

# Plant-Microbe Interfaces: Phytobiome and transcriptional adaptation of *Populus deltoides* to acute progressive drought and cyclic drought

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## Abstract

Plant drought stress causes systematic changes to photosynthesis, metabolism, and it's phytobiome. Additionally, drought effects plants in both a species-specific and water deficit driven manner, causing the response to drought to be dependent both on how much drought is being experienced and on any adaptation to prior drought exposure. As such, to understand the effect of drought on plants requires assessing drought response in multiple conditions, such as progressive acute drought and recurrent cyclic drought, and at different levels of severity. In this study, we have utilized RNA sequencing to identify changes to the plant transcriptome and the phytobiome during both acute progressive drought and cyclic drought at multiple severities. We have identified that the drought response ranges from increased transcripts related to photosynthesis and metabolic activity in mild acute drought to decreased transcripts related to photosynthesis and metabolic impairment in severe drought. Moreover, while water deficit is a main driver of transcriptional responses in severe drought, there are increases in reactive species metabolism and photosynthetic transcripts in cyclic severe drought compared to acute severe drought, independent of water deficit. Lastly, the phytobiome exhibits a different response to drought compared to the transcriptome, being more separated by the cyclic or acute nature of the drought rather than the severity of the drought based upon hierarchical clustering, with the phytobiome having an increase in organisms in cyclic drought that are usually reported to have beneficial effects on the plants.

## Drought Response

*Populus deltoides* WV94 plants were subjected to two different drought conditions. The first is an acute drought experiment where plants were left unwatered for eight days, with transcriptomics taken from mature leaves on day 0, day 5, day 7, and day 8. The second is a cyclic drought experiment where plants were subjected to four drought and three rewatering cycles with transcriptomics taken from mature leaves in plants well watered throughout the experiment, mild drought plants, and severe drought plants. Transcriptomics showed a strong water deficit response in the host with some differences between cylic and acute drought; however, the phytobiome is highly dependent on the cyclic or acute nature of the drought (Figure 3).

