Periodontitis

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1 Introduction

1.1 Microbiome

Microbiome is consist of microbiota, the micro-organisms which live inside and on humans (Turnbaugh et al., 2007). Microbiome is also about 10^{13} micro-organisms whose which collective genome (Gill et al., 2006).

1.2 Ribosomal RNA

Ribosomal RNA (rRNA) is well-known as a key to phylogeny (Olsen & Woese, 1993).

1.3 16S rRNA Gene Sequencing

1.4 Periodontitis

Periodontitis is an inflammatory conditions which effecting periodontium, tissues which surround and support teeth. Major components of periodontitis are clinical attachment loss and bone loss (Flemmig, 1999). Previous study found risk factors of periodontitis such as smoking, diabetes, genetic factors and host response (Van Dyke & Dave, 2005).

2 Materials

2.1 16S rRNA Gene Sequencing

- 100 Healthy samples
- 50 Chronic Early Periodontitis Sample
- 50 Chronic Moderate Periodontitis Sample
- 50 Chronic Severe Periodontitis Sample

3 Methods

3.1 QIIME2 Workflow

QIIME2 is a capable, expandable and distributed microbiome analysis package with transparent analysis (Bolyen et al., 2019, 2018). A theoretic overview of QIIME2 workflow is shown as figure 2.

3.1.1 Denoising techniques

There are two denoising techniques provided by QIIME2: DADA2 (Callahan et al., 2016) and Deblur (Amir et al., 2017). Major difference between DADA2 and Deblur, as shown as figure 3, is a strategy, the strategy used to divide as different species. DADA2 uses amplicon sequence variants (ASVs), strictly divides sequences even one-base mismatch. However, Deblur uses operational taxonomic units (OTUs), considers as same sequence when sequences are 97 % or more matched.

3.1.2 Taxonomy Classification

There are two taxonomy classification databases which provided by QIIME2: Greengenes (GG) (DeSantis et al., 2006) and SILVA (Pruesse et al., 2007). Major difference between Greengenes and SILVA is resolution. Resolution of Greengenes is from kingdom to species; however, resolution of SILVA is from domain to genus. Note that a higher accuracy at taxonomic levels above genus level; but accuracy drops at species level (Gihawi et al., 2019).

3.1.3 Rarefaction

Rarefaction is a statistical method of estimating the number of species expected in a random sample which taken from a collection (James & Rathbun, 1981). Moreover, rarefaction allows comparisons of the species richness among communities. Thus, rarefaction is a good choice for normalization (Weiss et al., 2017).

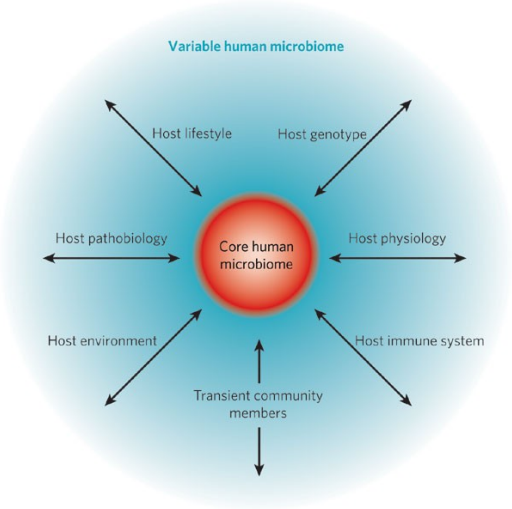

Figure 1: Concept of a Core Human Microbiome (Turnbaugh et al., 2007)

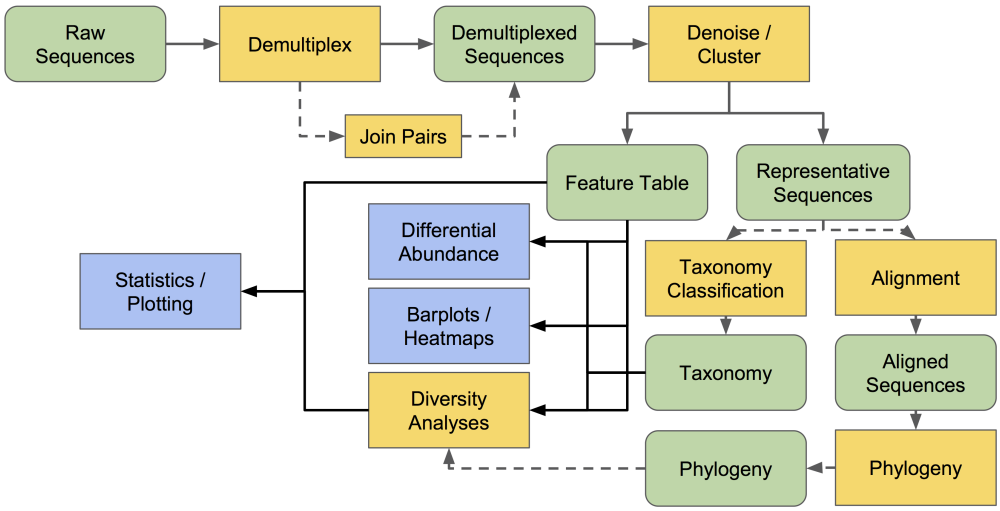

Figure 2: A Theoretic Overview of QIIME2 Workflow (Bolyen et al., 2019, 2018)

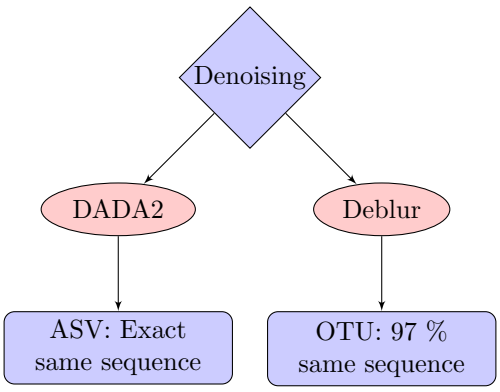

Figure 3: Denoising Techniques which provided by QIIME2

3.1.4 Alpha-diversity

Alpha-diversity is a metric which shows the richness of taxa at a single community. There are four alpha-diversity indices which provided from QIIME2:

- Evenness index.
- Faith's phylogenetic diversity.
- Observed features.
- Shannon's diversity index.

Shannon's diversity index shows a quantitative measure of community richness; Observed features, however, is a qualitative measure of community richness. Faith's phylogenetic diversity index indicates a qualitative measure of community richness which assimilates phylogenetic relationship among features. Finally, evenness index, as its name, shows a measure of community evenness.

3.1.5 Beta-diversity

Beta-diversity is a metric which indicates the taxonomic differentiation between multiple communities. There are four beta-diversity indices which provided from QIIME2:

- Bray-Curtis distance.
- Jaccard distance.
- Unweighted UniFrac distance.
- Weighted UniFrac distance.

Bray-Curtis distance shows a quantitative of community dissimilarity; Jaccard distance, however, indicates a qualitative measure of community dissimilarity. UniFrac distances reveal a measure of community dissimilarity which consolidates phylogenetic relationship among features. Difference between unweighted UniFrac distance and weighted UniFrac distance is a qualitative and a quantitative, respectively.

3.1.6 ANCOM

ANCOM (Analysis of composition of microbiomes) can be used for analyzing the composition of microbiome in multiple populations (Mandal et al., 2015). Example ANCOM volcano plot is shows as figure 5.

3.2 Python Packages

3.2.1 Pandas

Pandas is a Python package of rich data structures and tools for analyzing with structured data sets (McKinney et al., 2011).

