Periodontitis

Seunghoon Kim Jaewoong Lee Semin Lee

Ulsan National Institute of Science and Technology

jwlee230@unist.ac.kr

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Overview

1. Introduction
2. Materials
3. Methods
4. Results
5. Discussion

References

Introduction

Microbiome

- Microbiota: the micro-organisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome: about 10^{13} micro-organisms whose which collective genome (Gill et al., 2006)

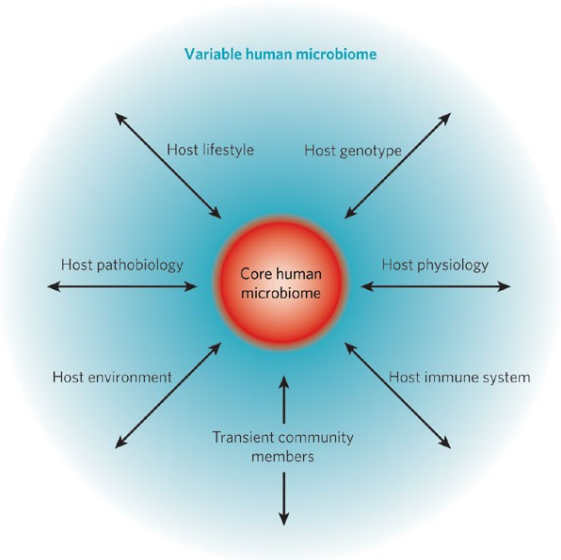


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

rRNA

- Ribosomal RNA
- Well-known as a key to phylogeny (Olsen & Woese, 1993)

Periodontitis (Periodontal disease)

- CAL (Clinical Attachment Loss) & BL (Bone Loss) (Flemmig, 1999)
- Risk Factors (Van Dyke & Dave, 2005)
  1. Smoking
  2. Diabetes
  3. Genetic factor
  4. Host response

Materials

16S rRNA Sequencing

- 100 Healthy people
- 50 Chronic periodontitis – Early
- 50 Chronic periodontitis – Moderate
- 50 Chronic periodontitis – Severe

Methods

QIIME2 Workflow

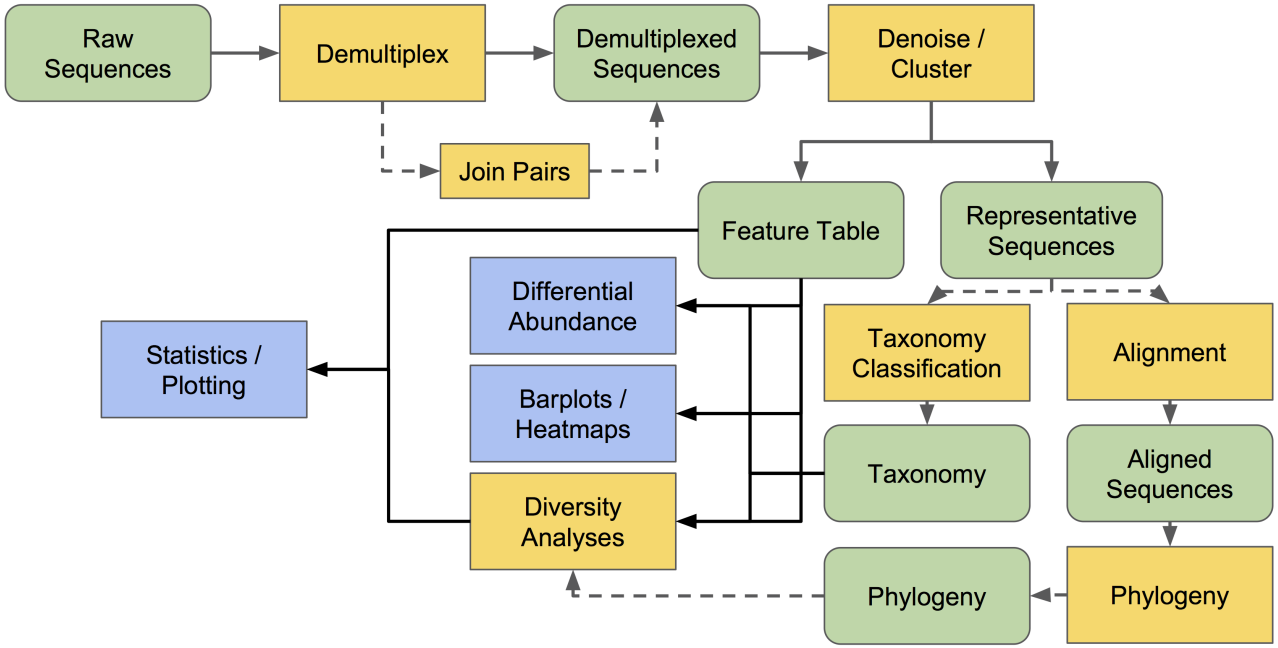


Figure: QIIME2 Workflow (Bolyen et al., 2019, 2018)

Denoising techniques

- DADA2: Amplicon Sequence Variants (ASVs) (Callahan et al., 2016)
- Deblur: Operational Taxonomic Units (OTUs) (Amir et al., 2017)

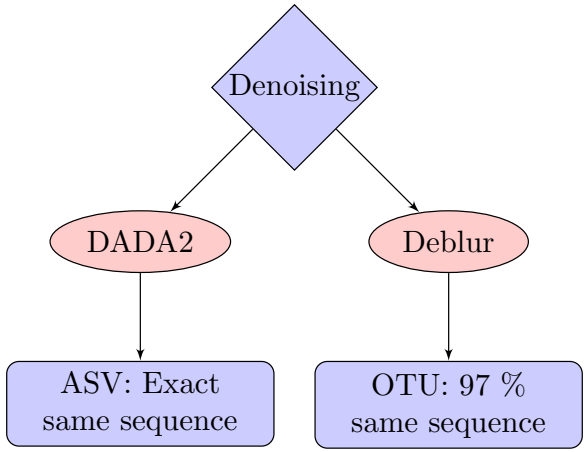


Figure: Denoising Techniques

Taxonomy Classification

- Greengenes (GG) (DeSantis et al., 2006)
- SILVA (Pruesse et al., 2007)

