Understanding disease suppressive soils: molecular and chemical identification of microorganisms and mechanisms involved in soil suppressiveness to Fusarium culmorum of wheat

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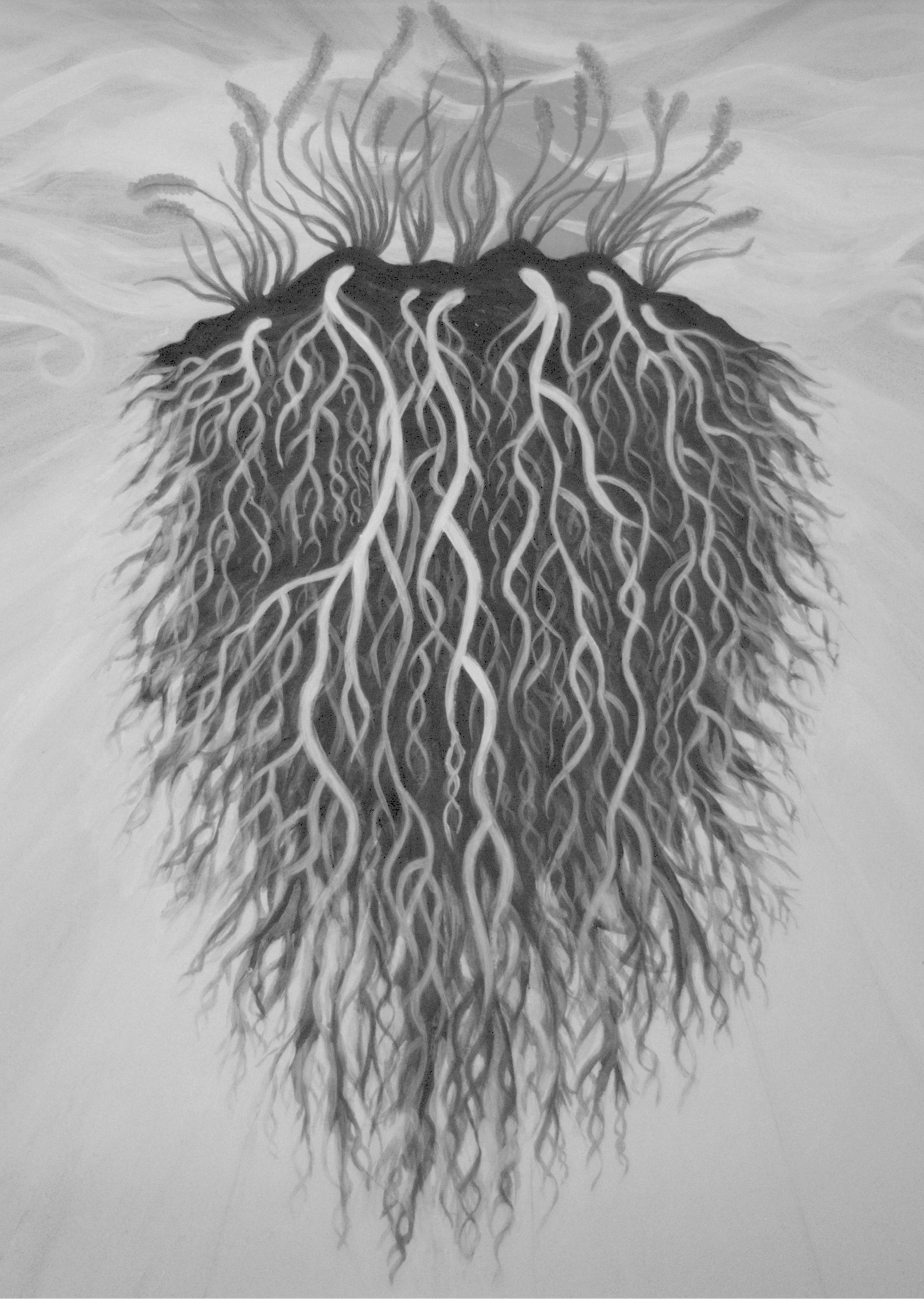

