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Review

Modulators or facilitators? Roles of lipids in plant root–microbe interactions

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Lipids have diverse functions in regulating the plasma membrane's cellular processes and signaling mediation. Plasma membrane lipids are also involved in the plant's complex interactions with the surrounding microorganisms, with which plants are in various forms of symbiosis. The roles of lipids influence the whole microbial colonization process, thus shaping the rhizomicrobiome. As chemical signals, lipids facilitate the stages of rhizospheric interactions – from plant root to microbe, microbe to microbe, and microbe to plant root – and modulate the plant's defense responses upon perception or contact with either beneficial or phytopathogenic microorganisms. Although studies have come a long way, further investigation is needed to discover more lipid species and elucidate novel lipid functions and profiles under various stages of plant root–microbe interactions.

Highlights

Lipids have diverse functions across the different stages in the formation of plant root–microbe interactions, commencing from the shaping of the rhizomicrobiome to the establishment of symbiosis.

Plant rhizodeposition and microbe-generated signals involve lipid substances, which act as chemical signals that are exchanged for successful microbe recruitment or phytopathogen defense.

The plasma membrane, as the interface for plant roots and microbes, is where lipids' intrinsic functions modulate and facilitate interactions.

How lipids are involved in rhizosphere interactions

Lipids (see Glossary), a ubiquitous class of biomolecules and major constituents of eukaryotic and prokaryotic cell membranes, are essential for a complete study of cell biology and functions. Lipids serve as structural components of plasma and intracellular membranes, provide energy and carbon storage, mediate cell signaling pathways, and regulate stress responses [1–3]. Because of their diverse roles, lipid homeostasis and lipid metabolism have systemic effects that can influence plant development and performance [4].

Numerous studies on aboveground plant–microbe interactions have already been documented; however, investigations of the **rhizosphere** have been restricted due to limitations in the application of 'omics' techniques [5] and difficulties in simulating a natural underground ecosystem [6]. These interactions have been investigated mostly at metabolic and transcriptomic levels, but there are still many questions on the biochemical exchange and communication between the involved organisms. Rhizosphere-related phenomena such as rhizodeposition and plant–microbe signaling are critical underground processes that still need to be further explored [7] and in which lipids are highly involved [8,9]. In the rhizosphere, whenever plant roots communicate with or contact microbes, molecular information is exchanged. Microbe recognition occurs at the plasma membrane (PM), which acts as the interface, either allowing advantageous resource exchange or inhibiting interaction through downstream signaling cascades [10,11]. Although the cell wall is the outermost border of the plant body that provides general resistance upon microbe penetration [12], the PM serves as the critical player in signaling responses to external stimuli, initial microbe recognition, and multiple downstream responses, which the microbes attempt to manipulate to suppress plant defense responses to colonize and procure nutrients [10,11,13]. As major components of the PM, lipids establish the physical barriers on the living cell surfaces, influence the communication between the host and microbe, and serve as signaling molecules or providers of elicitors for recognition, thereby influencing the establishment or prevention of microbial colonization [8,14–16].

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This review highlights the various roles of lipids *in planta* and at the different stages of plant root–microbe interactions, from (i) signaling and resource exchange; to (ii) pattern recognition, signal transduction, and downstream defense mechanisms during perception and contact; and to (iii) the establishment of **symbiosis** that can either impart plant growth promotion and stress tolerance or cause death. For reference to specific lipid species mentioned throughout the review, we follow the LIPID MAPS comprehensive classification system for lipids. This classification organizes lipids, covering eukaryotic and prokaryotic sources, into eight well-defined categories (Box 1 and Figure 1) [17] and hereafter is referred to in italicized square brackets (‘*[ ]*’) with abbreviated names to classify uncommonly known lipids.

Plant root PM and the lipids regulating its functions

The PM is a highly ordered fundamental biological structure that separates the interior of a living cell from the extracellular environment. It functions like a sensor that regulates cellular activities with an intricate pathway that orchestrates reception, signal transduction, and appropriate response mechanisms against a continuously changing environment [18].

Lipids, which compose and maintain the structural integrity of the PM, largely contribute to maintaining its essential processes and facilitating abiotic stress adaptation, intra- and intercellular communication, and nutrient exchange during an interaction [18]. The main lipid

Box 1. Lipid categories and description (refer to structure in Figure 1)

Fatty acyls *[FA]* represent the primary building blocks of complex lipids and therefore are one of the most fundamental categories of biological lipids. They are characterized by a series of methylene groups imparting their hydrophobic characteristic and are subdivided depending on double bonds in their hydrocarbon chains. Common examples include fatty acid esters such as wax monoesters and diesters and lactones.

Glycerolipids *[GL]* are mainly composed of mono-, di-, and trisubstituted glycerols, the most well known of which are the fatty acid esters of glycerol or acylglycerols. They comprise the bulk of oil storage in plant tissues.

