**List of plausible journals for submission**

**Selected journal**

**https://www.frontiersin.org/research-topics/42731/beneficial-microbe-plant-interactions-under-bioticabiotic-stress-conditions**

Journal: frontiers in microbiology

topic: Beneficial Microbe-Plant Interactions Under Biotic/Abiotic Stress Conditions

abstract deadline: 21/sept

manuscript Deadline: 15 December 2022

Max size: 12.000 words, 15 figures/tables

Author guidelines: https://www.frontiersin.org/journals/microbiology/for-authors/author-guidelines

Based on the below list I suggest the following sequence:

1. ISME, 2- New Phytologist, 3- PNAS??, 4- Plant Cell Env

* ISME (10.3) - [https://www.nature.com/ismej/](https://www.nature.com/ismej/" \t "_blank) - I like this suggestion and think we have a good story for this journal
* ~~Frontiers in Microbiology (0.06) - [https://www.frontiersin.org/journals/microbiology](https://www.frontiersin.org/journals/microbiology" \t "_blank)~~
* Environmental Microbiology (5.49) - <https://sfamjournals.onlinelibrary.wiley.com/journal/14622920>
* FEMS microbial ecology( 4.19) - <https://academic.oup.com/femsec>
* Microbiome - (5.29) - [https://microbiomejournal.biomedcentral.com/](https://microbiomejournal.biomedcentral.com/" \t "_blank)
* Phytobiomes (3.24) - <https://apsjournals.apsnet.org/toc/pbiomes/current>
* New Phytologist – also good suggestion
* Plant Cell and Enviroment – good suggestion
* Trends in Microbiology (17.07) <https://www.sciencedirect.com/journal/trends-in-microbiology> - only for reviews, not for data papers
* PNAS (12.29) - [https://www.pnas.org/](https://www.pnas.org/" \t "_blank)
* Science of the total enviroment (7.96) - <https://www.journals.elsevier.com/science-of-the-total-environment>
* mBio (7.867) - [https://journals.asm.org/journal/mbio#](https://journals.asm.org/journal/mbio)
* PLOS biology (7.07) - https://journals.plos.org/plosbiology/
* Molecular ecology (6.18) - <https://onlinelibrary.wiley.com/joufrnal/1365294x> - good suggestion
* Soil biology and biochemistry (5.29) - <https://www.journals.elsevier.com/soil-biology-and-biochemistry>
* Biology and fertility of soils (5.5) - <https://www.springer.com/journal/374?gclid=CjwKCAjw-ZCKBhBkEiwAM4qfF72rOmZkBm0ybpgfnc10tJEldzRdise64Mwj4MUk4eyKBkW3If8-_BoCsxMQAvD_BwE>
* applied and environmental microbiology (4.93) - <https://journals.asm.org/journal/aem>
* Plant and soil - (4.01) - <https://www.springer.com/journal/11104/?gclid=CjwKCAjw-ZCKBhBkEiwAM4qfF88g0ntjZoTn4v_jYBsz4z4U9eeFlMXtYXafxvdvDmSaYHO-L4KYaRoCF78QAvD_BwE>
* Molecular Plant-Microbe Interactions (3.69) - <https://apsjournals.apsnet.org/page/mpmi/about>
* Rhizosphere (3.12) - <https://www.journals.elsevier.com/rhizosphere>
* FEMS microbiology letters (2.77) <https://academic.oup.com/femsle>

***Essential paper outline***

**Introduction**

* plant-insect-microbe interaction
* Holobiont
* data deluge

**Methodology**

* Partially written, repository gets published/open
  + 1 figure showing the experimental design [MAIN]
  + 1 fluxogram figure showing code & data [SUP]

**Results**

* Plant growth (show plants were stressed)
  + 1 figure with 4 pannels, raw plant biomass values [ MAIN?]
  + 1 figure, Cohen’s D effect size across species and variables [SUP ]
* Beta & alpha diversity (Basic analysis present in any paper)
  + 1 Figure, rarefaction curve [SUP]
  + 1 Figure, full community beta diversity [MAIN]
    - 3 tables, PERMANOVAs and pairwise comparison [3 SUP]
  + 1 figure, full community Shannon diversity [MAIN]
    - 2 tables, ANOVA and post-hoc [2 SUP]
* Random forest (show ASVs that matter on prediction)
  + 1 Figure with 4 panels, ASV abundance per treatment [SUP]
  + 3 tables: sample size + precision + kappa, confusion matrix, ASV taxonomies [3 SUP]
* Network analysis (describe networks, ASVs with importance tags, )
  + 1 figure with 4 panels showing the networks [SUP?]
  + 1 table showing differences to 1000 random networks [SUP]
  + 1 figure showing PCA of network metrics [SUP]
* Differential abundance (keep *very* short)
  + 1 Fig, bi-plot heatmap [SUP ]
* Fisher Summary (describe figure, highlight comamonadaceae)
  + 1 figure with 4 pannels, showing fisher results on tree
* Neutral models [3 figures, 2 sup tables]:
  + 1 Figure of above-selected ASBs in a venn diagram[MAIN]
  + 1 Scheme figure of data partitions, neutral modeling, ASV selection, and re-joining [SUP]
  + 1 Figure of histogram of 100 PERMANOVA results, compared to full dataset and above-selected
    - 2 tables, PERMANOVA table and pairwise comparisons [2 SUP]
  + Above-expected differential abundances (heat tree focus on comamonadaceae)
    - 1 figure with 2 pannels, has complex matrix of heat trees [MAIN]
  + Alpha diversity regression (focus on comamonadaceae)
    - 3 figures with 6 pannels each (observed, Shannon and simpson diversty at Family, Order and Class level for BO and AT) [2 SUP]
  + **Discussion**

*Methyl Jasmonate triggers plant defense*

* Validate Experimental approach
  + MeJA impacts the microbial community similarly to Oral Secretion, thus MeJA can be used in place of real insects
  + Show methodological variation on MeJA applications (foliar spray and sealed chamber)

*“Fishing” with Fisher: 1 out of 1.111 taxa highlighted by 3 different methods*

* + Show how the excess of information complicates analysis
  + Show that after using several methods we still have a complex dataset
  + the fisher test in a heat tree helps summarizing findings (approach to data deluge)

*Stress treatments were subtle on the structure of the full community but clear on the taxa occuring above neutraility*

* Advantages Methodological approach on neutral models
  + Compare base alpha and beta diversity to other references
  + the neutral spliting-and-joining can help see subtle treatment effects ;
  + the neutral tree can help locate a diversity hotspot (mention rhizobiales but limit discussion)

*Family Commonadaceae was relevant across treatments, plant species, sample types, and analysis methods*

* + show that comamonadaceae was highlighted independently in both approaches
  + Show how common they are on the roots
  + Show how common they are in the insect gut
  + Fit them in the holobiont approach (insects do more than giving a ride to Bac)

CONCLUSION:

* + AG herbivory and MeJA influence rhizosphere and endosphere microbiome in 2 brassicaeceous species
  + Comamonadaceae are important in insect-plant interactions

Glossary and abbreviations

Treatments:

* Control
* MeJA 0.1mM
* MeJA 1.0mM
* *P. brassicae* OS

Species:

* *A.thaliana*
* *B.oleracea*

Plant Compartment:

* Rhizosphere / Rhizospheric communities
* Endosphere / Endospheric communities
* Rhizosphere samples
* Root samples
* Full community (when refering to a complete dataset)

**Introduction**

In these neutral models, ASVs are classified as above the neutral (selected by the environment), as predicted by neutrality (the expectation is that ASVs that have many sequences should be found in many samples) or bellow expected (underdispersed, thus missing in some samples) . the slope defining the data’s fit to the model are based on a data-derived migration parameter, which quantifies the chance of samples being re-sampled from the same environment after random removal from the OUT table.

MeJA was already used by other authors to simulate insect attack. On the Brassicaceae *Cardamine cordifolia*, 1mM Jasmonic Actid was applied as a spray in the field, with 50ml per 0.25m² patch (<https://www.nature.com/articles/s41559-019-1085-x>)

**Materials and methods**

**We wanted to find the right concentration of MeJA that causes stress and in return induce changes in the root microbiome. For this we measured both JA-dependent gene expression and sequenced root microbiome (endosphere and rhizoplane). We chose our 2 model species *Arabidopsis thaliana* and *Brassica oleracea* Riviera as representatives for lineage I and lineage II, respectively. A brief** [**literature review**](https://wageningenur4.sharepoint.com/:x:/r/sites/MiCRop-WUR/Gedeelde%20documenten/Papers,%20citations%20and%20references/Literature_methodology.xlsx?d=w6e2546180eda41a8991a4d373a11d3bb&csf=1&web=1&e=xpDCYx) **was done to check MeJA concentrations and taking this, and the Berendsen *et al* (2018) paper as reference, it was decided to test 0.1mM and 1mM. The lower concentration was used in the Berendsen paper and 1mM was the uppermost limit if we still wanted to mimic an ecologically-relevant situation of insect herbivory (Marcel Dicke and Rita Gols, personal communication + still found in other papers). Negative controls consisted of dipping plants in 0.015% Silwet only, and positive controls consisted of 10 ul of *Pieris brassicae* caterpillar (L4-L5 instar) oral secretion (OS) applied on freshly-wounded plants using a pattern wheel (methodology recommended by Eric Poelman + literature review in next slide). Plants treated with caterpillar OS were dipped in the Silwet only solution first and left to dry before the wounding + OS application took place. For each combination of plant species (2) and treatment (4), six replicates were used consisting of an individual plant in a 1L pot.**