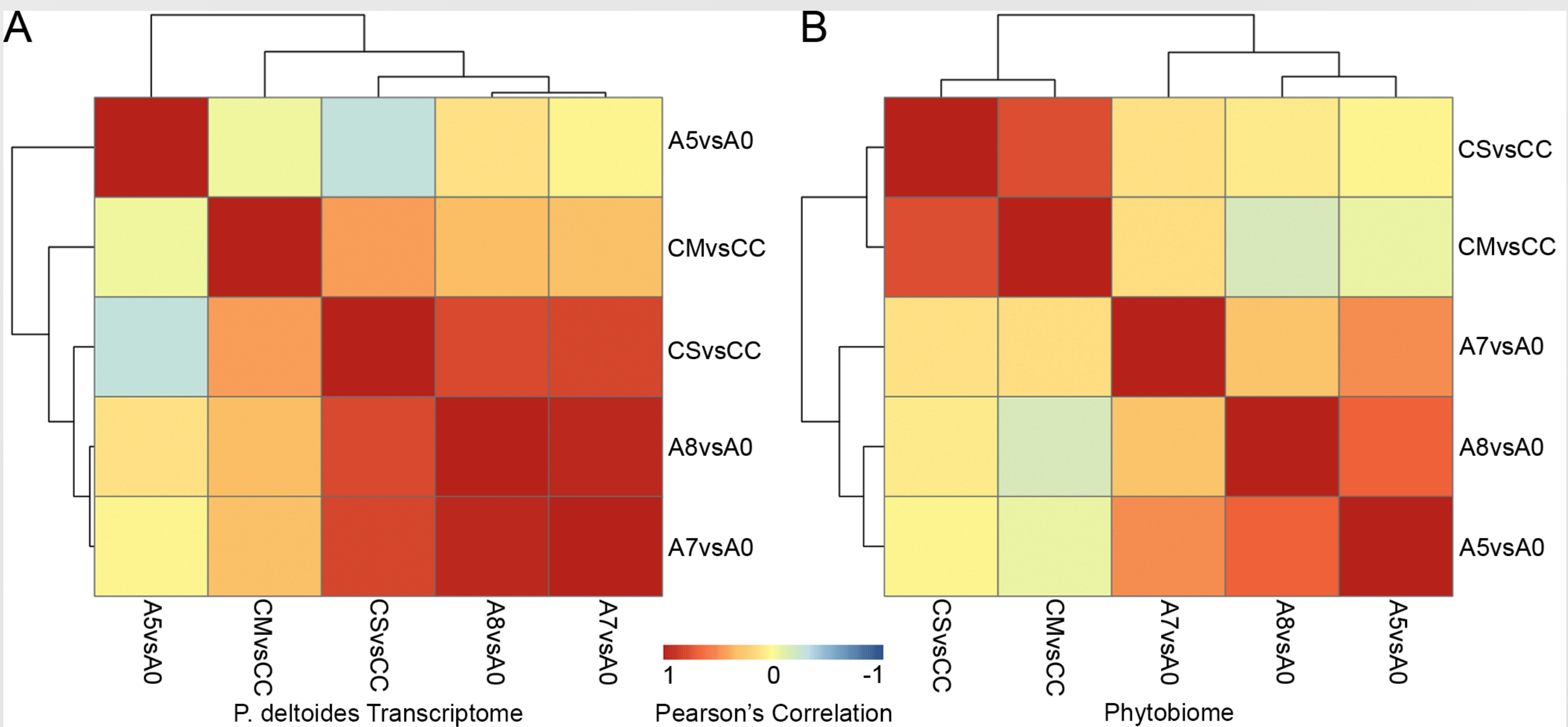


Figure 3. Correlations of differential transcript expression and taxa abundance between each drought condition relative to its control. Plant transcriptional changes are largely dependent on water deficit while the phytobiome is more separated between cyclic and acute drought

Drought, expectedly, causes decreases in photosynthesis and metabolic activity transcription in more severe drought (which also parallels the proteome<sup>2</sup>); however, in very mild drought conditions, we see an increase in photosynthesis transcripts. Additionally, cyclic drought plants have less of a decrease in photosynthesis, higher reactive oxygen species (ROS) metabolism, higher cutin, and higher aquaporin transcription relative to acute drought plants (Figure 4). The changes in transcription suggest that cyclic drought may be better acclimated to drought, which is further supported by differences in taxa abundances in the phytobiome.

