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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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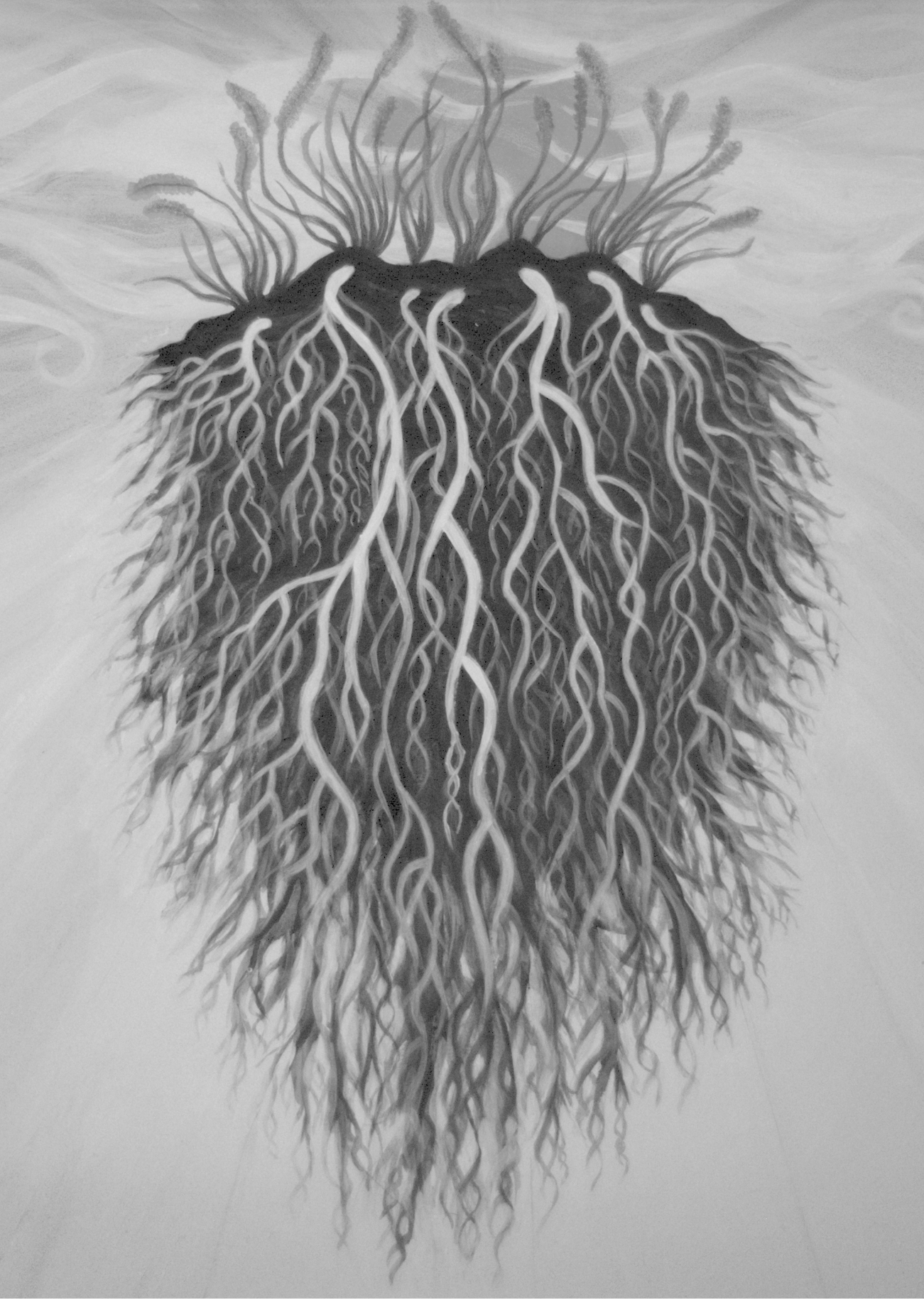