Chapter 6

General and summarizing discussion

In 2019, the European Union launched a strategy towards a zero pollution and toxic-free environment ("A European Green Deal," 2019). One of the targets in the so-called "From Farm to Fork" actions for the year 2030 is to reduce the use of chemicals and hazardous pesticides in agriculture by 50%. Hence, one of the great challenges of our generation is to increase agricultural yields and enhance nutritional quality of food crops while reducing the input of fertilizers and pesticides. Soil-borne diseases caused by pathogenic fungi form a major threat for crop production worldwide. Resistance breeding, chemical and cultural control measures have been largely unsuccessful to date for most fungal root pathogens. Hence, 'Learning from Nature' by exploring and exploiting the natural mechanisms of soil disease suppressiveness gives us a profound opportunity to develop effective and sustainable strategies to protect crops from fungal root pathogens. In my thesis, I explored the microbiome-driven phenomenon of soil suppressiveness to *Fusarium culmorum* in wheat, building a basis to identify the responsible microorganisms and to understand the underlying mechanisms of disease suppression (Chapters 2-4). Moreover, I evaluated the impacts of microplastic pollution on disease suppressiveness and the rhizosphere microbiome of wheat (Chapter 5). The main findings of each of the chapters are summarized in figure 1 and discussed in more detail below.

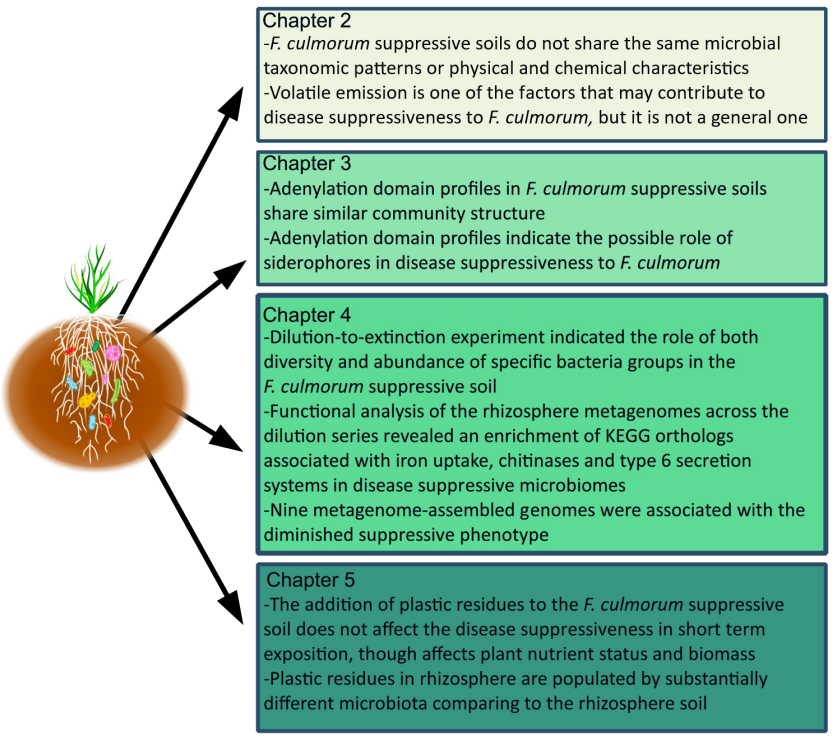

Figure 1. Summary of the main findings presented in the four experimental chapters of this PhD thesis.

