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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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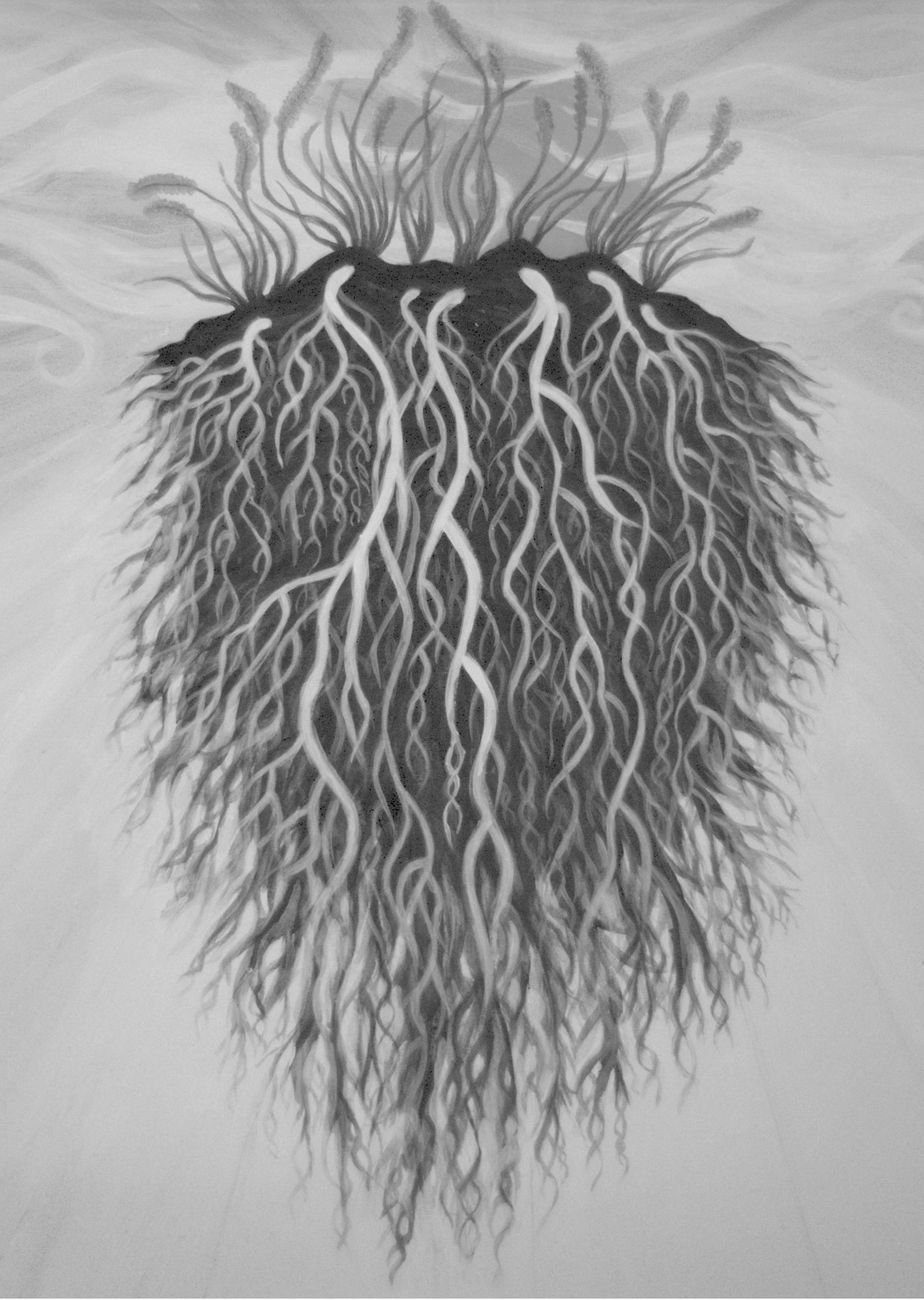


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Chapter 1

General introduction and thesis outline

A part of this chapter in modified version was submitted for publication

Soil – a habitat of microbes

Soil – the thin top layer of the Earth’s surface – is so common that often we do not think about it as an ecosystem with diverse life support functions. On a global scale, soil supports life on our planet by cleaning our drinking water, degrading toxic compounds, recycling of nutrients, and protecting plants from pests and diseases. These functions of this fascinating yet highly complex and heterogeneous world beneath our feet are largely governed by microorganisms with an estimated number of around 10^9 bacteria cells and up to 50,000 bacterial taxa in just one gram of soil (Roesch et al., 2007; Wagg et al., 2014). Together with other (micro)organisms like fungi, archaea, protists and viruses they constitute an enormous richness of microbial life (Tecon and Or, 2017). Soil environments can be remarkably diverse depending on their geographic location and the history of land use, but they are typically composed of a solid fraction (minerals), organic matter, live organisms, and water or gas-filled pores (Díaz-Zorita et al., 2002; Tecón and Or, 2017). A large contribution to the heterogeneity of the soil environments comes from the formation of aggregates with different physical and chemical characteristics (Rillig et al., 2017). Is it easy to be a microorganism in the soil? Certainly, living in the soil offers a variety of resources and diverse niches that are colonized cooperatively or in competition with other organisms (Pande and Kost, 2017; Vos et al., 2013). For more than three hundred years, we isolate and grow soil bacteria under laboratory conditions. Already thousands of bacterial species have been catalogued and preserved, but this is just the tip of the iceberg: most likely the vast majority of soil microorganisms do not survive extraction from their natural environments or can be cultivated in artificial media (Hofer, 2018; Pande and Kost, 2017). Despite these limitations, however, new sequencing technologies have enabled us to explore the unculturable and yet unknown fraction of the soil microbiome.

