



Review

The soybean rhizosphere: Metabolites, microbes, and beyond—A review

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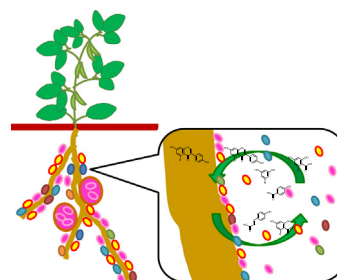
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HIGHLIGHTS

- Rhizosphere microbial communities are important for plant health.
- Specialized metabolites in the rhizosphere influence the microbial communities.
- Isoflavones and saponins are major specialized metabolites secreted by soybean.
- Secretion is regulated developmentally and nutritionally.
- Possible links between specialized metabolites and microbial communities are highlighted.

GRAPHICAL ABSTRACT



Effect of root-secreted metabolites in the rhizosphere

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ABSTRACT

The rhizosphere is the region close to a plant's roots, where various interactions occur. Recent evidence indicates that plants influence rhizosphere microbial communities by secreting various metabolites and, in turn, the microbes influence the growth and health of the plants. Despite the importance of plant-derived metabolites in the rhizosphere, relatively little is known about their spatiotemporal distribution and dynamics. In addition to being an important crop, soybean (*Glycine max*) is a good model plant with which to study these rhizosphere interactions, because soybean plants have symbiotic relationships with rhizobia and arbuscular mycorrhizal fungi and secrete various specialized metabolites, such as isoflavones and saponins, into the soil. This review summarizes the characteristics of the soybean rhizosphere from the viewpoint of specialized metabolites and microbes and discusses future research perspectives. In sum, secretion of these metabolites is developmentally and nutritionally regulated and potentially alters the rhizosphere microbial communities.

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Introduction

Soybean (*Glycine max*) is a major crop worldwide, with over 300 million tonnes produced globally. In contrast to cereals such as corn (maize; *Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*), soybean produces seeds containing many proteins and lipids, which make soybean particularly nutritious. In Japan, soybean is used as a raw material for tofu, natto, soy sauce, and miso, but elsewhere the seed is used mainly for oil and cattle feed.

Soybean also contains various plant specialized (secondary) metabolites, such as isoflavones and saponins, as functional ingredients [1,2]. Because soybean plants establish symbiotic relationships with rhizobia and arbuscular mycorrhizal fungi, the crop does not require much fertilizer to produce seeds. In reality, however, a large amount of fertilizers is supplied to soybean fields to maximize yield. Intensive use of fertilizers can lead to environmental problems such as eutrophication of rivers and lakes and global warming. Sustainable agricultural production requires that both yield and environmental issues be considered. Thence, the recruitment of rhizosphere microbes is necessary for sustainable soybean production.

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The rhizosphere is defined as “the zone of soil surrounding the root which is affected by it” [3,4]. Roots exert both physical influences, such as by root structure or heat generation, and chemical influences, such as by the secretion of a wide variety of plant-derived metabolites. Plant roots secrete metabolites into the rhizosphere actively using the energy from ATP and passively through diffusion [5,6]. Metabolites are also released into the rhizosphere as root tissues such as border cells become detached from the main root body [7].

Plants secrete both low-molecular-weight compounds, such as amino acids, sugars, phenolics, terpenoids, and lipids, and high-molecular-weight compounds, including proteins, polysaccharides, and nucleic acids, depending on the growth stage and environmental conditions [6]. Upon secretion into the rhizosphere, most metabolites are rapidly degraded by soil microbes, but some, especially specialized metabolites, remain in the soil and mediate biological communication [8,9]. The distribution of these metabolites in the rhizosphere varies depending on their chemical properties, with a relatively long-distance distribution of volatile compounds such as sesquiterpenes [10].

Metabolites secreted by soybean roots that function in biological communication in the rhizosphere are shown in Fig. 1. Isoflavones and strigolactones are signal molecules for symbioses with rhizobia and arbuscular mycorrhizal fungi, respectively [5]. Glyceollin is biosynthesized as a disease-responsive phytoalexin. Glycinoeclepin A, which promotes hatching of soybean cyst nematodes [11], has potential functions in communication.

