



Microbiome Differences in Control and Disease Inoculated

Acropora Corals

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Introduction

- *Acropora cervicornis* and *Acropora palmata* were once the most prevalent branching corals throughout the Caribbean reef system. Today both species are classified as critically endangered due to a 90% reduction in coral cover.
- As a result of the immense loss reefs are experiencing, coral “gardening” and outplanting restoration have become prominent methods to alleviate loss (4).
- Despite success, there are still variations in success rates due to continued exposure to disease, heat stress, and differing environmental conditions.
- In both the summers of 2016 (July-September) and 2017 (July-August) researchers investigated and characterized the disease susceptibility among different genotypes of both *A. palmata* and *A. cervicornis* (2).
- An analysis of the microbiome of these samples can not only indicate the beneficial bacteria that may be part of a “resistant” microbiome, but it can also help identify potential pathogens.
- The continued investigation into corals, their microbiome and diseases is vital to conservation.
- In this study the relationship between the microbiome and disease exposure will be analyzed and compared between *A. palmata* and *A. cervicornis* corals.

Methods

- Samples used for this study came from the coral disease assay experiments run in 2016 and 2017 in the Florida Keys (2)
- The field experiment took healthy *A. palmata* and *A. cervicornis* fragments of known genotypes and had zip-tied them to diseased *A. cervicornis* fragments. Exposure lasted for a 7-day period.
- Genomic samples were taken prior to disease exposure, and on day 7.
- 16s libraries were prepared following the same protocol as (1).
- All bioinformatic analysis occurred in R and Rstudio using DADA2, phyloseq, and DeSeq2.
- Identified ASVs were taxonomically classified using the Silva database. ASVs were removed from this analysis if
 - No annotation from the Silva database
 - Annotated to chloroplast or mitochondria
- For the alpha-diversity analysis, both coral species were evaluated together and separately.
- Beta-diversity was assessed between species, and within each species. Further ASV filtering was used ASVs were removed if they had (less than 1 count in greater than 2 samples for all samples).

