**Additional file**

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**Materials and methods**

***Arabidopsis thaliana* genotypes and *Fusarium* isolate**

We used the Arabidopsisecotypes*Columbia-0*(Col-0) and *Landsberg erecta*-0 (Ler-0) lines in this study. While Col-0 is a natural accession and maintained as a clean homozygous line, the Ler-0 carries mutations that are caused by X-ray irradiation in the ERECTA gene [1]. These accessions have been shown to exhibit distinct root morphologies [2, 3], chemical profiles [4], and disease resistances against *F. oxysporum* [5]. Both accessions were supplied by the Nottingham Arabidopsis stock centre (NASC), United Kingdom. FOM isolate 726 [6] was kindly provided by Dr. H. Corby Kistler at USDA ARS CDL-University of Minnesota, USA.

**Experimental design**

Arabidopsis seeds were sown in pots (8x8x6 cm) containing field soil (fine sand 32.2%, coarse sand 52.8%, humus 4.7%, clay 3%, silt 7.3%) [7] and pH 5.95, collected from the Jyndevad Experimental station (54.9023° N, 9.1511° E) Denmark in 2016. We sowed approximately 20 seeds per each pot. In total, we maintained 100 pots allocated to the 2 genotypes, (Col-0 and Ler-0), 2 treatments (FOM-inoculated or water-inoculated (non-inoculated), 5 replicates of each treatment and destructively sampled at 5 different time points.

The pots were arranged in trays, loosely covered with plastic wrap, and the seeds were stratified in the dark at 4 ºC for 3 days. Thereafter, the pots were completely randomized and maintained under greenhouse conditions (16h light, 8h dark and 18-23 ºC) for the entire duration of the experiment. After germination, thinning-out was done, leaving 10 seedlings in each pot. Seedlings were watered (100 mL/pot) two times per week and weeds removed regularly upon emergence.

**Pathogen culture and inoculation**

FOM was cultured on sporulation-induced synthetic nutrient-poor agar (SNA) medium: 1 g KH2PO4, 1 g KNO3, 0.5 g MgSO4•7H20, 0.5 g KCI, 0.2 g glucose, 0.2 g sucrose, 20 g agar, 1 L distilled water) and incubated 1 week under day/night light conditions at 20-23 ºC. Mycelial plugs (6 plugs/100mL from 7-day old FOM plates were transferred into a 400 mL sterile liquid carboxymethylcellulose (CMC) medium: 15 g CMC sodium salt (high viscosity, #C5013: Sigma Aldrich, St. Louis, MO, USA),1g NH4NO3, 1g KH2PO4, 1g yeast extract, 0.5 g MgSO4•7H2O and 1 L of distilled water and cultured for 3 days at 22 ºC in the dark with gyratory shaking (125 rpm). We harvested fungal spores by filtering through sterile Miracloth to remove mycelia and centrifuged (4500 *g*) the filtrate containing FOM spores for 15 minutes at room temperature to pellet spores. The spores were washed twice with sterile deionized distilled H2O, followed by centrifugation at 7500 *g* for 5 minutes at room temperature before discarding the supernatant. We resuspended the spore pellet in sterile water and estimated spore concentrations using a hemocytometer before adjustment to 1.1 x106 spores/uL. The soils surrounding the two-week old seedlings were inoculated each with 300 uL of the adjusted spore suspension by carefully pipetting into soils close to the roots of the seedlings (50 pots), while the 50 uninoculated pots received 300 uL distilled water.

**Sample collection**

Root samples were collected at 5 different sampling times at intervals of 5 days with the first sampling performed at 5 days after inoculation (DAI) with FOM. Roots were harvested by pressing the sides of each pot to loosen the soil around the roots. Subsequently, each root system was carefully pulled out and shaken gently to remove loosely attached soil, then cut with sterile scissors and roots with adhering rhizosphere soil of the ten plants in the individual pots were pooled into sterile 2 mL collecting tubes. The root samples were divided to allow for both metabarcoding and chemical analysis. Samples for chemical analysis were placed into pre-chilled tubes. Half of the root samples determined for chemical analysis were used in this study, the other half used in another study [8]. Shoot (stems and leaves of plants) samples were taken for FOM quantification. Harvested samples were immediately snap-frozen in liquid nitrogen and stored at -20 ºC (samples for metabarcoding) or -80 ºC (samples for chemical analysis). Root and shoot samples were lyophilized for 3 days. All lyophilized samples were stored at -20 ºC prior to downstream processing.

**DNA extraction**

Lyophilized samples were ground using sterile steel beads in a Geno/Grinder2000 (Spex, Metuchen, NJ, USA) at 1500 rpm for 3 × 30 s. For both root and shoot DNA extraction, we used 250 mg of ground sample. Root DNA extraction was done using the PowerLyzer™ PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). Shoot DNA was extracted using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The extracted shoot and root DNA samples were stored at − 20 ºC and subsequently used for FOM quantification or sequencing library preparation.