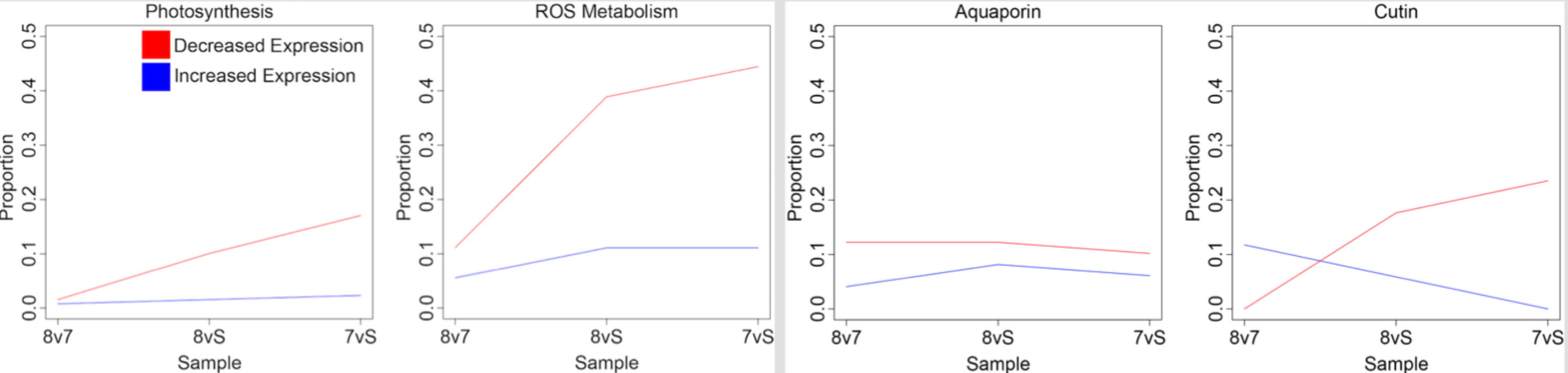


Figure 4. Categorical changes between cyclic drought and acute drought in the plant transcriptome. While the transcriptome is highly dependent on water deficit, there are functional differences relating to photosynthesis, ROS metabolism, cutin, and aquaporin transcription, suggesting cyclic drought plants may be better acclimated to drought conditions

Fungi are the most differentially abundant species in the drought, likely responding to a combination of environmental and host effects. Acute drought relative to controls shows an increase in species that have potential pathogens. Conversely, cyclic drought relative to controls has an increase in taxa that are associated with beneficial microorganisms. When comparing acute drought to cyclic drought, acute drought has higher taxa associated with potential pathogens while cyclic drought has higher potential growth promoters such as *Rhizophagus*, *Mesorhizobium*, and *Streptomyces*. The aforementioned organisms are associated with increased phosphorus availability, increased potassium availability, decreased ROS stress, increased nitrogen availability, and increased disease/pathogen resistance.

## Transcriptome-Phytobiome Comparison

There is an interplay between the host and its phytobiome that influences the response to drought stress experienced by both the host and organisms in the phytobiome. To assess potential relationships between the host and the organisms living on the leaves we both looked at co-differential expression/abundance (Figure 5 and Figure 6) and associations created by DUO (Figure 5, 6, and 7), an algorithm that identifies positive and negative relationships between taxa and genes.