Microbial life at the interface between soil and plants

Plant roots live in a close relationship with soil microbes and both sides of this relationship rely on each other. Plants recruit microorganisms from the reservoir present in the soil, creating a specialized environment around their roots. This specific ecosystem, called the rhizosphere, is strongly influenced by root exudates containing various substrates that can constitute up to 40% of the plant’s fixed carbon (D. L. Jones et al., 2009; Rillig and Mummey, 2006). Compared to the bulk soil (i.e. soil without plant roots), the microbial communities in the rhizosphere are substantially more numerous (approximately 10 to 100-fold higher) but taxonomically less diverse; this is called the rhizosphere effect (Bakker et al., 2013; Mendes et al., 2013; Minz et al., 2013). The rhizosphere communities are a subset of the microbes residing in the soil possibly complemented with endophytic microorganisms residing inside the germinating seeds, and their composition depends on the plant species or even genotype within a given plants species (Berg and Smalla, 2009; Hartmann et al.,

2009). What kinds of microbes do plants select in the rhizosphere? From an evolutionary perspective, plants are expected to recruit and support microorganisms that are beneficial for their growth, development and health. Hence, various rhizosphere microorganisms have been identified that exhibit beneficial functions for plants such as promoting germination of seeds (13), nutrient acquisition (Brussaard, 2012; *Soil Microbiology, Ecology and Biochemistry*, 2015), growth promotion via the production of phytohormones (Babalola, 2010; MacMillan, 2001; Saleem et al., 2007; Sousa and Olivares, 2016), inducing the plant's systemic resistance to infection (Bakker et al., 2003; Haas and Défago, 2005; Harman et al., 2004; Pieterse et al., 2014; Sousa and Olivares, 2016; Zehnder et al., 2001), or protecting directly against infectious root pathogens (Haas and Défago, 2005; Raaijmakers et al., 2002; Raaijmakers and Mazzola, 2012; Sousa and Olivares, 2016). This latter group of pathogenic microorganisms also responds to root exudates as cues for locating a host plant for infection. Hence, it has been postulated that soil-borne pathogens and parasites have hijacked the signals that the plant developed to recruit beneficial microbes (Al-Babili and Bouwmeester, 2015; López-Ráez and Bouwmeester, 2008). Thus, there is a fragile equilibrium between the attraction of pathogenic and beneficial microorganisms. The major challenge of modern agriculture is to steer soil and rhizosphere microbiomes in such a way that they provide beneficial functions for crops while at the same time they ward off the pathogenic (micro)organisms. To this end, we need a deeper understanding of soil functioning, microbe-microbe and microbe-plant interactions (Mendes et al., 2013).

Plant pathogenic microorganisms – a threat to food security

Wheat constitutes more than 40% of all cultivated cereals in Europe and, in 2018, approximately 700 million tons of a total of 2,7 billion tons of cereals produced worldwide, providing approximately 20% of the calories intake by humans ("Food and Agriculture Organisation of the United Nations," 2020). To meet the demand of a growing human population, farming has become even more intensified with less diversity and more fertilizers and pesticides. Multi-hectare monocultures are still the preferred crop production system, but make food security highly vulnerable to pests and diseases (Ramankutty et al., 2018). Infections, once initialized, can quickly spread, taking a serious toll on yield because of genetically uniform crop species (McDonald and Stukenbrock, 2016). For wheat, it is estimated that up to 23% of the yield is lost due to diseases (Savary et al., 2019). Plant pathogenic fungi have a great share in these economic losses. In particular fungal pathogens from the genus *Fusarium* are major yield-limiting factors and also cause hazards to human and animal health via the contamination of food products with mycotoxins (Antonissen et al., 2014; Dean et al., 2012; Scherm et al., 2013).

