Fusarium Oxysporum Disrupts Microbiome-Metabolome Networks in *Arabidopsis Thaliana* Roots

Enoch Narh Kudjorjie

Aarhus University: Aarhus Universitet

Kourosh Hooshmand

Aarhus University: Aarhus Universitet

Rumakanta Sapkota

Aarhus University: Aarhus Universitet

Inge S. Fomsgaard

Aarhus University: Aarhus Universitet

Mogens Nicolaisen (✉ mn@agro.au.dk)

Aarhus Universitet <https://orcid.org/0000-0002-0407-2488>

Research

Keywords: plant pathogens, root microbiome, glucosinolates, phenylpropanoids, phytohormones, microbial co-occurrence networks, plant differential resistance

Posted Date: September 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-845639/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Although it is well established that plant metabolomes mediate microbiome assembly, the question of how metabolome-microbiome interactions may prevent pathogen invasion remains to be answered. To address this question, we studied microbiome and metabolome profiles of two *Arabidopsis thaliana* accessions, Columbia-0 (Col-0) and Landsberg erecta (Ler-0) with differential resistance profiles to the fungal pathogen *Fusarium oxysporum* f.sp. *mathioli* (FOM). We used amplicon sequencing to characterize bacterial (16S) and fungal (ITS2) communities, and we used targeted metabolite analysis across 5 stages of FOM host progression.

Results

We found that microbiome and metabolome profiles were markedly altered in FOM-inoculated and non-inoculated samples of resistant Col-0 and susceptible Ler-0. Co-occurrence network analysis revealed robust microbial networks in the resistant Col-0 compared to the susceptible Ler-0, during FOM infection. Specific metabolites and microbial OTUs (including indicator and hub OTUs) correlated in both non-inoculated and inoculated Col-0 and Ler-0. The glucosinolates 4-hydroxyglucobrassicin, neoglucobrassicin and indole-3 carbinol, but also phenolic compounds were active in structuring the *A. thaliana*-microbiome.

Conclusions

Our results highlight the interactive effects of host resistance and its associated microbiota on *Fusarium* infection and progression. These findings shed significant insights into plant inter-omics dynamics during pathogen invasion and could possibly facilitate the exploitation of microbiomes for plant disease control.

Background

Plant-microbe interactions are of great agronomic importance due to their beneficial or detrimental effects on plant growth and productivity. For an improved holistic understanding of these interactions, many recent studies have attempted to unravel the complex interactions occurring between plants and their associated microbiota. Together with environmental factors, plant host traits including host-synthesized metabolites mediate the assembly of the plant microbiota [1, 2]. Those metabolites serve as a carbon and energy source, or as chemical signaling molecules that ultimately mediate plant-microbe interactions [3].

Strong evidence already exists on how plant metabolites affect host-associated microbiomes [4–6], and coumarins, flavonoids, glucosinolates, benzoxazinoids and phytohormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene play profound roles in modulating the microbiomes [5, 7–10]. During

pathogen invasion, host metabolic reconfiguration mostly follows immune signaling events triggered by the detection of pathogenic signatures [11]. For instance, changes in the metabolome, including phenolic and indolic compounds, were observed in the leaves of *Arabidopsis thaliana* (hereafter, *Arabidopsis*) after infection with *Pseudomonas syringae* [12], and changes in the rhizosphere microbiomes were triggered by alterations in plant exudation patterns [13]. In another study, the fungal pathogen *Zymoseptoria tritici* caused systemic shifts in the wheat metabolome followed by changes in the microbiome composition [14].

The composition and diversity of the host-associated microbiota determine community invasion resistance [15]. Highly complex and diverse microbial communities are characterized by a web of cooperative and antagonistic interactions among microbial members [16, 17] that have been shown to be more resistant to pathogenic perturbations [18, 19]. The host-associated microbiota suppresses fungal or bacterial pathogen invasion by directly antagonizing pathogens or through the activation of host resistance mechanisms such as physical barriers, the innate immune system, salicylate-mediated systemic acquired resistance, induced systemic resistance and defense compounds [20, 21]. Because resistance to pathogen infection is likely a combination of plant and microbiota traits, it is equally important to gain insights into microbial community responses to pathogen invasion. Ecological network analyses of plant-associated microbiomes have been used to explore microbial community dynamics and to predict outcomes of pathogen invasion [22], and network metrics are used to explain the mechanisms of persistence and stability of microbial communities [15, 23]. For example, Wei et al., (2015)[23] studied resource competition networks and showed that bacterial community networks having low nestedness and high connectance reduced invasion success and growth of the pathogen *Ralstonia solanacearum* in tomato roots. Moreover, network analysis has been used to highlight ecologically important microorganisms such as indicator species or hub members in microbial communities [17, 22, 24, 25]. For instance, hub species acting as keystone species have, by definition, many network connections, and their removal destabilizes the overall network structure [24] and thus affect community resilience to pathogen perturbations.

Novel plant-mediated avenues for designing pathogen-resilient plant microbiomes require in-depth knowledge of the dynamic interactions occurring between the complex metabolic and microbial communities. However, studies deciphering links between soil-borne fungal pathogens, the microbiota, and plant-metabolites are limited.

The *Arabidopsis* ecotypes Col-0 and Ler-0 have differential resistance responses to pathogenic species in the fungal genus *Fusarium* [26–29]. The RESISTANCE TO *FUSARIUM OXYSPORUM* (RFO) locus found in Col-0 confers resistance to a broad spectrum of *Fusarium oxysporum* races in *Arabidopsis* [26, 30]. We hypothesized that disease severity would be affected not only by the RFO gene but also by modulation of the microbiome. We further predicted that a successful FOM infection would result in more dramatic global shifts in the microbiome and metabolome of the susceptible Ler-0 than in the resistant Col-0. The objectives of our study were to (i) understand how FOM influences root-associated microbial community structures and *Arabidopsis* metabolite profiles, (ii) examine the inter-omics dynamics during FOM

infection in the two *Arabidopsis* accessions, and (iii) assess microbiome-metabolome associations occurring in the inoculated and non-inoculated Col-0 and Ler-0 lines. We studied omics profiles in a time series of *Fusarium oxysporum* f.sp. *mathioli* (FOM) infection in the resistant Col-0 and the susceptible Ler-0 accessions (Fig. 1A).

Results

FOM colonizes the susceptible Ler-0 faster than the resistant Col-0

The most remarkable symptoms of wilting were observed in Ler-0, and for both accessions, symptoms were strongest at 25 DAI. Symptoms of wilting disease were likewise observed in non-inoculated Ler-0. (Fig. 1A). qPCR confirmed that *F. oxysporum* DNA amounts were highest in inoculated plants and that *F. oxysporum* DNA levels were lower in the FOM-resistant Col-0. *F. oxysporum* DNA was detected at low levels in the non-inoculated plants, most likely due to the presence of *F. oxysporum* in the soil used for the experiments. *F. oxysporum* DNA levels increased at a slower rate in Col-0 and declined at 25 DAI (Fig. 1B).