Glycerophospholipids *[GP]*, also known as ‘phospholipids’, are amphipathic molecules with a polar head consisting of glycerol and a phosphate group and a nonpolar tail made of hydrocarbon chains. As critical components of the PM bilayer, they act as binding sites for intra- and intercellular proteins and are involved in cell metabolism and signaling.

Sphingolipids *[SP]* are a family of compounds sharing a common structural feature – a sphingoid base backbone synthesized *de novo* from serine and a long-chain fatty acyl–coenzyme A then converted into products such as ceramides, phosphosphingolipids, glycosphingolipids, and other derivative species. They have protective functions and play important roles in cellular signaling.

Sterol lipids *[ST]* are important components of the cell membrane, participating in signal transduction. The most well-known examples are phytosterol in plants and cholesterol and derivatives, such as steroids with specific roles as hormones and signaling molecules.

Prenol lipids *[PR]* are synthesized from the 5-carbon precursors, isopentenyl diphosphate and dimethyl diphosphate, which are mainly produced via the mevalonic acid pathway. They are essential for immune response and some regulatory functions, such as carotenoids that function as antioxidants and precursors of vitamins A and E.

Saccharolipids *[SL]* are composed of a fatty acid linked to a sugar backbone, forming structures that are compatible with membrane lipid bilayers. They can be found in the lipid A component of lipopolysaccharides in gram-negative bacteria as acylated glucosamine precursors.

Polyketides *[PK]* have great structural diversity and compose many secondary metabolites. They are often cyclic molecules whose backbones are further modified by glycosylation, methylation, hydroxylation, oxidation, and other processes. PKs are found in many antimicrobial, antiparasitic, and anticancer agents, including PK derivatives such as erythromycins, tetracyclines, and avermectins [17,84,85].

Glossary

Induced systemic resistance (ISR): plant defense response induced by beneficial and mutualistic colonizing microbes such as plant growth–promoting rhizobacteria and fungi against foliar pathogens and leaf-feeding insects, which initiate JA and SA signaling pathways.

Lipidomics: the science that analyzes the complete set of lipid species in a cell, tissue, or biological system (called the ‘lipidome’) through the application of analytical chemistry principles and techniques such as chromatography, spectroscopy, and mass spectrometry.

Lipids: various organic compounds that are insoluble in water but soluble in nonpolar solvents such as ether and chloroform. They are one of the macrobiomolecules (carbohydrate, protein, nucleic acid, containing hydrocarbons), which are the principal structural components of living cells.

Lipid peroxidation: the oxidative degradation of lipids, which occurs when free radicals abstract an electron from an unsaturated fatty acid in the cell membrane, creating unstable lipid radicals that can react with oxygen, leading to cell damage. This process can be mediated via enzymatic pathways (lipoxygenase) or nonenzymatic pathways (reactive oxygen species).

Mass spectrometry (MS): a powerful technique used to quantify known and identify unknown compounds by elucidating the structure and chemical properties of different molecules by measuring the mass and charge of those molecules or their fragments after ionization. Mass spectrometry (MS) often is hyphenated with chromatographic separation techniques. A typical MS-based workflow consists of biological sample collection, sample pretreatment and extraction, data acquisition, data processing and analysis, and biological interpretation.

Priming: a plant’s innate immune and physiological response triggered by beneficial microbes that helps the plant react more rapidly and/or robustly against future biotic and abiotic stimuli.

Quorum sensing (QS): a cell density–dependent signaling mechanism that regulates important bacterial biological processes, including bioluminescence, DNA transfer, antibiotic resistance, motility, biofilm formation, and virulence.

