**Results**

*16S amplicon sequencing*

Sequencing of 16S rRNA in the V3-V4 region generated 10.6M pair-ended reads with an average depth of 113±10.5 thousand sequences per sample for 94 samples, and average phred score 35.1±02. The No-template DNA extraction blank control had only 755 sequences. 2.509 million sequences of 434±11bp were kept after denoising. After removing 88 thousand plastid and mitochondrial sequences, the dataset was filtered by removing ASVs that occurred less than 8 times in the dataset. This reduced the total number of taxa from 9.838 to 4.943, but retained 99.02% of sequences. The mean library size was of 25.974 ± 8.390 sequences per sample. Rarefaction was performed at 8.678 sequences per sample, and removed only 43 out of 4.943 input taxa (figure\_rarefaction)

*Beta diversity for the whole community*

Root samples show significant interaction effects between MeJA treatments and plant species, but soil samples neither this interaction nor the MeJA treatments have a significant effect on microbial community composition (Permanova\_table\_b). When considering the 4 partions independently, it is clear that MeJA effects were not a significant contributor to the microbial community structure in the soils of *B. oelraceae* (permanova\_table\_c).

Neutral heat trees

It can also be noticed that the 15 genera overrepresented in *A.thaliana* controls belong to 11 different phyla, suggesting that stress could reduce diversity of selected bacteria at a phylum level. In *B.oleraceae* samples however, the 8 genera consistently overrepresented in controls are found in 5 different phyla. Thus, if this effect exists, it is species-specific.

The alpha diverisy metrics of above-neutral ASVs corroborates the hypotheses that diversity in these taxons is responsive to the stress treatments. Note that Order Burkholderiales and Class Gammaproteobacteria are either bellow or within all fitted curves, showing that these taxa are not relatively more diverse in the selected subset than on the full dataset. Class Alphaproteobacteria is above the fitted curve of observed number of taxa and shannon diversity, but not simpson diversity, while Class Bacteroidia and family Chitinophagaceae is above the fitted curve for the observed number of taxons only. It is also interesting to note that Order Micrococales is the furthest point form the curve for B. oleraceae, suggesting it is under strong selection pressure by retaining a large portion of it’s full diversity (9 out of 28 compared to 43 out of 165 in order Rhizobiales). With this approach we could thus pinpoint the taxa levels whose diversity is more likely to respond to the applied stresses by being selected by the plants.

Alpha diversity

This effect is more clear when testing each of the 4 data partitions individually. In this case, stress treatments cause significant changes of Shannon diversity only in the roots of B.oleraceae, with controls presenting less diversity than MeJA 0.1mM and Oral secretion, but similar diversity to MeJA 1.0.

The Shannon diversity pattern found in the soil samples is very similar to that of the ASVs selected by the neutral models. Treatment with MeJA 0.1mM increases diversity for both plant species (Figure\_alpha\_diversity\_above\_expected, table\_alpha\_diversity\_above\_expected). When considering only Family Commonadaeae, we can see an increase of Shannon diversity in this family according MeJA concentration on *B.oleracea*, but the diversity on oral secretion treatments is lower. For *A. thaliana*, diversity of Family Commonadaeae is highest at oral secretionand MeJA 0.1mM. When considering only Order rhizobiales, we can see an increase of Shannon diversity in this family according MeJA concentration on B.oleracea,and diversity is also high in oral secretion. for A. thaliana, diversity of Order rhizobiales is high at MeJA 0.1, but similar across other treatments. PEDRO YOU SHOULD ADD A PLOT FOR ORDER XHANTOMONADALES AND MICROCOCALES, SHOWING BOTH THE ABOVE-EXPECTED AND FULL DIVERSITY

Differential abundance

(obs: Deseq2 comparisons focused on Control VS treatment comparisons, not treatment VS treatment pairwise comparisons. Fisher tests will later include any ASV tagged as differentially abundant in any treatment-control comparisons. No ASV differentially abundant in treatment-treatment comparisons are added to the fisher test)

Random Forest

Here, accuracy refers to how often the model correctly classifies a sample according the stress (MeJA 1.0, Meja 0.1, Control or Oral secretion) when compared to the test set. These sample classifications are based on the abundances of the ASVs tagged as important in each sample partition. Kappa refers to a measure of agreement by two classification models (in this case, the ground truth and the RF-based classifications) by considering the observed accuracy and an expected accuracy based on random classifications. A higher Kappa indicates high agreement, and thus, better model performance. Without feature selection by Boruta, the RF models performs far worse, with accuracy between 0.328 and 0.489 and kappa of 0.090 and 0.335. Model Overfitting could occur, but as slight differences in experimental design can change the community structure, it is not so relevant to anticipate finding the same taxa as important in another dataset. It can be seen that several important ASVs have low total abundances, such as ASV\_1367 with only 16 occurrences in *A.thaliana* roots

Netowrk analysis

There were 8 ASVs tagged as keystone taxa according degree, betweness centrality and closeness centrality. Four of them are found in the rhizosphere communities of *B. oleraceae*. From these four keystone taxa, one ASV from order Burkholderiales, genus Massilia, was also classified as a module hub and was occurring above the predictions of the neutral model in all stress treatments. One ASV from genus Flavobacterium was also tagged as a module connector. A Methylobacterium-Methylorubrum ASV from order Rhizobiales was both a module connector and above predictions of the neutral models in all stress treatments. Finally, ASV\_410, from genus Nocardioides, was classified as above neutral model predictions in all treatments and as a predictor of treatment classification by the random forest algorithm. These independent importance tags suggests that those 4 keystone taxa, which together account for only 0.27% of the total reads in B.oleraceae rhizosphere samples, may strongly influence the rest of the microbial communities.

