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RESEARCH ARTICLE

Belowground pathogens rewire the phyllosphere microbiome in tomato plants

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Abstract

Background and aims Plant-associated microbiomes play a critical role in host health, yet the effects of belowground pathogens on aboveground microbiomes remain poorly understood. Since systemic plant responses can alter microbial recruitment across organs, we hypothesized that plant-pathogen interactions belowground can trigger specific shifts in the phyllosphere microbiome.

Methods Here, we used a tomato microcosm system to test whether the presence of three pathogens in soil —*Pseudomonas syringae* pv. *tomato*, *Fusarium oxysporum* f.sp. *lycopersici*, and *Alternaria alternata*— alter the plant phyllosphere bacterial community. We characterized the phyllosphere bacterial community using 16S rRNA amplicon sequencing and inferred the effect of pathogens on microbial diversity, community structure, ecological strategies, co-occurrence network robustness, and assembly processes.

Results While overall diversity remained unchanged, we observed pathogen-specific signatures in community structure, ecological strategies, and assembly processes. In addition, exposure to belowground pathogens led to a reduction in microbial network robustness, a shift from specialist to generalist and competitor taxa, and pathogen-specific taxa enriched through selection.

Conclusions Our findings suggest that plants are able to modulate their leaf microbiome in response to different belowground pathogens, even in the absence of visible symptoms. While this helps us to better understand the interactions within the holobiont, our results contribute to the development of microbiome-based diagnostic tools, and the targeted design of beneficial microbial consortia for plant protection.

Keywords Microbial community · *Pseudomonas syringae* pv. *tomato* · *Fusarium oxysporum* f.sp. *lycopersici* · *Alternaria alternata* · 16S rRNA · Amplicon sequencing

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Introduction

Plant microbiomes are fundamental in regulating plant health and productivity in both agricultural and natural environments (Trivedi et al. 2020; Malacrinò et al. 2022; Compant et al. 2025). The microbial component of the holobiont plays, indeed, a key role in helping plants to counteract potential pathogens (Turner et al. 2013; Liu et al. 2021b; Bordenstein et al. 2024). Several studies have shown that the structure and function of microbiomes are altered when plants are attacked by a pathogen (Gao et al. 2021; Arnault et al. 2023), mainly resulting from the recruitment of beneficial microorganisms (Spooren et al. 2024), although such changes can also be consequential to the disease (e.g., increases in saprophytes or secondary pathogens). Changes in microbiome composition and function can, in turn, have direct negative effects on the pathogen (e.g., reduced niche space, antibiosis), or they can trigger resistance mechanisms in the host plant (Compant et al. 2025), ultimately reducing the ability of pathogens to cause disease. Yet, we still know little about how microbiomes assemble when the host experiences biotic stress, which is key for planning microbiome management strategies to maximize plant health, but also for providing tools for the early detection of potential pathogens.

The outcome of plant-microbiome interactions on pathogens are well studied within the same compartment (e.g., rhizosphere and soil-borne pathogens), both belowground (Ping et al. 2024; Li et al. 2024; Duret et al. 2024), and aboveground (Sohrabi et al. 2023). On the other hand, microbial communities inhabiting aboveground and belowground compartments are not disconnected, but variation in one compartment (e.g., belowground) in response to an external input (e.g., biotic stress) reflects in the composition of microbiomes in the other compartment (e.g., aboveground). For example, previous studies have shown that the diversity and composition of the phyllosphere microbiome is strongly driven by the belowground microbiome and, at the same time, leaf herbivory can influence the belowground microbiome composition (Wolfgang et al. 2023; Malacrinò and Bennett 2024). Similarly, only few other studies found that the attack of a pathogen in a compartment influences the microbial composition in another compartment. This has been observed, for example, in leaves of *Agathis australis* grown on *Phytophthora agathidicida*-infected soils (Murray et al. 2024), and in leaves of soybean plants infected by *Phytophthora sojae* and *Septoria glycines* (Díaz-Cruz and Cassone 2022). However, we have only little evidence on the impact of a pathogen on the microbiome of an organ (or compartment) other than the one they attack, and the consequences for microbiome assembly.