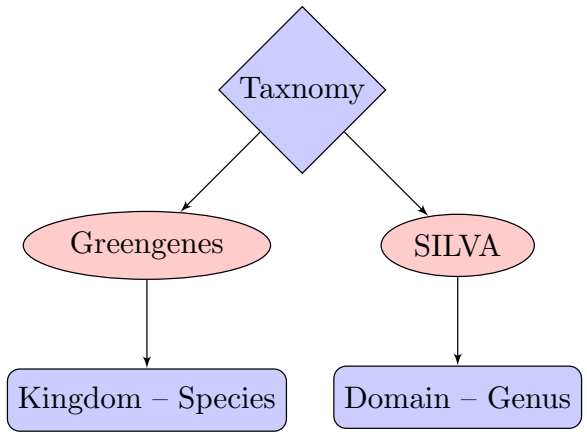


Figure: Taxonomy Classification

"A **higher** performance at taxonomic levels above *genus* level; but performance appears to drop at *species* level" (Gihawi et al., 2019)

- Analysis of composition of microbiomes
- ANCOM can be used for analyzing the composition of microbiomes in multiple populations (Mandal et al., 2015)
- Differential abundance testing

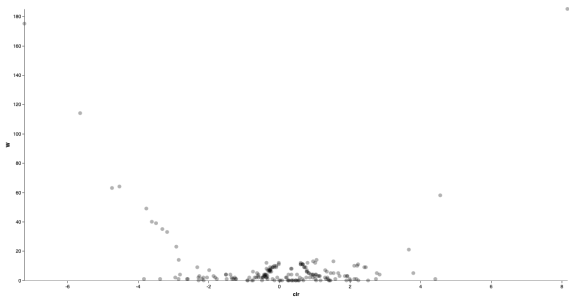


Figure: Example ANCOM Volcano Plot (Bolyen et al., 2019, 2018)

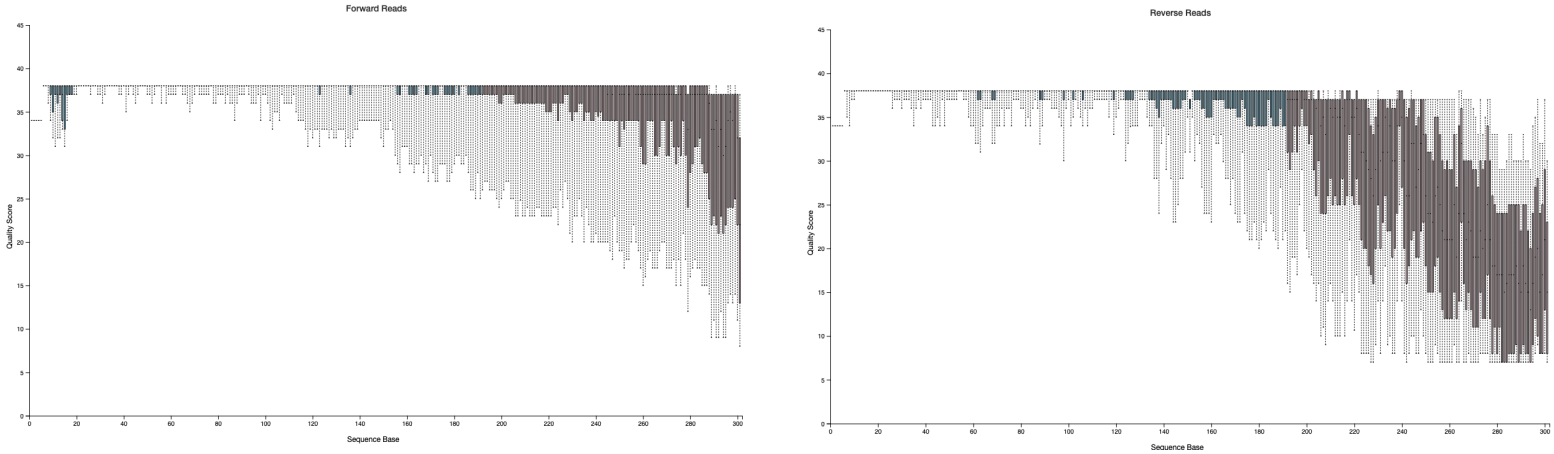
- clr: Centered log Ratio
- W: a count of the number of sub-hypothesis which have passed for given species

Python Packages

- Pandas (McKinney et al., 2011)
- Scikit-learn (Pedregosa et al., 2011)
- Matplotlib (Hunter, 2007; Barrett, Hunter, Miller, Hsu, & Greenfield, 2005)
- Seaborn (Waskom & the seaborn development team, 2020)

