activity. In turn, rhizosphere microorganisms can influence the quantity and composition of rhizodeposits by modifying root physiology, nutrient availability, and plant metabolic processes.

- **Exudates**

Exudates comprise mainly low-molecular-weight compounds released from roots, including sugars such as glucose and fructose, amino acids such as proline and lysine, organic acids such as citric, malic, and oxalic acids, as well as vitamins, hormones, and secondary metabolites. Their release occurs through passive diffusion and, for specific compounds, transporter-mediated processes.

- **Secretions**

Secretions are substances actively released by root cells through processes involving metabolic energy. They include polymeric carbohydrates, particularly mucilage, and enzymes such as phosphatases and proteases. Their release involves cellular secretion pathways, including membrane transport proteins and vesicular transport.

Mucilage facilitates root penetration through soil, promotes soil aggregation, and improves water retention around roots. Enzymes contribute to nutrient acquisition by breaking down complex organic substrates. For example, phosphatases release phosphate from organic compounds, increasing the availability of phosphorus to plants.

The production and release of secretions are influenced by plant physiological status and environmental conditions. Nutrient deficiencies, particularly phosphorus deficiency, can stimulate the release of enzymes and organic compounds involved in nutrient mobilization.

- **Lysates**

Lysates consist of cellular materials released when root cells undergo damage, autolysis, or senescence. They include cellular contents, cell wall components, sloughed-off root cells, and materials derived from dead root tissues. Unlike the release of many soluble exudates and active secretions, lysate release is generally associated with cell breakdown and turnover.

Lysates contribute to soil organic matter by supplying complex organic materials, including structural polysaccharides and other cellular components, which are subsequently decomposed by soil microorganisms. This process supports microbial activity, carbon cycling, and nutrient recycling in the rhizosphere.

The release of lysates is particularly important in older root zones and areas undergoing cell turnover. Its intensity can vary with plant age, developmental stage, and environmental stresses, including water stress and mechanical impedance.

❖ **Gases**

Root-derived gases include carbon dioxide (CO_2), produced during root respiration, and volatile compounds such as ethylene and hydrogen cyanide. These substances enter the surrounding soil or diffuse from root tissues through processes associated with respiration and plant metabolism.

Carbon dioxide influences rhizosphere pH and microbial activity, whereas ethylene plays a role in regulating root growth and plant responses to environmental stress. Other volatile compounds can participate in chemical communication, defense responses, or interactions with neighboring organisms.

In addition to these four categories, rhizodeposition can influence soil chemistry through the release or movement of inorganic ions, protons (H^+), water, oxygen, and electrons. These components contribute to changes in soil pH, nutrient availability, and redox conditions.

Sites of Rhizodeposit Release

- Root tips : The root cap and elongation zone are major sites of exudate release due to high metabolic activity and the permeability of young cells. Sugars, amino acids, organic acids, and mucilage are released, facilitating root growth and interactions with soil microorganisms.

- Root hairs and maturation zone : Root hairs increase the root surface area and release low-molecular-weight compounds, such as sugars, amino acids, and organic acids, as well as enzymes. These substances support microbial activity and contribute to nutrient availability.

- Older root zones and cortex : Cell senescence, autolysis, and cell damage lead to the release of lysates, sloughed cells, and cell wall components. These materials contribute to soil organic matter formation.

- Entire root length : Although exudation is generally more pronounced near root tips, sugars and other soluble compounds can also diffuse through the root surface along its entire length, contributing to microbial activity in the rhizosphere.

1.4. Root Exudates

Function of Root Exudates in the Rhizosphere

Root exudates play a fundamental role in shaping the rhizosphere by attracting and supporting beneficial soil microorganisms. They provide readily available carbon sources that stimulate the growth and activity of plant-associated microorganisms, including plant growth-promoting

rhizobacteria and arbuscular mycorrhizal fungi, thereby enhancing nutrient acquisition and plant development.

In addition to influencing microbial communities, root exudates modify the chemical and physical properties of the soil. They contribute to soil aggregation, facilitate metal chelation, influence soil pH, and affect water retention. Certain exudates also exhibit allelopathic activity, whereby the release of specific secondary metabolites inhibits the germination or growth of competing plant species.

Root exudates further contribute to plant adaptation to environmental stresses, particularly water scarcity, by influencing root architecture and interactions with beneficial microorganisms. Some exudates promote root growth and branching, while others support deeper root development, enabling plants to access water from deeper soil layers. Arbuscular mycorrhizal fungi enhance water and nutrient acquisition through their extensive hyphal networks, whereas beneficial rhizobacteria can improve nutrient availability, regulate plant hormonal responses, promote growth, and strengthen resistance to environmental stresses and diseases.

- **Mechanisms of Root Exudation**

Plants release a wide range of compounds into the rhizosphere through passive and active transport mechanisms. Passive release, often referred to as diffusion, mainly involves low-molecular-weight organic compounds. Small, polar, uncharged molecules can cross root cell membranes by passive diffusion, depending on membrane permeability, the chemical properties of the compounds, and cytosolic pH.

In contrast, active secretion involves membrane-associated transport proteins that facilitate the export of specific compounds, including secondary metabolites, polysaccharides, and proteins. Several transporter families contribute to these processes, including ATP-binding cassette (ABC) transporters, multidrug and toxic compound extrusion (MATE) transporters, members of the major facilitator superfamily (MFS), and aluminum-activated malate transporters (ALMTs).

ABC transporters use energy derived from ATP hydrolysis to transport a variety of substrates across cellular membranes. MATE transporters, on the other hand, generally rely on electrochemical gradients to drive the export of diverse compounds, including alkaloids, organic acids such as citrate, phenolic compounds, and other metabolites. These transport systems contribute to the selective release of root-derived compounds into the surrounding soil, influencing nutrient availability, microbial activity, and interactions between plants and soil microorganisms.