Results

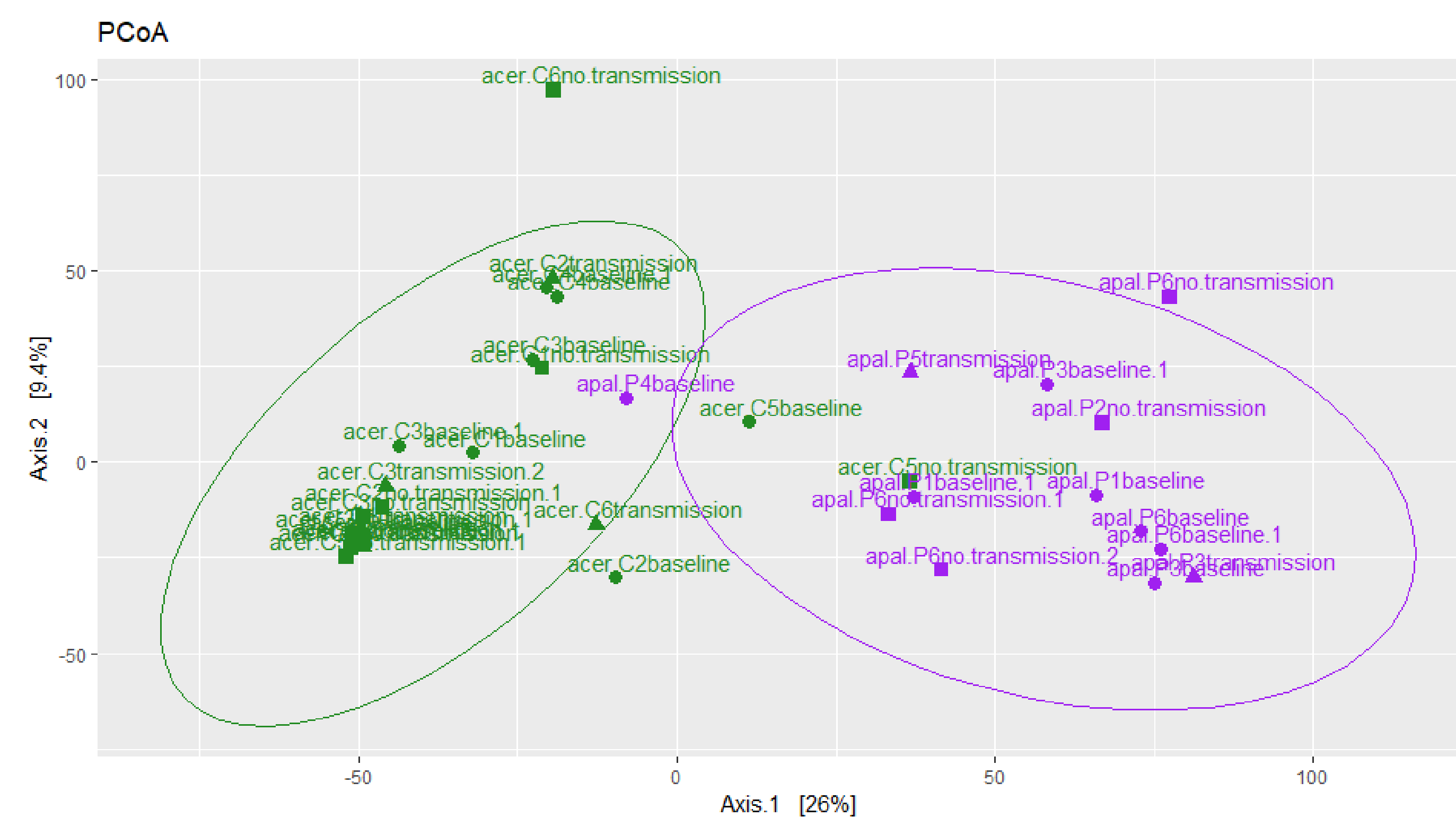


Figure 1: Principal coordinate analysis shows a clear split in the microbiomes of *Acropora palmata* (purple) and *Acropora cervicornis* (green). This hierarchical clustering of samples taken from both *A. palmata* and *A. cervicornis* shows distinct grouping, despite a few outliers, of each species separate from the other. This confirms and reinforces the statistically significant difference found between both species upon microbiome analysis.

