

# Periodontitis

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# Overview

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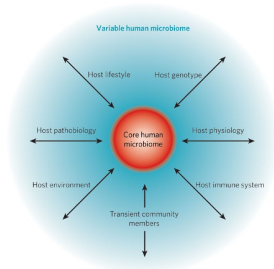
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References

# 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)

- 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

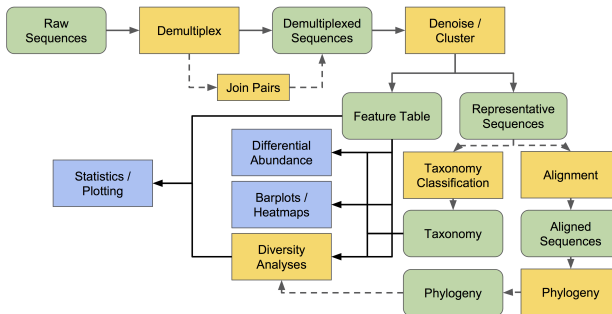


Figure: QIIME2 Workflow (Bolyen et al., 2019, 2018)

# 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)



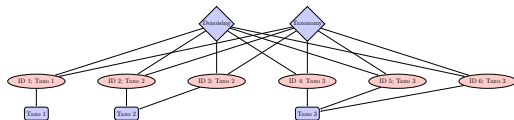
Figure: Taxonomy Classification

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

# Merging Denosing and Taxonomy Classification

Merging multiple IDs (ASVs and OTUs) into one, which have:

- Different IDs.
- Identified as same taxonomy.



**Figure:** Example Diagram for Merging Denosing and Taxonomy Classification

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

# Alpha- & Beta-diversity

- alpha-diversity: the richness of taxa at a single community
- beta-diversity: the taxonomic differentiation between communities

- Shannon's diversity index: a quantitative measure of community richness
- Observed Features: a qualitative measure of community richness
- Faith's Phylogenetic Diversity: a qualitative measure of community richness which incorporates phylogenetic relationship between the features
- Evenness: a measure of community evenness

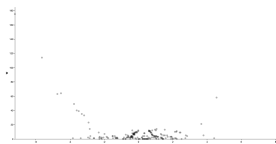
(Bolyen et al., 2019, 2018)



- Bray-Curtis distance: a quantitative measure of community dissimilarity
- Jaccard distance: a qualitative measure of community dissimilarity
- Unweighted UniFrac distance: a qualitative measure of community dissimilarity which incorporates phylogenetic relationships between the features
- Weighted UniFrac distance: a quantitative measure of community dissimilarity which incorporates phylogenetic relationship between the features

(Bolyen et al., 2019, 2018)

- 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

- 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)

- t-distributed stochastic neighbor embedding
- reveals high-dimensional data a location in two-dimensional map (Maaten & Hinton, 2008)

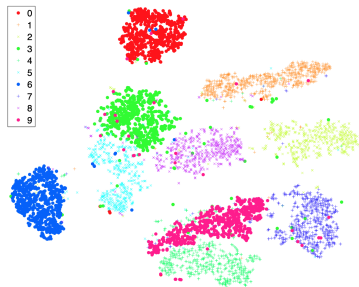


Figure: Visualization by t-SNE (Maaten & Hinton, 2008)



Figure: Workflow of Classification

## Classification Metrics:

- Accuracy
- Balanced Accuracy
- Sensitivity
- Specificity
- Precision

# Classification II

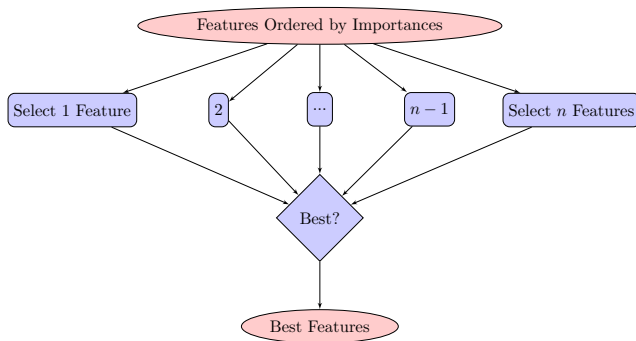
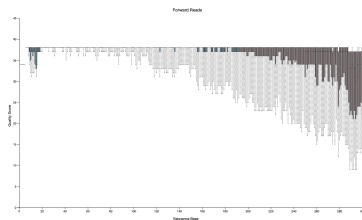