Microbiome structures

We characterized Col-0 and Ler-0 root-associated microbiomes during FOM progression. We obtained a total of 2,138,612 bacterial sequence reads (range 1,420–72,161; median 16,985 per sample) resulting in 8359 operational taxonomic units for bacteria (bOTUs). Fungal community profiling yielded 2,995,948 reads (range 11,192–37,255; median 18,122 per sample) resulting in 438 fungal OTUs (fOTUs) (**Additional file 1: Figure S2-4, Table S2**). Relative abundances at class and genus levels for bacteria and fungi, respectively are shown (**Additional file 1: Figure S3A,B**). Fungal reads from pathogen-inoculated samples were, not surprisingly, dominated by *Fusarium* (fOTU1 identified as FOM), as also supported by qPCR (**Additional file 1: Figure S3C,D**), and the appearance of symptoms.

Next, we assessed the impact of DAI, host genotype, and FOM treatment on bacterial and fungal community structures. Bacterial alpha diversity was generally lower in infected samples (**Additional file 1: Figure S4A**). Bacterial and fungal communities showed distinct clustering across the different DAIs in PCoA plots according to genotype and FOM inoculation (Fig. 1C and **Additional file 1: Figure S4B**). Bacterial communities were clearly separated based on FOM treatment in Ler-0 at early DAIs but became indistinguishable at later stages. A permutational multivariate analysis of variance (PERMANOVA) showed that in the whole dataset, DAI had the highest effect on bacterial communities (Adonis; $R^2 = 0.17$, $P < 0.001$, Table 1), while FOM treatment, not surprisingly, explained the highest variation on the fungal communities (Adonis; $R^2 = 0.37$, $P < 0.001$, Table 1). By sub-setting datasets, DAI was having a stronger effect on bacterial communities (Adonis; $R^2 = 0.42$, $P < 0.001$, **Additional file 1: Table S3**) in the inoculated Col-0, and on fungal communities (Adonis; $R^2 = 0.59$, $P < 0.001$, **Additional file 1: Table S3**) in the inoculated Ler-0. Using datasets partitioned for individual DAIs revealed the strongest effect of FOM

treatment at 5 and 10 DAI (Table 1). The effect of FOM treatment diminished with time, whereas genotype effects generally increased with DAI (Table 1).

FOM infection and host resistance affect OTU dynamics

To obtain an overview of microbial relative abundances in the treatments, we performed a heatmap visualization of the 50 most abundant bacterial and fungal OTUs (**Additional file 1: Figure S5A,B**). In the bacterial dataset, we found notable enrichment of bOTU6 belonging to *Rhizobium* and bOTU10 assigned to the genus *Pseudomonas protegens* in inoculated samples (**Additional file 1: Figure S5C**). Likewise, a differential abundance analysis revealed distinct microbial enrichment between genotypes and treatments. *Pedobacter* (bOTU248) and *Flavobacterium* (bOTU248) were strongly enriched in Col-0 and Ler-0, respectively. Similarly, the fungal taxa *Cladosporium*, *Colletotrichum* and *Fusarium* were enriched in Ler-0, while Dothidiomycetes were enriched in Col-0 (**Additional file 1: Figure S6, Table S4**). The bacterial OTUs including *Stenotrophomonas* (bOTU94), *Delftia* (bOTU65), *Pseudomonas* (bOTU10) and *Rhizobium* (bOTU6) were the most highly enriched in inoculated samples of both genotypes. *Uliginosibacterium* (bOTU765) and *Luteolibacter* (bOTU375) were highly enriched in non-inoculated Col-0, while Deltaproteobacteria (bOTU849) was strongly enriched in non-inoculated Ler-0. *Fusarium* (fOTU1) was enriched in inoculated samples of both genotypes (**Additional file 1: Figure S6, Table S4**).

Indicator species analysis identified the highest number of indicator bOTUs in Col-0, and in the non-inoculated samples, while the lowest number of indicator fOTUs was found in non-inoculated Col-0, (**Table 2 and Additional file 1: Figure S5**). Most of the indicator OTUs belonged to the bacterial phyla Proteobacteria and Actinobacteria and the fungal phylum Sordariomycetes. We assessed the dynamics of these taxa across different DAIs and found distinct patterns of enrichment of indicator OTUs, particularly bOTUs (Fig. 2A,B, **Additional file 1: Table S5**). In Col-0, indicator bOTUs were most actively recruited at 25 DAI, both in inoculated and non-inoculated roots, while indicator bOTU enrichment in Ler-0 was monotonous. The dominant indicator bOTUs in both Col-0 and Ler-0 belonged to Delta/Gamma-proteobacteria and Planctomycetes. bOTU10 (*P. protegens*) was the most abundant indicator in inoculated Col-0 and Ler-0, while indbOTU 61 (*Duganella*) and indbOTU 6 (*Rhizobium*) were depleted in inoculated Col-0 and Ler-0, respectively (**Additional file 1: Table S5**). Indicator fOTU enrichment showed weaker, albeit similar, patterns, with recruitment peaking at 25 DAI in Col-0, while uniform across DAI in Ler-0 (Fig. 2C,D, **Additional file 1: Table S5**).

Bacterial networks are stronger in the resistant Col-0, and breaks down in the susceptible Ler-0 after FOM inoculation

Co-occurrence networks visualized microbial co-occurrences and highlighted indicator OTUs in the overall OTU networks (Fig. 3, **Additional file 1: Table S6**). Network robustness determined by node degrees was higher in non-inoculated networks (Col-0: 8.49; Ler-0: 7.24) than in inoculated networks (Col-0: 7.06; Ler-0: 5.02). Communities in non-inoculated samples had more nodes and edges, while inoculated Ler-0 had highest numbers of negative edges (Fig. 3). Networks of Col-0 had a large core microbial cluster with

most of the indicator bOTUs found within this cluster (Fig. 3A,B). In contrast, Ler-0 networks were less dense and had smaller microbial clusters especially in inoculated samples. Indicator fOTUs were mostly located outside the main clusters, particularly in inoculated samples (Fig. 3C,D).

We found both positive and negative inter-kingdom correlations between bacteria and fungi, whereas all fungal-fungal correlations were positive (**Additional file 1: Table S6**). The number of positive bacteria-bacteria co-occurrences were notably smaller in inoculated Ler-0. A higher number of positive co-occurrences were observed in inoculated (1064) than in non-inoculated (558) networks of Ler-0. Similarly, a higher number of positive bacterial-fungal co-occurrences were observed in Col-0 non-inoculated and in Ler-0 inoculated. The number of negative bacterial-bacterial co-occurrences were higher in Col-0 non-inoculated (378) than in inoculated (129) and Ler-0 inoculated (262) compared to non-inoculated (48). In contrast, the highest number of negatively correlated bacterial-fungal OTUs were found in Col-0 inoculated (41) and Ler-0 non-inoculated (94) (**Additional file 1: Table S6**).

Hub nodes in microbial networks represent species having the highest numbers of connections to other species. The number of hub OTUs (5 % OTUs having the most connections) were highest in the networks of non-inoculated samples. (Fig. 3, **Additional file 1: Table S6**). A distinct number of OTUs serving as both indicators and hubs were identified in the inoculated and non-inoculated genotypes, with the highest (25) and lowest (7) numbers detected in inoculated and non-inoculated networks of Col-0, respectively (**Additional file 1: Table S6**). The highest number (11) of hub fOTUs was detected in inoculated Ler-0 networks. Finally, we identified a higher number of negatively correlating bOTUs and fOTU1 (FOM) (Spearman's $\rho > 0.05$, $p < 0.05$) in non-inoculated Ler-0 samples. The bOTUs that correlated negatively with fOTU1 mostly belonged to Proteobacteria, Actinobacteria, and Planctomycetes in Ler-0 (**Additional file 1: Table S7**).