The distribution of disease suppressiveness of soils to *Fusarium culmorum*

Natural disease suppressiveness of soils to soil-borne pathogens was recognized worldwide already more than a century ago (Atkinson, 1892; Baker and Cook, 1982; Gomes Exposito et al., 2017; Hornby, 1983; Schlatter et al., 2017a). For most soil-borne pathogens, however, the distribution of disease suppressiveness across multiple agricultural soils is unknown because of a lack of simple diagnostic tools. In *Chapter 2,* we conducted for the first time a large-scale screening of soils from different regions in the Netherlands and Germany for suppressiveness to root rot of wheat caused by the fungal pathogen *Fusarium culmorum*. This economically important pathogen causes significant losses in cereals, mostly in wheat and barley (Dean et al., 2012; Scherm et al., 2013). Because of mycotoxins production, it also contaminates grain making it unfit for human and animal consumption or malting (Antonissen et al., 2014; Nielsen et al., 2014). We found a baseline level of disease suppressiveness to *F. culmorum* in many of the screened soils, but a high level of suppressiveness was found in 4 out of 28 soils tested. In all four, the suppressiveness was eliminated by sterilization and could be transferred to a non-suppressive soil by transplantation (*Chapter 2*). Based on the results presented in *Chapter 2* we concluded that the distribution of *F. culmorum* suppressive soils does not follow a specific geographic pattern or field history. Moreover, we did not find any significant correlation with physical or chemical soil properties. To look for enriched bacteria populations in *Fusarium culmorum* suppressive soils, we performed 16S-based taxonomic profiling of all 28 soils. Based on our results we did not find any specific taxonomic group of bacteria that was significantly enriched in the suppressive soils; however, we found *Acidobacteria* to be hub taxa in some of the suppressive soils based on co-occurrence network analysis. The results presented in *Chapter 3* indicate that co-occurrence patterns of A-domains (functional amplicons) are much better predictors of soil suppressiveness than microbial taxonomy based on 16S amplicon sequencing. This is in line with studies revealing that functional genes involved in secondary metabolite production can be characteristic to disease suppressive microbiomes. For example, the *take-all decline* (TAD) soils show an overrepresentation of 2,4-diacetylphloroglucinol (*phl*) functional genes (Kwak and Weller, 2013; Raaijmakers and Weller, 1998), and *Rhizoctonia solani* AG8 suppressive soils show greater expression of polyketide cyclase genes (Hayden et al., 2018). Additionally, Zhao et al. found that the overrepresentation of non-ribosomal peptide synthases (NRPS) genes in Fusarium wilt suppressive soil is more characteristic for disease suppressiveness than taxonomic microbial community structure and chemical properties (Zhao et al., 2018), which is in line with our results for *F. culmorum* suppressive soils. More specifically, we found an enrichment of NRPS gene clusters in suppressive soils based on co-occurrence networks, and many of these clusters were predicted to encode siderophores. We also predicted, based on

substrate specificity profiles of A-domains, possible enrichment of cyclic and branched lipopeptides in the suppressive soils.

It would be instrumental to be able to diagnose disease suppressiveness in agricultural soils for the purpose of field and crop selection. The perfect diagnostic tool for soil disease suppressiveness should ideally be precise, fast, and easy for interpretation. The sequencing of one or more functional amplicons could possibly meet those criteria but still a more in-depth fundamental understanding of soil suppressiveness is required to develop such tools. The main obstacle is understanding disease suppressiveness at a mechanistic level, which is especially challenging since the phenomenon is complex and diverse. Different approaches to predict soil suppressiveness was presented in the work of Hayden et al. where authors used metabolomics to discriminate between *Rhizoctonia* suppressive and non-suppressive soils (Hayden et al., 2019). Metabolomics allows to study metabolites and their concentrations in biological systems, unlike DNA sequencing that reflects a potential to produce metabolites. Future studies should integrate both NGS sequencing and metabolomics to understand in depth the phenomenon of soil suppressiveness.

Is *Fusarium culmorum* soil suppressiveness general or specific?

Two main models of suppressiveness have been recognized: specific suppression associated with the activity of enriched populations of specific microbes, and general suppression associated with the metabolic activity of the microbial community as a whole. Both types of suppressiveness can be eliminated by soil sterilization but only the specific suppressiveness can be transplanted, by adding small amounts of suppressive soil to conducive soil (Baker and Cook, 1982; Schlatter et al., 2017a).

We show that suppressiveness to *F. culmorum* of the identified soils can be transplanted to non-suppressive soil (Chapter 2) and that rhizosphere suspensions of wheat grown in suppressive soils S11 can be transplanted to a sterile soil (Chapter 4). These results indicate that the observed suppressiveness is specific and associated with the rhizosphere microbiome. Subsequent comparison of the profiles of A-domain functional amplicons between *F. culmorum* suppressive and conducive soils revealed the association of siderophore BGCs with suppressiveness (*Chapter 3*). Soil microorganisms produce siderophores to sequester and solubilize iron that is essential for the functioning of a number of enzymes. It is one of the mechanisms of competition for this limited resource and overall, competition for resources is considered as a general soil suppressiveness phenomenon. Though we have not found any enriched bacterial taxa in *F. culmorum* suppressive soils based on 16S amplicon analyses (*Chapter 2*), we can not exclude the involvement of specific groups of other organisms that we have not tested like, fungi, oomycetes, nematodes, protists or viruses. We also cannot exclude that enriched groups of

microorganisms in the suppressive soils are not taxonomically coherent but rather these are groups of microorganisms collectively expressing certain functions.
All in all, in *F. culmorum* suppressive soils analyzed in this thesis, we found some putative traits associated with specific disease suppressiveness (transferability) but also traits typical for general suppressiveness (competition for iron via siderophores). These results therefore need to be interpreted with caution because they are based on predictions and need future validation experiments. We also can not exclude other traits or involvement of other groups of organisms that we have not tested. It is emphasized in the work of Schlatter et al. that a particular disease suppressive soil can operate in the whole spectrum between specific to general suppressiveness (Schlatter et al., 2017a) and based on our experiments we postulate that *F. culmorum* suppressiveness is one of the examples of intermediate models where both the density of microbial populations and diversity play a role.