Results

Quality Filter



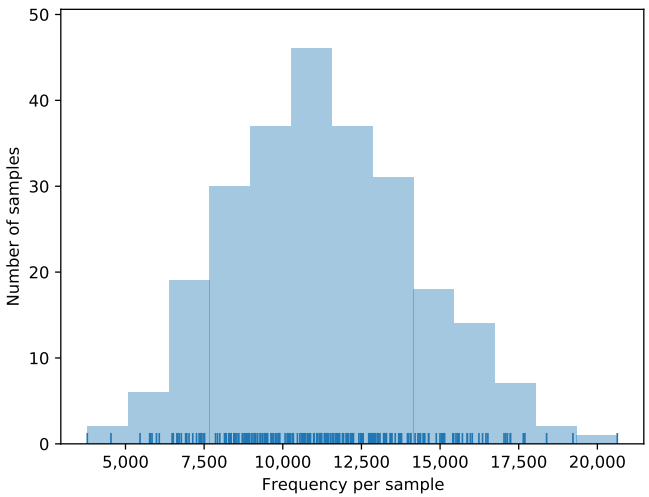
(a) Forward Reads (b) Reverse Reads

Figure: Sequence Quality Plot

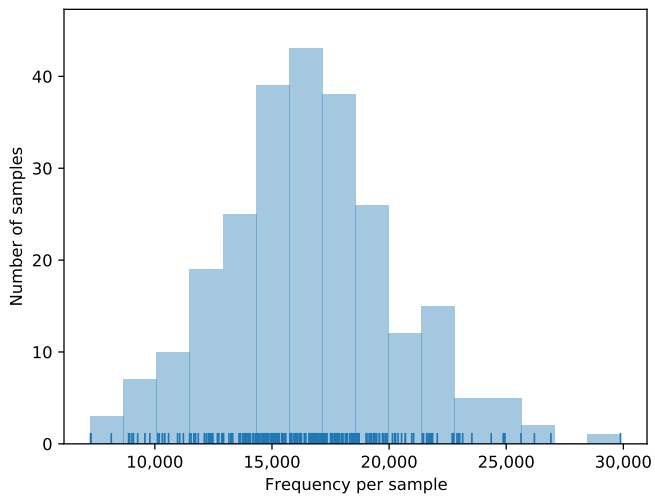
$\therefore$ Maximum Sequence Length $n_{forword} = 300$, $n_{reverse} = 265$

$\because$ The longest length which has sequence quality ≥ 30 at middle.

Rarefaction



(a) DADA2

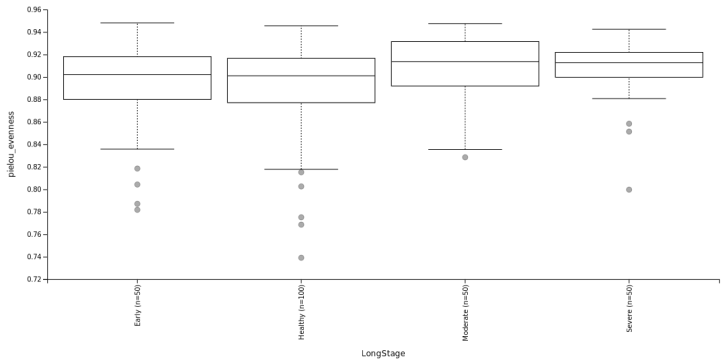


(b) Deblur

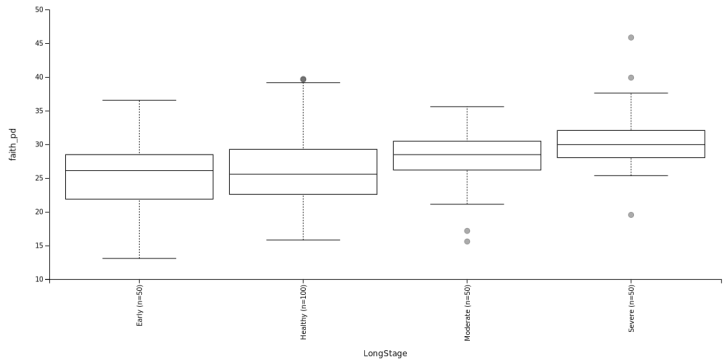
Figure: Frequency per sample

$\therefore$ p-sampling-depth $n_{DADA2} = 3786$ and $n_{Deblur} = 7253$

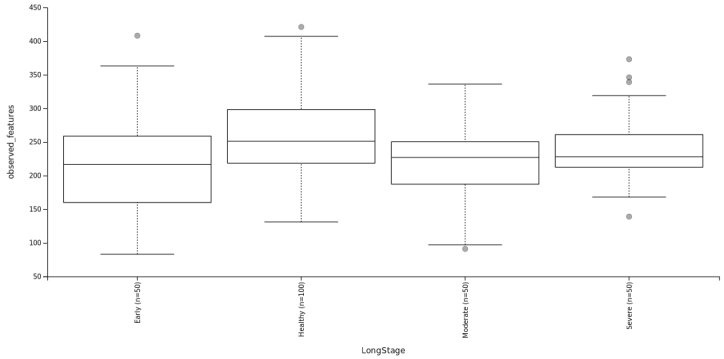
Alpha-diversity I



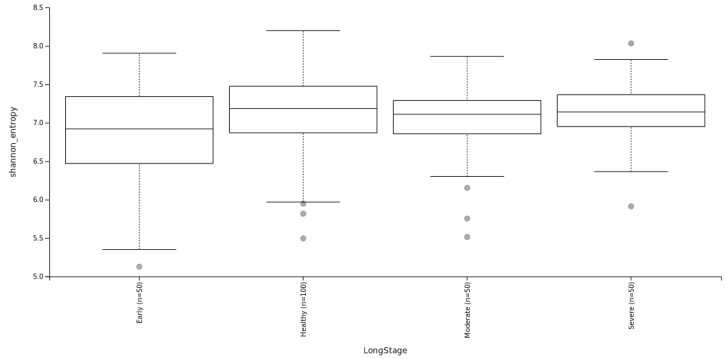
(a) Evenness ($p < 0.01$)



(b) Faith PD ($p < 10^{-6}$)