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Summary

Soil holds an enormous diversity of microbial life that is essential for the functioning of terrestrial ecosystems. Bacteria, fungi, protists, viruses and other organisms living in the soil colonise plant roots in response to root exudates and form a rhizosphere – a remarkable ecosystem where microorganisms live in close association with plant roots. The rhizosphere microbiome is known to provide beneficial services to plants like nutrient acquisition, growth promotion or directly and indirectly protecting against biotic and abiotic stresses. In contrast to beneficial plant-associated microorganisms, plant pathogens are a major threat causing reduced yields and contaminated food products. Their control requires the application of environmentally hazardous pesticides. Cereals and also other crops suffer severely from pathogenic fungi from the genus *Fusarium*. Those pathogens not only significantly reduce the yield but contaminate the harvest with mycotoxins making it unfit for consumption. In my thesis, I studied soils that are suppressive to *Fusarium culmorum* of wheat. These soils provide a natural protection against root infections which is mediated by the activity of soil microbial community. Those soils are recognised worldwide but, in most cases, the exact mechanisms of microbiome-based crop protection are unclear. The overall aim of my thesis was to disentangle the microorganisms and mechanisms involved in disease suppressiveness to *F. culmorum* of wheat.

We started the investigation on soil suppressiveness by performing a phenotypic screening of 28 field soils from the Netherlands and Germany to identify suppressive soils. Four soils exhibited a high level of disease suppressiveness to *F. culmorum* and via soil sterilization and soil transplantation we showed that the protective effect observed in the four suppressive soils is (micro)biological in nature. After comparing bacterial taxonomy profiles, physical and chemical parameters we found that different *F. culmorum* suppressive soils do not share the same microbial taxonomic patterns nor physical or chemical characteristics. Our results further showed that the emissions of volatiles from the soil microbiome may contribute to disease suppressiveness of some soils, but not for all. Subsequently we analyzed the profiles of adenylation domain (A-domain) functional genes. Those genes are part of biosynthetic genes clusters encoding secondary metabolites like polyketides, non-ribosomal peptides or terpenes, metabolites often associated with microbial interactions and suppression of plant pathogens in suppressive soils. Based on A-domain profiles we found that the *F. culmorum* suppressive soils share a similar A-domain community structure. These bioinformatic analyses further suggested a possible role of siderophores and competition for iron in disease suppressiveness. The microbiome of suppressive soil showing the highest level of protection – soil S11, was then subjected to perturbation by dilution-to-extinction by transferring serial dilutions of an extracted suppressive soil S11

microbiome to a sterile soil. In the wheat-Fusarium bioassays with diluted microbiomes, we showed the transferability of suppressiveness and a non-linear relationship between suppressiveness and microbiome dilution. Subsequent analysis of wheat rhizosphere metagenomes along the dilution trajectory revealed a significant reduction of genetic diversity and significant changes in microbial community composition. Bacterial taxa previously associated with suppressiveness and soil fertility (ex. Streptomyces, Acidobacteria and Verrucomicrobia) significantly decreased in relative abundance with higher dilutions of the microbiome. Moreover, in low dilutions that still conferred suppressiveness, we found an enrichment of genes related to iron uptake, chitinases and elements of type six secretion system. Furthermore, we discovered an association between suppressiveness and nine metagenome-assembled bacterial genomes carrying various novel biosynthetic gene clusters. Altogether, the dilution-to-extinction approach pointed to several mechanisms of disease suppression that need to be validated in future research. Finally, we evaluated the impact of plastic film residues on the soil microbiome, disease suppressiveness and plant growth. We found that the addition of plastic to the soil in short term does not affect the soil suppressiveness to *F. culmorum* but has a negative impact on plant biomass and nutrient status. We also found that plastic residues in soil create a new niche populated by significantly different microbiota that, in a longer term, may affect soil microbiome diversity and functioning.

In conclusion, this thesis presents the identification of disease suppressive soils, putative microbes, mechanisms, genes and metabolites associated with suppressiveness, as well as the environmental impact of microplastics on the soil microbiome, plant growth and plant health. Based on the results obtained, several new hypotheses were generated to further disentangle plant-associated microbiome functioning in the context of protection against biotic stress and the exposure to microplastic pollution of soil ecosystems.