*Plant, soil and insects*

*Brassica oleracea* var *alba* cultivar Riviera seeds were obtained from Bejo Zaden BV, and *Arabidopsis thaliana* Col-0 seeds were obtained from XX. Seeds from both species were stratified on wet filter paper and kept at 4 °C for 48h. Stratified seeds were sown in seedling trays containing semi-natural soil and transplanted 1 week later to 1L pots containing natural soil.Plants were grown in a greenhouse compartment at 23 °C, L16:D8 photoperiod, 50-60% RH, watered two times a week and fertilized with 50 ml of ½ Hoagland solution once per week. Plants were harvested on 27/nov/2020 at five weeks old. The semi-natural soil used in this experiment was sampled from a large (100 m³) soil heap which was coll*e*cted in 2014 from the surface of an organic agricultural field in Wageningen, the Netherlands (N 51.996250 E 5.659375). Since the collection in 2014, this soil heap was kept outdoors and unmanaged. At the time of sampling in 2020 this soil heap was fully covered by approximately 58 plant species from 21 plant families, with Asteraceae as the most common family and *Atriplex* sp. dominating the area sampled for this experiment. Due to the presence of these plant species, various insects, mushrooms, and nodulating legumes we considered this soil as functional and semi-natural.

*Pieris brassicae – short description of its rearing*

*Experimental design*

The plants were exposed to four treatments three weeks after transplantation. To stress the plants, we submerged the aerial parts in either a 0.1mM methyl jasmonate (MeJA) solution, or a 1mM MeJA solution. As positive control, plants were treated with *Pieris brassicae* caterpillar oral secretion, which is an effective mimic of *P. brassicae* herbivory. For this treatment, plants were damaged with a 1cm pattern wheel, and then 10µl of oral secretion from 4th instar *P. brassicae* larvae was immediately pipetted onto the fresh wounds. As negative control, plant aerial parts were submerged in water. This treatment was repeated every four days thereafter until four applications had been made in total. To dip the plants in MeJA solutions, first a 0.05 mm mesh was placed around the base of the plants on the soil, which was left in place until the end of the experiment. Then, a plastic cover with a large central opening was placed on the surface of the pot, fully covering the edge of the pots and the edge of the mesh. With this cover of the soil the plants could be turned upside down without significant losses of soil. This allowed the aerial part of the plants to be fully submerged in the MeJA solution for 2-3 seconds without application of MeJA on the soils. The 1 mM and 0.1mM MeJA solutions were prepared from a 1M stock of MeJA 95%(Sigma 392707-5ML) diluted with 96% ethanol. The final 1L solution has 0.1% MeJA solution in ethanol 96% and 0.015% silwet. Control water solutions received only 0.1% ethanol 96% and 0.015% silwet. Positive controls were also submerged in the control solution, approximately 30 minutes before wounding and caterpillar OS pipetting. Plants were harvested 2 days after the last MeJA application. We utilized a complete randomized block design with 6 blocks and 6 replicates/treatment (figure\_experimental\_design).

*RNA extraction and RT-qPCR/Expression of JA pathway/defense signaling genes*

Leaf tissue was sampled 3 h after the last of four MeJA dippings, between 2 and 4pm.  
For *A. thaliana*, two whole leaves were collected from each plant. Older leaves were avoided.  
For *B. oleracea*, we collected four leaf punches of 1 cm ⌀ from leaves 3 and 4. For the 1mM MeJA Treatmentment we sampled leaf 3 and the most damaged leaf (usually leaf 1 or 2). All sampling materials were cleaned with ethanol in between samples. The leaf puncher and collection tubes were kept in liquid nitrogen during the sampling process until storage in -80ºC. RNA was extracted with the Isolate II RNA Plant Kit (Bioline), treated with RQ1 RNase-Free DNase (Promega), and assessed for purity and concentration with a DeNovix DS-11 FX Spectrophotometer. Some samples with OD value below …. were purified with a Monarch RNA Cleanup Kit (New England Biolabs). cDNA was synthesized with the SensiFast Reverse Transcriptase (Bioline) and RT-qPCR performed with a SensiFAST SYBR No-ROX kit (Bioline). We used the primers Lox2-s267f and Lox2-1779r to quantify LOX2 transcripts and primers BoMyc\_For and MYC2-s204r to quantify MYC2 transcripts (SUP\_table\_Primers\_qPCR\_assays). Temocycling was performed at ….. 62 anneling....

*Plant phenotyping*

Plants were harvested two days after the last dipping (35 DAS). For this, belowground tissue was dry harvested for microbiome analysis and aboveground tissue was removed to measure different traits according to the plant species. For *B. oleracea* total leaf area was measured with a leaf area meter (brand) and leaves were dried at 70◦C for 72 hours to obtain dry weight. For *A. thaliana* was not possible to measure leaf dry weight as two whole leaves were removed for qPCR analysis. Instead, as all plants were flowering, the number of siliques per plant and the dry weight of the full inflorescence were measured to characterize phenotypic effects of the treatments.

*DNA extraction and 16S amplicon sequencing*

Rhizosphere and endosphere harvesting was based on (Thiergart et al., 2019). Briefly, roots were gently shaken, and stored in a 50 ml Falcon tube with 20 ml of sterile 0.85% NaCl solution. After gently inverting the roots 10 times, they were collected with an ethanol-cleaned tweezer to a 10 ml tube with 6 ml of autoclaved 1x Tris-acetate-EDTA ( TAE) buffer with 0.05% Tween 20. The pre-washed roots were then incubated sideways on the orbital shaker for 2 min at 400 RPM. Washed roots were then collected with an ethanol-cleaned and flame-sterilized tweezer, and transferred to another tube (1x TAE with 0.05% Tween 20) for additional washing. The process was repeated for three washes in total. Fully washed roots were then transferred to a 2 ml tube, flash-frozen, and stored at –20 °C. All three washes were stored at –20 °C for later consolidation and centrifugation (15 min at 5400 *g*) to collect a rhizosphere sample. 300 µl of resuspended pellet was used as template for rhizosphere DNA extraction. DNA extraction was performed with Qiagen PowerSoil Pro kit. Roots in 2 ml tubes were lyophilized during 48h, and then they were ground to dust with bead-beating on a painshaker for 150 sec. Powdered root DNA was extracted with Qiagen dneasy plant pro. DNA extracts were submitted to 16S rRNA sequencing of the V3-V4 region after amplification with primers 341f (5’-CCTACGGGNGGCWGCAG) and 806r (5’-GGACTACHVGGGTATCTAATCC). Sequencing was performed in the Illumina MiSeq platform (PE300bp) at Baseclear (Leiden, the Netherlands). Libraries for root samples were prepared with PCR blockers to prevent amplification of plant DNA according to (Fitzpatrick et al., 2018). Raw sequence data was deposited on NCBI SRA with the access number PRJNA873942.

*Microbiome data pre-processing*

Adapter removal and demultiplexing of sequencing data was performed by Baseclear (Leiden, the Netherlands). Trimmomatic 0.39 was used to trim the ends of the sequences, and Cutadapt pluing in QIIME2 (v 2021.2) was used to remove primer sequences. QIIME2 was used to apply the DADA2 pipeline for merging, denoising, and clustering of Amplicon Sequencing Variants (ASVs). A re-trained naive bayes classifier was used to classify taxonomies with sklearn in QIIME2. Taxonomies are based on the SILVA 138 SSU release after filtering with rescript. This data was processed in the High Performance Computing Cluster Anunna (Wageningen University). All following steps were perfomed in R 4.1.2. The decontam package (Davis et al., 2018) was used to remove contaminating ASVs based on blank DNA extraction samples. After plastid and mitochondrial sequences were removed, the feature table was filtered to only contain ASVs with more than eight occurrences in the dataset.

*Statistical analysis*

Beta diversity analysis was performed with NMDS ordination and PERMANOVA testing with the phyloseq and vegan packages, respectively. Shannon diversity indices were tested with two-way ANOVA tests followed by Tukey’s HSD test. For the application of Sloan’s Neutral community models (Sloan et al., 2007), four neutral models were generated for *B. oleraceae* rhizosphere samples, and fourneutral models were generated for *A.thaliana* rhizosphere samples. Each model contains only the samples from a single stress treatment, but includes all samples from the particular plant species and compartment as a pooled source of microbes for the model’s migration parameter. This ensures that only a subset of highly related samples could be within the pool of microbes for selection in each treatment. Neutral models were not constructed for root samples because of uneven sample sizes, which can affect results (Li et al., 2018). To check for artifacts on the PERMANOVA based on ASVs selected by the neutral models, a bootstrapping approach was utilized. First, random ASVs were selected from each treatment. In each treatment, the number of selected ASVs was equal to the number of ASVs that were classified as above expected. Then, these ASVs were joined in the same dataset and tested for treatment effects in community similarity with a PERMANOVA. This process was repeated 100 times for each plant species. Finally, the p, F and R2 values of these 100 PERMANOVA's were compared with the actual p, F and R2 values of the above-expected subset and of the full community. CSS-normalized data was used for this test. The loess regression on diversity indexes between the full dataset and the above-neutral dataset was performed with the stats package. The heat trees were created with the Metacoder package, which includes differential abundance testing with Wilcoxon’s test (Foster et al., 2017). As our goal with this part of the analysis was to compare the taxonomies of ASVs occurring above expectations from neutral models in each treatment, we remove counts of ASVs if they had other neutral classifications inside a treatment. This means that if, for example, ASV\_1 is present in both Control and MeJA 0.1mM samples, but it is only classified as above-expected in Control samples, the counts of ASV\_1 in MeJA 0.1mM samples would be set to zero. This is necessary to directly compare taxonomic groups that were classified as above expected in different treatments. If all counts of such ASVs were preserved, the differential abundance testing that was already performed with Deseq2 would be simply repeated on a smaller ASV subset. In addition, the information on whether the taxonomic groups of an ASV were selected by the neutral model in either one treatment or another, which is what we aim to analyze, would be lost.