Mechanisms of suppressiveness to *Fusarium culmorum*

Up to date, several mechanisms in different disease suppressiveness systems were found (for more details see *Chapter 1*). In *Rhizoctonia solani* suppressive soils, the production of chlorinated lipopeptides nunamycin and thanamycin by *Pseudomonas* was identified as a key mechanism of plant protection (Mendes et al., 2011; Michelsen et al., 2015; Watrous et al., 2012). For the sugar beet – Rhizoctonia suppressive soil, endophytic bacterial genera *Flavobacterium* and *Chitinophaga* provided an additional line of defense (Carrión et al., 2019). In wheat, enriched populations of fluorescent pseudomonads producing 2,4-diacetylphloroglucinol were found responsible, at least in part, in *take-all* suppressive soils (Kwak and Weller, 2013; Raaijmakers and Weller, 1998). For *R. solani* AG8 in wheat, higher frequencies of *Acidobacteria* and *Gemmatimonas* and higher expression of stress related genes and polyketide cyclases were related to suppressiveness (Hayden et al., 2018; Yin et al., 2013). Our work on wheat indicated several possible mechanisms of suppressiveness to *F. culmorum*. These mechanisms are schematically summarized in figure 2 and further described below. We do not know if there is only one dominant mechanism of suppressiveness in this system, but identification of several possible modes of action suggests that the suppressiveness is a synergistic or additive effect of multiple mechanisms that either work simultaneously, in sequence, or in spatially different compartments along the developing plant root system.

