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Underground alarms: volatile-mediated recruitment of beneficial soil bacteria by plants under biotic stress

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Citation

Rizaludin, M. S. (2026, January 21). *Underground alarms: volatile-mediated recruitment of beneficial soil bacteria by plants under biotic stress*. *NIOO-thesis*. Retrieved from https://hdl.handle.net/1887/4287295

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CHAPTER 3

Foliar infections by *Botrytis cinerea* modulate the tomato root volatilome and microbiome

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This chapter is published in:

FEMS Microbiology Ecology 2025, *101*(5) ; https://doi.org/10.1093/femsec/fiaf042

Abstract

The fungal pathogen *Botrytis cinerea* causes significant damage to aboveground plant parts, but its impact on root chemistry and microbiome composition is less understood. This study investigated how *B. cinerea* foliar infection influences the root volatilome and microbiome of two tomato genotypes: the wild *Solanum pimpinellifolium* and the domesticated *Solanum lycopersicum* var. Moneymaker. In the absence of infection, the wild tomato roots emitted higher levels of monoterpenes such as α-pinene and terpinene compared to domesticated tomato roots. The fungal infection induced elevated levels of benzyl alcohol and benzofuran in the root headspace and/or rhizosphere of both genotypes, alongside genotype-specific changes. Multivariate analyses revealed that *B. cinerea* significantly altered bacterial and fungal community compositions in the rhizosphere and rhizoplane, with stronger bacterial community shifts in the rhizoplane. Taxa depletion and enrichment were observed, particularly among Proteobacteria and Ascomycota. Mantel tests showed significant correlations between rhizoplane bacterial community compositions and root-associated volatilome. Notably, enriched bacterial taxa such as *Pelomonas* and *Comamonadaceae* positively correlated with benzyl alcohol and benzofuran levels in the root volatilome. These findings demonstrate that *B. cinerea* foliar infection might induce profound changes in root-associated volatilome and microbiome composition, highlighting its systemic effects on plant root chemistry and microbiome composition.

Keywords: foliar infection, plant systemic response, root volatilome, root microbiome

Introduction

Botrytis cinerea is a foliar fungal pathogen that infects a wide range of crop plants leading to decay of leaves, stems, flowers and fruits. While the direct effects of *B. cinerea* and other foliar fungal pathogens on the aerial parts of plants are well-documented, their impact on plant root chemistry and microbiome assembly remains largely unexplored. Foliar infections by fungal or bacterial pathogens can trigger systemic effects in plants, which primes the plant for defense against subsequent attacks (Berendsen et al., 2018; Luo et al., 2022). Interestingly, foliar stresses can trigger belowground plant responses by altering root exudate composition both quantitatively and qualitatively, which in turn selectively enriches or depletes specific root-associated microbial taxa leading to induction of resistance (Yang, et al., 2011). For instance, when *Arabidopsis thaliana* was infected with the downy mildew pathogen *Hyaloperonospora arabidopsidis,* bacterial consortia were recruited which induced resistance against the same foliar pathogen (Berendsen et al., 2018). Similarly, foliar infection of *Panax notoginseng* with *Alternaria panax* affected the composition and concentrations of root exudate constituents such as specific sugars and organic acids, which coincided with the enrichment of specific beneficial microbes in the rhizosphere and concomitant positive plant-soil feedback (Luo et al., 2022). *B. cinerea* has been reported to induce systemic responses in tomato plants by enhancing root secretion of gluconic acid, which was associated with an enrichment of beneficial fungi in the rhizosphere (Fernández et al., 2017).

The objective of our study was to investigate how foliar infection of tomato leaves by *B. cinerea* influences the root and rhizosphere volatilome, as well as the composition of root-associated bacterial and fungal communities. Specifically, we examined whether pathogen-induced changes in volatile organic compounds (VOCs) emissions coincide with shifts in microbial community structure. We hypothesized that *B. cinerea* infection alters both root and rhizosphere volatile profiles, as well as the composition of the root-associated microbiome, and that these effects may depend on tomato genotype. We focused on VOCs as they can serve as important belowground chemical signals for short and long-distance plant-microbe communications (Schulz-Bohm et al., 2018; Sharifi et al., 2022). Their unique chemical nature as small molecules with a low boiling point and high vapor pressure allow these compounds to diffuse through soil matrices and convey messages distances away from the emitters (Wenke et al., 2010). Soil microbes, such as

bacteria and fungi can perceive these volatiles and be attracted or repelled. Reciprocally, bacteria and fungi can emit VOCs which may affect plant growth and resistance to environmental stresses (Weisskopf et al., 2021). To date, however, little is known about the impact of aboveground foliar stress on this chemical interplay.

To investigate the impact of *B. cinerea* on the tomato root volatilome and microbiome composition, we used two different tomato genotypes: *Solanum pimpinellifolium* and *Solanum lycopersicum* var. Moneymaker representing a wild and domesticated tomato, respectively. We investigated whether foliar infections of these tomato genotypes change the root volatilome and if these changes coincide with specific changes in the composition of the root-associated bacterial and fungal communities. We developed non-destructive rhizosphere-root volatile sampling techniques using HiSorb traps to collect root VOCs in *in vitro* (sterile) and in greenhouse (non-sterile) assays. To achieve this, we conducted untargeted volatilome analyses using gas chromatography–mass spectrometry (GC/MS), along with 16S rRNA and ITS gene amplicon sequencing, to characterize changes in tomato root chemistry and microbiome in response to foliar infection by *B. cinerea*.

Materials and methods

Soil collection and preparation

Soil samples were obtained from an organically managed agricultural plot in Nergena, Bennekom, The Netherlands, with coordinates 51.996250, 5.659375. Collected in 2014, the top 80 cm layer of soil was excavated from the field and then stored outdoors without intervention, allowing the natural soil ecosystem, including wild plants, to remain intact. The soil was air-dried, sieved through a 5 mm mesh to eliminate larger particles and plant debris, and subsequently stored in dark, room temperature conditions until further use.

Seed sterilization and pre-germination

Two tomato genotypes: *Solanum lycopersicum* var. Moneymaker (purchased from Bingenheimmer Saatgut AG, Echzell, Germany) and its wild counterpart *Solanum pimpinellifolium* (provided by Wageningen Seed Science Centre, Wageningen, The Netherlands) were used. These genotypes have been extensively used in phenotyping studies, particularly regarding characteristics

such as seed, seedling, and root traits (Kazmi et al., 2017; Khan et al., 2012). Tomato seeds from both genotypes were surface sterilized by immersion in 70% (v/v) ethanol for 2 minutes, and 1.5% (v/v) sodium hypochlorite for 15 minutes. Subsequently, the seeds underwent three rinses with ample volumes of sterile demineralized water. To ensure surface sterility, 100 µL of demineralized water from the final rinse were plated onto 1/10th strength tryptic soy agar (TSA) and 1/2 strength potato dextrose agar (PDA) to check for bacterial and fungal growth, respectively. For the greenhouse assay, the sterilized seeds were placed on sterile, pre-moistened filter paper inside sterile Petri dishes and incubated at 25°C in the dark until germination.

Fungal strain preparation

Botrytis cinerea strain B05.10 was kindly provided by Dr. Jan van Kan from the Department of Phytopathology, Wageningen University and Research (WUR). The fungal strain was cultured on 1/2 strength malt extract agar (MEA, Difco) and incubated at 20°C in darkness for 14 days until sporulation. The fungal spores were harvested and adjusted to a concentration of 10^6 spores/mL for the infection assay.

Greenhouse experimental set-up and plant growth conditions

Pre-germinated tomato seeds were transplanted into PVC pots (Desch Plantpark, The Netherlands) with dimensions of 7x7x8 cm, containing 300 grams of previously mentioned agricultural soil with an initial moisture content of 20% (v/w). A total of 10 pots were prepared for each tomato genotype for the collection of rhizosphere and rhizoplane samples, while 4 pots were left unplanted for the collection of bulk soil. Additionally, 5 additional pots were prepared per genotype to assess the disease severity index following *B. cinerea* infection. Tomato seedlings were cultivated inside a controlled greenhouse environment at 23°C with a 16-hour light/8-hour dark photoperiod and 80% humidity. Plants received 20 mL of 1/2 strength Hoagland solution and water weekly to maintain optimal growth. Upon reaching the 5th true leaf stage, 5 biological replicates of each tomato genotype were inoculated with *Botrytis cinerea* by inoculating 10 µL of the spore suspension (diluted in half strength PDB, 10^6 spores/mL) onto the surface of tomato leaflets originating from the 3rd and 4th true leaves. Foliar infection was allowed to develop for another two weeks, during which the leaves were re-inoculated weekly with the same concentration of spore solution to sustain the infection. Fungal infection was quantified by measuring the area of the lesions formed on the surface of inoculated leaflets. Five replicates of each genotype were

mock-inoculated with 10 µL sterile 1/2 strength PDB, representing the healthy control treatment.

Fungal infection bioassay and disease scoring

Two weeks after fungal spore inoculations, tomato plants (including those in the healthy treatment) were harvested for measurement of shoot biomass (dry), and for collection of rhizosphere, rhizoplane, and bulk samples. Additionally, 5 additional plants for each genotype were harvested for the detached leaf bioassay. For this, leaflets originating from the 4th true tomato leaves were detached (5 biological replicates and 3 technical replicates per genotype), immersed in 70% (v/v) ethanol for 5 minutes, washed with sterile demineralized water, and placed onto sterile filter paper inside a 9-cm-Petri dish before being treated with 10 µL of spore solution (10^6 spores/mL) or mock-inoculated with 1/2 strength potato dextrose broth. The plates were then incubated at 23°C, and the lesions were allowed to develop for four days post-inoculation. WINFOLIA™ software was utilized to determine the lesion area (discoloured tissue), to quantify the percentage (%) of infected over the non-infected leaflet areas.

Rhizosphere volatilome profiling *in planta*

For the collection of volatiles from the soil-root interface in the greenhouse assay, HiSorb probes (Markes International Ltd., Llantrisant, UK) were inserted into cylindrical metal holders previously planted approximately 5 cm deep in the soil, closely positioned to the tomato root, to trap the root-associated volatilome (Supplementary Fig.S1). These metal holders, made of stainless steel with perforations around the surface, allow volatile diffusion into the cylinder while preventing direct contact between the probes and the soil, thereby creating a headspace. The top of the metal holders was equipped with screw caps enabling the cylinder to be closed when the probes were inside. The HiSorb probes were placed inside the metal holders overnight before recollection, 13 days after fungal spore application. The probes were also placed inside the holders to trap volatiles produced by the healthy control group, as well as the unplanted bulk soils. After recollection, HiSorb traps were placed into clean empty closed metal holders with screw caps at both ends (Markes International Ltd., Llantrisant, UK), and stored at room temperature until measurement.

Root volatilome profiling *in vitro*

We designed an *in vitro* setup using two-compartment plates to profile root volatiles of two tomato genotypes exposed to *Botrytis cinerea* under sterile conditions. This system ensured optimal trapping of only root volatiles and minimized potential contamination from shoot volatiles (Supplementary Fig. S2). In summary, surface-sterilized tomato seeds were placed inside tubes connected to one side of two-compartment Petri plates containing 20 mL of solidified 1/2 strength Murashige and Skoog (MS) medium. The other side of the compartment was left empty for the placement of the HiSorb probe. The plates were tightly sealed with parafilm, then placed inside a UV-sterilized box, and incubated in a climate chamber with a constant temperature of 23°C and a 16-hour light/8-hour dark photoperiod. Once the plants reached the 3rd to 4th true leaves growth stage, two conditions were applied for each tomato genotype: the foliar stress with 5 biological replicates exposed to *B. cinerea* by inoculating 10 µL of a spore suspension in 0.5X potato dextrose broth (PDB, 10^6 spores/mL) onto the surface of each leaflet from the 3rd true leaves. The remaining 5 replicates were mock-inoculated using 10 µL sterile 0.5X PDB, serving as the healthy control. Each set of replicates from the same treatment group was placed in one UV-sterilised box to prevent volatile cross-contamination. *Botrytis* infections were allowed to develop for 48 hours before root volatile trapping.

Volatile measurement using GC/Q-TOF

The volatiles were measured using GC/Q-TOF following the protocol outlined by Lee Diaz et al. (2022) (Díaz et al., 2022). In summary, volatile organic compounds were desorbed from HiSorb probes using an automated thermal desorption unit (Unity TD-100, Markes International, Llantrisant, UK) with helium gas at 240 °C for 8 minutes. The released volatiles were then injected into the GC/Q-TOF system (Agilent 7890B GC and the Agilent 7200 Q-TOF, Santa Clara, CA, USA) with a DBms ultra-inert column (Agilent Technologies, Inc., Santa Clara, CA, USA). Mass fragments were detected by the GC/Q-TOF system operating at 70 eV in electron ionization (EI) mode with a source temperature of 230 °C. A standard n-alkane mixture (C8-C20) was spiked at the beginning of the measurement to create a calibration curve. This curve was utilized to calculate the retention index (RI) of a compound based on its retention time (RT) relative to the known RT and RI of the alkane standard. The GC/Q-TOF raw data (.D) acquired at a full scanned mode (*m/z* 300-400,4scans/s, 2 GHz extended dynamic range).

