This is a draft manucript for the MICROP MeJA concentration pilot

SUMMARY

Material and methods

References

**Matherial and methods**

*Plant and soil preparation*

Plants planted on 20/oct/2020 (MeJA concentration Pilot)*.* Plants harvested on 27/nov/2020 (MeJA concentration Pilot), at 5 weeks old. Complete randomized block design with 6 blocks and 6 replicates/treatment.

CFU counting: 200ul inoculation of serially diluted soil in 0.85% NaCl. Plated in LB media, amended with 50ug/L cycloheximide to suppress fungi LB: 10g Tryptone, 5g yeast extract, 5g NaCl, 15g Agar (per Liter) Ciclohexamide: 50mg/ml stock, diluted in 96% ethanol. Utilized 1 ml/L media.

*MeJA stress application*

The 1 mM and 0.1mM Methyl Jasmonate (MeJA) solutions were prepared with from a 1M stock of Methyl jasmonate 95%(Sigma 392707-5ML) diluted with ethanol 96%. The final 1L use solution has 0.1% MeJA solution in Ethanol 96% and 0.015% silwet. Control solutions received only 0.1% ethanol 96% and 0.015% Silwet. To dip the plants on MeJA solutions, first a 0.05mm mesh was placed around the base of the plants, and 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 internal edge of the pots and the edge of the mesh. With this the plants could be turned upside down without significant losses of soil. This allowed the aerial part of the plants to be fully submerged on the MeJA solutions for 2-3 seconds without application of MeJA on the soils. MeJA was first applied when plants were 3 weeks old, and then every 4 days thereafter until 4 applications had taken place. Plants were harvested 2 days after the last MeJA application.

*RNA extraction and qPCR*

we sampled leaf tissue 3 hours after the last MeJA dipping (sampling between 2:20 and 4pm)  
For Arabdopisis, we collected 2 whole leaves from each plant, avoiding old leaves  
For Brassica, we collect 4 leaf punches (leaves 3 and 4. for the 1mM MeJA treatmentment we sampled leave 3 and the most damaged leaf (1 or 2))  
All materials were cleaned in between samples. we also chilled the leaf puncher and kept all tubes on liquid nitrogen during the sample loading process. We pre-cooled the tubes in liquid N, and kept the samples in liquid nitrogen until storage on -80C.

*Plant phenotyping*

*DNA extraction and 16S amplicon sequencing*

For harvesting, roots were gently shaken, and then stored in a 50ml falcon with 20ml of autoclaved 0.85% NaCl solution. They were then gently inverted 10 times. After the 10 inversions, roots were colected with a ethanol-cleaned tweezer to a 10ml tube with 6ml of autoclaved 1x TAE with 0.05% tween 20. The pre-washed roots (free of rhizopsheric soil) where then incubated sideways on the orbital shaker for 2 minutes, 400RPM. washed roots were then collected with a ethanol-cleaned and flame-sterilized long tweezer, and transferred to another tube (1x TAE with 0.05% tween 20.)  for additional washing. The process was repeated another time for 3 washes in total. Fully washed roots were then transfered to a 2ml tube, flash-frozen, and stored at -20.  
All 3 washes were stored on -20 for later consolidation into (2 tubes of 9ml) and centrifugation (15 min 5400 g) to generate a soil sample. up to 300ul of ressuspended pellet was used as template for soil DNA extraction. DNA extraction was performed with QIAGEN PowerSoil Pro kit. Roots in 2ml tubes were left to lyophilize for 48h, and then they were ground to dust on a painshaker for 150 sec. Powdered roots DNA was extracted with Qiagen dneasy plant pro. DNA extracts were submitted to 16S rRNA sequencing on the V3-V4 region with a Illumina MiSeq (PE300bp) at Baseclear (Leiden, the Netherlands). Libraries for root samples were prepared with PCR blockers to prevent amplification of plant DNA.

*Data analysis*

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 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 taxonomy against the SILVA 138 SSU release with the sklearn option in QIIME2. This data was processed in the High Performance Computing Cluster Anunna (Wageningen University) and used scripts are available at <https://github.com/PedroBeschoren/WUR_HPC_Annuna>.

**Results**

*16S amplicon sequencing*

Sequening of 16S rRNA in the V3-V4 region generated 10.6M PE reads with an average depth of 113±10.5k for 94 samples and average phred score 35.1±02. The No-template blank control only had 755 reads.

*Detecting portions of the microbial community that are relevant to the stress response*

We could observe that a subset of ASVs from each environment, when selected by the Sloan neutral model (sloan ref 2007), can strongly separate the different treatments of MeJA (Figure beta\_div\_neutral\_splitjoined). These subsets are expected to represent ASVs that thrive in a particular environment and are selected (although not necessarily for the benefit of the host; the environment could simply be optimal for a specific pathogen over another). It’s well understood that microbial consortia and subcommunities will have synergistic function effects as syntrophy and other metabolic routes are shared by co-evolved microorganism. we could observe on moule 12 of AT\_soil networks the positive correlation and MYX2 expression along with a 80% dominance of OTUs selected by both oral and MeJA treatments but not selected by controls. This is evidence by different methods that this subset of bacteria can have actual impacts on the MeJA response.