From the root of variation: A metabolomics perspective to plant soil-feedback

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Chapter 6

General discussion

The overarching aim of this thesis was to study if and how soil-mediated feedbacks can influence the metabolome of plants growing in these soils. In Chapter 2 we studied how plant soil feedbacks influence metabolomes of plants. We created soils of six grasses and six forbs and tested the metabolomic response of these same plant species when grown in sterilized soil that was inoculated with soils from each of the species. Furthermore, we investigated if the changes of the metabolome upon herbivory were dependent on the soil legacies that the host plants experienced. Most of the tested plant species were sensitive to the soil effects. In Chapter 3 we collected soils from different spatial scales (different sites and different locations within each site) and investigated how those soils influenced the metabolome of *Jacobaea vulgaris*. Here, we showed that the similarity in metabolomics profiles is higher if plants grow with soil inocula collected closely together. This work also shows that there is a connection between soil microbial communities and aboveground plant metabolomic responses. In Chapter 4, we investigated how soils of six different plant species influenced the metabolome of *J. vulgaris* and the effects of those plant-induced changes on the soil on herbivore performance of *J. vulgaris* over the course of one year. We also measured the microbiome of the soils at different time points and showed that metabolomic variation in the plant is related to microbiome changes in the soil. This demonstrates that the impact of soil on the metabolome changes over time but, again, show that metabolome changes in aboveground plant tissues are linked to microbial changes in the soil. Lastly, in Chapter 5 we applied diverse analytical platforms to the metabolomics of a model plant and investigated how they can be integrated in order to enlarge metabolic coverage and strengthen identification power. We suggest a concept of multiplatform metabolomics in integrated manner: each method is employed in each stage (overviewing by NMR, targeted analysis by LC-MS, fingerprinting and preparative works by HPTLC).

In the following, I will address the findings of all chapters and discuss them in a broader context to conclude how this thesis advances the knowledge about PSF effects on metabolomes and the implications that these will have on science in the future.

Soil effects on the metabolome of plants

The main goal of this thesis was to study if plant-mediated changes in the soil, here called soil legacies, influence the metabolome of plants aboveground, and how this relates to the performance of insects that feed on those plants. Earlier studies in our group observed performance changes of insects due to soil legacies (Heinen et al., 2019; Kos et al., 2015c; Kostenko and Bezemer, 2013a). Further, I studied if a broad range of plant species is sensitive to soil legacies or if these soils induced changes occur only in certain species. Recently, several studies were published that examined the effects of soils on the metabolome of plants. For example (Ristok et al., 2019) showed that the diversity of the plant community that was grown in a soil can influence the belowground and aboveground metabolome of four different plant species and that those changes resulted in differences in the performance of a herbivore. Further Badri et al. (2013) showed that inoculation with soils with a different legacy of plants growth (either agricultural or not) influenced the metabolome of *Arabidopsis thaliana* and that those changes resulted in herbivore performance differences. Zhu et al. (2018) used a subset of the plants used in Chapter 2 of this thesis, and showed that the soil legacies of 12 plant species lead to changes in defence-related genes in the plant *Plantago lanceolata* and two iridoid glycosides were changed due to the different legacies. Specifically, Zhu et al. (2018) showed that genes related to the jasmonic acid (JA) pathway in the plant were altered by soil legacies whereas the expression of genes related to salicylic acid (SA) signalling were not altered by soil legacies. In other studies, one component of the soil (bacteria, fungi, chemical composition-exudates) was specifically changed and such changes also resulted in altered metabolomes of response plants (Hu et al., 2018; Mahmood and Kataoka, 2020; Schweiger et al., 2014; Zhou et al., 2018). These recent studies show that soils are factors determining plant metabolomes and that there is rising interest in these soil-mediated effects.