Stressed plants activate metabolic pathways that lead to the production of exudates and volatile organic compounds used to recruit beneficial microorganisms (Raza et al. 2021; Yang et al. 2025). This response is not solely triggered locally in response to stress, but it is systemic in different plant organs (Vlot et al. 2021). Indeed, belowground stressors (e.g., drought, pathogen, herbivory) influence the chemistry of plant microbiomes aboveground, ultimately altering the composition of those microbial communities (Hou et al. 2021). This mechanism might be a key component of the recruitment of beneficial microorganisms, and it can be exploited to design microbiome-based strategies to reduce the impact of pathogens on plant health. We might also expect that different pathogens can trigger different physiological responses in the plants they interact with, which might lead to pathogen-specific responses in the plant metabolism. To the best of our knowledge, this has not been directly tested outside a few meta-analyses (Jiang et al. 2017; Wang et al. 2022), so it is still unclear whether we can detect pathogen-specific signatures in plant microbiomes, as it has been reported for other biotic (Malacrinò et al. 2021) and abiotic (Vescio et al. 2021) stressors. This pathogen-specific response in the plant microbiome can be key in identifying and assembling microbial consortia with high efficacy against specific pathogens, but it can be also used for the early detection of biotic stressors.

The goal of this study is to investigate whether the presence of belowground pathogens generate specific changes in the phyllosphere microbiome. We set up a microcosm system using tomato as a model plant to test the response of the bacterial leaf microbiome to the presence of three different pathogens inoculated belowground (*Pseudomonas syringae* pv. *tomato* – PSTO, *Fusarium oxysporum* f. sp. *lycopersici* – FOL, and *Alternaria alternata* – ALT). We hypothesized that infections in the belowground compartment can affect the structure and assembly of the phyllosphere microbial communities, and that

different pathogens would generate pathogen-specific changes in the leaf microbiome.

Material and methods

Microcosm experiment

The microcosm experiment included 30 plants divided in 3 groups of 10 plants, each assigned to a different pathogen treatment, and a control group of 15 plants, for a total of 45 plants. Plants were then inoculated with three different pathogens (two fungi and one bacterium): *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Alternaria alternata* (ALT), and *Pseudomonas syringae* pv. *tomato* DC3000 (PSTO).

Seeds of tomato (*Solanum lycopersicum* L.) cv. 'Money Maker' were germinated on sterilized peat moss (autoclaved twice at 121 °C for 3 h). Seedlings were regularly monitored to ensure healthy growth and were watered as needed, before being transplanted into larger pots (H 12 cm × 10 cm base Ø, 1L volume) filled with a 2:1 mixture of sand and soil. A full-spectrum artificial lighting system was used to simulate a 16:8 light:dark cycle. Approximately two months after transplanting, all the plants were treated with 10 mL of sterile Hoagland's nutrient solution (0.5X). After approximately two weeks, plants were inoculated with pathogens as described below.

Pure cultures of FOL and ALT isolates were grown on PDA plates at 24 °C for 7 days. For each fungal isolate, conidial suspensions were collected by scraping conidia from the fully grown mycelium using a sterile spatula and transferring them to 15 mL of sterile PBS 1X. The conidial suspensions were adjusted to approximately 10^6 conidia/mL utilizing a Thoma cell counting chamber, and resuspended in PBS 1X (Zahoor et al. 2022; Devi et al. 2022). The PSTO inoculum was prepared by transferring 100 µL of PSTO culture stock (40% glycerol stored at −80 °C) into four flasks containing 200 mL of LB broth, and incubating them for 24 h at 28 °C with gentle shaking (120 rpm). After that, the bacterial suspension was centrifuged at 3,200 rcf for 15 min to pellet bacterial cells, which were then resuspended in PBS 1X after discarding the supernatant. The bacterial concentration was estimated by measuring the optical density (OD600) with an EasyVis spectrophotometer (Mettler Toledo), and adjusted it to a value of 0.2 (corresponding to a concentration of 10^8 CFU/mL) (Jacob et al. 2017) with PBS 1X. Inoculums (10 mL) were applied directly to the crown of each plant. Control plants were inoculated with 10 mL of sterile PBS.