1.5. Root-Microbe Interactions

Root-microbe interactions encompass a broad spectrum of relationships, ranging from mutualistic symbiosis and commensalism to pathogenic associations. Beneficial microorganisms can enhance nutrient availability, promote plant growth, and protect roots against soilborne pathogens. In contrast, pathogenic microorganisms may impair root function, disrupt nutrient uptake, and cause plant diseases. These interactions collectively influence plant health, soil microbial communities, nutrient cycling, and the overall functioning of the rhizosphere.

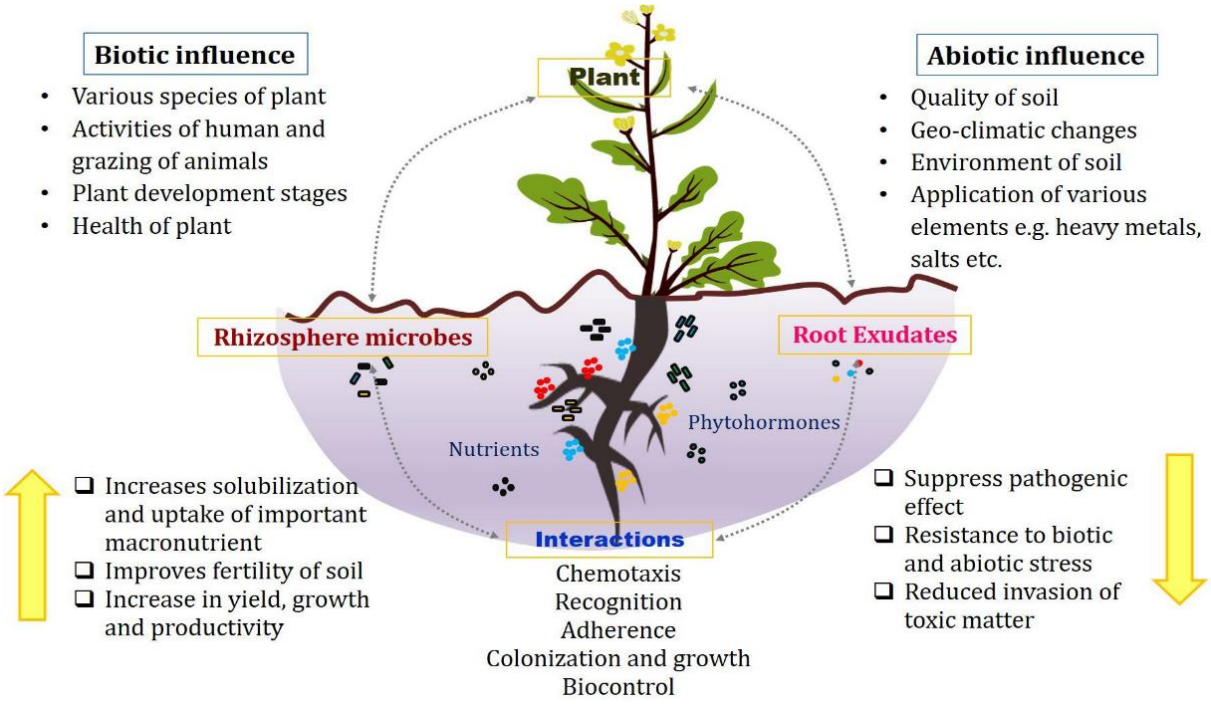

Figure 3 : Interactions between plant root, rhizosphere microbes and root exudates.

1.6. Organic Acids in Soil : Origins and Functions

Types and Concentrations of Organic Acids in Soils

Soils contain a wide variety of organic acids (OAs), among which low-molecular-weight organic acids (LMWOAs), such as oxalic, citric, malic, acetic, formic, tartaric, malonic, succinic, maleic, and fumaric acids, play important roles in soil processes and plant nutrient acquisition. These compounds influence nutrient solubilization, mineral weathering, metal complexation, and microbial activity. Their effectiveness in soil biological and chemical processes depends on their molecular structure, particularly the number and arrangement of carboxyl groups, as well as their chemical properties and interactions with soil constituents.

The concentrations of organic acids in soil solutions vary considerably according to their chemical characteristics and environmental conditions. Di- and tricarboxylic acids, including

oxalic, malonic, malic, succinic, tartaric, and citric acids, are generally detected at micromolar concentrations, often ranging from 0 to 50 µM. In contrast, monocarboxylic acids, such as formic, acetic, propionic, butyric, valeric, and lactic acids, may occur at concentrations ranging from 0 to 1 mM. These values are indicative rather than universal, as organic acid concentrations vary substantially among soil types, plant species, and environmental conditions.

The concentration and distribution of organic acids in soil are governed by several interacting factors, including their production by plant roots and microorganisms, their release during organic matter decomposition, and their removal through microbial degradation and plant uptake. Soil properties also play a major role in determining their availability. In particular, soil texture, mineral composition, pH, and the adsorption capacity of soil colloids influence the proportion of organic acids that remains dissolved in the soil solution.

Organic acid concentrations may also differ considerably depending on the plant species and the intensity of biological activity in the rhizosphere. For example, variations in citric and malic acid concentrations can reflect differences in microbial activity and root metabolism.

- **Origins of soil OAs**

Organic acids (OAs) in soil originate primarily from plant roots, soil microorganisms, and the decomposition of organic matter. These sources contribute to the accumulation and transformation of organic acids in the rhizosphere and surrounding soil, influencing nutrient availability, microbial activity, soil pH, and mineral weathering.

-Organic Acids of Plant Root Origin : As discussed in the section on root exudates, plant roots release organic acids into the rhizosphere, where they contribute to nutrient mobilization and influence soil chemical properties.

-Organic Acids of Microbial Origin : Soil microorganisms, particularly bacteria and fungi, produce and release organic acids as part of their metabolism and adaptation to environmental conditions. These compounds help microorganisms acquire essential nutrients by dissolving or mobilizing poorly soluble mineral compounds. Organic acid production is especially important in phosphorus solubilization, as certain microorganisms release acids that increase the availability of otherwise inaccessible phosphorus.