Soils influence the metabolome of most plant species

My thesis now adds to this body of knowledge by testing the effects of the inoculation with whole soil communities on the metabolome of various response plants. We could clearly show that the metabolome of the majority of the tested plant species was influenced by the legacy present in the soil in which they were grown. In Chapter 2, the metabolomic response of 12 plant species to soil legacies created by each of those plant species was studied and we found that only four of the 12 tested plants did not show metabolomic changes due to the different

soil legacies **(Fig. 2.3)**. However, what also appears from my work is that the outcome of such experiments can differ, as we demonstrate e.g. in Chapter 4, where we show that the leaf metabolome of *J. vulgaris* did only differ significantly depending on the plant species which grew in the soil previously, after 8 months of conditioning **(Table 4.2)**. Similarly, in Chapter 3 we show that the metabolome of *J. vulgaris* is sensitive to the soil in which the plant growth, but that this is only true for some sites and not always **(Table S3.2).** All together this indicates that it is still difficult to predict if the metabolome of a plant will responsive to changes in the soil and which factors in the soil explain this pattern.

Which plant metabolites change due to soil-mediated effects?

In our studies we show that the soil in which a plant grows can influence the sugar content, and the concentration of amino acids and secondary compounds of the plant. In Chapter 2 and 3 we found that tyrosine and sugars concentration changed due to soil legacies. These compounds are related to cell wall processes (Loewus and Murthy, 2000; Walling, 2000). Future PSF studies should therefore investigate the impact of PSFs on cell wall components in plants. Cell wall thickness and composition can influence herbivore performance, and cell wall adaptations are a stress response that is commonly observed across the monocotyledon/dicotyledon barrier (Houston et al., 2016). Furthermore, we found that sugar and amino acid concentrations change depending on the soil, in which the plant grows. Although plant-herbivore research has focused recently on the role of plant defence compounds, primary compounds such as sugars can greatly influence herbivore performance on plants (Berenbaum, 1995). In Chapter 3 and Chapter 4, we also showed that the concentration of secondary compounds (e.g. Pyrrolizidine alkaloids) can change depending on the soil legacy, and hence, also the defence status of a plant can depen d on the soil in which it grows. This gives rise to many possible exploitations which are described in more detail in the future directions section of this chapter.

Chemical variation

Although we recorded strong effects of soil legacies and herbivory on the metabolome of twelve different plant species in Chapter 2, the part of the variation explained by soil and herbivory largely differed between species **(Fig. 2.3)**. This is interesting as it raises the question why certain plant species, such as *Taraxacum officinale*, are more sensitive to soil legacies, than others like *Geranium molle*. So far, it is unknown why sensitivity of plants to soil varies and we can only speculate about the underlaying reasons. It is plausible that soils conditioned by other plant species will have stronger effects for the unresponsive species. Maybe some of the species do occur more often together than others and are therefore more prone to the soil legacies that they were exposed to in nature. Another possibility is that the metabolome of some plants is overall more sensitive in, or specifically to changes that occur in the soil. It is notable that for some plant species only 20% while for others up to 70 % of the variation of the metabolome is not explained by soils and herbivory. It is important that PSFs explain so much of the variation for some plant species, but there also is an urgent need to further explore the relevant factors that cause chemical variation in the plant species that were relatively insensitive to soil. How do these species respond to other environmental stresses? Could their metabolomic capacity for changes to different stresses be measured? Do they rely less on induced defences upon herbivory than the others? All these questions are surely interesting and should be addressed in future studies.

Effects of metabolome changes on insect performance

Soil legacies can impact the metabolome of a broad range of plant species and via soils, plants potentially influence other plants growing in the same soil at a later timepoint. This can be a reason for effects of PSF on insect performance on those plants as found in several recent studies (Hannula et al., 2019b; Heinen et al., 2019; Pineda et al., 2020). However, when we tested if changes in the metabolome explained the performance of *M. brassicae,* we could only find such a relationship in soils conditioned for 8 months **(Table 4.4)**. Numerous previous studies have found a relationship between specific metabolites and insect performance (Hu et al., 2018; Kos et al., 2015c; Ma et al., 2017; Zhu et al., 2018). One possible reason why we did not find such relationship could be linked to the holistic approach we choose. We related the whole metabolome structure to the performance of the herbivore, something which, to our knowledge, has not been done so far. Most studies either apply untargeted metabolomic