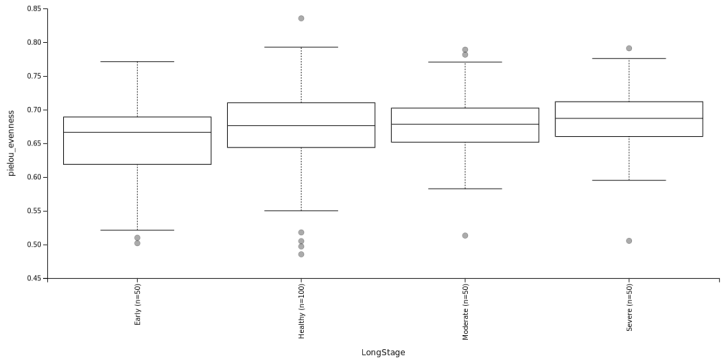
(c) Observed features ($p < 10^{-3}$)



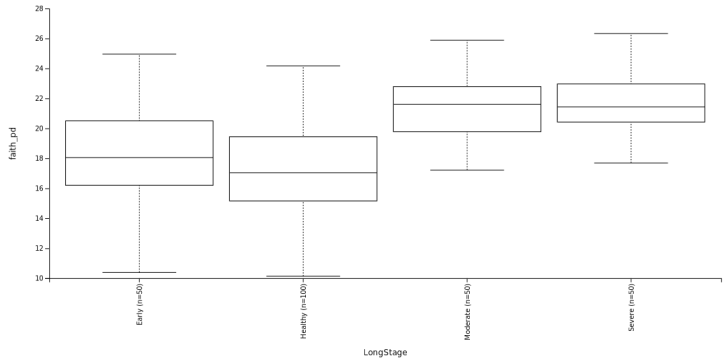
(d) Shannon ($p > 0.05$)

Figure: Alpha Diversity from DADA2 with Kruskal-Wallis among All Groups

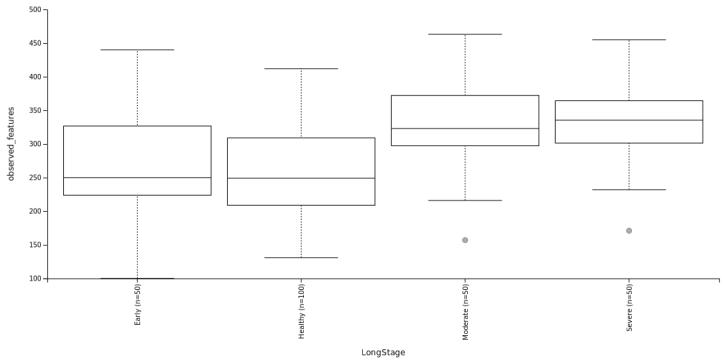
Alpha-diversity II



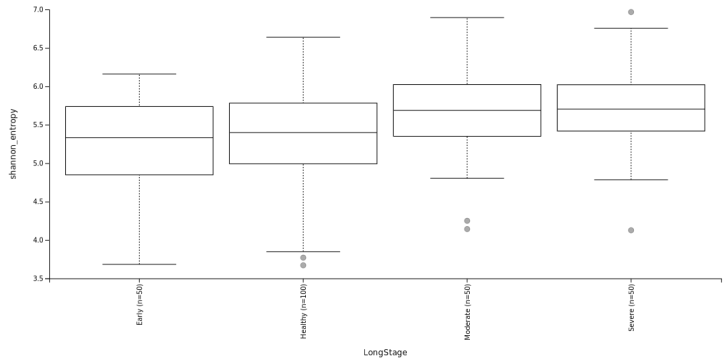
(a) Evenness ($p < 0.05$)



(b) Faith PD ($p < 10^{-18}$)



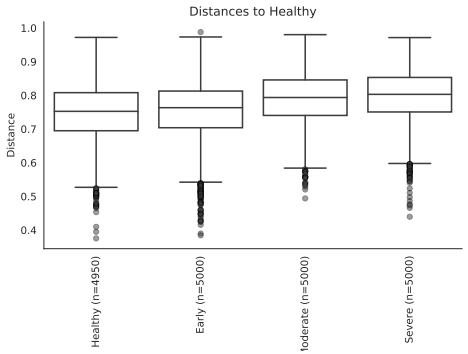
(c) Observed features ($p < 10^{-12}$)



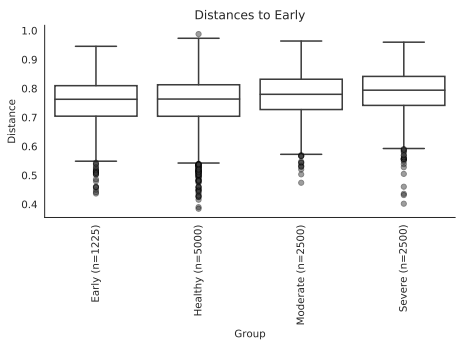
(d) Shannon ($p < 10^{-4}$)