After inoculation, plants were randomized within the greenhouse. Ten days after exposure to pathogens, shoot samples were collected and stored at −80 °C. This timeframe allowed for plant-pathogen interactions without the development of any significant symptom, which would mask the actual phyllosphere microbiome response to the presence of pathogens belowground. Subsequently, samples were freeze-dried and then powdered using a bead mill homogenizer 10 min at 30 Hz with 2–3 glass beads (4 mm Ø).

DNA extraction, library preparation and sequencing

DNA extraction was performed with the DNeasy PowerMax Soil Kit (Qiagen), according to the manufacturer's instructions. The DNA concentration and quality were estimated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Bacterial communities were characterized by amplifying a portion of the 16S rRNA gene (V3-V4) through PCR using the primers 515F/806R (Caporaso et al. 2012) as previously reported (Malacrinò and Bennett 2024). Besides PCR negative controls, amplifications were also performed on negative controls and non-template controls, in which the sample was replaced with nuclease-free water to detect potential contamination from instruments, reagents, or consumables used during DNA extraction. Libraries were quantified using a Qubit fluorometer (Thermo Fisher Scientific), pooled together at equimolar ratios, and sequenced on an Illumina NovaSeq 6000 platform (Illumina, CA, USA).

Data processing and analysis

Reads were processed with the *nf-core/ampliseq* v2.7.1 pipeline (Di Tommaso et al. 2017; Ewels et al. 2020; Straub et al. 2020) for quality control, adaptor/primer trimming, error correction, clustering of ASVs, chimera removal, and taxonomic identification using the SILVA database v138 (Quast et al. 2013). Amplicon Sequence Variants (ASV) sequences were aligned using *MAFFT* v7.525 (Katoh et al. 2002) and used to build a phylogenetic tree using *FastTree*

v2.1.11 (Price et al. 2009). All subsequent data processing and analysis was performed in *R* v4.4.1 (R Core Team 2022). The ASV table, taxonomic identification table, metadata, and phylogenetic tree were merged using *phyloseq* v1.48 (McMurdie and Holmes 2013) before subsequent analyses. Likely contaminants were removed using the package *decontam* v1.24 (Davis et al. 2018) and the data from negative control PCRs. Then, singletons and samples with less than 10,000 reads were discarded from the dataset. This resulted in a reduction of replicates for each group ($n=7$ for ALT, $n=6$ for FUS, $n=7$ for PSTO, and $n=13$ for control), but still maintaining a minimum level of replication to perform downstream analyses, and a depth of $57{,}367.52 \pm 6{,}954.32$ sequences/sample clustering into 7,167 ASVs.

Diversity metrics were calculated using the packages *microbiome* v1.26 (https://github.com/microbiome/microbiome/) and *picante* v1.8.2 (Kembel et al. 2010), and tested by fitting individual linear models using the package *lme4* v1.1 (Bates et al. 2015) using the variable "treatment group" (PSTO, ALT, FLO, CTRL) as fixed factor, and post-hoc contrasts were extracted using the package *emmeans* v1.10.7 (Lenth 2022). Differences in microbiome structure were tested using PERMANOVA (999 permutations) and visualized using a NMDS, both performed on unweighted and weighted UniFrac distance matrices using the package *vegan* v2.6 (Dixon 2003). PERMANOVA post-hoc contrasts were performed using the package *RVAideMemoire* v0.9 (Hervé 2022). The LEfSe (Linear discriminant analysis Effect Size) analysis (Segata et al. 2011) was performed using the package *microeco* v1.13 (Liu et al. 2021a), keeping taxa with an adjusted p-value >0.05 and an LDA score >3. Networks were built and analyzed using the package *microeco* v1.13 (Liu et al. 2021a). Each ASV was classified into "specialist" or "generalist" using the package *EcolUtils* v0.1 (https://github.com/GuillemSalazar/EcolUtils), and into "competitor", "stress tolerant", and "ruderal" through a null-modeling approach using the package *MicroEcoTools* v0.4 (Neshat et al. 2025). The relative contribution of different ecological processes in the assembly of the phyllosphere microbiomes for each treatment group was estimated using the package *iCAMP* v1.5.12 (Ning et al. 2020). Fit to the Sloan null-model was performed for each treatment group using the package *tyRa* v0.1 (https://github.com/DanielSprockett/tyRa). The random forest classification was performed using the package *randomForest* v4.7 (Breiman et al. 2002), and the relative abundance of the top 1% taxa contributing to the variation in microbiome composition was visualized by plotting the CLR (centered log-ratio) transformed data from the package *mia* v1.12 (https://github.com/microbiome/mia).