Rhizodeposits: a collective term for C-containing compounds released by the

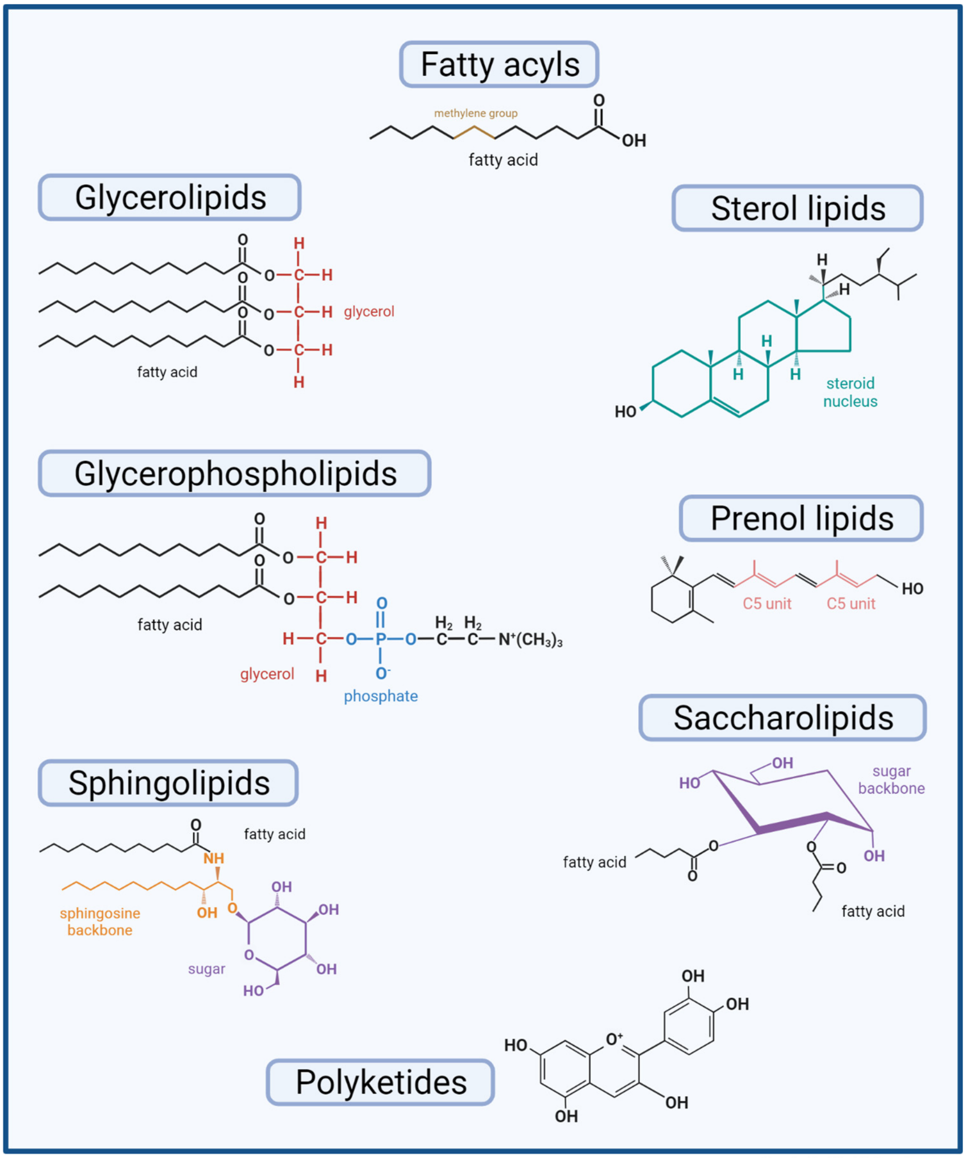

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Figure 1. Representative structure for each lipid category. The LIPID MAPS consortium has subdivided lipids into eight principal categories based on their functions, chemical characteristics, and specificities. A more consistent format for representing lipid structures has been proposed by Fahy *et al.* [84], in which, in the simplest case of the fatty acid derivatives, the acid group (or equivalent) is drawn on the right hand and the hydrophobic hydrocarbon chain is on the left, with some notable exceptions. Fatty acyls *[FA]* have an aliphatic chain of methylene groups and can be subdivided into saturated or unsaturated. Glycerolipids *[GL]* are characterized by the number of glycerol groups. Glycerophospholipids *[GP]* are amphipathic with a polar glycerol and phosphate group and nonpolar hydrocarbon. Sphingolipids *[SP]* have a sphingoid backbone. Sterol lipids *[ST]* have a sterol nucleus composed of four tightly fused carbon rings and a hydroxyl group attached to the first ring. Prenol lipids *[PR]* are synthesized from the five-carbon precursors (isopentyl diphosphate and dimethylallyl diphosphate). Saccharolipids *[SL]* have fatty acid and a sugar backbone. Polyketides *[PK]* have various structural forms, often as cyclic molecules with modifications such as methyl group or hydroxyl groups. Descriptions of each lipid category are provided in Box 1. Image created with BioRender.com.

roots, which can have various origins: at root apices, from sloughed-off root cells, border cells, and tissues; mucilage released from the root caps and root hairs; root exudates; volatile organic compounds; lysates released by senescing epidermal and cortical cells; and altered root-derived C by microbes or symbionts.

Rhizosphere: soil influenced by roots in terms of the microorganisms, caused by chemical changes in the soil due to the presence of the root and its secretions, including primary metabolites (e.g., carbohydrates, organic and amino acids) and secondary metabolites (e.g., alkaloids, terpenoids, and phenolics).

Symbiosis: any type of close and long-term ecological interaction between organisms from two different species, which usually benefits one or both organisms involved, be it mutualistic, commensalism, parasitic, or pathogenic.