3.2.2 Scikit-learn

Scikit-learn grants state-of-the-art implementation of many machine learning algorithms, while controlling an easy-to-use interface tightly integrated the Python code (Pedregosa et al., 2011).

3.2.3 Matplotlib

Matplotlib is a Python graphics package which used for application development, interactive scripting and publication quality image generation (Barrett, Hunter, Miller, Hsu, & Greenfield, 2005). Matplotlib, also, is designed to create simple plots with a few commands (Hunter, 2007).

3.2.4 Seaborn

Seaborn is a Python data visualization package which based on matplotlib, allows a high-level interface for displaying engaging and descriptive statistical graphics (Waskom & the seaborn development team, 2020).

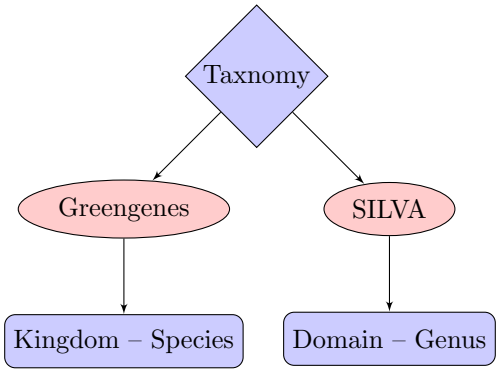

Figure 4: Taxonomy Classification which provided by QIIME2

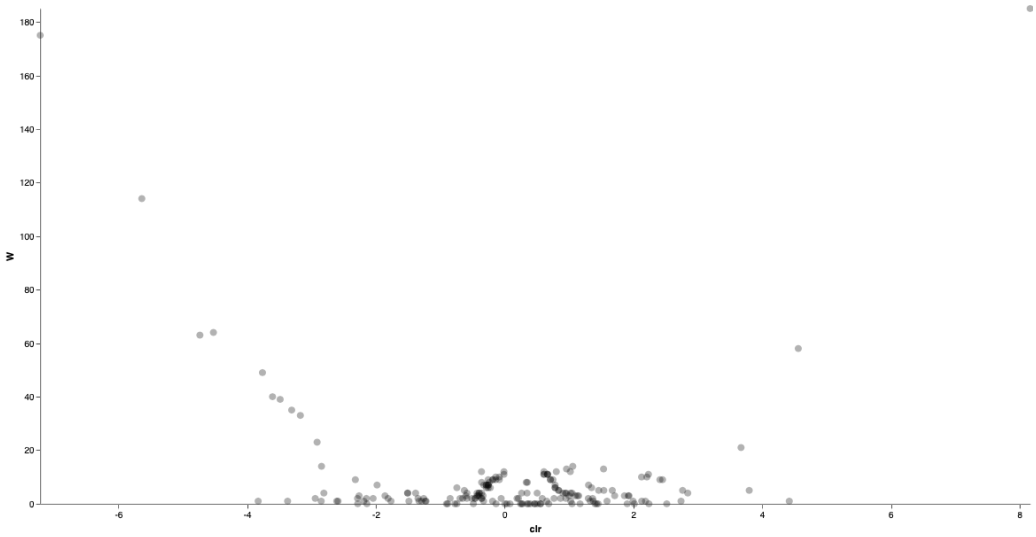

Figure 5: Example ANCOM Volcano Plot which Provided by QIIME2 (Bolyen et al., 2019, 2018)

4 Results

4.1 Quality Filter

Longer sequences have more fallen sequence quality than shorter. Thus, sequences which longer than threshold should be trimmed out due to their low quality. However, gold-standard strategy for deciding the threshold does not exist; the threshold is set as longest sequence length which have half of sequences have greater than 30 quality score. Hence, sequence quality plot is shown as figure 6; trimmed length in forward reads is 300, and trimmed length in reverse reads is 265.

4.2 Rarefaction

Sampling depth should be decided for rarefaction. Gold-standard method for determining sampling depth is minimum frequency in the samples. Hence, sampling depth with DADA2 is 3786 (Figure 7), and sampling depth with Deblur is 7253 (Figure 8).

4.3 Alpha-diversity

4.4 Beta-diversity

4.5 ANCOM

5 Discussion

6 References

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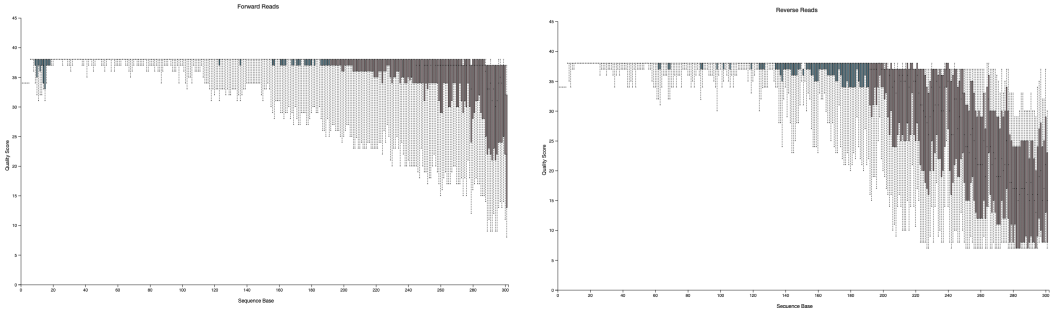