Figure: Alpha Diversity from Deblur with Kruskal-Wallis among All Groups

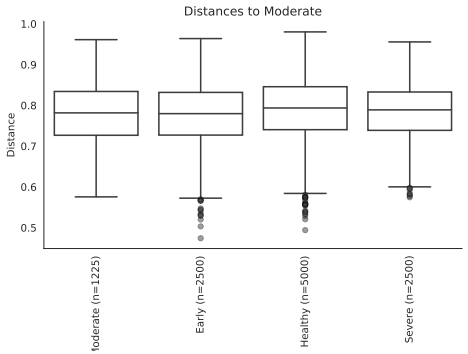
Beta-diversity I



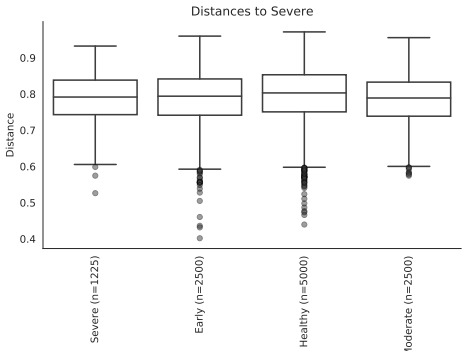
(a) Healthy



(b) Early



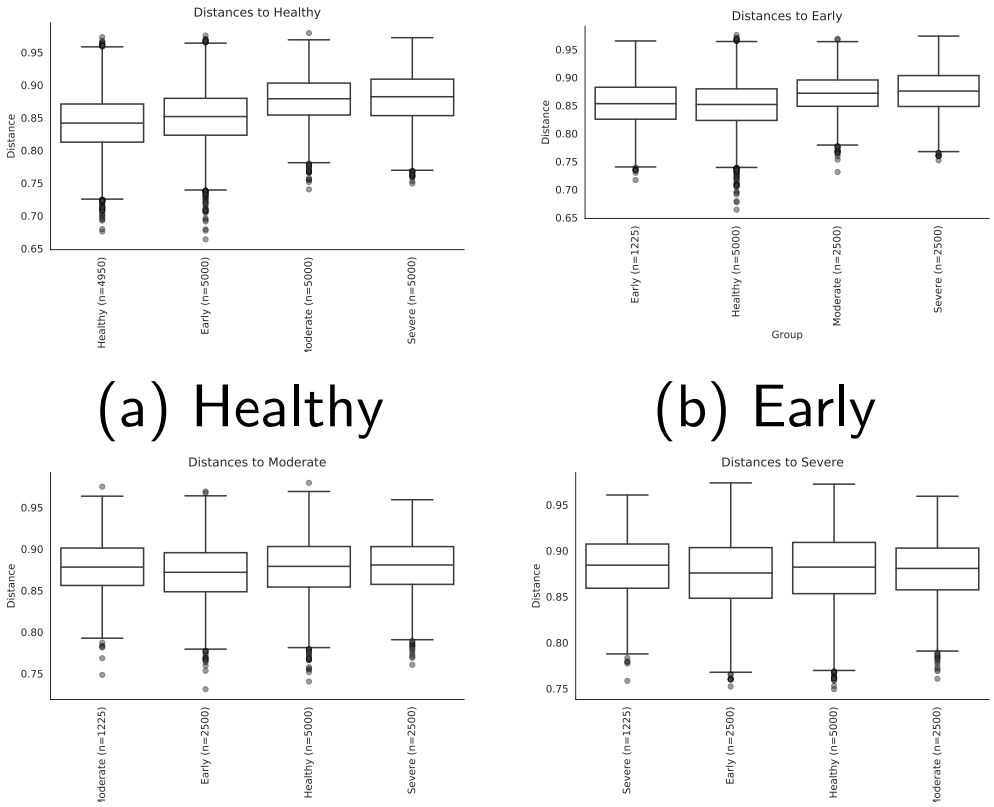
(c) Moderate



(d) Severe

Figure: Bray Curtis Distance with DADA2

Beta-diversity II

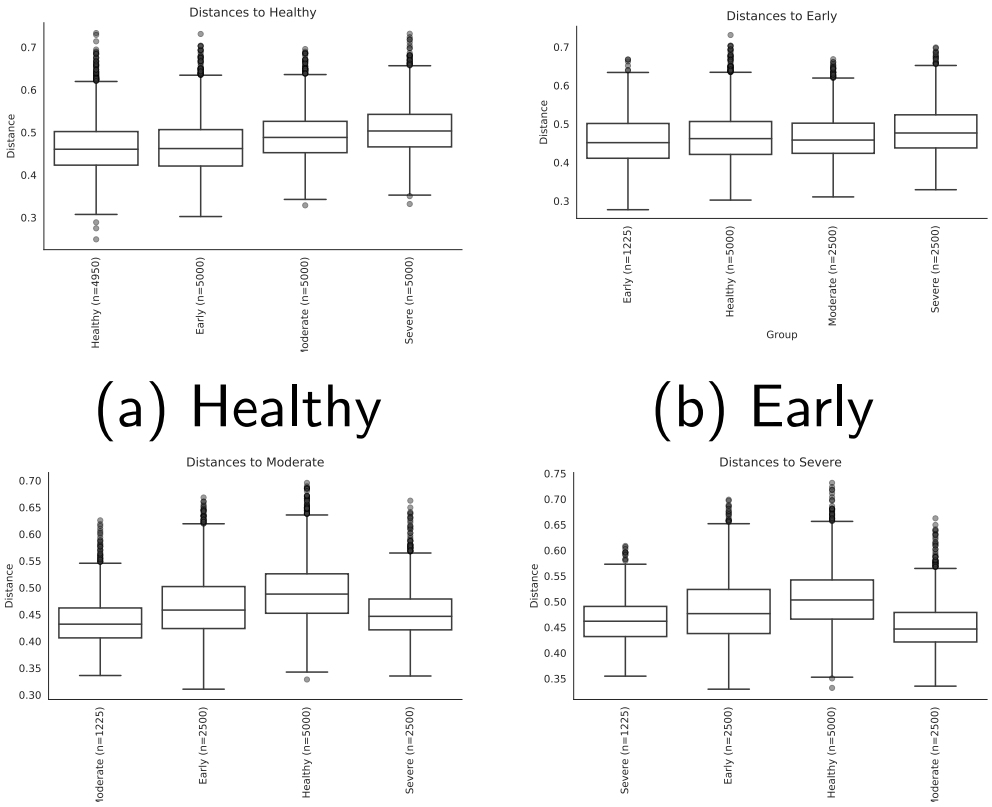


(a) Healthy (b) Early

(c) Moderate (d) Severe

Figure: Jaccard Distance with DADA2

Beta-diversity III