Systemic acquired resistance (SAR): a ‘whole-plant’ resistance response that is activated by exposure to elicitors of virulent, avirulent, or nonpathogenic microbes or artificial chemical stimuli such as chitosan that triggers SA signaling.

components found in the PM are glycerophospholipids *[GP]*, glycerolipids *[GL]*, sphingolipids *[SP]*, and sterol lipids *[ST]* [14]. GP, which have different head groups, make up its principal constituent, with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) as major components and phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidic acid (PA), and phosphatidylserine (PS) as minor components [14]. Phosphatidylinositol phosphates (PIPs), composed of a PI backbone with up to three phosphorylations on the inositol moiety, represent a minor fraction of GP. They are involved in many regulatory processes such as cell signaling and intracellular trafficking. GP are characterized by different lengths and the degree of unsaturation of their fatty acyl chains [14]. GL neutral lipids such as diacylglycerols (DAGs) have been found to be present at the PM of root epidermal cells in the transition zone and the apex of growing root hairs [19], whereas digalactosyldiacylglycerols (DGDGs) in the PM are found particularly as a phosphate deprivation response [20]. SP in plants are grouped into four classes: glycosyl inositolphosphoceramides (GIPCs), glucosylceramides, ceramides, and free long-chain bases, representing 64%, 34%, 2%, and 5% of total sphingolipids in Arabidopsis, respectively [21]. Just recently, GIPC SP have been shown to have essential roles in PM organization as necrosis and ethylene-inducing peptide 1-like (NLP) toxin receptors [22]. Over 250 different phytosterols have been identified in plants, with sitosterol being the dominant form in most of them, followed by isofucosterol (delta-5 avenasterol) also found in many plant species, and a few notable examples such as stigmasterol in tobacco and spinasterol in *Medicago* [23].

Lipids are vital components in the plant PM's physiological functions, such as regulating hormone signaling and transport, abiotic stress responses, plasmodesmata functions, and plant–microbe interactions [14]. For example, sterols are involved in endocytosis and recycling at the PM of PIN-FORMED (PIN) auxin carriers. This subcellular localization of sterol-mediated auxin carriers impacts the auxin distribution at the tissue level, affecting plant development such as the response of roots to gravity [24]. The length of the SP acyl chains (very-long-chain fatty acids) is also involved in the secretory sorting of the efflux phytohormone auxin carrier PIN2 and auxin redistribution during root gravitropism [25]. During abiotic stress responses, certain lipids function as secondary messengers. Phospholipase-derived PA, oxylipins, PIPs, SP, fatty acids, lysophospholipids, *N*-acylethanolamines, and galactolipids have been found to function in this manner [26,27]. A study on plasmodesmata by Grison [28] identified the role of sterols in modulating cell-to-cell connectivity by potentially establishing the positional specificity of callose-modifying glycosylphosphatidylinositol-anchored proteins at the PD, which also brought attention to the potential roles of GIPC and GL. Plants detect microbes by sensing non-self and modified-self molecules via cell surface and intracellular localized immune receptors [29]. Plant PM lipids and lipid-derived metabolites have been shown to facilitate the plant immune signaling response. For example, after sensing a pathogen, phospholipid-hydrolyzing enzymes are mobilized to trigger signaling cascades vital for cellular responses. As such, crucial messenger molecules, such as oxylipins, jasmonates, and notably PA, that regulate the activity of defense-associated proteins are generated by phospholipases [30]. Other PM lipid compounds involved in plant defense are phosphoinositides and lysophospholipids, which include lysophosphatidic acid (LPA), lysophosphatidylcholine, sphingosylphosphorylcholine, and sphingosine-1-phosphate. Several factors control the signaling activity or specificity of these compounds, such as the length and position of the acyl chain, the degree of saturation, and the presence of the phosphate head group [31]. As core components of membranes accumulating in the PM, plant sterols serve as conserved regulators of its organization, such as an increase in membrane stability and a decrease in membrane permeability, both associated with defense induction against phytopathogenic fungi [32].

Lipids in action

Rhizospheric interactions and signaling: Lipids as chemical language

Plant roots are contained within and highly influenced by the rhizosphere [33]. Because of their organotrophic nature, microorganisms such as bacteria, fungi, and protists populate this area – potentially extending the plant's performance and capacity to adapt to the environment or using nutrients at the plant's expense through niche colonization [8]. The behavior and impact of these microbes are believed to rely heavily on soil compounds in a process known in general terms as 'signaling or communication highways', 'underground interactions', 'rhizosphere chemical language', or 'complex plant–microbe interactions' [7,9]. The rhizospheric interactions can be grouped into three signaling categories: signaling from plant roots to microbes, microbial intra- and interspecies signaling, and signaling from microbes to plants [16] (Figure 2A, Key figure).