(a) Forward Reads (b) Reverse Reads

Figure 6: Sequence Quality Plot

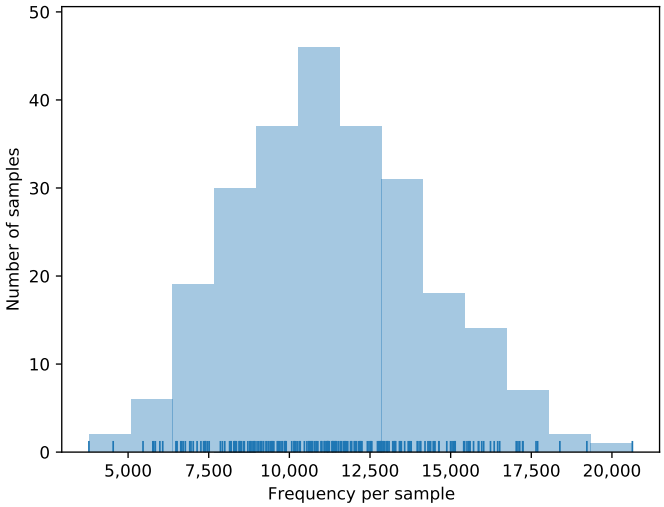


Figure 7: Frequency per Sample by DADA2

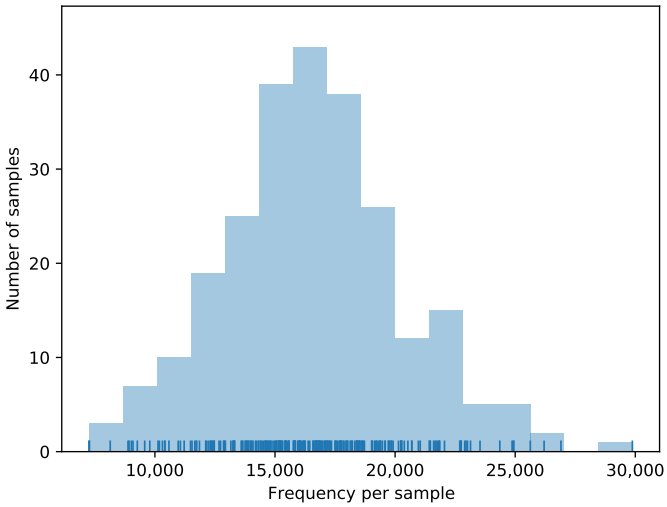


Figure 8: Frequency per Sample by DADA2

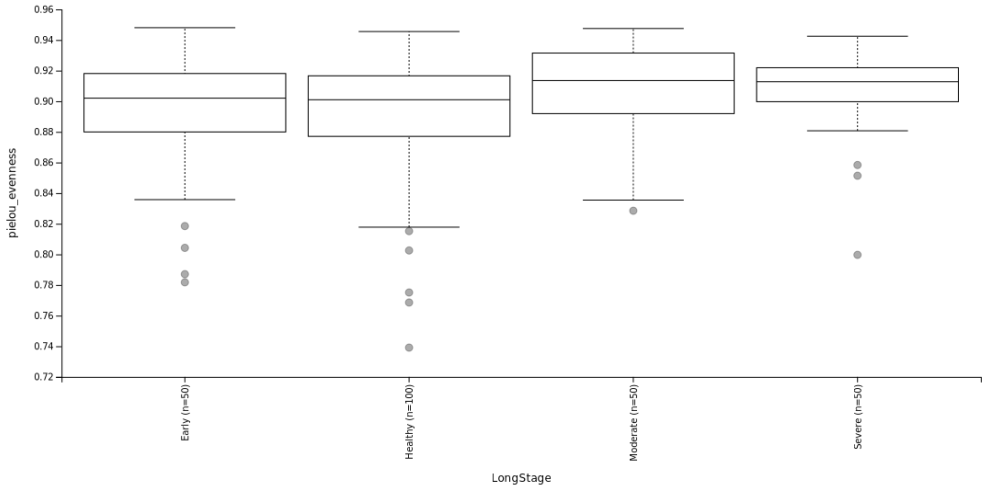


Figure 9: Evenness Index from DADA2

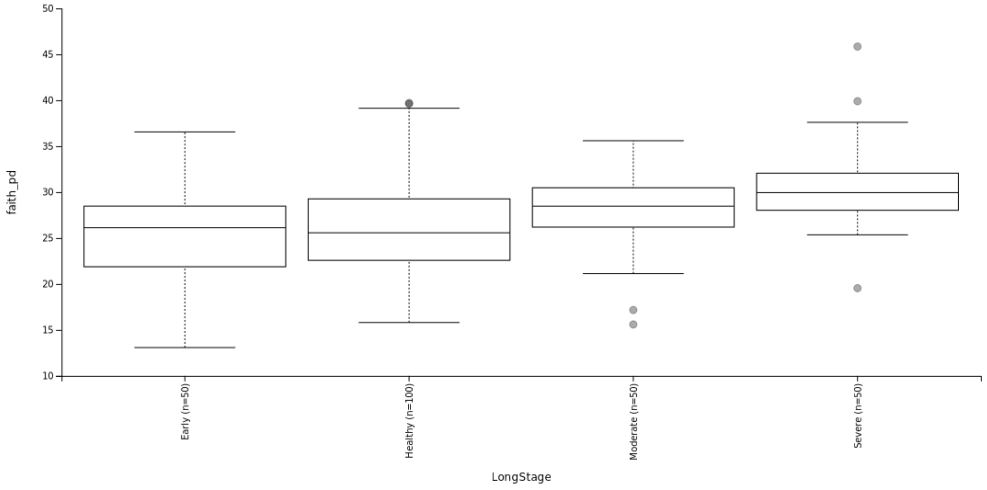


Figure 10: Faith PD Index from DADA2

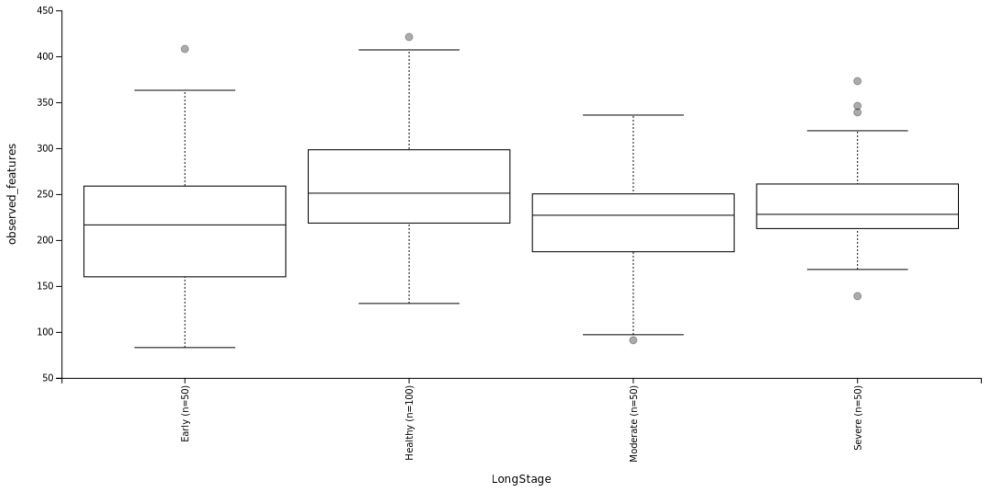


