

Comparative transcriptomic assay of *Phellinus tremulae* isolates to identify the genetic responses of environmental stress

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INTRODUCTION

Saprotrophic fungi are a diverse group of fungi that can decompose organic matter to obtain carbon.¹ Mimicking real world situations and using transcriptomic analysis can allow us understanding on how saprotrophic basidiomycetes respond to changes and what genetic mechanisms they utilize to thrive under those conditions.

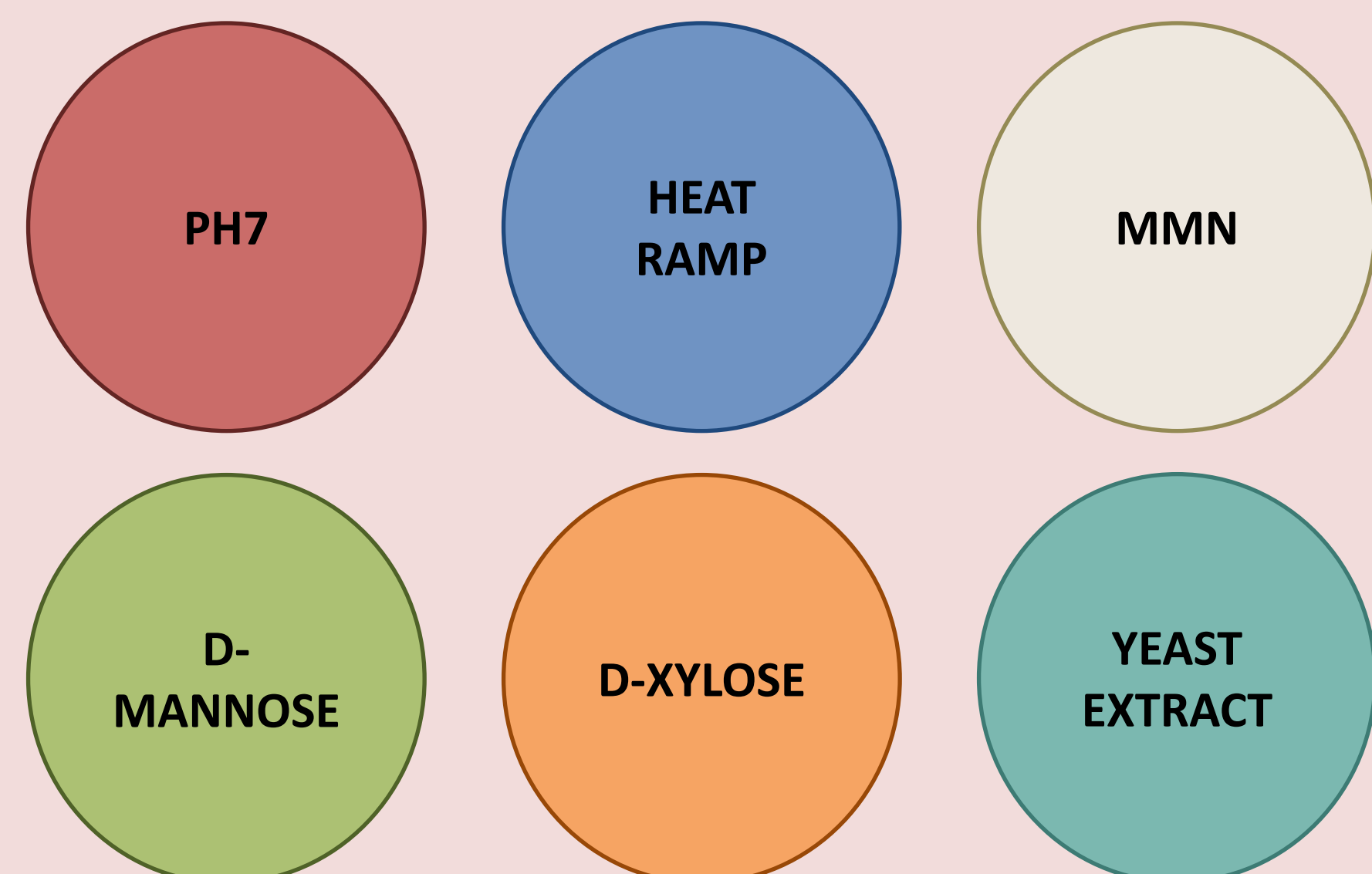
This research utilizes cultures of a parasitic fungus *Phellinus tremulae*, which is known to cause white trunk rot found among quaking aspen trees (*Populus tremuloides*). As temperatures rise due to climate change, the abundance, distribution, and impact of *P. tremulae* may change, which will change its impacts on aspen trees.²

This study can help us further understand the effects of climate change on saprotrophic fungi, including their decomposition abilities and their interactions with plants.