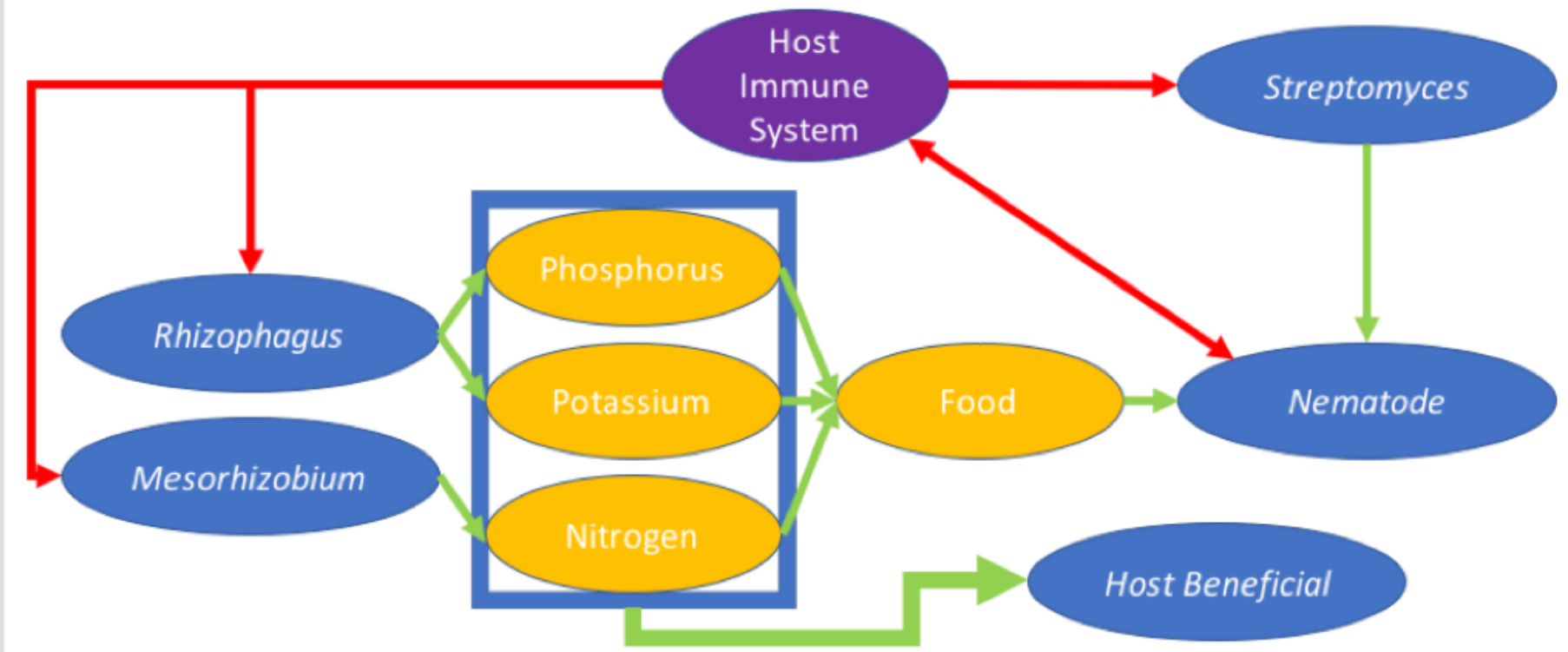


Figure 5. *Mesorhizobium* and *Rhizophagus* are associated with increased phosphorus, potassium, and nitrogen availability, providing nutrients for both host and other organisms in the phytobiome. Both the organisms have potential commensal/mutualistic/parasitic relationships with a nematode, suggesting that the increase in nutrient availability positively effects nematode growth, either directly or indirectly through promoting the growth of the nematode's food source. Additionally, *Streptomyces* is associated with promoting growth of nematodes that consume plants and fungi. Lastly the nematode is able to suppress the plant immune system, which may potentially affect the the growth of other organisms

