The impact of defense hormones on the interaction between plants and the soil microbial community

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Chapter 1

General introduction

The soil ecosystem consists of the largest reservoir of biodiversity on Earth (Zak et al., 2003; Decaëns, 2010; Bardgett and Van der Putten, 2014). Microbial communities are unseen drivers in soil ecosystems, and they play an important role in determining a wide variety of soil processes in terrestrial ecosystems (Van der Heijden, et al., 2008; Fester et al., 2014; Delgado-Baquerizo et al., 2016; Singh and Gupta, 2018). Soil microbial communities can influence plant performance and can drive plant species composition on a particular soil. Soil microbes are associated with an extensive range of ecosystem processes, such as nitrogen (N) and carbon (C) cycling, organic matter decomposition, soil structural formation and stability and these processes, in turn, affect plant growth (Beare et al., 1992; Ehrenfeld et al., 2005; Schimel and Schaeffer, 2012; Bardgett et al., 2014). While these processes can benefit plant growth, the soil microbial community also harbors microbes that compete with plants for nutrients or are pathogenic and impair plant growth. This leads to the question if plants can manipulate the composition of the soil microbial community to their advantage.

In agriculture, the physical structure of the soil is often altered to improve crop production and this modifies biological components and microbial properties of the soil (Mousavi and Eskandari, 2011; Van der Heijden et al., 2013; Van der Putten, et al., 2013). Moreover, green crop management technologies, such as regulation of soil microbial biodiversity, application of beneficial microbial agents and induction of plant hormonal resistance, are regarded as promising approaches against pests and microbial pathogens (Chung et al., 1988; Kennedy and Smith, 1995; Neher, 1999; Sturz and Christie, 2003). Although many experiments have shown that activation of hormonal signaling pathways can boost a plant's immunity against pathogenic microbial attacks, whether and how these hormonal signaling pathways affect the soil microbial community and consequently plant growth is still poorly understood (Berendsen et al., 2012; Graham, et al., 2016). Therefore, to better understand the roles of soil microbial communities at both taxonomic and functional level, studies from a plant defensive perspective are timely and needed, and this is the main focus of this thesis.

1. The relationship between plants and soil microbial communities

In the early middle ages, under Charles the Great, cropping and fallow rotations were already applied in Europe (van der Putten et al., 2013). Chinese historical books record that cropping and fallow rotations in China began even in the ancient Warring States period, which ran from 475 BC to 221 BC (Zhang and Yu, 2006). In agriculture, all these ancient practical actions aimed to overcome the same problem: a soil becomes less suitable for a crop if this crop is grown in that soil repeatedly.

We have since become aware that the negative impact of soil on plant growth is dependent on the role of soil microbes. Particularly, in the early 2000s, a large number of studies began to emphasize that the relationship between plants and soil microbial communities is bidirectional, rather than unidirectional. Plants can affect the microbial communities in the soil, and in turn, soil microbial communities also influence plant growth. Nowadays it is clear that the interactions between plants and soil microbial communities are extremely complicated (Van der Heijden, et al., 2008; Fester et al., 2014; Singh and Gupta, 2018).

1.1 Effects of soil microbial communities on plants

Generally speaking, plant-microbial interactions can be broadly subdivided into three basic groups of effects. Firstly, in many cases plants and specific microbes do not affect each other strongly. Secondly, there are negative effects on plants through root-associated organisms that form pathogenic relationships with plants. These pathogenic organisms in the rhizosphere include parasitic nematodes, fungi, Archaea, bacteria and invertebrate herbivores. Soil pathogens can reduce plant productivity, thus impacting ecosystem processes. Ecologists are long aware that soil microbes can cause serious reductions in plant growth (Nijjer et al., 2007). Among a myriad of soil-borne microbial pathogens, *Phytopathora, Pythium, Fusarium* and *Verticillium* are well-known genera and they have been widely reported to have negative effects on the production of many crops and economically important tree species, such as potato, wheat, radish, pea and oaks (Harman et al., 1980; Nirenberg, 1981; Jung et al., 1999).

Thirdly, root-associated organisms can have positive effects on plant growth. For example, several plant growth-promoting *rhizobacteria* (PGPR), like *Pseudomonas* and *Burkholderia*, residing in the rhizosphere may repress the growth and activity of soil-borne pathogens and other attackers (Bhattacharyya and Jha, 2012). PGPR commonly reside in the rhizosphere, where they are important regulators involved in numerous biological processes affecting host plants, e.g. solubilizing phosphate, fixing available soil nitrogen, producing siderophores, phytohormones, producing volatile organic compounds (VOCs), inducing host systemic resistance and systemic acquired resistance and stimulating antifungal compounds (Wei et al., 1991; Nelson, 2004; Esitken et al., 2010; Bhattacharyya and Jha, (2012). Arbuscular mycorrhizal fungi (AMF) can act as a natural extension of the host root system, to increase the possibility of plants to obtain resources from the soil, and plants provide carbon (C) to the AMF in exchange (Azcón-Aguilar et al., 1992; Barea, 2000). Moreover, some proteobacteria, such as legume-nodulating *Burkholderia* strains, and species of the genus *Azoarcus* and *Sinorhizobium meliloti*, are well known for their functions in fixing soil atmospheric nitrogen, which also benefits host plants (Reinhold-Hurek et al., 1993; Chen et al., 2003; Hayat et al., 2010; Schlüter et al., 2010). Additionally, root endophytes can play an important role in enhancing both biotic and abiotic stress tolerance in plants (Dimkpa et al., 2009), while some rhizosphere bacteria produce antibiotic compounds or protective biofilms that prevent the plant from attack by pathogenic soil bacteria.

Many studies report that the overall net effect of soil microbial communities on plant performance is negative (Nijjer et al., 2007). Inoculation of soil-borne microbial communities into sterilized soil often causes a reduction in plant growth. Negative effects of the soil microbial community on plant growth can be due to nutrient competition between plants and microbes and due to soil-borne plant-pathogenic microbes. Soil microbes, such as AMF, phosphorus-solubilizing bacteria, proteases and nitrogen-fixing bacteria can assist plants in taking up more nutrients from the surrounding soil. However, plants and microbes also depend largely on the same inorganic nutrients and therefore compete for these nutrients.

Plant-soil feedback studies, mostly show that inoculation of sterilized soil with microbial communities collected from underneath conspecific plants has a stronger negative effect on plant growth than inoculation with microbial communities from other plant species, suggesting that plant species-specific pathogenic or plant growthinhibiting microorganisms build up in the rhizosphere (Pendergast et al., 2013; Dawson et al., 2017; Howard et al., 2020). Studies with pure "home soil" and "away soil" also generally report negative effects of "home soil" on plant growth (Manning et al., 2008; Ayres et al., 2009). All these studies suggest that pathogenic effects of micro-organisms play an important role in the interaction between plants and the soil microbial community. These studies furthermore suggest that many of the interactions are species-specific and that plants affect the microbial community in a species-specific way and vice versa. Moreover, it is worth noticing that soil microorganisms can also affect the above and belowground defense system of a plant (Huberty et al., 2020). For instance, soil-borne microorganisms affect the composition of pyrrolizidine alkaloids (PAs) and the total PA concentration in the plant Jacobaea vulgaris (Joosten et al., 2009; Kostenko et al., 2012; Kos et al., 2015). Studies that explore the mechanisms behind the interactions between plants and the soil microbial community nowadays are boosted by the molecular tools that make it possible to study the composition and functions of microbial communities. In this thesis, I will concentrate on the effects of the microbial communities on plant growth.