Genera such as *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Enterobacter* can produce various organic acids, including citric, oxalic, lactic, acetic, and succinic acids. Mycorrhizal fungi can also influence organic acid concentrations and modify root exudation, thereby affecting microbial communities and nutrient cycling in the mycorrhizosphere.

Microorganisms release organic acids through active secretion, which requires energy, and through passive processes, including diffusion and leakage from damaged or lysed cells. Once released, these compounds may be rapidly consumed by microorganisms, absorbed by plants, or adsorbed onto soil particles. Their persistence and availability depend on soil properties, organic acid characteristics, and microbial activity.

-Organic Acids Derived from Plant Litter and Organic Matter : The decomposition of plant litter and other organic materials represents another important source of organic acids in soil. During decomposition, microorganisms break down complex organic compounds into simpler substances, including low-molecular-weight organic acids, phenolic acids, and other soluble organic compounds.

These decomposition products contribute to soil nutrient cycling by providing carbon and energy for microorganisms and influencing the availability of mineral nutrients. Organic matter also contains humic substances, which possess functional groups capable of binding metal ions and forming complexes. This process can influence metal mobility and reduce the toxicity of certain metals under specific soil conditions.

Organic matter decomposition can modify soil pH in different ways. The release and dissociation of organic acids may increase soil acidity, whereas microbial degradation of organic acid anions can consume hydrogen ions and potentially increase pH. The overall effect depends on the chemical composition of the organic matter, microbial processes, and soil conditions.

Under anaerobic conditions, organic matter can also be converted into organic acids and other intermediate products through anaerobic digestion. This process involves four principal stages : hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During these stages, complex organic materials are progressively converted into simpler compounds, including volatile fatty acids, acetate, hydrogen, and carbon dioxide, with methane produced during the final stage.

- **Roles of Organic Acids**

Organic acids (OAs) play important roles in agricultural systems by modifying the physicochemical properties of the rhizosphere, improving nutrient availability, facilitating mineral weathering, and contributing to plant tolerance to metal toxicity. Their effects depend on their chemical structure, concentration, soil properties, and interactions with plant roots and soil microorganisms.

- **Nutrient Mobilization and Mineral Weathering**

Organic acids contribute to nutrient acquisition by promoting the dissolution of minerals and mobilizing nutrients that are present in poorly soluble forms. Their carboxyl groups enable them

to bind metal ions through chelation, facilitating the release of essential nutrients such as phosphorus (P), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), zinc (Zn), and copper (Cu). In addition, the release of protons during organic acid dissociation can lower the pH of the surrounding soil and enhance mineral dissolution.

The production and release of organic acids may increase when plants or microorganisms experience nutrient deficiencies, particularly phosphorus or iron limitation. For example, citric and malic acids can contribute to phosphorus mobilization, while organic acids involved in iron acquisition help improve iron availability under certain soil conditions. Phosphate-solubilizing microorganisms, including species of *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Enterobacter*, can enhance phosphorus availability through organic acid production and other mechanisms.

Interactions between organic acids, plants, and beneficial microorganisms can consequently improve nutrient uptake and plant growth. However, the effectiveness of these processes depends on soil pH, mineral composition, microbial activity, and the quantity and type of organic acids released.

- **Metal Detoxification and Plant Tolerance**

Organic acids also influence the mobility, bioavailability, and toxicity of metals in soil. Through chelation, they form complexes with metal ions, potentially reducing the concentration of free, toxic ions in the soil solution. This mechanism is particularly important in acidic soils, where aluminium (Al) toxicity can inhibit root elongation, damage root tissues, and impair water and nutrient uptake.

The release of organic acids, especially citrate and malate, can help protect root tissues by binding aluminium in the rhizosphere and reducing its toxic effects. This mechanism contributes to aluminium tolerance in several crop species, including wheat and maize. The effectiveness of detoxification depends on the organic acid involved, its concentration, the chemical properties of the soil, and the metal concerned.

Organic acids can also increase the mobility and uptake of certain potentially toxic metals, including cadmium (Cd), lead (Pb), and chromium (Cr). This property may be useful in phytoremediation, where plants are used to remove contaminants from soil. However, increased metal mobility can also raise the risk of metal uptake by crops and entry into the food chain. Therefore, the effects of organic acids on metal contamination must be evaluated according to the specific soil conditions and intended agricultural application.

