**Supplementary material part I**

**Material and methods**

**Soil physical and chemical parameters**

Soil physical and chemical parameters were measured in triplicate using three independent sub-samples. The soil pH was measured in water and soil organic matter content was determined by loss of ignition, both using standard laboratory methods. Bioavailable iron, potassium, magnesium, phosphorus and sulfur were extracted using 0,01M CaCl2 (VWR, Netherlands) and concentrations were measured with ICP-OES (iCAP 6500 ICP-OES Duo ,Thermo Scientific). Carbon and nitrogen content were determined using an Element Analyser (Flash EA 1112 ,Thermo Scientific).

**Disease symptoms assessment**

To assess disease symptoms, the wheat plants were carefully removed, the excess of soil was shaken and roots were cleaned in water. The root system was visually inspected for brown/black lesions or rotting, and the stem base/coleoptile was inspected for rotting and the presence of pink-white fungal hyphae. Plants were scored for disease symptoms from 0-5: 0- healthy plants; 1- one or two dark lesions on the roots up to 10 mm; 2- lesions bigger than 10 mm; 3- large lesions on the roots and/or stem base rot; 4- visible pink-white fungal hyphae on the stem with shoot discoloration, extensive root lesions, or stem base rot (at least two of this symptoms); and 5- dead plants (for examples see figure S6). Statistical differences in disease symptoms between treatments and control were assessed using the chi-square test, with an alpha cutoff of p<0.05.

**Rhizosphere DNA extraction and sequencing**

At the end of the experiments, plants were gently removed from the trays. Soil loosely adhering to the roots was removed by shaking. The roots with surrounding soil were placed on a sterile filter in a laminar flow cabinet and the soil particles adhering to the roots were collected using a brush. DNA was immediately isolated using a DNeasy PowerSoil Kit (QIAGEN, the Netherlands) according to manufactures protocol using 0.25 g of rhizosphere soil. Samples were additionally purified using DNeasy PowerClean cleanup kit (QIAGEN, the Netherlands). Sequencing of the bacterial V3-V4 region of the 16S rRNA gene (with primers 16S\_V3-341F 5’-CCTACGGGNGGCWGCAG and 16S\_V4-785R 5’-GACTACHVGGGTATCTAATCC [1]) was performed at BaseClear (Leiden, the Netherlands) using Illumina MiSeq in 4 biological replicates per treatment.

**Amplicon assembly and taxonomical assignment**

The 16S rRNA gene sequencing yielded a total of 4,966,035 reads, sequencing depths for all samples is reported in table S2. Reads were quality-filtered using a sliding window approach of five nucleotides and a Phred score threshold of 30 [2]. Forward and reverse reads were then truncated at 280 and 220 bp in length, respectively. The Dada2 [3] pipeline from Qiime2 [4] was used to denoise and obtain amplicon sequence variants (ASVs) from demultiplexed reads for all 112 samples. Reads that did not overlap were removed from the rest of the analysis. To remove sequences derived from errors that were not corrected during the DADA2 denoising step, ASVs that had fewer than 20 amplicons mapped across all samples and that appeared in less than 4 different samples were removed from further analysis. This resulted in 4,322 reliable ASVs across the 112 samples, while still retaining over 80% of the sequencing information. Taxonomic assignment was performed in Qiime2 using a Bayesian classifier [5, 6] built for the primer pairs used in this study (341F and 805R) on the SILVA release 132 database ref NR 99. The resulting feature count tables were logCSS-normalized in R using the metagenomeSeq library [7]. Alpha/beta diversity and clustering of samples using different metrics were calculated with Qiime2 and python skbio library.

**Principal coordinate analysis of the amplicon data**

Principal coordinate analysis (PCoA) was performed in Qiime2 using Bray-Curtis dissimilarity, Jaccard index, weighted UniFrac and unweighted Unifrac [8]. Weighted and unweighted UniFrac distances were calculated in a Python script for all individual bacterial taxonomy groups to reveal existing correlations within taxonomic groups that were being masked by other features. Code for these analyses is available at (https://git.wageningenur.nl/users/traca001/groups).

**Identification of suppressive rhizosphere-associated ASVs**

ASVs enriched in suppressive and conducive soils were determined with FitZig [7] using the suppressive phenotype as model matrix for hypothesis testing. A strict filter was applied to select ASVs of interest; we consider enriched only ASVs which yielded a p-value below (p<10-10) and which occur in at least

two different suppressive soil samples (replicates).

**Random forest prediction of suppressive soil**

Multiple random forest classifiers were built to predict soil phenotype based on its community composition. The classifiers are built with python scikit-learn library bootstrapping the selection of suppressive and conducive samples used to train and test the model. For each classifier, 3 suppressive and 12 conducive samples are randomly chosen and the remaining samples are used to test the classifier predictions. None of the suppressive sample phenotype could be correctly predicted by the classifier.

**Co-occurrence network analysis**

Spearman's rank correlation coefficient was calculated using the normalized feature table for all ASV pairs across all samples. Clusters of highly correlated ASVs were obtained by applying a static threshold that preserved only correlations above 0.8, corresponding to approximately 1% of the nonzero interactions. Clusters which contained suppression-associated ASVs as determined by FitZig were further investigated to identify suppression-specific ASV clusters. Network visualization was performed in Cytoscape [9]; nodes in the network represent the different ASVs and edges were drawn between pairs of ASVs having a Spearman correlation score above the 0.8 threshold.