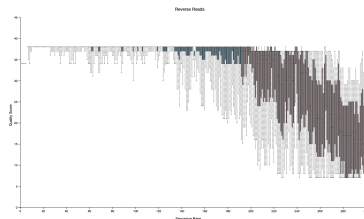
Figure: Deciding the Best Features

## Results

# Quality Filter



(a) Forward Reads



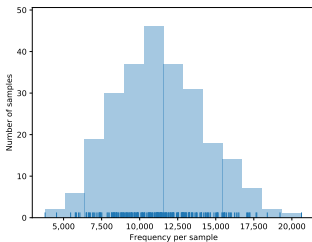
(b) Reverse Reads

Figure: Sequence Quality Plot

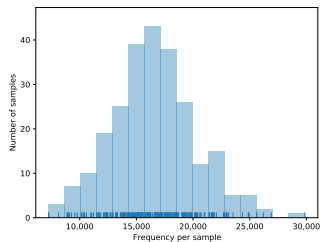
- ∴ Maximum Sequence Length  $n_{forward} = 300$ ,  $n_{reverse} = 265$
- ∴ The longest length which has sequence quality  $\geq 30$  at middle.



# Rarefaction



(a) DADA2

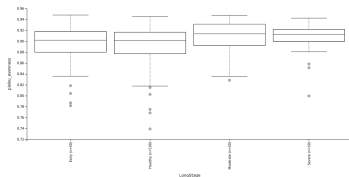


(b) Deblur

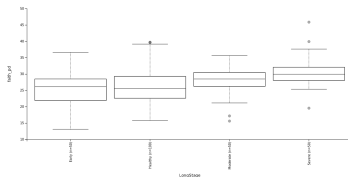
Figure: Frequency per sample

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

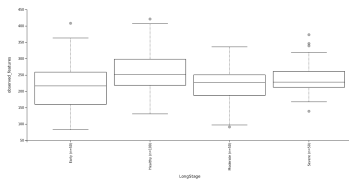
# Alpha-diversity I



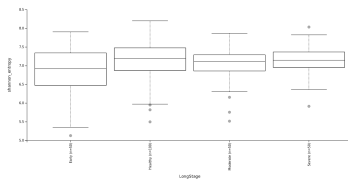
(a) Evenness ( $p < 0.01$ )



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



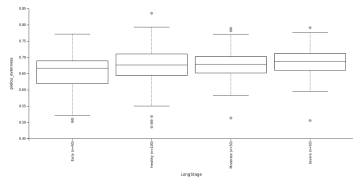
(c) Observed features ( $p < 10^{-3}$ )



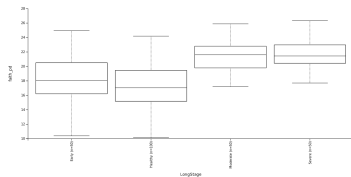
(d) Shannon's diversity ( $p > 0.05$ )

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

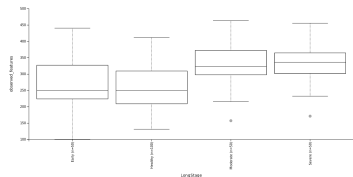
# Alpha-diversity II



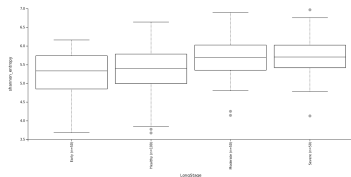
(a) Evenness ( $p < 0.05$ )