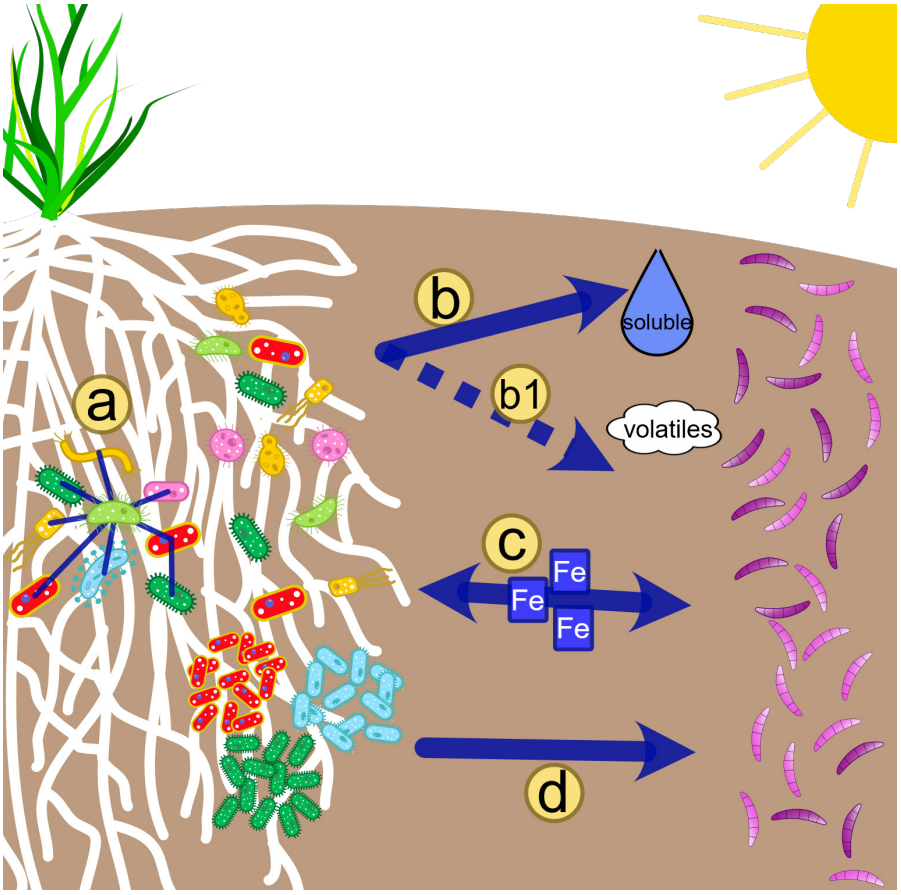

Fig. 2. Proposed mechanisms of Fusarium culmorum disease suppressiveness. The wheat root microbiome is depicted on the left side of the figure and the pathogen is depicted on the right side as spores of F. culmorum. a – Collective activity of the whole microbial community with Acidobacteria as hub taxa (Chapter 2), b- production of secondary metabolites, both soluble and b1 - volatile (Chapters 2, 3 and 4), c- competition for iron (Chapters 3 and 4), d- activity of specific bacteria taxa (Chapter 4).

Collective activity of the microbial community in suppressiveness with Acidobacteria as hub taxa

The high connectivity of Acidobacteria in *F. culmorum* suppressive soils found in the taxonomic analysis (*Chapter 2*) suggests the importance of its interactions with other species for the functioning of the whole bacterial community. The role of Acidobacteria in soil ecosystems is largely elusive despite its ubiquity in soil, mostly because of the difficulties of isolation and *in-vitro* cultivation methods (Eichorst et al., 2018; Kielak et al., 2016).

Nevertheless, sparse cultivation-based and culture-independent functional studies highlight the enormous metabolic potential of this genus, especially in complex carbohydrate metabolism, nitrogen and sulfur turnover and siderophores production (Costa et al., 2020; de Chaves et al., 2019; R. T. Jones et al., 2009; Kalam et al., 2020; Kielak et al., 2016; Lladó et al., 2016). In particular, its role in soil aggregation and in the turnover of exopolysaccharides can have a tremendous effect on soil functioning (Costa et al., 2018). We speculate that in *F. culmorum* suppressive soils Acidobacteria are involved in plant protection by modulating ecological processes and providing crucial ecological services like, nutrients turnover and soil matrix formation. That indirect role is more probable than direct antagonism against the pathogen but considering vast metabolic potential of Acidobacteria we cannot exclude the second scenario. It will be very interesting to further explore the yet unknown roles, if any, of *Acidobacteria* in disease soil suppressiveness.

Involvement of secondary metabolites in disease suppressiveness

Microorganisms execute their beneficial functions through the production of specialized secondary metabolites. These metabolites do not take part in essential cell processes but allow the microorganism to perform auxiliary functions. The synthesis and secretion of secondary metabolites is an energy and resource investment and that is why their production is most of the times controlled by regulation systems in response to external signals. Soil microorganisms are known to produce a plethora of various types of secondary metabolites that constitute a largely untapped resource for novel compounds (Cragg and Newman, 2013). Secondary metabolites allow microorganisms to communicate, acquire nutrients and interact with other (micro)organisms (Raaijmakers et al., 2002; Sharrar et al., 2020). In several cases, the production of microbial secondary metabolites or the potential to produce them (biosynthetic genes) was found to be linked to disease suppressiveness (more in *Chapter 1*). In my thesis (*Chapters 3* and *4*), several gene clusters associated with soil suppressiveness to *F. culmorum* were identified based on sequencing of adenylation domains and based on untargeted shotgun metagenomics. These gene clusters encoded mostly lipopeptides, polyketides and siderophores, groups of compounds that are known for their antifungal activities through direct antagonism or through competition for resources. Substantial genetic diversity of the gene clusters combined with limited prediction of their functions, in most of the cases, allows us only to speculate about their precise roles. Nevertheless, the association with the disease suppressive phenotype makes them a good target for further studies.

In general, the identification of gene cluster functions requires isolation of the microorganism carrying that cluster followed by site-directed mutagenesis or by cloning and expression of the gene cluster in a different host, in case the corresponding microbial species is difficult to culture or to transform (Cui et al., 2018; Gomez-Escribano and Bibb,

2011). With the mutants at hand, these can then be compared to their wild type strain for their ability to suppress the target fungal pathogen *in vitro* and/or *in planta*. One of the best examples of functional identification of a gene cluster followed by identification of its function in suppressive soils comes from the work on thanamycin. The gene cluster encoding this compound was identified to be crucial for antifungal activity against *R. solani* by transposon mutagenesis in *Pseudomonas* sp. strain SH-C52 isolated from a suppressive soil (Mendes et al., 2011). Later the encoded metabolite was identified as a chlorinated 9-AA lipopeptide and characterized for its antifungal activity and for its production by nanoDESI mass spectrometry in live microbial colonies (Watrous et al., 2012). Our findings about the biosynthetic genes in *F. culmorum* suppressive soils in *Chapters 3* and *4* allow us to generate a number of hypotheses about the role of secondary metabolites in the soil suppressiveness that we can further validate in a similar manner.