Fusarium species are commonly found in soils worldwide and are often associated with plants. While most of the members are saprophytes, several species are considered the

most devastating soil-borne plant pathogens (Dean et al., 2012, p. 10; Moretti, 2009). *Fusarium* pathogens can infect more than 100 plant species but few pathogens, mostly *F. graminearum*, *F. pseudograminearum* and *F. culmorum*, are widespread in cereals, mostly in wheat and barley. Among the disease symptoms caused by *Fusaria* one can find Fusarium head blight (FHB), Fusarium root rot (FRR), stem rot, stunting, wilting due to fungal colonization of the vascular tissues or due to enzymatic degradation of the plant cell wall. One of the most important problem in cereals affected by *Fusaria* is the contamination with mycotoxins that are poisonous for humans, animals, and not suitable for malting (Antonissen et al., 2014; Dean et al., 2012; Goswami and Kistler, 2004; Nielsen et al., 2014). The species used in experimental work described in this thesis is *Fusarium culmorum*. The most common symptoms caused by this pathogen in cereals are FRR and FHB (Fig. 1). Root rot occurs already in the early stages of plant development, one or two weeks after germination, but *Fusarium* infection may even lead to pre-emergence seedling blight. The most prevalent mycotoxins produced by *F. culmorum* are deoxynivalenol, nivalenol, fusarenone and their derivatives. The mycotoxin profile is a basis of dividing the pathogen into chemotypes. In severely affected plant tissues, the fungus produces characteristic pink-red coloration caused by sporulating mycelium, a phenotype that is also observed in *in-vitro* cultures (Fig. 1). The macroconidia produced by *F. culmorum* are curved, between 25 to 50 μm long (Fig. 1) and easily spread by rain, wind, insects, and animals. *Fusaria* are difficult to eradicate because they stay viable in post-harvest plant debris in the soil even after a few seasons, waiting for a suitable host.

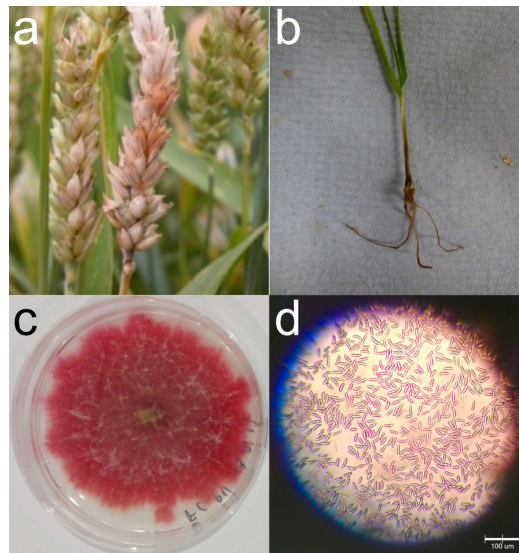


Figure 1. Characteristic disease symptoms on wheat plants caused by *Fusarium culmorum*: *Fusarium* Head Blight (a) and *Fusarium* Root Rot (b). *Fusarium culmorum* PV growing on agar media (c) and its macroconidia under light microscope (d). Photo (a) adopted from fyi.extension.wisc.edu

Soil suppressiveness - a sustainable alternative for controlling fungal diseases

The environmental impact of agriculture is already pushing the limits of Earth's resource boundaries (Campbell et al., 2017) and conventional, high-input agriculture aggravates this even further (Ramankutty et al., 2018). One of the alternatives and a big step towards sustainable agriculture is using what nature has to offer. Already for more than 100 years (Atkinson, 1892) it has been recognized that some agricultural soils are suppressive to specific soil-borne diseases including diseases caused by *Fusarium* (Alabouvette, 1986). In such soils, susceptible plants do not get diseased or only little despite the presence of a virulent pathogen and conditions that allow infection (Deacon, 1984). The nature of this phenomenon is, in most cases, microbiological and the physical and chemical properties of these soils may affect the onset and magnitude of the suppressiveness (Andrade et al., 1994; Carrion et al., 2018; Cha et al., 2016; Cook and Rovira, 1976; Duijff et al., 1999; Weller et al., 2002a; Wiseman et al., 1996). Soil disease suppressiveness has been defined as general and specific depending on the range of pathogens it protects from. General suppressiveness reduces the disease development of various pathogens and is present at some level in most soils as a result of the metabolic activity of the microbial community. The protective effect of general suppression is not transferable by soil transplantation. Specific disease suppressiveness, on the other hand, targets a specific pathogen and can be

transferred to a nonsuppressive (i.e. conducive) soil by transplantation. The protective effect is the result of the activity of select members within the soil or root-associated microbiome that are enriched in response to a disease outbreak (for review: (Alabouvette, 1986; Garbeva et al., 2004; Gomes Exposito et al., 2017; Hornby, 1983; Kwak and Weller, 2013; Schlatter et al., 2017a)). Understanding the microorganisms and mechanisms involved in disease suppressiveness can be exploited for developing new and sustainable management practices in agriculture. To this end, we need to disentangle the network of interactions in the soil- or root-associated microbiome and find the responsible microorganisms and underlying microbial traits.

