# Underground alarms: volatile-mediated recruitment of beneficial soil bacteria by plants under biotic stress

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# 1

# CHAPTER 1

## General introduction and thesis outline

## Rhizobiome, root exudates and plant health: an integrated perspective

Plant roots are highly dynamic belowground structures that interact with the diverse soil microbial community. These interactions are primarily mediated by the release of an array of chemically diverse compounds into the surrounding soil environment, collectively known as the rhizosphere. The rhizosphere is a hotspot of microbial activity and biological interactions, involving bacteria, fungi, archaea, and other microorganisms that colonize the root and the adjacent soil matrix (Philippot et al., 2013). In the past decade, the rhizobiome has gained significant attention due to its role in plant growth and health (Feng et al., 2024). Similar to the human gut microbiome, the rhizobiome supports its host plant by enhancing nutrient acquisition, promoting growth, and strengthening immunity, and is therefore often referred to as the second plant genome (Berendsen et al., 2012)

A major driver of rhizobiome composition and function is the release of root exudates, which represent a significant allocation of plant resources. It is estimated that up to 40% of photosynthetically fixed carbon in plants is secreted into the rhizosphere (Badri & Vivanco, 2009; Canarini et al., 2019). Root exudates include sugars, alcohols, amino acids, organic acids as well as volatile organic compounds (VOCs) (Baetz & Martinoia, 2014; Massalha et al., 2017; Wang et al., 2024; Wenke et al., 2010). These compounds can serve multiple functions, providing nutrients for microbial growth, acting as signaling molecules that influence microbial behavior or as chemical deterrents to ward off pathogens and pests (Baetz & Martinoia, 2014; Liu et al., 2024 ; Wenke et al., 2010). In this context, specific attention has been given to secondary metabolites such as benzoxazinoids, terpenoids, and alkaloids with antimicrobial properties (Ehlers et al., 2020; Koprivova & Kopriva, 2022).

Plant-microbe interactions become especially critical when plants are exposed to biotic stresses (Savary et al., 2019). When under siege, plants can dynamically adjust their exudate profiles, allowing them to select for microbial allies that bolster biotic stress resilience (Friman et al., 2021; Hu et al., 2018; Liu et al., 2021). Hence, plants can actively recruit beneficial microbes by modulating the composition of their root exudates, thereby affecting rhizobiome assembly as well as the densities and activities of specific rhizobiome members to enhance stress tolerance (Pantigoso et al., 2022; Park et al., 2023). Gaining a deeper understanding of how plants tailor their rhizobiome composition through root exudation, especially under fluctuating environmental conditions, holds significant promise for the development of sustainable agricultural practices, such as plant microbiome engineering and the use of bioinoculants to enhance crop productivity and stress resilience.

## Ecological significance of root-emitted volatile organic compounds (rVOCs)

While much attention has been given to water-soluble root exudates, root-emitted volatile organic compounds (rVOCs) represent an equally important yet less understood component of belowground chemical interactions (Delory et al., 2016). Most plant VOCs belong to chemical groups such as terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and sulfur-containing compounds (Baldwin, 2010; Pichersky et al., 2006; Schenkel et al., 2015). These small molecules (typically <300 Da) exhibit high vapor pressures, allowing them to evaporate and diffuse readily at low temperatures (Wenke et al., 2010). rVOCs operate in a complex belowground environment of soil particles, gases, water, and organic matter (van Dam et al., 2016). This heterogeneity affects the diffusion and stability of rVOCs (Sharifi et al., 2022), yet studies show that rVOCs can travel long distances through soil matrices. For example, the sesquiterpene (E)-β-caryophyllene, emitted by maize roots, was shown to diffuse over 10 cm through sandy soil, suggesting that rVOCs can mediate long-range interactions (Chiriboga et al., 2017; Hiltpold & Turlings, 2008; Rasmann, Köllner et al., 2008). However, studying rVOCs presents some challenges. Soil is a highly heterogeneous environment with multiple VOC emitters, making it difficult to accurately determine the contribution and ecological significance of rVOCs. Additionally, transport, diffusion, and perception of rVOCs are strongly influenced by soil characteristics such as pH, humidity, and pore size (Som et al., 2017; Tholl et al., 2021).