Figure 11: Observed Features Index from DADA2

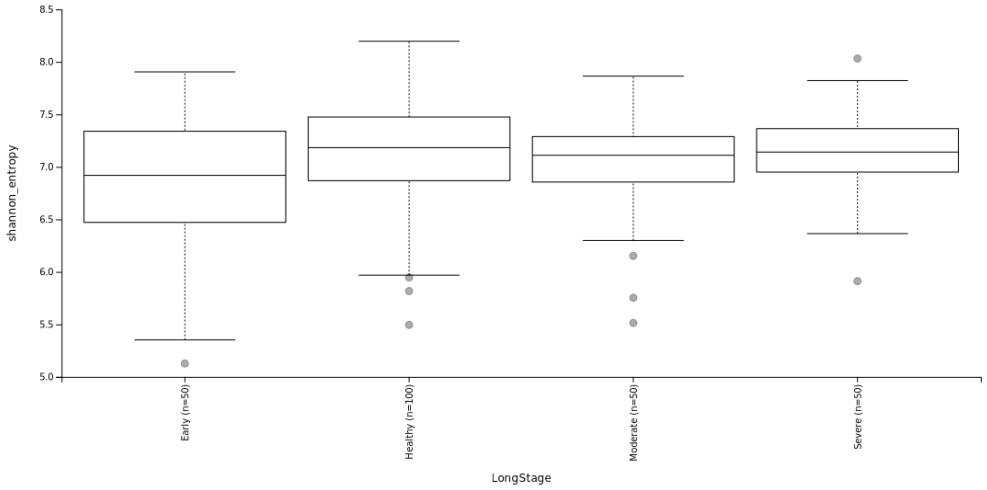


Figure 12: Shannon's Diversity Index from DADA2

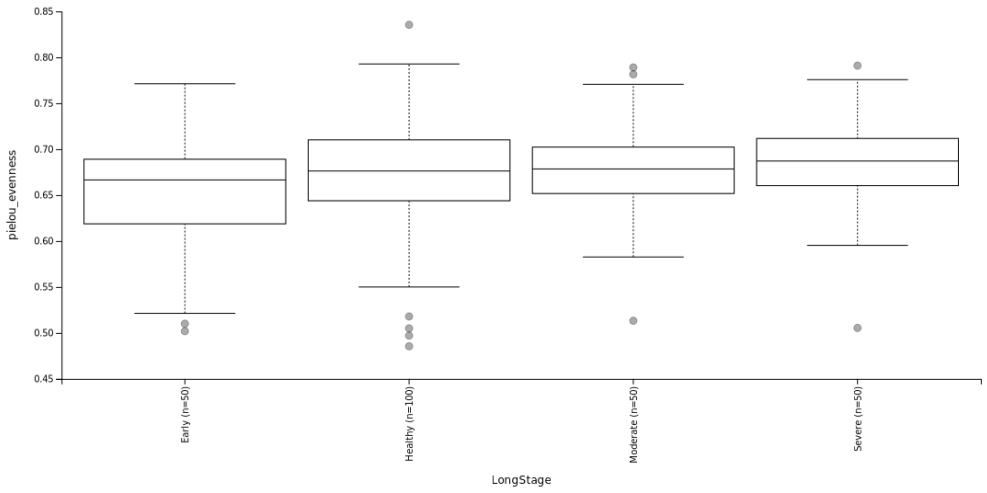


Figure 13: Evenness Index from Deblur

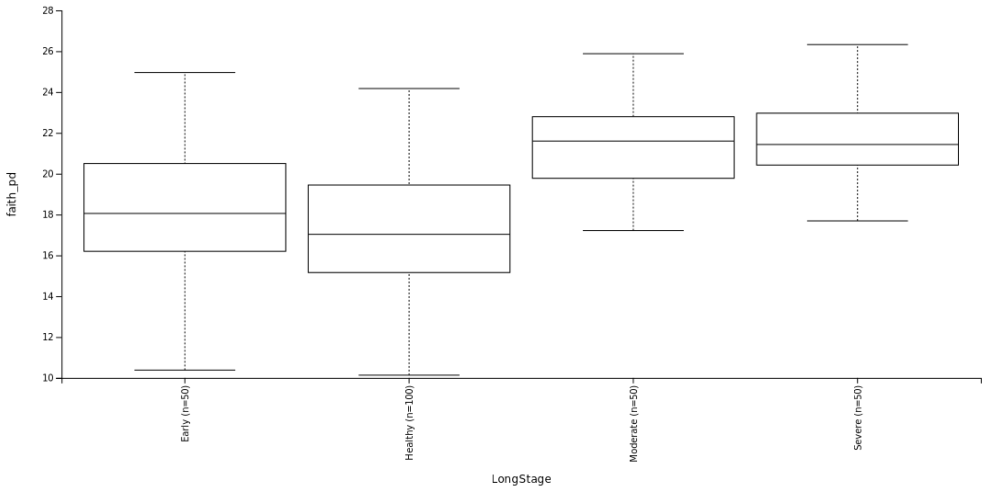


Figure 14: Faith PD Index from Deblur

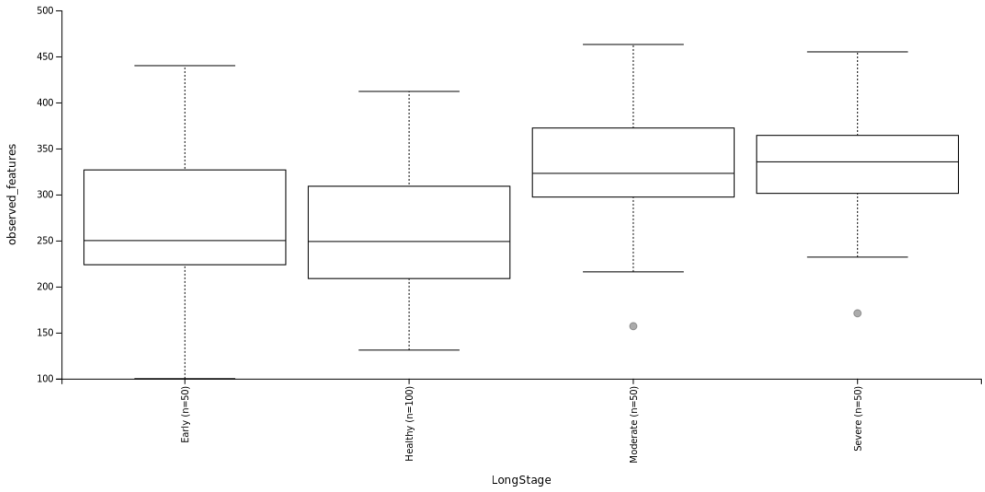


Figure 15: Observed Features Index from Deblur

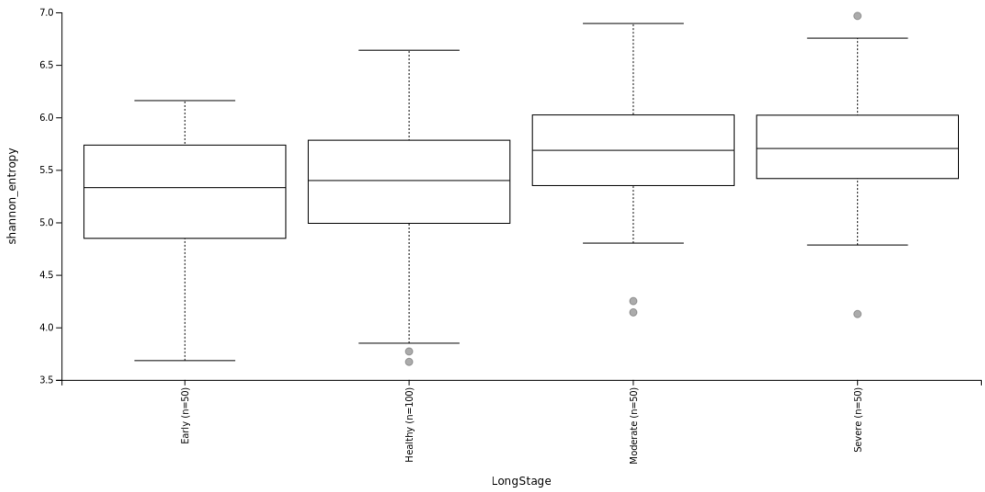
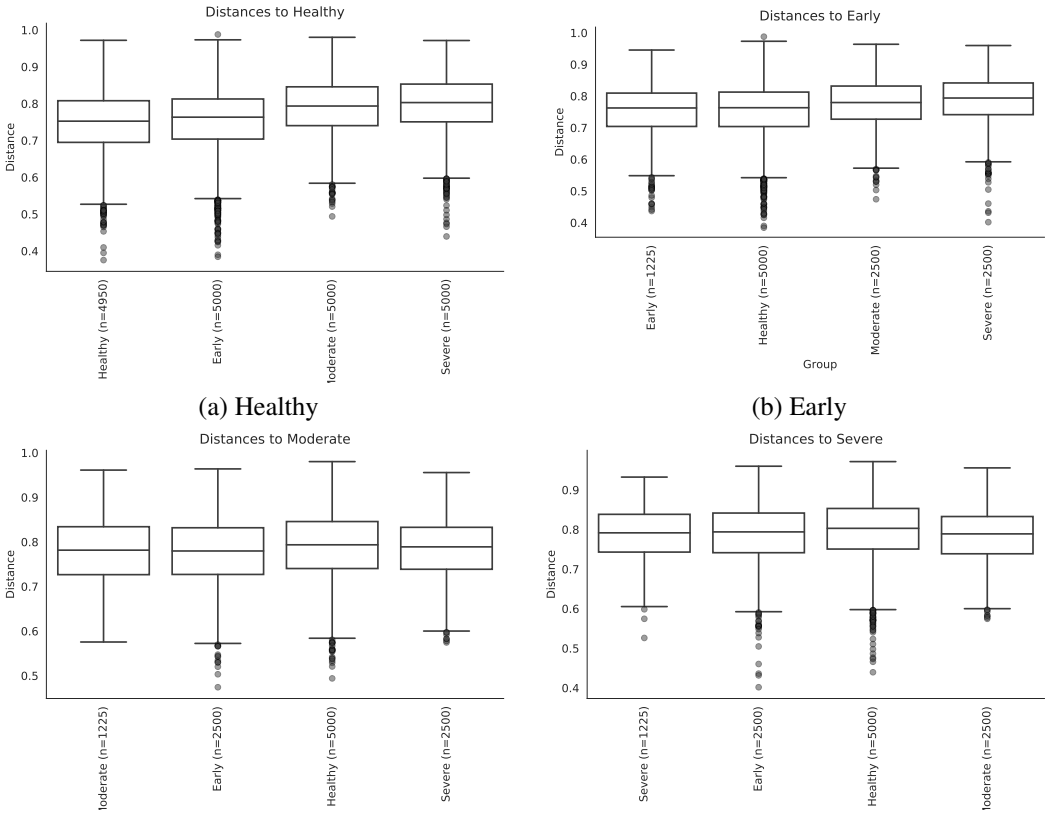