The communities and functions of rhizosphere microbes are distinct from those in bulk soils. Microbial diversity is reduced nearer to roots, with further reduction in the endosphere [12–15]. Accumulating evidence suggests that plants affect minerals and microbes in the rhizosphere [16–19]. The enhancement of the destabilization, solubilization, and accessibility of minerals in

the rhizosphere by plants is summarized elsewhere [20,21]. This review focuses on the metabolites and microbes of the rhizosphere of soybeans grown in hydroponic culture and in fields. The characteristics of the soybean rhizosphere in relation to sustainable agriculture are also discussed.

The keywords used in the search strategy, include rhizosphere, microbiome, metagenome, soil microbe, root exudate, secondary metabolite, specialized metabolite, and soybean field. The extracted information was collected from PubMed, Web of Science, and Google Scholar.

Metabolites

Plants produce a wide variety of low-molecular-weight compounds. These metabolites include a diverse range of bioactive compounds used in defence against both biotic and abiotic stresses and as attractants or repellents of other organisms. From an evolutionary perspective, most of these compounds are produced by certain species within a plant lineage and are called specialized metabolites. Researchers have estimated that more than 200,000 specialized metabolites are produced by plants [22,23]. During their evolution, plants acquire the ability to synthesize new metabolites, which confer adaptive advantages in ecosystems [24].

Two classes of specialized metabolites dominate the root exudates of soybean [25,26], namely, isoflavones and saponins. As dietary components, soybean isoflavones have important functions in reducing the risk of breast and prostate cancers [27], promoting bone health [28], relieving menopausal symptoms [29], and preventing coronary heart disease [30]. Soybean saponins also have bioactive functions [2], such as anti-inflammatory effects [31], free-radical scavenging activity [32], anti-allergic activity [33], and immune modulatory activities [34]. This section focuses on