FOM infection alters metabolite profiles in Arabidopsis

We profiled 34 root metabolites (**Additional file 1: Table S8**) and OPLS-DA results showed increasingly distinct clustering of the treatment groups across time (Fig. 4). For instance, FOM inoculation did not notably affect Col-0 metabolites at early time points but formed separate clusters later (Fig. 4A). A heatmap shows significant differences in metabolite concentrations in the samples (Fig. 4B).

Ler-0 and Col-0 varied in their glucosinolate (GLS) content and after FOM inoculation. Almost 98% of the total GLS detected in Ler-0 were indolic GLSs (iGLS), whereas Col-0 contained higher concentrations of aliphatic GLSs (aGLS) (10 to 30% of the total GLS) (Fig. 4B, **Additional file 1: Table S8**). FOM inoculation of Col-0 increased levels of glucoraphanin, sulforaphane, glucoerucin, and glucoiberin in roots at 5 DAI (approximately 4-fold increases) as well as at 25 DAI (4, 10, and 3.5-fold increases, respectively) (Fig. 4B, **Additional file 1: Table S8**). In contrast, concentrations of these compounds were not significantly different among the different treatments in Ler-0 (Fig. 4B, **Additional file 1: Table S8**). These results suggest that co-accumulation of structurally related aGLS in Col-0 could play a critical role in limiting FOM infection.

In Col-0-inoculated roots, neoglucobrassicin and 4-hydroxyglucobrassicin concentrations increased 2-3-fold at the early stage of infection (5 DAI), while levels of glucobrassicin, 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin decreased at 15 DAI (approximately 2-fold). A significant increment in the levels of several indole glucosinolates occurred in Ler-0 inoculated roots at 10 DAI (Fig. 4B, **Additional file 1: Table S8**). Camalexin was present in both genotypes at all time points. However, an increase in concentrations in response to FOM inoculation occurred at a much greater extent in Ler-0 roots, especially at 10 DAI (8-fold), 20 (2.5-fold), and 25 DAI (4-fold) (Fig. 4B, **Additional file 1: Tables S8**). Among the quantified lignans and lignan precursors, sinapaldehyde, sinapyl alcohol, and pinoresinol diglucoside showed an increase in inoculated Ler-0 roots at 15 DAI, while sinapaldehyde, sinapyl alcohol, pinoresinol diglucoside, lariciresinol, and pinoresinol decreased at 20 DAI. By contrast, the concentrations of coumaric acid, sinapaldehyde, coniferyl aldehyde, and syringin increased in inoculated Col-0 at 20 DAI. Among the hormones detected, a significant increase (2.5-fold) in the concentration of abscisic acid in Col-0 occurred at the early stages of infection (5 DAI). In addition, the level of SA decreased significantly in roots of inoculated Ler-0 at the later stages (25 DAI).

Distinct metabolite-OTU correlations in FOM resistant and susceptible *Arabidopsis*

We examined and visualized potential microbial-metabolite correlations in heatmaps (Fig. 5, **Additional file 1: Table S9**). Coniferyl alcohol, glucoiberin, indole-3-acetic acid, scopolin, sinapic acid, sinapyl alcohol, and sulforaphane were correlating with bOTUs exclusively in Ler-0, while 4-hydroxyglucobrassicin, abscisic acid, ferulic acid, glucobrassicin, neoglucobrassicin, and vanillic acid correlated with bOTUs in Col-0. Similarly, aesculetin, fraxin, glucobrassicin, and lariciresinol correlated with a number of fOTUs in Col-0, while several other metabolites, such as cinnamic acid, camalexin, indole-3-carbinol, sulforaphane, sinapyl alcohol, and coniferyl alcohol, correlated with fOTUs in Ler-0 (Fig. 5B, **Additional file 1: Table S9**). In the Col-0 dataset, vanillic acid and 4-methoxyglucobrassicin were positively correlating with several OTUs (Fig. 5A, **Additional file 1: Table S9**). In Ler-0, cinnamic acid, glucoerucin, indole-3-carbinol, SA, sinapyl alcohol, and sulforaphane had the highest numbers of correlations with bOTUs (Fig. 5B, **Additional file 1: Table S9**). A higher number of negative metabolite-fOTU correlations were identified in Col-0 and neoglucobrassicin, glucoraphanin, SA and ferulic acid negatively correlated with fOTUs in both Col-0 and Ler-0. (Fig. 5B, **Additional file 1: Table S9**). Neoglucobrassicin and scopolin correlated positively and negatively with fOTU1 (FOM) in Col-0 and Ler-0, respectively (**Additional file 1: Table S10**).

Three of the bOTUs that negatively correlated with fOTU1 showed positive correlations with metabolites in non-inoculated Ler-0. While both bOTU11 and bOTU39 were assigned to the genus *Massilia*, bOTU11 positively correlated with 4-hydroxyglucobrassicin and glucobrassicin, while bOTU39 positively correlated with syringin. Moreover, bOTU27 belonging to the family Methyloligellaceae positively correlated with cinnamic acid.

Specific metabolites affect overall community diversity and distinctively associate with major microbiota members of resistant and susceptible *Arabidopsis*

We found that 4-methoxyglucobrassicin, indole-3 carbinol, syringin and glucobrassicin had the strongest effects on bacterial communities, each explaining app. 2–4 % of the variation (Fig. 6A). Similarly, neoglucobrassicin, camalexin, and coumaric acid had the strongest effects on fungal communities explaining 4–6 % of the variation, respectively (Fig. 6B).

We postulate that OTUs acting as both indicators and keystone OTUs would have the strongest influence on microbiome structure. We thus examined whether indicator/keystone OTUs were affected by specific metabolites and found that some OTUs correlated with metabolites including several of the metabolites that had the strongest general effects on microbial community structures (Fig. 6C,D, **Additional file 1: Table S11**). For example, 4-methoxyglucobrassicin had mostly positive correlations with indicator b/fOTUs (Fig. 6C, **Additional file 1: Table S11**), and the hubOTUs fOTU35 (Ascomycota) and fOTU48 (Clavicipitaceae) in inoculated Col-0 (Fig. 6D, **Additional file 1: Table S11**), while neoglucobrassicin negatively correlated with indicator fOTUs assigned to Sordariomycetes (Fig. 6C, **Additional file 1: Table S11**). Similarly, indole-3-carbinol positively correlated with several indicator and hubOTUs: for example, bOTU281 (*Paenibacillus*), bOTU12 (*Nocardioides*), bOTU4664 (Xanthobacteraceae), and bOTU27 (Methyloligellaceae), in infected Ler-0 (Fig. 6D, **Additional file 1: Table S11**). Surprisingly, camalexin did not associate with indicator b/fOTUs or hubOTUs, although it had notable effects on fungal communities. Strikingly, metabolite and hubOTU correlations were all negative in the inoculated while positive in the non-inoculated Ler-0.