**FOM quantification in shoots**

We estimated *F. oxysporum* biomass in inoculated and non-inoculated shoot samples, using quantitative PCR (qPCR). We used the *F. oxysporum* specific primers F 5’- CCTGTTCGAGCGTCATTTCA-3’ and R 5’- GAATTAACGCGAGTCCCA ACAC -3. The PCR reaction consisted of 2.5 μl template, 0.375 μl each of forward and reverse primers (10 μM stock), 3 μl of water, and 6.25 μl of Bio SyGreen Mix Lo-Rox (PCR Biosystems, Ltd., London, UK). For positive and negative controls, 2 μl of template (dilutions of FOM DNA or sterile water) were added. Amplification reactions were performed with 2 replicates per sample using a ViiA TM 7 Real-time PCR system (Life Technologies, CA, USA). Thermal cycling conditions included an initial denaturation at 95°C for 10 minutes followed by 40 cycles of 95 ºC for 15 seconds and 58 ºC for 1 minute. Standards were included in the run using a 10x dilution series of FOM DNA with an initial concentration of 2.05 ng/µl. A standard curve was obtained by plotting the cycle threshold (*C*T) values as a function of log10 of the amount of fungal DNA added in a 10-fold serial dilution (1 to 10−9).

**Library preparation**

For the microbiome analysis, we followed the 16S and ITS library preparation procedures as previously described [9]. Briefly, the bacterial 16S rRNA V3/V4 amplicon library was generated using the PCR primers (S-DBact-0341-b-S-17 / S-D-Bact-0785-a-A-21) [10]. For amplification of the fungal internal transcribed spacer 2 (ITS2) region, we used the fITS7 [11] and ITS4 [12] primer pair. Both bacterial and fungal libraries for Illumina MiSeq sequencing were generated by a two-step dual indexing strategy as previously described [9] and sequenced at Eurofins MWG (Ebersberg, Germany). The raw sequence files were deposited at the National Centre for Biotechnology Information (NCBI) sequence read archive with the SRA accession number PRJNA756534.

**Metabolite extraction**

Metabolic pathways of the quantified metabolites are shown (**Figure S1**). Prior to metabolite extraction, approximately 5 mg of dried tissue was ground to a fine powder by using a mechanical disrupter Geno/Grinder 2010 (Spex, Metuchen, NJ, USA). Metabolites from root material were extracted by the addition of 1 ml of 70 % (v/v) methanol/water solution to the plant material [8]. The tubes were vortexed for 20 seconds. Subsequently, the tubes were heated at 72 ºC for 10 min, to avoid myrosinase-mediated glucosinolate breakdown [13]. Samples were cooled to room temperature and placed in a sonication bath for 5 minutes. Next, the samples were shaken at 30 rpm for 15 minutes at 4 ºC. The samples were centrifuged for 5 minutes at 15000 g, and the supernatant was transferred into new tubes. The supernatant was diluted in Milli-Q water, filtered through a 0.22-μm KX syringe filter (PTFE 13-mm diameter) (Mikrolab, Aarhus, Denmark) and injected into the LC-MS/MS system.

**Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis**

Samples were analyzed in multiple reaction mode (MRM) on an Agilent 1260 infinity HPLC system (Santa Clara, CA, USA) connected to an AB Sciex 4500 triple-quadrupole trap mass spectrometer (QTRAP/MS) (AB Sciex, Framingham, USA) equipped with electrospray ionization (ESI) source in negative and positive ion mode. MRM-transitions and compound-dependent parameters are summarized in **Table S1**. The information with respect to mass spectrometry parameters for multiple reaction monitoring can be found in the Supporting Information together with additional information about the LC-MS/MS method. Chromatographic separation for glucosinolates and plant hormones (negative mode) (**Table S1**) was performed at 40 ºC on a reversed-phase Synergi Fusion-RP C18, 80A column (250 mm × 2 mm i.d., 4 μm, Phenomenex) equipped with a Security Guard Cartridge (KJ0-4282, Phenomenex) [14]. For compounds related to lignin and lignan biosynthetic pathway (**Table S1**), plant hormones (positive mode), coumarins, and phenolic acids, the separation was carried out on a Kinetex EVO C18 (150 × 2.1 mm i.d., 5 μm, Phenomenex) protected by a Security Guard ULTRA Cartridge (AJ0-9298, Phenomenex). Further details on the stepwise gradient used in the LC-MS/MS analysis can be found in the Supporting Information. All data were collected using ABSciex Analyst software (version 1.6.2). Quantitation was performed using ABSciex MultiQuant software (version 3.0.2). Samples were run in randomized order.

**Sequence data and statistical analysis**

Bacterial and fungal sequence reads were analyzed as described earlier [9]. Briefly, paired-end reads were demultiplexed for internal barcodes, using Mr\_Demuxy using command pe\_demuxer.py (https://pypi.org/project/Mr\_Demuxy/). Subsequently, paired-end reads were assembled and joined reads were processed, using vsearch v.2.6 [15]. Primers were removed, using the cutapdapt [16]. Dereplication, chimera screening, and clustering of sequences were performed using vsearch v.2.6 [15]. Extraction of fungal ITS reads was carried out prior to clustering, using ITSx extractor version 1.0.6 [17]. Taxonomy assignments were performed using the SILVA 132 [18] and UNITE (v7.2) [19] reference databases, respectively for bacteria and fungi, in QIIME (v1.9) using assign\_taxonomy.py [20]. Unassigned OTUs at kingdom level or OTUs assigned as chloroplast or mitochondrial sequences were removed. Also, OTUs found in less than 3 samples in the total dataset were removed.