Results

Phyllosphere bacterial communities were mostly dominated by taxa within the phyla Proteobacteria and Actinobacteriota (Fig. 1a-b). While no significant effect of the exposure to belowground pathogens was detected on the diversity of the phyllosphere bacterial communities (Tab.S1), multivariate analyses suggested differences between treatment groups (Fig. 1c-d). Post-hoc contrasts clarified that while we observed pairwise differences when using a UniFrac distance matrix (ASV presence/absence; Tab**.** S2), no significant difference between treatments was identified when using a weighted UniFrac matrix (incorporating relative abundance; Tab. S2). Thus, differences in overall phyllosphere community structure might be due to presence/absence of specific ASV in each treatment group, rather than variation in their relative abundance. In addition, the LEfSe analysis identified 60 ASVs contributing to unique microbiome signatures in response to the exposure to pathogens belowground (Fig. 1e).

We also tested the influence of pathogens belowground on the stability of the microbial network in the phyllosphere. The co-occurrence network of control plants was more highly connected compared to all the other groups (Fig. 2a-d). We tested the vulnerability of these networks as a decrease in network connectivity when removing either edges or nodes. When removing elements randomly, we observed a linear decrease in connectivity for all groups, with networks from control plants always having a higher connectivity than inoculated plants (Fig. 2a-b). When removing edges (i.e., connections between taxa) by their weight (i.e., connection strength) within the network, we observed that all groups exposed to pathogens experience a steady decrease in connectivity, while the connectivity within the control group network remains unchanged even when 25% of the most important edges were removed, and decreasing