We found 17 OTUs acting as both indicator and keystone OTUs correlating with metabolites. Indole-3-acetic acid and sinapic acid negatively correlated with indicator/hub bOTU14 (Saccharimonadales) and bOTU70, respectively (**Additional file 1: Table S11**). Indole-3-carbinol correlated negatively with bOTU14 and positively with bOTU538 (Solirubrobacteraceae). SA correlated positively with bOTU24 (Gemmatimonadaceae) or negatively with bOTU61 (Duganella). Negative correlations between bOTU14 and indole-3-acetic acid and indole-3-carbinol were found in non-inoculated Col-0, while negative correlations between SA and bOTU61, and sinapic acid and bOTU70 (*Leptothrix*) occurred in inoculated Ler-0 (**Additional file 1: Table S11**). Cinnamic acid correlated positively with bOTU24 and bOTU27 (Methyloligellaceae) and negatively with several indicator/hub OTUs such as bOTU4 (*Streptomyces*), bOTU259, bOTU885 or bOTU70, while 4-Methoxyglucobrassicin positively correlated with fOTU35 and fOTU48. Indole-3-acetic acid and indole-3-carbinol correlated with several of the indicator/hub OTUs.

Discussion

The dynamic nature of pathogen invasion of plant tissues and the onset of changes in plant metabolic profiles require a time series approach to critically analyze inter-omics relationships [31–33]. By following

FOM progression over time, we tracked the orchestration of metabolites and root-associated microbiome assembly in two genotypes of *Arabidopsis* with different FOM susceptibilities.

Microbiome profiling revealed that Col-0 and Ler-0 exhibited distinct microbial compositions in the different FOM treatments across DAIs. The loss in bOTU richness in most inoculated samples could be a direct effect of FOM niche displacement [34]. The significant effects of DAI, genotype, and FOM on bacterial and fungal beta diversities confirm previous studies [35–37]. In both genotypes, the effect of FOM inoculation on microbial communities was strongest at the early sampling times, which could be attributed to high FOM densities. Genotype effects on microbial communities increased with DAI, as also genotype x FOM treatment effects. Although the relative importance of genotype on microbial communities has been observed to decline over time [38], our results showed the opposite, mostly in bacterial communities. These genotype response trajectories on the microbiome could possibly be a cause of the differences in disease resistances, as predicted.

Indicator species represent microbes that are mostly affected by treatments and could therefore be important in host responses to FOM [39, 40]. The identified indicator OTUs and their dynamics at different DAIs revealed (i) a large diversity of the *Arabidopsis* root-associated microbiota that was distinctively affected by the physiological status of the host, and (ii) a time-dependent assembly of microbial communities as previously reported [35, 36, 41]. For example, Edward et al., (2018)[35] found a highly dynamic host-associated microbiota in field grown rice, which was affected by host developmental stage and plant age. Thirdly, our results demonstrated a genotype-specific assembly of microbiota [42]. Notably, the enrichment of indicator bOTU10 assigned to *Pseudomonas protegens* in inoculated samples of both genotypes suggest that *Arabidopsis* recruit microorganisms to enhance FOM resistance. *P. protegens* is a well-known plant growth-promoting bacterium with broad-spectrum antifungal activity against phytopathogens [43]. The genus *Streptomyces* was strongly enriched in Col-0. *Streptomyces* is known for its unparalleled synthesis of antibiotics [44], and could thus contribute to FOM resistance [44, 45]. Moreover, the enrichment of more diverse indicator taxa in Col-0 at 25 DAI further supports distinct genotype effects.

We assessed the overall robustness of Col-0 and Ler-0 microbial networks to FOM invasion. A combination of network variables is best for explaining microbial community resilience and robustness [15]. We found node degree, hub numbers, and link densities to be highest in Col-0, indicating robust microbial communities. In contrast, connectance was higher in Ler-0, a result that corroborates earlier studies where high connectance promoted *Ralstonia solanacearum* host colonization [23]. In our study, all the measured properties were highest in the networks of non-inoculated Col-0 and Ler-0, suggesting a network breakdown during FOM invasion. Node degree breakdown during FOM infection was higher (30.7%) in the susceptible Ler-0 compared to Col-0 (16.8%). Altogether, these results support our initial hypothesis of a stronger network resistance in Col-0 and that stronger networks are indicative of pathogen-resilient microbial communities.

We found both positive and negative intra- and inter-kingdom co-occurrences, except that we did not identify any negative fungi-fungi co-occurrences, plausibly due to weaker antagonistic interactions among fungi. These results support previous studies of cooperative and antagonistic microbial interactions among microbial kingdoms [16, 22]. The differences in co-occurrence network structures underscore the distinct interactions in inoculated and non-inoculated samples of Col-0 and Ler-0 and are also essential in explaining aspects of host invasion resistance [15, 22]. The microbial networks further highlighted how indicator species are affected during FOM invasion. Surprisingly, keystone species and indicator species were mostly observed in the networks of the inoculated Col-0, and we speculate that enrichment of these species could serve as an important factor in resilience towards pathogen invasion [46]. Taken together, these results largely support the hypothesis of distinct microbiome structuring in resistant and susceptible genotypes across different DAIs.

Surprisingly, we only found negative co-occurrences between fOTU1 and bOTUs, and these were observed mostly in non-inoculated Ler-0. Among these bOTUs several belonged to Proteobacteria and Actinobacteria, which are dominant taxa of the Arabidopsis microbiome [47]. In contrast to Col-0 (having the RFO gene), Ler-0 could possibly be deploying other mechanisms to resist pathogens by recruiting antagonistic microorganisms. This putative defense mechanism, however, was broken down by the abrupt invasion caused by FOM inoculation. Similarly, Snelders et al., [48] demonstrated the ability of the fungal pathogen *Verticillium dahliae* to manipulate tomato and cotton microbiomes by suppressing antagonistic bacteria. The ability of FOM to cause this disruption could be explained by disturbance via resource competition and secretion of antifungal compounds [16, 48].

Plant chemical compounds are strong modulators of microbial communities, and variations in their diversity and quantities can be used to predict resistance profiles of plants towards specific pathogens [49]. We observed a clear separation of metabolites (in OPLS-DA) at individual DAIs and these coincided with the marked assembly of host-associated microbiota, confirming that metabolites have a regulatory role in shaping the microbiome [5, 8]. Several metabolites were found in higher amounts in Ler-0, mostly at 5 and 10 DAI, that were induced in response to FOM infection. The observed differences in iGLS, aGLS, camalexin, and phenylpropanoid concentrations in Col-0 and Ler-0 could contribute to the differential resistances observed in the two genotypes. For example, aGLSs were induced at higher levels in inoculated Col-0 compared to Ler-0 at 5 DAI, which could potentially affect microbial communities [7, 42]. aGLSs and their hydrolysis products have been reported to have higher effects on microorganisms compared to iGLSs [50]. Thus, we further predict that the high aGLS content could inhibit FOM in Col-0. We did not find any strong evidence of inhibitory effects on FOM of camalexin, which is in line with other studies [51, 52].

Changes in concentrations of a number of phenolic compounds, including cinnamic acid, coumarins, lignin precursors and lignans notably in the inoculated samples have been observed earlier [53, 54]. The elevated concentrations of a number of these phenols (sinapyl aldehyde, syringin, sinapyl alcohol, and coniferyl aldehyde) in infected samples support a previous study where soluble derivatives of the phenylpropanoid pathway increased defense in an *Arabidopsis-Verticillium longisporum* interaction [53].