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



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



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

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

# Beta-diversity I

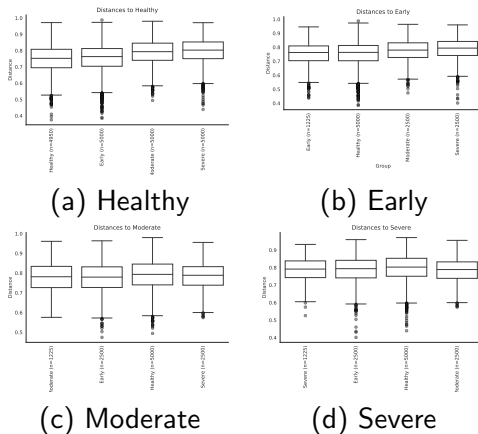


Figure: Bray-Curtis Distance with DADA2

# Beta-diversity II

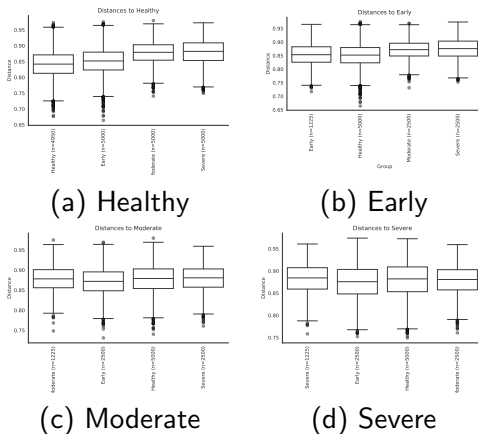


Figure: Jaccard Distance with DADA2

# Beta-diversity III

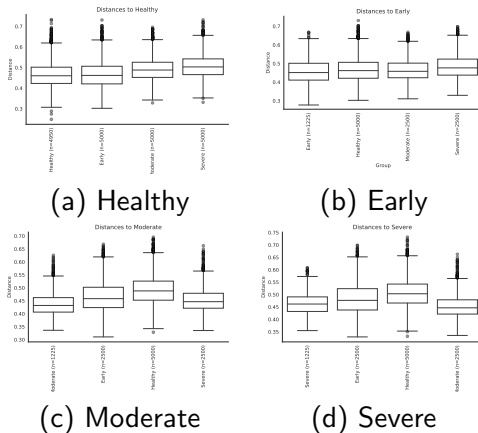


Figure: Unweighted Unifrac Distance with DADA2

# Beta-diversity IV

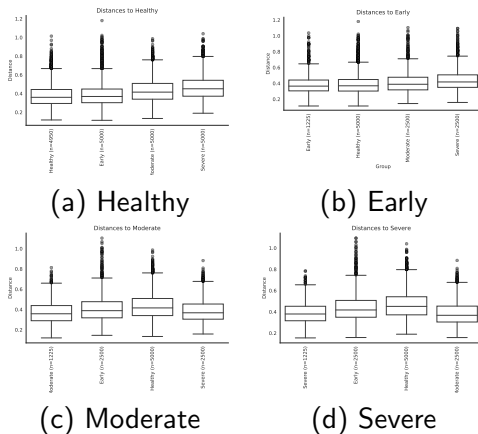


Figure: Weighted Unifrac Distance with DADA2

# Beta-diversity V

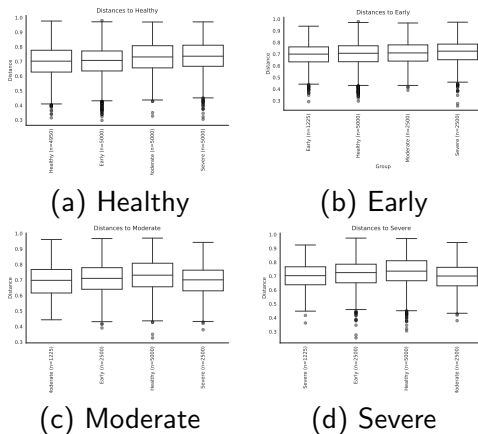


Figure: Bray-Curtis Distance with Deblur