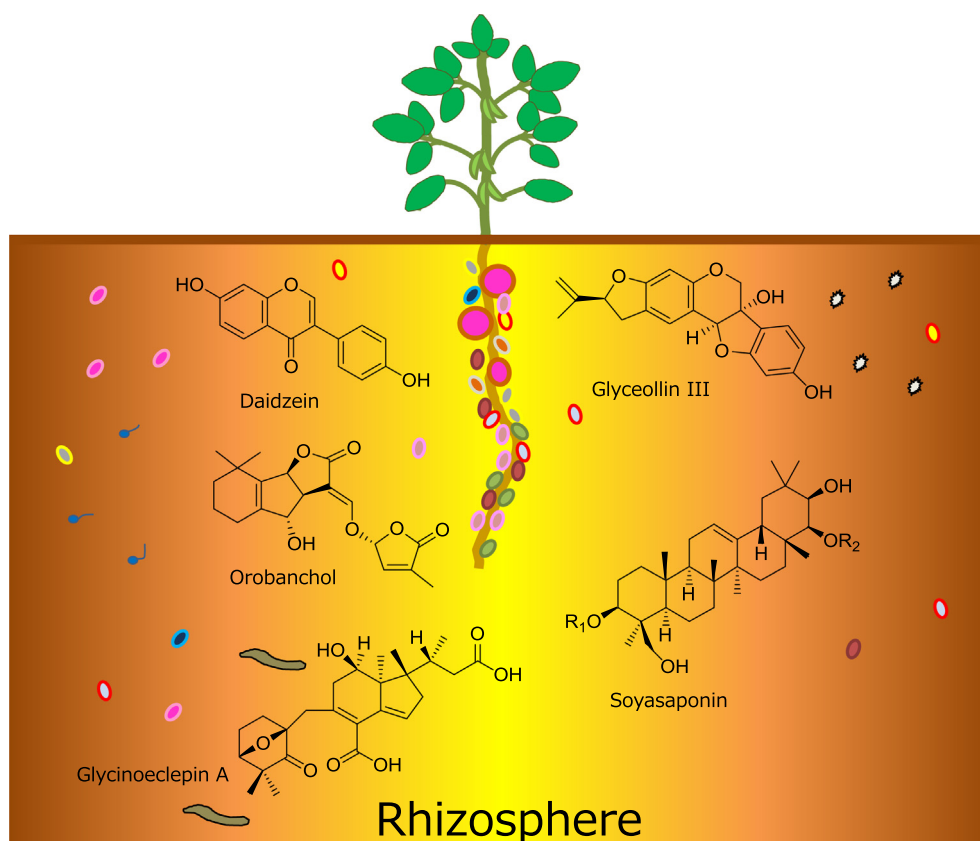


Fig. 1. Metabolites in the soybean rhizosphere.

isoflavones and saponins, but the recent findings on the secretion of other metabolites and their potential functions in the rhizosphere are also summarized.

Isoflavones

Isoflavones are a subgroup of flavonoids found predominantly in legume plants [35]. These flavonoids are produced via isoflavone synthase. Isoflavones are well known for their function in plant-microbe interactions, particularly in symbiosis and defence. In symbiosis, soybean roots secrete isoflavones such as daidzein and genistein into the rhizosphere as signal compounds for rhizobia to establish nodulation [36]. In defence, daidzein serves as a precursor for the biosynthesis of glyceollins and phytoalexins that have antimicrobial and/or anti-herbivore activities, and are induced upon infection by pathogens such as *Phytophthora sojae* and *Macrophomina phaseolina* [37]. Rhizosphere isoflavones also play various roles in biological communication with soil microbes [38,39]. Researchers have proposed two pathways for the secretion of isoflavones in soybean: (1) ATP-dependent active transport of isoflavone aglycones [40], and (2) secretion of isoflavone glucosides (possibly stored in vacuoles) into the apoplast, followed by the hydrolysis of glucosides with isoflavone conjugate-hydrolysing beta-glucosidase (ICHG) [41] (Fig. 2).

In hydroponic culture, daidzein was the predominant isoflavone in soybean root exudates throughout growth, with greater secretion during vegetative stages than during reproductive stages [25]. During reproductive stages, the secretion of malonylglucosides and glucosides increased to levels similar to those of aglycones. Under nitrogen deficiency, when nodule symbiosis occurred, the secretion of daidzein and genistein into the rhizosphere increased approximately 10-fold [25].

In field culture, both daidzein and genistein were found in the rhizosphere soil; the daidzein content was higher than that of genistein, as was the ratio of daidzein to genistein in the roots [42]. The isoflavone contents in rhizosphere soils were more than 100 times those in bulk soils at both the vegetative and reproductive stages. The degradation rate constant for daidzein in the soil was calculated to be $9.15 \times 10^{-2} \text{ (d}^{-1}\text{)}$, which corresponded to a half-life of 7.5 days [42]. The degradation rates for malonyl-

daidzein and daidzin were $8.511 \text{ (d}^{-1}\text{)}$ and $11.62 \text{ (d}^{-1}\text{)}$, respectively, both of which corresponded to a half-life of less than 2 h [42]. From the degradation kinetics and the amount of isoflavones secreted in hydroponic culture during all growth stages, the rhizosphere daidzein concentration in the field was estimated to be maintained during the growth stages of soybean [42].

Saponins

Saponins occur widely throughout the plant kingdom and have various functions [43,44]. The typical structure of saponins is a combination of a hydrophobic aglycone to various functional groups and hydrophilic sugar moieties, which results in surface-active amphiphathic compounds. Saponins appear to have physiological functions in defence against pathogens, pests, and herbivores [44].