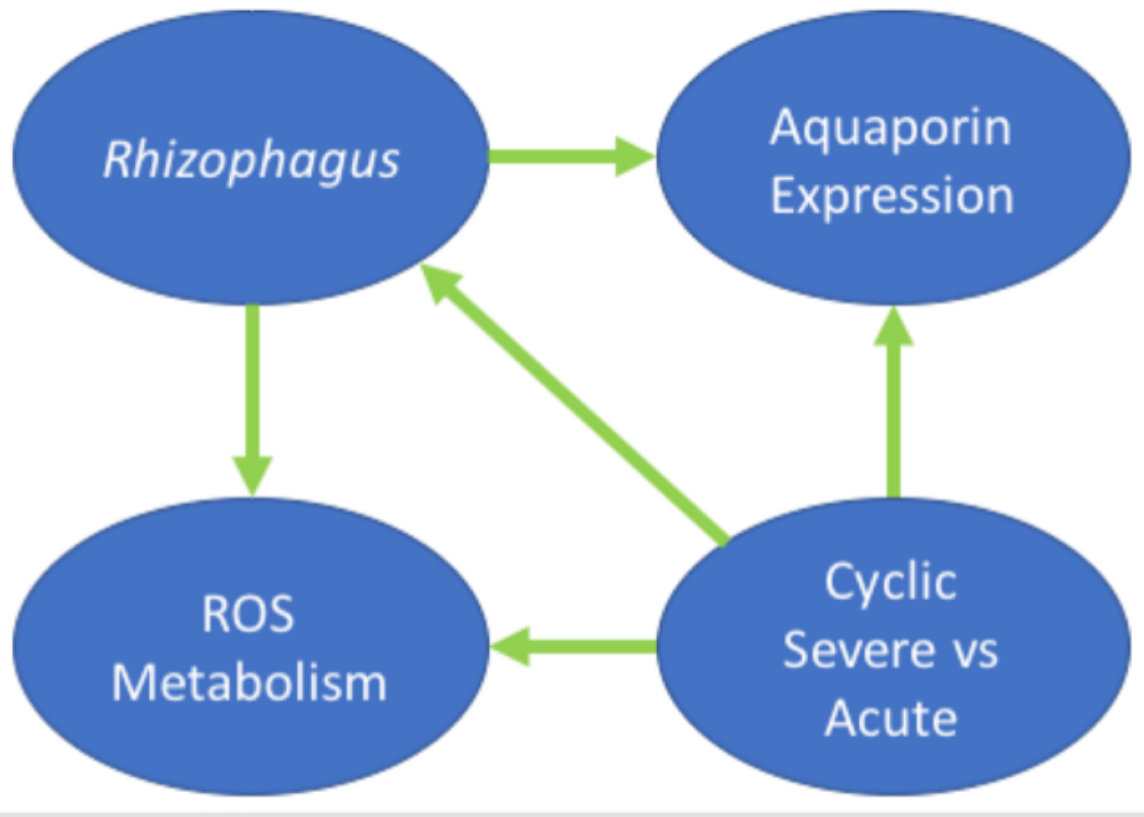


Figure 6. Cyclic severe drought has higher aquaporin and ROS metabolism expression and higher *Rhizophagus* abundance relative to acute drought. *Rhizophagus* has been shown to be associated with alternative oxidases and increased aquaporin expression in other studies and also has a positive DUO association with host ROS metabolism genes, suggesting *Rhizophagus* may alleviate drought stress