analyses, identify the signals which differ most between herbivory and non-herbivory, or carry out targeted metabolomics and correlate changes in these selected compounds to herbivore performance (Ma et al., 2017; Marti et al., 2013). In untargeted metabolomics, it is possible that the effect of a group of compounds or one specific compound is missed since the extraction and detection are targeted to detect as many compounds as possible which might result in less fine scaled results. On the other hand, several publications have pointed out that is highly likely that for interactions between plants and insect herbivores, many compounds may play a role and these compounds can interact in how they influence the herbivore (Berenbaum and Zangerl, 1993; Houston et al., 2016; Liu et al., 2017; Nelson and Kursar, 1999). Therefore, the approach we used might reveal the real interaction between a plant and a herbivore more closely than correlations based on one or a few specific compounds. It is important to note that in Chapter 4, the influence of PSFs on herbivore performance was surprisingly weak and this could be another reason why the metabolomic changes were not linked to the performance of the herbivore in most of the experimental PSF rounds. Other studies found strong effects of PSF on insect performance, but these effects strongly depend on the plant species and the insect species used (Pineda et al., 2017). Furthermore, soil-mediated effects were found to considerably differ depending on the conditioning plant species. An earlier study conducted in our group showed that PSF effects on growth, defence and susceptibility to soil-borne diseases differed among 37 conditioning plant species (Ma et al., 2017).

Therefore, it is possible that the choice of the herbivore and plant species, in this study, resulted in the finding that the metabolome and herbivory performance were not linked.

Ideally, studies should explore the metabolomic differences for a multitude of plant species grown in differently conditioned soils and treated with different herbivore species. Of course, reality applies restrictions to the dimensions of an experiment, and a trade of between complexity of the model and feasibility of the execution needs to be found. One solution for this could be to build a network of researchers working on PSF. Samples from the different experiments could be collected and combined for data analysis. However, such large-scale collaborative actions require standard protocols and procedures in how to carry out PSF experiments, and these are hard to agree on. The idea of standardized protocols for PSF research has been advocated several times in the recent literature (Brinkman et al., 2010; Gundale et al., 2019). Scientists that focus on investigating the biomass responses of plants,

for example, could freeze-dry the harvested material instead of drying it in the oven, so that the samples could also be used for metabolomics analyses. This would offer the possibility to explore the reproducibility of the effect of soils on the metabolome across different experiments and the samples sizes will be much larger and conditions much broader than what a single study can provide at the moment. Different handling and procession of samples as well as additional data acquisition is always associated to additional costs. It is therefore important to agree on such an endeavour in the scientific community. This would be an important step towards making the soil-metabolome connection applicable for agriculture and horticulture and therefore can also greatly increase its relevance for society.

Different plant species lead to different microbiomes

Not only do the plant species differ in their response to PSF, they also have distinct species-specific effects on the soil in which they grow. For example, studies have shown that the microbiome of the soil differs depending on the species and/or the functional group of the plant species grown in it (Bezemer et al., 2018; Hannula et al., 2019a; Heinen et al., 2018; Latz et al., 2016, 2015). The reasons for these differences between the functional groups can be manifold. Grasses and forbs do not only differ morphologically but also in their root architecture. Grasses produce finer but denser roots compared to forbs. Therefore, grasses might have a larger area of contact with soil microorganisms than forbs. This might lead to a more distinct influence on the microbiome of the soil (Brundrett, 2002). Several studies have also reported that plant-growth-promoting-rhizobacteria accumulate in soils of grass species (e.g. Latz et al., 2012). These bacteria have the potential to change the composition or concentration of defence compounds in aboveground tissues (Pangesti et al., 2015; Van Oosten et al., 2008). Not only does the effect that plants have on soils differ between functional groups, often soils in PSF are categorized in conspecific and heterospecific soils. Most plants grow less well in their own soil and hence better in soils in which a plant that belongs to another species has grown before (Kulmatiski et al., 2008), probably due to the accumulation of species-specific pathogens in the soil (Reynolds et al., 2003; Van der Putten et al., 2013). Furthermore, some soils might contain a higher density of mycorrhizal fungi than other soils due differences in the capacity to accumulate mycorrhizal fungi by different plant species. Based on my work and that of others we can conclude that PSFs are highly plant species-dependent and, hence, this should always be taken into account in PSF studies.

What are the driving forces of soil-mediated effects on plants?