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Network modules, groups of nodes forming a highly connected cluster, can be correlated to sample metadata once the abudance of the ASVs that compose the module are summarized in a single principal component in eingengene analysis. In our analysis, several network modules had significant or near significant (p<0.1) correlations with leaf dry weight, MYC2 expression, and LOX2 expression (figure Module\_eingengene\_correlations\_to\_plant\_metadata). We highlight module 3 in both endosphere and rhizoplane of *A. thaliana*. These modules had positive correlations to MYC2 and negative correlations to plant dry weight. These two *A. thaliana* modules have different ASV abudances when compared to other modules. The endosphere module has lower medias for class actinobacteria and Gammaproteobacteria, but higher abudnace in class Bacteroidia. The rhizosphere module instead has higher abundance in Class Verrucomicrobiae, Thermoleophilia and Bdellovibrionia. The endosphere module also has low abundance of Order Burkholderiales, Family Commonadaceae, and Genus Massilia. Meanwhile, while the rhizosphere module has high abundance in order Solirubrobacterales, family Rhizobiaceae and genus Mesorhizobium. It is also possible that the ASVs found in these modules are more present in the MeJA 1.0mM treatment, where MYC2 and LOX2 expression was high for A. thaliana.

On *B. oleracea*e rhizoplane samples, module 5 positively correlated (r = 0.425, p = 0.054) with LOX2 expressions. We note that this module had 2 keystone taxa, 5 ASVs tagged as important in the random forest algorithm and 7 ASVs tagged as module connectors. Thus the sub-community of ASVs in this module might indeed have a major role in stress-impacted community structure. This compared to other modules in the same network, this module presented high frequency of ASVs in class Alphaproteobacteria, orders Rhizobiales and Sphingobacteriales, family Xanthobacteriaceae, and genus Mesorhizobium.

While our analysis focuses in the connected components of the network, the 3-nodes fragmented module 13 in the A. Thaliana endophyte network requires mention. The 3 ASVs in that modedule have been tagged as important by boruta, are positively correlated between each other and negatively correlated to MYC2 and LOX2 expression ( r = -0.488 and -0.465; p = 0.033 and 0.044, respectively). These 3 ASVs have low abundances in the MeJA 1.0 Treatment (figure\_ASV\_mod13)

AT\_soil module 2 has multiple relevant nodes and all the keystones from that network, but as the module correlates positively with plant biomass these may be simply beneficial bacteria, not directly related to MeJA stress responses. However, as it is well established, plants must balance defense and growth regulation.

Finally, we note a significant role of ASVs from genus Mucilaginibacter, composing 12 nodes across 8 modules (27 edges total) for AT\_soil and in 13 nodes across 5 modules in BO\_soil (67 edges total) but only 2 nodes across 2 modules (5 edges total) in AT\_root and 8 nodes croos 5 modules (19 edges total) in BO\_roots. given their potential role in formation of biofilms…..

Supplementary discussion (?)

(Liu et al., 2017) also indicates both increase and decreases in relative abundances of *Streptomyces* in roots of wheat due to MeJA. Here, we report neutrally-selected *Streptomyces* as increasing in all treatments of A. thaliana when compared to controls. The study highlights that all differentially abundant OTUs are part of Class Actinobacteria. Here, we see that the diversity of actinobracteria si relatively high in the neutrally-selected ASVs when compared to the full dataset. As per (Liu et al., 2017)*“Streptomycetaceae* are key components of endophytic communities in *Arabidopsis thaliana* roots”

This report (Liu et al., 2017) also shows that bulk soil alpha diversity was not affected by MeJA. our results show that the full rhizosphere communities have, also, not been affected by MeJA treatments. The neutrally-selected microbial communities, however, show that MeJA 0.1 increases diversity of the rhizospheric communities in both plant species. The report also mentions that JA signaling had no effect on alpha diversity of rhizosphere communities.

As mentioned in(Liu et al., 2017) “When grown in soil collected from areas where A. thaliana grows naturally, stimulation of the A. thaliana JA pathway led to a significant alteration in rhizosphere bacterial community composition (Carvalhais et al., 2013). However, when grown in ‘non-native’ soils, induction of the A. thaliana JA pathway had no effect on the composition of rhizosphere bacterial communities (Doornbos et al., 2011)

As we can see in this JA-microbiome review by a brazilian author (Carvalhais et al., 2017)… It talks most about the mechanisms and is a bit outdated

Pedro also has to read this one, which says that MeJA increases glucosinolates production and exudation (Rios et al., 2021)

Some microbes produce JA with unknown pathways. Fungi and bacteria can directly influence JA synthesis pathways for increased virulence (Nakano et al., 2022)

As mentioned in (Liu et al., 2017), “Effects of JA signalling on root and shoot biomass: Relative to the controls, MeJA treatment led to a 14% reduction in root dry weight (P = 0.015) but shoot biomass was not affected (Fig. S7). This is consistent with previous studies in Arabidopsis thaliana and sunflower (Helianthus annuus L.), which reported root inhibition upon activation of JA signaling”

Use of cohens D to compare multiple experiments/controls in (Giauque et al., 2019)

Indole-3-acetic acid originated in ancient bacterial and was, apparently, horizontally transferred to land plants by algeae metabolism (Bowman et al., 2021)

Comamonadaceae (and rhizobiaceae) acting as antifungal agents in root networks of A.thaliana**[already cited in manuscript]** in this eference negative co-occurences between comamonadace and pathogenic fungi were specially high. Follow-up strain based interkingdom reconstruction shows that strains from these groups have a significant, but not exclusive role in suppressing a wide range of fungal pathogens. Still, they can be classified as biocontrol candidates that steractc strongly with the rest of the bacterial community to, together, suppress fungal pathogens.