Differential abundance analysis between treatments and controls was performed with Deseq2 (Love et al., 2014). Feature selection by random forest was performed with the Boruta package. Model performance was evaluated on a 5-fold cross validation repeated 100 times (Kim, 2009) with the caret package. We generated four independent models, separating samples according to plant species and compartment. Network construction was performed with the SpiecEasi package, with network metrics calculated with the igraph package. Four independent networks were generated, according plant species and compartment. The global metrics of the networks were compared to global metrics of 1000 random networks with the same number of nodes and edges. Nodes were defined as keystone taxa according to degree, betweeness centrality, and closeness centrality. These metrics were log-transformed and z-scored, and then underwent a one-tail test against the network’s average. Pi and Zi classifications were based on (Shi et al., 2016) and calculated with the ZiPi function of the jtclaypool/microbiome package.

The analysis that summarizes the results of differential abundance, network analysis and random forest analysis is fully available online. Briefly, all ASVs that were highlighted by any of these 3 methods (tagged as “important” taxa) were put together into a single phyloseq object. Then, the proportions in the occurrence of every taxa level in these “important” ASVs were compared to all other ASVs that were not highlighted by any of the three methods (“unimportant” ASVs). These proportions were compared with the Fisher test using the the stats package. The output of these Fisher tests were then inserted into a metacoder object for visualization in a heat tree. For simplification this analysis is unweighted, therefore ASVs with more than one importance tag are treated identically to an ASV that has only been tagged only one method. Likewise, there is no discrimination between ASVs tagged by the different methods. Sources and versions of all utilized R packages were managed and recorded by the renv package for ease of reproduction. A flowchart mapping the analysis steps and tools to code chunks is available in Figure\_analysis\_fluxogram. All code scripts and full data are available at <https://github.com/PedroBeschoren/MeJA_Pilot>.

**Results**

*Plant phenotype*

Leaf dry weight and area were strongly affected by the stress treatments, with MeJA 1.0mM exposure significantly reducing *B. oleracea* biomass. For *A. thaliana*, the number of fruits MeJA 1.0mM exposed plants was significantly reduced, while the reduction of inflorescence dry weight was not significant. Nonetheless, MeJA 1.0mM exposure could clearly impact plant development when compared to controls (figure\_plant\_phenotype), and necrotic spots could be observed in the surface of leaves after the first MeJA 1.0mM dipping event (figure\_plant\_pictures). These lesions were clearer in *B. oleracea* than in *A. thaliana*. Interestingly, such lesions only occurred after the first dipping with MeJA 1.0mM: subsequent dippings with MeJA 1.0mM did not cause further lesions. When exposing *B.oleraceae* to intermediate MeJA concentrations in another experiment (data not shown), we observed that a 0.5mM MeJA application did not cause these necrotic spots, but prevented their appearance on a subsequent dippings of MeJA 1.0mM. Finally, *B.oleraceae* leaves surface had a noticeable higher amount of wax when exposed to higher concentrations of MeJA

*Beta and Alpha diversity for the whole microbial community shows limited treatment effect*

As expected, the factors plant compartments(endo- and rhizosphere) and plant species (*B. oleraceae* an *A. thaliana*), explain most of the variance in the dataset, with R² of 0.16 and 0.10, respectively (SUP\_Permanova\_table\_a). These factors clearly separated the samples on the ordination space (Figure\_Beta\_diversity\_all\_samples). The effects of stress treatment are dependent on the plant species, as noted in significant interactions, but explain little variance (F = 1.841, R² = 0.04, p = 0.001). When analyzed separately, we found significant effects of stress treatment in endophytic communities of both plant species and in the rhizospheric communities of *A. thaliana* (SUP\_Permanova\_table\_b). After FDR correction for multiple testing in pairwise treatment comparisons, endosphere microbial communities were significantly different between 0.1 and 1mM MeJA treated *A.thaliana* plants. No other pairwise comparison was significant. (SUP\_Pairwise\_permanova\_table).

*~~Alpha diversity~~*

The Shannon diversity index indicated a clear interaction between plant species and root compartments (p = 0.0004, SUP\_table\_alpha\_diversity\_tests). As expected, rhizosphere communities were more diverse than endosphere communities. *A.thaliana* had higher diversity than *B.oleracea* on rhizosphere communities, while *B.oleracea* had more diversity than *A.thaliana* on endsophere communities (Figure\_alpha\_diversity, SUP\_table\_alpha\_diversity\_pairwise).There was a significant stress treatment effect, as diversity was generally overall lower in controls and MeJA 1.0mM applications than Meja 0.1mM and oral secretion treatments.

*Random Forest*

The Boruta function tagged 41 ASVs as important to predict the stress treatment in endosphere and rhizosphere communities of both *A. thaliana* and *B. oleracea*. We observed that models based on endophytic communities had a higher number of ASVs classified as important when compared to rhizosphere communities. While accuracy and Kappa for the *A.thaliana* endophytic and rhizospheric models were quite similar, it was much lower in the rhizosphere communities of *B. oleraceae* when compared to endophytic communities (SUP\_Table\_RF\_output). As can be seen in the confusion matrixes, *P. brassicae* OS treatments had the overall lowest prediction accuracy (SUP\_table\_confusion\_matrix). The abundance of the 41 ASVs selected by random forest can be seen in figure Figure\_rf\_ASVs. The taxonomies of these ASVs and their importance for the Boruta model are shown in SUP\_table\_rf\_taxonomies. The most common genera is *Massilia* (4 occurences), the most common family is Commonadaceae (9 occurences), and the most common order is Burkholderiales (18 occurences). The highest mean ASV importance is found in ASVs from genus Mucilaginibacter for B. oleraceae rhizosphere; genus Leptothrix for *B. oleraceae* endosphere; genus Asticcacaulis for *A.thaliana* rhizosphere; and genus Niastella for *A.thaliana* endosphere. There were no ASVs that were important in more than one sample partition.

*Network Analysis*

We created four co-variance networks, according to root compartment and plant species, which could be differentiated from random networks with the same number of nodes and edges. (SUP\_table\_random\_networks). Networks based on rhizosphere communities were more complex than networks based on endosphere communities. They presented a higher number of nodes, number of edges, average degree, and maximum module size. Endosphere communities presented higher modularity, and higher ratio of positive to negative edges. The node degree of *B.oleraceae* samples presented a higher fit to power law than *A. thaliana samples*. The total number of keystone nodes, module connector nodes, and module hub nodes were also higher in rhizosphere community networks than in endosphere community networks (SUP\_figure\_network\_metrics\_PCA). There was a total of 34 ASVs tagged as keystone taxa, module connectors, or module hubs for *A.thaliana* and 47 for *B.oleracea.* From the 8 keystone taxa, four were found in the rhizosphere communities of *B. oleraceae*, including ASV\_410. This ASV, from genus Nocardioides, was also classified as above neutral model predictions in all treatments and as a predictor of treatment classification by the random forest algorithm. It was the only ASV in with 3 different “tags” of importance from different methods.

*ASV Differential abundance per treatment*

Differential abundance analysis performed by deseq2 indicated 117 unique ASVs as differentially abundant across all pairwise comparisons between controls and treatments. Endosphere samples clearly had more differentially abundant ASVs than rhizosphere samples, and *A.thaliana* had more differentially abundant ASVs than *B. oleracea* While root compartment and plant species were separated in a bi-cluster heatmap, there was no clear pattern for groups of ASVs occurring in the same treatments (SUP\_Figure\_deseq2\_heatmap). The most represented families are Oxalobacteriaceae and Comamonadaceae, with 18 and 13 ASVs, respectively.

*A visual summary of differential abundance, network analysis and random forest*

When combined, differential abundance, random forest and network analysis tagged 346 different ASVs as important across the sample partitions. This pool of selected ASVs was represented in a heat tree, where we also represent fisher tests to compare proportions of each taxonomic level in the important ASV subset against the rest of the community (Fisher\_test\_relevance). The test essentially returns, after a p adjustment, whether a specific taxa is significantly more represented in the important ASV subset than on the rest of the community. For example, 8 of the 53 important ASVs in *B.olerceae* soil samples are from the genus Mucilaginibacter, while 57 out of 2499 ASVs in the non-important ASV set are from the genus Mucilaginibacter. A one-tailed fisher test indicates there is a significant difference in these proportions (padj = 0.0228) with an odds ratio of 4.18. Thus the proportion of Mucilaginibacter in the important ASV set is significantly higher than the proportion of Mucilaginibacter in the non-important ASV set. This approach allows us to summarize the output of the 3 methods across all taxonomic levels of both species and root compartments at a glance.