1.2 Effects of plants on rhizosphere microbial communities

The term rhizosphere was introduced by Hiltner in 1904 to describe the layer of soil that was influenced by the root of a plant (Hiltner, 1904). In comparison to root-free soil, the rhizosphere is an area where plant roots and soil microorganisms are mutually interacting. Soil properties (pH, humidity, chemical composition, texture and structure) play an important role in the modulation of rhizosphere microbial communities (Börner, 1960; Bach et al., 2010). However, plants also greatly influence the structure and function and diversity of microbial communities, especially in the rhizosphere (Grayston et al., 1998; Girvan et al., 2003; Nunan et al., 2005; Berg and Smalla, 2009; Dennis et al., 2010). Plant species differ in their effect, and in the strength of this effect, on microbial communities (Zak et al., 2003; Mangan et al., 2010). Other studies showed that bacterial communities in the soil of grass and forb species differ (Hannula et al., 2019) and that the structure and function of soil microbial communities of exotic plant species are different from those of native species (Kourtev et al., 2003). A prediction of how specific crops will influence the soil microbial community may help to reduce risks and yield losses in agriculture, but, so far, this is poorly understood and remains a long-term challenge.

Plants synthesize a vast array of secondary metabolites (SMs) and more than 100,000 are reported (Dixon, 2001; Quiroga et al., 2001; Bartwal et al., 2013). Many studies have demonstrated that these compounds are involved in the chemical defense of plants against pathogenic microbes (Van Loon, 2007; Boller and He, 2009). In particular, root exudates are key drivers of microbial diversity and composition in the rhizosphere. For example, sugars, organic acids and amino acids are well-known nutrients for microbes (Canarini, et al., 2019). The composition and concentration of these metabolites in the rhizosphere depend upon the plant species and overall environmental conditions (Broeckling et al., 2008; Zahar et al., 2008).

Plant chemical defenses play an important role in plant-pathogenic microbe interactions. It has been suggested that the diversity of defensive compounds has evolved as a result of an evolutionary arms race between the plants and their potential attackers (Ehrlich and Raven, 1964). In particular, those defenses based on molecules with low molecular weight, and long-distance communicating molecules, such as volatile organic compounds (VOCs) (Insam and Seewald, 2010; Frankenberger and Arshad, 2020). Moreover, SMs, such as citronellal, berberine and pyrazines are also functioning in plant defense against pathogenic microbes (Wink, 1988; Tyc et al., 2017).

In addition, plants can regulate the production of protease inhibitors to defend themselves (Lawrence and Koundal, 2002; Habib and Fazili, 2007). Microbial pathogens can secrete extracellular protease enzymes, and those enzymes can digest some proteins in the tissues of plants (Ryan, 1990). Plants can defend themselves from protease-related pathogens through expressing protease inhibitors and also regulate them to accurate and strict concentrations. Researchers have generated some transgenic plants with high expression of protease inhibitors, such as transgenic rice, potato, soybean, and these plants exhibit increased resistance against various pathogens (Cowgill et al., 2002; Rahbé et al., 2003).

Moreover, induced defense responses in the plant influence the chemical composition of root exudates and through that the bacterial community structure in the soil. Salicylic acid (SA), ethylene (ET), abscisic acid (ABA) and methyl jasmonate (MeJA) hormonal signaling pathways can alter the bacterial community composition in the soil (Carvalhais et al., 2015; Lebeis et al., 2015). Van der Meij et al. (2018) showed that application of SA to endophytic actinobacteria stimulates antibiotic production. Altogether these findings suggest that activating hormonal signaling may not only boosts the plant's defense system directly but also can affect the microbial composition on the soil thereby potentially mitigating the negative effects of the soil microbial community on plant growth.

2. Plant hormonal induced defense against soil-borne pathogens

To counteract the effects of microbial pathogens, plants have evolved a broad range of defensive mechanisms, which are partly regulated via hormonal signaling pathways (Fujita et al., 2006). Defense, as an essential and effective strategy for terrestrial plant species against pests and pathogens has been broadly developed in plants (Wesson and Wesson, 1993; Bronstein, 1998; Agrawal, 2011; Turley et al., 2013; Zhang et al., 2015). Induced defenses are defenses that are activated after infection occurs and enhance plant fitness (Boots and Best, 2018). Phytohormones are a group of natural plant compounds with low molecular weight that play an important role in the regulation of plant growth and development and induced plant resistance against pests and pathogens. SA, ET, abscisic ABA, MeJA, auxin, cytokinins (CKs), gibberellic acid (GA) and brassinosteroids (GAs) are commonly studied phytohormones. Besides these, there are also several other compounds (e.g. karrikins, triacontanol and nitric oxide) that can be involved in induced plant defense, but their functions are still under debate.

Each phytohormone has clear functions, however, they can also exhibit strong interactive effects. For instance, JA and SA are well-known for their negative cross-talk (Munné-Bosch and Müller, 2013). Upregulating the SA signaling can lead to downregulation of the JA signaling and vice versa. Such cross talk is one of the mechanisms that can explain why plant pathogens in the soil can e.g. affect herbivory above ground (Aljbory and Chen, 2018). Although most hormones have been implicated to be involved in defense pathways, the key regulator against pathogens and pests, in particular, to defend plants against biotrophic, necrotrophic pathogenes and herbivores, are the phytohormones JA and SA (Bari and Jones, 2009). In the following sections, JA and SA induced resistance in plants against soil-borne pathogens is described separately.

2.1 JA-induced resistance

JA is associated with several biological processes in plants. Specifically, JA can stimulate the germination of seeds, negatively impacts root growth, and invokes tuber formation and fruit ripening. Apart from these functions, JA is well-known for being involved in induced resistance against herbivores and for being a regulator of the activation of induced systemic resistance (ISR) of plants against necrotrophic microbial pathogens.

The biosynthesis of JA has been mostly studied in the model plant species *Arabidopsis thaliana* and tomato (*Lycopersicon esculentum*) (Ruan et al., 2019). Both biotic and abiotic stresses can induce the synthesis of JA. In plant tissues JA can be converted into JA-isoleucine conjugate (JA-Ile) by JAR1 (an auxin-induced gene), JA-Ile is a bioactive state of JA. MYC is a family of regulator genes that code for transcription factors and JA-Ile activates the MYC transcription factors by directly binding to the jasmonate zim-domain (JAZ) and a *coronatine insensitive1* (COI1) protein, which results in the degradation of JAZ transcriptional repressor proteins through the proteasome pathway. These processes result in the activation of transcription factors and the regulation of JA-responsive genes (e.g. MYC2, ERF1 and ORA59), which are associated with plant responses against environmental stresses from pathogens, wounding, and insect herbivory, biosynthesis of secondary metabolites, and with plant growth and development. For example, Carvalhais et al. (2015) demonstrated that the JA signaling pathway affects the composition of root exudates by enhancing the production of ornithine and that ornithine can be used by plant growth-promoting bacteria such as *Pseudomonas fluorescens*, which in turn has a positive effect on plant growth.