The mechanisms underlaying PSFs can be numerous and reach from changes in the microbiome of the soil and abiotic conditions of the soil to extracellular-self DNA of plants (Dostálek et al., 2016; Mazzoleni et al., 2015; Wang et al., 2019a). In this thesis we investigated the microbiome and the abiotic conditions of the soil as causal agents of PSFs, although we only analysed the microbiome of the soils in Chapter 4. The changes in the metabolome that we recorded in response to the soil inocula used in our studies, are likely due to differences in the microbiomes of the soils, since we mixed a small portion of the inoculum with a large portion of sterilized soil, in order to reduce the differences among the conditioned soils in nutrient availability. This is common practice in PSF studies, and in Chapter 3 **(Table S 3.4)** we compared the nutrient content of soils after *J. vulgaris* had been grown in these soils and did not detect differences in nutrient contents. Furthermore, we also studied the nutrient content of the soils used as inocula in Chapter 4 and did not detect significant effects of soil nutrients on the metabolome **(Table 4.3)**. Therefore, we conclude that the differences in the plant metabolomes are caused by differences in the microbiota in the soils. This was actively tested in Chapter 4 where we show that the metabolome composition is influenced by the bacterial and fungal community. We correlated the fungal and microbial communities sequenced in the soil samples, with the metabolome composition of the plants which grew in sterilized soil inoculated with theses soil samples. This experiment demonstrated that the many metabolome changes are linked to specific bacteria and fungi in the soil. However which bacteria/fungi led to which alterations differed between the experimental rounds and more research is needed to better understand the influence of particular bacteria and fungi in the soil on the plant metabolome. For this it might be a good approach to start with a simple experiment in which sterilized soil is inoculated with cultures of the bacteria or fungi that were related to changes in the metabolites and then examine the metabolome response under these more controlled conditions.

How do soil microbiome changes lead to metabolome changes?

Differences in the metabolome of a plant due to the distinct soil legacies can be caused by direct or indirect effects of the microbiome in the soil on the plant. Bacteria and fungi in the soil can directly influence the metabolome of a plant by activating immune responses such as JA and SA pathways in the plant and trigger the induction of inducible defences. However, there are also many possibilities for how the microbiome of the soil can indirectly influence the metabolome. Certain bacteria and fungi are known to enhance growth of plants and that can result in changes in the metabolome of the plants or cause changes in the concentrations of compounds in shoots and roots (Hol, 2011). Plants with more biomass, for example, often contain higher concentrations of sugars than smaller plants. Endophyte communities in the plant also depend on the microbiome of the soil (Lundberg et al., 2012). Endophytes interact with the plant in various ways. They can produce beneficial compounds, helping the plant to protect itself, or themselves can process compounds produced by the plant. By this, compounds used by the plant for defence can become more toxic. Furthermore, a plant's hormone signalling pathway can be manipulated and by that plants increase the resistance to pathogens (Khare et al., 2018; Vandenkoornhuyse et al., 2015). Furthermore, through enzyme exudation, certain communities of bacteria and fungi increase the availability of nutrients in the soil, and thereby change the nutritional status of the plant. Other microbes are known to control the abundance of pathogenic bacteria and fungi in the soil by the production of antibiotics, hydrogen cyanide or enzymes (Ferreira et al., 2019). It was beyond the scope of this thesis to pinpoint the specific mechanisms causing the changes in plant metabolomes. Future research is needed to identify these mechanisms. Multi -omics has great potential to investigate the transcriptional and genetic changes in the plant, and will make it possible to more exactly pinpoint specific mechanisms.