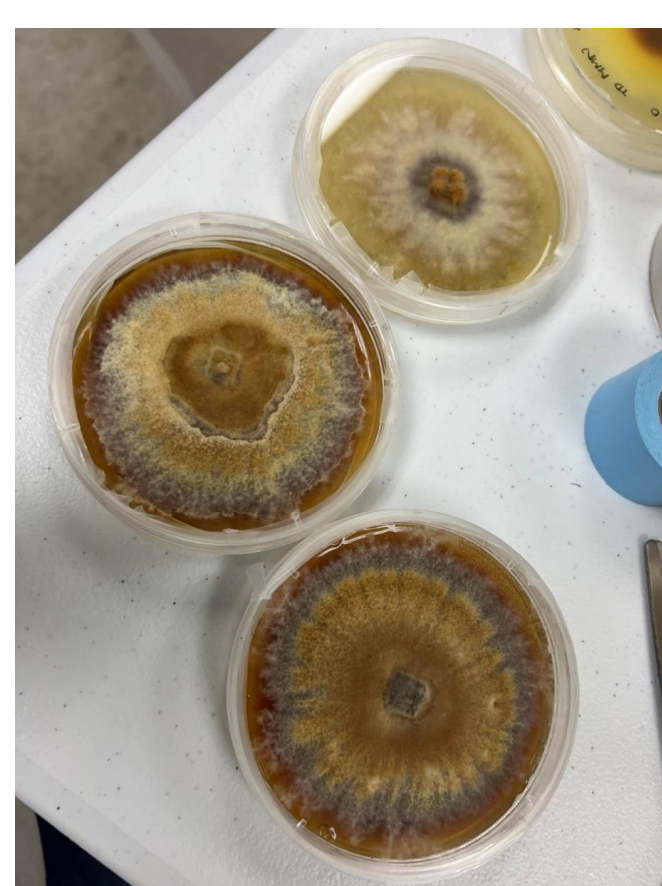
As climate change continues to become an issue, studying these stressors can create a better understanding of the stress tolerance mechanisms involved and how it can lead to evolutionary adaptation.

METHODS

• Conditions:



- Heat Ramp: Gradual increase from 24°C to 40°C
- RNA was extracted from the tissue.
- Bioinformatic workflow with fastp, hisat2, and Salmon was used for trimming and sequence alignment.
- A reference transcriptome for BD265, *Phellinus tremulae*, was constructed with a meta-transcriptome of all from the BD265 isolates sequences combined utilizing Trinity-v2.9.1. After assembly we removed redundant transcripts with CD-HIT-est using default settings.
- We interpreted the sequence data with R package DESeq2 to statistically analyze and find the differentially expressed genes.³
- After assemble, transcripts were annotated using eggNOG-mapper.⁴