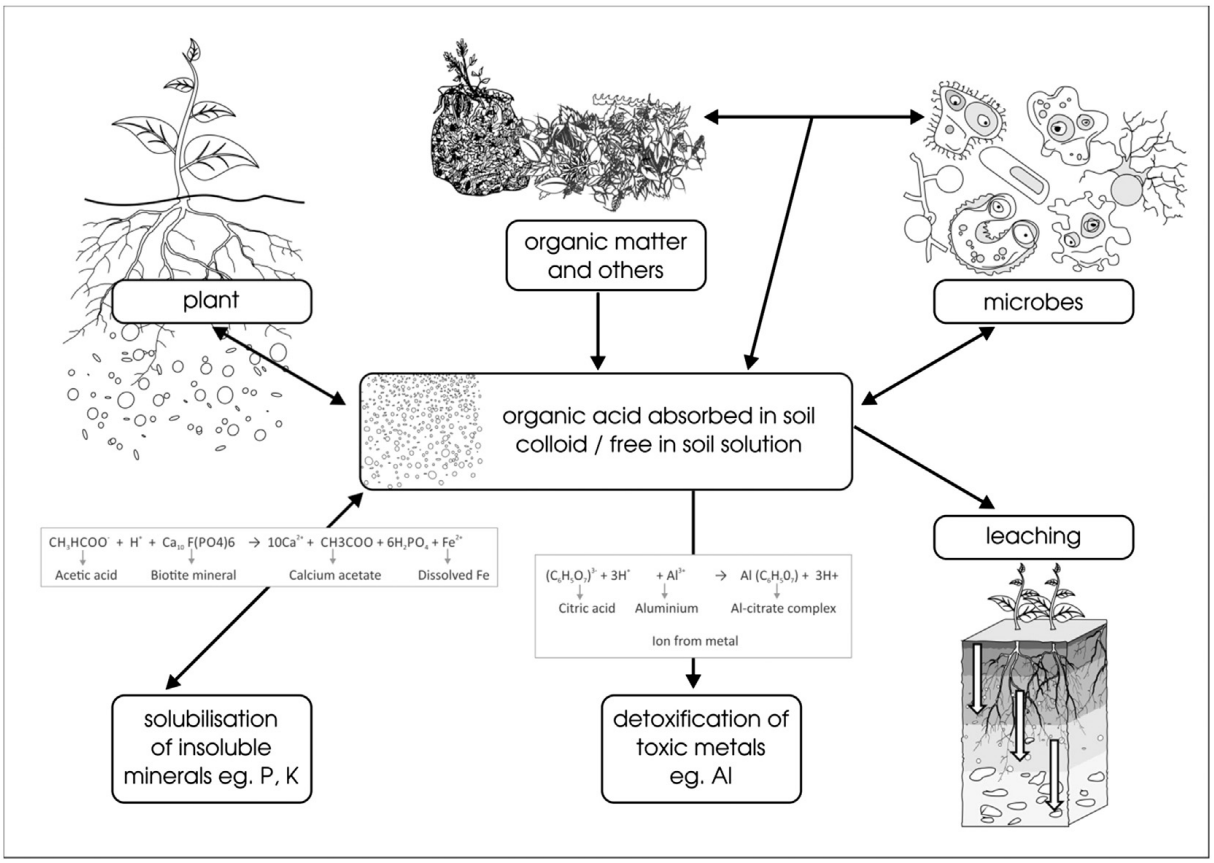

Figure 4: Sources and roles of soil organic acids.

2. Soil Microbiome : Plant–Beneficial Microorganism Interactions (PGPR) and Nutrient Cycling

2.1. The Soil Microbiome and Rhizosphere Microbial Diversity

A microbiome is a community of microorganisms inhabiting a specific environmen. Microbiomes are found in a wide range of environments, including humans, animals, plants, soils, and oceans. Among these environments, soil hosts one of the most complex and diverse microbial communities on Earth. A single gram of soil may contain tens of thousands of microbial species, reflecting the remarkable genetic diversity of the soil microbiome and its essential role in ecosystem functioning, nutrient cycling, and plant-soil interactions.

The rhizosphere hosts a diverse microbial community comprising bacteria, fungi, archaea, viruses, protozoa, nematodes, and other soil microfauna. These organisms form complex and dynamic networks of interactions that influence plant health, soil functioning, and ecosystem processes.

Bacteria are major components of the rhizosphere microbiome and include groups such as *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*. They contribute to nutrient cycling, nutrient solubilization, phytohormone production, plant growth promotion, and disease suppression. Fungi, particularly members of the *Ascomycota* and *Basidiomycota*, contribute to organic matter decomposition, nutrient cycling, and the establishment of mycorrhizal symbioses with plant roots.

Archaea and viruses also contribute to rhizosphere dynamics. Certain archaea, particularly those belonging to *Thaumarchaeota*, participate in nitrogen cycling, especially ammonia oxidation, and in other biogeochemical processes, including methane oxidation. Viruses influence microbial community composition and nutrient cycling through their interactions with bacterial and fungal hosts and may contribute to microbial genetic diversity through horizontal gene transfer.

2.2. Plant Growth-Promoting Rhizobacteria (PGPR)

2.2.1. Definition

Plant growth-promoting rhizobacteria (PGPR) are beneficial microorganisms that colonize the rhizosphere and enhance plant growth through direct and indirect mechanisms. Their plant-growth-promoting effects include improved nutrient acquisition, phytohormone production, suppression of plant pathogens, and increased tolerance to biotic and abiotic stresses.

For example, *Azospirillum brasilense* promotes root elongation and contributes to nitrogen fixation in a range of crops, thereby improving plant nutrient status and development. Similarly, *Bacillus subtilis* enhances plant growth by stimulating host defense responses, producing antifungal compounds, and increasing the availability of nutrients in the rhizosphere. In addition, *Rhizobium* species establish mutualistic symbioses with leguminous plants, forming root nodules in which atmospheric nitrogen is fixed and made available to the host.