It can be observed that family Commonadaceae was highlighted as overrepresented in the important ASV sets in *B. oleraceae* endosphere and rhizosphere, as well as rhizosphereof *A. thaliana*. We also highlight order Rhizobiales as important in endosphere and rhizosphere of *B. oleraceae*, and genus Flavobacterium in endosphere- and rhizosphere of *A. thaliana*. Finally, Genus Streptomyces is very clearly highlighted as relevant in the endosphere of *A.thaliana*, and family Xanthobacteriaceae as important in the rhizosphere of *B. oleraceae.*

*Sloan’s Neutral models indicated taxa that separate treatments in beta diversity plots*

Rhizosphere communities from each plant species were used to construct one neutral model for each treatment. We could observe that the model fit across treatments was similar between both species (mean r² = 0.603 ± 0.018 for *A. thaliana* and mean r² = 0.597 ± 0.017 for *B. oleracea*). Plant species and treatments presented similar proportions of ASVs classified as above neutrality (11.18±1.06%), as expected by neutrality (85.51±1.25%), and below neutrality (3.30±0.33%). There were 377 ASVs for *A. thaliana* and 336 ASVs for *B. oleraceae* that were classified as above-expected by the neutral model. Half of these ASVs were exclusive to the different treatments, and 17 to 20% were shared between all treatments (figure\_venn\_diagram). To explore these ASVs, the microbial communities were first filtered to only include ASVs that were classified as above expected in at least one treatment, and then re-joined (SUP\_NeutralModels\_scheme).When these subsets were evaluated in a PERMANOVA, role of stress became more evident than in the full community. The R2 values for treatment effects increased from 0.1416 to 0.2259 in *A. thaliana* and 0.1442 to 0.2278 in *B. oleracea* (Table\_Permanovas\_above\_neutral, SUP\_Permanova\_table\_b). Pairwise comparisons between treatments were highly significant (SUP\_table\_pairwise\_comparisons\_neutral), and ordinations indicate a slightly clearer distinction between some treatments (NeutralModel\_ordinations\_provisory\_SUP).

To check whether the observed increases in R2 of stress treatment effects were due to ASV picking from within the 4 different treatments followed by re-joining, we used a bootstrapping approach by picking random ASVs from the same samples 100 times and then performing a PERMANOVA. The above-neutral ASV subset presented lower treatment effect p values, and higher treatment effect R2 and F values, than the random ASV subsets or the full community model. The p, R2, and F values of the full community fall within the distribution of the random ASV subsets, indicating that picking ASVs present in different samples and later re-joining the dataset does not cause the observed changes in p, R2, and F values (NeutralModel\_100\_permanovas\_histogram) This approach indicates that differences in community composition due to stress effects are better represented by the above-neutral subset than by the full community. These improvements of statistical metrics are important in our analysis as treatment effects are subtle, thus requiring in-depth exploration to notice relevant differences. *Taxonomic groups of ASVs occurring above expected values of a neutral model*

To evaluate the diversity of the above-neutral ASV subsets, we fitted alpha diversity indexes of every taxonomic group in the above-neutral ASV subsets against the same taxonomic groups in the full community (SUP\_figure\_alpha\_correlation\_Family, SUP\_figure\_alpha\_correlation\_Order\_Class). Class Actinobacteria, Order Xanthomonadales, and Family Commonadaceae are all above the confidence interval of a loess regression between the diversity of above-selected ASVs and the diversity of the complete dataset. This pattern holds for the observed number of taxa, Shannon diversity, and Simpson diversity for both plant species. Family Comamonadaceae was the most diverse family in the above-neutral ASV subset for *A. thaliana* and the second most diverse family for *B. oleracea* and in all diversity metrics ( SUP\_figure\_alpha\_correlation\_Family,)

Some taxonomic groups occurring above expectation of the neutral model were differentially abundant in pairwise comparisons between treatments. This can be shown with the hierarchical taxonomies of ASVs in a large phylogenetic tree, with the small “heat trees” highlighting differentially abundant ASVS of each pairwise comparison in a matrix (figure Neutral\_heat\_trees). This matrix of heat trees can be interpreted by looking for (dis)similarities across rows and columns. For example, some taxa are consistently more abundant in all the treatments when compared to controls. This is the case for the genera *Nitrospira*, *Caulobacter*, *Pedobacter* and *Streptomyces* in *A. thaliana* and the genera *Bosea*, *Pelomonas*, *Sporocytophaga*, and genus 67-14 from order Olirubrobacterales in *B. oleraceae*.

In addition, hotspots of differential abundance, where different members of a supertaxon are being highlighted in different treatments, can be found in some taxons. This is the case for Family Comamonadace in *B.oleracea*, as all pairwise comparisons between treatments include genera that are differentially abundant in both compared treatments. This also occurs in the Order Rhizobiales and Burkholderiales, and phylum Actinobacteriota. In *A. thaliana*, Order Rhizobiales, Class Alphaproteobacteria, Class Gammaproteobacteria, and Class Bacteroidia also act as hotspots of differential abundance. We consider Family Comamonadaceae to be also acting as a hotspot of differential abundance in *A. thaliana*, even as Control and MeJA 1.0mM treatments do not have a Comamonadaceae genus that is more abundant than in *P. brassicae* OS. This sets Family Comamonadaceae as the highest resolution taxonomy that acts as a hotspot of diversity by differentially responding to different treatments. Members of this family are highlighted in figure Neutral\_heat\_trees.

**Discussion**

*Methyl Jasmonate triggers plant defense*

The observed increase in LOX2 and MYC2 expression for *A.thaliana*, the occurence of necrotic spots for *B. oleraceae*, and reduction in plant dry weight for both species indicates that the plants were stressed by the dippings with Methy Jasmonate. This was expected. There are many studies that use MeJA as an stress inducer in different plants. Wheat (Liu et al., 2017) and Arabidopsis (Carvalhais et al., 2013) have been induced by applying MeJA in cotton balls, and then sealing the atmosphere in growth trays. Foliar MeJA sprays were used to induce stress in the pine tree *Larix olgensis* (Jiang and Yan, 2018) and the tallow tree *Triadica sebifera* (Xiao et al., 2019) and tomato (Zygadlo et al., 2022). Arabidopsis has been exposed by dipping the plants in MeJA solutions in four-day intervals (Roeland L. Berendsen et al., 2018). it is well known that plants must choose between different defense pathways, more specifically methyl jasmonate for chewer herbivores and necrotrophic phatogens and Salicilic Acid for sucker herbivores and biotrophic pathogens (Smets and Koskella, 2020). In this work we avoided foliar sprays as abaxial stomata may be unable to absorb the hormone, while dipping the plants was more convenient than sealing atmosphere in trays.

*“Fishing” with Fisher: 1 out of 1.111 taxa highlighted by 3 different methods*

A major challenge in microbiome analysis is addressing the complexity and diversity of microbial communities. Several analysis methods, each with their own advantages and limitations, have been developed to address this issue. Random forest, network analysis, and differential abundance can all identify ASVs of special relevance for an experimental system. While applying different methods may help alleviate their individual drawbacks, it is still difficult to summarize their output, especially when considering the hierarchical taxonomic levels across plant species and sample types. Therefore, focusing on the 346 ASVs detected as important in these 3 methods is a challenge on its own, without tools designed to address it. Our approach was to use a statistical test to check if a taxa level has been overly represented in the list of “important” ASVs, considering the microbial community in the same dataset as a background. Once this is performed for all taxa levels in all “important” ASVs, we plot the results in heat trees from the metacoder package, a recent tool on visualizing taxonomies of microbial communities. A relevant feature of our approach is that it is independent of the tools that tag ASVs as relevant or not. For example, network analysis could be replaced by indicator species analysis, or random forest could be substituted by genetic algorithms. As long there is a list of “important” ASVs drawn from a full microbial community, our approach can be applied to summarize results from different methods. In our data, this approach highlighted family Comamonadaceae as overly represented in the important ASV set in 3 our of 4 sample partitions, which facilitated further analysis and in depth discussion in only 1 out of the 1.111 different taxonomic groups evaluated in this report.

This visualization also gives us a taxonomic pathway: instead of stating that proteobacteria will be important in soil systems, like has been done many times before, we could find the right taxonomic level that responds to our experimental system. The Suprataxons for family Commonadaceae (order Burkholderiales, class Gammaproteobacteria, and phylum proteobacteria) are also all highlighted in these data partitions. However, the heat tree visualization suggests that Commonadaceae could be driving the relevance of these suprataxons in this analysis. An infrataxon to family Commonadaceae, the plant pathogenic genus *Acidovorax* (Fujiwara et al., 2022), presented a very high fold ratio in the rhizosphere communities of *b. oleraceae*. This indicates that this genera is clearly much more present in the important ASV subset than on the non-important ASV subset, and could be driving the weight given to family Commonadace in this sample partition. Genus Streptomyces is also clearly highlighted as overrepresented in the important taxa of A. thaliana roots. This genus has also been highlighted on the neutral model analysis, as above-expected Streptomyces are more abundant in the treatment conditions than in the controls.