Left: Cultures of *P. tremulae* isolates

Right: *P. tremulae* on a tree.⁵

RESULTS

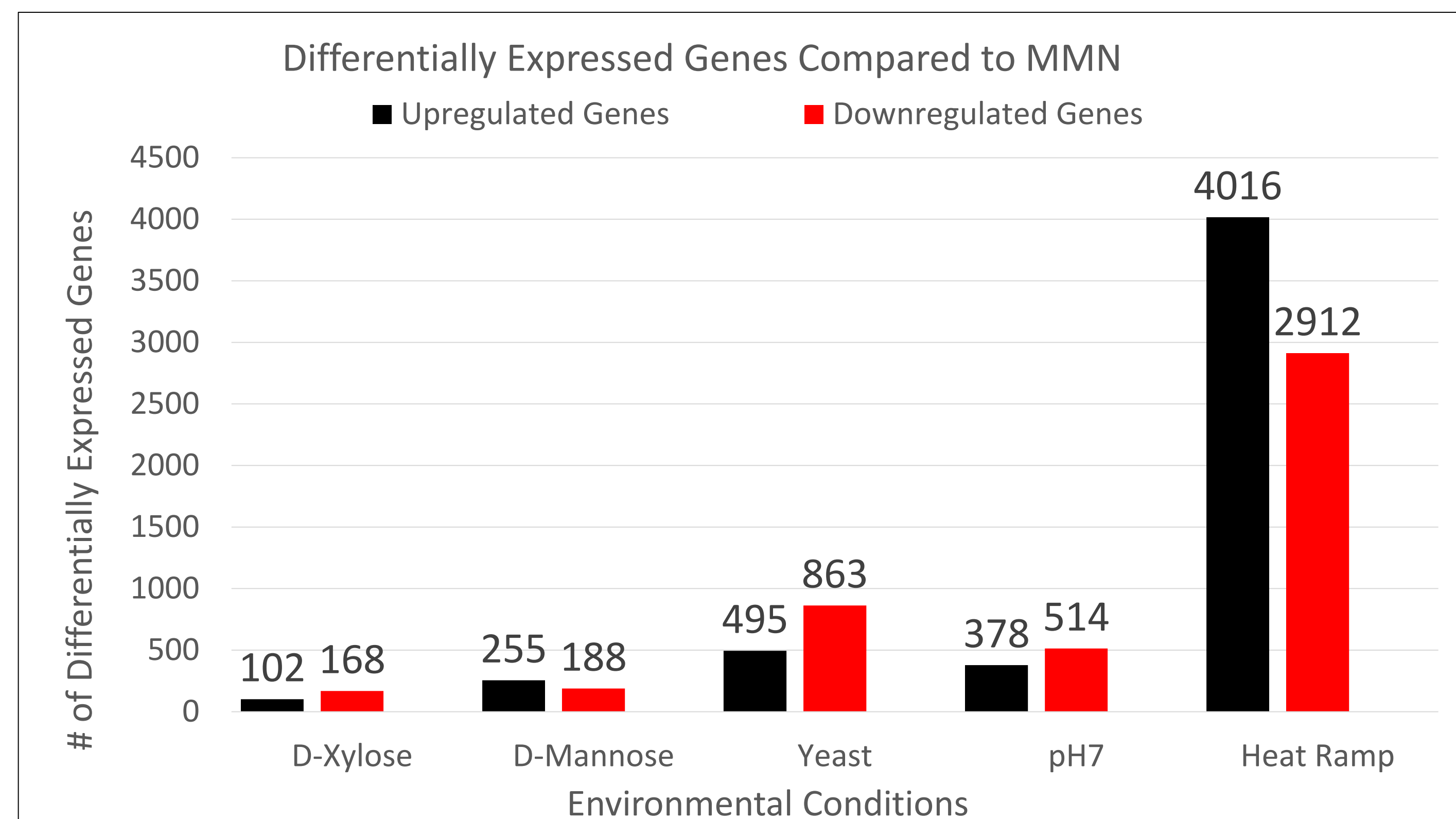


Figure 1: Bar graph depicts the number of differentially expressed genes from each of the five conditions found using a Wald test with a adj. p-value of 0.05.