Plant root to microbe signaling through rhizodeposition

The first stage in a rhizospheric interaction is the recruitment of the rhizobiome into the plant vicinity, which is mediated by **rhizodeposits**. The release of rhizodeposits comes with a wide variety of substances, such as sugars, amino acids, organic acids, enzymes, growth factors and vitamins, flavanones and purines/nucleotides, and miscellaneous substances [34]. Lipids in various forms, such as fatty acids and sterols, are also among these compounds that enrich the rhizosphere chemistry [34]. Plants initiate an interaction by secreting these chemical signals into the rhizosphere. Some examples are plant-produced flavonoids *[PK]* such as 2-phenyl-1,4-benzopyrone derivatives involved in root nodule formation [35]; inhibitory flavonoids such as phytoalexin, medicarpin, and glyceollin [36]; and the volatile organic compound (VOC) (E)-β-caryophyllene *[PR]* that functions as a plant bioprotectant against herbivores and pathogens and as an attractant for organisms preying on root-feeding herbivores from maize roots [37,38]. These chemotactic attractors can facilitate the recruitment, nutrition, shaping, and tuning of the microbial communities from a reservoir of microorganisms present in the soil by encouraging, limiting, or inhibiting microbial activity and proliferation [8,9,36] (Figure 2A-1).

Microbe perception of plant root–released compounds and other microbial signals

The second stage of the rhizosphere interaction is the perception or detection of low-molecular-weight compounds, such as lipid molecules, released by the plant roots (or other microbes) by microbes, resulting in catabolism, transformation, or rejection of the perceived compound [16]. Perception of these compounds then leads to the stimulation of regulatory or signaling cascades that cause various responses in the microbes. Root-secreted substances have been thought to influence the gene expression of different microorganisms in the rhizosphere, which are not in proximity to and associated with plants [36]. Chemical communication (cell–cell signaling) with signal molecules called 'autoinducers' that increase in concentration as a function of cell density, found in bacteria [39] and recently in fungi [40], coordinate a wide range of activities within and between different species as a function of population density. The regulation of gene expression in response to fluctuations in microbial cell population density is done via **quorum sensing (QS)** [41]. QS is a widespread process using autoinducing chemical signals that coordinate diverse co-operative functions [39]. This governing mechanism is instrumental in regulating a wide array of physiological activities and microbial phenotypes such as biofilm formation, pathogenicity, conjugation, secretion of hydrolytic enzymes, and production of antibiotic and secondary metabolites, increasing rhizosphere competence that leads to successful colonization [42]. Common examples of lipids that act as QS signals are N-acyl homoserine lactones (AHL) *[FA]* [39], diffusible signal factor (DSF) family *[FA]*, and the more recently discovered pyrones and dialkylresorcinols *[PK]* from gram-negative bacteria [43–46]. There are also antimicrobial lipids released at low concentrations, as well as alcohols from fungal species (mostly *Ascomycetes*), which are associated with developmental processes [34,47]. Some VOCs, such as aldehyde and ketone *[FA]* and terpene