*Stress treatments were subtle on the structure of the full community but more clear on the taxa occurring above neutrality*

Plant compartment and genotype effects are well known to shape microbial communities (Berendsen et al., 2012), thus the clear clustering observed in the full community (Figure\_Beta\_diversity\_all\_samples**)**  was expected. Previous studies indicate that microbial communities of MeJA-treated plants can be indistinguishable from controls in *A. thaliana* (Roeland L Berendsen et al., 2018; Doornbos et al., 2011), different in endophytic but not rhizospheric or bulk soil communities in wheat (Liu et al., 2017), different in rhizospheric communities but not on bulk soils of *A. thaliana* (Carvalhais et al., 2013), and different in phylosphere tomato communities in plants that were cultivated with Selenium (Zygadlo et al., 2022). We found that community differences due to the experimental stress treatment could be significant depending on plant species and plant compartment, but were subtle overall. It has been previously reported that the root endophytic communities of wheat have lower diversity when exposed to MeJA (Liu et al., 2017). In the phylosphere of tomato plants exposed to Selenium, MeJA could increase Chao-1 and Shannon diversity (Zygadlo et al., 2022). We report that the effect of MeJA stress treatments on Shannon diversity depends on plant species and plant compartment, with slightly higher diversity in MeJA 0.1mM treated *B. oleracea* plants*.* Previous studies on MeJA effects in plant microbiomes did not test more than one plant species, nor applied neutral models, random forest or network analysis to microbiome data.

When analysis is focused on the ASVs classified as above expected by the Sloan neutral community model, the variance on microbial communities explained by stress treatment effects increases by 50% when compared to the full communities (NeutralModel\_100\_permanovas\_histogram). In the context of subtle treatment effects taking place in microbial communities that respond strongly to plant species and plant compartment, such increase in explained variance indicates that parts of the community are more tightly related to experimental treatments than others. We show that the ASVs selected by each treatment were very different (figure\_venn\_diagram), indicating that each treatment selects for a particular community subset. This is further substantiated by the PERMANOVA pairwise treatment comparisons, which are almost all significant (SUP\_Pairwise\_permanova\_table\_neutral). It is also relevant to notice the overlap between *P. brassicae* OS treatment and MeJA 0.1mM treatments in the above-expected ordinations (NeutralModel\_ordinations\_provisory). Although they will be selecting for different ASVs, their community profile is rather similar. These also indicates that MeJA 1mM might cause community effects that are too extreme and too different from the *P. brassicae* OS treatment. Taken together, we consider that the effects of MeJA 0.1mM treatments on the neutrally-selected microbial communities are similar enough to the *P. brassicae* OS treatment to be used as a proxy for insect herbivore pressure in Brassicaceae plants. Previous work using MeJA as a proxy (Roeland L. Berendsen et al., 2018; Carvalhais et al., 2013; Doornbos et al., 2011; Liu et al., 2017) did not apply neutral community models. More importantly than filtering the data to obtain better p, R2 or F values, focusing the analysis on the above-expected ASVs helps detect the differences between the treatments that could otherwise be missed.

The neutral model expectation is that ASVs occurring in high mean relative abundance (that is, have a large number of reads) should also have high frequency (are present in many samples) because they are neutrally or stochastically distributed. It has already been reported that the majority of ASVs fits the neutral model, from fish guts (Burns et al., 2015; Heys et al., 2022), to human lungs (Venkataraman et al., 2015), bacterioplankton (Wang et al., 2020), and geographically distant soils (Barnett et al., 2020). A deterministic process could shift the community away from neutrality (Barnett et al., 2020), with microbes more fit to the environment occurring more often than predicted by the model. These above-expected ASVs may represent “positively selected” microbes (Burns et al., 2015). Higher selection pressure in the community can potentially be seen as lower fits to the model, such as lower neutrality fit in fish gut microbes as the animal develops (Burns et al., 2015; Heys et al., 2022), alterations in bacterioplankton during cyanobacterial blooms (Wang et al., 2020), or in soil microbes in later successional stages after a disturbance (Barnett et al., 2020). In our study, both plant species had similarly high fits to the neutral model, and roughly the same proportion of ASVs occurring above expected. This means that much of the community is neutrally assembled, and while treatments may select different ASVs they do not drastically change the intensity of selection pressures for the microbial community. More dramatic stress conditions might change this scenario.We consider that this approach of detailing above-selected ASVs is very useful when treatment effects are subtle. Our bootstrapping approach to detect artefacts show that a same number of ASVs picked random from the same samples cannot explain as much variance as the above-selected ASV subset . At the same time, the randomly picked ASVs show PERMANOVA metrics that fall within the full community, indicating that the data splitting and re-joining did not cause the observed differences. It is worth mentioning that the input data for Sloans’ neutral models should be rarefied. Rarefaction can normalize different sequencing library sizes, but it is currently under criticism and disuse (McMurdie and Holmes, 2014). While rarefaction level seems to be of little relevance in neutral models (Weiland-bra et al., 2019), approaches like repeated rarefaction (Cameron et al., 2021) could help alleviate the issue.

*Family Commonadaceae was relevant across treatments, plant species, sample types, and analysis methods*

The summarization of differential abundance, network analysis, and random forest indicates family Commonadaceae as over-represented in the set of ASVs tagged as important when compared to the ASVs not tagged by these methods. We also fitted the diversity of families within the above-neutral subset against the diversity of families in the full datasets (**figure\_alpha\_correlation\_shannon**), showing that Comamonadaceae were overly diverse in the above-neutral subset. Family Comamonadaceae was ralso elevant because there is always a different member in this taxa being selected by the different treatments in pairwise comparisons (**Neutral\_heat\_trees**). Note that the fisher summarization does not consider any results from the neutral model, therefore, both analyses are independent. Although the endosphere of *A.thaliana* does not highlight Comamonadaceae as an important taxa in the fisher summary, we focus the discussion in this family.

Comamonadaceae have a very diverse physiology and live in a wide range of habitats. They have been detected in plants both as a pathogenic and as beneficial microorganism (Willems, 2014), and are have been described as enriched inside root systems (Bulgarelli et al., 2015; Edwards et al., 2015; Hacquard et al., 2015; Li et al., 2020). Members of the family have been associated to N nutrition (Cope-Selby et al., 2017; Pagé et al., 2019) including N fixation in rice (Han et al., 2005), heavy metal tolerance in phytoremediation (Chen et al., 2018), soil disease suppression (Li et al., 2015), suppression of phytopathogenic fungi trough negative network correlations (Durán et al., 2018), infestation of withe grubs (Geng et al., 2018), causation of fruit blotch in cucurbitaceae (Fujiwara et al., 2022), and also resistance to Fusarium wilt disease in cucurbitaceae. ~~As environmental contexts like soil diversity levels can determine if the same strain can be detrimental or beneficial for plant development (Beschoren et al., 2020), having both pathogenic and beneficial microorganisms on the same family is unsurprising.~~

Comamonadaceae are also important in digestive systems of animals with poor diets (Willems, 2014), such as herbivorous insects and even algae-consuming carnivorous plants (Sirová et al., 2018). Comamonadaceae have been found to be abundant in the guts of the herbivore beetles *Dactylispa xanthospila* (Cui et al., 2021), cycad-feeding Rhopalotria furfuracea (Salzman et al., 2018), *Cryptocephalus* (Montagna et al., 2015)*,*  all life stages of the poor-diet dung beetle *Onthophagus Taurus* (Estes et al., 2013), besides larval stages of Lepidoptera *Trichoplusia ni* (Leite-Mondin et al., 2021), cycad-feeding *Chilades pandava* (Salzman et al., 2018), pyralid moths (Zhu et al., 2021), the sap-feeding white fly *Bemisia tabaci* (Santos-Garcia et al., 2020), and the springtail *Orchesella cincta* (Bahrndorff et al., 2018). Therefore, it is likely that members of this family are actively shared and transferred between plants and herbivores, having significant roles in health and disease for both hosts. This could increase Comamonadaceae’s niche breath and dispersal, similarly to what happens in Enterobacteriaceae and Pseudomonadaceae, which are also very common and highly functional in both plant roots and animal guts (Brennan et al., 2022; Costa et al., 2014). For example*, Enterobacter ludwigii* was isolated from oral secretion of the fruitworm *Helicoverpa zea*, and its inoculation on tomato plants increased plant fruit and seed production compared to non-inoculated controls (Pan et al., 2019). A Pseudomonas strain isolated from *Plutella* moths larvae guts has shown to increase tomato growth and exhibit trains like tricalcium phosphate solubilization and production of indole 3-acetic acid (Indiragandhi et al., 2008). Indole-3-acetic acid, a key plant hormone that interferes with JA and SA signaling (Wielkopolan and Obrępalska-Stęplowska, 2016), can be produced in high quantities by endophytic Enterobacteriaceae (Costa et al., 2014) but also has a role on gut metabolism of humans (Roager and Licht, 2018) and insects (Dolan et al., 2022). As per the holobiont concept, it is not surprising that the microbiomes of plants and herbivores will actively interact. It is difficult, however, to define specific microbial players with precision (beyond phylum level) or consistency (across analysis pipelines) in these complex interactions. Here we pinpoint family Comamonadaceae as excessively present in community subsets that only includes ASVs of interest with different methods, in different plant species, and in different plant compartments. This finding calls for strain-level resolution and functional screening of Comamonadaceae in both plants and herbivorous insects. It has already been established that soil fauna can transport microbes (Yang and van Elsas, 2018), and that feeding can introduce bacteria to insect gut (Wielkopolan and Obrępalska-Stęplowska, 2016). While insect oral secretions can also introduce microbes into the plants, like putative pathogenic *Pseudomonas* species (Humphrey and Whiteman, 2020), our results show that MeJA expositions can also recruit Comamanadaceae, and RF shows their presence is not an exclusive indication of oral secretion**.** Therefore Comamonadaceae was stimulated by the plant, from the soil, as a response to herbivore stress signals.

