

# The microbial communities associated with the leaves and roots of turtle grass and manatee grass differ from seawater and sediment communities, but are similar between species

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## Introduction

Seagrasses are vital members of coastal ecosystems providing services such as shelter, food and nursery to many species including economically important fish. Recent seagrass die-offs have occurred in Florida due to microbes that reduce sulfate into phyto-toxic sulfide, causing a cloud of sulfur as seen in the image below. Other microbes, such as algae, prevent sunlight from reaching the seagrass leaves. Investigating the microbial communities of seagrasses can provide us with information about the key members of the seagrass core-microbiome. This information might allow us to explore the possibility of targeting specific microbes to help seagrasses endure environmental stress, and subsequently help the conservation of the ocean and fauna that inhabit the seagrass beds.



Cloud of sulfur surrounding dying and dead seagrass (FFWCC. Miami Herald).

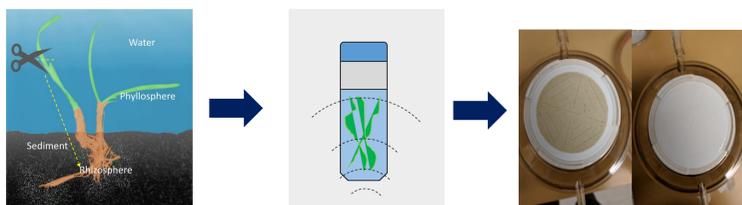
## Goals

- To identify consistencies and/or differences in the microbiome of two seagrass species in the same meadow
- To elucidate the possible functions of the abundant microbes for targeting in future studies.

## Methods

Three replicates of turtle and manatee grass were collected from Hobie Island Beach Park in Key Biscayne, FL. Collection sites were between 0.1 and 1 km apart. We collected seawater and sediment from all three locations using sterile carboys and 50 ml Falcon tubes, respectively.

### Sample Processing



- Cut leaves and roots
- Sonicate
- Filter (5 µm and 0.22 µm)

### Molecular Analysis

- DNA was extracted from water, seagrass filters, and 3 g of sediment using commercial kits
- PCR amplification of the 16S rRNA V4 region followed by amplicon sequencing on the Illumina MiSeq platform
- QIIME 2, SILVA taxonomy, R, and the MicrobiomeAnalyst Shotgun Data Profiling (SDP) module were used for bioinformatics

## References

- Guide to Unique Wildlife of the Florida Keys. (2017) Marine Life Around the World. Key West Aquarium. (Background image).
- Ugarelli, K.; Chakrabarti, S.; Laas, P.; Stingl, U. (2017) The Seagrass Holobiont and Its Microbiome. *Microorganisms*, 5, 81.
- The dying seagrass feed a cycle of elevated sulfide in bay water. FFWCC (2016). Will Florida Bay survive the summer? Miami Herald.
- 3 QIIME2 homepage: <https://qiime2.org>
- 4 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41, 590-596.
- 5 R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

## Conclusions

- The microbial community of the roots, leaves, sediment and water are distinct from one another
- Microbes involved in sulfur oxidation and nitrogen fixation were identified in the sediment and plant samples
- Some sequences are unique to each species while many overlap, indicating a possible core microbiome
- No significant differences in predictive metabolism

## Results

### Clustering of samples based on microbial composition

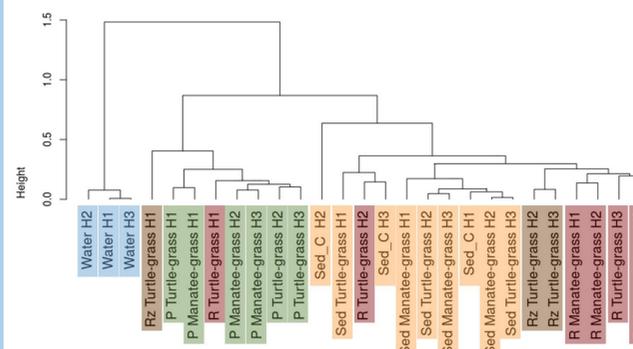


Fig 1. Cluster analysis of the microbial communities of the samples show that the roots, leaves, sediment and water microbes are distinct from one another. This indicates a possible difference in symbiotic relationships between the microbes and the seagrass at each part of the plant.