Legumes commonly synthesize triterpenoid saponins called soyasaponins, which are composed of aglycones, soyasapogenols, and oligosaccharides. Soyasaponins are classified into four groups depending on the aglycone structure: glycosides of soyasapogenol A (Group A), glycosides of soyasapogenol B (Group B), glycosides of soyasapogenol E (Group E), and glycosides of soyasapogenol B, the C₂₂ of which is bound to 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) residues [2] (Fig. 3). Saponins may play roles in allelopathy in alfalfa (*Medicago sativa*) [45–47]. However, the secretion of saponins into the rhizosphere and their functions in biological communication remain largely unknown, except for the recent identification of soyasaponins in root exudates of legume species [26].

In hydroponic culture, the amount of soyasaponins secreted into the rhizosphere per plant peaked at the V3 growth stage (3 weeks of age) and decreased in reproductive stages. The composition of soyasaponins in hydroponic culture medium varied with growth stage, with predominant secretion of Group A soyasaponins at stages V3 and V7 (5 weeks of age) and higher secretion of Group B soyasaponins at reproductive stages. At the VE stage (1 week of age), when soyasaponin secretion was the highest per amount of root tissue (dry weight), the soyasaponin composition differed from that of other growth stages, with greater secretion of deacetyl soyasaponin Af, soyasaponin Ab, and soyasaponin Bb [26]. DDMP saponins were

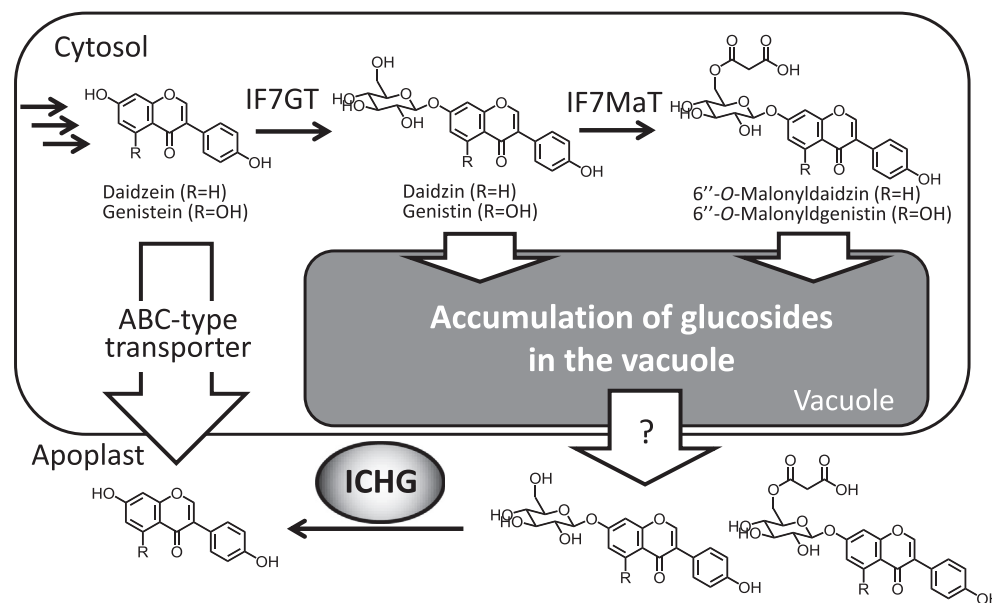


Fig. 2. Synthesis of isoflavones in soybean root and their secretion. Aglycones (daidzein and genistein) are glucosylated by UDP-glucose:isoflavone 7-O-glucosyltransferase (IF7GT), and further malonylated by malonyl-CoA:isoflavone 7-O-glucoside 6''-O-malonyltransferase (IF7Mat). These (malonyl)glucosides accumulate in vacuoles. The arrows show two possible pathways for isoflavone secretion.