There are also other taxa highlighted as relevant in our summary and neutral approach, like Nitrogen-fixing family Rhizobiales, that can be interacting with insect guts. Rhizobium has been found in the gut of herbivorous insects (Leite-Mondin et al., 2021), sometimes associated relevant nutritional roles like methane degradation (Montagna et al., 2015) or N nutrition in termites (Fröhlich et al., 2007), ants (Jackson et al., 2022) and *Plutella* moths (Indiragandhi et al., 2008). As members of Comamonadaceae can also participate of nitrogen cycling in plants (Bahulikar et al., 2021; Yi et al., 2022), it becomes a possibility that some Comamonadaceae may have roles in N nutrition in insects.

**Conclusion**

Here we could show that the bacterial family Comamonadaceae is important in the response to herbivore stress. To demonstrate this we applied several different microbiome analysis methods, each with their own principles and biases. These methods however are similar in their ultimate output: they tag ASVs as being relevant or not. Both the neutral model and fisher summary analysis pipelines indicates that of all taxonomic levels and groups Family Comamonadaceae is strongly represent in the relevant ASV set. This occurred in the rhizosphere of both *A. thaliana* and *B. oleracea* and in the endosphere of *B. oleracea*. Visualization and testing at all taxonomic levels was critical to notice this family as relevant. As we provide full data with commented code, this approach can be implemented and improved by other researchers. We did not demonstrate a mechanism by which Comamonadaceae interacts with plant herbivore response, but literature suggests that nitrogen fixation and indole-3-acetic acid production by bacteria could play a role. This should be investigated with strain-level resolution metagenomics, isolation of Comamonadaceae strains from plants and insects, and inoculation and tracking of Comamonadaceae in the insect-plant holobiont.

OTHER NOTES AND POSSIBLY INTERESTING REFERENCES

Arabdopsis mutants unable to produce JA (including a MYC2 mutant) have showed distinct exudation patterns, including lower amounts of asparagine, ornithine, and tryptophan, as well as distinct bacterial and archaeal community composition, as illustrated by an increased abundance of Streptomyces, Bacillus, and Lysinibacillus taxa in the med25 rhizosphere and an Enterobacteriaceae population in myc2. Alternatively, the Clostridiales population was less abundant in the rhizosphere of both mutants. (Carvalhais et al., 2015)

Comamonadace abudances in soybeans are known to be affected by isoflavonoids (Pang et al., 2021). Silencing of isoflavone synthase increased the relative abudnaces of Commonadaceae (White et al., 2017), while soils treated with the isoflavone daidzein soils increased the abundances of comamonadaceae (Okutani et al., 2019).

~~Comamonadaceae originating from plants have been found in the gut of Pyralidae (chewer)~~ but not cicada (sucking) insects. a large portion of the Pyralidae gut microbiome was estimated to be sources from the host plant microbiome, which did not happen in Cicada and mammalian herbivores. The more aerobic insect gut environment could play a role in the survival of aerobic plant-associated microbes (Zhu et al., 2021)

~~The Lepidoptera~~ *~~Trichoplusia ni~~* ~~also has comamonadacea in the gut, whether it is fed with A.thaliana or tomato leaves (Leite-Mondin et al., 2021)~~

~~The guts of cycad-feeding Chilades pandava (Lepidoptera)and Rhopalotria furfuracea (Coleoptera) are partly dominated by Comamonadaceae (Salzman et al., 2018)~~

~~Presence of Comamonadaceae differentiates the gut of the whitefly Bemisia tabaci (Hemiptera) when it is feeding from the sap of watermelon (suitable host) and pepper (less suitable host) (Santos-Garcia et al., 2020)~~

~~In (Humphrey and Whiteman, 2020) putative phatogenic pseudomonas were more abundant in the phylosphere of brasiceaceease plants that were attacked by herbivores, likely because of oral secretions. Thus our oral treatment positive control may have introduce microbes we would never be able to find in the MeJA treatments~~

~~Comamonadaceae is part of the main taxa in the gut of the springtail Orchesella cincta (Bahrndorff et al., 2018)~~

~~Comamonadace abudances are affected by isoflavones  and daidzein  (Pang et al., 2021). Isoflavonoids might be inhibiting commonadaceae but increasing Xanthomonads; acidovorax is from this family and is a plant-pathogenic genus (White et al., 2017). Dadzein-treat soils increased the abundances of comamonadaceae (Okutani et al., 2019)~~

~~Comamonadaceae as more abundant in root samples than on stool samples; also shows no overlap in most abundant taxa between roots & mammal guts (Hacquard et al., 2015). Pedro has to read this more in-depth!~~

Comamonadaceae (and fusarium) are recognized by Arabidopsis immune system as it shares a motiff (SCOOP) very similar to a brassiceaceae **[ paper with details of molecular interactions has to be thoroughly read, but this might be better off in Karen’s hands]** (Hou et al., 2021)

Indole acts as an inter-kingdom communication component that affects plants, insects and microbes from parasitoid recruitment to adhesion of intestinal cells (Tomberlin et al., 2017).

Plant-insect-microbiome review, by auther present in mICROPe: (Dolan et al., 2022) obligate insect endosymbiontes have smaller genomes, so this could filter out shared insect-plant holobionts. Phloem and xylem sucking insects relying on microbiomes for essential aminoacids. Generalist insects might transmit more microbes than specialists due to host range and microbe pickup. Auxin signaling is used in insect metabolism for nutrient metabolism. The review also extensively details the JA-SA balance and how this is exploited by insects and their microbes. Family comamonadaceae, however, is not highlighted at all.

Comamonadaceae seems to be quite prevalent in the gut of different insect species (Bahrndorff et al., 2018; Estes et al., 2013; Leite-Mondin et al., 2021; Santos-Garcia et al., 2020) suggesting the insect and plant could be alternating hosts, helpling on bacterial dispersal and survival. It can even be abundant in the lumen of algae-consuming aquatic carnivorous plants (Sirová et al., 2018): (“Members are considered important for the digestion of nutritionally poor diet of animal hosts”). In fact, members of this family are good competitors This is similar to what occurs in human entorobacteria found in plant roots.

Bahrndorff, S., De Jonge, N., Hansen, J.K., Lauritzen, J.M.S., Spanggaard, L.H., Sørensen, M.H., Yde, M., Nielsen, J.L., 2018. Diversity and metabolic potential of the microbiota associated with a soil arthropod. Scientific Reports 2018 8:1 8, 1–8. doi:10.1038/S41598-018-20967-0

Bahulikar, R.A., Chaluvadi, S.R., Torres-Jerez, I., Mosali, J., Bennetzen, J.L., Udvardi, M., 2021. Nitrogen Fertilization Reduces Nitrogen Fixation Activity of Diverse Diazotrophs in Switchgrass Roots. Phytobiomes Journal • 2021 • 5, 80–88. doi:10.1094/PBIOMES-09-19-0050-FI

Barnett, S.E., Youngblut, N.D., Buckley, D.H., 2020. Soil characteristics and land-use drive bacterial community assembly patterns. FEMS Microbiology Ecology 96, 194. doi:10.1093/FEMSEC/FIZ194

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends in Plant Science 17, 478–486. doi:10.1016/J.TPLANTS.2012.04.001

Berendsen, Roeland L., Vismans, G., Yu, K., Song, Y., De Jonge, R., Burgman, W.P., Burmølle, M., Herschend, J., Bakker, P.A.H.M., Pieterse, C.M.J., 2018. Disease-induced assemblage of a plant-beneficial bacterial consortium. The ISME Journal 2018 12:6 12, 1496–1507. doi:10.1038/s41396-018-0093-1

Berendsen, Roeland L, Vismans, G., Yu, K., Song, Y., Jonge, R. De, Burgman, W.P., Burmølle, M., Herschend, J., Bakker, P.A.H.M., Pieterse, C.M.J., 2018. Disease-induced assemblage of a plant-bene fi cial bacterial consortium 1496–1507. doi:10.1038/s41396-018-0093-1

Beschoren, P., Dirk, J., Elsas, V., Mallon, C., Gustavo, L., Maria, L., Passaglia, P., 2020. Efficiency of probiotic traits in plant inoculation is determined by environmental constrains. Soil Biology and Biochemistry 148, 107893. doi:10.1016/j.soilbio.2020.107893

