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

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Summary

Soil is not only a substrate for plants to grow in, it is a substantial part of the system in which plants live and defines the identity of plants. While growing in a soil, plants change the abiotic and biotic properties of the soil. These effects on the soil are species specific and can influence, mainly through changes in the bacterial and fungal soil communities, the properties of plants growing in this soil later on. Those so-called plant mediated changes in the soil, or plant-soil feedback, are known to change the biomass of plants as well as the concentration of specific chemical compounds within the plant. This is probably also directly related to the performance of insects interacting with these plants. Up to today, studies that investigate the changes in the chemistry of plants due to soil legacies mostly use targeted approaches, focusing on one or a few specific compounds in plants. However, the plant metabolome is composed of hundreds to thousands of metabolites. Untargeted metabolomics aims to measure all metabolites within an organism and by that provides an overall view of its chemical state. Modern metabolomics provides the possibility to investigate the full metabolome response to ecological changes plants face, such as plant mediated changes in the soil. In this thesis, we harnessed the power of these metabolomics techniques to answer the question, if and how plant metabolomes change, depending on the soil.

First, we investigated if the effects of soils on metabolomes are a general phenomenon and common across different plant species. Additionally, we investigated if the effect which soils have on the metabolome are as pronounced as herbivory, which is well known to have strong impact on the metabolome. We set up a fully crossed design, in which we tested the metabolic response of 12 plant species to soil mediated plant legacies of all 12 plant species as well as herbivory. To evaluate the changes in the metabolome due to soil legacies and herbivory, the shoots of all plants were analysed with ^{1}H nuclear magnetic resonance (NMR). For 7 out of 12 plant species, soil explained most of the variance in the metabolome. This effect was even stronger than herbivory. We were able to show that the influence of soil on the metabolome of plants is common across different plant species and therefore soil might be one of the relevant factors to understand the often-unexplained intraspecific variation in the chemical composition of plants.

Bacterial and fungal communities in the soil do not only depend on the plant species growing in the soil, but they can also differ on spatial scales of millimetres to centimetres. In nature, these soil communities are known to be one relevant factor to explain composition of plants species and the insects that interact with them. However, so far it was unclear how this spatial variation of microbiomes affects the chemical composition of plants. To address this question, we set up an experiment, in which sterilized soil was inoculated with soils collected from different fields according to a spatial gradient. Identical clones of *Jacobaea vulgaris* were grown in these differently inoculated soils, weighed for biomass and analysed with ^{1}H NMR to assess the foliar metabolome. Metabolomic profiles of plants were more similar if the soil samples were collected closer together. However, only in samples from one of the four grasslands did the metabolome significantly differ from that of plants grown in sterilized soil. This proofs that soils of different spatial scales can indeed have a distinct effect on the metabolome, but also that not all soil inocula have an effect on the metabolome.

The effect of soil legacies does not only differ within space but the properties of the soil also depend on the time of conditioning, referring to the time of growth of the first plant species. To explore the variation of soil legacies over time, we repeatedly examined plant-soil feedback by measuring biomass, herbivory and metabolomic composition for genetically identical *J. vulgaris* plants in monoculture soils. This data was acquired for a full year. While plant biomass in monoculture soils was similar over the course of the year, the effects of the soils on the metabolome of plants varied greatly. As causal agent of these effects we assessed the fungal and bacterial communities in the soil. Bacteria communities manly differed between the time and fungal community composition did differ strongest between the different monocultures. The metabolome changes in the plants could be related to these community shifts in the soil. Variation in the metabolome of plants was mostly related to changes of bacterial communities in the soil and the effect of soils on biomass was probably due to changes in the fungal communities of the soil. This study provides evidence that there is a distinct difference between the effect of fungal and bacterial communities on plants over time.

In previous chapters of this thesis we used one common metabolomics technique - ^{1}H NMR, a platform that excels at giving a broad overview of the metabolome and detecting abundant compounds within the metabolome. However, the ultimate goal of metabolomics is to extract and detect all metabolites within an organism. For now, this is ambitious goal cannot be reached by one technique. In this last chapter we give an overview of the currently available platforms for metabolomics and explain on the example of *Taraxacum officinale* how the strengths of the different platforms can be combined. We used a combination of ^{1}H NMR, high-performance thin-layer chromatography (HPTLC) and liquid chromatography with tandem mass spectrometry (LC-MS-MS) to examine herbivory effects. We introduce the concept of HPTLC and demonstrate how it can be used for metabolomic fingerprinting as well as for the separation of compounds, which can be analysed subsequently with other methods. With HPTLC we were able to concentrate and identify a chemical compound, campesterol, which was not detected with conventional methods, but showed a significant response to herbivory treatment. This highlights the importance of translating cutting-edge metabolomics tools to ecology-based research.

In conclusion, in this thesis I have shown that changes, belowground, in the soil can have implications on the language of plants – the metabolome aboveground. It is important to note that these effects differ in time and space. I provide evidence that soils can be one important factor to explain the variation in plant chemistry in nature. However, this thesis only represents a first step towards a better understanding of how microbial communities influence the chemistry of plants, and with that also the insects interacting with this plant in nature. We demonstrate that there is large variation in the effects of soils on plant metabolomes and with this thesis I set the ground for future studies to explore this variation and the underlaying mechanisms. This will help us to get a better understanding of plant-soil interactions in nature and how to utilize these effects to overcome ecological and environmental challenges of the future.