Potential role of volatile metabolites in suppressiveness

A number of studies have demonstrated the role of volatile compounds emitted by soil bacteria in antagonism towards plant pathogens *in-vitro* (Garbeva et al., 2014b; Hammerbacher et al., 2019; Hunziker et al., 2015; Ossowicki et al., 2017) including strains isolated from suppressive soils (Carrion et al., 2018; Cho et al., 2017; V. Cordovez et al., 2015; Gómez Expósito et al., 2015). Nevertheless, the exact role of this group of compounds in disease suppressiveness is still unclear. In the work presented in *Chapter 2* we have made the first attempt to elucidate this role. Based on our results the overall volatile emissions by the soil microbiome provided some level of protection against the pathogen. Volatile-mediated soil suppressiveness was observed for four soils, including two suppressive and two conducive soils. Hence, volatile emission is one of the factors that may contribute to disease suppressiveness to *F. culmorum*, but it is not a general one. In our work we did not focus on isolating pure cultures of bacteria and testing their *in-vitro* activity but rather tested the suppressive effect of overall soil volatile emissions on plant health. There are often discrepancies between *in-vitro* experiments with single isolates and experiments including soil and plants. Gómez Expósito et al. described the antifungal *in-vitro* activity (including volatiles) of *Lysobacter* spp. isolated from a *Rhizoctonia*-suppressive soil showing plant growth-promoting traits of these strains, but when introduced into the soil the *Lysobacter* isolates were not able to protect plant against infection (Gómez Expósito et al., 2015). Recent *in-vitro* and *in-situ* studies indicated that the production of volatiles in natural environment is related to the composition of microbial communities and that reduced microbial diversity increased the volatile emission but decreased the number of emitted volatiles (Abis et al., 2020). In a perspective article, Brilli et al. highlighted the need to further explore volatile emissions in soil to improve sustainable plant protection strategies for agriculture (Brilli et al., 2019). Our findings indicating the potential role of volatiles in soil

suppressiveness to *F. culmorum* require follow-up experiments to identify the volatile compounds with suppressive activity and the microorganisms emitting them.

Competition for iron

The availability of iron is soils is generally low and while this element is essential for living organisms it is often a limiting resource in this environment. Microorganisms, under low iron conditions, produce siderophores allowing them to sequester and take up iron. Production of siderophores is often recognized as a mechanism of competition between (micro)organisms (Ahmed and Holmström, 2014; Saha et al., 2016). The profound example of the key role of competition for iron in soil suppressiveness comes from the work on *Fusarium oxysporum* suppressive soils in the Châteaurenard region in France. The central role of competition for iron and involvement of siderophore-producing Pseudomonads was demonstrated as one of the key mechanisms of suppression (summarized in *Chapter 1*). The pathogen used in the French work is related to *F. culmorum*, which is why we presumed some similarities between these two suppressiveness soil systems. Indeed, we found overrepresentation of siderophore biosynthetic gene clusters (BGC) in *F. culmorum*-suppressive soils based on adenylation domain profiles (*Chapter 3*) and enrichment of iron uptake functional genes based on a dilution-to-extinction experiment (*Chapter 4*). Iron-limiting conditions, where the production of siderophores is triggered, are most likely to prevail in soil environments. That is why the production of siderophores is often identified in soil microorganisms, including the microorganisms related to disease suppression and plant growth promotion (for review: (Ahmed and Holmström, 2014; Höfte and Bakker, 2007)). It has also been shown that some siderophores can trigger systemic resistance in plants, constituting another line of defense against pathogens (Audenaert et al., 2002; Chae et al., 2020; Pieterse et al., 2014; Sousa and Olivares, 2016). In three out of four *F. culmorum* suppressive soils found in our work the concentration of bioavailable iron is very low (0.02 to 0.11 mg/kg) and on the remaining one it reaches 0.45 mg/kg what is related to a low pH of this soil (*Chapter 2)*. That suggests that in most of the suppressive soils tested here, we can expect competition of iron via siderophores. That is in line with the data obtained in *Chapter 3* where we see the overrepresentation of siderophore BGCs in suppressive soils. All in all our results suggest that siderophores take part in direct antagonism against pathogens causing iron starvation, but also may trigger ISR in wheat, making it a possible dual action mechanism of soil suppressiveness. Additional experiments are needed to validate the role of competition for iron in disease suppressiveness. In previous works on other soil suppressiveness systems the involvement of this competition was tested by modifying iron availability in soil using surplus of iron or chelators (Almario et al., 2014; Scher and Baker, 1982). This simple yet elegant approach is also planned in the continuation of our work.