Volatile data analysis

The GC/Q-TOF raw data (.D) were first converted into content definition file (.cdf) format using GC AIA translator B.07.04 (Agilent Technologies, Santa Clara, CA, USA). Subsequently, the translated data were imported into Mzmine 2.53 to extract a mass feature table (Pluskal et al., 2020). The Automated Data Analysis Pipeline (ADAP) algorithm was employed to perform chromatogram building, peak and spectral deconvolution, as well as alignment steps (Du et al., 2020) (detailed parameters are available in the supplementary table). The mass feature table (.csv) was then subjected to filtering to eliminate features were not biologically relevant including silica fragments prior to statistical analyses. The normalization of volatilome data was done using Metaboanalyst v6.0 (Pang et al., 2024). Briefly, the mass feature table was subject to normalization using log10 and Pareto-scalling. The Principle Component Analysis (PCA) was performed based on this normalized data. PERMANOVA with 999 permutations was conducted to see if there was any factor significantly affecting the volatile profile. Multiple pairwise comparison was then performed to obtain significantly different mass feature (p-value<0.05) between treatments using Metaboanalyst v6.0 for the normalized dataset. Heatmap was built to visualize the statistically significant mass features between treatments and clustered based on the Euclidean distance. Spearman's rank correlation coefficients (calculated using MetaboAnalyst v6.0) were applied to identify rVOCs that differentiate infected tomato plants from healthy ones.

Rhizosphere and rhizoplane sampling

Tomato plants, both healthy and *Botrytis*-infected, were uprooted from pots and roots were separated from the shoot. The roots were vigorously shaken to remove the loosely adhering soil. To collect rhizosphere and rhizoplane samples from the same root fragment, we modified a fractionation and detachment protocol previously described by Richter-Heitmann, et al (Richter-Heitmann et al., 2016) and Attia et al (Attia et al., 2022). In brief, the roots with attached soil were placed into 50 mL Falcon tubes containing 35 mL of sterile 10 mM $MgSO_4$ buffer. The solution was vortexed at maximum speed for 1 minute, and the root was removed, leaving the soil suspension, which is considered as rhizosphere samples. The same roots were then transferred to new 50 mL Falcon tubes containing 35 mL sterile 10 mM MgSO4 buffer, where the solution was subjected to 1 minute vortex at maximum speed, followed by 1 minute sonication. This process was repeated twice to further detach the remaining soil particles from the roots. After cleaning, the roots

were removed, resulting in rhizoplane suspension. Bulk soil samples were collected from unplanted pots and then mixed directly with the same buffer as mentioned above. Bulk, rhizosphere, and rhizoplane suspensions were then centrifuged at 4500 rpm for 10 minutes to separate the soil pellet from the supernatant. The pellets were transferred to 2 mL tubes and then stored at -80°C until DNA extraction

DNA extraction, 16S and ITS-amplicon sequencing

Total DNA from different compartments (bulk, rhizosphere and rhizoplane) was extracted using DNeasy PowerSoil Pro kit (Qiagen, USA) according to the manufacturer protocol. The quantity and quality of the extracted DNA were checked using Nanodrop One (Thermo Fisher Scientific, USA) before sending the extract to BaseClear (BaseClear, B.V., Leiden, The Netherlands) for Illumina MiSeq sequencing. The primer set 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') was used to amplify the V3–V4 region of the bacterial 16S rRNA gene.

Meanwhile, the primer set of ITS1F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') was employed for amplification of the ITS1–ITS2 region of the fungal Internal Transcribed Spacer (ITS). Each 25 µL PCR reaction contained 12.5 µL of 2× KAPA HiFi HotStart ReadyMix, 5 µL of each primer (1 µM), and 2.5 µL of template DNA. Thermal cycling conditions included an initial denaturation at 95°C for 3 minutes; followed by 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 5 minutes. The paired-end sequence reads (2×250 bp) generated by the Illumina MiSeq were then converted into FASTQ files using bcl2fastq (v2.20, Illumina) and were checked for quality with the FastQC tool (v0.12.0)

Bioinformatic and statistical analyses

The demultiplexed paired-end files were processed to amplicon sequence variances (ASVs) using DADA2 pipeline 1.16 (Callahan et al., 2016) run in R studio (version 2023.06.0+421). Before processing with the DADA2, primers were removed from the sequences using the Cutadapt function (v3.4). Sequencing error was checked and chimera were removed before assigning the reads for taxonomic identification using Silva (v1.38.1) and Unite database (v8.2) for bacteria and fungi respectively. The resulting ASV abundance, taxonomy table and the sample metadata were combined into a phyloseq object using phyloseq package (v1.42). Bacterial ASVs were firstly filtered out

from affiliated with “eukaryote,” “archaea,” “mitochondria,” and “chloroplast.” To reduce noise and improve statistical power, the ASV table was further filtered to include only those with a minimum of 10 reads in at least four samples. Alpha diversity, based on the Shannon index, was calculated on a rarefied ASV table (minimum read count of 5,849) using the `get_alphaindex` function from the MicrobiotaProcess package (v1.10.3). One-way ANOVA was performed to test the significance. Furthermore, beta diversity analysis was performed to assess differences in microbial composition between treatments. For this, Principal Coordinate Analysis (PCoA) based on Aitchison distance was performed on the filtered ASVs (center-log ratio (clr)-transformed) using the microViz package (v0.10.8). PERMANOVA with 999 permutations was also conducted to investigate factors (compartment, genotype, and treatment) and significantly influenced microbial beta diversity. This was followed by a pairwise comparison using the pairwiseAdonis package (v0.4). To further investigate the difference in beta diversity of the two tomato genotypes under stress treatment without bulk soil effect, we repeated the previously mentioned analyses only on the ASVs enriched in the rhizosphere and rhizoplane. For this, a contrast test using the DESeq2 package (v1.38.3) was performed between bulk and rhizosphere, as well as between bulk and rhizoplane, to obtain ASVs enriched in the rhizosphere and rhizoplane, respectively (Benjamini-Hochberg adjusted p-values (FDR) < 0.05 and log-fold change (LFC)>2). The same package was also used to conduct differential abundance analyses to determine ASVs significantly enriched or depleted upon *Botrytis cinerea* infection of each of the two tomato genotypes corrected for the bulk soil effect.The Mantel test based on Spearman’s correlation method with 999 permutations was conducted to investigate the association between fungal or bacterial communities and rhizosphere-associated volatilome. Spearman’s correlation analysis was further used to assess the correlation between differentially abundant ASVs and differential metabolites using the rcorr function from Hmsic package (v5.1-2). Significant correlations ($p<0.05$ *, $p<0.01$**) were plotted in the heatmap using pheatmap package (v1.0.12).

Results

Susceptibility of tomato plants to *Botrytis cinerea*

Leaves of *S. lycopersicum and S. pimpinellifolium* were inoculated with spores of *Botrytis cinerea*. Infection progressed more in the modern *S. lycopersicum* based on the higher percentage of lesion area compared to that of wild relative *S. pimpinellifolium* (Supplementary Fig. S3a). Furthermore, the dry shoot biomass of *Botrytis*-infected *S. lycopersicum* was substantially lower compared to the healthy plants, whereas in *S. pimpinellifolium,* the dry shoot biomass was slightly reduced in the infected plants compared to the non-infected (healthy) plants (Supplementary Fig. S3b).

Impact of *Botrytis cinerea* infection on tomato rhizosphere and root volatilome

A total of 189 mass features correspoding to volatile compounds were detected from the five different treatments (unplanted bulk soil, rhizosphere of healthy and *Botrytis*-infected *S. pimpinellifolium* and *S. lycopersicum,* Supplementary Data 1). Principal coordinate analysis (PCoA) indicated that the unplanted bulk soil clustered separately from the rest of the treatments suggesting that soil emitted a unique blend of volatiles (Supplementary Fig. S4a). We then filtered mass features that were significantly enriched relative to those detected in unplanted bulk soil (>2log-fold change, FDR<0.05). In total, peak areas of 86 mass features were found to be significantly higher in the rhizosphere of the two tomato genotypes (with and without *Botrytis cinerea* infection) compared to unplanted bulk soil (Supplementary Data 1). Subsequent multivariate analysis for this filtered dataset showed that both the tomato genotype and treatment significantly affected the rhizosphere volatile profile (Supplementary Figure S4a, Table 1). Tomato genotype explained the variation seen in the volatile profile more than the stress treatment (Table 1). In the absence of *Botrytis* infection (healthy plants), higher peak areas of monoterpenes such as alpha-pinene and terpinene were detected in the rhizosphere of wild *S. pimpinellifolium* than the modern *S. lycopersicum* var. Moneymaker (Supplementary Fig. S5a,b, Supplementary Data 2). Furthermore, we observed a significant interaction effect between the tomato genotype and stress treatment, suggesting that foliar infection by *B. cinerea* enhanced emission of genotype-specific volatile compounds in the rhizosphere (Table 1). For infected *S. pimpinellifolium,* the volatile 1.2 tridecadiene, 1-ethyldecyl benzene and triacetin were detected at higher abundance, whereas 3-octanone, 5-methyl 3-hexanone were detected at

lower abundance compared to non-infected *S. pimpinellifolium* (Figure 1a, Supplementary Fig. S6a,b, Supplementary Data2). Infected *S. lycopersicum* showed reduced emission of (1-methyldecyl)benzene but an increase of terpinene emission (Supplementary Fig. S7a,b, Supplementary Data 2). Interestingly, emission of benzyl alcohol, benzofuran, and n-butyl benzene was found to be higher in the rhizosphere of both tomato genotypes following *B. cinerea* leaf infections (Fig. 1a, Supplementary Fig. S6,S7, Supplementary Data 2). This finding was in line with the results of the Spearman's correlation coefficient showing that these volatile compounds were among the top three significant compounds discriminating the volatile profile of infected from that of non-infected tomato plants, regardless of the genotype (Fig. 1b).

Table 1. Pairwise PERMANOVA test based on the 999 permutations on the effect of tomato (genotype) and *B.cinerea infection* (treatment) and their interaction (genotype:treatment) on the profile of root/rhizosphere-associated volatiles. In the pairwise panel, the following abbreviations apply; CBP: healthy *S.pimpinellifolium*, CBM: healthy *S.lycopersicum* var. Moneymaker, BP: Botrytis-infected *S.pimpinellifolium*, BM *Botrytis*-infected *S.lycopersicum* var. Moneymaker. The star (*) symbol indicated statistical difference (FDR<0.05).

	Rhizosphere volatilome	
PERMANOVA	**R2**	**p adjusted**
genotype	0.092	0.01*
treatment	0.077	0.042*
genotype:treatment	0.099	0.004*
Pairwise	**R2**	**p adjusted**
CBP vs CBM	0.21	0.048*
CBP VS BP	0.21	0.03*
CBM VS BM	0.177	0.07
CBP VS BM	0.211	0.03*
CBM VS BP	0.153	0.192
BP VS BM	0.2	0.03*