# Beta-diversity VI

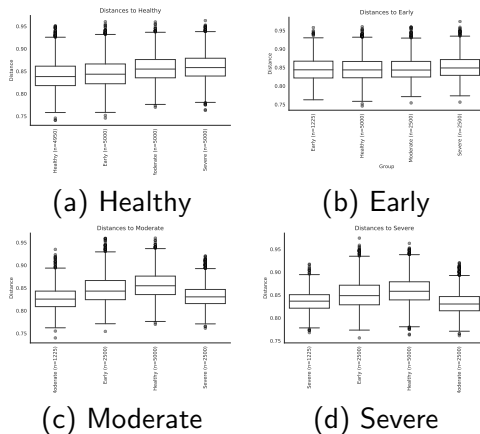


Figure: Jaccard Distance with Deblur

# Beta-diversity VII

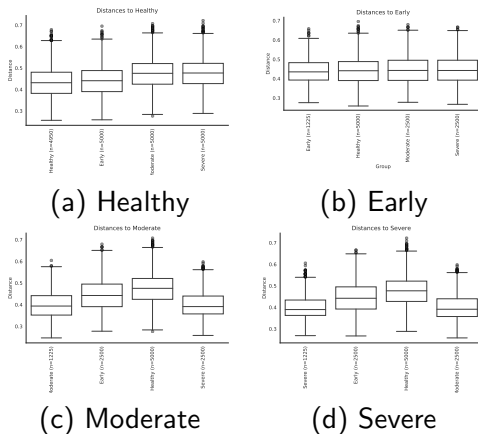


Figure: Unweighted Unifrac Distance with Deblur

# Beta-diversity VIII

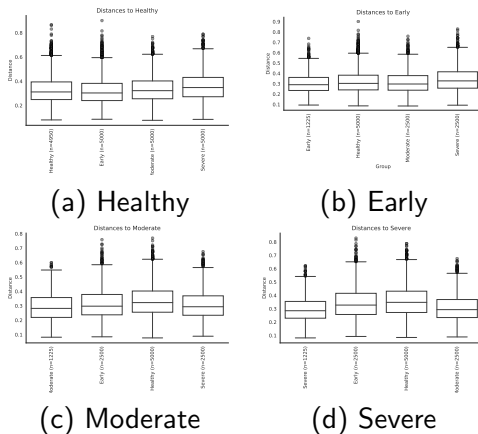
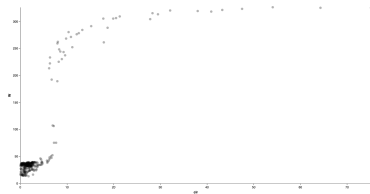
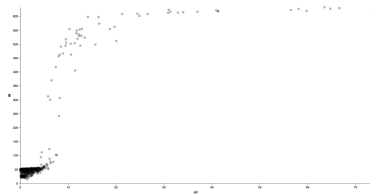


Figure: Weighted Unifrac Distance with Deblur

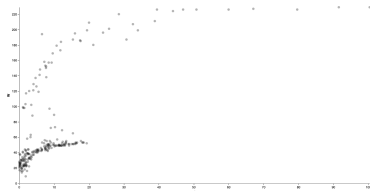


(a) Greengenes

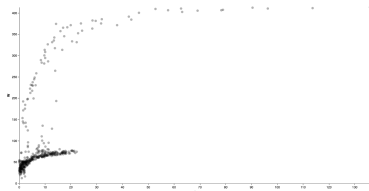


(b) SILVA

Figure: ANCOM Volcano Plot with DADA2



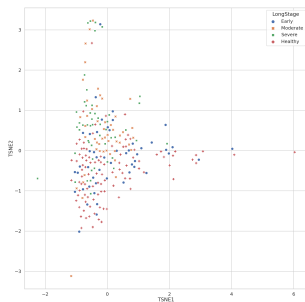
(a) Greengenes



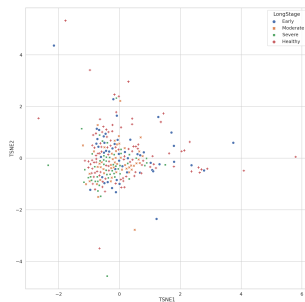
(b) SILVA

Figure: ANCOM Volcano Plot with Deblur

# t-SNE with Whole Microbiome I



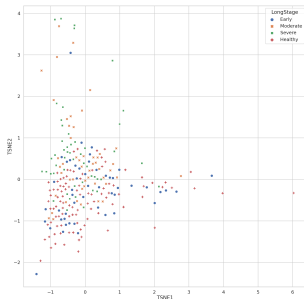
(a) Greengenes (328 Taxa)