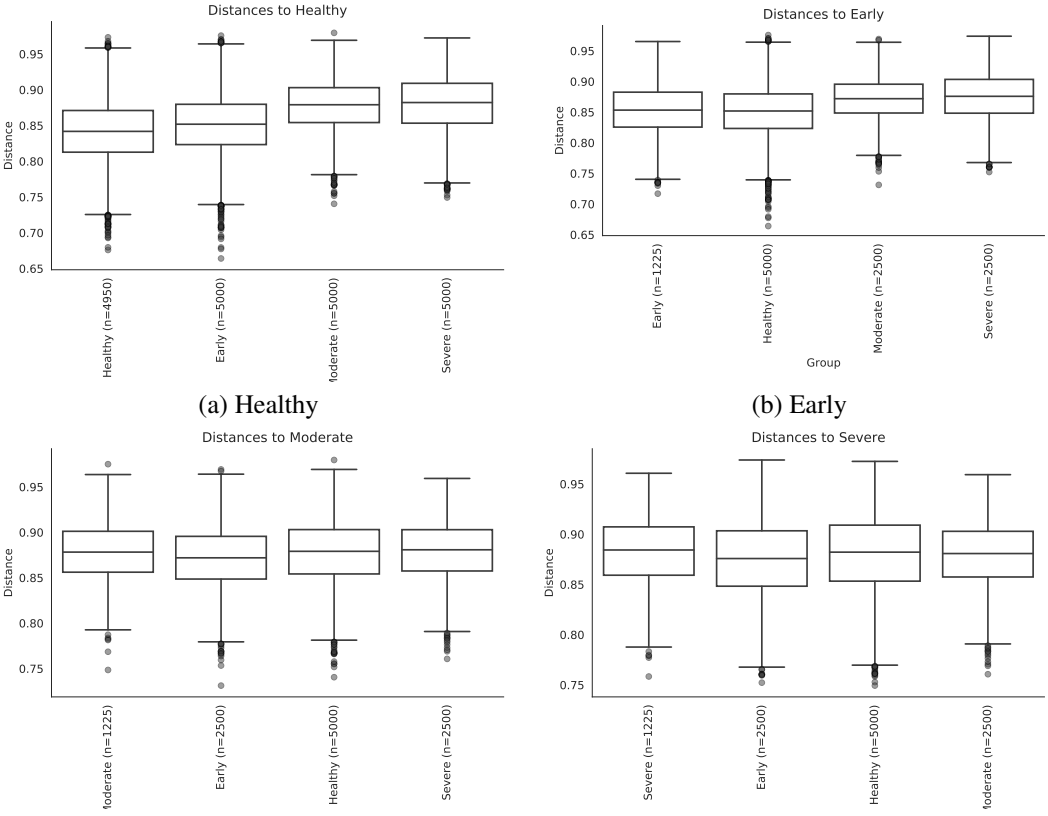
Figure 16: Shannon's Diversity Index from Deblur



(a) Healthy (b) Early

(c) Moderate (d) Severe

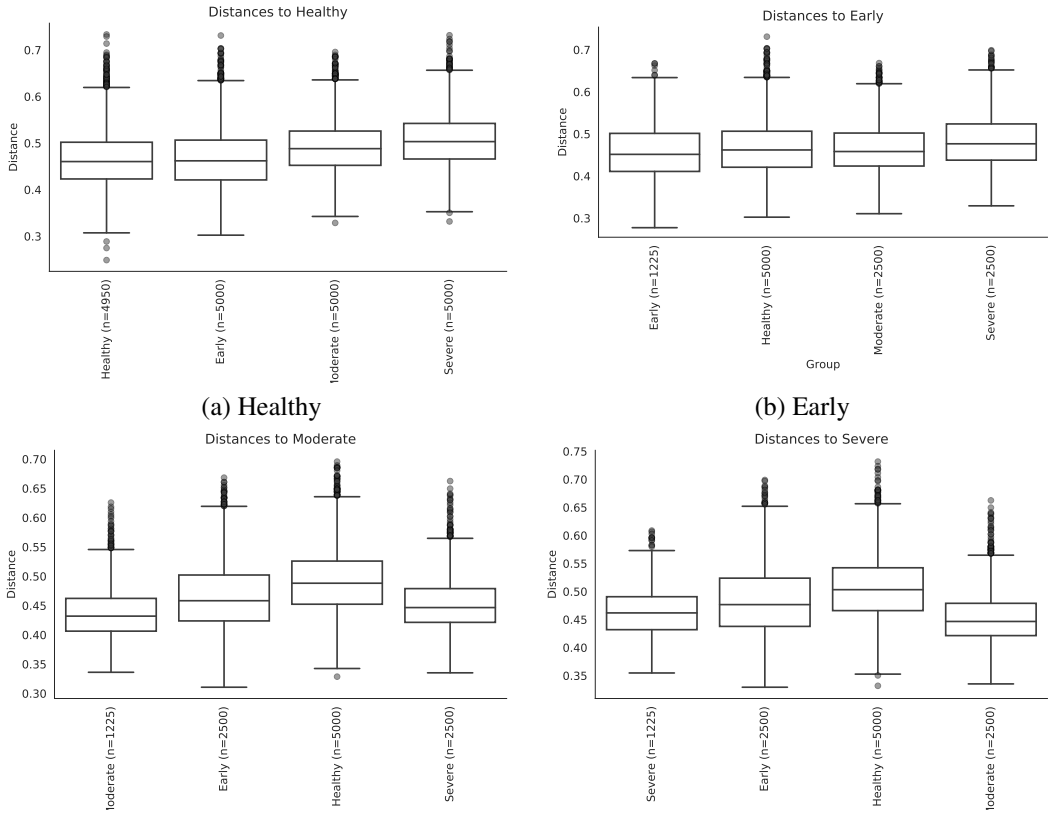
Figure 17: Bray-Curtis Distance Index with DADA2