Activity of specific bacteria taxa

The screening of 28 soils described in *Chapter 2* did not reveal any common enriched bacterial taxa within suppressive soils but the extensive analysis of suppressive soil S11 in *Chapter 4* suggested that several taxa possibly play a role in disease suppressiveness (Acidobacteria, Verrucomicrobia and Streptomycetes). There could be two main reasons for this apparent discrepancy: soil suppressiveness in different soils may not share the same mechanisms or the microbial basis of the activity may be more similar functionally than taxonomically. Moreover, in *Chapter 4* we used a deep shotgun metagenomic that can provide more detailed taxonomic representation of microbial community than 16S amplicon sequencing used in *Chapter 2*. Using high volume metagenomes, we can often find the differences between datasets at lower taxonomic levels (Schöler et al., 2017; Tessler et al., 2017). Comparison of shotgun metagenomes obtained in a dilution-to-extinction experiment revealed that several bacterial taxa significantly decreased in abundance as the soil lost disease suppressiveness upon dilutions of the extracted microbiome (*Chapter 4*). Among these taxa we found again Acidobacteria, but also Streptomycetes, previously associated with suppressiveness, and Verrucomicrobiota, previously associated with soil fertility (Navarrete et al., 2015). *Streptomyces* isolated from *Streptomyces scabies* suppressive soils (Liu et al., 2011), from *Fusarium oxysporum* suppressive soils (Cha et al., 2016) and from *Rhizoctonia* suppressive soil (V. Cordovez et al., 2015) exhibited antagonistic activities against pathogens. In general, the biosynthetic potential and biocontrol traits of *Streptomyces* are prominent (Belknap et al., 2020; Nicault et al., 2020; Sousa and Olivares, 2016; van der Meij et al., 2017). In the metagenomic analysis of suppressive soil S11 (*Chapter 4*) we found a number of biosyntetic gene clusters taxonomically assigned to Streptomycetes encoding siderophores (discussed above), melanins that appear to play a role in colonization of plant roots by Streptomycetes (Chewning et al., 2019) and possibly novel compounds of unknown functions belonging to terpenes, polyketides and non-ribosomal peptides.

The future efforts in this study will be focused on the integration of the metagenomic approach, with metabolomics and culturomics. In the course of the dilution-to-extinction experiments on suppressive soil S11 (*Chapter 4*) from the collected rhizosphere samples, we have additionally extracted metabolites for mass spectrometry analysis (MS) and preserved a bacterial collection of 184 strains, which were also subjected to sequencing. Successive steps will be identifying the changing trends in the abundance of natural products across the dilution series and linking these products to BGCs and taxa identified in this study. Having this information, we can further use selective isolation and molecular identification approaches (PCR, DNA probes or sequencing) to recognize producers of these natural products in the bacterial isolate collection from soil S11. Following this path, we can further identify functions and mechanisms provided by key taxa and elucidate their role in

soil suppressiveness. Extensive knowledge about the microbial community of suppressive soil S11 and bacterial collection will allow us to construct synthetic microbial communities that we can test for protection against Fusarium and possibly other pathogens in wheat or other plants. Altogether, the follow up studies will allow us to not only predict the mechanisms of the soil disease suppression but also validate the findings.