Despite the challenges of detecting and characterizing VOCs in soil, the ecological significance of rVOCs is becoming increasingly evident. rVOCs play key roles in interactions with larger soil organisms, including neighboring plant roots, insects, and nematodes (Delory et al., 2016; Wenke et al., 2010). For instance, spotted knapweed (*Centaurea stoebe*) constitutively emits sesquiterpenes that promote the germination and growth of nearby plants, indicating a potential role of this rVOC in root-to-root communication (Gfeller et al., 2019). Similarly, rVOCs such as (E)-β-caryophyllene, dimethyl disulfide and methyl salicylate act as chemical cues for soil-dwelling arthropods and entomopathogenic nematodes, guiding them towards roots or their preys (Lin et al., 2017; Turlings et al., 2012).

Yet, the influence of rVOCs on soil microorganisms, particularly bacteria and fungi, remains less well understood. Given their diverse structures and functions, rVOCs may serve as signaling compounds, defense metabolites, or even carbon sources (Raza et al., 2021; Schulz-Bohm et al., 2017; van Dam et al., 2016). For instance, the degradation of monoterpenes like geraniol has been documented in the rhizosphere of *Populus tremula* (Owen et al., 2007), while rhizobacteria such as *Pseudomonas fluorescens* and *Alcaligenes xylosoxidans* have been shown to metabolize alpha pinene (Chalkos et al., 2021; Kleinheinz et al., 1999). Similarly, microbial families like *Enterobacteriaceae* and *Pseudomonadaceae* associated with *Vetiveria zizanioides*, can utilize volatile sesquiterpenes as sole carbon sources (Del Giudice et al., 2008). Additionally, the constitutive emission of methyl jasmonate from roots of diverse plant species recently suggests a conserved signaling role that can trigger changes in soil microbiome composition and biofilm formation (Kulkarni et al., 2024), further highlighting the significance of rVOCs in shaping microbiome assembly and functions belowground.

## Cry-for-Help: shoot–root communication and microbial recruitment

Plant are inevitably exposed to distinct environmental stresses both above- and belowground. As a result, plants have evolved efficient and localized responses to these stresses. However, growing evidence suggests that plants can also coordinate their responses through long-distance signaling along the shoot–root axis (Hou et al., 2021). This systemic communication allows one organ to influence the stress response of the other. For example, in *Arabidopsis thaliana*, the mobile peptide signal CEPD-like 2 is transported from the shoot to the root to regulate nitrate uptake (Ota et al., 2020).

One emerging dimension of this shoot–root communication is the plant’s ability to recruit beneficial microbes through a phenomenon referred to as the “cry-for-help” (Rolfe et al., 2019; Yi et al., 2011). Insect pests and pathogens are major biotic stressors in agriculture, causing substantial leaf damage and significant yield losses. Increasing evidence suggests that aboveground infestation by these biotic agents can reshape root-associated microbial communities, primarily through stress-induced changes in the root exudation profile (Friman et al., 2021; Rolfe et al., 2019). Intriguingly, these microbiome shifts have been linked to enhanced plant resistance to the same stresses, supporting the notion of active microbial recruitment as an indirect defensive strategy (Berendsen et al., 2018). In *A. thaliana* infected with *Pseudomonas syringae* pv. *tomato* DC3000, root exudation of long-chain carbon compounds increases, leading to a compositional shift in the root microbiome marked by an enrichment of *Firmicutes* and a depletion of *Proteobacteria* (Yuan et al., 2018). These microbiome alterations have been linked to enhanced resistance, mediated through induced systemic resistance (ISR), a primed defense where plants exhibit improved responsiveness to subsequent stressors (Pieterse et al., 2014). Similarly, in *Ambrosia artemisiifolia* (common ragweed), infestation by *Spodoptera litura* leads to increased exudation of fatty acids and decreased phenolic secretion, resulting in a higher abundance of arbuscular mycorrhizal *Glomus* species across root compartments. Interestingly, root exudates from infested plants can promote arbuscular mycorrhizal colonization even in uninfested plants, reinforcing the concept of root-mediated microbiome recruitment as an indirect defense mechanism (Xing et al., 2024). Recently, it was demonstrated that maize leaves infested with *Spodoptera frugiperda* emit a blend of green leaf volatiles (GLVs), which can enhance the resistance of neighboring plants against the same stress by actively recruiting beneficial rhizobacteria in the rhizosphere, possibly through changes in root exudate composition (Hu et al., 2025). However, while the impact of biotic stress on water-soluble root exudates is well documented, less is known about if and how such stress influences rVOC emissions and their potential roles in shoot-root communication and microbial recruitment in rhizosphere.