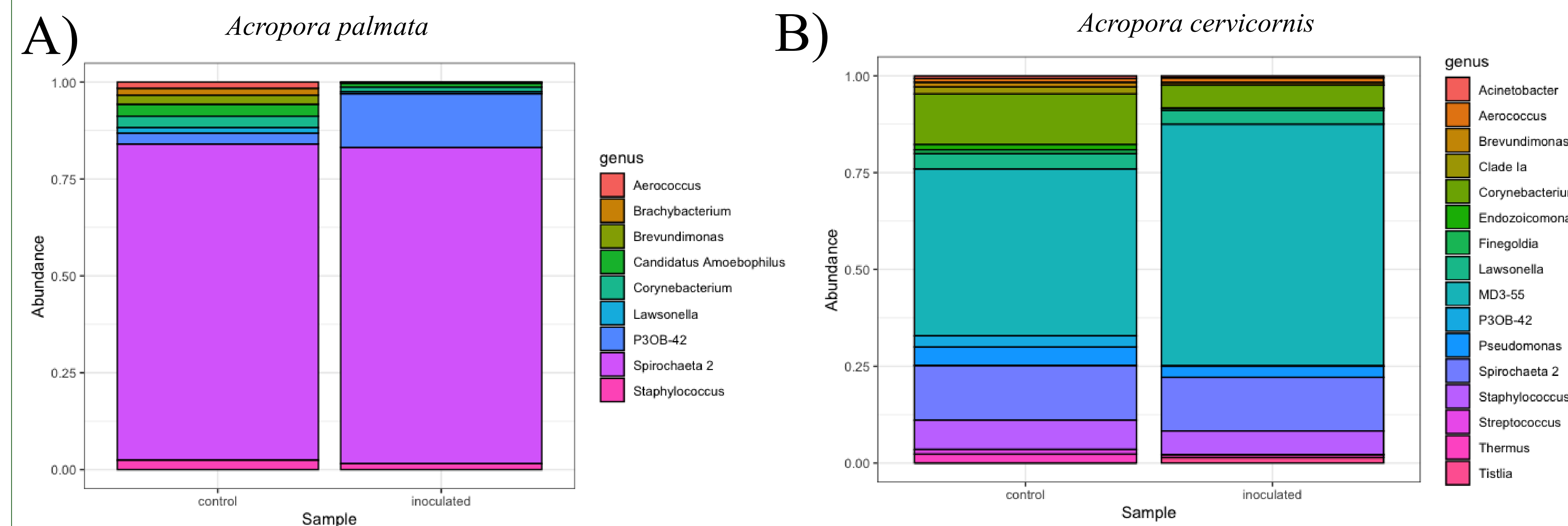


Figure 2: Alpha and beta analysis identified significant differences between the two species (*A.pal* vs *A.cer*) but not within the species (control vs inoculated).

A = density plot showing the most relative abundant genera in *Acropora palmata*; Spirochaeta 2 (phylum *Spirochaetota*) and P3OB-42 (phylum *Proteobacteria*)
 B = density plot showing the most relative abundant genera *Acropora cervicornis*; MD3-55 (phylum *Proteobacteria*) and Spirochaeta 2 (phylum *Spirochaetota*).
 For A) and B); samples are grouped as control (samples taken before disease grafting) or inoculated (samples taken after 7-day disease exposure). The most abundant taxa at the genera level for each species were identified by retaining any taxa that had a proportion > 0.05 with all taxa present. For each species, density plot fills are identified to the right.
 C = the three alpha metrics calculated between the two species. The only identified significant difference between species was identified by the Chao1 index (alpha 0.05).