The impact of plastic on soil microbiomes and disease suppressiveness

Agriculture is a substantial producer of waste products, and in the last decade, also of plastic waste (Millati et al., 2019). Nowadays, accumulation of plastic is becoming a global threat, but the use of plastic in agriculture is becoming increasingly popular. One of the main sources of plastic in agriculture is the use of mulching films. It was demonstrated by Zhao et al. that fields that use mulching contain significantly more plastic residues then fields that do not (Zhou et al., 2020). The impact of plastic on terrestrial ecosystems is not only the problem for agriculture but our knowledge on how it affects farming is insufficient (Rillig, 2020; Rillig and Lehmann, 2020). In the last years the answer from the industry for the demand of more environmentally friendly solutions has been manufacturing all kinds of "bioplastics". In principle these materials have similar physical properties to conventional, fossil fuel-based plastic, but they degrade in the environment much faster. In practice, there are no clear international guidelines defining what is a "bioplastic", which causes a lot of misconceptions. Often "bioplastics" are composed of bio-based (for example cellulose-based) polymers mixed with conventional plastic. Recent work of Qi et al. show that both conventional plastics and "bioplastic" have a negative impact on the growth of wheat and a significant impact on rhizosphere microbial communities (Qi et al., 2019, 2018). We have found that the soil suppressiveness to *F. culmorum* has a microbial basis (*Chapter 2*) and subsequently we wanted to evaluate if the addition of plastic to a suppressive soil can influence the ability of the microbiome to protect the plant. Although we have not seen a significant impact on the protective effect of suppressive soil in our short-term experiment, we have found an impact on the plant nutrient status and microbiome. We see in our experiments that the "bioplastic" we tested, may cause a significant shift in the microbial community as it creates a new niche in the soil inhabited by different microbiota. The difference in this microbiota manifested mainly as the high relative abundance of fungal genera such as *Rhizoctonia* and *Fusarium.* We can speculate that in the long term it can have an impact enrichment of economically relevant pathogens as many of them belong to these genera, but further experiments are needed to investigate this (Chapter 5). What seems to be an environmentally friendly alternative to conventional plastic may be actually harmful to soil health. This problem may get more pressing as global climate changes affect the ability to grow crops (Godfray et al., 2010; Wang et al., 2017). Increasing temperature and drought may force farmers to use more plastic mulching to maintain food production at the

same level. Global warming and drought can also directly have an impact on soil microbiomes, causing unforeseen effects in food production (Ochoa-Hueso, 2017). Our work highlights the importance of healthy soil microbiomes and gives the basis for improving the sustainability of agriculture in the future.

Concluding remarks and future perspectives

Overall, the research presented in this thesis constitutes the first extensive screening of suppressiveness to *Fusarium culmorum* across numerous agricultural field soils, the essential groundwork to establish the microbial basis of disease suppressiveness, and the first integration of a dilution-to-extinction approach with untargeted metagenomics to deconstruct the mechanistic basis of suppressiveness. We also evaluated the impact of plastic on disease suppressiveness, plant growth and soil microbial communities that addresses current global environmental concerns. This work builds a foundation for further research on this intriguing phenomenon and a methodological base for plant-associated microbiome studies. We suggested several possible mechanisms of soil suppressiveness and proposed the ways to validate them. In the course of our work on disease suppressiveness to *F. culmorum*, we have also pointed out several putative mechanisms of suppressiveness that were not yet experimentally validated. Considering the high abundance of various pathogenic and non-pathogenic Fusaria in soils (Moretti, 2009; Summerell et al., 2010) and that the main distinction between these groups is the ability of producing mycotoxins and other pathogenicity factors (Perincherry et al., 2019; Wachowska et al., 2017), studying the effects of a disease suppressive microbiome on the production of pathogenicity factors by *F. culmorum* will be part of these validation experiments.

Another step in research on disease suppressiveness is the implementation of metatranscriptomics and metabolomics. The current study indicated a metabolic potential of the rhizosphere microbiome of wheat to produce a number of metabolites that may play an important role in soil suppressiveness, nevertheless linking this data with information about produced metabolites necessitates confirmation and validation. Moreover, the data on bacterial taxa that were proposed to play a role in suppressiveness in *Chapter 4* should be used for selective isolation and characterization including site-directed mutagenesis and functional bioassays. Altogether, these approaches can be used to further pinpoint key microbial taxa and traits which in turn provides a framework to design a synthetic microbial community that recreates the natural protection against *F. culmorum* observed in the field soils (Carrión et al., 2019; Tsolakidou et al., 2019). Having a deep understanding of functioning of soil suppressiveness, we can utilize this knowledge in designing biological plant protection agents or in steering soil microbial communities towards developing disease suppressiveness (Arif et al., 2020; Kumar and Dubey, 2020; Orozco-Mosqueda et al., 2018).