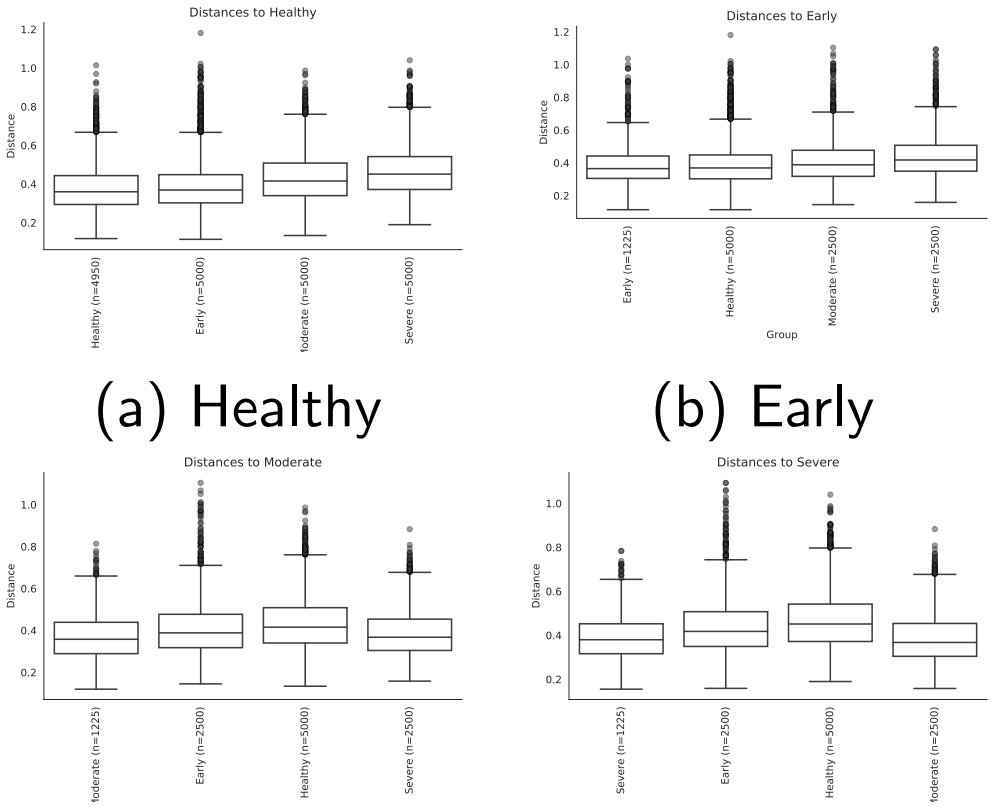


(a) Healthy (b) Early

(c) Moderate (d) Severe

Figure: Unweighted Unifrac Distance with DADA2

Beta-diversity IV

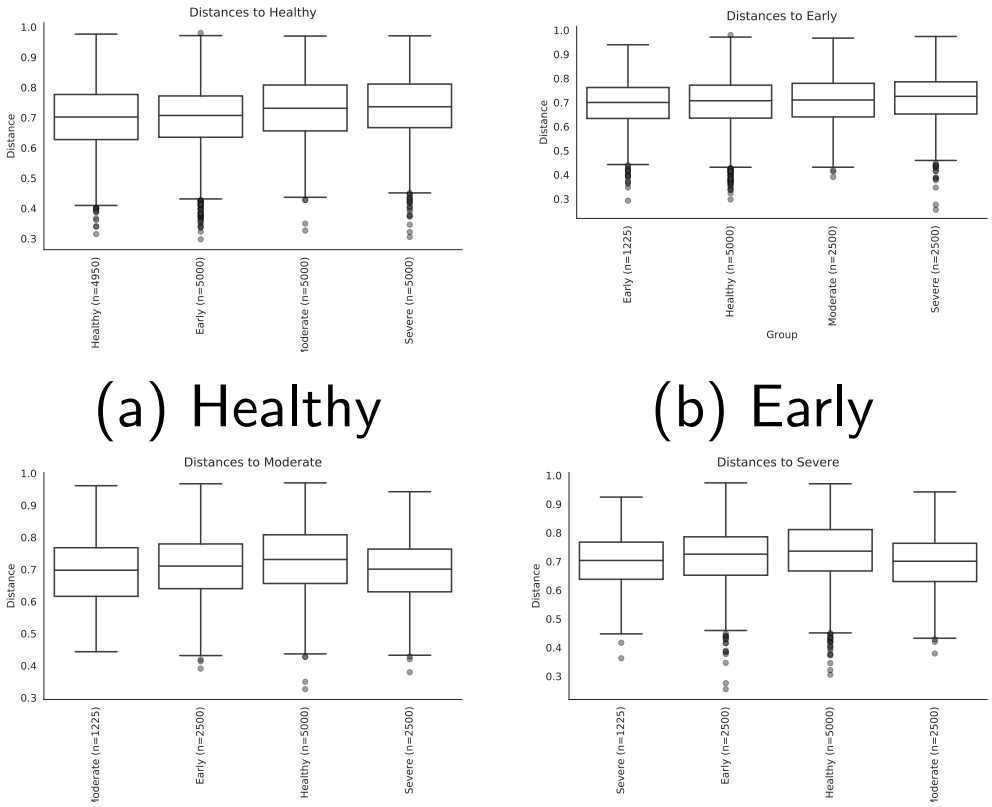


(a) Healthy (b) Early

(c) Moderate (d) Severe

Figure: Weighted Unifrac Distance with DADA2

Beta-diversity V



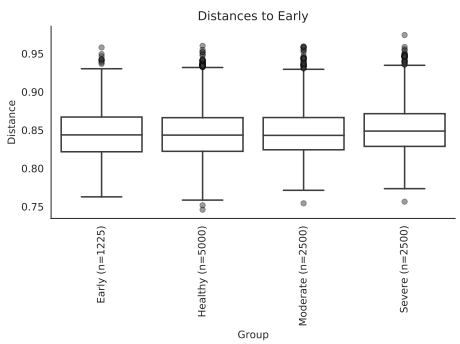
(a) Healthy (b) Early

(c) Moderate (d) Severe

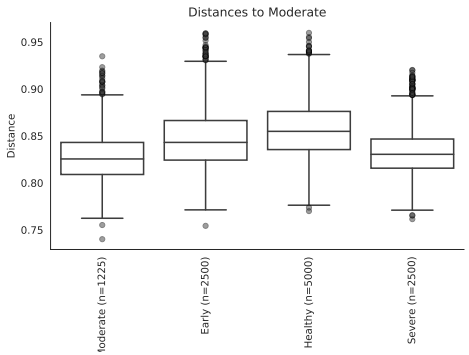
Figure: Bray Curtis Distance with Deblur