The positive and negative correlations between metabolites and OTUs, including a number of indicator and keystone members, support the selective effects of metabolites on specific microbial members of root microbiomes [6–8, 33]. We further identified metabolite-OTU correlations that uniquely occurred in either Col-0 or Ler-0, underpinning the different resistances and recruitment of specific microbial taxa [55–58]. For instance, the iGLSs glucobrassicin, neoglucobarassicin, and 4-hydroglucobrassicin correlated with specific bOTUs in Col-0, while the aGLSs glucoiberin and sulforaphane correlated with bOTUs in only Ler-0. Genotypic variation in chemical diversity affected fungal and bacterial communities associated with plants [59]. Moreover, the observation that aGLS mostly correlated with bOTUs and iGLS mostly correlated with fOTUs is indicative of differential effects of GLSs on microbial communities. Similarly, phenolic compounds displayed genotype-specific relations as they were correlating with bOTUs only in Ler-0, supporting previous studies in which phenolic compounds were found to selectively inhibit *F. oxysporum* and *Verticillium dahliae* [33].

We found 4-methoxyglucobrassicin, indole-3-carbinol, and syringin had the strongest overall effects on bacterial communities, while neoglucobrassicin, camalexin, and coumaric acid highly affected fungal communities. Our findings consolidate these compounds as highly bioactive that could be of interest in future studies. Moreover, the results suggest a dominant role of iGLSs on the Arabidopsis root microbiota. GLS have been widely studied due to their well-known role in plant-microbe interactions, and Zeng et al., (2003)[60] reported 4-methoxyglucobrassicin as a growth stimulator of ectomycorrhizal fungi, while indole-3-carbinol is known for its broad antimicrobial activity against bacteria and yeasts [61]. The iGLSs 4-methoxyglucobrassicin and indole-3-carbinol displayed strong positive correlations with specific OTUs, while aGLSs, including glucoerucin, glucoraphanine, and sulforaphane, were strongly negatively correlating with indicator taxa such as Gammaproteobacteria and Bacteroidia. These results suggest that aGLSs display higher toxicity to major microbial members compared to iGLSs, as was also found in previous studies where the effect of aGLSs on *Alternaria brassicicola* was stronger than the effect of iGLSs [62]. The strong inhibition of microorganisms by aGLSs is attributed to degradation products such as isothiocyanates, thiocyanates, oxazolidinethiones, and nitriles produced from enzymatic cleavage by myrosinase [63–65].

Hub microbes are important for maintaining network structure and function [22]. Interestingly, all the identified hub OTUs were also indicator species in our analysis, reaffirming their importance in the microbiome. Most of these indicator/hub OTUs were bOTUs and were mostly found in Ler-0, thus corroborating our hypothesis that inter-omics interactions depend on both host genotype and host infection status. The indicator/hub OTUs were affected negatively or positively by specific metabolites and these were further observed to be physiologically dependent. Specifically, we observed unique metabolite-indicator/hub OTU correlations, mostly positive in non-inoculated Ler-0 and negative in inoculated Ler-0, suggesting that the host utilizes its metabolites to selectively recruit specific microbes under different physiological conditions [66]. The indicator/hubs bOTU259 (*Niastella*) and bOTU538 (Solirubrobacteraceae) had mostly negative and positive correlations, respectively, with metabolites in Ler-0. While Solirubrobacteraceae has been found to suppress common scab disease of potatoes [67], the *Niastella* is reported to improve soil health and promote root growth [68, 69]. In non-inoculated Ler-0,

the indicator/hub OTUs *Paenibacillus* (bOTU281), *Nocardioides* (bOTU12), Xanthobacteraceae (bOTU4664), and Methyloligellaceae (bOTU27) exclusively correlated positively with metabolites. Interestingly, these taxa are considered ecologically important, either involved in nitrogen fixation or acting as antagonists against pathogens. For example, the plant growth-promoting *Paenibacillus polymyxa* induces host defense responses against *F. oxysporum* [70, 71]. Altogether, these results support our hypotheses of genotype specific metabolome-microbiome interactions primarily due to the differential FOM resistances. Specifically, we speculate that Ler-0, in the lack of the RFO resistance gene, is recruiting antagonistic microorganisms to prevent infection by synthesizing certain metabolites. However, after inoculation with FOM this pathogen resilient network is broken down.

The observation that cinnamic acid and glucoerucin had the highest numbers of positive and negative correlations with indicator/hub OTUs suggest their bioactivity. Both inhibitory and chemoattracting effects of cinnamic acid has been demonstrated [72, 73]. Glucoerucin is toxic against *Xanthomonas campestris*, *Pseudomonas syringae* [74] *Pythium irregulare* and *Rhizoctonia solani* [75]. Sulforaphane negatively correlated with *Niastella* (bOTU259) and *Leptothrix* (bOTU70) in inoculated Ler-0 and correlated positively with *Nocardioides* (bOTU12) and *Duganella* (bOTU61) in non-inoculated Ler-0. Sulforaphane is known for its selective effects towards *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* [76]. Indole-3-carbinol correlated positively with several indicator/hub OTUs in non-inoculated Ler-0. These findings further support differential effects of GLS and their hydrolysis products [77] on host-associated microbiomes [13, 14]. While sinapyl-alcohol showed both positive and negative correlations with several taxa, its glucoside syringin, as well as pinorexinol and fraxin, only correlated positively with indicator/hub taxa. Importantly, the results of distinctive interactions, for instance, sinapyl-alcohol negatively correlating with the family Chitinophagaceae in inoculated Ler-0 and positively with the family Xanthobacteraceae, are indicative of host-dependent effects of these compound on the microbiota. The potential of other phenylpropanoids, for example coumarins, to differentially inhibit both beneficial and pathogenic microorganisms has been reported [8, 78]. Together, these findings suggest an active role of phenylpropanoids in shaping microbiota and should thus be prioritized in future studies.

Although most metabolite-OTU correlations were identified in Ler-0, an important observation in Col-0 was the positive correlation of syringin with the beneficial *Streptomyces* (bOTU4). 4-methoxyglucobrassicin only correlated with fungal indicator/hub OTUs in Col-0, indicative of a higher effect on fungal communities [60]. In addition, the phytohormone SA and the phenols sinapic acid and sinapyl alcohol showed negative correlations with a high number of indicator OTUs, suggestive of their modulating effect on the microbiota. Contrasting correlations between indicator/hub OTUs and phytohormones were observed, suggesting distinctive effects of these hormones on microbial communities.

Conclusions

This study characterized root-associated microbiota - metabolome dynamics during FOM infection in two genotypes of Arabidopsis with different FOM susceptibilities. We show evidence of the dynamic relationships that exists between the root-associated microbiome and the plant-metabolome of FOM-

inoculated and non-inoculated *Arabidopsis*. We demonstrate that the microbiome and metabolome in the two *Arabidopsis* genotypes are distinct and markedly shift during FOM infection. Col-0 microbial networks were more robust during *Fusarium* infection. *Pseudomonas* (bOTU10) and *Rhizobium* (bOTU6) were strongly enriched in inoculated samples of both genotypes, indicating a prominent role of these OTUs in response to FOM infection. Changes in the microbiome and metabolome were more dramatic, with stronger effects on major indicator/hub taxa in the susceptible Ler-0 than in the resistant Col-0. Correlations between metabolites and OTUs were host genotype and FOM dependent. Our study identified metabolites with the strongest effects on microbiome structure and these compounds, including the GLS glucoerucin, or its hydrolysis products indole-3 carbinol and sulforaphane, cinnamic acid, sinapyl-alcohol and phytohormones SA and indole-3 acetic acid correlated with indicator/hub OTUs. Considering the major role of indicator/hub microbial taxa in overall microbiome composition, we infer that these compounds are pivotal in plant microbiome assembly. It is worth emphasizing that, although correlation-based analyses are not definitively causal, the identified interactions deepen our understanding of inter-omics interactions in resistant and susceptible plant microbiomes. In future studies, it will be interesting to study the possible microbiome-mediated effects of RFO gene resistance by using RFO gene disrupted or complemented mutants.