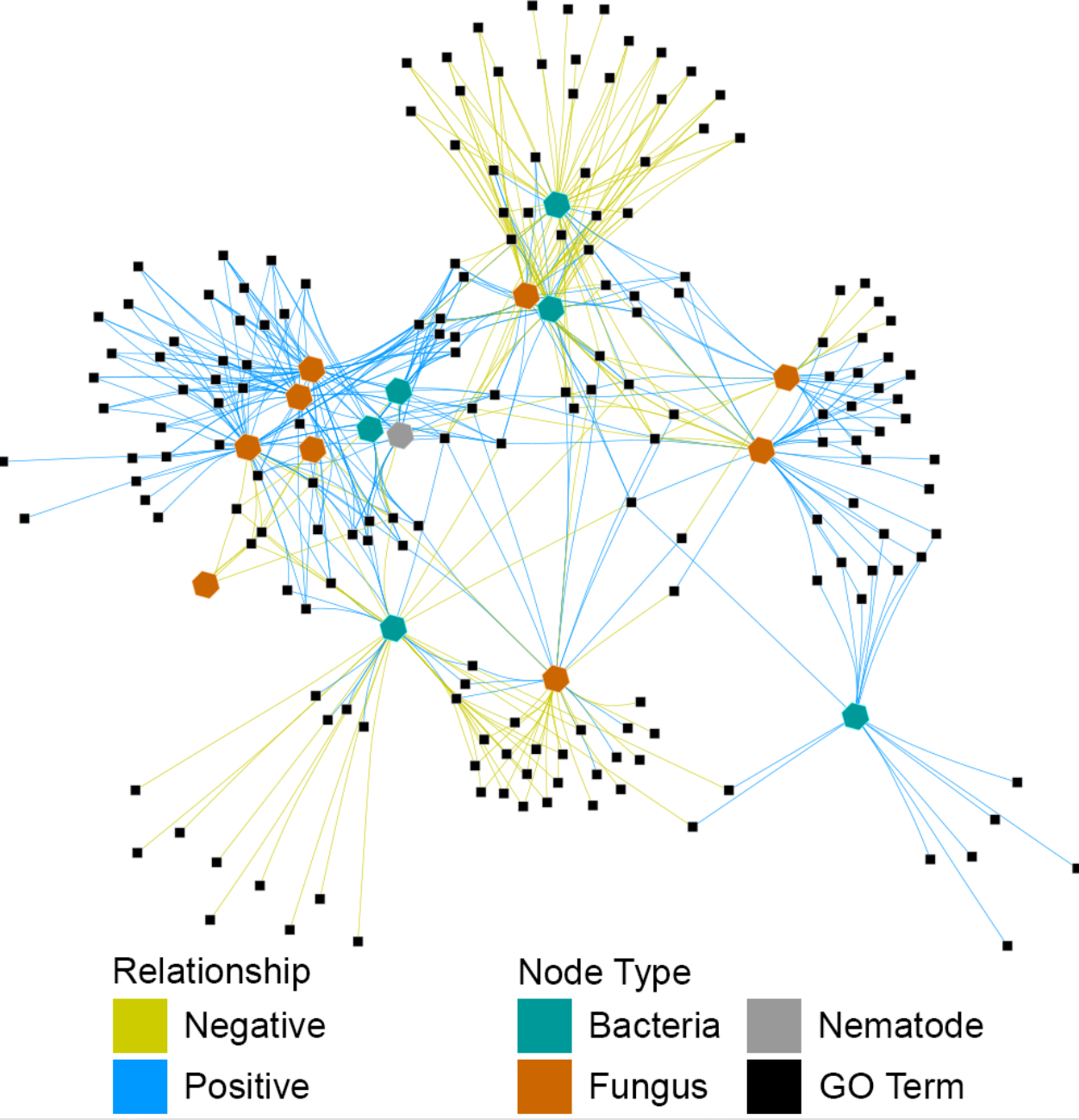


Figure 7. DUO associations between taxa and GO terms. *Rhizophagus* and *Trichinella* are associated with host ROS metabolism, further supporting *Rhizophagus* may be mitigating drought stress and *Trichinella* may be modulating the host immune system. Additionally, *Streptomyces* and *Pseudomonas* are positively associated with disease resistance genes, which may help protect plants from disease during drought stress. Also, acute drought and cyclic drought have non-overlapping associations of disease resistant genes and taxa suggesting that both conditions are experiencing different types of disease stresses.

## Characterizing the Phytobiome

Traditional identification of microbiome abundance still often relies on marker-based operational taxonomic units (OTU), such as 16s rRNA, which both limits the scope of organisms identified and requires a separate sequencing run. However, RNA-Seq offers the opportunity to identify both the host transcriptome and the high abundance organisms using whole genome sequences of all available sequenced organisms, and not just the organisms that contain the marker of interest. To achieve this, we have developed a pipeline (Figure 1) that combines traditional RNA-Seq with modified parallelized version of Kraken<sup>1</sup> (ParaKraken).