Brennan, F.P., Alsanius, B.W., Allende, A., Burgess, C.M., Moreira, H., Johannessen, G.S., Castro, P.M.L., Uyttendaele, M., Truchado, P., Holden, N.J., 2022. Harnessing agricultural microbiomes for human pathogen control. ISME Communications 2022 2:1 2, 1–6. doi:10.1038/s43705-022-00127-2

Bulgarelli, D., Garrido-Oter, R., Münch, P.C., Weiman, A., Dröge, J., Pan, Y., McHardy, A.C., Schulze-Lefert, P., 2015. Structure and Function of the Bacterial Root Microbiota in Wild and Domesticated Barley. Cell Host & Microbe 17, 392–403. doi:10.1016/J.CHOM.2015.01.011

Burns, A.R., Stephens, W.Z., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K., Bohannan, B.J.M., 2015. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. The ISME Journal 10, 655–664. doi:10.1038/ismej.2015.142

Cameron, E.S., Schmidt, P.J., Tremblay, B.J.M., Emelko, M.B., Müller, K.M., 2021. Enhancing diversity analysis by repeatedly rarefying next generation sequencing data describing microbial communities. Scientific Reports 2021 11:1 11, 1–13. doi:10.1038/S41598-021-01636-1

Carvalhais, L.C., Dennis, P.G., Badri, D. V., Tyson, G.W., Vivanco, J.M., Schenk, P.M., 2013. Activation of the Jasmonic Acid Plant Defence Pathway Alters the Composition of Rhizosphere Bacterial Communities. PLOS ONE 8, e56457. doi:10.1371/JOURNAL.PONE.0056457

Carvalhais, L.C., Dennis, P.G., Badri, D. V, Kidd, B.N., Vivanco, J.M., 2015. Linking Jasmonic Acid Signaling , Root Exudates , and Rhizosphere Microbiomes 28, 1049–1058.

Chen, Y., Ding, Q., Chao, Y., Wei, X., Wang, S., Qiu, R., 2018. Structural development and assembly patterns of the root-associated microbiomes during phytoremediation. Science of The Total Environment 644, 1591–1601. doi:10.1016/J.SCITOTENV.2018.07.095

Cope-Selby, N., Cookson, A., Squance, M., Donnison, I., Flavell, R., Farrar, K., 2017. Endophytic bacteria in Miscanthus seed: implications for germination, vertical inheritance of endophytes, plant evolution and breeding. GCB Bioenergy 9, 57–77. doi:10.1111/GCBB.12364

Costa, P.B., Granada, C.E., Ambrosini, A., Moreira, F., Souza, R., Passos, J.F.M., Arruda, L., Passaglia, L.M.P., 2014. A Model to Explain Plant Growth Promotion Traits : A Multivariate Analysis of 2 , 211 Bacterial Isolates. PloS One 9, 1–25. doi:10.1371/journal.pone.0116020

Cui, L., Guo, Q., Wang, X., Duffy, K.J., Dai, X., 2021. Midgut bacterial diversity of a leaf-mining beetle, Dactylispaxanthospila (Gestro) (Coleoptera: Chrysomelidae: Cassidinae). Biodiversity Data Journal 9, 1–16. doi:10.3897/BDJ.9.E62843

Davis, N.M., Proctor, Di.M., Holmes, S.P., Relman, D.A., Callahan, B.J., 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6, 1–14. doi:10.1186/S40168-018-0605-2/FIGURES/6

Dolan, J.R., Forster, D., Dunthorn, M., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverie, J., Decelle, J., Edvardsen, B., Egge, E., Eikrem, W., Kooistra, W.H.C.F., Logares, R., Massana, R., Montresor, M., Not, F., Ogata, H., Pawlowski, J., Pernice, M.C., Romac, S., Shalchian-tabrizi, K., Sarno, D., Simon, N., Richards, T.A., Siano, R., Vaulot, D., Wincker, P., Zingone, A., Vargas, C. De, Stoeck, T., Csic, M., Mar, P., 2022. The secret life of insect-associated microbes and how they shape insect-plant interactions. FEMS Microbiology Ecology. doi:10.1093/FEMSEC/FIAC083

Doornbos, R.F., Geraats, B.P.J., Kuramae, E.E., Van Loon, L.C., Bakker, P.A.H.M., 2011. Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of Arabidopsis thaliana. Molecular Plant-Microbe Interactions : MPMI 24, 395–407. doi:10.1094/MPMI-05-10-0115

Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., Hacquard, S., 2018. Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. Cell 175, 973-983.e14. doi:10.1016/J.CELL.2018.10.020

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.A., Sundaresan, V., Jeffery, L.D., 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proceedings of the National Academy of Sciences of the United States of America 112, E911–E920. doi:10.1073/PNAS.1414592112

Estes, A.M., Hearn, D.J., Snell-Rood, E.C., Feindler, M., Feeser, K., Abebe, T., Dunning Hotopp, J.C., Moczek, A.P., 2013. Brood Ball-Mediated Transmission of Microbiome Members in the Dung Beetle, Onthophagus taurus (Coleoptera: Scarabaeidae). PLOS ONE 8, e79061. doi:10.1371/JOURNAL.PONE.0079061

Fitzpatrick, C.R., Lu-Irving, P., Copeland, J., Guttman, D.S., Wang, P.W., Baltrus, D.A., Dlugosch, K.M., Johnson, M.T.J., 2018. Chloroplast sequence variation and the efficacy of peptide nucleic acids for blocking host amplification in plant microbiome studies. Microbiome 6, 1–10. doi:10.1186/s40168-018-0534-0

Foster, Z.S.L., Sharpton, T.J., Grünwald, N.J., 2017. Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. PLOS Computational Biology 13, e1005404. doi:10.1371/JOURNAL.PCBI.1005404

Fröhlich, J., Koustiane, C., Kämpfer, P., Rosselló-Mora, R., Valens, M., Berchtold, M., Kuhnigk, T., Hertel, H., Maheshwari, D.K., König, H., 2007. Occurrence of rhizobia in the gut of the higher termite Nasutitermes nigriceps. Systematic and Applied Microbiology 30, 68–74. doi:10.1016/J.SYAPM.2006.03.001

Fujiwara, S., Toshio, M., Nakayama, E., Tanaka, N., Tabuchi, M., 2022. Host-specific activation of a pathogen effector Aave\_4606 from Acidovorax citrulli, the causal agent for bacterial fruit blotch. Biochemical and Biophysical Research Communications 616, 41–48. doi:10.1016/J.BBRC.2022.05.071

Geng, L.L., Shao, G.X., Raymond, B., Wang, M.L., Sun, X.X., Shu, C.L., Zhang, J., 2018. Subterranean infestation by Holotrichia parallela larvae is associated with changes in the peanut (Arachis hypogaea L.) rhizosphere microbiome. Microbiological Research 211, 13–20. doi:10.1016/J.MICRES.2018.02.008

Hacquard, S., Garrido-Oter, R., González, A., Spaepen, S., Ackermann, G., Lebeis, S., McHardy, A.C., Dangl, J.L., Knight, R., Ley, R., Schulze-Lefert, P., 2015. Microbiota and Host Nutrition across Plant and Animal Kingdoms. Cell Host & Microbe 17, 603–616. doi:10.1016/J.CHOM.2015.04.009

Han, J., Sun, L., Dong, X., Cai, Z., Sun, X., Yang, H., Wang, Y., Song, W., 2005. Characterization of a novel plant growth-promoting bacteria strain Delftia tsuruhatensis HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Systematic and Applied Microbiology 28, 66–76. doi:10.1016/J.SYAPM.2004.09.003

Heys, C., Cheaib, B., Busetti, A., Kazlauskaite, R., Maier, L., Sloan, W.T., Ijaz, U.Z., Kaufmann, J., Mcginnity, P., Llewellyn, M.S., 2022. Neutral Processes Dominate Microbial Community Assembly in Atlantic Salmon, Salmo salar. doi:10.1128/AEM.02283-19

Hou, S., Liu, D., Huang, S., Luo, D., Liu, Z., Xiang, Q., Wang, P., Mu, R., Han, Z., Chen, S., Chai, J., Shan, L., He, P., 2021. The Arabidopsis MIK2 receptor elicits immunity by sensing a conserved signature from phytocytokines and microbes. Nature Communications 2021 12:1 12, 1–15. doi:10.1038/S41467-021-25580-W

Humphrey, P.T., Whiteman, N.K., 2020. Insect herbivory reshapes a native leaf microbiome. Nature Ecology & Evolution 4. doi:10.1038/s41559-019-1085-x

Indiragandhi, P., Anandham, R., Madhaiyan, M., Sa, T.M., 2008. Characterization of plant growth-promoting traits of bacteria isolated from larval guts of Diamondback moth Plutella xylostella (Lepidoptera: Plutellidae). Current Microbiology 56, 327–333. doi:10.1007/S00284-007-9086-4/TABLES/3

Jackson, R., Monnin, D., Patapiou, P.A., Golding, G., Helanterä, H., Oettler, J., Heinze, J., Wurm, Y., Economou, C.K., Chapuisat, M., Henry, L.M., 2022. Convergent evolution of a labile nutritional symbiosis in ants. The ISME Journal 2022 16:9 16, 2114–2122. doi:10.1038/s41396-022-01256-1

Jiang, D., Yan, S., 2018. MeJA is more effective than JA in inducing defense responses in Larix olgensis. Arthropod-Plant Interactions 12, 49–56. doi:10.1007/S11829-017-9551-3/TABLES/4