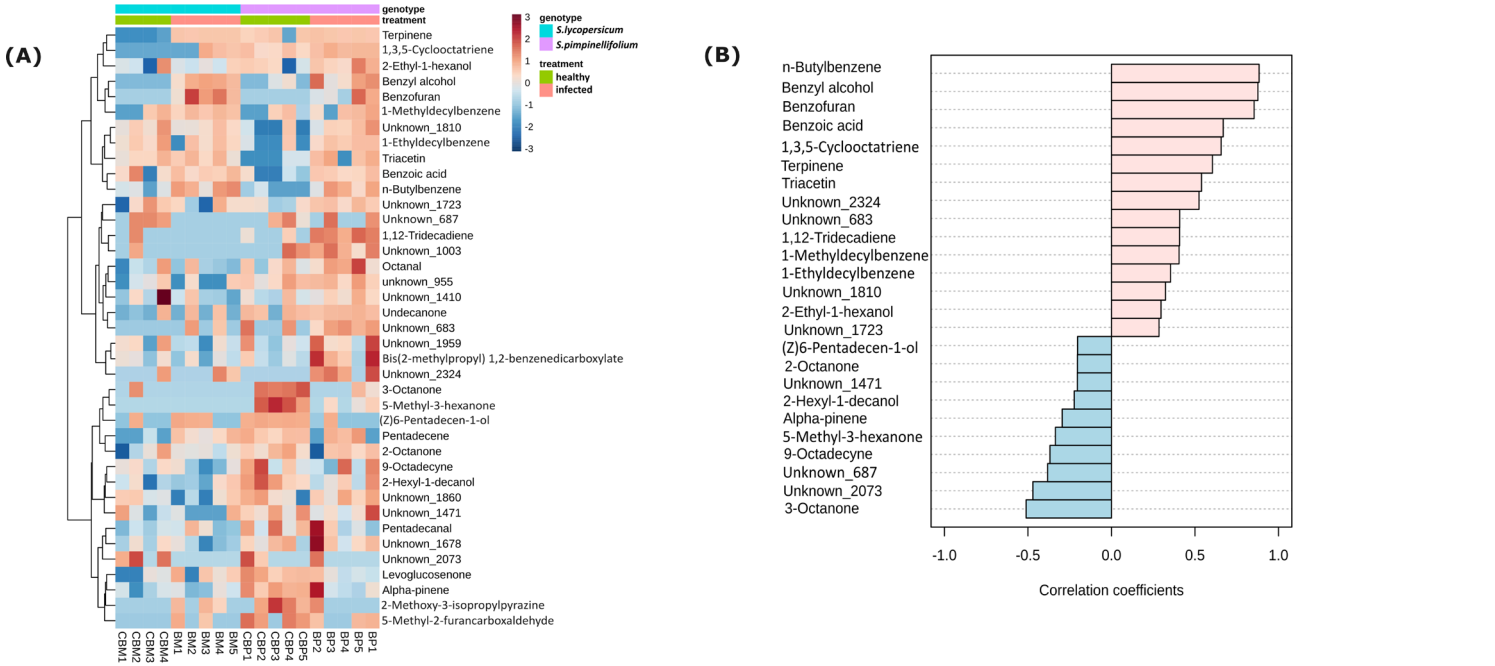


Figure 1. (A) Heatmap showing a comparison of normalized peak areas (log-transformed and Pareto-scaled) of selected mass features across different treatments. Mass features were selected based on their pairwise comparisons across treatments [healthy *S. lycopersicum* var. Moneymaker (CBM), healthy *S. pimpinellifolium* (CBP), *Botrytis*-infected *S. lycopersicum* var. Moneymaker (BM), and *Botrytis*-infected *S. pimpinellifolium* (BP), using multiple testing correction (FDR). Only statistically significant features (FDR < .05) in at least one comparison are included in the heatmap. Clustering of the samples was performed using the Euclidean distance. The intensity of normalized peak area is indicated by the color gradient legend, with darker blue color suggesting a lower intensity while the darker red color indicating the higher peak area intensity. The bottom column codes refer to different treatments, with numbers referring to the number of replicate for each treatment. Some unidentified compounds are referred to unknowns followed by numbers indicating their calculated retention indices. (B) Spearman's rank correlation coefficients of the top 25 root-associated volatile compounds differentially detected upon *B. cinerea* infection in tomato plants. Red bars and blue bars indicate compounds with positive and negative association with the infected plants, regardless of the genotype. N-butyl benzene, benzyl alcohol, and benzofuran are among the top three compounds positively associated with the stress responses of tomato plants (regardless of genotype) upon the infection of B. cinerea. Some unidentified compounds are referred to unknowns followed by numbers indicating their calculated retention indices.

To confirm if some volatile compounds detected in the rhizosphere originated from the roots, we compared the volatile data from the greenhouse bioassays (non-sterile system) to our *in vitro* experiments (Supplementary Fig. S2), where we collected the root volatiles from two tomato genotypes (control and *Botrytis*-infected) under sterile conditions. Similar findings were also observed in our *in vitro* system where, in the absence of *Botrytis cinerea* infection, root volatile profile differs between two tomato genotypes with higher peak areas of monoterpenes including alpha pinene, beta-pinene, terpinene and limonene were detected in the roots of the wild tomato *S.pimpinellifolium* (Supplementary Fig. S8). Interestingly, despite system differences (different plant age and growing conditions), the stress-associated root volatiles detected in the greenhouse bioassays, in particular benzyl alcohol and benzofuran, were also detected *in vitro* for both tomato genotypes infected with *B. cinerea* (Supplementary Fig. S8a,b) suggesting that the same compounds were also induced in these genotypes following the infection of the same foliar pathogen.

Impact of *Botrytis cinerea* leaf infection on root-associated bacterial community

A total of 3,293 unique bacterial amplicon sequence variants (ASVs) were detected in bulk soil, rhizosphere and rhizoplane of the two tomato genotypes. The bacterial alpha diversity (Shannon index) was significantly lower in the rhizoplane compared to rhizosphere and bulk soil, whereas no significant differences in alpha diversity were observed between bulk soil and rhizosphere (Supplementary Fig. S9a). The genotype and infection did not reveal any significant effects on the alpha diversity for both rhizosphere and rhizoplane samples (Supplementary Fig. S9c). In contrast, Principal coordinate analysis (PCoA) showed clustering based on the compartment: bulk soil, rhizophere and rhizoplane (Supplementary Fig. S10a). In general, the majority of bacterial ASVs belonged to the phyla *Proteobacteria* [currently known as *Pseudomonadota*], *Cyanobacteria*, *Actinobacteriota* [*Actinomycetota*], *Chloroflexi*, *Bacteroidota* [*Bacteroidetes*], and *Firmicutes* [*Bacillota*] (Supplementary Fig. S10c), differing in their relative abundances across the compartments (bulk soil, rhizosphere, and rhizoplane). The relative abundance of *Proteobacteria* [currently known as *Pseudomonadota*] gradually increased from bulk soil to rhizosphere and rhizoplane. Similarly, *Cyanobacteria* and *Bacteroidota* [*Bacteroidetes*] had higher relative abundances in the rhizoplane than in the rhizosphere and bulk soil. In contrast, the relative abundances

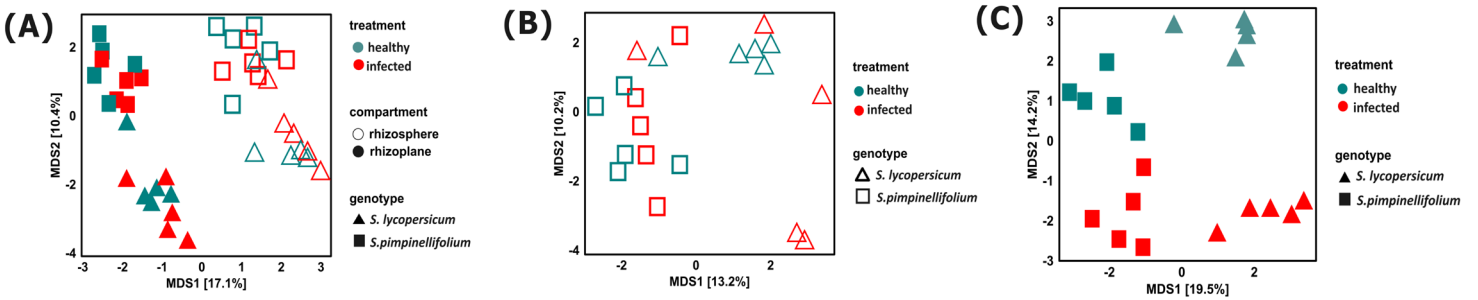


Figure 2. (A) Principal coordinate analysis (PCoA) based on Aitchison distance of bacterial community profile (of pooled data set) that were enriched in rhizosphere (filled triangle) and that of rhizoplane (empty triangle). The PCoA plot was then regrouped based on two compartments; rhizosphere **(B)** and rhizoplane **(C)**. Each plot includes data for *S. lycopersicum* var. Moneymaker (indicated by smaller triangles) and *S. pimpinellifolium* (indicated by larger triangles), with different colors representing different stress treatments; red for *Botrytis*-infected tomato plants and green for non-infected (healthy) tomato plants.

of *Firmicutes* [*Bacillota*], *Actinobacteriota* [*Actinomycetota*], and *Chloroflexi* decreased in the rhizoplane (Supplementary Fig. S10c)

To further investigate the impact of the foliar infection on the rhizosphere and rhizoplane bacterial community of the two tomato genotypes, we selected those bacterial ASVs that were significantly enriched in the rhizosphere and rhizoplane compared to that of bulk/unplanted soil samples (>2 fold change, adjusted $p<0.05$). Multivariate analyses on this filtered dataset showed that the compartment (rhizosphere vs rhizoplane) remains explaining the biggest variations seen in the bacterial community diversity, followed by the tomato genotype, and stress treatment (Fig.2a, Table 2). We therefore repeated the multivariate analyses for the rhizosphere and rhizoplane separately to look into the contribution of the genotype and stress factor to the bacterial community diversity in each compartment (Fig. 2b,c, Table 3). The bacterial community diversity was significantly influenced by tomato genotype for both rhizosphere and rhizoplane. The genotype factor better explained the variation seen in the beta diversity in the rhizoplane (approximately 17%) compared to the rhizosphere (approximately 10%). The foliar infection by *B. cinerea* had only a significant effect in shaping the bacterial community diversity in the rhizoplane, not in the rhizosphere.

Table 2. Pairwise PERMANOVA test based on the 999 permutations to investigate the contribution of root compartment (rhizosphere and rhizoplane), tomato genotype and treatment (fungal infection) on bacterial and fungal community composition.

	bacterial community		**fungal community**	
PERMANOVA	***R2***	***p adjusted***	***R2***	***p adjusted***
compartment	0.155	0.001*	0.085	0.001*
genotype	0.093	0.001*	0.048	0.002*
treatment	0.053	0.003*	0.071	0.001*

Table 3. Pairwise PERMANOVA test based on the 999 permutations on the effect of tomato genotype (genotype) and the fungal infection (treatment) as well as their interaction (genotype:treatment) on bacterial community diversity enriched in rhizosphere and rhizoplane. In the pairwise panel, the following abbreviations apply; CBP: healthy *S.pimpinellifolium*, CBM: healthy *S.lycopersicum* var. Moneymaker, BP: *Botrytis*-infected *S.pimpinellifolium*, BM; *Botrytis*-infected *S.lycopersicum* var. Moneymaker. The star (*) symbol indicated statistical difference (FDR<0.05).

	16S rhizosphere		16S rhizoplane	
PERMANOVA	**R^2**	**p adjusted**	**R^2**	**p adjusted**
genotype	0.102	0.001*	0.175	0.001*
treatment	0.056	0.169	0.133	0.001*
genotype:treatment	0.062	0.07	0.084	0.005*
Pairwise	**R^2**	**p adjusted**	**R^2**	**p adjusted**
CBP vs CBM	0.186	0.042*	0.324	0.013*
CBP VS BP	0.129	0.142	0.237	0.013*
CBM VS BM	0.136	0.145	0.289	0.013*
CBP VS BM	0.194	0.042*	0.358	0.013*
CBM VS BP	0.145	0.042*	0.316	0.013*
BP VS BM	0.162	0.058	0.274	0.013*

Differential abundances of bacterial taxa associated with *Botrytis* infection

To identify changes in the abundance of specific baterial ASVs associated with the tomato genotype and foliar stress, we conducted a differential abundance analysis of all possible pairwise comparisons for rhizosphere and rhizoplane (Table 3). In line with our multivariate analysis, we found that rhizoplane bacterial communities showed larger changes in diversity than the rhizosphere indicated by more ASVs were differentially abundant (between treatments) in the rhizoplane than in the rhizosphere (Fig. 3a,b).

In general, foliar infection caused depletion and enrichment of several taxa in rhizoplane (Supplementary Data 3). Some of these changes were unique to each tomato genotype, while others were shared between the two. These changes were mainly associated with the phyla *Proteobacteria* (currently known as *Pseudomonadota*), *Actinobacteria* [*Actinomycetota*], and *Bacteroidota* [*Bacteroidetes*] (Figure 3b, Supplementary Data 3). In *S. pimpinellifolium*, the foliar stress reduced the abundances of several ASVs belonging to the genera *Massilia* (ASV351, ASV367, ASV390, and ASV378), *Duganella* (ASV379, ASV317, ASV678), *Devosia* (ASV186),

Burkholderia (ASV455), *Flavobacterium* (ASV65), *Enterobacter* (ASV59), *Actinoplanes* (ASV177). On the other hand, the relative abundances of *Cylindrospermum* (ASV61) and *Pantoea* (ASV135) were higher in the *Botrytis*-infected *S. pimpinellifolium*. For *S.lycopersicum,* we observed significantly lower abundances of *Flavobacterium* (ASV642), *Sphingomonas* (ASV454)*, Stenotrophomonas* (ASV243), *Rahnella* (ASV33) and *Tychonema* (ASV78). Meanwhile, the relative abundances of *Leifsonia* (ASV776,ASV493), *Nakamurella* (ASV496), *Pantoea* (ASV198), *Duganella* (ASV74 and ASV180) and *Pseudomonas* (ASV375) were higher in the rhizoplane of plants infected by *Botrytis.*

Interestingly both *S. pimpinellifolium* and *S. lycopersicum* also showed similar patterns (Figure 4b, Supplementary Table S5,6) particularly for the lower abundances of ASVs belonging to *Enterobacteriaceae* (ASV6, ASV9), *Pantoea* (ASV73), *Pseudomonas* (ASV119, ASV259), *Burkholderia* (ASV455) and *Massilia* (ASV378). On the other hand, the rhizoplane of both tomato genotypes showed higher relative abundances of *Pelomonas* (ASV615), *Streptomyces* (ASV443), *Cylindrospermum* (ASV61) and an ASV from the *Comamonadaceae* (ASV261).