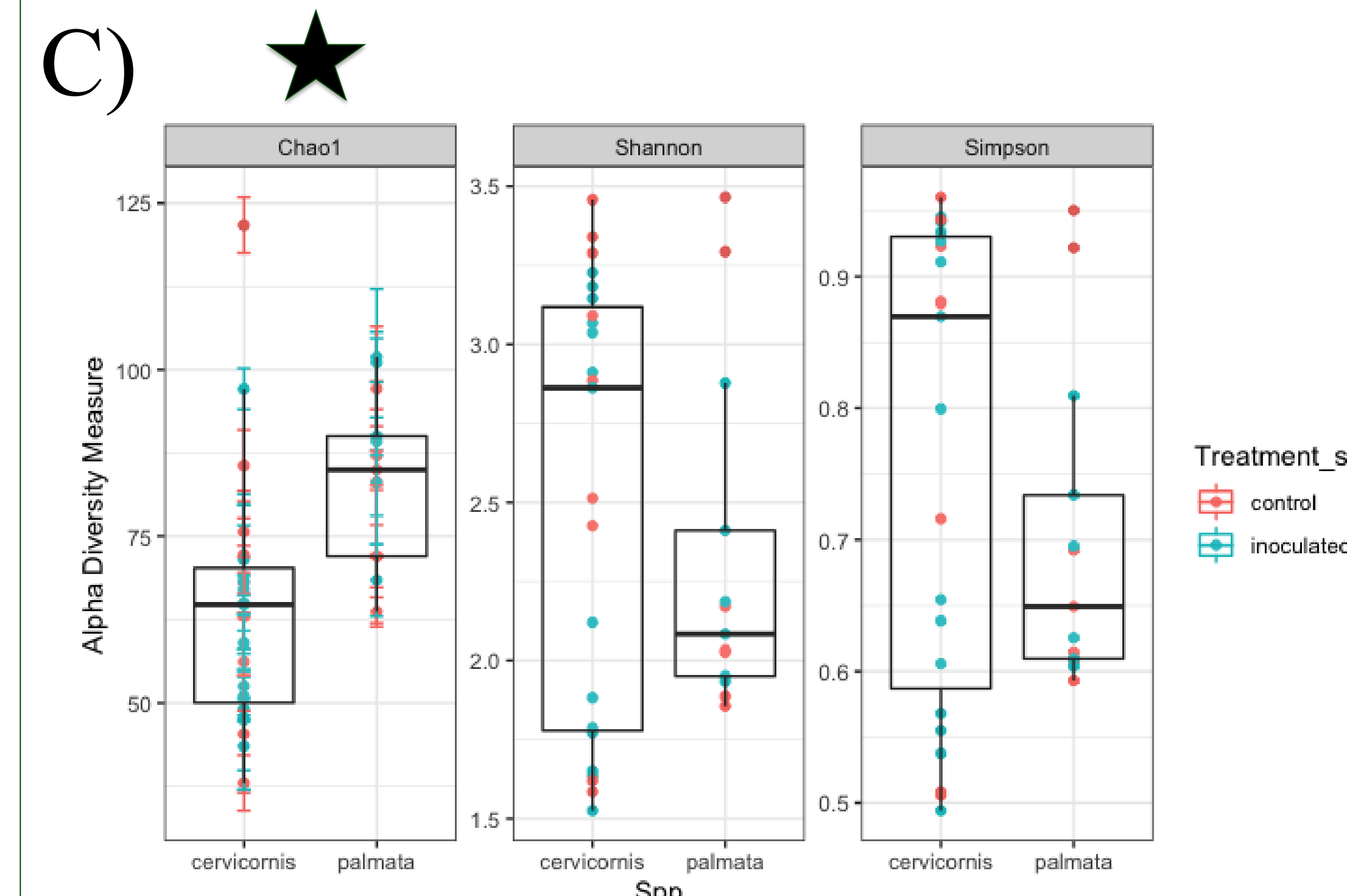


Figure 3: There are 6 significant differentially abundant taxa identified between *Acropora palmata* and *Acropora cervicornis*:

For *A. cervicornis*: *Pseudomonas* (phylum *Proteobacteria*) and one only classified to domain *Bacteria* decreased after disease exposure; *Rhizobiaceae* (phylum *Proteobacteria*), *Corynebacterium* (phylum *Actinobacteria*), and *Schlegella* (phylum *Proteobacteria*) increased after disease exposure.

For *A. Palmata*: *Brevundimonas* (phylum *Proteobacteria*) was less abundant in diseased coral samples.

Heatmap showing the differentially significant abundance taxa between *A. cervicornis* and *A. palmata*. *A. cervicornis* samples are shown at the very top in black, and *A. palmata* samples are shown in grey. Red scale colors indicate taxa that are more highly significant within the dataset than the neutral-cool colors that indicate lower abundance significance. Samples are clustered by hierarchical clustering identifying the clear split between the two species.

Discussion

- This overall showed that *A. cervicornis* and *A. palmata* are host to distinct microbial communities, which is in agreement with past microbiome studies.
- Spirochaeta 2 was significant in *A. palmata* and *A. cervicornis* and also found in high abundance in the 2017 *A. Palmata* samples; this taxa has nitrogen-fixing abilities and documentation in microbiomes of other coral species, leading to the possibility that it is a beneficial microbe.
- MD3-55, which was identified in control and inoculated *A. cervicornis* samples, was also found in the 2017 samples (1), this bacterium has been previously identified as a parasitic pathogen often associated with WBD.
- Not much is known about P3OB-42 (present in *A. palmata*), however in the 2017 analysis, this ASV was significantly in the visually unaffected samples, leading to the possibility that this microbe plays a role in disease resistance(1).
- *Rhizobiaceae* (phylum *Proteobacteria*) was identified to be a potential pathogen, especially in *A. cervicornis*, and *Brevundimonas* (phylum *Proteobacteria*) was identified in *A. palmata* as a potentially beneficial microbe.

Acknowledgments

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References

1. Rosales et al. 2019
2. Miller et al 2019
3. Lirman et al. 2010
4. Lirman et al 2016