Beta-diversity VI

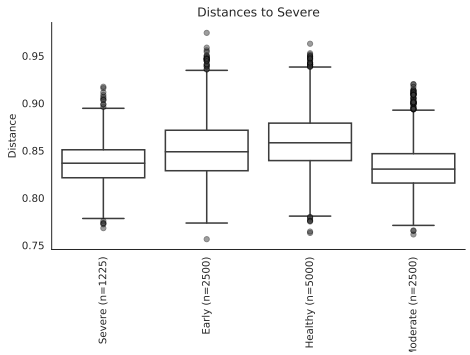
(a) Healthy



(b) Early



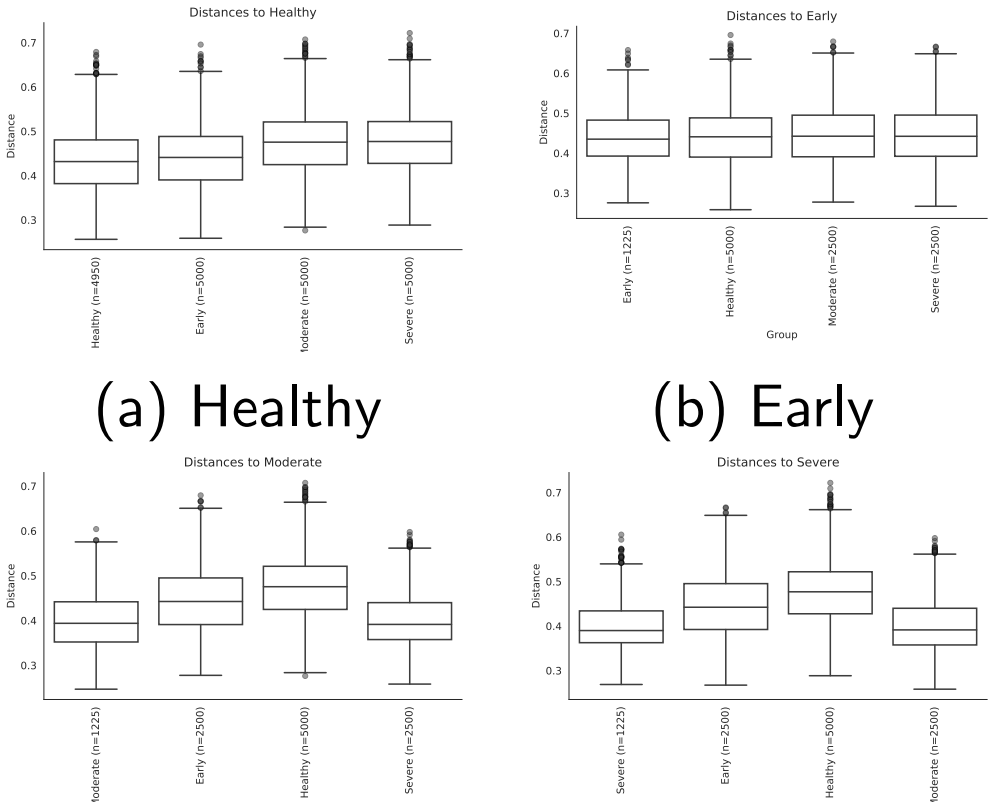
(c) Moderate



(d) Severe

Figure: Jaccard Distance with Deblur

Beta-diversity VII

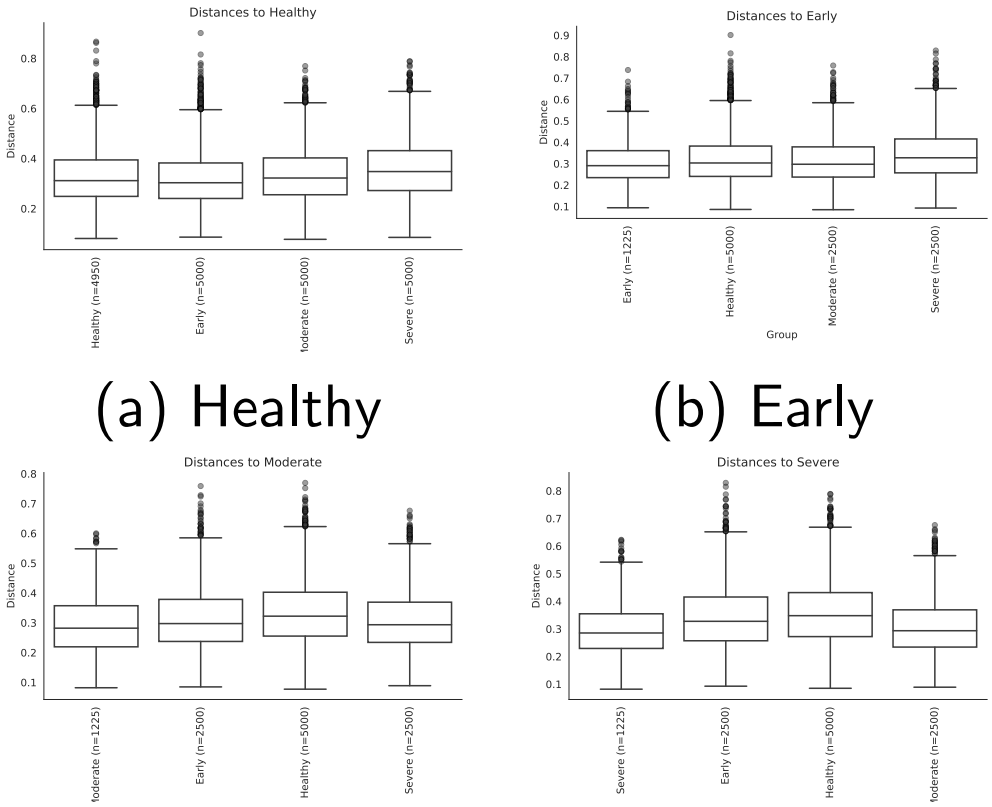


(a) Healthy (b) Early

(c) Moderate (d) Severe

Figure: Unweighted Unifrac Distance with Deblur

Beta-diversity VIII