Impact of *Botrytis* infections on root-associated fungal community

A total of 1,968 unique fungal ASVs were identified by ITS sequencing of the bulk soil, rhizosphere and rhizoplane samples of the two tomato genotypes. Unlike the bacterial community, the Shannon diversity index showed no statistically significant differences in fungal alpha diversity between the three compartments (Supplementary Fig. S9b). Also, the fungal alpha diversity was hardly influenced by the tomato genotype and foliar infection by *B. cinerea* (Supplementary Figure S9b, d). The compartment factor (including bulk soil), however, significantly influenced the beta diversity as shown in the PCoA clustering (Supplementary Fig. 10b). In general, *Ascomycota*, *Basidiomycota* and *Mortierellomycota* were among the most dominant phyla with slightly differing their abundances across compartments (Supplementary Fig. 10d)

We re-conducted the multivariate analysis on the fungal ASVs that were only significantly enriched in rhizosphere and rhizoplane to normalize for the bulk soil effect (Fig. 4a,b,c). We found that compartment, tomato genotype, and stress treatment had significant effects on the fungal community diversity (Table 3). We then performed multivariate analysis for rhizosphere and rhizoplane separately to investigate the contribution of genotype and the

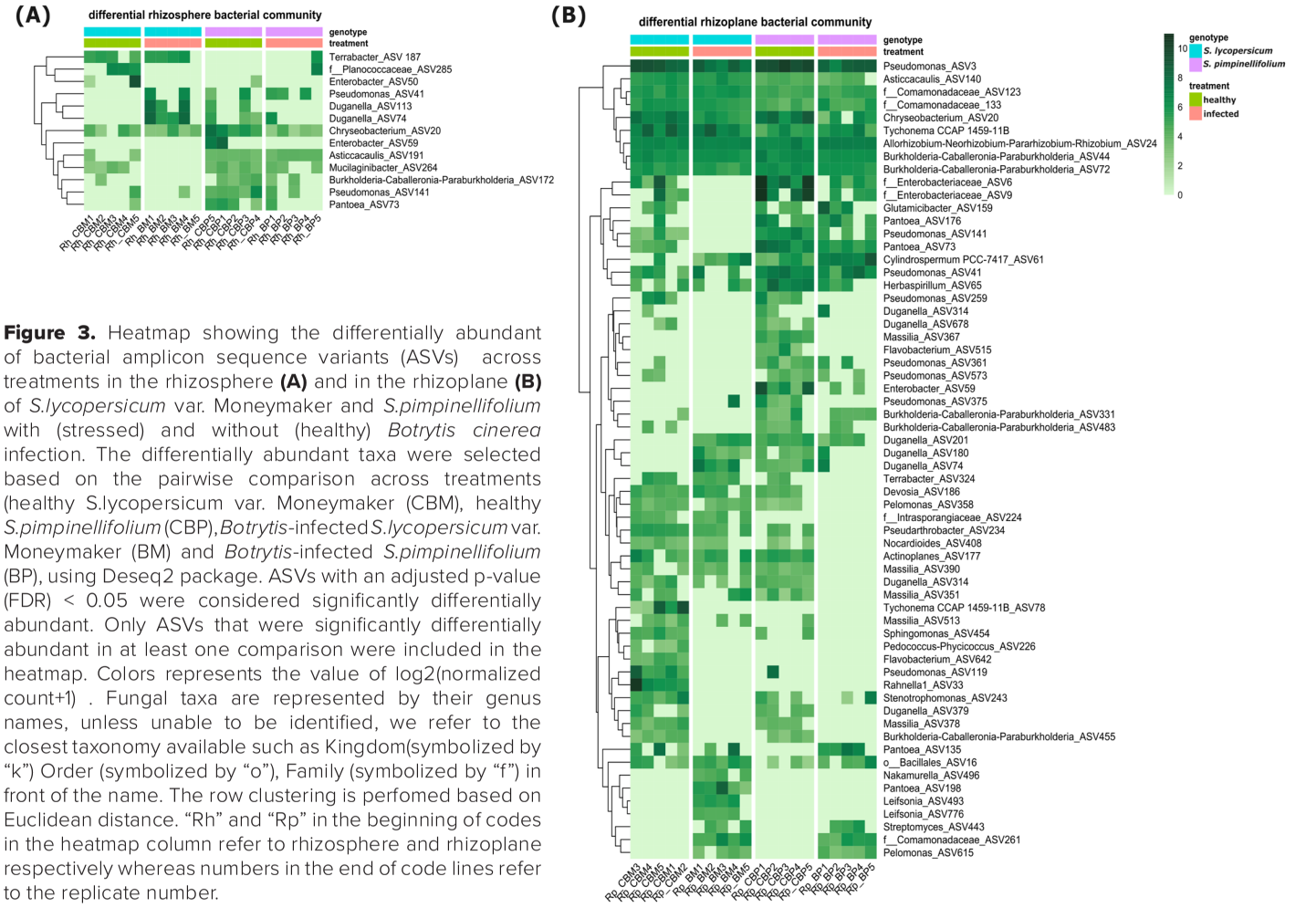


Figure 3. Heatmap showing the differentially abundant of bacterial amplicon sequence variants (ASVs) across treatments in the rhizosphere **(A)** and in the rhizoplane **(B)** of *S.lycopersicum* var. Moneymaker and *S.pimpinellifolium* with (stressed) and without (healthy) *Botrytis cinerea* infection. The differentially abundant taxa were selected based on the pairwise comparison across treatments (healthy S.lycopersicum var. Moneymaker (CBM), healthy *S.pimpinellifolium* (CBP), *Botrytis*-infected *S.lycopersicum* var. Moneymaker (BM) and *Botrytis*-infected *S.pimpinellifolium* (BP), using Deseq2 package. ASVs with an adjusted p-value (FDR) < 0.05 were considered significantly differentially abundant. Only ASVs that were significantly differentially abundant in at least one comparison were included in the heatmap. Colors represents the value of log2(normalized count+1) . Fungal taxa are represented by their genus names, unless unable to be identified, we refer to the closest taxonomy available such as Kingdom(symbolized by "k") Order (symbolized by "o"), Family (symbolized by "f") in front of the name. The row clustering is perfomed based on Euclidean distance. "Rh" and "Rp" in the beginning of codes in the heatmap column refer to rhizosphere and rhizoplane respectively whereas numbers in the end of code lines refer to the replicate number.

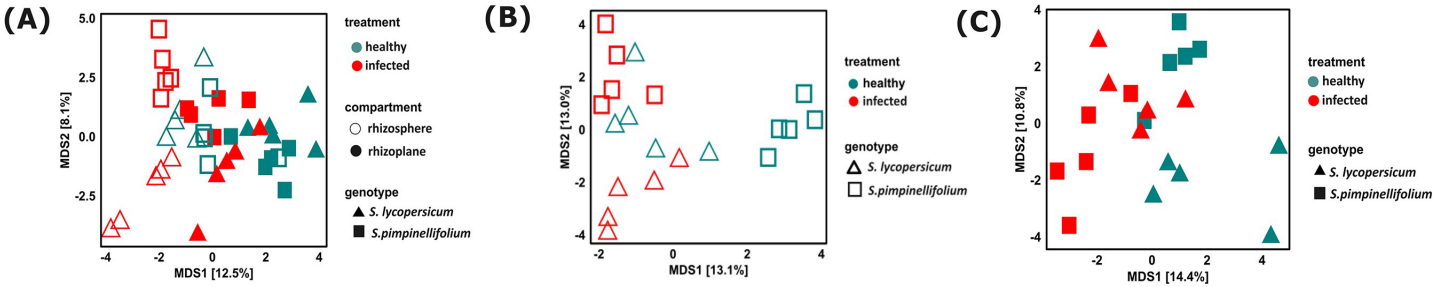


Figure 4. (A) Principal coordinate analysis (PCoA) based on Aitchison distance of fungal community profile (of pooled dataset) that were enriched in rhizosphere (filled triangle) and rhizoplane (empty triangle). The PCoA plot was then regrouped based on two compartments; rhizosphere **(B)** and rhizoplane **(C)**. Each plot includes data for *S. lycopersicum* var. Moneymaker (indicated by smaller triangles) and *S. pimpinellifolium* (indicated by larger triangles), with different colors representing treatment conditions: red for *Botrytis*-infected tomato plants and green for non-infected (healthy) tomato plants.

foliar stress treatment on the beta diversity. Interestingly, both genotype and the stress factors significantly affected the fungal beta diversity for both rhizosphere and rhizoplane (Table 4). The influence of foliar stress on the fungal community, however, was slightly stronger in rhizoplane compared to that in the rhizosphere. Furthermore, the foliar stress treatment better explained the variation seen in the rhizoplane fungal community than the tomato genotype.

Table 4. Pairwise PERMANOVA test based on the 999 permutations on the effect of tomato genotype (genotype) and the fungal infection (treatment) as well as their interaction (genotype:treatment) on fungal community diversity enriched in rhizosphere (ITS rhizosphere) and rhizoplane (ITS rhizoplane). In the pairwise panel, the following abbreviations apply; CBP: healthy *S.pimpinellifolium*, CBM: healthy *S.lycopersicum* var. Moneymaker, BP: *Botrytis*-infected *S.pimpinellifolium*, BM; *Botrytis*-infected *S.lycopersicum* var. Moneymaker. The star (*) symbol indicated statistical difference (FDR<0.05).

	ITS rhizosphere		ITS rhizoplane	
PERMANOVA	**R^2**	**p adjusted**	**R^2**	**p adjusted**
genotype	0.088	0.002*	0.083	0.003*
treatment	0.088	0.002*	0.118	0.001*
genotype:treatment	0.104	0.001*	0.093	0.001*
Pairwise	**R^2**	**p adjusted**	**R^2**	**p adjusted**
CBP vs CBM	0.190	0.02*	0.206	0.01*
CBP vs BP	0.236	0.02*	0.237	0.01*
CBM vs BM	0.189	0.02*	0.224	0.01*
CBP VS BM	0.245	0.02*	0.221	0.01*
CBM VS BP	0.132	0.17	0.222	0.01*
BP VS BM	0.231	0.02*	0.193	0.01*

Differential abundances of fungal taxa associated with foliar stress

Differential abundant analysis was performed at the ASV level to identify changes between different treatments as indicated by the pairwise PERMANOVA (Table 4). We found that both rhizosphere and rhizoplane fungal communities exhibited similar numbers of differentially abundant ASVs across treatments (Fig. 5a, b). Changes in abundances of fungal ASVs to the stress treatment were different for the root compartments and tomato genotypes, but predominantly driven by taxa from Ascomycota (Supplementary Data 4, 5). In the rhizosphere of *S. pimpinellifolium*, among other ASVs, *Purpureocillium* (ASV444), *Fusarium* (ASV61), *Mortierella* (ASV143), *Myrmecridium* (ASV186) and *Fusicola* (ASV219) were unique for the healthy plants but their

abundances reduced following the foliar stress (Figure 5a, Supplementary Data 4). Furthermore, ASVs belonging to the genus of *Trichoderma* (ASV73), *Olpidium* (ASV193), *Myceliophthora* (ASV259) and an unidentified ASV of the Order Hypocreales (ASV248) were uniquely enriched in the infected *S. pimpinellifolium*.

In the rhizoplane of infected *S. pimpinellifolium* (Fig. 5b, Supplementary Data 5), ASVs with reduced abundances were noticeable such as *Chaetomium* (ASV39), *Setophoma* (ASV135), *Mortierella* (ASV42, ASV153), and *Penicillium* (ASV191). On the other hand, the foliar infection increased the abundances of ASVs classified as *Annulohpoxylon* (ASV138), *Trichoderma* (ASV73), and an undentified genus belonging to Order Hypocreales (ASV248).