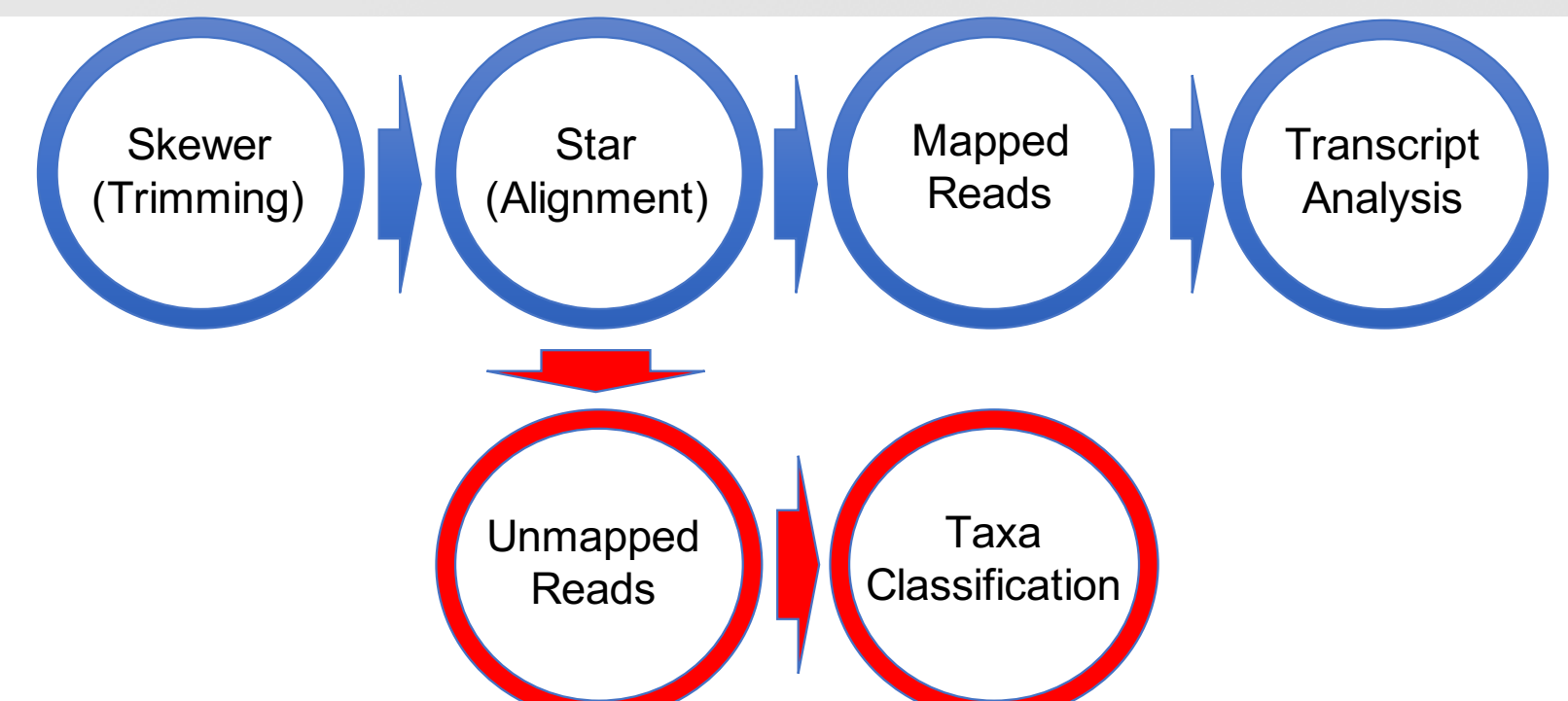


Figure 1. RNA-Seq pipeline that utilizes the mapped reads for transcriptome analysis while running the unmapped reads through ParaKraken to identify organisms in the phytobiome

As it is impractical to store every single whole genome in a single database, we have developed ParaKraken (Figure 2), allowing us to utilize whole genome sequences without subsetting the genomes and losing accuracy. Kraken<sup>1</sup> uses a kmer approach, which breaks up genomes into short nucleotide sequences (31 mers) that allows high precision with fast matching speeds. For this drought study, we have created databases that contain whole genomes from 33k+ bacteria, 734 archaea, 571 fungi, 25 nematodes, 2 aphids, 7k+ viruses, and *P. deltoides* with the ability and future plans to expand the databases to every known sequenced organisms allowing for unprecedented classification ability.