## Can rVOCs signal a 'Cry-for-Help' from aboveground attack?

rVOCs can mediate indirect plant defenses, particularly in belowground trophic interactions by attracting beneficial organisms such as predatory or parasitic species of root herbivores. A well-studied example is in maize (*Zea mays*), where infestation by larvae of the western corn rootworm (*Diabrotica virgifera virgifera*) enhances root emission of the sesquiterpene (E)-β-caryophyllene (EβC) (Hiltpold et al., 2011). In turn, this rVOC attracts entomopathogenic nematodes that locate and kill the larvae, thereby reducing subsequent herbivore pressure. Such interactions exemplify how rVOCs function in the rhizosphere as part of a plant's "cry-for-help" strategy.

Apart from interactions with macro-organisms such as insects and nematodes, rVOCs may also shape microbial dynamics belowground. Recent findings suggest that plants can recruit beneficial microbes through stress-induced rVOCs. For instance, roots of *Carex arenaria* infected with the fungal pathogen *Fusarium culmorum* emitted a distinctive volatile blend containing (Z)-limonene-oxid. This volatile blend, not detectable in healthy plants, selectively attracted specific bacterial strains from a synthetic microbial community over distances of more than 12 cm (Schulz-Bohm et al., 2018). These attracted bacteria strains suppressed the pathogen, illustrating the plant's ability to fine-tune rVOC profiles to engage microbial allies. While the local role of rVOCs in root defense is well established, their involvement in systemic signaling, particularly in response to aboveground stressors such as herbivory or foliar pathogen attack remains largely unexplored. The possibility that foliar stress can modulate rVOC biosynthesis and release belowground introduces intriguing questions about shoot-to-root communication. Such systemic responses could alter both the composition of water-soluble root exudates and the intensity or specificity of rVOC emissions. Understanding these dynamics is crucial for developing a comprehensive model of integrated plant defense. In addition to local pathogen suppression, rVOC-recruited microbes may also prime systemic immune responses, such as induced systemic resistance (ISR). While ISR has traditionally been associated with water-soluble metabolites (Rolfe et al., 2019), the potential involvement of rVOCs in ISR-induction represents an exciting and underexplored area of research. Investigating how aboveground stressors reshape rVOC-mediated microbial recruitment could significantly expand our understanding of plant–microbe interactions under siege.

## Thesis aim and outline

This PhD thesis is conducted within the framework of the **MiCRop** (Microbial Imprinting for Crop Resilience) project, which aims to uncover the mechanisms by which plants recruit beneficial root microbiota under stress. The overall aim of my PhD thesis is to elucidate biotic stress-induced bacterial recruitment via rVOCs and to resolve the chemistry, specificity, and underlying mechanisms. **Chapter 2** reviews plant responses to various environmental stresses through belowground "cry for help" strategies. Specifically, we summarize the current understanding of how plants recruit beneficial root-associated microbiota to enhance resistance against both biotic and abiotic stressors. Specific emphasis is given to the roles of water-soluble and rVOCs in mediating the recruitment and establishment of protective microbial communities. In experimental **Chapter 3,** I investigated the impact of *Botrytis cinerea* infection of tomato leaves on the composition of rhizosphere and rVOCs and associated changes in the composition of the root-associated microbiota. We studied two tomato genotypes as model species: *Solanum pimpinellifolium* (a wild species) and *Solanum lycopersicum* var. Moneymaker (a modern cultivar). Our objective was to assess changes in the root and rhizosphere volatilome, as well as in the diversity of bacterial and fungal communities in the rhizosphere and rhizoplane, in response to foliar infection by the fungal