In the rhizosphere of *S. lycopersicum*, foliar stress specifically increased the abundances of *Monocillium* (ASV127, ASV201), *Humicola* (ASV231), *Chaetomium* (ASV94), an ASV belonging to the phylum Rozellomycota and three unidentified fungal ASVs (ASV59, ASV47, ASV67). Meanwhile the abundances of *Fusarium* (ASV174), *Eucasphaeria* (ASV171), and *Acrostalagmus* (ASV93) decreased following the foliar stress (Fig 5a, Supplementary Data 4). In the rhizoplane of *S. lycopersicum,* the foliar stress increased the abundances of *Chaetomium (*ASV39*)*, *Acremonium* (ASV204) and few unidentified fungal ASVs (ASV67, ASV145, ASV202) but decreased the abundances of *Fusarium* (ASV133), *Lachnum* (ASV128) and *Acrostalagmus* (ASV93). Interestingly, few ASVs responded similarly for both tomato genotypes: higher relative abundances of *Trichoderma* (ASV73, ASV74) and lower relative abundance of *Setophoma* (ASV135) were detected in the rhizoplane of both tomato genotypes following foliar infection by *Botrytis* (Fig 5b, Supplementary Table S7,8).

Correlations between changes in root-associated volatilome and microbiome

The Mantel test was performed to investigate whether changes in the root volatilome correlate with changes in the bacterial or fungal community composition in the rhizosphere and rhizoplane. Results revealed that only rhizoplane bacterial community had a significant correlation with the root volatilome (Table 5). In contrast, neither rhizoplane nor rhizosphere fungal communities showed statistically significant correlations with the root volatilome (Table 5). Subsequently, we performed Spearman's correlation analysis to determine the association between individual bacterial taxa and a certain volatile compound. In general, we found significant positive and

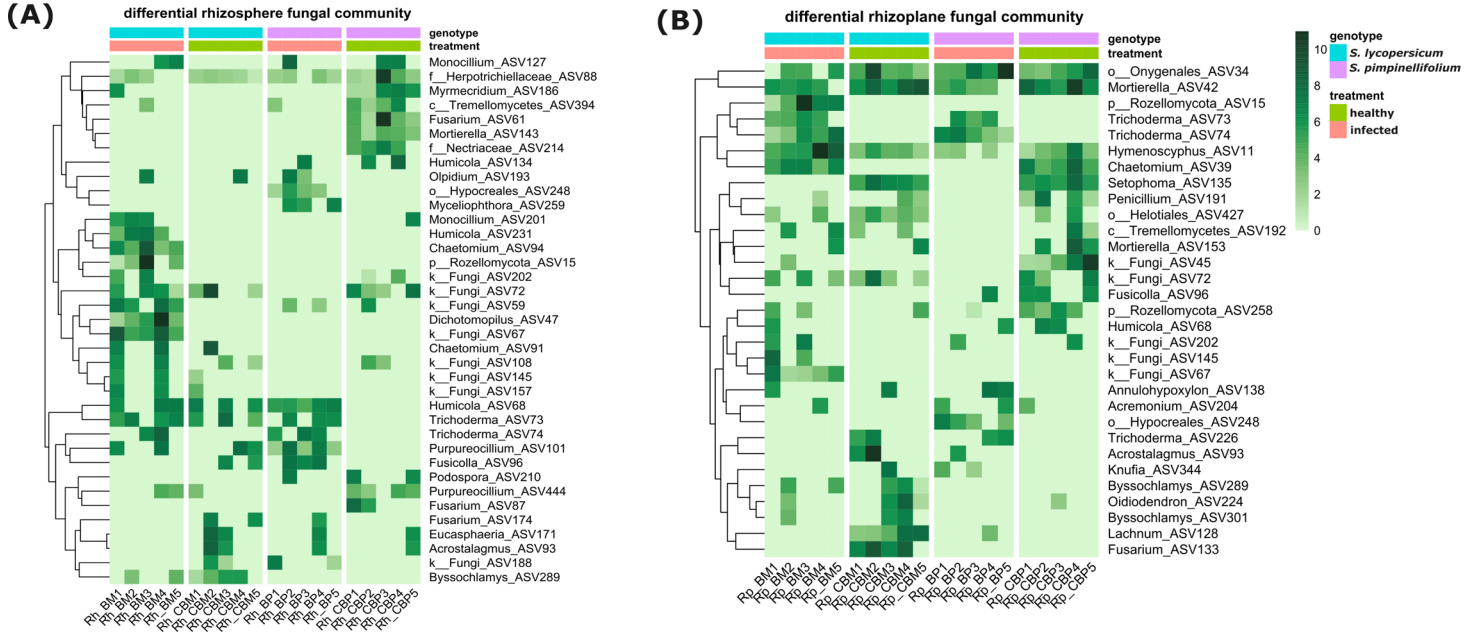


Figure 5. Heatmap showing the differentially abundant of fungal amplicon sequence variants (ASVs) across treatments in the rhizosphere (A) andin the rhizoplane (B) of *S.lycopersicum* var. Moneymaker and *S.pimpinellifolium* with (stressed) and without (healthy) *Botrytis cinerea* infection. Thedifferentially abundant taxa were selected based on the pairwise comparison across treatments (healthy *S.lycopersicum* var. Moneymaker (CBM),healthy *S.pimpinellifolium* (CBP), *Botrytis*-infected *S.lycopersicum* var. Moneymaker (BM) and *Botrytis*-infected *S.pimpinellifolium* (BP), using Deseq2package. ASVs with an adjusted p-value (FDR) < 0.05 were considered significantly differentially abundant. Only ASVs that were significantly differently abundant in at least one comparison were included in the heatmap. Colors represents the value of log2(normalized count+1). Fungal taxa are represented by their genus names, unless unable to be identified, we refer to the closest taxonomy available such as Kingdom(symbolized by “k”) Order (symbolized by “o”), Family (symbolized by “f”), Class (symbolized by “c”)in front of the name. The row clustering was performed based on Euclidean distance. “Rh” and “Rp” in the beginning of codes in the heatmap column refer to rhizosphere and rhizoplane respectively whereasnumbers in the end of code lines refer to the replicate number.

negative correlations of several bacterial taxa with root-associated volatiles (Fig. 6). Specifically, higher levels of volatile compounds associated with the stress response in tomato to *B. cinerea* (regardless of the genotype), particularly benzofuran, benzyl alcohol and n-butyl benzene (Fig. 1B), negatively correlated with *Enterobacteriaceae* (ASV6), *Chryseobacterium* (ASV20), *Pantoea* (ASV73), *Pseudomonas (*ASV259, ASV3, ASV141), *Duganella* (ASV317, ASV678, ASV379) and *Massilia* (ASV378), bacterial taxa with relative abundances decreasing in the rhizoplane of both tomato genotypes upon foliar infection. In contrast, the abundance of these stress-associated volatile compounds positively correlated with the relative abundance of *Pelomonas* (ASV615) and an ASV from the *Comamonadaceae* (ASV261), bacterial taxa that are enriched in the rhizoplane of both tomato genotypes following foliar infection (Figure 4b, Supplementary Figure S11).

Table 5. The Mantel test using Spearman's correlation method with 999 permutations to assess the correlation between root-associated volatilome and bacterial and fungal communities in the rhizopshpere and rhizoplane. The star (*) symbol indicated significant correlation ($p<0.05$).

Correlation	**Mantel R**	***p* value**
Volatile*rhizosphere bacteria	0.05	0.265
Volatile *rhizoplane bacteria	0.27	0.002*
Volatile *rhizosphere fungi	0.108	0.123
Volatile *rhizoplane fungi	-0.037	0.609

Figure 6. Spearman correlations between differentially abundant rhizoplane bacterial ASVs and root/rhizoshere-associated volatilome. Asterisks indicate significant correlations (FDR<0.05) and (FDR<0.01) are marked with (*) and (**) respectively. Significantly positive correlations are purple colored whereas significantly negative correlations are green colored. No significant (p>0.05) correlations was letf uncolored/blank.

Discussion

Our study revealed that for both the wild and domesticated tomato genotypes, foliar infection by *Botrytis* led to compositional changes in the root-associated microbiota. These compositional changes in bacterial communities were more distinct for the rhizoplane than for the rhizosphere. This compartment-specific differentiation in microbiome response is consistent with results of earlier studies on tomato as well as *Arabidopsis* and rice plants (Bulgarelli et al., 2012; Edwards et al., 2015; French et al., 2020). Overall, the root compartment poses selective pressure on the composition of microbial communities creating niches with specific microbial taxa. Such niche-specificity can be explained by the multiple selection processes imposed by the plants, and/or by the ability of soil microorganisms to occupy only certain rhizo-compartments (Liu et al., 2024). For the fungal community, however, no compartment-specific changes were observed, consistent with earlier studies on other plant species such as *Broussonetia papyrifera* and *Lycium ruthenicium* (Chen et al., 2020; Li et al., 2022). Collectively these results confirm and extend earlier observations of differential responses between the two microbial domains across compartments in the soil-root interface.

Proteobacteria [currently known as *Pseudomonadota*] gradually increasingly became a dominant bacterial phylum as it moved closely to the root surface, with its highest abundance in rhizoplane. Previous studies on tomato plants showed a similar trend with the higher abundance of this phyla in the rhizosphere and rhizoplane relative to bulk soil (French et al., 2020; Lee et al., 2019). Our result also showed higher abundance of Cyanobacteria in the tomato rhizoplane. Although their presence across rhizo-compartment generally has received less attention, this phyla was reported to actively colonize the rhizoplane of rice and hairgrass species *Deschampsia antartica,* which may be associated with their competitive ability to form biofilms on root surfaces (Ahmed et al., 2010, 2014; Znój et al., 2022). Different concentrations of root metabolites on root surface than the adjacent soil layers may give additional selection pressure on the rhizoplane-inhabiting microbes due to distinct capability of the individual taxa to metabolize or tolerate certain compounds. Also differences in root architecture between the plant genotypes (not studied in this research) can shape rhizoplane microbial communities (Herms et al., 2022). A previous study on wild and domesticated common beans revealed that root phenotypic traits (e.g. specific root-length)

contributed to 11.4% of the variation in rhizobacterial community composition (Pérez-Jaramillo et al., 2017).

Foliar stress caused by *Botrytis* generally induced bacterial ASV changes in a genotype-specific manner. *Botrytis* infection reduced the abundance of Proteobacteria in the rhizoplane of *S. pimpinellifolium* which was exemplified by the lower abundances of ASVs classified as *Pseudomonas*, *Duganella*, *Devosia*, and *Massilia*. Strains from these genera have been commonly described for their plant growth-promotion and disease suppressive effects in various plant species (Yin et al., 2013, 2021). For example, the genus *Massilia* was previously found to be enriched in healthy *S. pimpinellifolium* across several growth cycles suggesting that such genera might be actively recruited by this wild tomato genotype under optimal growth conditions (Cordovez et al., 2021). *Botrytis* infection of *S. lycopersicum* caused depletion of several ASVs classified as *Flavobacterium* and *Sphingomonas*,. The genus *Sphingomonas* has been previously reported to promote tomato growth and induce tomato resistance to oxidative stress under salinity(Halo et al., 2015; Khan et al., 2014) , while *Flavobacterium* can suppress bacterial wilt incident caused by *Ralstonia solanacearum* in *S. lycopersicum* var. Moneymaker (Kwak et al., 2018). Apart from the taxa depletion, we also observed a higher relative abundance of several taxa in the rhizoplane of two tomato genotypes infected by *B. cinerea*, in particular ASVs classified as *Pelomonas*, *Cylindrospermum*, *Streptomyces*, and unknown genera belonging to the family *Comamonadaceae*. *Streptomyces* strains are known to induce systemic resistance of chickpea plants against *B. cinerea* and of tomato plants against leaf-curl virus (Chen et al., 2022; Vijayabharathi et al., 2018). Interestingly, *Cylindrospermum* has been recently reported to be a key rhizosphere taxon responsible for systemic resistance in *S. lycopersicum* (Ketehouli et al., 2024). Furthermore, the rhizosphere-enriched *Comamonadacae* family has been reported to induce resistance against *Fusarium oxysporum* in cucumber plants (Ketehouli et al., 2024).

Taxa depletion was also found for fungal ASVs, mainly from Ascomycota and Mortierellomycota, with a reduced relative abundance of *Mortierella* and *Chaetomium* in the rhizosphere and rhizoplane of *Botrytis*-infected *S. pimpinellifolium*. *Mortierella* was previously described as a plant growth-promoting fungus and biocontrol agent against *Verticillium dahliae* in tomato plants (Nouri et al., 2024; Ozimek & Hanaka, 2021). This genus was also

enriched in the rhizosphere of maize and was responsible for soil carbon and phosphorous cycling. Strains from the genus *Chaetomium* are often referred to as biocontrol agents for *Fusarium* wilt in tomato plants due to their ability to produce antimicrobial compounds and to induce systemic resistance (Madbouly et al., 2017; Singh et al., 2021). Infection of *S. lycopersicum* by *Botrytis*, on the other hand, led to a reduced abundance of ASVs classified as *Fusarium* and *Lachnum*. *Fusarium* genera are commonly regarded as soil-borne pathogens of different plant species and their presence can negatively impact on crop growth and productivity. The genus *Lachnum* was previously studied for its colonization and plant growth-promoting effects on blueberry plants (Bizabani & Dames, 2015). Interestingly, we also found enrichment of ASVs classified as *Trichoderma* in the rhizosphere and rhizoplane of both tomato genotypes. In line with our finding, a previous study demonstrated the enrichment of *Trichoderma* strain T34 in the rhizosphere of tomato plants infected with *B. cinerea*. When inoculated on tomato, this strain induced systemic resistance against the same foliar pathogen (Fernández et al., 2017), exemplifying that upon infection the plant may recruit or enrich microbiome members that trigger a resistance response for protection against subsequent infections. To what extent this combination of taxa depletion and enrichment affects plant growth and stress resistance remains to be investigated.