Due to the effectivity of JA-induced resistance against herbivores, in agriculture, foliar application of JA or MeJA has been considered as an alternative approach to control pests rather than using chemical pesticides. This theory has been tested in several crop plant species, including corn, tomato and wheat (Mandal et al., 2006; War et al., 2011; Chen et al., 2018).

In addition, to increased resistance to pests, JA-mediated defense also regulates the plant's response to necrotrophic microbial pathogens, e.g. *Pseudomonas syringae, Fusarium oxysporum and Hyaloperonospora parasitica* (Antico et al., 2012; Wasternack and Strnad, 2018; Li et al., 2019). The activation of JA-signaling pathways in the plant can result in changes in the composition of bacteria in the rhizosphere as was shown for the plant *A. thaliana* (Carvakhais et al., 2013). The mechanisms behind this are not yet fully resolved. JA signaling may directly affect microbial species or through the interaction with SA signaling.

2.2 SA-induced resistance

SA is another well-studied hormonal compound, which plays an important role in the activation of SA-induced resistance against biotrophic microbial pathogens. Hypersensitive response (HR) is a primary manifestation of a plant to pathogenic attack, e.g. due to cell death of the tissues surrounding the infection, to control the spread of pathogens. Cultivars that are highly sensitive to SA are often more tolerant of microbial pathogens (Seskar et al., 1998).

Infection of plant tissues with biotrophic pathogens leads to the accumulation of SA, as well as monomerization of NPR1 via SA-mediated redox changes in the cell. Later, monomeric NPR1 is relocated into the nucleus, at which the monomeric NPR1 interacts with TGA transcription factors, as a result, SA-responsive genes are activated. A large number of WRKY genes are induced by SA, among which some can regulate SA-responsive gene expression (Van der Does et al., 2013).

SAR is associated with the expression of pathogenesis-related proteins (PRPs). PRPs include proteins like β-1, 3-glucanase and chitinases (Van Loon and Van Strien, 1999). Commonly, both chitinases and glucanases show antimicrobial activities. Once PRPs are induced by SA, they take actions in several ways to assist plants against pathogens. These actions can be direct and indirect (Edreva, 2005). PRPs can direct breakdown or damage pathogens based on their antifungal and antibacterial activities. In addition, PRPs can indirectly boost host defensive abilities through hydrolytic released compounds of fungal cell walls, e.g. ochitin and glucan fragments. These released oligosaccharides could further stimulate a series of defensive responses in host plants (Lawrence et al., 2000; Edreva, 2005).

SA induced resistance can interact with beneficial bacteria and fungi, such as *Pseudomonas*, plant growth-promoting bacteria (PGPR), arbuscular mycorrhiza fungi (AMF). Those beneficial microbes interact with SA-induced resistance through species-specific microbe-associated molecular patterns (MAMPs) (Bittel and Robatzek, 2007; Choi and Klessig, 2016). MAMPs are special components on the surface of general microbes, such as, *Trichoderma*, *Bacillus* and *Pseudomonas* (Pieterse et al., 2014). Once a host plant recognizes these MAMPs, the plant will activate its innate systemic defensive system to be ready to cope with future pathogens.

Exogenous application of SA or Methyl SA to activate SAR is a way to control microbial pathogenic diseases. In many crops, like tomato, pepper and pea exogenous application of SA results in a suppressing effect on microbial pathogens (Esmailzadeh et al., 2008; Barilli et al., 2010; Choi and Hwang, 2011). Overall, in agriculture, improving SA-mediated resistance has become a promising strategy to control microbial pathogens and viruses.

3. Adaptation of microbes to plant defenses

Plants are not the only organisms that can produce hormonal compounds, which stimulate the activation of signaling pathways in the plant. For example, plant growth-promoting rhizobacteria can produce and/or degrade phytohormones, and in this way interfere with the regulation of plant growth (Dodd et al., 2010). Not only beneficial bacteria but also pathogenic microbes can produce hormones or compounds that impact plant growth.

Some microbial pathogens can mimic the production of plant hormones and are able to highjack the plant immune system (Cui et al., 2005; Laurie-Berry et al., 2006; Navarro et al., 2008). Cui et al. (2005), for example, reported that the bacterial pathogen (*Pseudomonas syringae*) activated induced systemic resistance in *A. thaliana* by producing coronatine (COR). This compound can mimic the function of the JA hormone, thereafter induce the JA-related signaling pathway in host plants. Besides, Laurie-Berry et al. (2006) also demonstrated that *P. syringae* could utilize COR to upregulate the JA pathway in host plants and suppress the SA-mediated signaling pathway in tomato plants, making *P. syringae* even more virulent.

Some bacteria can synthesize SA by converting their chorismate through isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) (Chen et al., 2009; Dempsey et al., 2011). This has been reported for several bacteria species, like *Pseudomonas aeruginosa* and *P. fluorescens* (Mercado-Blancoet al., 2001; Kerbarh et al., 2005; AI-Mustafaet al., 2009). The production of SA by bacteria can increase the plant's resistance against pathogens. For example, the production of SA by *P. aeruginosa* can enhance the resistance of plants against the bacterial pathogen *Botrytis cinerea*, which causes leaf diseases on bean (De Meyer and Höfte, 1997); *P. fluorescens* enhances plant defense in chickpea against *Fusarium* wilt (Saikia et al., 2003).

4. Contradictory observations of SA signaling on the soil microbial community

Many studies investigated the effects of hormonally induced defenses on single pathogens (El-Khallal 2007; Abo-Elyousr et al., 2009; Mandal et al., 2009). However, our knowledge about the impact of activating plant hormonal signaling pathways on the composition of the soil microbial community is still limited and contradictory (reviewed in Hacquard et al., 2017).

Lebeis et al. (2015) examined the effect of SA on isogenic *A. thaliana* mutants with altered immune systems and found that plants with an altered SA signaling pathway contained rhizospheres that differed in the relative abundance of specific bacterial families as compared to wild type plants. Kniskern et al. (2007) using *A. thaliana* mutants found that activation of SA signaling pathways reduced endophytic bacterial community diversity, whereas plants that were deficient in JA-mediated defenses experienced greater epiphytic bacterial diversity. When a plant is exposed to microbial pathogens or herbivory, the plant changes the composition of the primary and secondary metabolites that are produced, and this can impact the soil microbial community, and may result in a feedback to the plant (Rolfe et al., 2019).