(a) Healthy (b) Early

(c) Moderate (d) Severe

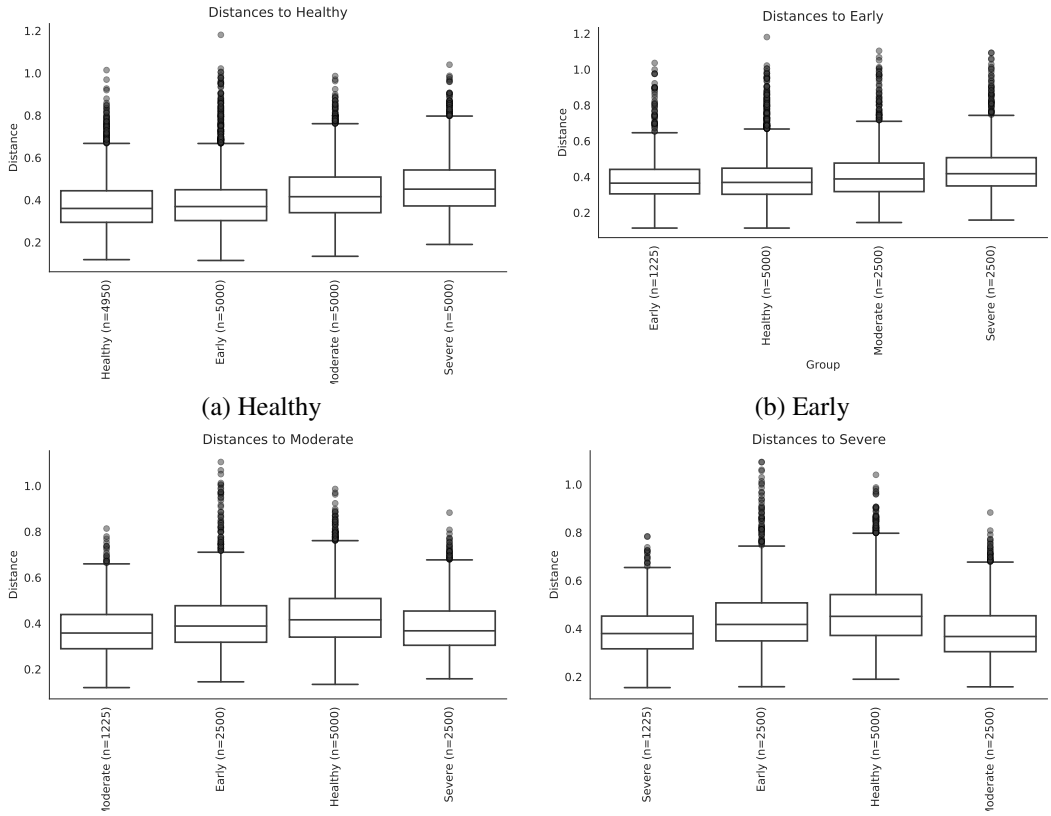
Figure 18: Jaccard Distance Index with DADA2



(a) Healthy (b) Early

(c) Moderate (d) Severe

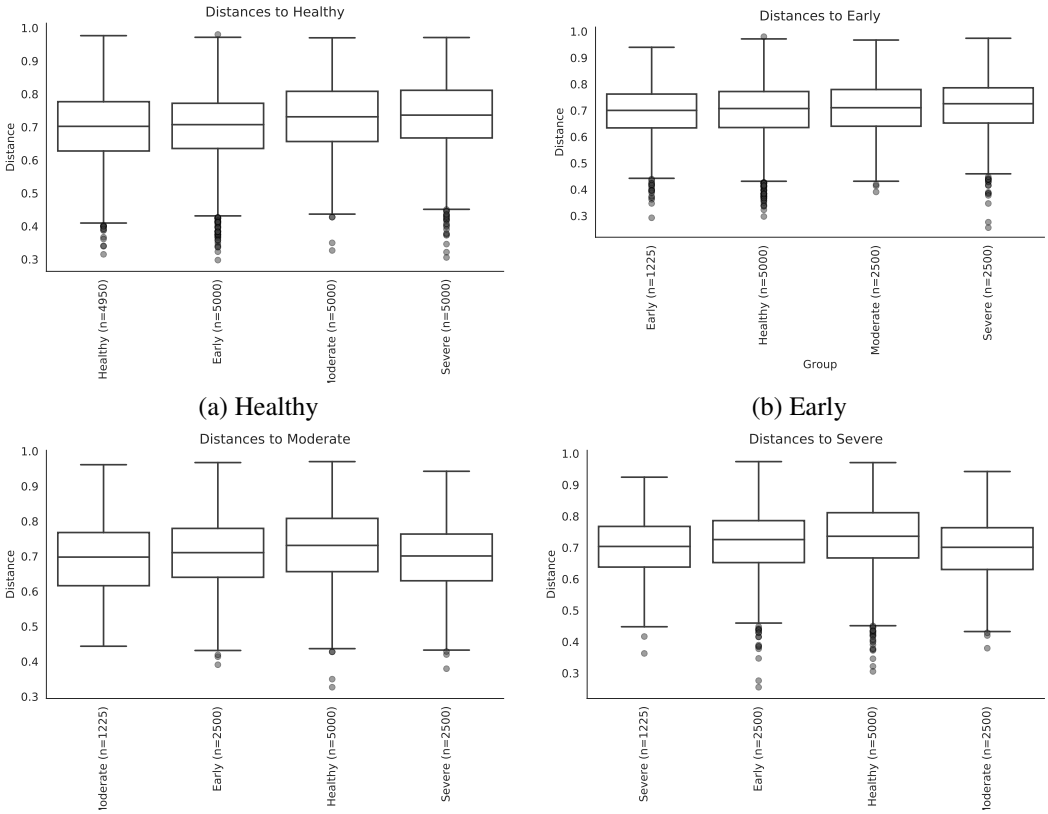
Figure 19: Unweighted Unifrac Distance Index with DADA2



(a) Healthy (b) Early

(c) Moderate (d) Severe

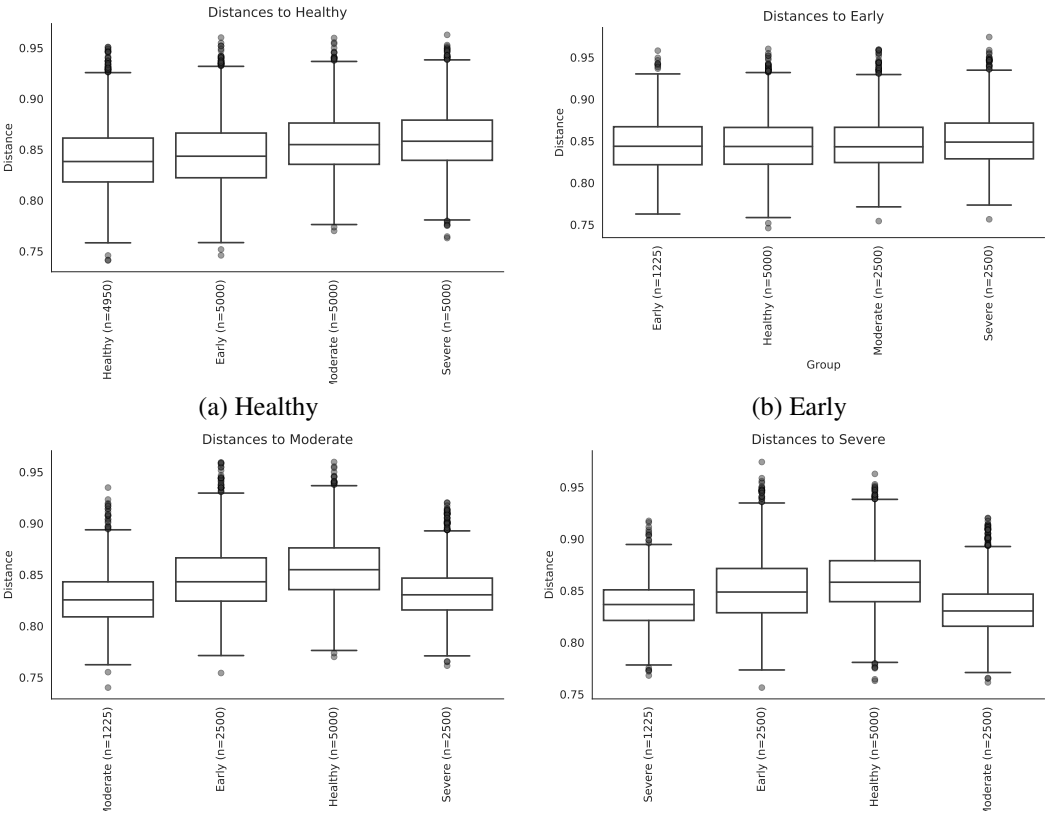
Figure 20: Weighted Unifrac Distance Index with DADA2