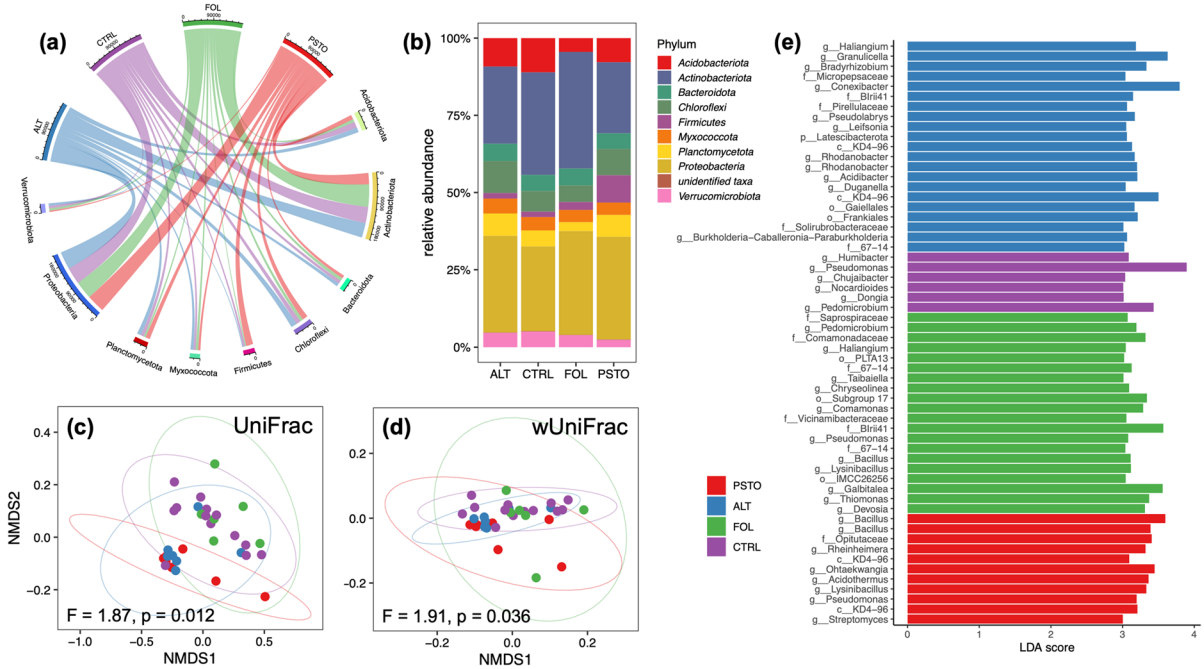

Fig. 1 (**a**) Chord plot and (**b**) barplot showing the relative abundance of bacterial phyla (relative abundance > 1%) for each treatment group (*Pseudomonas syringae* pv. *tomato* – PSTO, *Fusarium oxysporum* f. sp. *lycopersici* – FOL, *Alternaria alternata* – ALT, Control – CTRL). Non-metric multidimensional scaling (NMDS) plots built on UniFrac (**c**) and weighted UniFrac (**d**) distance matrices (each plot includes results from PERMANOVA). (**e**) LEfSe bar chart showing the ASVs (identified at the highest confidence taxonomic level) with the strongest association to each of the treatment groups, and the lengths of the bars (LDA score) indicate the influence of that ASV in discriminating between treatments

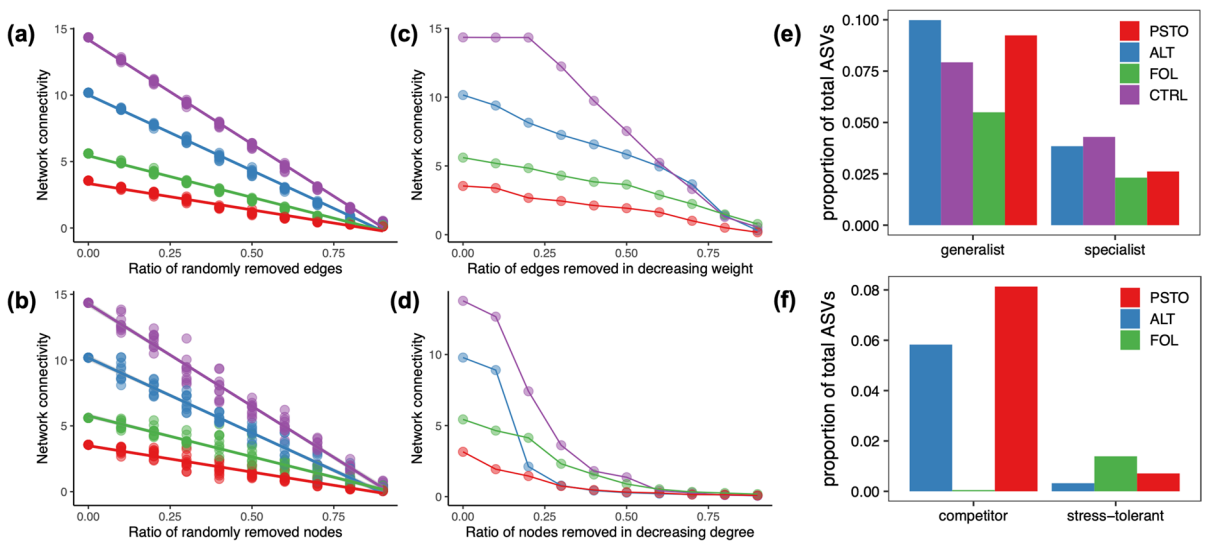

Fig. 2 Network connectivity as measure of network robustness as response to removing random edges (**a**) or nodes (**b**), and to removing edges in decreasing order of weight (**c**) or nodes in decreasing degree (**d**) for each treatment group (*Pseudomonas syringae* pv. *tomato* – PSTO, *Fusarium oxysporum* f. sp. *lycopersici* – FOL, *Alternaria alternata* – ALT, Control – CTRL). Proportion of generalist and specialist taxa in plants exposed to the different treatment groups (**e**). Proportion of competitor and stress-tolerant taxa (calculated by comparison towards the control group) in plants exposed to the different pathogens (**f**)