Materials And Methods

Details of the materials and methods used in this work, including qPCR, microbial community analysis (ref. 5) and metabolomics are described in **Additional file 1: Materials and Methods**.

Abbreviations

Col-0 Columbia - 0

Ler-0 Landsberg erecta

FOM *Fusarium oxysporum* f.sp. *mathioli*

ITS2 Internal transcribed spacer – 2

16S rRNA 16S ribosomal RiboNucleic Acid

GLS Glucosinolate

aGLS Aliphatic glucosinolate

iGLS Indolic glucosinolate

SA Salicylic acid SA

JA Jasmonic acid JA

RFO Resistance to *Fusarium Oxysporum* (RFO)

DAI Days after inoculation DAI

OTU Operational taxonomic unit

bOTU Bacterial operational taxonomic unit

fOTU Fungal operational taxonomic unit

qPCR quantitative PCR

PERMANOVA Permutational multivariate analysis of variance

HubOTU hub microbial operational taxonomic unit

OPLS-DA Orthogonal partial least squares discriminant analysis

NCBI National Center for Biotechnology Information

SRA Sequence Read Archive

QIIME Quantitative Insights Into Microbial Ecology

Declarations

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The MiSeq paired end reads for bacterial 16s rRNA gene (V3-V4) and fungal ITS2 regions have been deposited in NCBI SRA database using accession code PRJNA756534. Sequence processing in QIIME and data analysis in R were performed using the pipeline and scripts from Kudjordjie et al., (5).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was financially support by Aarhus University (project number 22550) and the Independent Research Fund Denmark DFF grant number 23844.

Authors' contributions

ENK, KH, RS, INF, MN conceived the study, participated in its design. ENK and KH conducted the experiment and analyzed the data. ENK and MN drafted the manuscript. All authors read, revised and approved the manuscript.

Acknowledgements

We thank H. Corby Kistler for providing the FOM isolate. We thank Mette Vestergård for editing the manuscript. Simone Ena Rasmussen, Bente Birgitte Laursen and Mathilde Schiøtt Dige are thanked for their excellent laboratory assistance.

Authors' information

Not applicable

References

1. Bulgarelli D, Schlaeppi K, Spaepen S, Ver E, Themaat L Van, Schulze-lefert P. Structure and functions of the bacterial Microbiota of Plants. *Annu Rev Plant Biol* 2013; **64**: 807–38.
2. Jacoby RP, Koprivova A, Kopriva S. Pinpointing secondary metabolites that shape the composition and function of the plant microbiome. *J Exp Bot* 2021; **72**: 57–69.
3. Jones DL, Nguyen C, Finlay RD. Carbon flow in the rhizosphere: carbon trading at the soil – root interface. *Plant Soil* 2009; 5–33.
4. Sasse J, Martinoia E, Northen T. Feed your friends: do plant exudates shape the root microbiome? *Trends Plant Sci* 2018; **23**: 25–41.
5. Kudjordjie EN, Sapkota R, Steffensen SK, Fomsgaard IS, Nicolaisen M. Maize synthesized benzoxazinoids affect the host associated microbiome. *Microbiome* 2019; 1–17.
6. Huang AC, Jiang T, Liu YX, Bai YC, Reed J, Qu B, et al. A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. *Science (364)* eaau6389.
7. Bressan M, Achouak W, Berge O. Exogenous glucosinolate produced by transgenic *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. *ISME J* 2009; **3**: 1243–1257.
8. Voges MJEEE, Bai Y, Schulze-Lefert P, Sattely ES. Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. *PNAS* 2019; **116**: 12558–12565.
9. Lebeis SL. The potential for give and take in plant-microbiome relationships. *Front Plant Sci* 2014; **5**: 1–6.
10. Carvalhais LC, Dennis PG, Badri D V, Kidd BN, Vivanco JM, Schenk PM. Linking jasmonic acid signaling, root exudates, and rhizosphere microbiomes. *Mol Plant Microbe Interact* 2015; **28**: 1049–

58.

11. Boller T, Felix G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu Rev Plant Biol* 2009; **60**: 379–406.
12. Ward JL, Forcat S, Beckmann M, Bennett M, Miller SJ, Baker JM, et al. The metabolic transition during disease following infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato*. *Plant J* 2010; **63**: 443–457.
13. Yuan J, Zhao J, Wen T, Zhao M, Li R, Goossens P, et al. Root exudates drive the soil-borne legacy of aboveground pathogen infection. *Microbiome* (2018) 6:156
14. Seybold H, Demetrowitsch TJ, Hassani MA, Szymczak S, Reim E, Haueisen J, et al. A fungal pathogen induces systemic susceptibility and systemic shifts in wheat metabolome and microbiome composition. *Nat Commun* 2020; **11**: 1–12.
15. Murall CL, Abbate JL, Touzel MP, Froissart R, Mccann K, Murall CL, et al. Invasions of host-associated microbiome networks. Research, BT-Advances in ecological networks of invasion: empirical evidence and case studies, 57, Academic Press, 2017, Advances in Ecological Research.
16. Hassani MA, Durán P, Hacquard S. Microbial interactions within the plant holobiont. *Microbiome* 2018; **6**: 58.
17. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant–microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol* 2020; 1–15.
18. Mccann KS. The diversity–stability debate. *Nature* 2000; **405**.
19. Dirk J, Elsas V, Chiurazzi M, Mallon CA, Elhottova D. Microbial diversity determines the invasion of soil by a bacterial pathogen. *PNAS* 2011; **109**: 1159–1164.
20. Jones JDG, Dangl JL. The plant immune system. *Nature* 2006; **444**: 323–329.
21. Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM. Induced systemic resistance by beneficial microbes. *Annu Rev Phytopathol* 2014; **52**: 347–75.
22. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S, Weigel D, et al. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol* 2016; **14**(1): 1–31.
23. Wei Z, Yang T, Friman V, Xu Y, Shen Q, Jousset A. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nat Commun* 2015; **6**: 1–9.
24. Berry D, Widder S. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Front Microbiol* 2014; **5**: 1–14.
25. Röttgers L, Faust K. From hairballs to hypotheses–biological insights from microbial networks. *FEMS Microbiol Rev* 2018; **42**: 761–780.
26. Diener AC, Ausubel FM. RESISTANCE TO *FUSARIUM OXYSPORUM* 1, a dominant *Arabidopsis* disease-resistance gene, is not race specific. *Genetics* 2005; **171**: 305–321.
27. Zapata L, Ding J, Willing EM, Hartwig B, Bezdan D, Jiao WB, et al. Chromosome-level assembly of *Arabidopsis thaliana* Ler reveals the extent of translocation and inversion polymorphisms. *PNAS* 2016; **113**: E4052–E4060.