Examples of suppressive soils and the microbes and mechanisms involved

Take-all decline soils

The fungus *Gaeumannomyces graminis* var. *tritici* (GGT) causes take-all disease in wheat, barley, and other cereals. Typical GGT-symptoms are brown to black lesions of root system, stunting and yellowing, the heads appear white, not producing seeds. Affected plants die prematurely within circular patches in the field. The pathogen is difficult to control by chemical pesticides, and there are also no GGT-resistant varieties of cereals yet. Soils suppressive to GGT are referred to as take-all decline (TAD) soils and are found worldwide (Kwak and Weller, 2013). TAD soils were found in the USA (Cook and Rovira, 1976; Raaijmakers and Weller, 1998), Netherlands (Bergsma-Vlami et al., 2005; Gerlagh, 1968), three different areas in Montana, USA (Andrade et al., 1994), in volcanic soils in Chile (Duran et al., 2017) and on the South Island, New Zealand (Chng et al., 2015). In most of these studies, disease suppressiveness was eliminated by pasteurization and could be transferred to a conducive soil by transplantation of 1% (w/w) of the suppressive soil. Microbiome analysis of TAD soils from Washington State (USA) and the Netherlands revealed that the suppressiveness is associated with enriched populations of fluorescent *Pseudomonads* that inhibit the growth of the pathogen by the production of 2,4-diacetylphloroglucinol (2,4-DAPG), also hydrogen cyanide and cyclic lipopeptides were suggested to have a role in suppressiveness (Kwak and Weller, 2013; Raaijmakers and Weller, 1998). Another proposed mode of action has its basis in the low abundance of bioavailable iron in soils where *Pseudomonads* compete with pathogen producing siderophores, nevertheless it was not experimentally proven. There is also evidence that 2,4-DAPG can induce systemic resistance of plants, which can constitute another mode of action of *Pseudomonads* in TAD soils. Nevertheless, so far this latter mechanism was only shown for *Arabidopsis thaliana* (Chae et al., 2020).

Rhizoctonia-suppressive soils

Dutch agricultural fields near Hoeven and Zwaagdijk in the Netherlands were identified as suppressive to *Rhizoctonia solani* AG2. The biological nature of suppressiveness was confirmed by heat sterilization and soil transplantation (Mendes et al., 2011). Comparative taxonomic and functional analyses between suppressive and conducive soils further revealed the importance of rhizospheric *Pseudomonadaceae* and *Burkholderiaceae*, and of endophytic *Flavobacteriaceae* and *Chitinophagaceae* in suppressiveness. Subsequent transposon mutagenesis of selected isolates from the suppressive soils led to the identification of the chlorinated lipopeptide thanamycin as a key metabolite in *Rhizoctonia* control by *Pseudomonas* strain SH-C52 (Mendes et al., 2011; Watrous et al., 2012), antifungal volatiles by *Burkholderia*, *Paraburkholderia* (Carrion et al., 2018) and *Streptomyces* (V. Cordovez et al., 2015), and a chemically unidentified compound of endophytic *Flavobacterium* (Carrión et al., 2019). Carrion et al. (2019) also reconstructed a synthetic community which was able to provide enhanced protection of sugar beet from the pathogen (Carrión et al., 2019). Moreover, in the Greenlandic potato fields, suppressiveness to *Rhizoctonia solani* AG3 was observed with no significant disease symptoms for even 50 years. Bioassays with agricultural soil from Inneruulalik demonstrated that the damping-off symptoms caused by *R. solani* on tomato were indeed minor and the disease suppressive effect was eliminated by sterilization and transferred to conducive soil by a soil transplant. Detailed *in-vitro* analysis of a bacterial strain collection from this suppressive soil revealed a *Pseudomonas fluorescens* strain In5 with strong antifungal activity towards *R. solani* AG3. In a microcosm experiment, where strain In5 was introduced to the soil, Michelsen *et al.* showed protective effects at In5 population densities less than 1% of the bacterial community (Michelsen et al., 2015). Genomic and metabolomic analysis of strain In5 revealed a gene cluster and corresponding metabolite, referred to as nunamycin, which were highly similar to the BGC and thanamycin compound identified in *Pseudomonas* SH-C52 from the Dutch *Rhizoctonia*-suppressive soil described above.

Different anastomosis group *Rhizoctonia solani* - AG8 is a pathogen of grain crops, mostly wheat and barley, causing root rot and stunting of seedlings called bare patch disease. The pathogen is difficult to control because of its wide host range, lack of resistant crop varieties and the survival structures referred to as sclerotia (Barnett et al., 2017; Cook et al., 2002). Suppressive soils to *R. solani* AG8 were identified for the first time in South Australia (Roget, 1995) and confirmed as (micro)biological in nature and transferable (Wiseman et al., 1996). Using a high throughput screening with an *in-vitro* pathosystem, Barnett et al. isolated a variety of fungal and bacterial strains assigned to *Aspergillus*, *Cylindrocarpon*, *Trichoderma*, *Bacillus*, *Paenibacillus*, *Microbacterium*, *Streptomyces*, *Pandoraea*, *Brevibacterium*, *Chryseobacterium*, *Phyllobacterium* and *Pseudomonas* able to provide protection against *R. solani* AG8 and growth promotion of wheat (Barnett et al., 2017). Later