### Relative abundance of the most common taxa in each sample

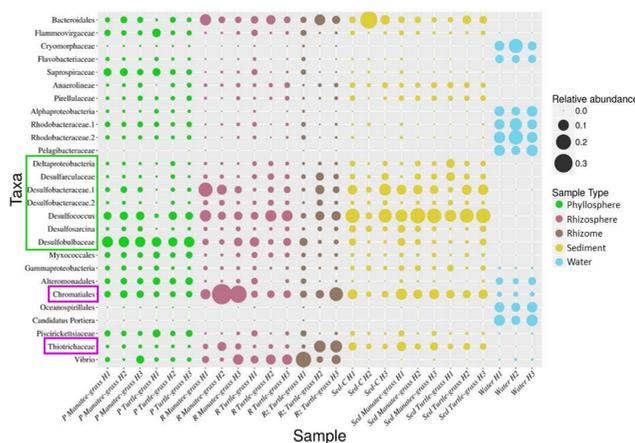


Fig 2. Some of the abundant taxa (> 1% of the sequences) are involved in biogeochemical cycles of sulfur (S oxidation: purple boxes; sulfate reduction: green boxes). Some taxa are more abundant on different segments of the plants and others are more abundant on one species than the other.

### Comparison of the number of sequence variants per species and site

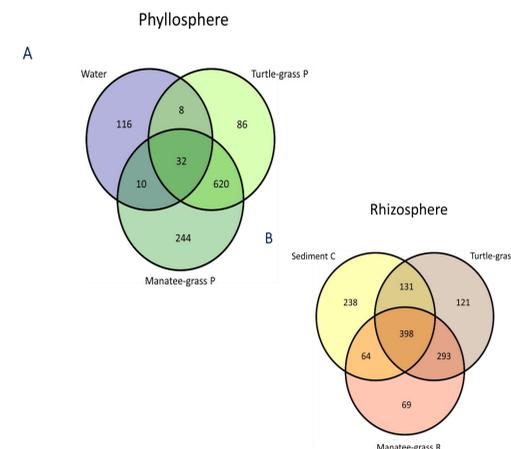


Fig 3. Comparison of sequence variants that make up > 0.01% of the total sequences. In both species, the phyllosphere communities resemble each other and not the water, while the rhizosphere communities resemble each other and the sediment. Several sequences are unique to each species.

### Significantly distinct genes based on predictive metabolism

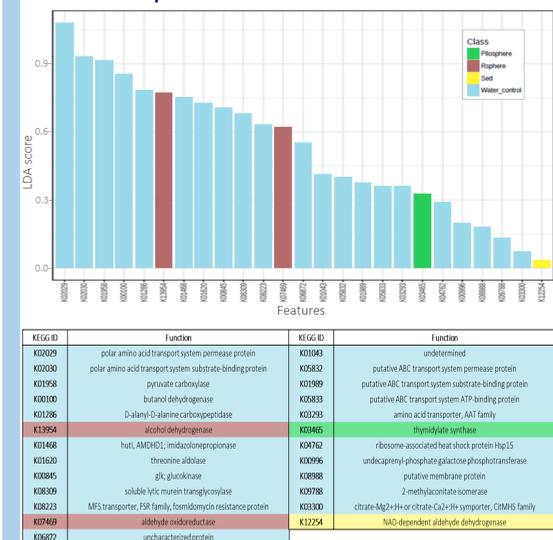


Fig 4. The most abundant significantly distinct genes per sample type are predominantly involved in basic metabolic functions. Seawater is more distinct than the other sample types.

## Future Directions

- Metagenomic sequencing for analysis of the metabolic functions of the microbiome with more replicates
- Nitrogen-fixing gene analysis of the microbiome
- Effects of environmental factors on the microbiome
- Cultivation of the key members of the microbiome