Analysis of Soil and eDNA from the Rhizosphere of a *Quercus virginiana*

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Introduction

- Ciliates are eukaryotic microorganisms that fall under one of the most controversial and diverse monophyletic supergroups, SAR [3].
- Soil microbiomes are largely unexplored and there is limited information regarding the connection between the biodiversity of ecosystems and the tree rhizosphere [2].
- The purpose of this experiment was to analyze the soil metadata collected from the *Quercus virginiana,* hypothesize how this connects to the overall community profile of the tree, and how organisms are impacted by the biodiversity.
- An eDNA sample from the rhizosphere of a *Quercus virginiana* was obtained due to the natural ability for ciliates to thrive in the rhizosphere [4].
- Methods were adapted through the use of silica beads in aims to isolate the eukaryotic 18SV4 region of terrestrial ciliates [2].
- The 18SV4 region was specifically chosen in attempts to analyze eukaryotic biodiversity and perform metabarcoding analysis.

Figure 1: The soil samples were collected from this *Quercus virginiana* in the Vara Martin Daniel Plaza on Baylor University's campus.

Methods

Soil Collection

Soil Metadata Analysis

Ciliate Isolation and DNA Extraction

Gel Electrophoresis

PCR Amplification

Results

Soil Metadata:	MNH Sample:	KRM Sample:	MAA Sample:
Percent Water Content	4.494%	16.47%	15.38%
pH	7.0	6.5	6.5
Soil Texture	Sandy Loam	Sandy Clay	Sandy Clay
Ciliate Cultures	2	2	1

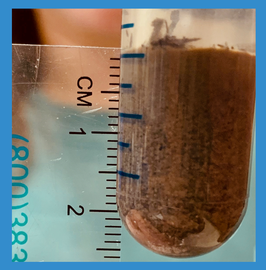

Figure 2: This image features the falcon tube containing the MNH sample. This sample was determined to be Sandy Loam based on percent composition.

Tree and Leaf Metadata	
Tree Identification:	*Quercus virginiana*
Shape and Arrangement	Linear
Margin	Entire
Venation	Pinnate

Figure 3: Leaves collected from a *Quercus virginiana* in the Vara Martin Daniel Plaza.

Figure 4: Collected leaf under microscope to better distinguish leaf metadata.

Sample	Concentration	A260/280:	A260/320:
MNH	33 ng/µL	1.3	0.05
KRM	326.3ng/µl	1.44	0.52

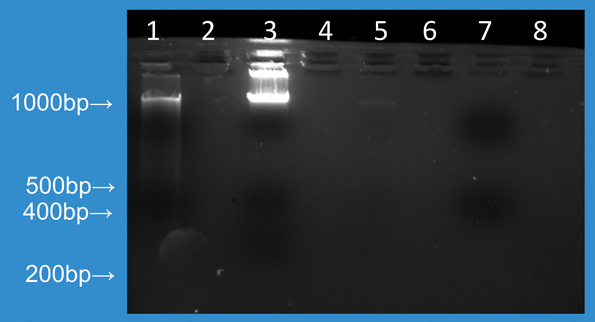

Figure 5: The soil sample was collected from the rhizosphere, near the shallow root of a tree. The DNA was extracted using a lysate method involving charcoal and silica beads. The DNA was then analyzed using a 1.5% agarose gel for electrophoresis; Lane 1: DNA Sample LL, Lane 2: blank, Lane 3: 5,000ng mass standard, Lane 4: blank, Lane 5: 15 ng mass standard, Lane 6: blank, Lane 7: DNA Sample MNH, Lane 8: blank. The image was taken by the MCB C305.

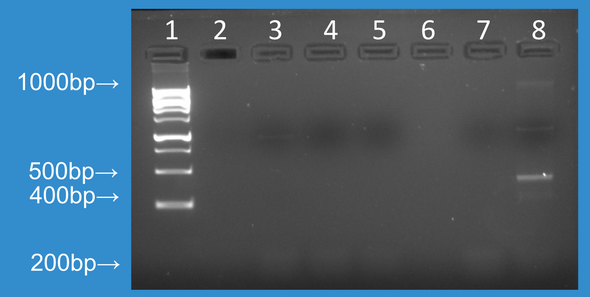

Figure 6: The DNA was prepared using the 2x Master Mix and the 18S V4 primer. The DNA was then analyzed using a 1.5% agarose gel for electrophoresis. Lanes from left to right, Lane 1: 5ul 1 kb ladder (company unknown), Lane 2: MM control, Lane 3: MM DNA, Lane 4: KRM control, Lane 5: KRM treatment, Lane 6: blank, Lane 7: LL control, Lane 8: LL DNA. The image was taken by the MCB C305.

Conclusion and Discussion

- Both MNH and KRM yielded an A260/280 concentration close to 1.8, indicating that there was a presence of eDNA.
- Results from PCR amplification of the KMR and MNH samples indicate that soil collected from the rhizosphere surrounding the *Quercus virginiana* did not contain quantifiable eukaryotic eDNA.
- Before eDNA was collected, ciliates were observed within the non-flooded plate sample. This could indicate that the V4 region of the ciliates could have been corrupted.
- This field experiment allowed research to be conducted analyzing soil metadata of the collected soil from the *Quercus Virginiana* rhizosphere and how this may affect the overall community profile of Live Oak trees.
- Although the results from both samples were negative, adapted methods from *A Rapid and Economical Method for Efficient DNA Extraction from Diverse Soils Suitable for Metagenomic Applications* [1] produced positive DNA results for silica bead extraction and can be used in future studies.

Acknowledgments

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