metatranscriptomics analysis revealed higher expression of oxidative stress related genes and polyketide cyclases in suppressive soil compared to the conducive soil, indicating possible mechanisms of suppression (Hayden et al., 2018). Moreover, a metabolomics approach using liquid chromatography and nuclear magnetic resonance to discriminate between suppressive and non-suppressive soils to AG8 indicated antifungal secondary metabolites macrocarpals to be soil suppressiveness biomarkers (Hayden et al., 2019). Another *R. solani* AG8 suppressive soil was recognized in the Pacific Northwest of the USA where Schillinger and Paulitz demonstrated that bare patch disease can be suppressed by natural microflora in long-term cropping systems (Schillinger and Paulitz, 2013). In a subsequent study the role of bacterial communities was found instrumental in developing disease suppressiveness with higher frequencies of *Acidobacteria* and *Gemmatimonas* in the rhizosphere of healthy plants (Yin et al., 2013).

Fusarium-suppressive soils

Fusarium oxysporum infects many vegetables and fruits causing wilting. Based on the host range, the pathogen is further classified as *formae speciales* (*f. sp.*). Symptomatic plants show chlorosis, necrosis, premature leaf drop, stunting and damping-off as a result of the fungus colonizing their vascular system (Bosland, 1988). *Fusarium* wilt suppressive soils of strawberry were identified in South Korea (Cha et al., 2016), and of banana in Northern Hainan Island, China (Ou et al., 2019). The *Fusarium*-suppressive soils in the Châteaurenard region in France has been studied since the 1970s. Also, in that case, suppressiveness was eliminated by sterilization and transferred to conducive soil by a transplant of 1-2% (w/w). Two of the key microorganisms associated with suppressiveness of the Châteaurenard soil are non-pathogenic *Fusarium oxysporum* and fluorescent *Pseudomonas* species. The major mechanisms are competition for organic carbon and for iron. These two mechanisms complement each other, the production of siderophores by *Pseudomonads* regulates the availability of iron, while the availability of iron is proportional to the intensity of competition for carbon between pathogenic and non-pathogenic *Fusaria* (Alabouvette, 1999, 1986; Alabouvette et al., 2009; Cha et al., 2016; Lemanceau et al., 1992; Mazurier et al., 2009, 2009; Siegel-Hertz et al., 2018a).

Disentangling the microbiome of suppressive soils by perturbation

To resolve the functioning of microbial communities in disease-suppressive soils is challenging because of the taxonomic and functional complexity as well as the spatial-temporal dynamics of root-associated microbiomes. Nevertheless, there is a fragile balance in their functioning in response to external factors that we can use to our advantage in studying the microbial and mechanistic basis of suppressive soils. Perturbation is a promising tool to disentangle microbial communities and understand their functions. By

applying a selective pressure that perturbs the microbiome, we can generate a compositional shift and a simplification of a complex microbial community in a controlled way. This simplification reduces the microbiome diversity, disrupts the activity of specific members of the microbiome and/or alters the communication and interactions within the microbiome and between the microbiome, the plant and the pathogen. Perturbation methods are culture-independent and therefore the preferred choice for soil microbiome analyses where the majority of taxa appear to be unculturable. Here, I present an overview of different microbiome perturbation approaches, in particular selective heat, selective biocides and dilution-to-extinction (Fig. 2).