thereafter (Fig. 2c). Similarly, the microbial network connectivity quickly decreases when removing nodes (i.e., taxa) with the highest degree (i.e., number of connections with other nodes), while this collapse was quicker in plants exposed to pathogens compared to control plants (Fig. 2d). We also found that plants exposed to ALT and PSTO had the highest proportion of generalist taxa (i.e., with wide habitat preferences and heightened resistance to disturbance), those exposed to FOL had the lowest proportion of generalists, while control plants had the highest proportion of specialist taxa (i.e., narrow habitat preferences and resistance to disturbance) (Fig. 2e). We also found that plants exposed to ALT and PSTO had higher proportion of competitor taxa (i.e., taxa that maximize resource acquisition in consistently productive niches) compared to FOL, while FOL showed the highest proportion of stress tolerant taxa (i.e., taxa that maintain metabolic performance in unproductive niches) (Fig. 2f). No taxa was identified as "ruderal".

We then tested the influence of the exposure to pathogens belowground on the assembly processes of the phyllosphere microbiome. We found that stochastic processes (i.e., dispersal and drift) dominated the assembly of those microbial communities (Fig.3a), with a higher contribution of homogenizing dispersal in plants exposed to ALT compared to those exposed to FOL and the control group (Fig. 3a, Tab.S3). However, selection processes contributed to ~ 20% of the assembly processes, so using a null-model analysis we identified the taxa that did not follow a random assembly process (Fig. 3b-e), and found that most of those ASVs were unique to each treatment (Fig. 3f). Considering the high total number of ASVs ($n = 874$)