Key Figure

Lipid roles in the microbial colonization of roots

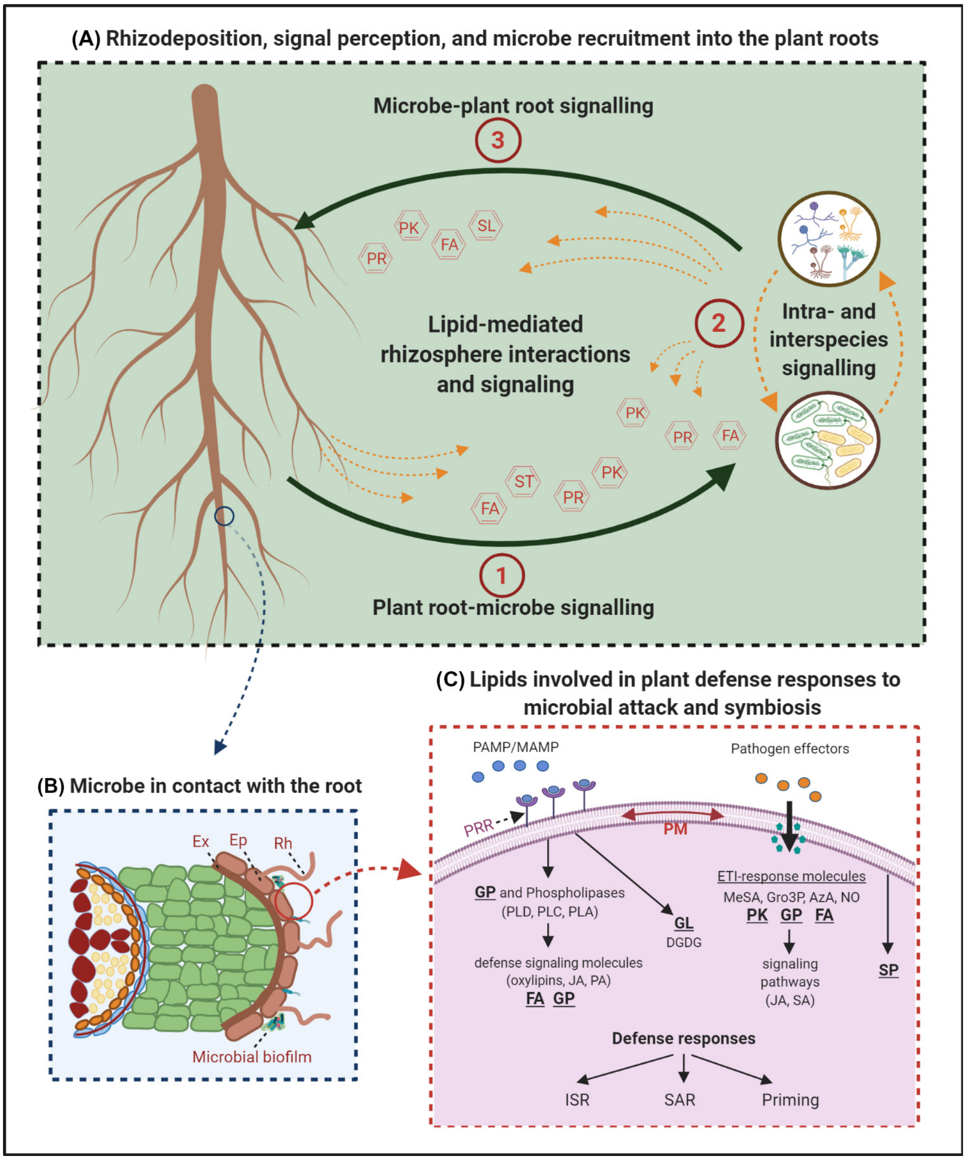

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Figure 2. (A) During the different stages in rhizosphere interactions (plant root to microbe, microbe to microbe, microbe to plant root), lipids act as chemical signals that facilitate signaling: from the release of rhizodeposits from the roots that comes with a variety of substances (through rhizodeposition), to the perception of these substances by microbes to regulate intra- and interspecies behavior, and to the release of microbial substances back to the plants that can influence plant gene expression, hormonal balance, development, metabolism, and stress responses. Some of the known lipid molecules involved in rhizosphere signaling belong to the categories of fatty acyls *[FA]*, sterol lipids *[ST]*, prenol lipids *[PR]*, saccharolipids *[SL]*, and polyketides *[PK]*. (B) Successful attachment and colonization of microbes lead to the formation of microbial biofilms on root surfaces. (C) Different lipid species perform various functions during the signal reception at the PM, transduction, and downstream defense mechanism of plants in response to microbial attack or beneficial symbiosis, which can be induced systemic resistance (ISR), systemic

(Figure legend continued at the bottom of the next page.)

[PR], which are released by a wide range of bacterial and fungal species, can play crucial roles in long-distance rhizosphere interactions [48]. Some can act like 'chemical weapons' that inhibit microbial activity or interfere with other QS systems (interspecies). Moreover, they can coordinate gene expression and influence intraspecies behaviors such as biofilm formation, virulence, and stress tolerance [49]. All these QS signals and VOCs released by microorganisms can also act as interkingdom signals, influencing plant gene expression and immunity and affecting plant root architecture, growth, and development [16,48] (Figure 2A2).

Signaling response from microbes back to the plant

The third stage in the rhizosphere interaction involves the release of diverse signaling molecules from microorganisms to their plant host as a response to signals perceived from among themselves (intraspecies), other microorganisms (interspecies), or plants. Conserved microbe-specific molecules known as pathogen- or microbe-associated molecular patterns (PAMP/MAMP), such as lipopolysaccharides (which have the active lipid A), peptidoglycans, flagellin, and chitin, are detected by dedicated pattern recognition receptors (PRR) from plants [50]. These signals trigger a local basal systemic defense response controlled by regulatory networks that involve signaling pathways via plant hormones, including salicylic acid (SA), jasmonic acid (JA), and ethylene [51,52]. The defense response of plants can be **induced systemic resistance (ISR)** [52], **systemic acquired resistance (SAR)** [51], or **priming** [53]. Although these mechanisms are designed to prevent potential attacks, beneficial rhizosphere microbes have developed countermeasures for immune recognition, leading to successful plant colonization [50]. Molecules such as QS signals and VOCs used for intra- and interspecies signaling also act as interkingdom signals, influencing plants [16,48]. Examples of microorganism–released QS molecules are AHL from plant growth–promoting rhizobacteria (PGPR) and DSF from *Xanthomonas.* Certain antimicrobials at sub-inhibitory concentrations, such as 2,4-diacetylphloroglucinol *[PK]* from rhizosphere pseudomonads, can induce ISR responses (via SA and ethylene signaling) against fungal and bacterial leaf pathogens [54] and affect root development via an auxin-dependent signaling pathway [55,56]. They can change the gene expression and protein profiles of plant roots and shoots; influence root development, plant defense, and stress responses; and regulate metabolic activity and hormonal balance [57,58]. Other microbial signaling molecules belong to VOCs, which can act as plant growth promoters or inhibitors and as priming agents or elicitors of plant defense and stress tolerance (Figure 2A3).