Several other studies showed that there was no effect of activation of SA signaling on the soil microbial community. For instance, Wang et al. (2015) found that higher concentrations of exogenously added SA inhibited the growth of grape plants, however, there was no direct correlation between the inhibitory effects of SA on plant growth and the diversity of the soil bacterial or fungal community. Similarly, Doornbos et al. (2011) found that chemical activation of JA- or SA-induced resistance did not significantly affect the composition and diversity of the rhizosphere bacterial community in *A. thaliana*. Hein et al. (2008) compared the effect of SA-induced resistance on the diversity of rhizosphere bacterial communities in several *Arabidopsis* mutants and found that changes in microbial composition were not caused by the induction of the SA signaling pathway. Even though the previous mentioned study showed that activation of SA-dependent defenses did not change the composition of soil microbial community in *A. thaliana* and the SA-independent defense was not induced by foliar application of SA, this still provides a great value to understand the interplay of activating SA-signaling pathways and microbial composition (Sonnemann et al., 2002; Doornbos, et al., 2012; Moccia and Lebeis, 2019).

All taken together, the role of induced resistance on the soil microbial community is still being debated, and more research on the impact of upregulated hormonal signaling in plants on the composition and functionality of the soil microbial community is necessary.

5. Characterizing microbial communities

Microbial communities can be characterized using metagenomics tools. Metagenomics aims at determining the microorganisms as a whole and allows us to extract the biological information of all the microbes from the environment directly

(Hugenholtz and Tyson, 2008). Up to date, metagenomics has been widely applied in various environments to investigate microbial communities ranging from soils, water, ocean and human gut (Handelsman, 2004; Daniel, 2005). However, the lack of reference sequences and genomes is a major drawback of metagenomics (Krehenwinkel et al., 2019).

The microbial community can be characterized on basis of the species present or on the basis on the genes and their functions that are expressed in the microbial community. While the first is highly relevant to understand the diversity and the dynamics of microbial populations and communities, the second is of great importance if we want to understand the mechanisms behind plant-microbial interactions and how a microbial community adapts to the environment. In other words: taxonomic information helps to answer the most primary question for soil microbial-ecologists: Who is there? Analysis of gene expression helps to understand what they are doing. Amplification of 16S rRNA barcode markers is commonly used to determine bacterial microbial communities and the costs of characterizing part of the genome are much lower than sequencing the whole genome. Pipelines for 16S rRNA gene sequencing and identifying operational taxonomic units (OTUs) by aligning the reads against known public databases (e.g. NCBI, EzBioCloud 16S database and MBGD) are available. Nowadays, high-throughput sequencing has become a vital and cost-effective tool for profiling functions of soil microbial communities. It can generate a high volume of data and long read lengths. Illumina short reads sequencing (up to 250 bp) has a high output and low read errors. In this thesis, the Illumina sequencing platform is used to examine the microbial composition and functional genes of rhizosphere soil microbial communities.

Also to process metatranscriptomics data existing pipelines can be used. These pipelines can be modified and applied to different experimental designs. For example, the IMP pipeline incorporates robust read preprocessing and is suitable for analyzing metagenomic and metatranscriptomics as it provides information on both microbial structure and functional genes (Narayanasamy et al., 2016). MetaTrans is an open-source pipeline developed for a paired-end RNA-Seq analysis (Martinez et al., 2016) while the functional mapping and analysis pipeline (FMAP) provides alignment, gene family abundance calculations and open-level statistical analysis (Kim et al., 2016). SAMSA2 is a standalone metatranscriptome analysis pipeline and is used on a supercomputing cluster, which is more flexible and reproductive in processing a large volume of sequence data (Ni et al., 2016). In this thesis I used a modified pipeline that can run on a regular computer and is easily customized. The pipeline that was used assists with the transcriptomic tools Trinity and Trinotate. Transcripts generated by Trinity can be annotated with Trinotate and Trinotate allows users to perform functional annotation with several selective methods, such as homology search, protein domain search, or protein peptide domain search. The combination of these two bioinformatic tools enabled us to explore the structure and the functionality of microbial communities (Haas et al., 2013).

6. Research questions

A number of studies have shown that the overall effect of the soil microbial community on the growth of *J. vulgaris* seedlings is negative (Bezemer and van Dam, 2005; Van de Voorde et al., 2012; Wang et al., 2019). In this project, we asked whether this negative effect of the soil microbial community could be mitigated by the activation of Me-JA and SA signaling pathways through an effect on the composition of the soil microbial community.

First, I studied in four plant species (*J. vulgaris*, *Cirsium vulgare*, *Trifolium repens* and *Daucus carota*) how the growth of these plants was affected by the presence of a live microbial community in the soil. Then, I examined, for *J. vulgaris* that grew less well in live soil than in sterilized soil and for which the negative effect of the live soil on plant growth was mitigated by exogenous application of SA on leaves of the plant, how the application of SA alters the soil microbial community on both taxonomic and functional levels through a multi-generational experiment where I analyzed mRNA of the soil. In addition, I studied for how long during the plant's life the negative effect of a live soil on plant growth is maintained.

Specifically, the following research questions are addressed in this thesis:

(1) Do the effects of the soil microbial community on plant growth differ among four plant species that occur in the same habitat? Does the foliar application of JA and SA alter the effects of the soil microbial community on plant growth of these four plant species? Does the negative effect of the soil microbial community increase or decrease over successive generations of plant growth in *J. vulgaris* inoculated with the soil of the previous generation, and how is this influenced by SA application?

(2) How does the application of SA on *J. vulgaris* affect the composition of the microbial community in the rhizosphere? How does the soil microbial composition change over plant generations, when in each generation sterilized soil is inoculated with soil from the previous generation for plants that are treated with SA and untreated control plants?

(3) Does the application of SA on *J. vulgaris* alter the gene expression in the rhizosphere? Does the application of SA impact microbial gene expression over generations? Which groups of genes are influenced by SA-treated soil samples compared to control over generations?

(4) How long does the effect of inoculum of 10% soil containing a natural microbial community on plant growth last? Does the timing of inoculation change the effect of soil microbial communities on plant growth in *J. vulgaris*?

7. Thesis outline

Many plant species grow better in sterilized soil than in soil that contains a live microbial community, this could be due to an overall net pathogenic effect of soil microbial communities. To find out if an overall negative effect on plant growth is a common phenomenon in nature, in **Chapter 2**, four plant species were grown in either sterilized soil or sterilized soil containing 10% of live soil. In addition, I exposed plant leaves to two hormonal treatments (jasmonic acid and salicylic acid) to examine if hormonal defense pathways can influence the microbial effects on plants.

In Chapter 3, I sequenced and analyzed the microbial communities from the experiment of **Chapter 2**, to investigate if SA-induced defense had an impact on the taxonomic composition of the microbial community in rhizosphere samples using Illumina sequencing. Since the application of SA mitigated the negative effect of soil microbial communities on the growth of *J. vulgaris*, I used this species to study the changes in the composition of the microbial community in response to SA application for four generations of plant growth.