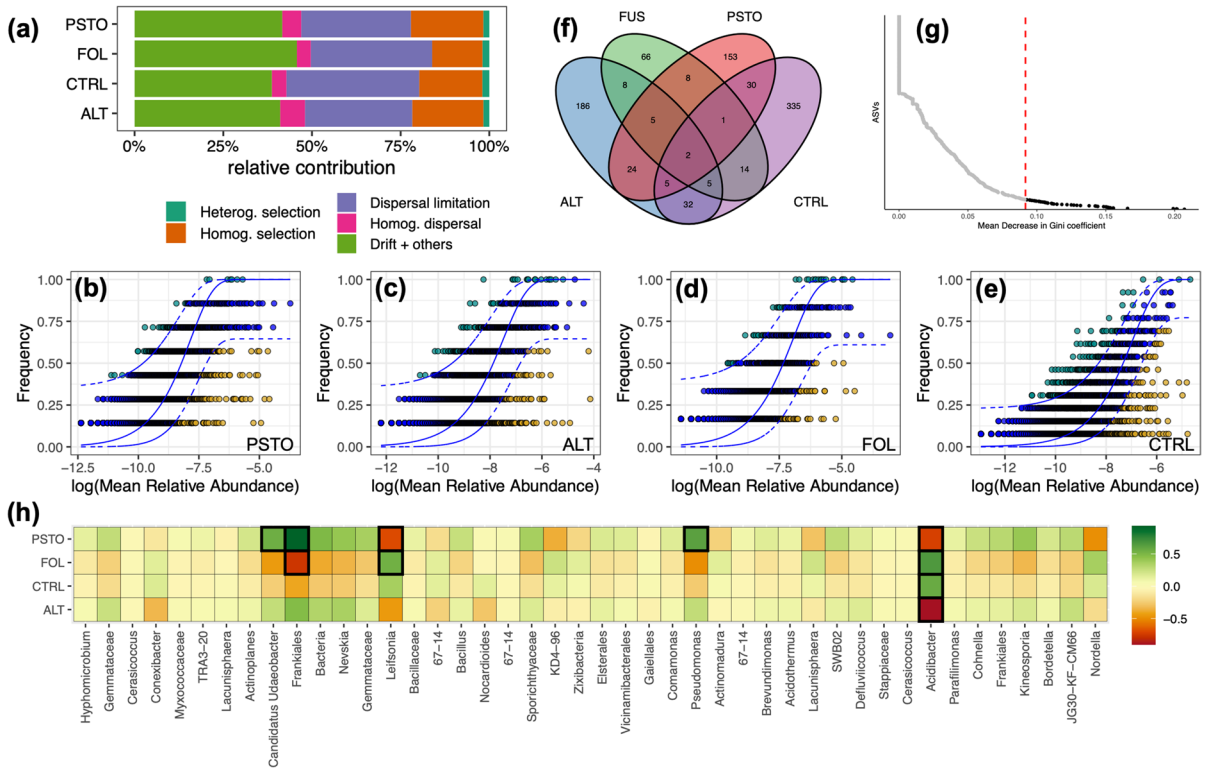

Fig. 3 (**a**) Null-model analysis showing the relative contribution of different processes in the assembly of phyllosphere bacterial communities for each treatment group (*Pseudomonas syringae* pv. *tomato* – PSTO, *Fusarium oxysporum* f. sp. *lycopersici* – FOL, *Alternaria alternata* – ALT, Control – CTRL). Fit to sloan null-model of bacterial taxa for each treatment: PSTO (**b**), ALT (**c**), FOL (**d**), control (**e**). (**f**) Venn diagram comparing the identity of the ASVs identified to not follow a null assembly model. (**g**) Random forest analysis, with the y-axis ranking the ASVs by their importance (Mean Decrease in Gini coefficient) for the group classification, with top 1% ASVs identified on the right of the vertical dashed line. (**h**) CLR (centered log-ratio) transformed abundance of taxa identified using the random forest analysis for each treatment group (boxes with thick border had a |CLR|> 0.5)

identified to not follow a stochastic assembly process (i.e., they were subjected to selection), we used a random forest algorithm to identify the top 1% ASVs explaining the variation between groups (Fig. 3 g-h). When looking at the variation of these ASV within the microbial community of each treatment, we found unique signature in the microbial community of each treatment, including some taxa (e.g., *Acidibacter*, *Pseudomonas*, *Leifsonia*, and *Candidatus* Udaeobacter) which had a stronger response of each treatment compared to the others (Fig. 3 h)

Discussion

In this study, we found that different pathogens belowground produce variation in the phyllosphere bacterial communities in tomato plants. While we found no changes in microbial diversity, and little changes in microbiome structure, we also found pathogen-specific signatures in the assembly of the phyllosphere microbiome and in the robustness of microbial networks. We also identified microbial markers associated with specific pathogens.

We found that pathogens inoculated belowground influence the structure of aboveground microbial communities and their assembly. To the best of our knowledge, no previous study investigated the effect of pathogens on plant microbiota across compartments, but previous studies found that the leaf microbial communities are altered in presence of pathogens within the phyllosphere in citrus (Li et al. 2022), cacao (Schmidt et al. 2023), rice (Dastogeer et al. 2022), kiwifruit (Purahong et al. 2018), sorghum (Masenya et al. 2021), tobacco (Huang et al. 2021), wheat (Liu et al. 2023), beech (Ewing et al. 2021), and others. Previous studies also found that pathogens have little or no influence on the leaf microbial diversity, but they alter the microbiota structure (Ewing et al. 2021; Masenya et al. 2021; Dastogeer et al. 2022), which aligns with our observations although in our case pathogens do not directly interact with the phyllosphere microbiota. The phyllosphere microbial communities, while shaped by host plant and soil, are also under the influence of the microbiomes of the surrounding environment (Laforest-Lapointe et al. 2016), which can serve as a continuous source of inoculum, for example from airborne communities and neighboring plants (Maignien et al. 2014; Meyer et al. 2022). This can buffer the variation in microbial diversity due to stressful conditions. This finds also support in stochastic factors being the major driver behind the assembly of bacterial communities in all our treatment groups, reflecting that a wide portion of the phyllosphere microbiome is not under ecological selection, and likely randomly assembled from the environment. While the dominance of deterministic and stochastic processes can vary between plant species and environmental conditions (Mohamed et al. 2024), individual taxa within a microbiome might assemble stochastically or be subjected to selection. Indeed, the portion of the phyllosphere bacterial community subjected to selection (i.e., taxa that do not follow a random assembly process) was unique for each of our treatment groups (i.e., pathogens inoculated belowground). This observation might be the result of pathogen-specific cry for help strategy to enrich beneficial microorganisms, which is commonly observed in the rhizosphere, but it has been reported in the phyllosphere (Zhang et al. 2023; Zhao et al. 2025). Indeed, we found ASVs enriched in plants exposed to pathogens identified within bacterial genera with potential beneficial effects on plant growth and health, including *Leifsonia* (Nordstedt et al. 2021), *Bacillus* (Borriss 2015), *Brevundimonas* (Wang et al. 2023), and others, although the specific recruitment of each taxa varied according to the pathogen plants were exposed to. Taken together, these results suggest that plants can deploy pathogen-specific strategies to modulate their overall microbial community, and recruit beneficial organisms that can help them to counteract specific pathogens.