Soyasaponin

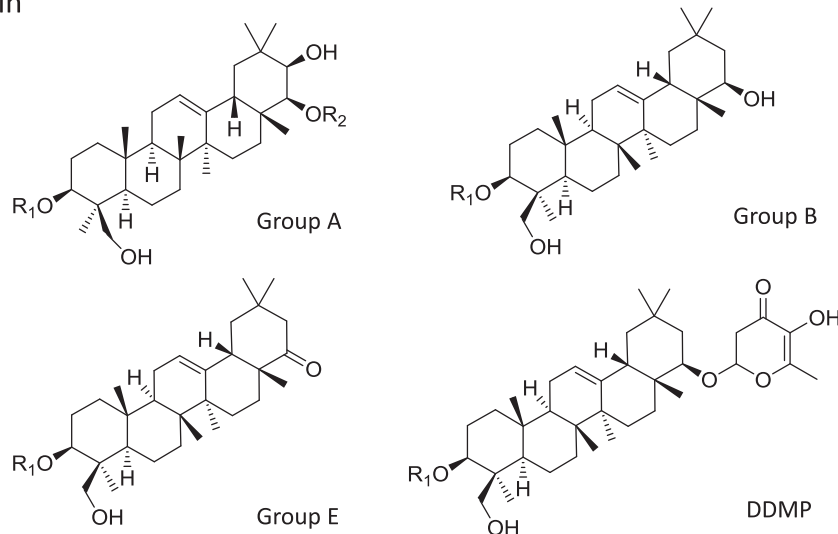


Fig. 3. Chemical structures of saponins in soybean root exudates.

detected only in trace amounts throughout the growth stages, although they are a major class of soyasaponin in root tissues [26]. These results suggest mechanisms that regulate soyasaponins secretion. The amounts and functions of saponins in the soybean rhizosphere are currently under investigation.

Other metabolites

Besides isoflavones and saponins, soybean roots secrete a diverse range of metabolites, but the function of most of these metabolites in the rhizosphere has not been thoroughly analyzed. Capillary electrophoresis mass spectrometry of soybean root exudates identified 79 metabolites belonging to organic and amino acids such as adipic acid, gluconic acid, glutaric acid, glyceric acid, glycine, L-alanine, L-asparagine, and L-serine [48]. Divergent responses of these metabolites were found during development and under phosphorus deficiency [48]. Highly variable forms of sugars, including glucose, pinitol, arabinose, galactose, sucrose, kojibiose, and oligosaccharides, were detected in soybean root exudates; these sugars are a potential carbon source for rhizosphere microbes [49]. Osmolytes such as proline and pinitol were found in soybean root exudates under drought stress [50].

Glyceollins are phytoalexins synthesized in response to pathogens such as *Phytophthora megasperma* and herbicides [51]. More than 50% of glyceollins synthesized in soybean roots are secreted into hydroponic solution [52], but their fate and function in the rhizosphere remain to be characterized. Glycinoeclepin A and related compounds from a root extract of common bean (*Phaseolus vulgaris*) stimulate hatching of soybean cyst nematodes [11,53]; however, the synthesis of these compounds in soybean and their identification in the soybean rhizosphere have not been reported. The *bona fide* functions of glycinoeclepin in plants as well as in the rhizosphere are still to be elucidated. Functions of strigolactones were identified as signals for arbuscular mycorrhizal fungi and phytohormones years after their identification as signals for parasitic weeds. Strigolactones are also secreted into the soybean rhizosphere, but their composition and dynamics in the rhizosphere have not been reported in soybean [5,54].

Microbes

Rhizosphere microbial communities have prominent effects on plant growth and health, including nutrition, disease suppression,

and resistance to both biotic and abiotic stresses [55–58]. Numerous studies support the idea that, in addition to the climate, soil type, plant species, plant genotype, and growth stage are among the factors that regulate the diversity and composition of rhizosphere microbial communities [59–61]. There have been several reports on the microbial communities (both bacterial and fungal) of the soybean rhizosphere [62–64], and most such communities show a higher abundance of symbiotic rhizobia than does bulk soil [65,66].