Statistical analyses and data visualizations were carried out in R v4.0.5 [21], using vegan (v2.5.7) [22], phyloseq (v1.34.0.) [23], ggplot2 (v3.3.2)[24] packages. Before diversity analysis, samples with less than 1000 reads were removed from the datasets. OTU tables were rarified 100 times at a depth of 1000 reads for both datasets, and the mean of the diversity estimates of 100 trials was used to estimate each alpha diversity metric (observed and Shannon diversity). Significant differences between alpha diversities were evaluated, using Kruskal-Wallis rank sum test. OTU tables were transformed to relative abundances (RAs) prior to beta diversity analysis. Bray-Curtis dissimilarity matrices were visualized, using unconstrained principal coordinates analysis (PCoA), and permutation analysis of variance (PERMANOVA) was performed for both bacterial and fungal communities, using the “adonis” function from the “vegan” package. We performed indicator species analysis, using the labdsv function in R [25] to determine abundance differentials of the bacterial (bOTUs) and fungal OTUs (fOTUs) associated with inoculated and non-inoculated samples of Col-0 and Ler-0. Datasets were partitioned and analyzed separately for Col-0 and Ler-0 for identification of indicator OTUs. Highly significant OTUs (p≤0.01) with indicator values > 0.4 were considered as indicator species [26].

We performed differential abundance analysis of microbial OTUs based on negative binomial distribution between inoculated and non-inoculated datasets Col-0 and Ler-0 using ‘DESeq2’ R package [23, 27].

# Microbial network analysis

Microbial interaction patterns in roots of non-inoculated and inoculated samples of Col-0 and Ler-0 were examined by Spearman’s rank correlation analysis. We pooled bacterial and fungal datasets generated for the 5 time points in the construction of the respective networks. Next, bacterial and fungal OTUs were pooled and normalized, using trimmed mean of M values (TMM) method using the BioConductor package EdgeR in R [28]. For microbial network construction, we used OTUs that were present in at least 10 samples with Spearman’s rank correlations > 0.7 for positive correlations and < −0.7 for negative correlations, and p values < 0.001. The correlated OTUs were visualized in a network with OTUs set as nodes and correlations as edges. OTUs that were identified in the indicator analysis and appeared in the co-occurrence analysis were shown as larger nodes. Network properties were computed, using the “igraph” package with defined parameters as described in [9]. We used Spearman’s rank correlations to determine associations between fOTU1 and bOTUs.

**Statistical analysis for targeted metabolomics**

The absolute concentrations (µg/g) from targeted metabolomics analysis were subjected to the time‐series and two‐factor data analysis by MetaboAnalyst 4.0 [29] with Bonferroni correction to determine the level of significance between the factors and their interaction. Bonferroni-corrected and adjusted p values less than 0.05 were considered significant. SIMCA-P (ver. 15.0.2, Umetrics AB, Umeå, Sweden) was used to perform orthogonal partial least square-discriminant analysis (OPLS-DA) to determine the metabolites that were contributing to the variation between treatment groups at different infection stages. OPLS-DA models were performed on cubic root-transformed and Pareto-scaled data to identify clustering behavior related to treatment groups. OPLS-DA models were validated by correlation (R2) and predictability (Q2) parameters. In addition, a permutation test (n = 100) was performed to validate the robustness and overfitting of the OPLS-DA models. The assessment of the validated models was performed by inspecting the intercept of the permutation plot (permQ2). The significance of the OPLSDA model was assessed by the cross-validated analysis of variance (CV-ANOVA). A heatmap was generated with MultiExperiment Viewer application [30] and visualized, showing log2-transformed significant differences between FOM inoculated plants and control. Fold changes between inoculated and non-inoculated plants were calculated and Student’s t-tests for two group-comparisons were performed by SigmaPlot software (version 11.0). The non-parametric Mann-Whitney Rank Sum test was used when the data violated the assumption of normality. Metabolites with a threshold of Fold Change (FC) > 1 as well as p < 0.05 were considered significant. Bar plots were generated, using ggplot2 in R to observe variation in metabolite concentrations shown as means ± standard error.

**OTU-metabolite correlation analysis**

We examined metabolite microbial OTU associations by performing Spearman’s correlation analysis, using the rcorr function. Prior to the analysis, we filtered low abundance OTUs by removing OTUs occurring in less than four samples and less than 50 read counts followed by a transformation to relative abundance. The metabolome data were also transformed to fit the normal distribution pattern. Metabolite-OTU associations with strong correlations (*ρ*>0.4 or <-0.4 and p <0.01) were visualized in heatmaps. In addition, relative abundance patterns of the correlated metabolite OTUs across different DAI were visualized, using bubble plots.

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