Plant root lipids during pathogenic and symbiotic interactions with microorganisms

Apart from acting as chemical signals in rhizosphere signaling, lipids are also involved in diverse functions during pathogen attack or infection and mutualistic interactions with beneficial microorganisms. Specific lipids in the PM have particular functions, such as in host-specific pathogen recognition, signaling in the cells from the site of infection or interaction, and transfer of infection signals to distal organs of the plants during defense responses [2,31,59,60]. Signal-inducing compounds from microbes or elicitors are recognized by the plant's innate immune system, which results in the induction of defense responses [59] or invasion of host tissues [13].

acquired resistance (SAR), and priming. Lipids that are particularly involved belong to the categories of glycerophospholipids *[GP]*, glycerolipids *[GL]*, sphingolipids *[SP]*, fatty acyls *[FA]*, and polyketides *[PK]*. Abbreviations: AzA, azelaic acid; Ep, epidermis; ETI, effector-triggered immunity; Ex, exodermis; Gro3P, glycerol-3-phosphate; JA, jasmonic acid; MeSA, methylsalicylic acid; NO, nitric oxide; PA, phosphatidic acid; PAMP/MAMP, pathogen-/microbe-associated molecular pattern; PLA, phospholipase A; PLC, phospholipase C; PLD, phospholipase D; PM, plasma membrane; PRR, pattern recognition receptor; RH, root hair; SA, salicylic acid. Image created with BioRender.com.

Lipids in the PM play essential roles in plant cell responses to microbial attack and interactions with beneficial microbes. These lipids are synthesized, modified, or reallocated upon the upregulation of genes encoding enzymes of lipid metabolism. Lipid-modifying enzymes regulate the spatial and temporal production of lipid metabolites involved in signaling and membrane proliferation to establish intracellular compartments or compositional changes of the bilayer [59]. During plant–pathogen interaction, phospholipid-hydrolyzing enzymes induce the production of defense-signaling molecules such as oxylipins, including JA and the potent second messenger PA [61]. For example, phospholipase D (PLD), which forms PA, is involved in lipid metabolism and hormone signaling in plant defense responses [62]. Plant-derived PA regulate a range of different physiological processes, such as activities of kinases, phosphatases, phospholipases, and proteins involved in membrane trafficking, Ca^{2+} signaling, or the oxidative burst [62]. They also act as precursors for lipid intermediates LPA, DAG, and free fatty acids, which are involved in plant defense signaling [63]. Activation of phospholipase C (PLC) or the DAG kinase pathway is triggered by PAMP recognition, also leading to the accumulation of PA [64]. Phospholipase A (PLA), which yields FA and lysophospholipids, the latter of which are involved in systemic responses after wounding, is involved in growth regulation, root and pollen development, stress responses, defense signaling, and plant immunity because of its roles in oxylipin and JA biosynthesis [65]. Phosphoinositides have also been implicated in the modulation of mutualistic interactions between plants and beneficial microorganisms and is known to involve ionic and cytoskeletal changes [26,66,67]. Lipid kinases in plants, particularly the phosphatidylinositol 3-kinase (PI3K) family, regulate various physiological functions, such as the innate immune response, intracellular trafficking, autophagy, and senescence, including symbiosis of leguminous plants with rhizobia and arbuscular mycorrhizal fungi [68–70].

At the site of infection during pathogen attack, small molecules that serve as initial signals of the effector-triggered immunity (ETI) response, such as methylsalicylic acid (MeSA), are produced and then moved to the distal plant organs, where they are hydrolyzed into SA that trigger SAR. Other signal molecules, such as glycerol-3-phosphate (Gro3P), azelaic acid (AzA), and nitric oxide (NO), function as inducers of SAR [42]. However, mutualistic or symbiotic interactions with beneficial plant growth–promoting microorganisms can stimulate the plant immune system, which results in ISR, mainly based on the activities of JA [52] that mediate resistance to a wide array of diseases. Galactolipids also have essential roles in signal transduction, cell communication, and pathogen response. For example, galactolipids have different functions in SAR, with monogalactosyldiacylglycerol (MGDG) regulating the biosynthesis of AzA and Gro3P and with DGDG affecting the biosynthesis of NO and SA [71]. In roots specifically, the accumulation of DGDG in the peribacteroid membrane of nodules helps to save phosphate because of reduced requirements for phospholipids during root–nodule symbiosis [72]. During arbuscular mycorrhizal formation (AMF), genes encoding enzymes for fatty acids from plastid and GL synthesis are upregulated, indicating the increased production of lipids during root mycorrhization [73]. This lipid demand might be explained by the requirements of phospholipids to establish a large surface of the periarbuscular membrane and by the accumulation of triacylglycerol in the fungus [59]. Lipid-derived signals by reduced arbuscular mycorrhiza 2 (RAM2) such as Gro3P acyltransferase (GPAT), involved in the synthesis of fatty acids associated with suberin and cutin, were also found to be involved in AMF formation [74,75].