As described in **Chapter 2** and **3**, certain groups of microbial species responded differently to the exogenous application of SA on plant leaves. However, the functions of those microbial species in the rhizosphere are largely unknown. Therefore, **in Chapter 4** I used metatranscriptomics to study the functional genes and clusters in the rhizosphere microbiome of both SA-treated and control samples. The changes in microbial functional genes over four generations were analyzed and compared.

Studies on plant-soil-interactions often address the soil microbial effect with measurements on plant absolute biomass. However, even if there is no difference in the relative growth rate (RGR), the absolute difference in plant growth can still increase. In **Chapter 5,** I studied for *J. vulgaris* how long the negative effect of live soil on plant growth is maintained. Also, I studied if the timing of inoculation affected the RGR of this species. Finally, the results described in this thesis and their implications are discussed in **Chapter 6**.

References

Abo-Elyousr, K.A., Hussein, M.A.M., Allam, A.D.A. and Hassan, M.H., 2009. Salicylic acid induced systemic resistance on onion plants against *Stemphylium vesicarium*. *Archives of Phytopathology and Plant Protection*, *42*(11), pp.1042-1050.

Agrawal, A.A., 2011. Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, *25*(2), pp.420-432.

Al-Mustafa, M.M., Badran, I.Z., Abu-Ali, H.M., Al-Barazangi, B.A., Massad, I.M. and Al-Ghanem, S.M., 2009. Intravenous dexmedetomidine prolongs bupivacaine spinal analgesia. *Middle East J Anesthesiol*, 20(2), pp.225-231.

Aljbory, Z. and Chen, M.S., 2018. Indirect plant defense against insect herbivores: a review. *Insect Science*, *25*(1), pp.2-23.

Antico, C.J., Colon, C., Banks, T. and Ramonell, K.M., 2012. Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens. *Frontiers in Biology*, *7*(1), pp.48-56.

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C. and Wall, D.H., 2009. Home-field advantage accelerates leaf litter decomposition in forests. *Soil Biology and Biochemistry*, *41*(3), pp.606-610.

Azcón-Aguilar, C., & Barea, J. M., 1992. Interactions between mycorrhizal fungi and other rhizosphere microorganisms. *Mycorrhizal functioning: an integrative plant-fungal process. Chapman and Hall, New York*, pp.163-198.

Bach, E.M., Baer, S.G., Meyer, C.K. and Six, J., 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biology and Biochemistry*, *42*(12), pp.2182-2191.

Bardgett, R.D. and Van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), pp.505-511.

Bardgett, R.D., Mommer, L. and De Vries, F.T., 2014. Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution*, *29*(12), pp.692-699.

Barea, J. M., 2000. Rhizosphere and mycorrhiza of field crops. In *Biological resource management connecting Science and Policy*. Springer, Berlin, Heidelberg, 2000, pp.81-92.

Bari, R. and Jones, J.D., 2009. Role of plant hormones in plant defence responses. *Plant molecular biology*, *69*(4), pp.473-488.

Barilli, E., Sillero, J.C. and Rubiales, D., 2010. Induction of systemic acquired resistance in pea against rust (*Uromyces pisi*) by exogenous application of biotic and abiotic inducers. *Journal of Phytopathology*, 158(1), pp.30-34.

Bartwal, A., Mall, R., Lohani, P., Guru, S.K. and Arora, S., 2013. Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. *Journal of plant growth regulation*, *32*(1), pp.216-232.

Beare, M.H., Parmelee, R.W., Hendrix, P.F., Cheng, W., Coleman, D.C. and Crossley Jr, D.A., 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs*, *62*(4), pp.569-591.

Berendsen, R.L., Pieterse, C.M. and Bakker, P.A., 2012. The rhizosphere microbiome and plant health. *Trends in plant science*, *17*(8), pp.478-486.

Berg, G. and Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS microbiology ecology*, *68*(1), pp.1-13.

Bezemer, T.M. and van Dam, N.M., 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology & Evolution*, *20*(11), pp.617-624.

Bhattacharyya, P.N. and Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, *28*(4), pp.1327-1350.

Bittel, P. and Robatzek, S., 2007. Microbe-associated molecular patterns (MAMPs) probe plant immunity. *Current opinion in plant biology*, *10*(4), pp.335-341.

Boller, T. and He, S.Y., 2009. Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science*, *324*(5928), pp.742-744.

Boots, M. and Best, A., 2018. The evolution of constitutive and induced defences to infectious disease. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883), p.20180658.

Börner, H., 1960. Liberation of organic substances from higher plants and their role in the soil sickness problem. *The Botanical Review*, *26*(3), pp.393-424.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K. and Vivanco, J.M., 2008. Root exudates regulate soil fungal community composition and diversity. *Appl. Environ. Microbiol.*, *74*(3), pp.738-744.

Bronstein, J.L., 1998. The contribution of ant-plant protection studies to our understanding of mutualism 1. *Biotropica*, *30*(2), pp.150-161.

Canarini, A., Wanek, W., Merchant, A., Richter, A. and Kaiser, C., 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*, *10*, p.157.

Carvalhais, L.C., Dennis, P.G., Badri, D.V., Tyson, G.W., Vivanco, J.M. and Schenk, P.M., 2013. Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. *PLoS One*, *8*(2), p.e56457.

Carvalhais, L.C., Dennis, P.G., Badri, D.V., Kidd, B.N., Vivanco, J.M. and Schenk, P.M., 2015. Linking jasmonic acid signaling, root exudates, and rhizosphere microbiomes. *Molecular Plant-Microbe Interactions*, *28*(9), pp.1049-1058.

Chen, Z., Zheng, Z., Huang, J., Lai, Z. and Fan, B., 2009. Biosynthesis of salicylic acid in plants. *Plant signaling & behavior*, *4*(6), pp.493-496.

Chen, W.M., Moulin, L., Bontemps, C., Vandamme, P., Béna, G. and Boivin-Masson, C., 2003. Legume symbiotic nitrogen fixation by β-proteobacteria is widespread in nature. *Journal of bacteriology*, *185*(24), pp.7266-7272.

Chen, G., Klinkhamer, P.G., Escobar-Bravo, R. and Leiss, K.A., 2018. Type VI glandular trichome density and their derived volatiles are differently induced by jasmonic acid in developing and fully developed tomato leaves: Implications for thrips resistance. *Plant Science*, *276*, pp.87-98.

Choi, H.W. and Hwang, B.K., 2011. Systemic acquired resistance of pepper to microbial pathogens. *Journal of Phytopathology*, *159*(6), pp.393-400.

Choi, H.W. and Klessig, D.F., 2016. DAMPs, MAMPs, and NAMPs in plant innate immunity. *BMC plant biology*, *16*(1), p.232.

Chung, Y.R., Hoitink, H.A.H. and Lipps, P.E., 1988. Interactions between organic-matter decomposition level and soilborne disease severity. *Agriculture, ecosystems & environment*, *24*(1-3), pp.183-193.