CONDITIONS	PATHWAYS		
D-mannose Downregulated	(RNA Methylation)	(Global Regulator of Gene Expression)	N/A
D-mannose Upregulated	Inhibitor of growth proteins N-terminal histone-binding (Inhibitory Enzymes)	Alpha beta-hydrolase (Decomposition)	O-methyltransferase family 3 protein (DNA Methylation)
D-xylose Downregulated	WD40 repeat-like protein (Biological Functions)	NACHT domain (Conserved Protein Domain)	ribosomal protein (rRNA)
D-xylose Upregulated	(Enzyme-Catalyzed Reactions)	chromatin remodeling protein (Cellular Processes)	2OG-Fe(II) oxygenase superfamily (Metabolic Pathways)
Yeast Extract Downregulated	Fungal specific transcription factor domain (Transcription Factor)	Sugar transporter (TC 2.A.1.1) family (Transporter)	Protein transport protein Sec61 subunit beta (Transport Protein)
Yeast Extract Upregulated	Dienelactone hydrolase family (Metabolite Degradation)	ATP-dependent DNA helicase (DNA Replication)	MAPK signaling pathway activated in response to high osmolarity. (Stress-activated MAPK pathway)
PH7 Downregulated	P-loop containing nucleoside triphosphate hydrolase protein (Nucleotide-Binding Protein Folds)	C2H2-type zinc finger (Transcription Factors)	WD40 repeat-like protein (Biological Functions)
PH7 Upregulated	Mitochondrial carrier (Metabolite Transporters)	(Membrane Protein Transportation/Cell Wall Synthesis)	WD40 repeat-like protein (Biological Functions)
Heat Ramp Downregulated	CorA-like Mg2+ transporter protein (Transporter)	Calcium proton exchanger (Exchanger)	RNA-binding, Nab2-type zinc finger (Transcription Factors)
Heat Ramp Upregulated	Cell cycle checkpoint (Cell-Division Cycle)	Signal recognition particle receptor (Docking Protein)	alpha/beta hydrolase fold (Decomposition)
Nutritional Stress Downregulated	LCCL domain-containing protein	cytosine-purine permease (Active Transporter)	N/A
Nutritional Stress Upregulated	N/A	N/A	N/A
Habitat Stress Upregulated	L-lysine 6-monooxygenase (NADPH-requiring)	Inhibitor of growth proteins histone-binding (Inhibitory Enzymes)	DASH complex subunit Dam1 (Biological/Cellular Functions)
Habitat Stress Downregulated	UDP-glucose:Glycoprotein Glucosyltransferase (Glucose Metabolism)	rRNA (uridine-2'-O-)-methyltransferase activity (RNA Methylation)	Ribosomal protein S6 (Translation)

Table 1: A table of top three hits that have predicted functions from differentially expressed genes. These are from genes that are unique to each condition.