Spatial variation in soil effects on plants

The microbiome of the soil can differ at scales from millimetres to centimetres. This heterogeneity in the microbiome of the soil is driven by abiotic as well as biotic factors (Fierer, 2017). In Chapter 3 we studied if such spatial differences in the microbiome lead to differences in the chemical composition of plants. We showed that the metabolome of plants grown in soils with inocula collected in close proximity is more similar than the metabolome of a plant grown with inocula collected at larger distances **(Fig. 3.4)**. These spatial differences are

probably linked to specific bacterial and fungal communities, known to strongly differ in abundance, even at small spatial scales. However, we can only speculate about the driving forces of these effects since we did not measure the microbiome of the soils used for inoculation. Nevertheless, the interaction of soil and plant at each specific spatial scale can be one explanation for the often-unexplained large chemical differences among plants of the same species in the field. This variation leads to several questions such as: is this also the case in intensively used agricultural landscapes such as monocultures of crops, and if so, can plants actively chose in which patch in the soil to grow their roots? This could be for example triggered by volatiles released by the bacterial communities in the soil and picked up by the plant. A first attempt to investigate this has been made by (Hendriks et al., 2015), who showed that spatial heterogeneity of PSFs influences root responses. However, in their setup it remains unclear if plants can actively choose the spots where to put their roots in a heterogenic environment. Finally, the spatial factor of the soil is enormously complex which makes it extremely challenging to measure, study and manipulate it.

The effect of temporal variation of plant soil feedbacks on plants

One of the common criticisms to PSF research has been its reproducibility and consistency over time. Two different phases of PSF studies can be influenced by time: the conditioning and the feedback phase. In this thesis we investigated how an increase in conditioning time influences the microbiome in the soil, the biomass and the metabolome of plants grown in these soils and their resistance to herbivory.

In Chapter 4 we investigated the changes of PSF over the course of a year with soils that were conditioned for different times while keeping the duration of the feedback phase constant **(Fig. 4.1)**. *J. vulgaris* produced most biomass in sterilized soil and least in its own soil **(Fig. 4.2).** This has been found also in other studies (Kos et al., 2015c; Van de Voorde et al., 2012, 2011). However, in Chapter 3, the biomass of *J. vulgaris* grown in 100% sterilised soil and inoculated soil did not differ **(Fig. 3.5)**. Another study in our group, showed that the positive effect of sterilized soil on the biomass of the plant (in this case Chrysanthemum) did reverse after one growth cycle and the plants performed better in soils which were inoculated with plant-conditioned inocula than in sterile soil (Ma et al., 2018). Also, in another study using *J. vulgaris* plants did not produce most biomass in sterilised soil (Joosten et al., 2009). Sterilized

soil is a medium that can be easily colonized by bacteria and fungi from the environment as there is no microbiome present in the soil that these colonizers have to compete with.

In Chapter 4 we further investigated how the plant metabolome changes when the conditioning period increases. It is difficult to predict the effect of an inoculum on the metabolome of a plant as the specific connections between the microbes in the soils and the metabolome changes differed between the rounds of the experiments **(Table 4.2)**. In Chapter 4, this can be caused by the different ages of the soils that were used. This stresses the importance to replicate PSF studies investigating the metabolome of the plants under exactly the same conditions to understand how universal the influence of soils or soil microbes on specific metabolites is. As causal agents of the changes over time we investigated the fungal and bacterial communities in the soil and their changes over time in Chapter 4. In Hannula et al. (2019a) these microbial changes were examined in much more detail. There we show that the bacteria greatly differ over time independent of the plant species that conditions the soil, but that and fungi mainly differ depending on the conditioning species. In Chapter 4 we show that the metabolome changes in the plant were mostly related to bacterial changes in the soil. It seems that the effects of the soils on the biomass of *J. vulgaris* are mainly driven by fungi in the soil and therefore stay more similar over time, whereas the effects of bacteria are mainly on the metabolome, and hence the metabolome of *J. vulgaris* changed greatly depending on when the soil was collected. Whether this is true remains to be confirmed, but the potential implications warrant that these contrasting effects of bacteria and fungi on plant growth and chemistry should be examined in more detail in future studies.

Metabolomics – a key tool for today's ecologists

In this thesis I showed that metabolomics is a tool which enables scientists to investigate a language that is important of many interactions between plants and their environment – the metabolome. Ecometabolomics is a promising and new strategy which uses a metabolomic approach to address and understand ecological problems (Peters et al., 2018). In Chapter 2 we showed that it is important to not only investigate concentrations of targeted groups of compounds but instead to investigate changes in the whole metabolome that occur due to interactions of the plant with the soil and herbivore. However, even today, it is not possible to reach the ultimate goal of metabolomics: to extract, detect and quantify all compounds in an

organism. To accomplish this goal, different platforms for metabolomics should be used together and their outcome linked. A study pointing in this direction is presented in Chapter 5.