The presence of pathogens belowground induced shifts in microbial ecological strategies. We observed an increase in generalist and competitor taxa in plants exposed to ALT and PSTO, alongside a decrease in specialist taxa compared to control plants. Also, plants exposed to FOL had a higher proportion of stress-tolerant bacterial taxa. This suggests that biotic stressors may trigger the selection for microbial traits that enhance survival in disturbed or fluctuating environments, and further support the idea that different pathogens elicit distinct effects on microbial assembly. These shifts could also have important consequences for microbiome stability and resilience (Laine and Leino 2025) and, thus, its capacity to support plant health. A reduction in specialist taxa and an increase in generalists may lower functional

redundancy and destabilize beneficial plant–microbe interactions (Philippot et al. 2021; Laine and Leino 2025). Indeed, the phyllosphere bacterial communities of plants inoculated with pathogens exhibited a less robust microbial network compared to control plants. This decrease in network robustness may compromise functions critical to plant health. This, together with the reduction of specialist taxa and the higher proportion of generalists could limit the ability of microbial communities to support plants during stress episodes (Chen et al. 2024; Laine and Leino 2025), and perhaps even lead to the assembly of dysbiotic microbial communities (i.e., communities with detrimental effects to plant health, Arnault et al. 2023).

While our results contribute to the understanding of how plant-associated microbial communities assemble under stress, our observations are also important for designing the next generation of diagnostic tools and microbiome-based protection strategies. Metagenomics tools are boosting our ability to identify known agents of plant diseases without isolating them (Abdelrazek et al. 2025), although they require a reference dataset that includes either the a barcode gene of the pathogen (e.g., ITS rRNA) or its full genome. At the same time, we are now dealing with new threats to plant health due to the alteration of plant–microbe interactions resulting in detrimental effects for the host plant (Berg and Cernava 2022; Trivedi et al. 2022), the (re)emergence of pathogens (Corredor-Moreno and Saunders 2020; Fones et al. 2020), and the development of complex diseases and syndromes (Boixel et al. 2024). However, metagenomics tools might fail in detecting these threats as, often, they do not follow the one pathogen–one disease paradigm (Bettenfeld et al. 2020) and, thus, we lack references to query sequencing data to. Thus, it is important that we identify alternative routes to detect signatures of biotic stress in the structure and assembly of plant microbiomes. Our results show pathogen-specific signatures in the assembly of phyllosphere microbiomes, even if plants were exposed to belowground pathogens. While this needs to be validated in a wider set of samples, host plant genotypes, and soil microbial community composition, it might represent an alternative way to detect complex diseases and syndromes. Similarly, the identification of recruited beneficial microorganisms can aid the design of small consortia of microorganisms to improve plant growth and health (Brisson et al. 2019; Martins et al. 2023; Yan et al. 2024; Kang et al. 2024).

Conclusions

In this study, we show that the aboveground microbiome assembly responds to the presence of belowground pathogens, and that a portion of the phyllosphere microbiome is selected differently according to the pathogen plants are exposed to. While this framework needs further validation, we can use this approach to develop new tools to identify current and new threats to plant health, and to design microbiome-based solutions for sustainable crop protection. This is particularly important considering that plant–microbe interactions play an essential role in the holobiont health and productivity (Vandenkoornhuyse et al. 2015; Sánchez-Cañizares et al. 2017; Bai et al. 2022; Berg et al. 2024; Beattie et al. 2024; Bordenstein et al. 2024).

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Author contributions Conceptualization: EF, LS, AM; Methodology: EF, MMA, LS, AM; Investigation: EF, MMA, MC; Visualization: EF, AM; Writing-original draft: EF, AM; Writing-review and editing: all co-authors.

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Data availability Data is available on NCBI SRA under BioProject PRJNA1297628.

Declarations

Competing interests All authors declare no financial or non-financial competing interests.

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