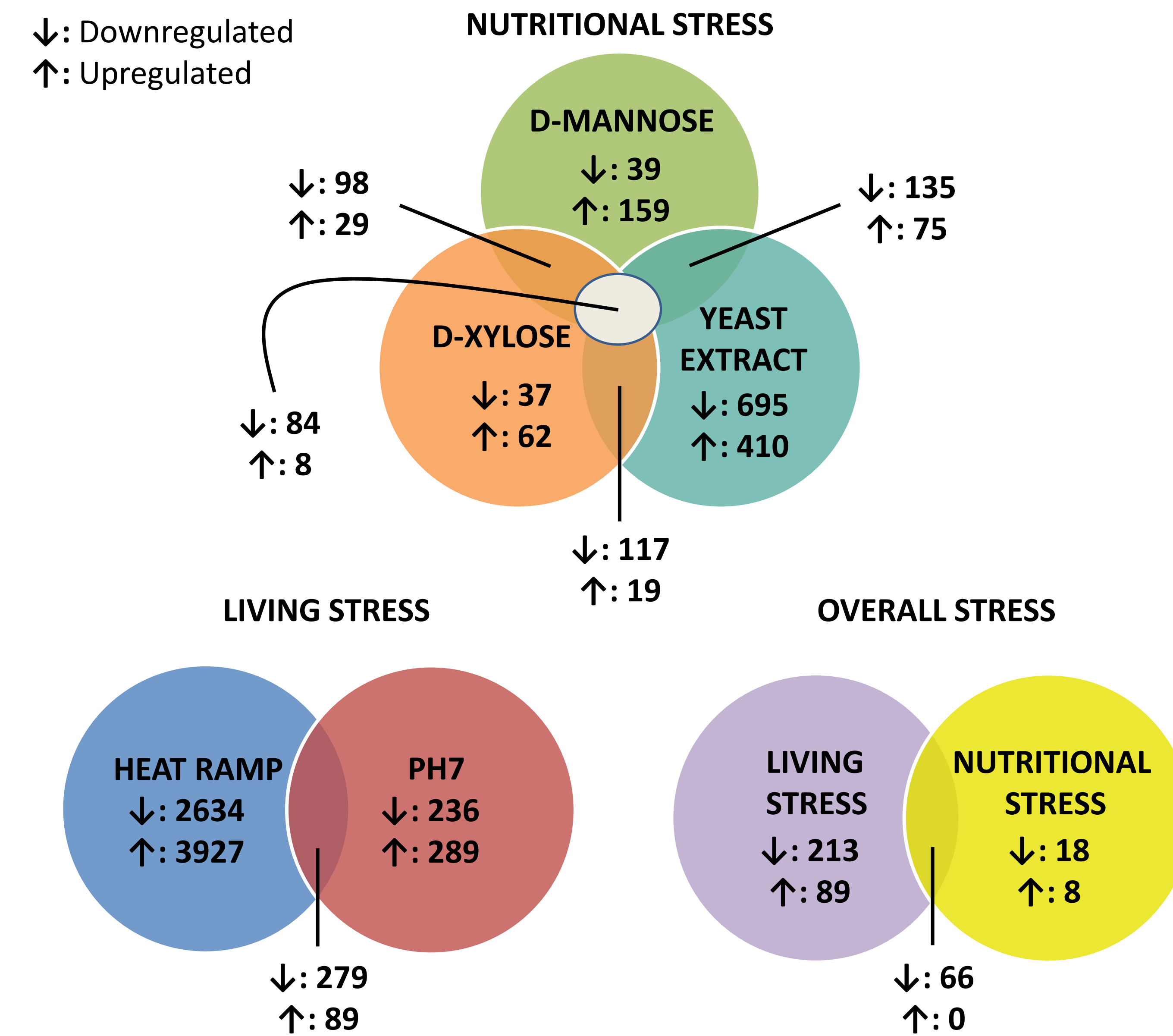


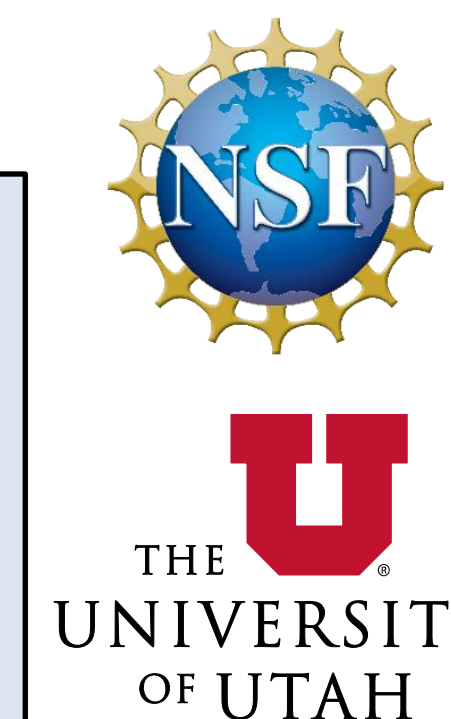
Figure 2: Venn Diagram of the Breakdown of Differentially Expressed Genes. Colors have no indication factor other than for differentiating the conditions.

CONCLUSIONS

- Five main conditions were analyzed against MMN to look at differentially expressed genes under environmental stress. Living stress had more impact on the isolates than those of nutritional stress. Out of the carbon sources, there were more differentially expressed genes in isolates grown on yeast than D-xylose and D-mannose.
- These results could be due to the complexity of living stresses and the wide variety of nutrients found in yeast extract.
- Decomposition enzymes were found in nutritional stress while growth-inhibiting pathways were found in habitat stress.
- As *P. tremulae* growth is impacted by climate change due to stressors, aspen tree parasitism rates can shift.
- This study can help us further understand the effects of climate change on saprotrophic fungi, including their decomposition abilities and their interactions with plants.
- Most fungal proteins remain functionally uncharacterized. Further research is needed to fully understand the role of these proteins across a range of environmental conditions.
- Future experimentation also includes repeating this on ECF.

REFERENCES

1. Várnai, Anikó, et al. (2014)
2. Koide et al. (2017)
3. Love et al. (2014)
4. Cantalapiedra et al. (2021); Huerta-Cepas et al. (2019)
5. Wikipedia: Phellinus Tremulae



ACKNOWLEDGEMENTS

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