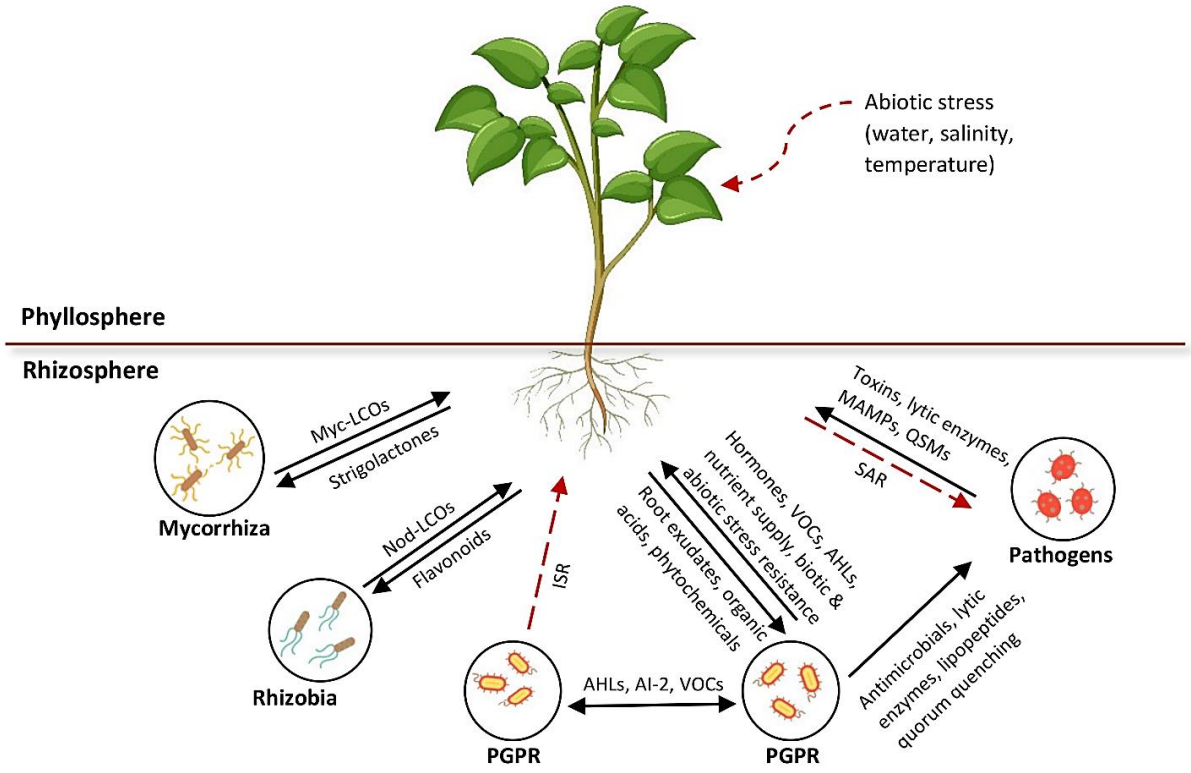

Figure 5 : Diverse communication networks operating in the rhizosphere, spanning three major signaling categories: intra- and interspecies microbial communication, and interkingdom dialogue between microbes and plants. Myc, mycorrhizal; LCOs, lipo-chito oligosaccharides; Nod, nodulation; VOCs, volatile organic compounds; Ais, autoinducers; AHLs, N-acyl homoserine lactone; QSMs, quorum sensing molecules.

2.2.2. Diverse modes of PGPR communication

- **Contact-dependent communication**

Contact-dependent communication allows bacteria to regulate neighboring cells—both kin and competitors—through direct physical interactions rather than diffusible signals. These direct contacts facilitate the exchange of DNA, RNA, nutrients, and metabolites without exposure to the external environment, while structures like type VI secretion systems deliver inhibitory molecules to hostile neighboring cells, safeguarding specific bacterial populations.

A key mechanism is Contact-Dependent Growth Inhibition (CDI), in which inhibitory cells rely on direct cell-to-cell contact to impair target cell growth. The CDI system operates through a two-partner secretion mechanism involving CdiA and CdiB proteins: CdiA is presented on the surface of the inhibiting bacterium and binds to specific receptors on target bacteria, upon which the C-terminal toxin domain (CdiA-CT) is delivered into the neighboring cell, leading to growth

inhibition. This direct toxin transfer confirms that CDI is fundamentally a contact-dependent process rather than one relying on chemical induction.

In the rhizosphere, plant growth-promoting rhizobacteria (PGPR) leverage these contact-dependent mechanisms to protect plants from pathogen invasion by forming physical barriers, creating denser root networks, and producing biofilms that prevent pathogenic microbes from colonizing plant roots. These combined efforts suppress pathogens and preserve plant health.

- **Chemical-dependent communication**

Chemical-dependent communication is a form of microbial communication in which organisms release and detect chemical signals in their environment. These signalling molecules, or autoinducers, allow nearby microbes to sense one another and coordinate their behaviour. In the rhizosphere, such communication is important for processes including biofilm formation, nutrient cycling, competition, and cooperation among plant-growth-promoting rhizobacteria (PGPR).

Quorum sensing (QS) is the best-known form of bacterial chemical communication. Bacteria continuously release small signal molecules into their surroundings; as the population grows, these molecules accumulate. Once a threshold concentration is reached, the signals bind to receptors and trigger coordinated changes in gene expression. This enables bacteria to behave collectively according to population density.

Through QS, bacteria can regulate traits such as:

- Biofilm formation and attachment to surfaces
- Production of extracellular polymeric substances (EPS)
- Antibiotic and virulence-factor production
- Genetic exchange through transformation or conjugation
- Motility, sporulation, and other survival-related behaviourspmc.ncbi.nlm.nih+1

Gram-negative bacteria mainly use N-acyl homoserine lactones (AHLs), also called autoinducer-1 (AI-1), as QS signals. AHLs diffuse across cell membranes and accumulate in the environment. At sufficient concentrations, they bind intracellular LuxR-type receptor proteins, which regulate the expression of target genes.

AHLs share a common homoserine-lactone structure but differ in their acyl side chains, particularly in chain length and substitutions at the third carbon. Many genera produce AHLs, including *Pseudomonas*, *Rhizobium*, *Agrobacterium*, *Burkholderia*, *Serratia*, and *Vibrio*.

A well-studied example is *Pseudomonas aeruginosa*. Its *lasI* gene produces the AHL signal 3-oxo-dodecanoyl-L-homoserine lactone (OdDHL). When OdDHL reaches a threshold level, it

binds the LasR receptor and activates genes involved in biofilm development and exopolysaccharide production.

Gram-positive bacteria generally use autoinducing peptides (AIPs) rather than AHLs. These short peptides are synthesized as precursors, processed, exported, and detected by membrane-bound sensor kinases. Signal detection activates a phosphorylation cascade that changes gene transcription.

AIP-mediated QS commonly regulates processes such as competence, sporulation, virulence, and biofilm formation. Unlike AHLs, which can diffuse freely across membranes, AIPs usually require active transport or specific export systems.