Metabolomics is a new tool to explore complex ecological interactions, introduced only just a decade ago. Although its application has often yielded new and promising results, most studies on plant ecology still focus on targeted compounds. There can be many reasons for this, but one is that access to metabolomics facilities is often unavailable to ecologists. Interdisciplinary approaches have led to great success in tackling problems in science, and in my opinion, more courage is required to combine ecology with cutting-edge techniques from other fields. Even though it is often challenging to find a consensus between the ecologist's and chemist's view on the design and interpretation of an experiment, this thesis proofs that the outcome can be very rewarding. By using metabolomics approaches we could show in this thesis that the changes in the metabolome of plants do not always occur in the metabolomic groups that we expect. I propose that there is a lot of potential in other ecological research to discover pathways and compounds in plants that are influenced by treatments, but have been neglected so far and this is essential to obtain a realistic and holistic view of the processes that occur within the plant.

Although the techniques and the sensitivity of the tools used in metabolomics have greatly improved over the past years, it is still not possible to detect and identify all metabolites within an organism with a single metabolomics platform and one extraction process. Ecologists often conduct experiments with many biological replicates but with relatively low amounts of sample available per replicate, Ample amount of sample, is however, one of the main requirements of metabolomics facilities, and therefore often there is only enough material for one extraction process. We faced this problem in chapters 2, 3 and 4, and therefore aimed to provide a framework to overcome this issue in the last experimental chapter. One way to approach this is to use one extraction method, analyse the sample with a non-destructive platform and recover the sample to analyse subsequently on another platform. However, this is often extremely time consuming and instead of a single dataset, multiple datasets from different platforms have to be analysed and combined. There are methods available to link datasets statistically, however, this remains a challenging task, especially since the different matrixes have different sizes. Consecutive analysis is also not applicable for all metabolomics platforms and may require extensive laboratory work before it can be applied. For example,

samples extracted for GC-MS cannot be used at another platform because derivatisation is required. Therefore, there is a clear need for a more optimized strategy for multi-platform metabolomics.

In general, NMR represents a method with a relatively simple sample extraction method, that still allows the identification of a large range of compounds. However, signals often overlap and it is difficult to identify secondary compounds with this method. In Chapter 5 we explore an alternative solution and use an intermediate step of HPTLC which can be used to examine global differences in the metabolome of plants between treatments. In this setup, compounds can be split according to their chemical properties and different reagents can be used to colour them according to their chemical group. Subsequently, data has to be statistically analysed to find groups which differ among the treatments. With the chemical information (e.g. polarity, chemical group) of these groups, the fractions can then be analysed with the suitable platform **(Fig. 5.1)**. Another method, to simplify metabolomics work in the future, especially to simplify the identification of compounds are MS-MS networks. These networks deploy similarities in information such as mass-to-charge ratio (m/z) and group compounds with similar chemical properties together which helps to group signals which would not have been identified otherwise **(Fig. 5.4)**. This is important since, for example, for LC-MS data a detailed library including all potential compounds is often not available.

Implications for ecology

Metabolomic changes are often related to a complex network of influencing factors. Regardless, in our experiments we could show the influence of soil on the metabolomes. Hence, soil characteristics may be one of the potential drivers of insect distributions observed in the field.

Practical implications

Although soil is acknowledged more and more as being important for plant growth, it is still often ignored in studies investigating plant responses. Greenhouse studies most often use potting soil and probably all plant ecologists – including myself – have done experiments using potting soil. But not only are the nutrient levels in potting soil often not representative for the nutrient levels occurring in natural soils but most certainly the microbial communities in these potting soils are also not comparable to naturally occurring bacterial and fungal communities in the soil. With this thesis I now show that the microbiome of the soil can not only influence the growth of plants but in most cases also impacts the metabolome of plants. Therefore, it is of great importance that ecologists investigating for example insect performance on plants take the microbiome of the soil into account. Furthermore, studies investigating the metabolome of plants often ignore soils as a component of the experiment and with that ignore the fact that the choice of the soil can influence the outcome of the study. The outcomes of my thesis exemplify that plant ecologists and plant chemists should think about the soils they use for experiments and consciously incorporate soils in the experimental design.