(a) Healthy (b) Early

(c) Moderate (d) Severe

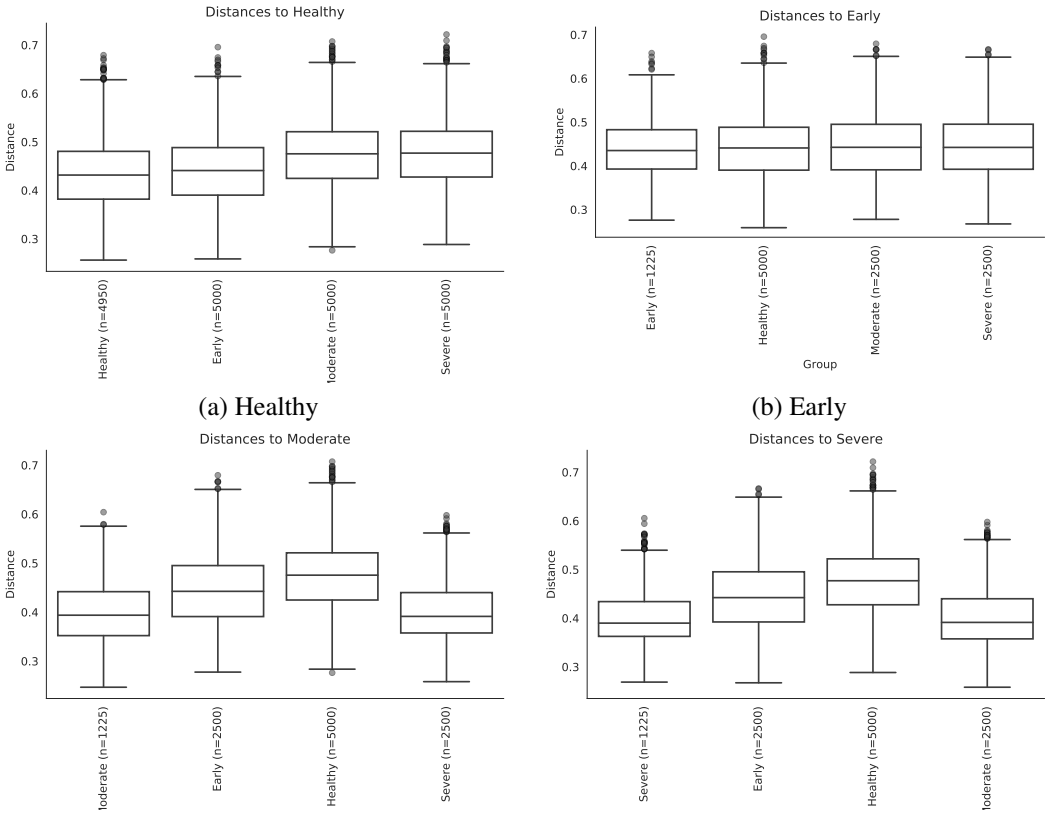
Figure 21: Bray-Curtis Distance Index with Deblur



(a) Healthy (b) Early

(c) Moderate (d) Severe

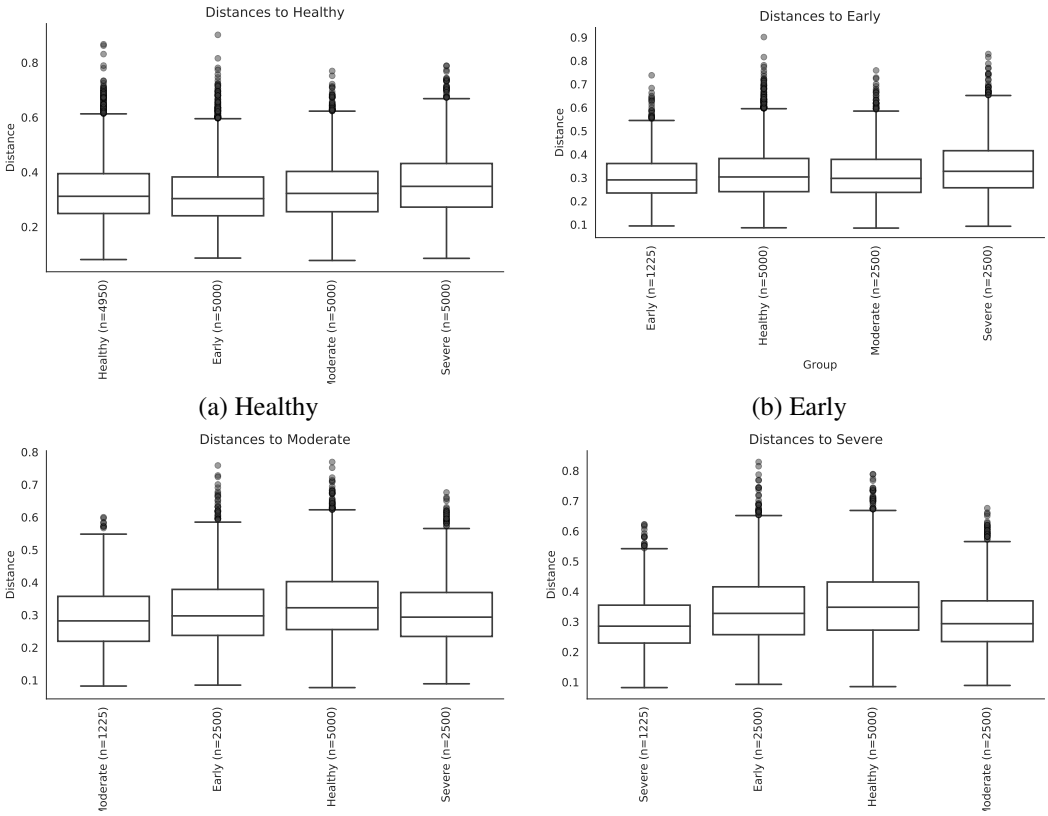
Figure 22: Jaccard Distance Index with Deblur