Recently, we showed that the root volatile profile differed between *S. pimpinellifolium* and *S. lycopersicum* var. Moneymaker, with higher abundance of monoterpene compounds detected in the wild tomato (Díaz et al., 2022). Our *in vitro* study further confirmed that roots of *S. pimpinellifolium* emit higher monoterpene compounds such as alpha pinene, camphene, limonene and terpinene. Furthermore, our previous study also showed that aboveground insect herbivory altered the rhizosphere volatilome of tomato (Lee Díaz et al. 2024). In the present study, we also showed that *Botrytis* leaf infection changed the tomato rhizosphere volatilome quantitatively as well as qualitatively. These changes were largely unique to each of the two tomato genotypes tested. For instance, a higher intensity (based on peak areas) of 1,2-tridecadiene was detected in the rhizosphere of infected *S. pimpinellifolium,* while a lower intensity of 5-methyl-3-hexanone was detected in the same treatment. Volatile analyses of infected *S. lycopersicum* showed an increased intensity of terpinene. Terpinene is a compound classified as monoterpene and is known to be emitted by several plant species including tomato and pine (Díaz et al., 2022; Lin et al., 2007). This compound was also emitted in response to phytohormone stress and exhibits antimicrobial

properties which in turn may impact microbial community structure (Alavi-Samani et al., 2015; Yang et al., 2022)

The Mantel test results indicate a significant correlation between the rhizosphere-associated volatilome and the rhizoplane rather rhizosphere bacterial community (r=0.27, p=0.002), while no such correlation was found for fungal communities in either the rhizoplane or rhizosphere. This suggests different responsiveness among microbial communities towards certain root metabolites. Higher exudate concentrations at the root surface compared to the adjacent soil layer may act as a stronger driver of bacterial community assembly, leading to the differing responsiveness of rhizoplane and rhizosphere bacteria to volatile compounds. However, while our study focused on volatile compounds, it is important to recognize that roots also release non-volatile, water-soluble compounds that can play crucial roles in shaping microbial assembly (Koprivova & Kopriva, 2022; Zhalnina et al., 2018). Therefore, rVOCs should be viewed as one of several drivers influencing rhizoplane community structure, rather than the sole determinant.

Whether the volatiles measured in this study are produced directly by plant roots or by rhizoplane microbes, requires further scrutiny. It is also plausible that both plants and microbes emit specific volatiles in response to their dynamic interactions, further complicating the source attribution of these compounds. Interestingly, benzyl alcohol and benzofuran were detected in the rhizosphere of both tomato genotypes upon foliar stress. These two compounds were also detected in our *in vitro* experiment, emitted by roots of both tomato genotypes. Previously, benzyl alcohol and benzofuran were also detected in root of tomato plants upon herbivory stresses (Díaz et al., 2022; Lee Díaz et al., 2024), suggesting that these compounds may be more generic markers of root volatiles of plants under biotic stresses.

Spearman's correlation analyses showed positive associations between stress-associated volatiles like benzyl alcohol, benzofuran and n-butyl benzene to bacterial taxa enriched in rhizoplane of both tomato genotypes such as *Pelomonas*, *Cylindrospermum* and *Comamonadaceae* , while taxa like *Enterobacteriaceae* and *Chryseobacterium* correlated negatively. These findings suggest root-associated volatiles might, in part, selectively shape bacterial populations, potentially influencing microbial dynamics and plant defense mechanisms (de la Porte et al., 2020; Rizaludin et al., 2021; Schulz-Bohm et al., 2018).

Overall, our results show that tomato plants systemically respond to foliar infection by altering the composition of root-associated microbial communities and the rhizosphere/root volatilome. Future research needs to be performed to understand the reciprocal effects of these microbial and volatile changes for plant growth and stress resilience.

Funding

This work was supported by the Dutch Research Council (NWO/OCW), as part of the MiCRop Consortium Programme, Harnessing the second genome of plants (grant number 024.004.014).

Data availability statement

Raw sequences data (both 16S and ITS amplicons) are available at SRA NCBI under BioProject number PRJNA1241739. Raw GC-MS data are publicly available at Zenodo: 10.5281/zenodo.14223861. The code used to analyzed both 16S and ITS amplicon data is available at Github: https://github.com/MuhammadRizaludin/Botrytis.

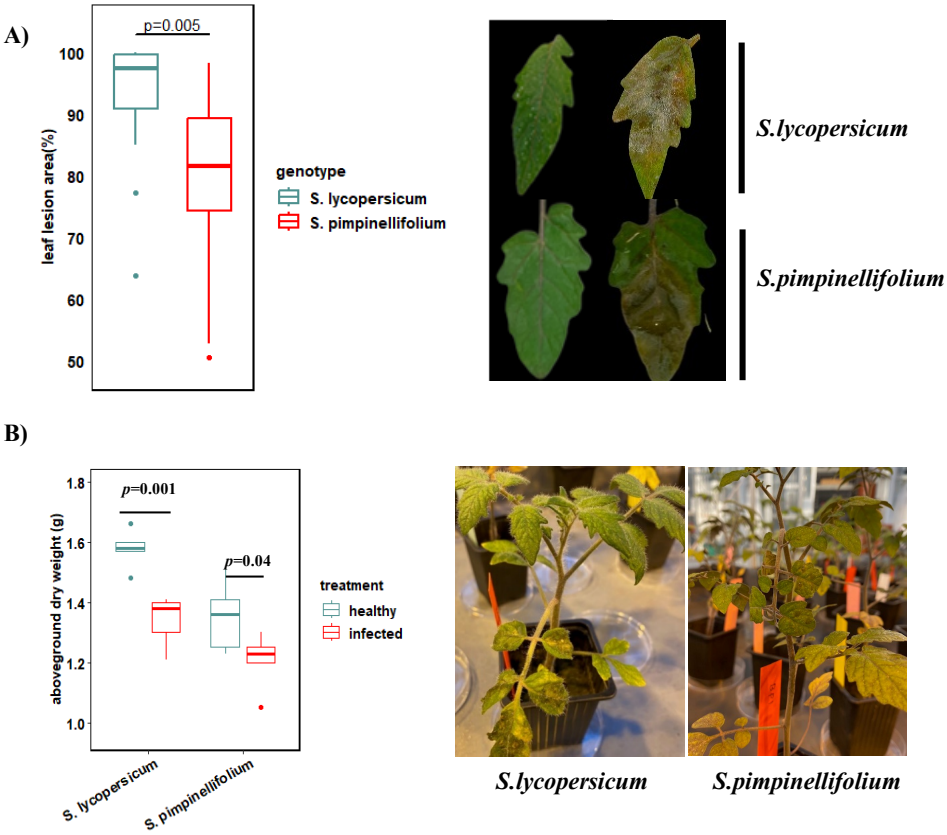
Acknowledgements

We would like to thank Stalin Sarango-Flores for his support with the sample collection and for giving the protocol for DNA extraction of rhizosphere and rhizoplane samples.

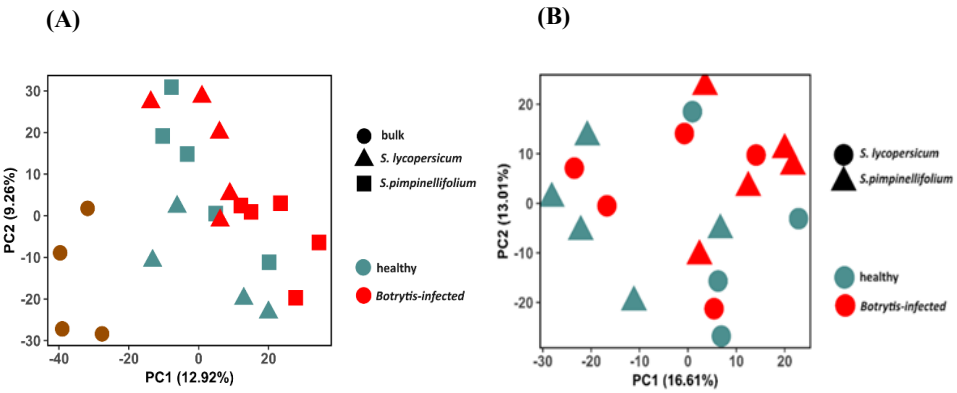
Supplementary information

Supplementary Figure S1. Non-invasive set-up to sample root/rhizosphere associated volatilome in planta. Hisorb probes were inserted into cylindrical holders made of stainless steels, and buried for approximately 5 cm deep in the soil near tomato roots to trap root-associated volatiles. The cylindrical holders have small perforated surface preventing soil contact, creating headspace and allowed passive diffusion of volatile compounds toward the probes inside the holders. Screw caps on the holders enabled secure closure to minimize contamination of volatile compounds emitted by aboveground plant tissue or by environmental backgrounds.

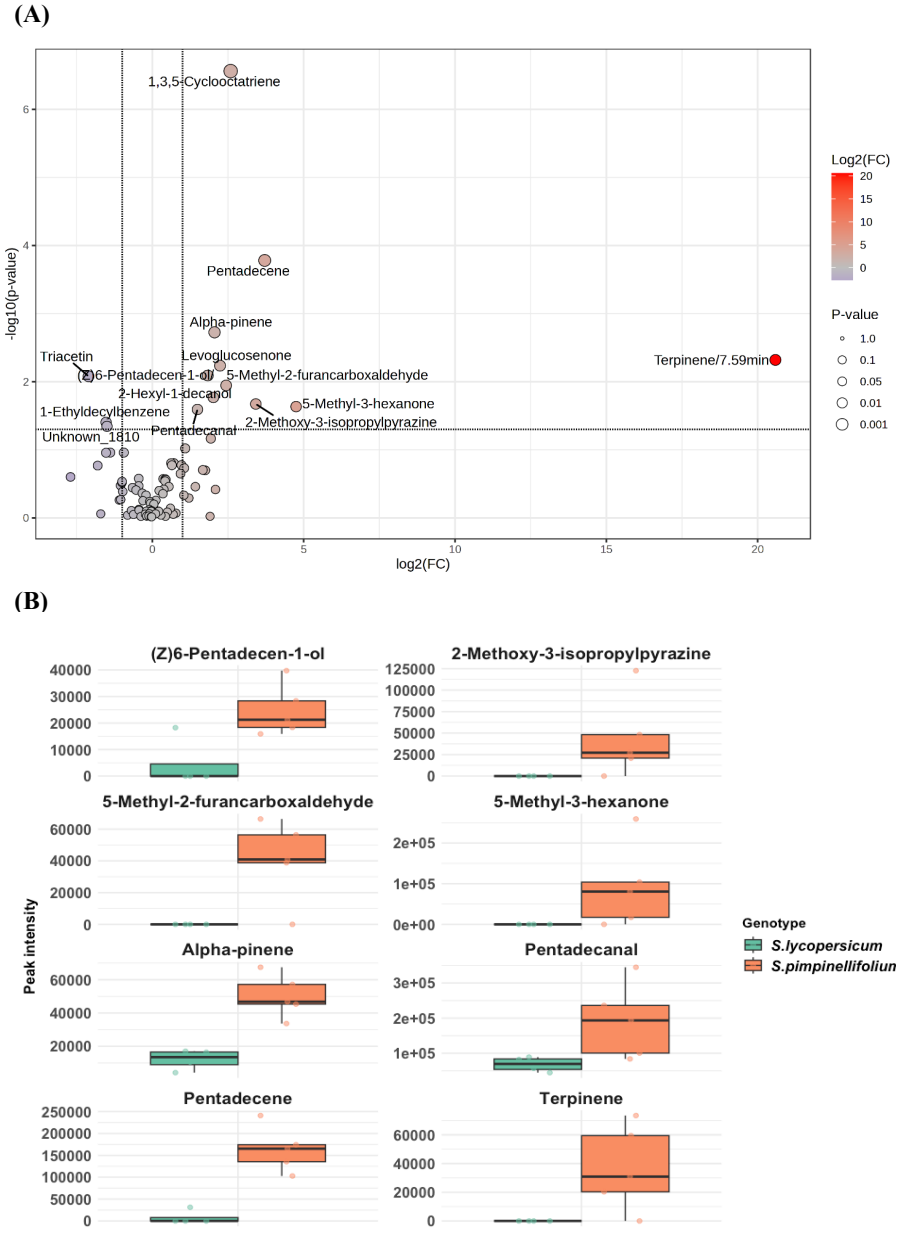
Supplementary Figure S2. Low-disturbance sampling of root volatile compounds in an *in vitro* system. Tomato plants were grown (under sterile condition) using Murashige and Skoog (MS) medium filled at one side of the two-compartment petri plate. The roots grew along the MS media inside the petri plate, while tomato shoots grew outside the petri plate. Another side of the compartment remained empty (headspace) for placing the sorbent material (HiSOrb). The two-compartment plate enabled volatile compounds emitted by roots (in one compartment) to passively diffuse toward the headspace containing HiSorb probe allowing the collection of root volatile compounds. 48h following VOC collection, tomato leaves were (un)infected with *B.cinerea*.