Kim, J.H., 2009. Estimating classification error rate: Repeated cross-validation, repeated hold-out and bootstrap. Computational Statistics & Data Analysis 53, 3735–3745. doi:10.1016/J.CSDA.2009.04.009

Leite-Mondin, M., DiLegge, M.J., Manter, D.K., Weir, T.L., Silva-Filho, M.C., Vivanco, J.M., 2021. The gut microbiota composition of Trichoplusia ni is altered by diet and may influence its polyphagous behavior. Scientific Reports 2021 11:1 11, 1–16. doi:10.1038/S41598-021-85057-0

Li, W., Yuan, Y., Xia, Y., Sun, Y., Miao, Y., Ma, S., Ma, S., 2018. A Cross-Scale Neutral Theory Approach to the Influence of Obesity on Community Assembly of Human Gut Microbiome 9, 1–8. doi:10.3389/fmicb.2018.02320

Li, X., Zhang, Y., Ding, C., Jia, Z., He, Z., Zhang, T., Wang, X., 2015. Declined soil suppressiveness to Fusarium oxysporum by rhizosphere microflora of cotton in soil sickness. Biology and Fertility of Soils 51, 935–946. doi:10.1007/S00374-015-1038-8/FIGURES/6

Li, Y., Yuan, L., Xue, S., Liu, B., Jin, G., 2020. The recruitment of bacterial communities by the plant root system changed by acid mine drainage pollution in soils. FEMS Microbiology Letters 367, 117. doi:10.1093/FEMSLE/FNAA117

Liu, H., Carvalhais, L.C., Schenk, P.M., Dennis, P.G., 2017. Effects of jasmonic acid signalling on the wheat microbiome differ between body sites. Scientific Reports 2017 7:1 7, 1–8. doi:10.1038/SREP41766

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15, 1–21. doi:10.1186/S13059-014-0550-8/FIGURES/9

McMurdie, P.J., Holmes, S., 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLOS Computational Biology 10, e1003531. doi:10.1371/JOURNAL.PCBI.1003531

Montagna, M., Gómez-Zurita, J., Giorgi, A., Epis, S., Lozzia, G., Bandi, C., 2015. Metamicrobiomics in herbivore beetles of the genus Cryptocephalus (Chrysomelidae): toward the understanding of ecological determinants in insect symbiosis. Insect Science 22, 340–352. doi:10.1111/1744-7917.12143

Okutani, F., Hamamoto, S., Aoki, Y., Nakayasu, M., Nihei, N., Nishimura, T., Yazaki, K., Sugiyama, A., 2019. Rhizosphere modelling reveals spatiotemporal distribution of daidzein shaping soybean rhizosphere bacterial community 1036–1046. doi:10.1111/pce.13708

Pagé, A.P., Tremblay, J., Masson, L., Greer, C.W., 2019. Nitrogen- and phosphorus-starved Triticum aestivum show distinct belowground microbiome profiles. PLOS ONE 14, e0210538. doi:10.1371/JOURNAL.PONE.0210538

Pan, Q., Shikano, I., Hoover, K., Liu, T.X., Felton, G.W., 2019. Enterobacter ludwigii, isolated from the gut microbiota of Helicoverpa zea, promotes tomato plant growth and yield without compromising anti-herbivore defenses. Arthropod-Plant Interactions 13, 271–278. doi:10.1007/S11829-018-9634-9/FIGURES/6

Pang, Z., Chen, J., Wang, T., Gao, C., Li, Z., Guo, L., 2021. Linking Plant Secondary Metabolites and Plant Microbiomes : A Review 12. doi:10.3389/fpls.2021.621276

Roager, H.M., Licht, T.R., 2018. Microbial tryptophan catabolites in health and disease. Nature Communications 2018 9:1 9, 1–10. doi:10.1038/s41467-018-05470-4

Salzman, S., Whitaker, M., Pierce, N.E., 2018. Cycad-feeding insects share a core gut microbiome. Biological Journal of the Linnean Society 123, 728–738. doi:10.1093/BIOLINNEAN/BLY017

Santos-Garcia, D., Mestre-Rincon, N., Zchori-Fein, E., Morin, S., 2020. Inside out: microbiota dynamics during host-plant adaptation of whiteflies. The ISME Journal 2020 14:3 14, 847–856. doi:10.1038/S41396-019-0576-8

Shi, S., Nuccio, E., Shi, Zhou He, Z., Zhou, J., Firestone, M., 2016. The interconnected rhizosphere : High network complexity dominates rhizosphere assemblages. Ecology Letters 19, 926–936. doi:10.1111/ele.12630

Sirová, D., Bárta, J., Šimek, K., Posch, T., Pech, J., Stone, J., Borovec, J., Adamec, L., Vrba, J., 2018. Hunters or farmers? Microbiome characteristics help elucidate the diet composition in an aquatic carnivorous plant. Microbiome 6, 1–13. doi:10.1186/S40168-018-0600-7/FIGURES/3

Sloan, W.T., Woodcock, S., Lunn, M., Head, I.M., Curtis, T.P., 2007. Modeling taxa-abundance distributions in microbial communities using environmental sequence data. Microbial Ecology 53, 443–455. doi:10.1007/s00248-006-9141-x

Smets, W., Koskella, B., 2020. Microbiome: Insect Herbivory Drives Plant Phyllosphere Dysbiosis. Current Biology 30, R412–R414. doi:10.1016/J.CUB.2020.03.039

Thiergart, T., Durán, P., Ellis, T., Vannier, N., Garrido-Oter, R., Kemen, E., Roux, F., Alonso-Blanco, C., Ågren, J., Schulze-Lefert, P., Hacquard, S., 2019. Root microbiota assembly and adaptive differentiation among European Arabidopsis populations. Nature Ecology & Evolution 2019 4:1 4, 122–131. doi:10.1038/S41559-019-1063-3

Tomberlin, J.K., Crippen, T.L., Wu, G., Griffin, A.S., Wood, T.K., Kilner, R.M., 2017. Indole: An evolutionarily conserved influencer of behavior across kingdoms. BioEssays 39, 1600203. doi:10.1002/BIES.201600203

Venkataraman, A., Bassis, C.M., Beck, J.M., Young, V.B., Curtis, J.L., Huffnagle, G.B., Schmidt, T.M., 2015. Application of a Neutral Community Model To Assess Structuring of the Human Lung Microbiome 6. doi:10.1128/mBio.02284-14.Venkataraman

Wang, K., Razzano, M., Mou, X., 2020. Cyanobacterial blooms alter the relative importance of neutral and selective processes in assembling freshwater bacterioplankton community. Science of The Total Environment 706, 135724. doi:10.1016/J.SCITOTENV.2019.135724

Weiland-bra, N., Dirksen, P., Wang, J., Id, M.S., Id, F., Schmitz, R.A., Baines, J.F., Id, B.M., Id, A.T., 2019. Neutrality in the Metaorganism 1–21.

White, L.J., Ge, X., Subramanian, S., State, S.D., 2017. Root isoflavonoids and hairy root transformation influence key bacterial taxa in the soybean rhizosphere 19, 1391–1406. doi:10.1111/1462-2920.13602

Wielkopolan, B., Obrępalska-Stęplowska, A., 2016. Three-way interaction among plants, bacteria, and coleopteran insects. Planta 2016 244:2 244, 313–332. doi:10.1007/S00425-016-2543-1

Willems, A., 2014. The Prokaryotes, in: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer Verlag Berlin Heidelberg, pp. 777–851. doi:10.11646/zootaxa.3263.1.2

Xiao, L., Carrillo, J., Siemann, E., Ding, J., 2019. Herbivore-specific induction of indirect and direct defensive responses in leaves and roots. AoB PLANTS 11. doi:10.1093/AOBPLA/PLZ003

Yang, P., van Elsas, J.D., 2018. Mechanisms and ecological implications of the movement of bacteria in soil. Applied Soil Ecology 129, 112–120. doi:10.1016/J.APSOIL.2018.04.014

Yi, M., Zhang, L., Qin, C., Lu, P., Bai, H., Han, X., Yuan, S., 2022. Temporal changes of microbial community structure and nitrogen cycling processes during the aerobic degradation of phenanthrene. Chemosphere 286, 131709. doi:10.1016/J.CHEMOSPHERE.2021.131709

Zhu, L., Zhang, Y., Cui, X., Zhu, Y., Dai, Q., Chen, H., Liu, G., Yao, R., Yang, Z., 2021. Host Bias in Diet-Source Microbiome Transmission in Wild Cohabitating Herbivores: New Knowledge for the Evolution of Herbivory and Plant Defense. Microbiology Spectrum 9. doi:10.1128/SPECTRUM.00756-21/SUPPL\_FILE/SPECTRUM00756-21\_SUPP\_1\_SEQ6.DOCX

Zygadlo, J.A., Li, C., Hu, C., Xie, J., Shi, G., Wang, X., Yuan, X., Li, K., Chen, S., Zhao, X., Fan, G., 2022. Selenium Combined with Methyl Jasmonate to Control Tomato Gray Mold by Optimizing Microbial Community Structure in Plants. Journal of Fungi 2022, Vol. 8, Page 731 8, 731. doi:10.3390/JOF8070731