Cowgill, S.E., Wright, C. and Atkinson, H.J., 2002. Transgenic potatoes with enhanced levels of nematode resistance do not have altered susceptibility to nontarget aphids. *Molecular Ecology*, *11*(4), pp.821-827.

Cui, J., Bahrami, A.K., Pringle, E.G. Hernandez-Guzman, G., Bender, C.L., Pierce, N.E. and Ausubel, F.M., 2005. *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores. *Proceedings of the National Academy of Sciences*, *U.S.A,* 102(5), pp.1791-1796.

Daniel, R., 2005. The metagenomics of soil. *Nature Reviews Microbiology*, *3*(6), pp.470-478.

Dawson, W., Hör, J., Egert, M., van Kleunen, M. and Pester, M., 2017. A small number of low-abundance bacteria dominate plant species-specific responses during rhizosphere colonization. *Frontiers in microbiology*, *8*, p.975.

De Meyer, G. and Höfte, M., 1997. Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathology*, *87*(6), pp.588-593.

Decaëns, T., 2010. Macroecological patterns in soil communities. *Global Ecology and Biogeography*, *19*(3), pp.287-302.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D. and Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature communications*, *7*(1), pp.1-8.

Dempsey, D.M.A., Vlot, A.C., Wildermuth, M.C. and Klessig, D.F., 2011. Salicylic acid biosynthesis and metabolism. *The Arabidopsis book/ American Society of Plant Biologists*, *9*.

Dennis, P.G., Miller, A.J. and Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS microbiology ecology*, *72*(3), pp.313-327.

Dimkpa, C., Weinand, T. and Asch, F., 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant, cell & environment*, *32*(12), pp.1682-1694.

Dixon, R.A., 2001. Natural products and plant disease resistance. *Nature*, *411* (6839), pp.843-847.

Dodd, I.C., Zinovkina, N.Y., Safronova, V.I. and Belimov, A.A., 2010. Rhizobacterial mediation of plant hormone status. *Annals of Applied Biology*, *157*(3), pp.361-379.

Doornbos, R.F., Geraats, B.P., Kuramae, E.E., Van Loon, L.C. and Bakker, P.A., 2011. Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of *Arabidopsis thaliana*. *Molecular plant-microbe interactions*, *24*(4), pp.395-407.

Doornbos, R.F., van Loon, L.C. and Bakker, P.A., 2012. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable Development*, *32*(1), pp.227-243

Edreva, A., 2005. Pathogenesis-related proteins: research progress in the last 15 years. *Gen Appl Plant Physiol*, *31*(1-2), pp.105-24.

Ehrenfeld, J.G., Ravit, B. and Elgersma, K., 2005. Feedback in the plant-soil system. *Annu. Rev. Environ. Resour*, 30, pp.75-115.

Ehrlich, P.R. and Raven, P.H., 1964. Butterflies and plants: a study in coevolution. *Evolution*, pp.586-608.

El-Khallal, S.M., 2007. Induction and modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid & salicylic acid): 2-changes in the antioxidant enzymes, phenolic compounds and pathogen related-proteins. *Aust J Basic Appl Sci*, 1(4), pp.717-732.

Esitken, A., Yildiz, H. E., Ercisli, S., Donmez, M. F., Turan, M., & Gunes, A., 2010. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Scientia Horticulturae*, *124*(1), pp.62-66.

Esmailzadeh, M., Soleimani, M.J. and Rouhani, H., 2008. Exogenous applications of salicylic acid for inducing systemic acquired resistance against tomato stem canker disease. *Journal of Biological Sciences*, 8(6), pp.1039-1044.

Fester, T., Giebler, J., Wick, L.Y., Schlosser, D. and Kästner, M., 2014. Plant-microbe interactions as drivers of ecosystem functions relevant for the biodegradation of organic contaminants. *Current opinion in biotechnology*, *27*, pp.168-175.

Frankenberger Jr, W.T. and Arshad, M., 2020. *Phytohormones in Soils Microbial Production & Function*. CRC Press.

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K., 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current opinion in plant biology*, *9*(4), pp.436-442.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M. and Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Appl. Environ. Microbiol.*, *69*(3), pp.1800-1809.

Graham, E.B., Knelman, J.E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J.M., Abell, G., Philippot, L., Prosser, J. and Foulquier, A., 2016. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Frontiers in microbiology*, *7*, p.214.

Grayston, S.J., Wang, S., Campbell, C.D. and Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, *30*(3), pp.369-378.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M. and MacManes, M.D., 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature protocols*, *8*(8), pp.1494-1512.

Habib, H. and Fazili, K.M., 2007. Plant protease inhibitors: a defense strategy in plants. *Biotechnol Mol Biol Rev*, *2*(3), pp.68-85.

Hacquard, S., Spaepen, S., Garrido-Oter, R. and Schulze-Lefert, P., 2017. Interplay between innate immunity and the plant microbiota. *Annual review of Phytopathology*, *55*, pp.565-589.

Handelsman, J., 2004. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol. Mol. Biol. Rev.*, *68*(4), pp.669-685.

Hannula, S.E., Kielak, A.M., Steinauer, K., Huberty, M., Jongen, R., Jonathan, R., Heinen, R. and Bezemer, T.M., 2019. Time after time: temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. *mBio*, *10*(6), e02635-19.

Harman, G.E., Chet, I. and Baker, R., 1980. *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium spp.* or *Rhizoctonia solani*. *Phytopathology*, *70*(12), pp.1167-1172.

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*, *60*(4), pp.579-598.

Hein, J.W., Wolfe, G.V. and Blee, K.A., 2008. Comparison of rhizosphere bacterial communities in *Arabidopsis thaliana* mutants for systemic acquired resistance. *Microbial ecology*, *55*(2), pp.333-343.

Hiltner, L., 1904. Über neuere erfahrungen und probleme auf dem debiete der bo denbakteriologie und unter besonderer berucksichtigung der grundund und brache. *Zbl. Bakteriol*, *2*, pp.14-25.

Howard, M.M., Muñoz, C.A., Kao-Kniffin, J. and Kessler, A., 2020. Soil microbiomes from fallow fields have species-specific effects on crop growth and pest resistance. *Frontiers in Plant Science*, *11*, p.1171.

Huberty, M., Choi, Y.H., Heinen, R. and Bezemer, T.M., 2020. Above-ground plant metabolomic responses to plant-soil feedbacks and herbivory. *Journal of Ecology*, *2020*(108), pp.1703-1712.

Hugenholtz, P. and Tyson, G.W., 2008. Metagenomics. *Nature*, *455*(7212), pp.481-483.

Insam, H. and Seewald, M.S., 2010. Volatile organic compounds (VOCs) in soils. *Biology and fertility of soils*, *46*(3), pp.199-213.

Joosten, L., Mulder, P.P., Klinkhamer, P.G. and van Veen, J.A., 2009. Soil-borne microorganisms and soil-type affect pyrrolizidine alkaloids in *Jacobaea vulgaris*. *Plant and Soil*, *325*(1-2), p.133.