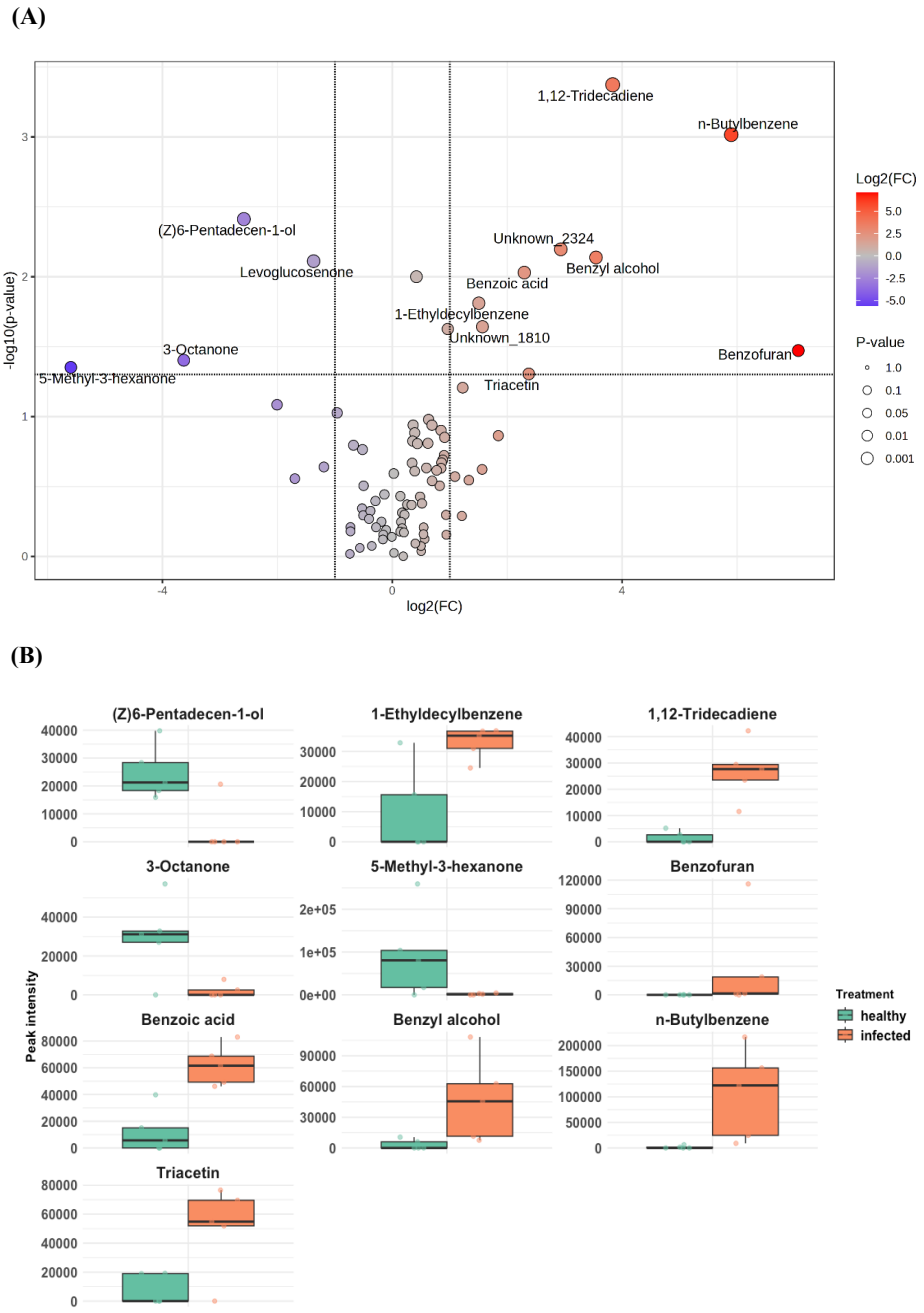
Supplementary Figure S3. Impact of the foliar infection by *B.cinerea* on the disease progression and aboveground biomass in a wild tomato *S. pimpinellifolium* and a domesticated tomato *S. lycopersicum*. **(A)** The y-axis shows lesion percentage (%) indicating the progression of disease in the leaflet of the wild and the modern tomatoes after four days post-inoculation with spore suspension of *B.cinerea* in a detached leaf bioassay. WINFOLIA™ software was utilized to determine the lesion area (discoloured tissue), to quantify the percentage (%) of infected over the non-infected leaflet areas. **(B)** The dry shoot biomass of healthy (n=5 independent biological replicates) and non -infected tomato plants (n=5 independent biological replicates) after two weeks of the fungal infection . The individual point in the bar plot refer to a single replicate in a treatment (A,B). Statistical difference values (p) were obtained following the t.test.



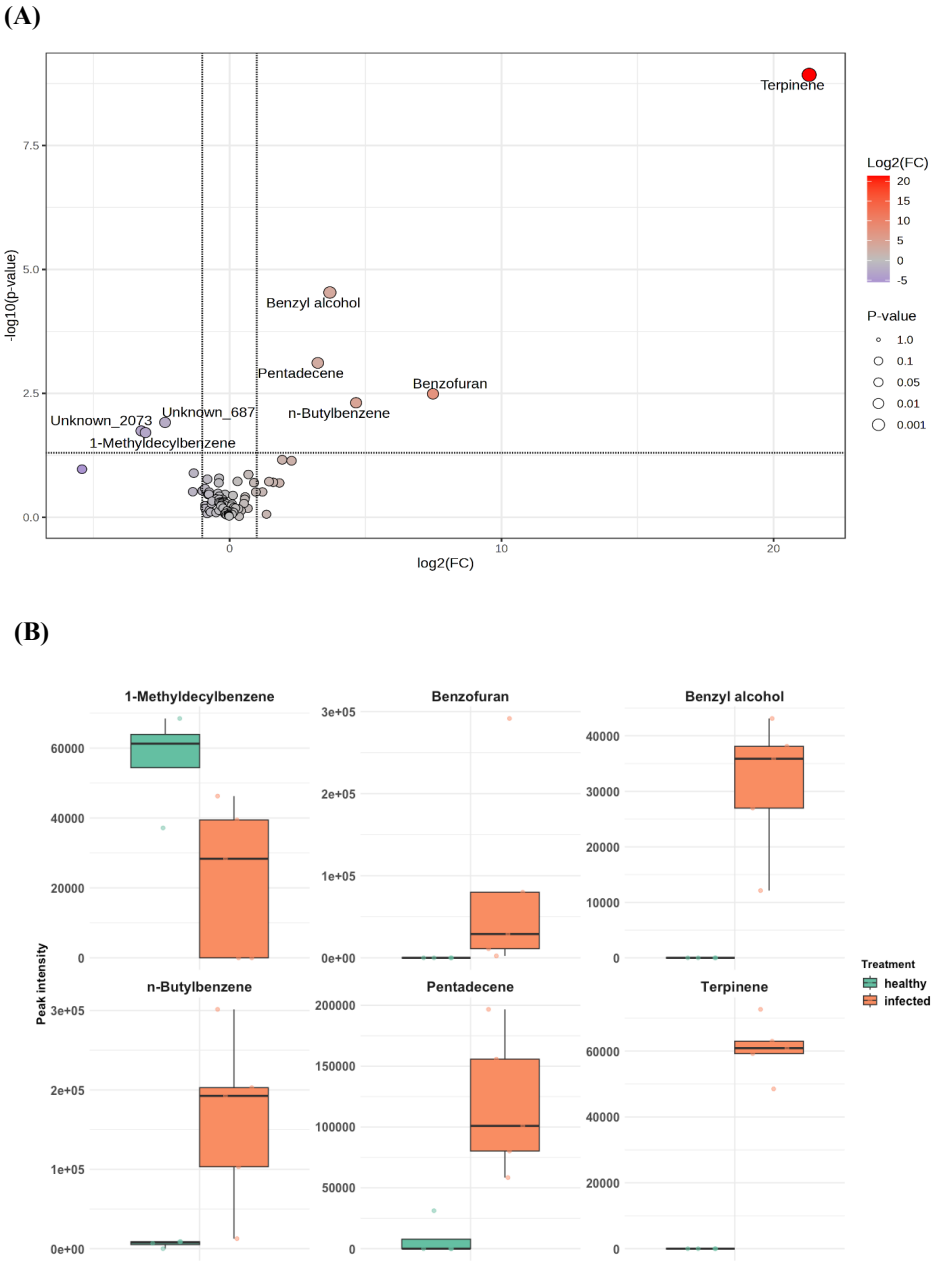
Supplementary Figure S4. (A) The principal coordinate analysis (PCoA) representing the dissimilarity (based on the Aitchison distance) of total volatilome (unsubstracted) originated from bulk (circle, brown colored) and from root/rhizosphere of *S.lycopersicum* cv. Moneymaker (triangle) and *S.pimpinellifolium* (triangle), with each genotype under infection with *B.cinerea* (*Botrytis*-infected, red colored) and without infection (healthy, green colored). **(B)** The principal coordinate analysis (PCoA) representing the dissimilarity (based on the Aitchison distance) of filtered data set (only rhizosphere-associated volatile profile) between two genotypes: *S.lycopersicum* cv. Moneymaker (circle) and *S.pimpinellifolium* (triangle), with each genotype under infection with *B.cinerea* (Botrytis-infected, red colored) and without infection (healthy, green colored)**.**



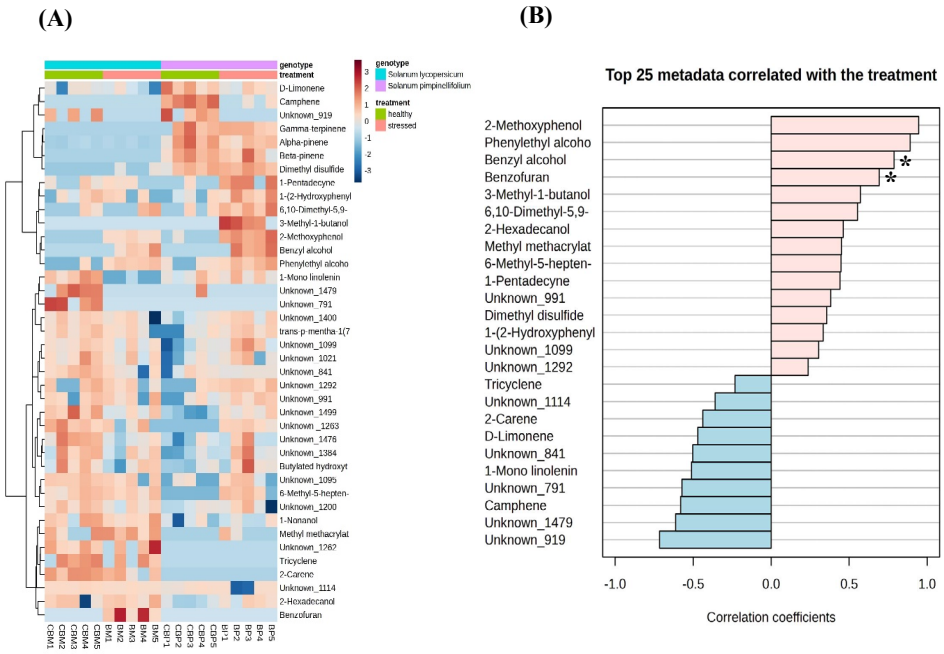
Supplementary Figure S5. (A) Volcano plot showing pairwise contrast VOCs enriched in *S.pimpinellifolium* (right side of the plot) and those that enriched in *S. lycopersicum* var. Moneymaker (left side of the plot) in the absence of *B.cinerea* infection (non-stressed condition). Alpha-pinene and terpinene (monoterpenes) were among the compounds emitted at significantly higher levels in healthy *S.pimpinellifolium* than in *S.lycopersicum* var. Moneymaker. (B) Boxplots illustrating the peak intensities (peak areas) of selected VOCs that were significantly detected in the rhizosphere of *S. pimpinellifolium* and *S. lycopersicum* in the absence of *Botrytis cinerea* (non-infected plants). Each boxplot represents the distribution and median of peak areas from five biological replicates (n = 5) per treatment