During the growth of soybean in the field, bacterial communities change in the rhizosphere [66] but they did not change in bulk soil. These findings suggest that variation in rhizosphere bacterial communities is more influenced by plant growth than by environmental factors. *Bradyrhizobium* spp. and other potential plant-growth-promoting rhizobacteria, such as *Bacillus* spp., are more abundant in the rhizosphere than in bulk soil. In one soybean field, both *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* were the predominant species that formed nodules on roots [67]. In another study, although the resolution of the sequence analysis was insufficient to distinguish members of *Bradyrhizobium* in the field at the species or strain level, *Bradyrhizobium* spp. showed differential responses at the operational taxonomic unit level [68].

Rhizosphere fungal communities are rather stable during soybean growth at the phylum level, with the highest abundance of Ascomycota and Basidiomycota [69], but community analysis based on the internal transcribed spacer region revealed that the growth stage of soybean determined the diversity of the fungal communities [70]. Fungal communities are also affected by fertilizer application and rhizobium inoculation [70]. Continuous cropping altered fungal composition, with 38 genera increased and 17 decreased; these genera include both potentially pathogenic and beneficial fungi [71].

A study of field-grown black soybean suggested the involvement of rhizosphere bacterial communities in soybean production [72]. Yields of black soybean grown in the mountainous region around central Kyoto have decreased with no clear symptoms of pathogen infection; therefore, the involvement of microbial communities was investigated [73]. Variations in the bulk soil bacterial communities among farms with similar climate suggested the effect of management practices on the communities. The rhizosphere bacterial communities at each farm differed significantly from those of bulk soil, with the dominance of *Bradyrhizobium* spp. and *Bacillus* spp. Network analysis using the Confeito algorithm showed a possible connection between rhizosphere bacteria and soybean growth, although more detailed analysis is necessary [72].

Linking metabolites and microbes

In vitro studies have been conducted to dissect the effects of metabolites on microbial communities. The effects of root exudates of three generations of *Arabidopsis thaliana* and *Medicago truncatula* on the soil fungal community were qualitatively and quantitatively similar to the effects of growing plants [74]. Root exudates of *Arabidopsis* fractionated to obtain natural blends of phytochemicals were also applied to soil. It was found that phenolic compounds from *Arabidopsis* root exudates showed positive correlation with the number of bacteria in soil [75]. The flavonoid 7,4'-dihydroxyflavone from alfalfa root exudates, which functions as a *nod*-gene-inducing signal, influenced the interaction with a diverse range of soil bacteria (not limited to rhizobia) when added to soil *in vitro* [76]. Linkage between root-secreted metabolites and microbial communities were also reported in the metabolome and microbiome analyses during development, which indicate a link between root-secreted metabolites and microbial communities [59,77]. Such a link is also suggested by the comparative genomics and exometabolomics analysis in *Avena barbata*, in which root-secreted aromatic organic acids are key factors for the assembly of the rhizosphere microbiome [78].

Root-secreted metabolites of soybean have been studied in the context of interaction with plant growth promoting rhizobacteria and degradation of hazardous pollutants, polycyclic aromatic hydrocarbons (PAHs) [79,80]. Inoculation of *Pseudomonas oryzae* affects the profiles of root exudates of soybean in genotype-dependent manner, with the decrease of sugars and amino acids [79]. Application of soybean root exudates to PAH-contaminated soil resulted in a significant enhancement in the degradation of PAHs by soil bacteria [80]. It has been also reported that in a 13-year experiment of continuous soybean monocultures daidzein and genistein concentrations in the rhizosphere of soybean has a correlation with soil microbial communities, especially the possible linkage between genistein and the hyphal growth of arbuscular mycorrhizal fungi [81,82]. Genetic link between the root exudation of flavonoid and the interaction with rhizobia has been suggested from the study on the identification of quantitative trait loci controlling both the affinity to rhizobacterial strains and genistein secretion [83]. The analysis of rhizosphere bacterial communities of hairy roots silenced in isoflavone synthetase revealed that isoflavones exert small but significant influence on the bacterial communities, especially for Comamonadaceae and Xanthomonadaceae [38]. Taken together the above literatures point out the linkage between root-secreted metabolites and microbes in the rhizosphere. The molecular basis on this linkage in the soybean rhizosphere is still to be elucidated.