A process that plays an important role in signal transduction and programmed cell death (PCD), which can be induced by both biotic and abiotic stresses, is **lipid peroxidation**. Lipids that can be subjected to peroxidation are galactolipids, free fatty acids, or acyl groups bound to triacylglycerol (TAG), leading to the generation of JA during defense response [76]. JA is mainly produced during wounding, such as after herbivore attack and microbe infection, and is vital for defense response to different fungal and bacterial pathogens [77]. Because some fungi, such as

Aspergillus spp., produce a set of oxylipins related to that of plants, it is speculated that plants and fungi 'communicate' via the oxylipin language [78]. AMF colonization leading to the accumulation of JA in barley roots also showed upregulation of the expression of JA biosynthetic genes (allene oxide cyclase, AOC; allene oxide synthase, AOS) [79]. SP have also been implicated in PCD, which is a defense reaction against pathogen attack [80]. For example, the expression of ceramide synthases in Arabidopsis (longevity assurance gene one homologs LOH1, LOH2, and LOH3) showed that although overexpression of LOH1 and LOH3 increases plant growth, overexpression of LOH2 results in dwarfing and the constitutive expression of hypersensitive response (HR) genes and PCD [81]. Moreover, an aspect in many studies is the accumulation of SA in SP mutants, which led to the proposal of a putative interaction between SP metabolism and SA signaling during PCD and HR [82].

The many essential functions of lipids can be assigned to distinct lipid categories (Figure 2) with unique chemical characteristics that allow a wide range of performances. This ability for specificity could be the reason that lipids evolved as signal molecules for communication between host plants and microorganisms in pathogenic and mutualistic interactions. Comprehensive lipid studies are necessary to further identify additional lipid chemistry that may be involved in plant root–microbe interactions. With substantial room for improvement in analytical techniques, much can still be done in plant lipid research, which can further our understanding of plant lipid metabolism and its interaction with the biotic environment.

Concluding remarks and future perspectives

Several research studies have shown the complex underground interactions that occur between plant roots and soil microorganisms through lipids. It has become apparent that plant lipids actively shape the microbiome inhabiting the rhizosphere and the subsequent colonization of their root tissues. We have presented lipid exchange in plant root–microbe interactions in three main stages: plant root to microbe, microbe to microbe, and microbe to plant root. On all accounts of these interactions, lipids have been shown to play essential roles as the 'chemical language' that facilitates the exchange of resources and modulates the cell responses by inhibiting pathogen attack or enhancing microbial symbiosis. There are many more currently unknown lipids with their corresponding signaling functions that are likely to exist which play a pivotal role in determining or shaping the rhizomicrobiome. Our understanding will become clearer through detecting, identifying, and quantifying the plant and microbial lipids *in situ* and while in interaction. This will shed light on their vital functions and how they behave, react, and transform in response to various stimuli. This knowledge will be greatly enhanced by continuous developments in analytical techniques, which will allow an effective qualitative and quantitative approach that can further our insights into lipid diversity and functions. **Lipidomics**, which is largely attributed to advances in its enabling technology, **mass spectrometry (MS)**, has been a powerful tool in the characterization of the structures of lipid species; quantification of the level of individual lipid species in biological samples; and determination of interactions of individual species with other lipids, metabolites, and proteins *in vivo* [83]. The large-scale analysis of the lipid compounds can then be combined with transcriptomic and genomic studies to begin to uncover the genes involved in the signaling and various stages of the interactions. These integrated 'omics' studies will be useful in the holistic interpretation of biological systems such as plants and how they respond to environmental biotic and abiotic stressors.

As part of rhizospheric signaling and interaction studies, research on lipids will therefore open new avenues to increase crop productivity and tolerance to environmental stresses. Harnessing the use of beneficial microorganisms and understanding the mechanisms of pathogenicity and symbiosis could reduce the use of agrochemicals. Microbial solutions can

Outstanding questions

Are specific interactions between plants and microbes common occurrences in the rhizosphere, or are they limited to a few organisms?

How do environmental abiotic stressors such as high or cold temperature, drought or flooding, metal toxicity, and salinity affect the underground, lipid-mediated plant–microbe interactions?

To what extent will lipidomics and other omic technologies contribute to discovering novel lipid molecules involved with *in planta* and *ex planta* functions and understanding the molecular mechanisms involved in signaling and rhizosphere interactions?

How conserved are lipid signaling mechanisms between various plant and microbial species?

How transferable will knowledge gained from the relatively few investigated cases of lipids in the rhizosphere be to application to improve plant performance in field conditions?

then be used for more sustainable agriculture to enrich the rhizomicrobiome for beneficial microbes, thereby improving plant performance and resistance to biotic and abiotic stresses. From a broader perspective, lipid research on plant–microbe interactions can also provide biotechnological solutions, as with the broad spectrum of antimicrobial lipids isolated from plants, which can be used as promising alternatives to control microbial infections. This can be applied in various economic sectors aside from agriculture, such as the pharmaceutical, cosmetic, and food industries (see Outstanding questions).

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Declaration of interests

The authors have no interests to declare.

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