(a) Healthy (b) Early

(c) Moderate (d) Severe

Figure 23: Unweighted Unifrac Distance Index with Deblur



(a) Healthy (b) Early

(c) Moderate (d) Severe

Figure 24: Weighted Unifrac Distance Index with Deblur

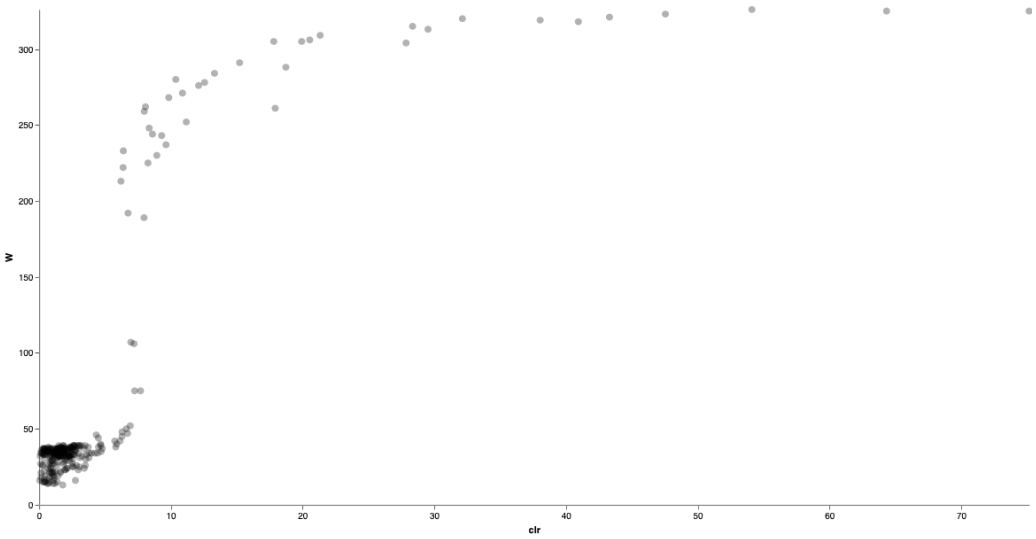


Figure 25: ANCOM Volcano Plot with DADA2 and Greengenes

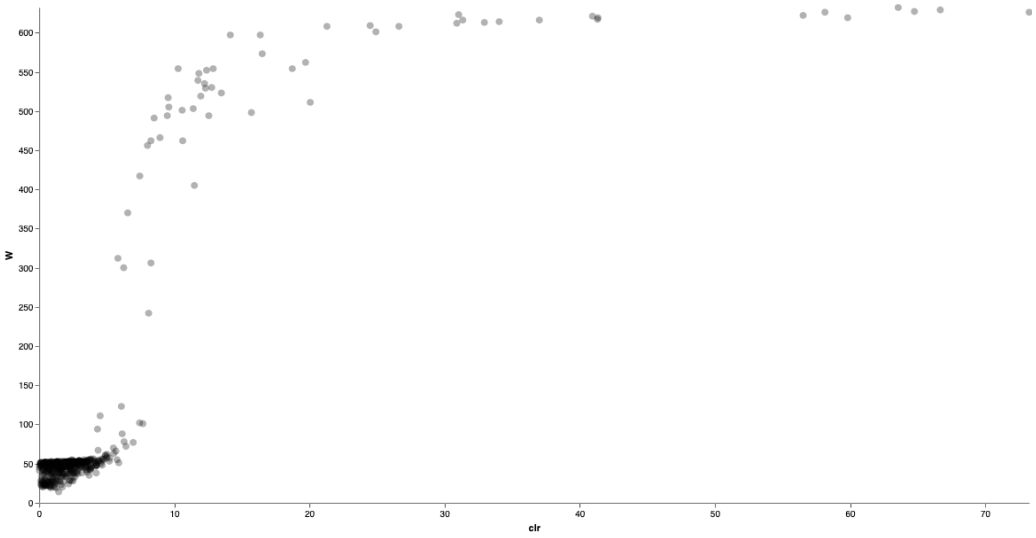


Figure 26: ANCOM Volcano Plot with DADA2 and SILVA

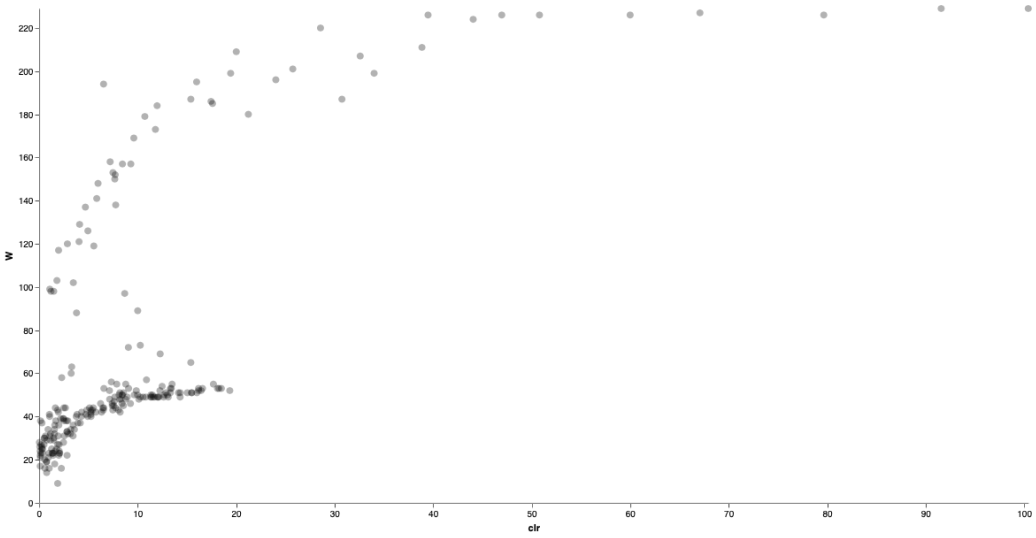


Figure 27: ANCOM Volcano Plot with Deblur and Greengenes

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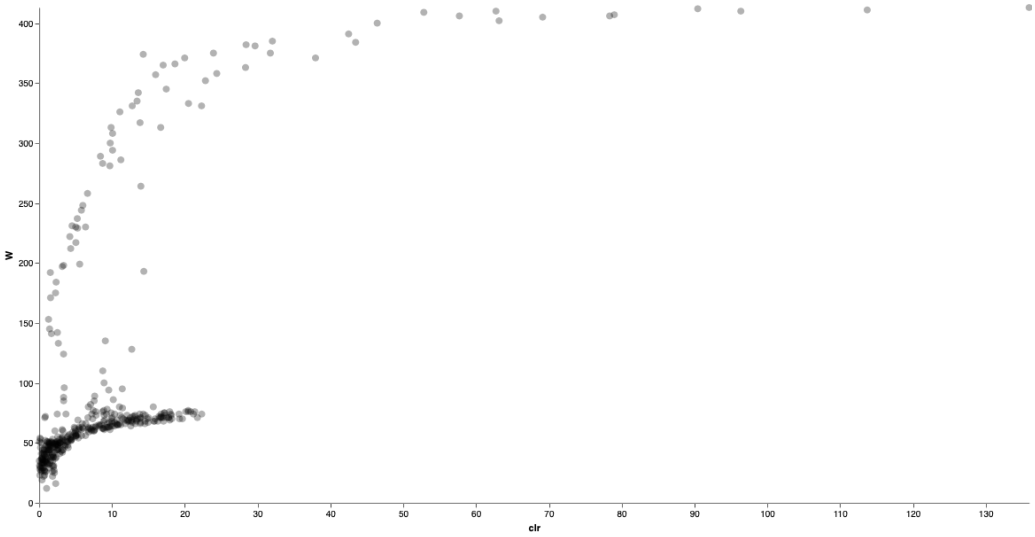

Figure 28: ANCOM Volcano Plot with Deblur and SILVA