Jung, T., Cooke, D.E.L., Blaschke, H., Duncan, J.M. and Oßwald, W., 1999. *Phytophthora quercina* sp. nov., causing root rot of *European oaks*. *Mycological Research*, *103*(7), pp.785-798.

Kennedy, A.C. and Smith, K.L., 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant and soil*, *170*(1), pp.75-86.

Kerbarh, O., Ciulli, A., Howard, N.I. and Abell, C., 2005. Salicylate biosynthesis: overexpression, purification, and characterization of Irp9, a bifunctional salicylate synthase from *Yersinia enterocolitica*. *Journal of bacteriology*, *187*(15), pp.5061-5066.

Kim, J., Kim, M.S., Koh, A.Y., Xie, Y. and Zhan, X., 2016. FMAP: functional mapping and analysis pipeline for metagenomics and metatranscriptomics studies. *BMC bioinformatics*, *17*(1), p.420.

Kniskern, J.M., Traw, M.B. and Bergelson, J., 2007. Salicylic acid and jasmonic acid signaling defense pathways reduce natural bacterial diversity on *Arabidopsis thaliana*. *Molecular plant-microbe interactions, 20*(12), pp.1512-1522.

Kos, M., Tuijl, M.A., de Roo, J., Mulder, P.P. and Bezemer, T.M., 2015. Plant-soil feedback effects on plant quality and performance of an aboveground herbivore interact with fertilisation. *Oikos*, *124*(5), pp.658-667.

Kostenko, O., van de Voorde, T.F., Mulder, P.P., van der Putten, W.H. and Martijn Bezemer, T., 2012. Legacy effects of aboveground-belowground interactions. *Ecology Letters*, *15*(8), pp.813-821.

Kourtev, P.S., Ehrenfeld, J.G. and Häggblom, M., 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology and Biochemistry*, *35*(7), pp.895-905.

Krehenwinkel, H., Pomerantz, A. and Prost, S., 2019. Genetic biomonitoring and biodiversity assessment using portable sequencing technologies: current uses and future directions. *Genes*, *10*(11), p.858.

Laurie-Berry, N., Joardar, V., Street, I.H. and Kunkel, B.N., 2006. The *Arabidopsis thaliana JASMONATE INSENSITIVE 1* gene is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. *Molecular Plant-Microbe Interactions*, *19*(7), pp.789-800.

Lawrence, C.B., Singh, N.P., Qiu, J., Gardner, R.G. and Tuzun, S., 2000. Constitutive hydrolytic enzymes are associated with polygenic resistance of tomato to *Alternaria solani* and may function as an elicitor release mechanism. *Physiological and Molecular Plant Pathology*, *57*(5), pp.211-220.

Lawrence, P.K. and Koundal, K.R., 2002. Plant protease inhibitors in control of phytophagous insects. *Electronic Journal of Biotechnology*, *5*(1), pp.5-6.

Lebeis, S.L., Paredes, S.H., Lundberg, D.S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., Del Rio, T.G., Jones, C.D., Tringe, S.G. and Dangl, J.L., 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*, *349*(6250), pp.860-864.

Li, N., Han, X., Feng, D., Yuan, D. and Huang, L.J., 2019. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: do we understand what they are whispering? *International Journal of Molecular Sciences*, *20*(3), p.671.

Mandal, M.K., Pandey, D., Purwar, S., Singh, U.S. and Kumar, A., 2006. Influence of jasmonic acid as potential activator of induced resistance against Karnal bunt in developing spikes of wheat. *Journal of biosciences, 31*(5), pp.607-616.

Mandal, S., Mallick, N. and Mitra, A., 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. lycopersici in tomato *lant Physiology and Biochemistry*, 47(7), pp.642-649.

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M., Valencia, M.C., Sanchez, E.I. and Bever, J.D., 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, *466*(7307), pp.752-755.

Manning, P., Morrison, S.A., Bonkowski, M. and Bardgett, R.D., 2008. Nitrogen enrichment modifies plant community structure via changes to plant-soil feedback. *Oecologia*, *157*(4), pp.661-673.

Martinez, X., Pozuelo, M., Pascal, V., Campos, D., Gut, I., Gut, M., Azpiroz, F., Guarner, F. and Manichanh, C., 2016. MetaTrans: an open-source pipeline for metatranscriptomics. *Scientific reports*, *6*, p.26447.

Mercado-Blanco, J., van der Drift, K.M., Olsson, P.E., Thomas-Oates, J.E., van Loon, L.C. and Bakker, P.A., 2001. Analysis of the pmsCEAB gene cluster involved in biosynthesis of salicylic acid and the siderophore pseudomonine in the biocontrol strain *Pseudomonas fluorescens* WCS374. *Journal of Bacteriology*, *183*(6), pp.1909-1920.

Moccia, K.M. and Lebeis, S.L., 2019. Microbial ecology: how to fight the establishment. *Current Biology*, *29*(24), pp.R1320-R1323.

Mousavi, S.R. and Eskandari, H., 2011. A general overview on intercropping and its advantages in sustainable agriculture. *Journal of Applied Environmental and Biological Sciences*, *1*(11), pp.482-486.

Munné-Bosch, S. and Müller, M., 2013. Hormonal cross-talk in plant development and stress responses. *Frontiers in plant science*, *4*, p.529.

Narayanasamy, S., Jarosz, Y., Muller, E.E., Heintz-Buschart, A., Herold, M., Kaysen, A., Laczny, C.C., Pinel, N., May, P. and Wilmes, P., 2016. IMP: a pipeline for reproducible reference-independent integrated metagenomic and metatranscriptomic analyses. *Genome biology*, *17*(1), pp.1-21.

Navarro, L., Bari, R., Seilaniantz, A., Nemri, A. and Jones, J.D., 2008. Roles of plant hormones in plant resistance and susceptibility to pathogens. In *Genomics of Disease*. Springer, New York, NY, pp.1-10.

Neher, D.A., 1999. Soil community composition and ecosystem processes: comparing agricultural ecosystems with natural ecosystems. *Agroforestry Systems*, *45*(1-3), pp.159-185.

Nelson, L.M., 2004. Plant growth promoting rhizobacteria (PGPR): Prospects for new inoculants. *Crop management*, *3*(1), pp.1-7.

Ni, Y., Li, J. and Panagiotou, G., 2016. COMAN: a web server for comprehensive metatranscriptomics analysis. *BMC genomics*, *17*(1), p.622.

Nijjer, S., Rogers, W.E. and Siemann, E., 2007. Negative plant-soil feedbacks may limit persistence of an invasive tree due to rapid accumulation of soil pathogens. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1625), pp.2621-2627.

Nirenberg, H.I., 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. *Canadian Journal of Botany*, *59*(9), pp.1599-1609.

Nunan, N., Daniell, T.J., Singh, B.K., Papert, A., McNicol, J.W. and Prosser, J.I., 2005. Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. *Appl. Environ. Microbiol*, *71*(11), pp.6784-6792.