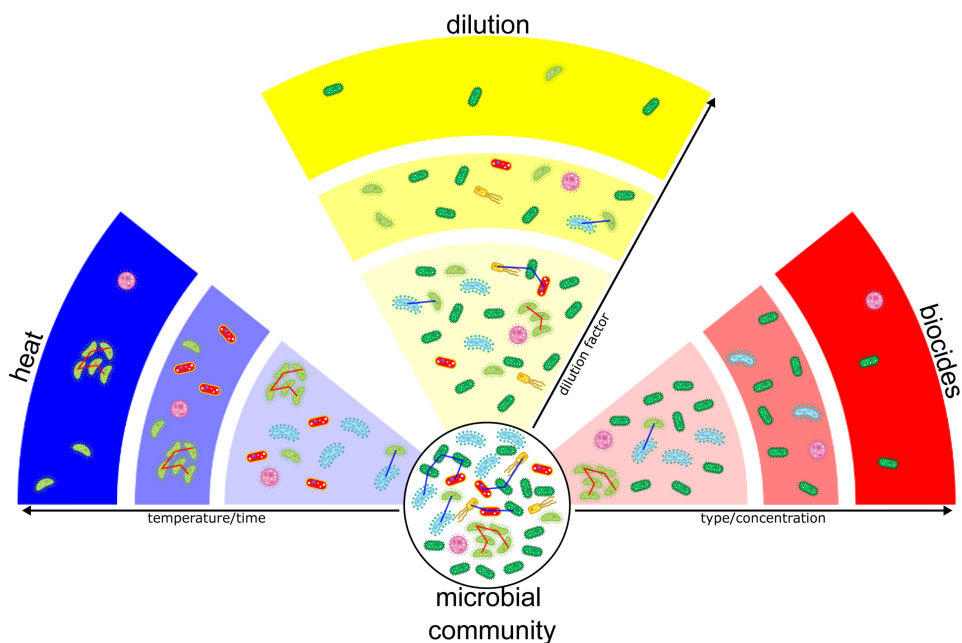


Figure 2. A schematic representation of three perturbation techniques and their effect on a soil microbiome. The original microbial community is presented in the circle. It is composed of six bacterial species with different abundance, from highly abundant (dark green), to rare species (pink). The interactions between bacteria are represented as lines connecting microbes, intraspecific with red and interspecific with blue lines. The intensity of the perturbation grows outwards from the inner circle and is represented in three levels with gradually darker colours. As we can see on the figure, regardless of the perturbation technique used, the microbial community gets simplified, the number of species decreases, and the interactions are disturbed. Blue part - heat can be used for sterilization, but when applied in sublethal temperature and exposure times it can result in a subset of the original community that is composed of mostly organisms with higher

resilience to changing temperature and organisms able to produce some heat resistance survival forms (e.g., spores). Red part - Some groups of microorganisms can be eliminated from the original microbial community by the application of biocides in specific concentrations. That treatment will eliminate the groups of bacteria that are susceptible to the compound. We can also attempt to engineer the microbiome using a combination of antibiotics if the presence of antibiotic resistance genes in a microbial community is known from molecular studies. Yellow part - The dilution-to-extinction technique where the microbial community is distributed stochastically in a series of dilutions. This technique is unspecific and the presence of the groups of microorganisms in consecutive dilutions mostly depends on their initial abundance.

Heat treatment

Elevated temperatures cause substantial mortality (30-60%) in a microbial community and leaves empty niches ready to be colonized by those microorganisms that survived the heat. The mortality rate depends on the method used to elevate the temperature and exposure time. Firmicutes, Actinobacteria and other Gram-positive bacterial phyla forming survival structures are typically the first to recover from elevated temperatures. The second bacterial phyla to colonize the new environment is Proteobacteria, which typically consists of fast-growing bacterial genera able to quickly multiply and use a broad spectrum of nutritional resources. The community structure in a heat-treated soil microbiome, measured as diversity and evenness, is unlikely to come back to the initial point even weeks after the disturbance (Jurburg et al., 2017a, 2017b). Riah-Anglet et al. (2015) further investigated the functions of microbial communities after a heat stress by measuring enzymatic activity. Their experiment showed substantial changes in functions and taxa composition. Soil microbiomes did not fully restore their β -glucosidase, cellulase, N-acetyl-glucosaminidase, and dehydrogenase activities within 4 weeks after the heat disturbance, which was associated with decreased abundance of Actinobacteria, Acidobacteria and Planctomycetes. They also showed, based on a marker-gene copy number study, that soil fungal communities are more susceptible to the heat stress than bacteria, and that the physical parameters and chemical composition of the soil have a major impact on the perturbed community (Riah-Anglet et al., 2015). The effect of heat treatment on microbiome assembly in a suppressive soil was investigated by Van der Voort et al (2016) indicating heat tolerance and growth rate of bacteria as key traits influencing rearrangement of microbial community after the perturbation. This paper also suggested that elimination of suppressiveness to *R. solani* by heat treatment was associated with a decrease in abundance of three Actinobacteria families: Streptomycetaceae, Micrococcaceae and Mycobacteriaceae (van der Voort et al., 2016). The effect of a heat shock on rare soil taxa was examined by Kurm et al., providing an indication that low

abundance taxa can benefit from the disturbance. In their study, taxa classified as rare (< 0,01% relative abundance) increased their relative abundance even to above 1% (Kurm et al., 2019).

Biocides

Chemical substances used for control of potentially harmful living organisms in medicine, agriculture and industry are collectively named biocides. We can include in that group pesticides (e.g., fungicides, insecticides) and antimicrobials (e.g., antibiotics). The majority of studies on biocides concerns the impact of antibiotics on gut microbiome and human health (for review: (Blaser, 2016; Francino, 2016; Schwartz et al., 2020)). The influence of biocides on soil and rhizosphere microbiomes has been mostly studied in the context of antibiotic and fungicide spills into the environment from anthropogenic sources, like agriculture and industry (Cycoń et al., 2019). Nevertheless, the application of biocides is also a valuable tool for investigating the functionalities of soil microbiomes. Recently, Dong et al. (2020) described the impact of different classes of antibiotics on soil bacteriomes and the stability of antibiotics in the soil (Dong et al., 2020). Their study showed the minor effect of easily degradable antibiotics on soil bacteria communities, the significant growth inhibition of phyla Firmicutes and Bacteroidetes by nalidixic acid and of the phyla Firmicutes, Bacteroidetes and Cyanobacteria by tetracycline. These two antibiotics also have a negative impact on overall bacteria diversity. It is also worth noting that tetracycline, ceftriaxone and nalidixic acid caused an increase in the relative abundance of some classes of *Proteobacteria*. In recent work Lee et al. investigated the effect of vancomycin on biological disease suppression to bacterial wilt disease caused by *Ralstonia solanacearum*. Their results show that vancomycin alter microbial community of suppressive soil via disturbing the protective effect conferred by Firmicutes and Actinobacteria (Lee et al., 2020). Using biocides to perturbate the soil microbiome in a controlled way depends a lot on experimental setup. One should take into consideration following factors: stability of the biocides, dispersion in the system, heat or light resistance, and impact of by-products of metabolism and degradation.