- **Communication through volatile organic compounds**

Volatile organic compounds (VOCs) are small, lipophilic microbial metabolites, typically about 100–500 Da, produced by many bacteria and fungi. Because they can diffuse through air-filled soil pores and liquid films, microbial VOCs (MVOCs) act as long-distance chemical signals that influence interactions among microorganisms even when they are physically separated.pmc.

MVOCs are produced through genotype-specific primary and secondary metabolic pathways, often from glucose oxidation and related intermediates. Major chemical classes include alcohols, ketones, alkenes, terpenes, benzenoids, pyrazines, acids, esters, and sulfur-containing compounds.pmc.

Their composition is often strain- or species-specific, meaning that VOC profiles can serve as chemical identifiers. This specificity allows microbes to distinguish nearby organisms and tailor their responses accordingly.

Many MVOCs have direct antimicrobial activity and help PGPR suppress competitors or plant pathogens. Examples include dimethyl disulfide, dimethyl trisulfide, hydrogen cyanide, 1-undecene, benzonitrile, and various terpenes.

For example:

-*Pseudomonas fluorescens* and *Serratia plymuthica* emit dimethyl disulfide, which inhibits *Agrobacterium tumefaciens* and *Agrobacterium vitis*.

-*P. fluorescens* WR-1 produces benzothiazole and 1-methyl naphthalene, which inhibit the tomato pathogen *Ralstonia solanacearum*.

-Several *Pseudomonas* and *Bacillus* strains used as biocontrol agents release VOCs with antibacterial or antifungal properties.

VOCs are not only weapons; they can also promote the growth and activity of neighbouring beneficial bacteria. VOCs from *Collimonas pratensis* and *Serratia plymuthica*, for instance,

stimulate growth of *P. fluorescens* Pf0-1 and activate genes involved in motility and antibacterial secondary-metabolite production.

Such effects may represent cooperation: one strain's volatiles can attract, nourish, or activate another strain, strengthening the community's collective defence against competitors or fungal pathogens.

Hydrogen cyanide (HCN) is a volatile secondary metabolite produced by some PGPR, including certain *Bacillus* and *Pseudomonas* strains. It can inhibit microbial enzymes, interfere with electron transport, reduce energy production, and ultimately suppress or kill competing microorganisms.

HCN therefore contributes to biocontrol by limiting plant pathogens and competing microbes, although its activity depends on concentration and environmental conditions.

PGPR can sense VOCs released by other organisms and respond by altering gene expression. In *Escherichia coli*, for example, the YpdB protein detects VOCs such as 2,3-butanedione and glyoxylic acid from *Bacillus subtilis*, leading to changes in motility-related genes.

These responses show that VOCs function as environmental cues, helping bacteria adjust their movement, metabolism, and competitive strategies to changing rhizosphere conditions.

- **Other forms of interactions and relationships among PGPR**

In the rhizosphere, plant-growth-promoting rhizobacteria (PGPR) interact through cooperation, nutrient exchange, signalling, and competition. These interactions include synergism, commensalism, and mutualism, and they help microbial communities function as coordinated systems that can improve plant health, nutrient availability, and ecosystem resilience.

Synergism occurs when two or more PGPR strains produce a combined effect greater than the sum of their individual effects. Such cooperation may involve one strain attracting or recruiting another through chemical signals.link.

A notable example involves *Bacillus subtilis* and *Paenibacillus dendritiformis*. *B. subtilis* releases surfactin, a lipopeptide with antimicrobial activity, which attracts *P. dendritiformis*. Rather than being harmed, *P. dendritiformis* degrades surfactin and accumulates the degradation products as territorial markers. This suggests that a strain can benefit from a partner's metabolites even if it does not produce those metabolites itself.

Microbial partnerships can also enhance growth directly. For example, *Bacillus cereus* can support high population densities of Cytophaga–Flavobacterium bacteria by supplying peptidoglycan and stimulating their reproduction.

Cross-feeding is a common form of metabolic cooperation in which one microorganism, the donor, converts an environmental substrate into a product that another microorganism, the

receiver, can use. These exchanged compounds are often described as “public goods” because they may benefit the producer, the immediate partner, and other nearby microbes.

Shared products can include:

- Enzymes that degrade antibiotics
- Surfactants that improve motility
- Biofilm matrix components
- Organic acids, amino acids, vitamins, and other secondary metabolites.

Cross-feeding is especially common among closely related microbes. By helping relatives, a donor may indirectly increase the transmission of shared genes, a process known as kin selection. However, cross-feeding also occurs between different species.

A synthetic coculture of *Escherichia coli* and *Rhodopseudomonas palustris* illustrates reciprocal cross-feeding under anaerobic conditions. *E. coli* ferments glucose and releases organic acids and ethanol; *R. palustris* uses most of these organic acids as carbon sources. In return, *R. palustris* fixes atmospheric nitrogen through nitrogenase and releases ammonium, which serves as the nitrogen source for *E. coli*.

Under these conditions, each species depends on the other for an essential nutrient: *E. coli* provides carbon, while *R. palustris* provides nitrogen. This reciprocal exchange promotes stable coexistence and demonstrates how metabolic specialization can create mutualistic microbial partnerships.

PGPR communication and cooperation involve more than simple nutrient exchange. They include the release of signalling molecules, activation of stress-resistance mechanisms, biofilm formation, and nutrient cycling. Together, these processes shape rhizosphere community structure and improve the efficiency with which microbes use available resources.

These cooperative interactions can benefit plants by increasing nutrient mobilization, suppressing pathogens, and enhancing tolerance to biotic and abiotic stress. Understanding PGPR communication therefore offers opportunities for developing more sustainable agricultural and environmental-management strategies.

2.3. Chemical Communication and Beneficial Plant-Microbe Interactions

2.3.1. Chemical Communication

Plants release secondary metabolites into the rhizosphere to attract and communicate with beneficial microorganisms. Two major signal classes are flavonoids and strigolactones.