Future directions

Active manipulation of chemical composition

The findings of this thesis are a first step in understanding how soils change the metabolomes of plants. Thinking about this concept it would be possible to change the chemical composition of plants by inoculation of soils with certain bacteria or fungi. Of course, to achieve this further research is needed that investigates combinations of bacteria of fungi and different plant species and that identifies their specific effects on the metabolome. Furthermore, the applicability of soil inoculation to soils that already contain a microbiome will need to be tested. If soil microbiomes can be steered, inoculation could for example be used to enhance the taste of fruits and crops in a natural way. Furthermore, an intriguing question is how volatiles of plants change in response to variation in certain components of the soil, and how this then influences predator host location, or pollination rates which currently decline due to a decreasing number of insects (Cardoso et al., 2020). In agriculture it is already common to inoculate fields with beneficial bacteria in order to increase the yield of plants or to make them

more resistant to herbivores (Compant et al., 2005). However not much is known about the effect of this on the metabolomes of plants. Therefore, it can be assumed that optimal conditions have not yet been discovered and that there is still room for improvement as well as targeted application of soils to trigger specific plant responses. With this thesis I provide more evidence that the soil microbiome is an important factor for the chemical composition of plants and this paves the way for future studies in the field.

Interacting spatial and temporal variation in PSF

In this thesis we investigated how soil - metabolome relationships change at different spatial and temporal scales in two separate chapters. The interaction of spatial and temporal variation in PSF is still lacking and this should be explored in future studies. One of the reasons for this is that long term experiments across large spatial scales are difficult to setup and maintain. However, the effort would be worth to learn more about the variation of the effects in nature and see if they act in similar or different ways at different spatial scales. A recent study (Zhang et al., 2020) investigated spatial dynamics in microbes in the soil over time. They showed that spatial or temporal heterogeneity is far more important for their composition than seasonality and this has been confirmed by other studies (Fierer and Jackson, 2006; Lauber et al., 2013). It is important to study if this also holds true for the metabolome of the plants that grow in these soils.

PSF under climate change

Climate change is impacting ecosystems all over the world, but what will happen to plant-soil interactions? To examine how climate change will influence the interactions of plants and microbes and the effect that they have on plant chemistry is an important future direction. Especially when those patterns are linked to how insects will perform on plants in the future climates. It is argued that genetic variability makes plants more resilient to climate change, however, a variation in the rhizosphere microbiome may also be important for creating plants that are resistant or well-adapted to climate change. Certain bacteria can increase resistance of plants to droughts or increase availability of nutrients to the plant (Vurukonda et al., 2016). Additionally, microbes in the soil differ in their sensibility towards temperature increases, which often go along with climates with increases in droughts (Oliverio et al., 2017). This

might lead to unforeseen changes in the microbiome of the soil tand in metabolome changes in the plants grown in these soils. Another possible effect of climate change might be the loss of biodiversity not only aboveground but also belowground (Bardgett and van der Putten, 2014). On this line of reasoning, a loss of biodiversity below ground could result in a loss of diversity in the metabolites of a plant, which can have far stretching consequences. Another recent study (Ristok et al., 2019) supports this proposition. To predict the effects of such losses on the metabolome of plants, and with that on their interactions with the environment, will be an important next step.

Combining omics techniques

With the recent advancements in omics techniques, mixed-omics is becoming more and more common and is slowly turning into a useful tool for ecologists. Linking, for example, genomics and transcriptomics to metabolomics would help in our study system to understand which chemical pathways in plants are activated through the different microbiota of the soil. In a way, this thesis is a first attempt in fusing two omic techniques for our study system by combining the microbiome in the soil with the metabolome of the plants that grow in these soils in Chapter 4. In my opinion omics techniques will become more readily available to a broader scientific community in the near future, which will aid these scientists to better understand specific plant-microbe interactions, such as which genes are switched on in the plant due to which microbes in the soil, and as a consequence which metabolites change in the plant. This can lead to a new approach in ecology and help to truly understand the mechanisms behind different ecological processes.