Dilution-to-extinction

The dilution technique allows us to perturbate the microbiome in an untargeted way. Diluted microbial communities contain a subset of the original one that is smaller as the dilution factor increases. Between consecutive dilutions, members of the microbiome (species) distribute stochastically, reducing the diversity and possibly losing some species. Most of the soil microbiome dilution experiments described to date use sterile water or water-based buffer to extract the microbial community and later as a diluent (Chen et al., 2020; Garland and Lehman, 1999; Hol et al., 2015; Korenblum et al., 2020; Peter et al., 2011;

Yan et al., 2017; Zegeye et al., 2019). This way, the communities are serially diluted up to 10^9 times depending on the study. Microbiome dilutions are mostly introduced to a sterile soil and incubated for a certain time before any further testing. It is also possible to dilute the soil microbiome without extraction using sterile soil as a diluent.

Studies published to date, show the decrease of taxonomic and functional diversity along the dilution series. For example, Yan et al. compared the taxonomic structure of the diluted soil microbial community before introducing it to the soil and after 8 weeks of incubation in the soil. They showed that microbiome dilution leads to a significant change in microbiome assembly over time, but its structure cannot be predicted based on the taxonomic composition of the inoculum (Yan et al., 2015). In the follow-up study, Yan et al. analyzed the functional potential of the microbiome over the dilution series using *in-silico* function prediction and showed the relative stability of functions in the rhizosphere. They indicated the strong selective power of the rhizosphere towards general functions (Yan et al., 2017). The changes in various soil/plant traits and functions were also measured in diluted microbial communities by sequencing-independent methods. The carbon source respiration tests showed a non-linear reduction in the number of the utilized carbon sources in diluted soil microbiomes. Serial dilution of a chitin-enriched soil microbial community revealed the importance of initial community complexity on the future stability of microbiome (Zegeye et al., 2019). The impact of the soil microbiome dilution on plants was also investigated. Chen et al. indicated that the loss of bacterial diversity, being a result of the dilution, may have a negative correlation with plant productivity, using lettuce as a model (Chen et al., 2020). Another study investigated how the root microbiome modulates the systemic induction of root exudation of metabolites in tomato. Authors showed that the reduction of microbiome diversity and composition, obtained by dilution-to-extinction, resulted in exudation of particular metabolite profiles. They demonstrated that the rhizosphere microbiome assembly drives the systemically induced exudation process at the molecular and chemical level (Korenblum et al., 2020). The only study, at least to our knowledge, that used the dilution-to-extinction approach in relation to plant protection against pathogens is the study by Hol et al. They diluted microbial communities from agricultural soils and tested these in the context of volatile-mediated inhibition of plant pathogens (Hol et al., 2015). They showed that emission of volatile compounds in soil was one of the factors contributing to overall soil disease suppressiveness. The role of volatiles in *Fusarium*-suppressive soils is also addressed in the second chapter of this thesis.

Dilution-to-extinction proved to be a good tool to manipulate natural soil microbiomes. Unlike other methods, it allows us to manipulate the diversity of a community and track diversity-related microbiome characteristics like, network structure or functional redundancy. In combination with -omics techniques dilution-to-extinction makes a powerful tool to investigate the mechanisms of soil functions.

Other methods used for microbiome perturbation

In recent years we see fast advances in genome editing tools based on CRISPR-Cas system. This method can be used to edit genomes microorganisms, control gene expression, modulate the production of metabolites and proteins and specifically eliminate members of the microbiome (Ramachandran and Bikard, 2019). So far, targeted microbiome perturbation using CRISPR-Cas editing capabilities were successfully used for microbiome studies for strain-specific depletion in mouse gut microbiome (Lam et al., 2020). In the field of plant-microbe interactions, it was used in the work of Carrion et al., where a specific gene cluster in a member of the plant protective synthetic community was perturbed in a targeted way using a SpyCas9-mediated system. This community was able to protect sugar beet from *Rhizoctonia solani*, but partially lost this ability after the elimination of a specific gene encoding a NRPS/PKS biosynthetic gene cluster (Carrión et al., 2019). A totally different approach to study soil microbiome functions was presented recently by Naylor et al. where a soil microbiome was enriched in functions by using different nutritional and growth conditions. This work presents an intriguing set of different “functional modules” and their implications for the bacteria community (Naylor et al., 2020).