Conclusions and future perspectives

In the past few decades, many studies have shown the importance of plant metabolites and microbes in the rhizosphere. Recent advances in sequencing technologies have further deepened the understanding of plant-microbe interactions in the rhizosphere. Despite this progress, however, most of the key metabolites that facilitate these interactions remain to be characterized at the molecular level, mostly owing to difficulties in the spatiotemporal analysis of metabolites in the rhizosphere. Traditionally, analyses of root exudates or metabolites that are functional in the rhizosphere have been performed in hydroponic culture or in plate media [7,84]. To utilize the functions of these molecules for sustainable agriculture, it is necessary to analyse them in the rhizosphere of field-grown plants [85].

For the spatiotemporal analysis of metabolites and microbes in the rhizosphere, non-destructive analysis using sensors is one

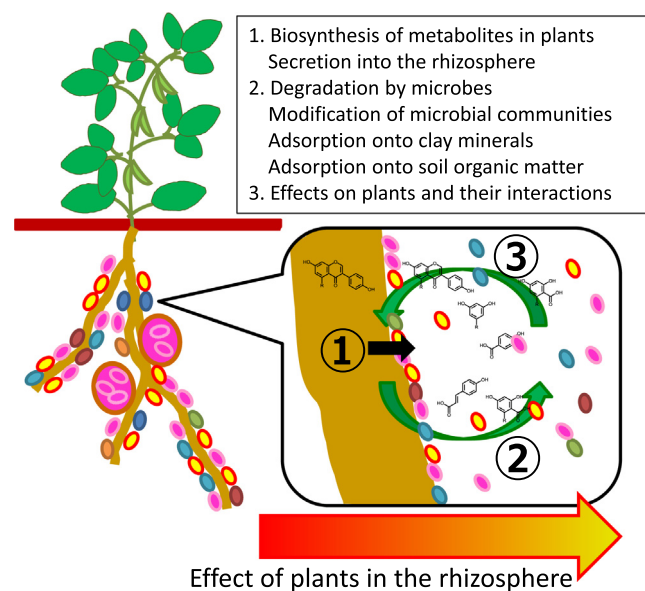


Fig. 4. Secretion and fate of metabolites in the rhizosphere and their effects on microbes.

promising possibility. Various sensors are used in rhizoboxes for the spatiotemporal analysis of metabolites, minerals, and oxygen [86–88]. Their use could be expanded to analyse the rhizosphere of field-grown plants to monitor the changes of rhizosphere conditions. The use of coloured molecules is another possibility. Shikonicin, a naphthoquinone biosynthesized by members of the Boraginaceae, exhibits a red colour in the rhizosphere [89] and has antimicrobial properties [90]. The production of shikonicin in cell cultures has been well characterized [91], and its function as an allelochemical in the rhizosphere of the invasive weed *Echium plantagineum* has been reported. Juglone from black walnut (*Juglans nigra*) is another prominent candidate, because it is yellow and is allelopathic [92].

The dynamics and their interactions of metabolites and microbes are of particular importance for improving our understanding of plant-microbe interactions (Fig. 4). The stability of metabolites varies with the composition of soils. To simulate metabolite dynamics in the rhizosphere, analysis of their movement, degradation, and adsorption onto soil organic matter and clay minerals is needed to be analysed in various soil types, because the stability of metabolites varies with the composition of soils. The spatiotemporal distribution of metabolites and chemicals can be validated and analysed using rhizoboxes in combination with various sensors. As the definition of the rhizosphere is not quantitatively rigorous, the area influenced by plant roots varies with soil conditions, such as the abundance of organic matter, water content, and types of minerals, in addition to the metabolites and microbes in the soil. Defining the functions and area of the rhizosphere at the molecular level could pave the way towards the use of these metabolites and microbes for sustainable agriculture in the era of climate change.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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