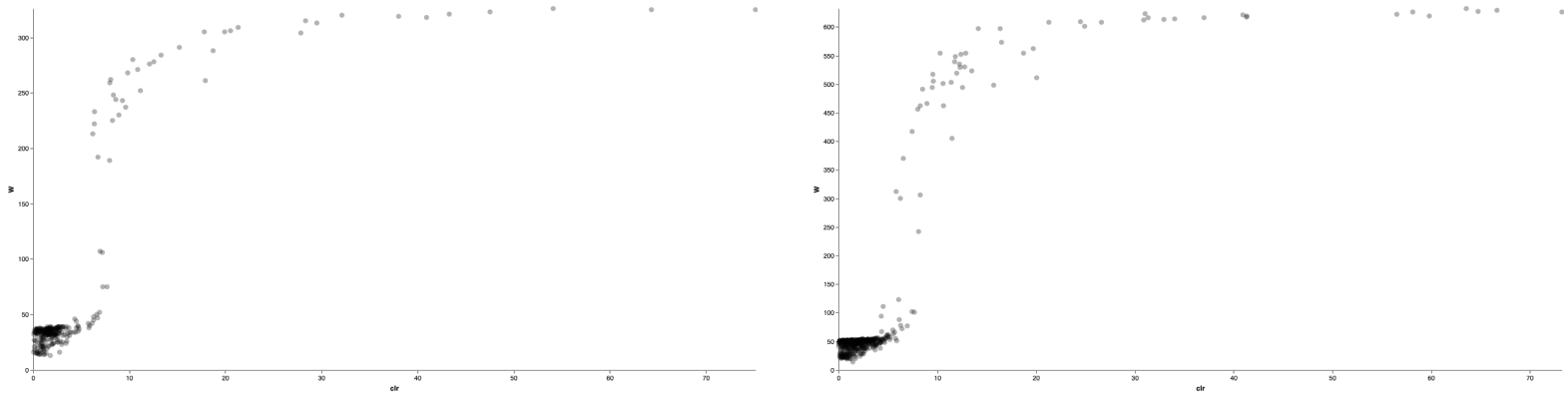


(a) Healthy (b) Early

(c) Moderate (d) Severe

Figure: Weighted Unifrac Distance with Deblur

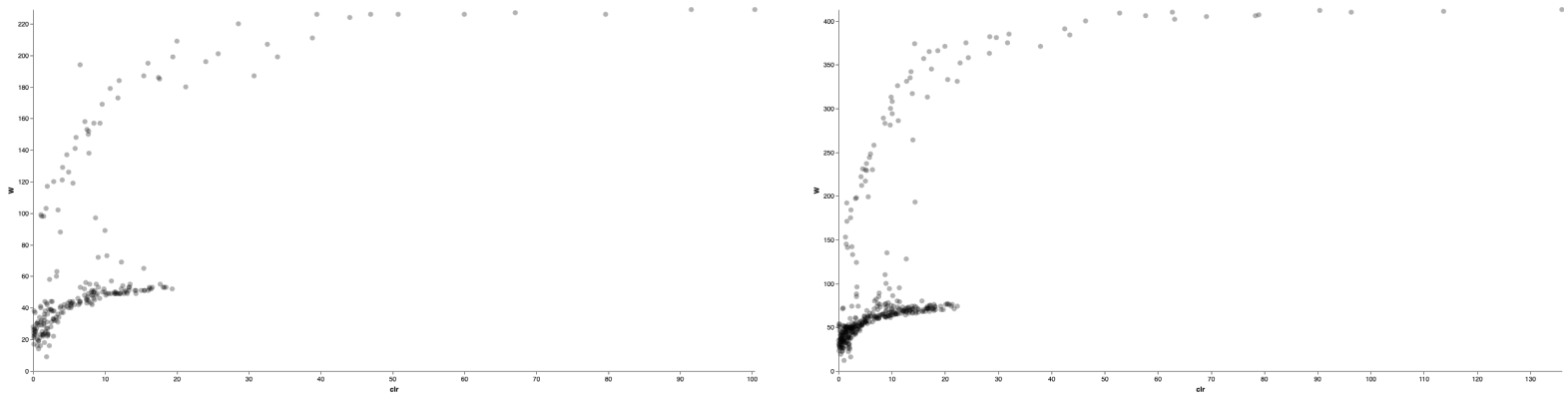
ANCOM I



(a) Greengenes (b) SILVA

Figure: ANCOM Volcano Plot with DADA2

ANCOM II



(a) Greengenes (b) SILVA

Figure: ANCOM Volcano Plot with Deblur

Discussion

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