In conclusion, various perturbation methods have proven to be a valuable tool to manipulate natural soil and plant-associated microbiomes. These methods allow us to manipulate the diversity of a microbial community and track diversity-related microbiome characteristics like, network structure or functional redundancy. In combination with different -omics techniques, perturbation presents a powerful tool to deconstruct the functionalities of soil and plant microbiomes and to disentangle the microbes and mechanisms responsible for specific soil functions, including disease suppressiveness.

Thesis outline

The overall aim of my thesis is to disentangle the microorganisms and mechanisms involved in disease suppressiveness to *Fusarium culmorum* of wheat. To this end I integrated various approaches and techniques to identify the taxonomic groups of bacteria, their biosynthetic gene clusters and metabolites associated with this intriguing microbiome-associated plant phenotype.

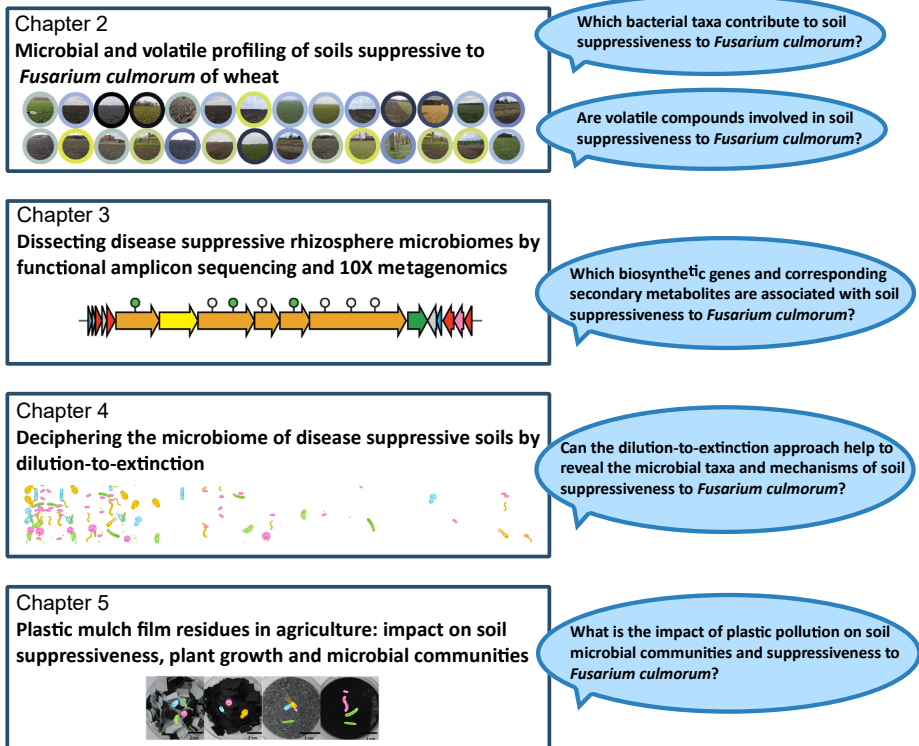


Fig. 3. Thesis outline with the main research questions addressed in each chapter

The first chapter of my thesis introduces the topic of suppressive soils and describes its importance for sustainable agriculture and food security. In **the second chapter**, we built and characterized a collection of diverse soil samples and evaluated their disease suppressiveness to *Fusarium culmorum*, an economically important fungal root pathogen of wheat. Subsequently, we analyzed the taxonomic diversity of the soil and rhizosphere microbiomes as well as the emitted volatile profiles of these soils. In **chapter 3**, we extended the approach of taxonomic profiling of bacteria in suppressive soils by adopting an extensive functional amplicon analysis focusing on adenylation domains of non-ribosomal peptide

synthetases and polyketide synthases. These domains are parts of biosynthetic gene clusters encoding modular enzymes used by microorganisms to synthesize non-ribosomal peptides and polyketides, metabolites that are often associated with disease suppressiveness. In **chapter 4**, we make use of the dilution-to-extinction approach to further deconstruct the microbiome of one of the *Fusarium*-suppressive soil. A combination of deep metagenomic sequencing and phenotypic analysis of diluted microbiomes gave additional insight into the microbes and putative functions by which the plants are protected from *Fusarium* root rot. In **chapter 5**, we evaluated the impact of plastic mulching film debris on wheat growth and suppressiveness to *Fusarium culmorum* and how this is reflected in changes in the microbial community of the suppressive soil.

Key questions addressed in this thesis:

- Which bacterial taxa contribute to soil suppressiveness to *Fusarium culmorum*?
- Are volatile compounds involved in soil suppressiveness to *Fusarium culmorum*?
- Which biosynthetic genes and corresponding secondary metabolites are associated with soil suppressiveness to *Fusarium culmorum*?
- Can the dilution-to-extinction approach help to reveal the microbial taxa and mechanisms of soil suppressiveness to *Fusarium culmorum*?
- What is the impact of plastic pollution on soil microbial communities and suppressiveness to *Fusarium culmorum*?