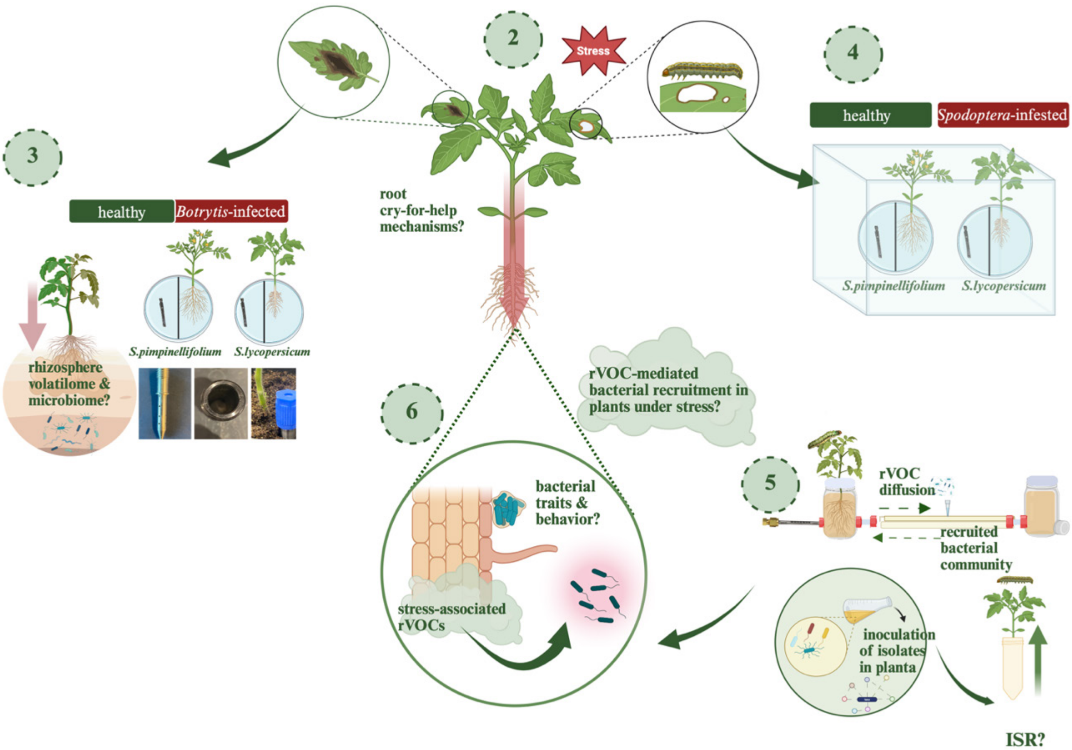


**Figure 1.** Schematic outline of the thesis chapters (indicated by chapter numbers) exploring the role of root-emitted volatile organic compounds (rVOCs) as belowground signals important for plant–microbe communication under aboveground biotic stress conditions.

pathogen *B. cinerea*. Adopting untargeted volatilome analyses and 16S and ITS amplicon sequencing, this chapter explores how tomato genotype and biotic stress influence the rVOCs composition and root microbiome assembly.

In **Chapter 4** we further investigated the diversity of rVOCs released by the two tomato genotypes in response to the leaf herbivore *Spodoptera exigua*. We developed a novel *in vitro* assay for low-disturbance of rVOC sampling and performed untargeted volatilome analyses to determine how the rVOC profiles of a wild and a domesticated tomato genotypes differed in response to foliar herbivory. Additionally, we compared the performance of two sorbent materials for rVOC trapping, assessing their sensitivity and overall suitability for rVOC profiling.

**Chapter 5** explores the functional potential of rVOCs in belowground "cry for help" signaling, with a specific focus on the mechanisms by which stressed plants recruit beneficial bacterial communities via rVOCs. To this end, we developed a belowground olfactometer system, to investigate whether domesticated tomato plants (*S.lycopersicum* var. Moneymaker) subjected to *S. exigua* herbivory can recruit specific subsets of bacterial communities via rVOCs. This olfactometer allows only volatile compounds to diffuse from the roots into arm tubes, effectively excluding the influence of water-soluble compounds. A mixed bacterial community was inoculated at the center of each olfactometer arm, and bacterial migration toward rVOCs emitted by infested or non-infested plants was assessed. We characterized the rVOC profiles and used 16S rRNA amplicon sequencing of the inoculum and of the soil near the root zone to identify recruited bacterial taxa. Targeted isolation of bacterial strains attracted by rVOCs from stressed plants was then performed. The recruited strains were subsequently tested for their ability to induce resistance in tomato plants against *S. exigua* herbivory, thereby evaluating their functional roles in plant defense.

In **Chapter 6**, we aimed to characterize the bacterial traits relevant for rVOCs recruitment and establishment in the rhizosphere. Using selected pure rVOC standards, we assessed their impact on bacterial surface motility, chemotaxis biofilm formation, and root colonization ability. These assays were performed using a range of *in vitro* assays, including liquid media, semi-solid media, and synthetic soil substrates, to comprehensively evaluate the functional roles of specific rVOCs in shaping bacterial behaviors relevant to rhizosphere colonization.

In **Chapter 7** I synthesize the main findings of the thesis, highlighting the emerging role of rVOCs in mediating belowground plant–microbe interactions under biotic stress. I discuss the implications of our results for the broader field of belowground chemical ecology and outline key directions for future research, particularly in understanding the role of rVOCs in plant defense, microbiome assembly, and sustainable agriculture.