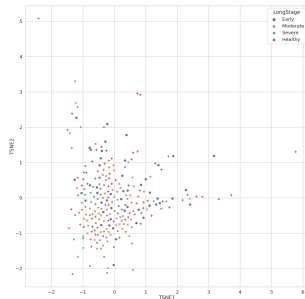
(b) SILVA (633 Taxa)

Figure: t-SNE Plot with Whole Microbiome from DADA2

# t-SNE with Whole Microbiome II



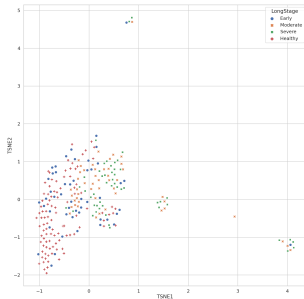
(a) Greengenes (232 Taxa)



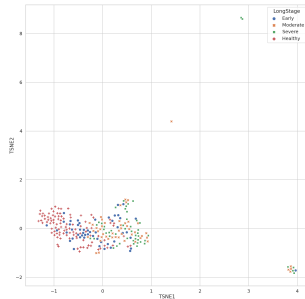
(b) SILVA (414 Taxa)

Figure: t-SNE Plot with Whole Microbiome from Deblur

# t-SNE with ANCOM Selected I



(a) Greengenes (15 Taxa)

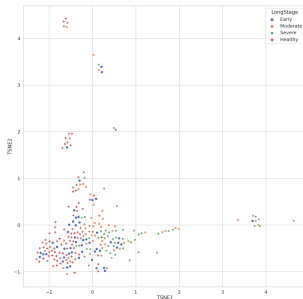


(b) SILVA (23 Taxa)

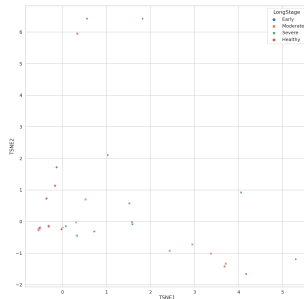
Figure: t-SNE Plot with ANCOM Selected from DADA2



# t-SNE with ANCOM Selected II



(a) Greengenes (27 Taxa)



(b) SILVA (20 Taxa)

Figure: t-SNE Plot with ANCOM Selected from Deblur

# RandomForest Classifier I

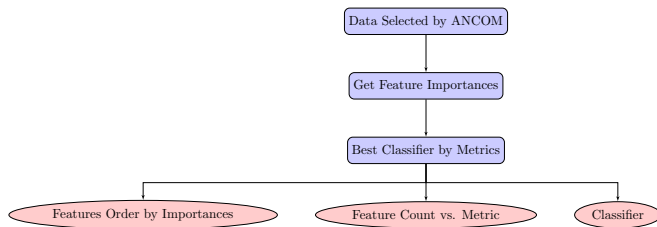


Figure: RandomForest Classifier Workflow

**DADA2 + SILVA** gives the best result with many metrics.

# RandomForest Classifier II

Table: Features Order by Importances

Order	Taxonomy (Family Genus [Species])
1	<i>Actinomycetaceae Actinomyces</i>
2	<i>Actinomycetaceae Actinomyces Schaalia-odontolytica</i>
3	<i>Prevotellaceae Prevotella Prevotella-intermedia</i>
4	<i>Peptostreptococcaceae Filifactor Filifactor-alocis</i>
5	<i>Lachnospiraceae Oribacterium</i>
6	<i>Tannerellaceae Tannerella Tannerella-forsythia</i>

# RandomForest Classifier III

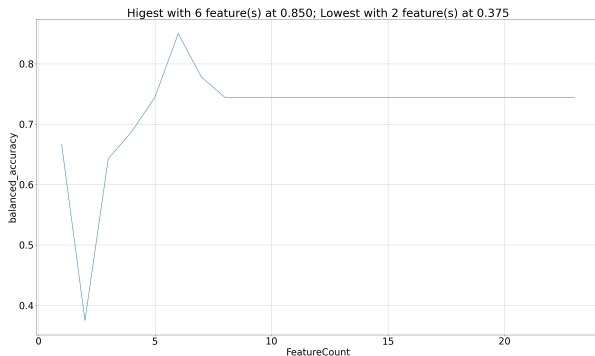


Figure: Balanced Accuracy by Feature Count



## Discussion

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