**Effects of soil-emitted volatiles on fungal growth**

To investigate the antifungal activity of the volatiles emitted by eight selected soils on the growth of Fusarium culmorum PV, the bottom-top approach was applied as described previously by Garbeva et al.,[10] with small modifications. The top part of the experimental system contained a Petri dish with 20 ml of 1/2 PDA medium and a 6 mm Fusarium culmorum PV plug placed in the center, whereas 30 g of soil was added at the bottom. The two parts where separated with a 30 µm sterile nylon mesh (Sefar, Switzerland) to allow exchange of volatile compounds but prevent exchange of spores and soil particles. The whole system was fixed together with paper medical tape (Kruidvat, the Netherlands). The scheme of the system is shown on figure 3A. After seven days at 20°C in the dark, fungal hyphae were harvested, lyophilized and weighed. Every treatment was replicated five times.

**Volatile trapping and GC-MS analysis**

Volatiles emitted by the eight suppressive and conducive soils were collected in a steel trap with 150 mg Tenax TA and 150 mg Carbopack B (Markes International Ltd, Llantrisant, UK). A perforated steel tube was placed in the glass pot with tested soil mixed with F. culmorum PV agar plugs. The pre-germinated wheat seedling was introduced to this system and after one week of growth, the steel trap was connected to tubes present in the soil for 16h. Every soil was tested in three independent biological replicates. After removing, traps were capped and stored at 4°C until GC-Q-TOF analysis. Volatiles were desorbed from the traps using an automated thermodesorption unit (model UnityTD-100, Markes International Ltd., Llantrisant, UK) at 210°C for 12 min (Helium flow 50 mL/min) and trapped on the cold trap at -10°C. The trapped volatiles were introduced into the GC-QTOF (model Agilent 7890B GC and the Agilent 7200AB QTOF, Santa Clara, USA) by heating the cold trap for 3 min to 280°C with the split ratio set to 1:10. The column used was a 30 × 0.25 mm DB-5MS UI, film thickness 0.25 μm (122-5532UI, Agilent J&W, USA). Temperature program used was as follows: 39°C for 2 min, from 39°C to 95°C at 3.5°C/min, then to 165°C at 6°C/min, to 250°C at 15°C/min and finally to 300°C at 40°C/min, hold 20 min. The volatiles were detected by the MS operating at 70 eV in EI mode. Mass spectra were acquired in full scan mode (30–400 amu, 4 scans/s). For the volatilomics analysis, the acquired raw mass spectrometry (MS) data was extracted to m/z format using MassHunter Qualitative Analysis Software V B.07.00 (Agilent Technologies, Santa Clara, CA, USA). The m/z data was processed with MZMine V 2.36 (Copyright © 2005–2012 MZmine Development Team, [11]) as described in [12] to create an m/z and peak intensity table that could be used as input file for MetaboAnalyst 4.0 software (http://www.metaboanalyst.ca/MetaboAnalyst [13] ). Before the statistical analysis, the data was filtered using Interquantile range (IQR) and normalized by the log transformation with automatic scaling.

References:

[1] Herlemann, D.P.R., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J.J. & Andersson, A.F. 2011 Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *Isme J* **5**, 1571-1579. (doi:10.1038/ismej.2011.41).

[2] Bolger, A.M., Lohse, M. & Usadel, B. 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120. (doi:10.1093/bioinformatics/btu170).

[3] Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. 2016 DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**, 581. (doi:10.1038/Nmeth.3869).

[4] Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., et al. 2010 QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**, 335-336. (doi:10.1038/nmeth.f.303).

[5] Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., et al. 2011 Scikit-learn: Machine Learning in Python. *J Mach Learn Res* **12**, 2825-2830.

[6] Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A. & Caporaso, J.G. 2018 Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2 ' s q2-feature-classifier plugin. *Microbiome* **6**. (doi:10.1186/s40168-018-0470-z).

[7] Paulson, J.N., Stine, O.C., Bravo, H.C. & Pop, M. 2013 Differential abundance analysis for microbial marker-gene surveys. *Nat Methods* **10**, 1200. (doi:10.1038/Nmeth.2658).

[8] Lozupone, C. & Knight, R. 2005 UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microb* **71**, 8228-8235. (doi:10.1128/Aem.71.12.8228-8235.2005).

[9] Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B. & Ideker, T. 2003 Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res* **13**, 2498-2504. (doi:10.1101/gr.1239303).

[10] Garbeva, P., Hordijk, C., Gerards, S. & de Boer, W. 2014 Volatiles produced by the mycophagous soil bacterium Collimonas. *Fems Microbiol Ecol* **87**, 639-649. (doi:10.1111/1574-6941.12252).

[11] Pluskal, T., Castillo, S., Villar-Briones, A. & Oresic, M. 2010 MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *Bmc Bioinformatics* **11**. (doi:10.1186/1471-2105-11-395).

[12] Lankenau, M.A., Patel, R., Liyanarachchi, S., Maharry, S.E., Hoag, K.W., Duggan, M., Walker, C.J., Markowitz, J., Carson, W.E., 3rd, Eisfeld, A.K., et al. 2015 MicroRNA-3151 inactivates TP53 in BRAF-mutated human malignancies. *Proc Natl Acad Sci U S A* **112**, E6744-6751. (doi:10.1073/pnas.1520390112).

[13] Chong, J., Soufan, O., Li, C., Caraus, I., Li, S.Z., Bourque, G., Wishart, D.S. & Xia, J.G. 2018 MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res* **46**, W486-W494. (doi:10.1093/nar/gky310).