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Please prepare an abstract consisting of a title (250 characters max.), up to 14 authors and an abstract body **(2500 characters max.)** . In the online editor you may use simple formatting elements such as bold, italic, underline, super & subscript and special characters in both the title and abstract.

TARGET AUDIENCE: international researchers, with in-depth knowledge of plant microbiomes

GOAL 1: attract people interested in the concepts, tools, MeJA biology to discuss the results (and anticipate reviewer’s concerns). Higher focus on analysis methodology.

GOAL 2: Showcase the results as part of professional networking

Abstract 1(Pedro's submission) - deseq2, Random Forest, network

Abstract 2 Marcela's submission) – neutral model fits

OBS: Marcela will work on neutral models with the family experiment data

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**Abstract 1 - deseq2, Random Forest, networks**

*Brassicaceae microbiome response to insect herbivory: summarizing random forest, network analysis, and differential abundance*

Pedro Beschoren da Costa, Marcela Gomez Aragón, Zulema Carracedo Lorenzo, Karen Kloth, Erik Poelman, Marcel Dicke

Plant microbiomes have been shown to respond to different stress conditions, including insect herbivory. However, reliably detecting members of the microbial community that are actually relevant to experimental treatments, and thus could be targets for use in agricultural applications, is difficult due to the complexity of microbial communities. One approach to address this is to integrate different methods to analyze the dataset and pinpoint bacterial taxa consistently tagged to be of importance. Here, *Arabidopsis thaliana* (*At*) and *Brassica oleracea* (*Bo*) were exposed to Methyl Jasmonate to mimic insect herbivory, and then had both rhizospheric and endophytic bacterial communities profiled with 16S rRNA gene sequencing. Then, ASVs were tagged as important according to random forest by Boruta, network analysis by SpiecEasi, and differential abundance by DESeq2. Finally, taxa tagged as important were summarized with a Fisher test and visualized in a heat tree. The random forest machine learning algorithm tagged 41 ASVs as reliable predictors of the stress treatments. Endosphere communities presented a larger number of predictors than rhizosphere communities and *Bo* communities presented more predictors than *At* communities. The model’s prediction accuracy was between 0.678 and 0.891. Network analysis shows that rhizosphere community networks were more complex but less modular than endosphere networks. There was a total of 34 ASVs tagged as keystone taxa, module connectors, or module hubs for *At* and 47 for *Bo.* Differential abundance analysis tagged 224 ASVs in treatment-control pairwise comparisons. Endosphere communities contained more differentially abundant ASVs than rhizosphere communities and *At* communities had more differentially abundant ASVs than *Bo* communities. These 3 analysis tools tagged ASVs 346 times in total. To reveal which taxonomic levels were consistently tagged as important, Fisher tests were performed at every taxa level. This test compared the proportions of a taxon in the important taxa subset with proportions of the same taxon in the full dataset. The output of this test was displayed in a heat tree. For example, results show that the Commonadaceaefamily is overrepresented in the important taxa subset in rhizospheres of both plant species, suggesting this family plays a role in plant stress response. This approach allows an integrated analysis of stress-induced microbiome recruitment with a statistical basis and phylogeny-wide visualization.

**Abstract 2 – neutral model fits**

*Brassicaceae microbiome response to insect herbivory: using a neutral theory model to explore microbial communities*

Marcela Aragón, Pedro Beschoren da Costa, Zulema Carracedo Lorenzo, Karen Kloth, Erik Poelman, Marcel Dicke

Insect herbivory has been shown to alter plant root-associated microbial community composition. This happens as the plant selects for microbes that could help to cope with such stress. However, it is challenging to reliably identify taxa responsive to stress when the microbial community differences between experimental treatments are subtle. Here, we applied Methyl Jasmonate to mimic insect herbivory to *Arabidopsis thaliana* (*At*) and *Brassica oleracea* (*Bo*) plants and sequenced the rhizospheric communities through 16S rRNA gene sequencing. For each species, there was one non-stressed control and 3 types of insect herbivory stress treatments. NMDS ordination of community distances indicated very clear differences between rhizospheric bacterial communities of *At* and *Bo*, but not between the 4 different stress treatments. Sloan’s Neutral Theory Model was then applied to define the ASVs as sub-communities occurring above, below, or as expected in conditions of neutrality and null selection. This was performed for each treatment in each plant species. Differences in sub-community composition of the taxa occurring above neutrality were highly significant in all pairwise comparisons between treatments. This indicates that the taxa selected by each treatment were quite different, even if this is not evident when looking at the full community level. Pairwise differential abundance of these taxa in a matrix of heat trees indicated that some taxa were consistently more present in all 3 stress treatments when compared to control. This was the case for four genera in *At* and three genera in *Bo*. The matrix of heat trees also showed diversity hotspots. Different members of the families Commonadaceaeand Chitinophagaceae, and the orders Rhizobialesand Burkholderialeswere highlighted as overabundant in every treatment. This suggests that diversity within these taxa could be important in the plant defense response against insect herbivory. To test if such hotspot taxa are not diverse in the selected subset simply because they were very diverse in the base dataset, diversity metrics of the ASVs selected by the neutral models were fitted against the total ASV pool. This analysis indicated that the order Rhizobiales and family Commonadaceaehave an overly enriched diversity in the neutrally-selected ASV subset, in response to the applied stress treatments.