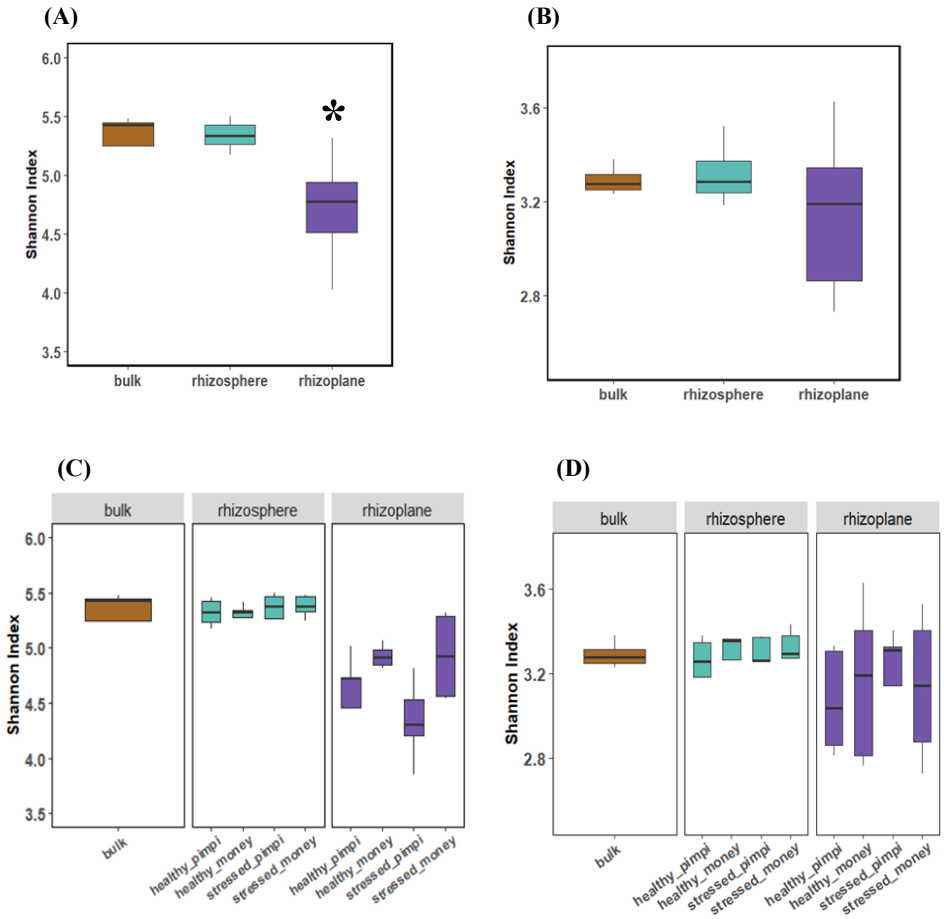
Supplementary Figure S6. (A) Volcano plot showing pairwise contrast of VOCs that are enriched (right side of the plot) and depleted (left side of the plot) in the rhizosphere of *Botrytis*-infected *S.pimpinellifolium*. (B) Boxplots illustrating the peak intensities (peak areas) of selected VOCs that were significantly detected in the rhizosphere of healthy and *Botrytis*-infected (infected) *S. pimpinellifolium*. Each boxplot represents the distribution and median of peak areas from five biological replicates (n = 5) per treatment



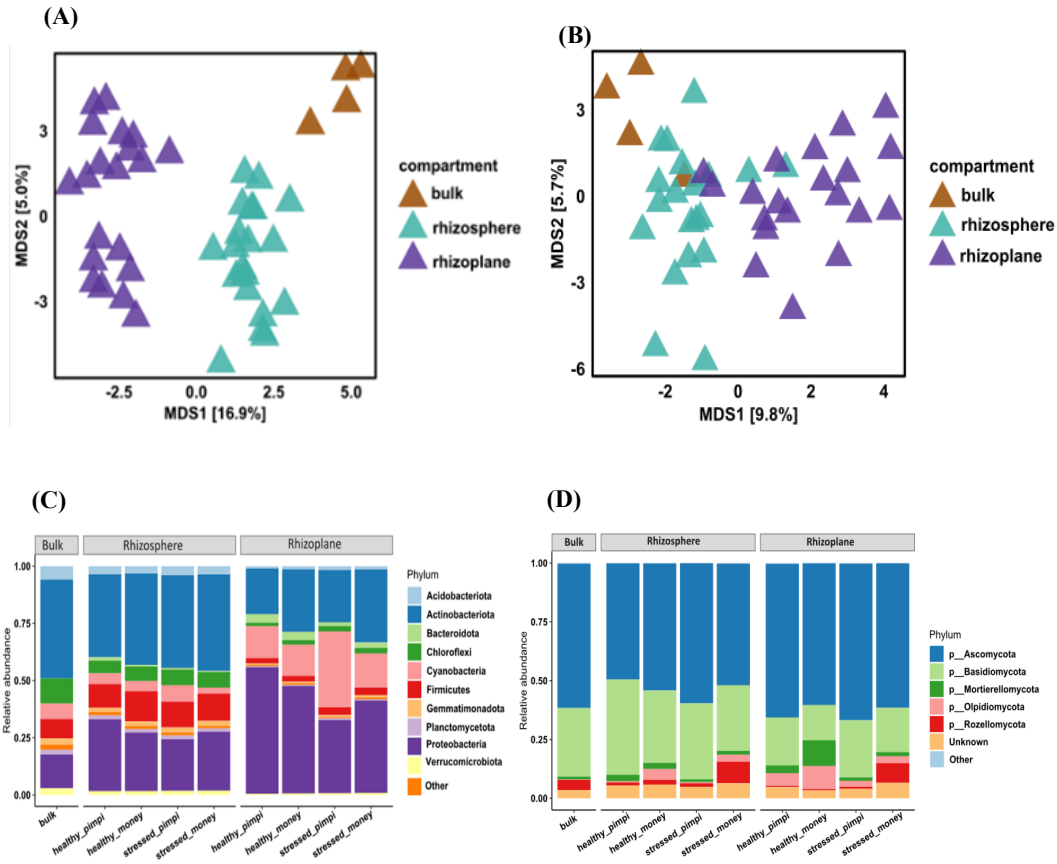
Supplementary Figure S7. (A) Volcano plot showing pairwise contrast of VOCs that are enriched (right side of the plot) and depleted (left side of the plot) in the rhizosphere of *Botrytis*-infected *S.lycopersicum* var. Moneymaker. (B) Boxplots illustrating the peak intensities (peak areas) of selected VOCs that were significantly detected in the rhizosphere of healthy and *Botrytis*-infected (infected) *S. lycopersicum* var. Moneymaker. Each boxplot represents the distribution and median of peak areas from five biological replicates (n = 5) per treatment



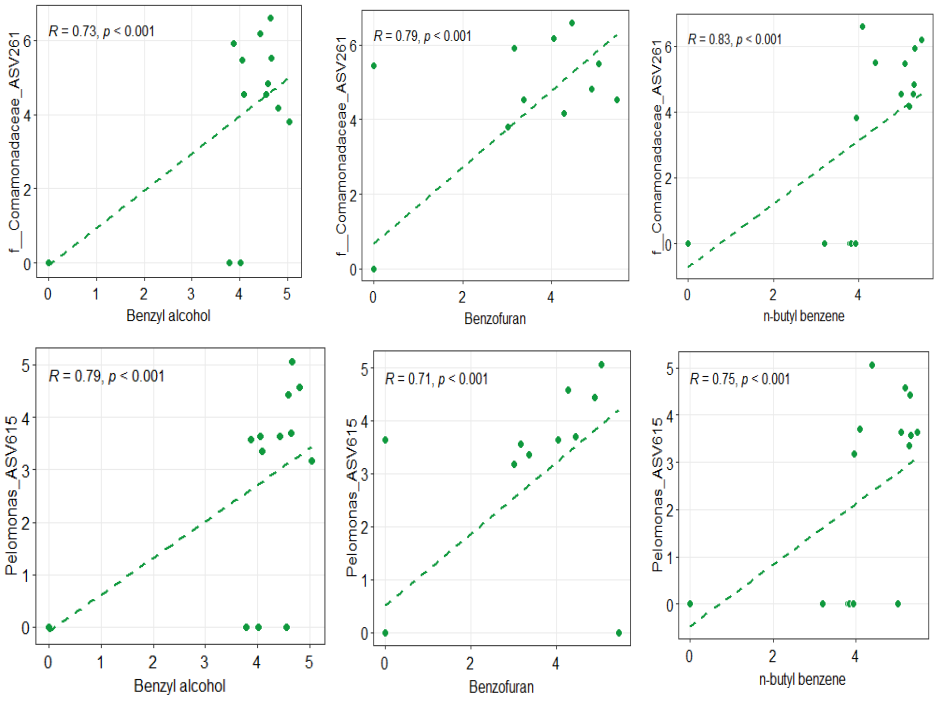
Supplementary Figure S8. (A) Root volatile profile of *S. pimpinellifolium* and *S.lycpersicum* var. Moneymaker (un)infected with *Botrytis cinerea* detected from an *in vitro* set up. A heatmap showing a comparison of normalized peak areas (log-transformed and Pareto-scaled) of selected mass features across different treatments. Mass features were selected based on their pairwise comparisons across treatments (healthy *S.lycopersicum* var. Moneymaker (CBM), healthy *S.pimpinellifolium* (CBP), *Botrytis*-infected *S.lycopersicum* var. Moneymaker (BM) and *Botrytis*-infected *S.pimpinellifolium* (BP) using multiple testing correction (FDR). Only statistically significant features (FDR<0.05) in at least one comparison are in included in the heatmap. Clustering of the samples was performed using the Euclidean distance. The intensity of normalized peak area is indicated by the color gradient legend with darker blue color suggesting a lower intensity while the darker red color indicating the higher peak area intensity. The bottom column codes refer to different treatments with numbers refer to the number of replicate for each treatment. (B) Spearman's rank correlation coefficients of the top 25 root-associated volatile compounds differentially detected upon *Botrytis cinerea* infection in tomato plants grown in the *in vitro* system. Red bars and blue bars indicate compounds with positive and negative association with the infected plants, regardless of genotype. Benzyl alcohol and benzofuran are among the volatile compounds positively associated with the stress responses of tomato plants (regardless the genotype) upon the infection of *Botrytis cinerea*. Some unidentified compounds are referred to unknowns followed by numbers indicating their calculated retention indices.



Supplementary Figure S9. Boxplots showing alpha diversity of pooled datasets for bacterial **(A)** and fungal community **(B)** as estimated by the Shannon diversity index. The boxplots were then regrouped into an individual treatment within each compartment for bacterial **(C)** and fungal community **(D)**. Except the unplanted bulk soil (bulk), the rhizosphere and rhizoplane compartments contain four conditions; uninfected *S.pimpinellifolium* (healthy_pimpi, n=5 independent biological replicates), *Botrytis*-infected *S.pimpinellifolium* (stressed_pimpi, n=5 independent biological replicates), uninfected *S.lycopersicum* var. Moneymaker (healthy_money, n=5 independent biological replicates), and *Botrytis*-infected *S.lycopersicum* var. Moneymaker (stressed_money , n=5 independent biological replicates). The star (*) symbol indicated statistical difference ($p<0.05$).



Supplementary Figure S10. Principal coordinate analysis (PCoA) of bacterial **(A)** and fungal community **(B)** at the ASV level based on the Aitchison distance for the pooled dataset, including bulk soil. Clustering was observed between the three compartments; bulk soil (brown triangle), rhizosphere (green triangle), and rhizoplane (purple triangle). The stack bar plot showing the relative abundances of most dominant bacterial **(C)** and fungal **(D)** phyla from different compartments. For each compartment, except the unplanted bulk soil(bulk), contains four treatments; uninfected *S.pimpinellifolium* (healthy_pimpi, n=5 independent biological replicates), *Botrytis*-infected *S.pimpinellifolium* (stressed_pimpi, n=5 independent biological replicates), uninfected *S.lycopersicum* var. Moneymaker (healthy_money, n=5 independent biological replicates), and *Botrytis*-infected *S.lycopersicum* var. Moneymaker (stressed_money, n=5 independent biological replicates)



Supplementary Figure S11. Scatter plots showing Spearman's correlation between rhizoplane bacterial taxa and root-associated volatile compounds. In y-axis, the relative abundances (%) of taxa enriched in rhizoplane of both tomato genotypes upon the foliar infection: f_*Comamonadaceae* (ASV261), and *Pelomonas* (ASV615) were positively correlated with stress-induced volatile compounds (normalized peak areas) in tomato plants; Benzyl alcohol, benzofuran and n-butyl benzene.

Supplementary data and table

Additional supplementary Data and Table (excel file) can be accessed via the following link: https://academic.oup.com/femsec/article/101/5/fiaf042/8116290#supplementary-data