28. Chen X, Steed A, Harden C, Nicholson P. Characterization of *Arabidopsis thaliana*-*Fusarium graminearum* interactions and identification of variation in resistance among ecotypes. *Mol Plant Pathol* 2006; **7**: 391–403.
29. Ziolkowski PA, Koczyk G, Galganski L, Sadowski J. Genome sequence comparison of Col and Ler lines reveals the dynamic nature of *Arabidopsis* chromosomes. *Nucleic Acids Res* 2009; **37**: 3189–3201.
30. Diener AC. Routine mapping of *Fusarium* wilt resistance in BC1 populations of *Arabidopsis thaliana*. *BMC Plant Biol* 2013; **13**.
31. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature* 2012; 489: 220–230
32. Ridenhour BJ, Brooker SL, Williams JE, Van Leuven JT, Miller AW, Dearing MD, et al. Modeling time-series data from microbial communities. *ISME J* 2017; **11**: 2526–2537.
33. Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC, et al. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc Natl Acad Sci* 2018; **115**: E5213–E5222.
34. Baquero F, Coque TM, Galán JC, Martínez JL. The Origin of Niches and Species in the Bacterial World. *Front Microbiol* 2021; **12**: 1–13.
35. Edwards JA, Santos-Medellín CM, Liechty ZS, Nguyen B, Lurie E, Eason S, et al. Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. *PLoS Biol* 2018; **16**: 1–28.
36. Wei Z, Gu Y, Friman VP, Kowalchuk GA, Xu Y, Shen Q, et al. Initial soil microbiome composition and functioning predetermine future plant health. *Sci Adv* 2019; **5**: 1–12.
37. Chapelle E, Mendes R, Bakker PAH, Raaijmakers JM. Fungal invasion of the rhizosphere microbiome. *ISME J* 2016; **10**: 265–268.
38. Morella NM, Weng FCH, Joubert PM, Jessica C, Lindow S, Koskella B. Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. *PNAS* 2020; **117**: 1148–1159.
39. Rivedal HM, Stone AG, Severns PM, Johnson KB. Characterization of the fungal community associated with root, crown, and vascular symptoms in an undiagnosed yield decline of winter squash. *Phytobiomes J* 2020; **4**: 178–192.
40. Severns PM, Sykes EM. Indicator species analysis: A useful tool for plant disease studies. *Phytopathology* 2020; **110**: 1860–1862.
41. Li X, Rui J, Mao Y, Yannarell A, Mackie R. Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. *Soil Biol Biochem* 2014; **68**: 392–401.
42. Wagner MR, Lundberg DS, Del Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nat Commun* 2016; **7**, 12151.

43. Andreolli M, Zapparoli G, Angelini E, Lucchetta G, Lampis S, Vallini G. *Pseudomonas protegens* MP12: A plant growth-promoting endophytic bacterium with broad-spectrum antifungal activity against grapevine phytopathogens. *Microbiol Res* 2019; **219**: 123–131.
44. Challis GL, Hopwood DA. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *PNAS* 2003; **100**: 14555–14561.
45. Essarioui A, LeBlanc N, Kistler HC, Kinkel LL. Plant community richness mediates inhibitory interactions and resource competition between streptomyces and *fusarium* populations in the rhizosphere. *Microb Ecol* 2017; **74**: 157–167.
46. Banerjee S, Schlaeppi K, van der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. *Nat Rev Microbiol* 2018; **16**: 567–576.
47. Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, et al. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 2012; 488: 91–5.
48. Snelders NC, Kettles GJ, Rudd JJ, Thomma BPHJ. Plant pathogen effector proteins as manipulators of host microbiomes? *Mol Plant Pathol* 2018; **19**: 257–259.
49. Pais AL, Li X, (Jenny) Xiang QY. Discovering variation of secondary metabolite diversity and its relationship with disease resistance in *Cornus florida* L. *Ecol Evol* 2018; **8**: 5619–5636.
50. Kirkegaard JA, Sarwar M. Biofumigation potential of brassicas I. Variation in glucosinolate profiles of diverse field-grown brassicas. *Plant and Soil* 1998; 71–89.
51. Kliebenstein DJ. Secondary metabolites and plant/environment interactions: A view through *Arabidopsis thaliana* tinged glasses. *Plant, Cell Environ* 2004; **27**: 675–684.
52. Kliebenstein DJ, Rowe HC, Denby KJ. Secondary metabolites influence *Arabidopsis*/Botrytis interactions: Variation in host production and pathogen sensitivity. *Plant J* 2005; **44**: 25–36.
53. König S, Feussner K, Kaefer A, Landesfeind M, Thürow C, Karlovsky P, et al. Soluble phenylpropanoids are involved in the defense response of *Arabidopsis* against *Verticillium longisporum*. *New Phytol* 2014; **202**: 823–837.
54. Lee M, Jeon HS, Kim SH, Chung JH, Roppolo D, Lee H, et al. Lignin-based barrier restricts pathogens to the infection site and confers resistance in plants. *EMBO J* 2019; **38**.
55. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, et al. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 2012; **488**: 86–90.
56. Schlaeppi K, Dombrowski N, Oter RG, Ver Loren van Themaat E, Schulze-Lefert P. Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. *PNAS* 2014; **111**: 585–92.
57. Grady KL, Sorensen JW, Stopnisek N, Guittar J, Shade A. Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops. *Nat Commun* 2019; **10**: 1–10.
58. Baltrus DA. Adaptation, specialization, and coevolution within phytobiomes. *Curr Opin Plant Biol* 2017; **38**: 109–116.

59. Pérez-Izquierdo L, Zabal-Aguirre M, González-Martínez SC, Buée M, Verdú M, Rincón A, et al. Plant intraspecific variation modulates nutrient cycling through its below ground rhizospheric microbiome. *J Ecol* 2019; **107**: 1594–1605.
60. Zeng R Sen, Mallik AU, Setliff E. Growth stimulation of ectomycorrhizal fungi by root exudates of Brassicaceae plants: Role of degraded compounds of indole glucosinolates. *J Chem Ecol* 2003; **29**: 1337–1355.
61. Sung WS, Lee DG. In vitro antimicrobial activity and the mode of action of indole-3-carbinol against human pathogenic microorganisms. *Biol Pharm Bull* 2007; **30**: 1865–1869.
62. Buxdorf K, Yaffe H, Barda O, Levy M. The effects of glucosinolates and their breakdown products on necrotrophic fungi. *PLoS One* 2013; **8**.
63. Bones AM, Rossiter JT. The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* 2006; **67**: 1053–1067.
64. Burel C, Boujard T, Kaushik SJ, Boeuf G, Mol KA, Van Der Geyten S, et al. Effects of rapeseed meal-glucosinolates on thyroid metabolism and feed utilization in rainbow trout. *Gen Comp Endocrinol* 2001; **124**: 343–358.
65. Kliebenstein DJ, Kroymann J, Mitchell-Olds T. The glucosinolate-myrosinase system in an ecological and evolutionary context. *Curr Opin Plant Biol* 2005; **8**: 264–271.
66. Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A. Plant microbiome—an account of the factors that shape community composition and diversity. *Curr Plant Biol* 2020; **23**: 100161.
67. Sarikhani E, Sagova-Mareckova M, Omelka M, Kopecky J. The effect of peat and iron supplements on the severity of potato common scab and bacterial community in tuberosphere soil. *FEMS Microbiol Ecol* 2017; **93**: 1–8.
68. Visioli G, Sanangelantoni AM, Vamerali T, Dal Cortivo C, Blandino M. 16S rDNA profiling to reveal the influence of seed-applied biostimulants on the rhizosphere of young maize plants. *Molecules* 2018; **23**: 1–18.
69. Randall TE, Fernandez-Bayo JD, Harrold DR, Achmon Y, Hestmark K V, Gordon TR, et al. Changes of *Fusarium oxysporum* f.sp. *Lactucae* levels and soil microbial community during soil biosolarization using chitin as soil amendment. *PLoS One* 2020; **15**: 1–17.
70. Du N, Shi L, Yuan Y, Sun J, Shu S, Guo S. Isolation of a potential biocontrol agent *Paenibacillus polymyxa* NSY50 from vinegar waste compost and its induction of host defense responses against *Fusarium* wilt of cucumber. *Microbiol Res* 2017; **202**: 1–10.
71. Dijksterhuis J, Sanders M, Gorris LGM, Smid EJ. Antibiosis plays a role in the context of direct interaction during antagonism of *Paenibacillus polymyxa* towards *Fusarium oxysporum*. *J Appl Microbiol* 1999; **86**: 13–21.
72. Wu HS, Raza W, Fan JQ, Sun YG, Bao W, Shen QR. Cinnamic acid inhibits growth but stimulates production of pathogenesis factors by in vitro cultures of *Fusarium oxysporum* f.sp. *niveum*. *J Agric Food Chem* 2008; **56**: 1316–1321.

73. Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse USR. De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. *New Phytol* 2010; 185: 577–588.
74. Sotelo T, Lema M, Soengas P, Cartea ME, Velasco P. In vitro activity of glucosinolates and their degradation products against Brassica-pathogenic bacteria and fungi. *Appl Environ Microbiol* 2015; **81**: 432–440.
75. Manici LM, Lazzeri L, Baruzzi G, Leoni O, Galletti S, Palmieri S. Suppressive activity of some glucosinolate enzyme degradation products on *Pythium irregulare* and *Rhizoctonia solani* in sterile soil. *Pest Manag Sci* 2000; **56**: 921–926.
76. Johansson NL, Pavia CS, Jen WC. Growth inhibition of a spectrum of bacterial and fungal pathogens by sulforaphane, an isothiocyanate product found in broccoli and other cruciferous vegetables. *Planta Med* 2008; **74**: 747–750.
77. Tierens KFM, Thomma BPHJ, Brouwer M, Kistner K, Porzel A, Mauch-mani B, et al. Study of the Role of antimicrobial glucosinolate-derived isothiocyanates in resistance of arabidopsis to microbial pathogens. *Plant Physiol* 2001, 125 (4) 1688-1699.
78. Lundberg DS, Teixeira PJPL. Root-exuded coumarin shapes the root microbiome. *PNAS* 2018; **115**: 5629–5631.

Tables

Table 1

Summary of permutational analysis of variance (PERMANOVA) using 'adonis' test on Bray-Curtis distance matrices for bacterial and fungal community dissimilarity assessment using 1000 permutations.

Dataset	Factor	Bacteria R2	Fungi R2
Whole	Genotype	0.02***	0.05***
	DAI	0.17***	0.08***
	Ftrt	0.06***	0.37***
	Genotype*DAI	0.10***	0.07***
	Genotype*Ftrt	–	0.02***
	DAI*Ftrt	0.05***	0.08***
	Genotype*DAI*Ftrt	0.04**	0.05***
DAI			
5	Genotype	0.13***	0.04*
	Ftrt	0.20***	0.69***
	Genotype *Ftrt	–	–
10			
	Genotype	0.13***	–
	Ftrt	0.15***	0.60***
	Genotype *Ftrt	–	–
15			
	Genotype	0.15***	0.11**
	Ftrt	0.14***	0.41***
	Genotype *Ftrt	0.07*	0.10**
20			
	Genotype	0.17***	0.16***
	Ftrt	0.09*	0.46***
	Genotype *Ftrt	–	0.10**
25			

Significance of test indicated as *** for $p < 0.001$, ** $p > 0.01$, * $p < 0.05$. R^2 is the proportion of variation explained.

Dataset	Factor	Bacteria R2	Fungi R2
	Genotype	0.14***	0.32***
	Ftrt	—	0.21***
	Genotype *Ftrt	0.08*	0.12**
Significance of test indicated as *** for p < 0.001, ** p > 0.01, *p < 0.05. R ² is the proportion of variation explained.			

Table 2: Summary of indicator species analysis identified in inoculated and non-inoculated Col-0 and Ler-0.

Sample	Bacteria	Fungi
Col-0 non-inoculated	128	36
Col-0 inoculated	115	50
Ler-0 non-inoculated	85	46
Ler-0 inoculated	65	43

Figures

Figure 1

Metabolites data-derived OPLS-DA and heatmap representation of the metabolite identified in the root of Arabidopsis accessions after being challenged with the FOM pathogen at different DAI. A) OPLS-DA score plots showing the discrimination between treatment groups of the root of Arabidopsis accessions at different infection phases after root inoculation with FOM (infected) and water (control). Hotelling's T2 statistical test cut-off was set at 95% to identify extreme outliers. B) Heatmap comparison of secondary metabolites (glucosinolates (aliphatic glucosinolates (aGLS)), indolic glucosinolates (iGLS), glucosinolates hydrolysis products, coumarins, phenolic acids, monolignols, lignans, and hormones) with log2 fold change (mean infected/mean control) and significance levels in the Arabidopsis Col-0 and Ler-0 root inoculated with FOM (n=5) compared to the control (n=5) at different infection phases. Yellow color intensity representing increased values compared to control, and blue decreased values compared to control. Significant differences between the root of control samples and those exposed to FOM are indicated by asterisks (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Figure 5

Heatmaps showing microbial OTU-metabolite correlations. Heatmaps showing A) Bacterial and B) fungal OTU-metabolite correlations heatmaps in Col-0. Heatmaps showing C) Bacterial and D) fungal OTU-metabolite correlations heatmaps in Ler-0. All microbiome and metabolite datasets generated for Col-0 and Ler-0 was used in this analysis. Metabolite-OTU associations with strong correlations ($\rho > 0.4$ or < -0.4 and $p < 0.01$) were visualized in heatmaps.

Figure 6

Metabolite-microbial OTU analysis. A) Effects of metabolites on bacterial (A) and fungal (B) community diversity. B) Indicator and metabolites co-occurrence network in inoculated and non-inoculated Col-0 and Ler-0. Numbers correspond to different metabolites shown in table. C) Indicator-hub OTUs and metabolites co-occurrence network in inoculated and non-inoculated Col-0 and Ler-0.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [MaterialsandMethods.docx](#)
- [SupplementaryFigures.pptx](#)
- [TableS1to3.docx](#)
- [TableS4DiffanalysisGenFrtBacteriaFungi.csv](#)
- [TableS5IndicSpeciesAnalysisBacFun.xlsx](#)
- [TableS6NetworkpropOTUMet.xlsx](#)
- [TableS7bOTUfOTU1Corr.xlsx](#)
- [TableS8rootmetaboliteconcentration.xlsx](#)
- [TableS9OTUMetaboliteCorrelations.xlsx](#)

- [TableS10fOTU1metaboliteCorr.xlsx](#)
- [TableS11indOTUHubOTUmetabolitecorre.xlsx](#)