Pendergast IV, T.H., Burke, D.J. and Carson, W.P., 2013. Belowground biotic complexity drives aboveground dynamics: a test of the soil community feedback model. *New Phytologist*, *197*(4), pp.1300-1310.

Pieterse, C.M., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C. and Bakker, P.A., 2014. Induced systemic resistance by beneficial microbes. *Annual review of phytopathology*, *52*, pp.347-375.

Quiroga, E.N., Sampietro, A.R. and Vattuone, M.A., 2001. Screening antifungal activities of selected medicinal plants. *Journal of ethnopharmacology*, *74*(1), pp.89-96.

Rahbé, Y., Deraison, C., Bonadé-Bottino, M., Girard, C., Nardon, C. and Jouanin, L., 2003. Effects of the cysteine protease inhibitor oryzacystatin (OC-I) on different aphids and reduced performance of *Myzus persicae* on OC-I expressing transgenic oilseed rape. *Plant science*, *164*(4), pp.441-450.

Reinhold-Hurek, B., Hurek, T., Gillis, M., Hoste, B., Vancanneyt, M., Kersters, K. and De Ley, J., 1993. *Azoarcus* gen. nov., nitrogen-fixing proteobacteria associated with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth), and description of two species, *Azoarcus indigens* sp. nov. and *Azoarcus communis* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *43*(3), pp.574-584.

Rolfe, S.A., Griffiths, J. and Ton, J., 2019. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. *Current opinion in microbiology*, *49*, pp.73-82.

Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., Cheng, J. and Zhang, K., 2019. Jasmonic acid signaling pathway in plants. *International journal of molecular sciences*, *20*(10), p.2479.

Ryan, C.A., 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual review of phytopathology*, *28*(1), pp.425-449.

Saikia, R., Singh, T., Kumar, R., Srivastava, J., Srivastava, A.K., Singh, K. and Arora, D.K., 2003. Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. ciceri in chickpea. *Microbiological Research*, *158*(3), pp.203-213.

Schimel, J. and Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. *Frontiers in microbiology*, *3*, p.348.

Schlüter, J.P., Reinkensmeier, J., Daschkey, S., Evguenieva-Hackenberg, E., Janssen, S., Jänicke, S., Becker, J.D., Giegerich, R. and Becker, A., 2010. A genome-wide survey of sRNAs in the symbiotic nitrogen-fixing alpha-proteobacterium *Sinorhizobium meliloti*. *BMC genomics*, *11*(1), p.245.

Seskar, M., Shulaev, V. and Raskin, I., 1998. Endogenous methyl salicylate in pathogen-inoculated tobacco plants. *Plant physiology*, *116*(1), pp.387-392.

Singh, J.S. and Gupta, V.K., 2018. Soil microbial biomass: a key soil driver in management of ecosystem functioning. *Science of the Total Environment*, *634*, pp.497-500.

Sonnemann, I., Finkhaeuser, K. and Wolters, V., 2002. Does induced resistance in plants affect the belowground community? *Applied Soil Ecology*, *21*(2), pp.179-185.

Sturz, A.V. and Christie, B.R., 2003. Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. *Soil and Tillage Research*, *72*(2), pp.107-123.

Turley, N.E., Godfrey, R.M. and Johnson, M.T., 2013. Evolution of mixed strategies of plant defense against herbivores. *New Phytologist*, *197*(2), pp.359-361.

Tyc, O., Song, C., Dickschat, J.S., Vos, M. and Garbeva, P., 2017. The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. *Trends in microbiology*, *25*(4), pp.280-292.

Van der Does, D., Leon-Reyes, A., Koornneef, A., Van Verk, M.C., Rodenburg, N., Pauwels, L., Goossens, A., Körbes, A.P., Memelink, J., Ritsema, T. and Van Wees, S.C., 2013. Salicylic acid suppresses jasmonic acid signaling downstream of SCFCOI1-JAZ by targeting GCC promoter motifs via transcription factor ORA59. *The Plant Cell*, *25*(2), pp.744-761.

Van der Heijden, M.G., Bardgett, R.D. and Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, *11*(3), pp.296-310.

Van der Heijden, M.G. and Wagg, C., 2013. Soil microbial diversity and agro-ecosystem functioning. *Plant and soil*, *363*(1-2), pp.1-5.

Van der Meij, A., Willemse, J., Schneijderberg, M.A., Geurts, R., Raaijmakers, J.M. and van Wezel, G.P., 2018. Inter-and intracellular colonization of *Arabidopsis* roots by endophytic actinobacteria and the impact of plant hormones on their antimicrobial activity. *Antonie Van Leeuwenhoek*, *111*(5), pp.679-690.

Van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A. and Suding, K.N., 2013. Plant-soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, *101*(2), pp.265-276.

Van de Voorde, K., Paauwe, J. and Van Veldhoven, M., 2012. Employee well-being and the HRM-organizational performance relationship: a review of quantitative studies. *International Journal of Management Reviews*, 14(4), pp.391-407.

Van Loon, L.C. and Van Strien, E.A., 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and molecular plant pathology*, *55*, pp.85-97.

Van Loon, L.C., 2007. Plant responses to plant growth-promoting rhizobacteria. In *New perspectives and approaches in plant growth-promoting Rhizobacteria research*. Springer, Dordrecht, pp.243-254.

Wang, B., Guo, X.W., Li, K., Han, X., Xu, S.J., Liu, Z.D., Guo, Y.S. and Xie, H.G., 2015. Effects of Salicylic acid on grape plants and the soil microbial community. *Allelopathy Journal*, *36*(1), pp.49-61.

Wang, M., Ruan, W., Kostenko, O., Carvalho, S., Hannula, S.E., Mulder, P.P., Bu, F., van der Putten, W.H. and Bezemer, T.M., 2019. Removal of soil biota alters soil feedback effects on plant growth and defense chemistry. *New Phytologist*, *221*(3), pp.1478-1491.

War, A.R., Paulraj, M.G., War, M.Y. and Ignacimuthu, S., 2011. Jasmonic acid-mediated-induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Journal of Plant Growth Regulation*, *30*(4), pp.512-523.

Wasternack, C. and Strnad, M., 2018. Jasmonates: News on occurrence, biosynthesis, metabolism and action of an ancient group of signaling compounds. *International journal of molecular sciences*, *19*(9), p.2539.

Wei, G., Kloepper, J.W. and Tuzun, S., 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. *Phytopathology*, *81*(11), pp.1508-1512.

Wesson, R.G. and Wesson, R., 1993. *Beyond natural selection*. MIT press.

Wink, M., 1988. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theoretical and applied genetics*, *75*(2), pp.225-233.

Zahar Haichar, F., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T. and Achouak, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. *The ISME journal*, *2*(12), pp.1221-1230.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D. and Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology*, *84*(8), pp.2042-2050.

Zhang, M. and Yu, JR, 2006. History of Chinese science and technology. Jilin Literature and History Press.

Zhang, S., Zhang, Y. and Ma, K., 2015. The equal effectiveness of different defensive strategies. *Scientific reports*, *5*, p.13049.