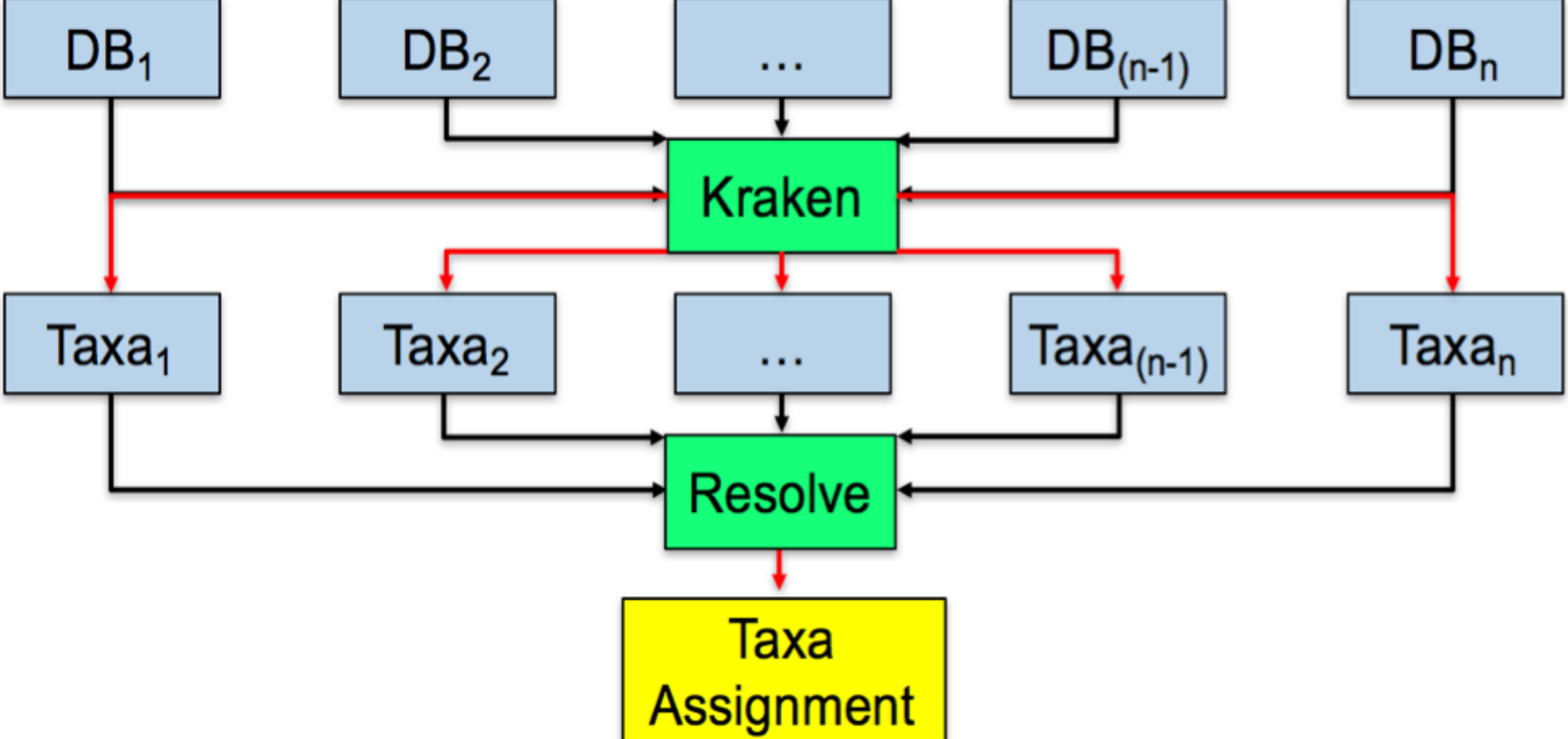


Figure 2. ParaKraken takes any number of RNA or DNA read files and runs them against all databases filled with whole genome sequences in parallel. Taxa assignments are generated for each database-read pair and then resolved using lowest common ancestor to assign a final taxa

## Conclusions and Future Directions

Effects of drought on both the plant and phytobiome are dependent on severity and prior drought exposure, with more severe drought causing severe metabolic impairment. However, despite this impairment, cyclic severe drought plants have relatively higher photosynthetic and ROS metabolism than severe acute drought, in addition to phytobiome changes that favor nitrogen production, potassium and phosphorus uptake, and lower plant oxidative stress. Conversely, severe acute drought plants have a phytobiome that is more associated with plant death and pathogenicity. Changes that occur during cyclic drought suggest that these plants are better acclimated to drought compared to those subjected to a progressive acute drought.

Future work includes expanding the ParaKraken databases to contain all known full genome sequences to allow for more complete identification of members of the phytobiome. Additionally, integrating meta-omics (metabolomics, transcriptomics, proteomics, phytobiomics) into a more complete analysis of stress, including drought, to identify more complete system and pathway responses that can then be modulated to alleviate stress and improve plant growth.

### References:

- Wood et al. Kraken: ultrafast metagenomics sequence classification using exact alignments. Genome Biology. 2014
- Abraham et al. Quantitative proteome profile of water deficit stress responses in eastern cottonwood (*Populus deltoides*) leaves. PLOS One 2018

### Acknowledgement:

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