The dialogue involved in establishing the interaction is not unidirectional. Microorganisms must also be recognized by the host in return. Nod-factor production by rhizobia is stimulated

by specific plant flavonoids, whereas Myc-factor signaling involves arbuscular mycorrhizal fungi and is associated with plant-derived strigolactones. However, the precise signaling mechanisms differ between these symbiotic systems. These two types of molecules share similar structural features.

As shown in figure 6, both Nod factors and certain Myc factors are lipochitooligosaccharides (LCOs) that activate signaling pathways in host plants. However, Nod factors exhibit considerable structural diversity in their chitin backbone and in the chemical modifications attached to it. LCOs generally consist of a short chain of β-1,4-linked N-acetyl-D-glucosamine residues, typically three to five units long, attached to a lipid chain.

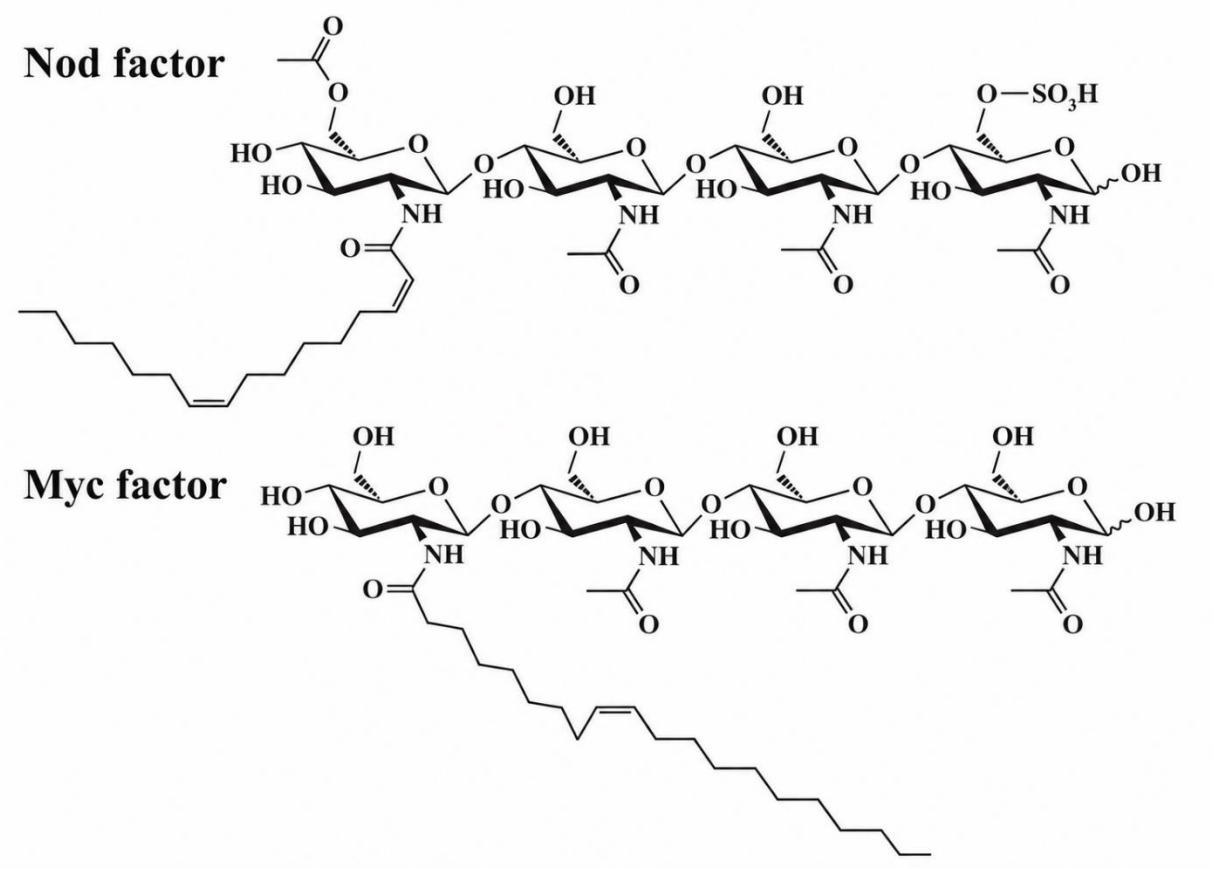

Figure 6 : Structures of Nod factors and Myc factors.

2.4. Beneficial Plant-Microbe Interactions

Nodulation of legumes by rhizobia

Rhizobia are soil bacteria that form nitrogen-fixing symbioses with many legumes. Inside root nodules, they convert atmospheric nitrogen into forms usable by the plant. However, this

partnership is highly host-specific: particular rhizobial strains usually nodulate only particular legume species or genera. Some interactions are narrow. For example, *Sinorhizobium meliloti* nodulates mainly *Medicago*, *Melilotus*, and *Trigonella*, while *Rhizobium leguminosarum* bv. *viciae* nodulates *Pisum*, *Vicia*, *Lens*, and *Lathyrus*. In contrast, *Rhizobium* strain NGR234 has a broad host range and can nodulate many legume species, as well as the non-legume *Parasponia andersonii*.

Nodulation is also not universal among legumes. Members of the basal subfamily Caesalpinioideae are largely non-nodulating, showing that nitrogen-fixing nodules are not found in every legume lineage.

The first step in recognition is the release of flavonoids from legume roots into the rhizosphere. These plant compounds attract compatible rhizobia and activate bacterial nodulation (*nod*) genes.pmc.

Different legumes release different flavonoids, which helps determine which rhizobia can establish a symbiosis. For example:

-Soybean (*Glycine max*) releases the isoflavonoids daidzein and genistein, which induce *nod* genes in *Bradyrhizobium japonicum*.

-The same compounds inhibit *nod* gene expression in *S. meliloti*.

-*S. meliloti* is instead activated by luteolin, a flavonoid associated with *Medicago*.

Rhizobia perceive flavonoids through NodD, a LysR-type transcriptional regulator. When a compatible flavonoid binds NodD, the protein changes shape and binds conserved promoter sequences called *nod* boxes, activating *nod* gene expression.

The activated *nod* genes produce enzymes that synthesize Nod factors, which are lipochitooligosaccharides (LCOs). Their basic structure consists of a short chain of β-1,4-linked *N*-acetylglucosamine residues, usually four or five units, with a fatty acyl chain attached to the non-reducing end.

Nod factors vary among rhizobial strains because of chemical decorations such as acetyl, sulfate, carbamoyl, or sugar groups, as well as differences in fatty-acid chain length and saturation. These structural variations determine whether a particular legume can recognize the bacterial signal.

Compatible Nod factors are detected by legume LysM receptor kinases on root cells. This recognition triggers root-hair curling, infection-thread formation, cortical cell division, and ultimately nodule development

Mycorrhizal associations

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of more than 80% of terrestrial plants. The fungus improves plant uptake of water and mineral nutrients, especially phosphate, while receiving lipids and carbohydrates from the host. Unlike the relatively specific legume–rhizobia interaction, mycorrhizal associations are widespread across plant groups.

AMF and rhizobia appear to use parts of an ancient, conserved plant–microbe signalling pathway. Both interactions involve plant recognition of microbial signals and activation of symbiosis-related genes, including *ENOD11* in *Medicago*.

Experiments in which fungal hyphae and roots were separated by a membrane showed that AMF release a diffusible signal capable of activating the *Medicago ENOD11* promoter. Because physical contact was prevented, this provided early evidence that AMF produce soluble signalling molecules that prepare the plant for colonization.onlinelibrary.

A key early step in mycorrhizal establishment is hyphal branching near the host root. Branching increases the chance that fungal hyphae will contact the root and form an appressorium, the structure used to enter root tissues.

The main plant-derived branching signals are strigolactones, carotenoid-derived sesquiterpene lactones released in root exudates. In *Lotus japonicus*, 5-deoxy-strigol was identified as a potent inducer of hyphal branching in *Gigaspora* spores.

Strigolactone production is strongly influenced by nutrient status. Phosphate-limited plants release more branching factors, which helps recruit AMF when the plant needs improved phosphorus acquisition. Flavonoids are not considered the primary branching factors, since maize mutants unable to synthesize flavonoids still produce exudates with branching activity.

After hyphal contact, AMF form appressoria and colonize the root cortex, where they develop highly branched structures called arbuscules. These structures are the main sites of nutrient exchange between the fungus and plant.

Although colonization involves fungal invasion, plants restrict fungal growth mainly to the cortex and prevent extensive spread into other tissues. This indicates that the host retains control over the symbiosis.

Mycorrhizal roots show only weak, transient, or localized defence responses compared with pathogen interactions. Some defence-related pathways are activated, including phenylpropanoid metabolism, oxidative-stress enzymes, and pathogenesis-related genes, but these responses generally do not prevent colonization.

Mycorrhizal roots also show altered activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and peroxidases. These enzymes help manage reactive oxygen species during colonization.

2.4. PGPR-mediated enhancement of soil nutrients

- **Macronutrient mobilization**

PGPR improve the availability of major plant nutrients-especially nitrogen, phosphorus, and potassium-by converting insoluble or atmospheric forms into compounds that roots can absorb. These processes can reduce dependence on synthetic fertilizers and support more sustainable crop production.

-Nitrogen fixation : PGPR supply nitrogen through two main routes: symbiotic fixation and associative or free-living fixation. Symbiotic diazotrophs, such as *Rhizobium* and *Bradyrhizobium*, form nodules on legume roots, where nitrogenase converts atmospheric N_2 into ammonia

- Phosphorus mobilization : Although phosphorus is abundant in soils, much of it is fixed in insoluble mineral or organic forms. Phosphate-solubilizing bacteria (PSB), including *Pseudomonas* and *Bacillus*, release low-molecular-weight organic acids such as gluconic, citric, and oxalic acids.

These acids lower rhizosphere pH and chelate metal cations such as Ca^{2+}, Al^{3+}, and Fe^{3+}, disrupting phosphate complexes and releasing soluble orthophosphate ($H_2PO_4^-$, and HPO_4^{2-}).

-Potassium mobilization : Potassium is often locked within silicate minerals such as mica, biotite, muscovite, feldspar, and illite. Potassium-solubilizing bacteria (KSB), including *Bacillus mucilaginosus* and some *Pseudomonas* species, release organic acids that weather these minerals and liberate soluble K^+.

Acidification, proton release, chelation, and the production of extracellular polysaccharides can destabilize mineral structures and increase potassium in the soil solution. This is particularly valuable in degraded or potassium-deficient soils.

- **Micronutrient availability enhancement**

PGPR also help plants overcome micronutrient deficiencies, especially iron deficiency. In alkaline and aerobic soils, iron commonly occurs as insoluble ferric iron (Fe^{3+}), which plants cannot easily absorb and which can lead to iron-deficiency chlorosis.

To acquire iron, many PGPR produce siderophores-small, high-affinity iron-chelating compounds. They bind ferric iron and form soluble Fe–siderophore complexes that can be taken

up by bacteria or made available to plant roots through receptor-mediated uptake or ligand exchange.

Siderophores are classified into three major groups: catecholates, hydroxamates, and carboxylates. By increasing iron solubility and uptake, they reduce chlorosis, improve chlorophyll formation, and support plant growth under iron-limited conditions.

Siderophore production also gives PGPR a competitive advantage. By strongly binding available iron, they limit its accessibility to pathogenic microorganisms, thereby contributing to biological control.

In addition, siderophores can bind other metals, including zinc, lead, and cadmium. This can reduce the bioavailability and phytotoxicity of heavy metals, helping plants tolerate metal-contaminated soils.