

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

Introduction

Microbiome

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

- Clinical Attachment Loss & 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

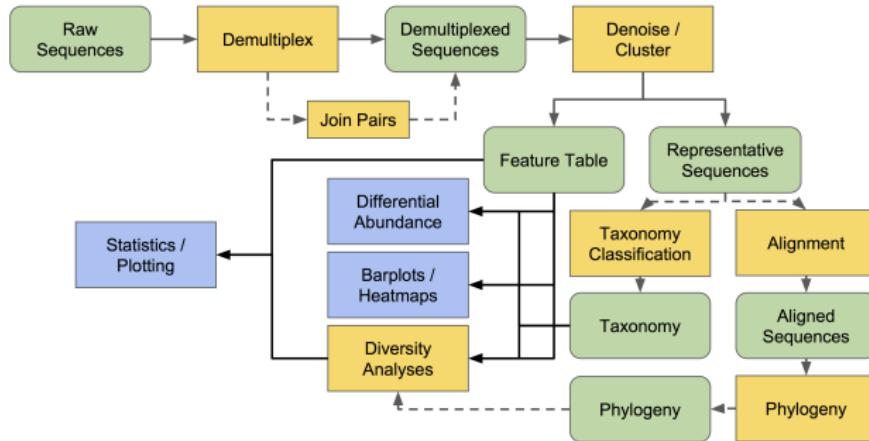


Figure: QIIME2 Workflow (Bolyen et al., 2019, 2018)

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)

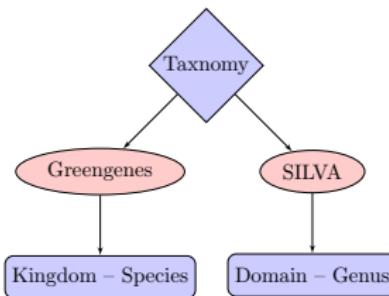


Figure: Taxonomy Classification

“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.

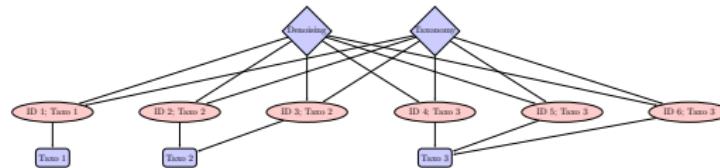


Figure: Example Diagram for Merging Denosing and Taxonomy Classification

Rarefaction

- 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**

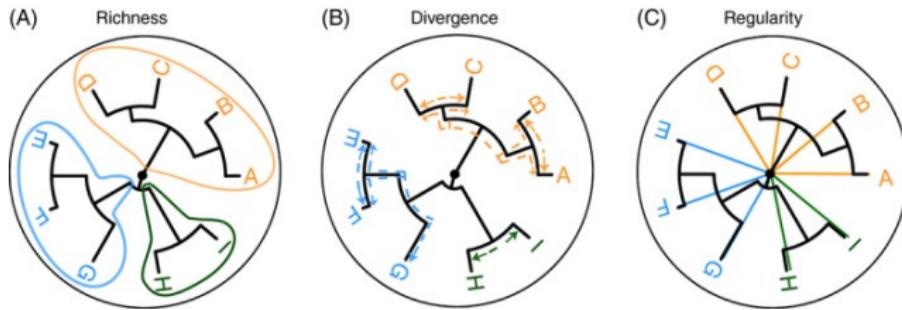


Figure: Three Dimensions of Phylogenetic Information (Tucker et al., 2017)

Alpha-diversity

- 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)

Beta-diversity

- 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)

ANCOM

- 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

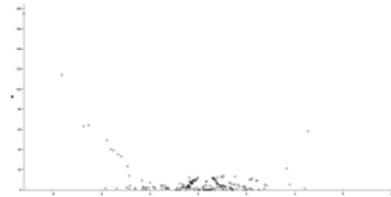


Figure: Example ANCOM Volcano Plot (Bolyen et al., 2019, 2018)

- 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)

t-SNE

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

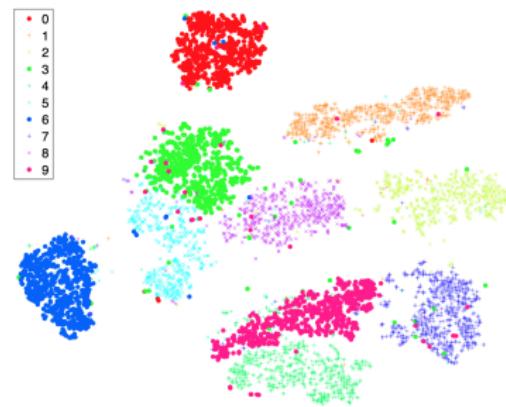


Figure: Visualization by t-SNE (Maaten & Hinton, 2008)

Classification I



Figure: Workflow of Classification

Classification Metrics:

- Accuracy
- Balanced Accuracy
- Sensitivity
- Specificity
- Precision

Classification II

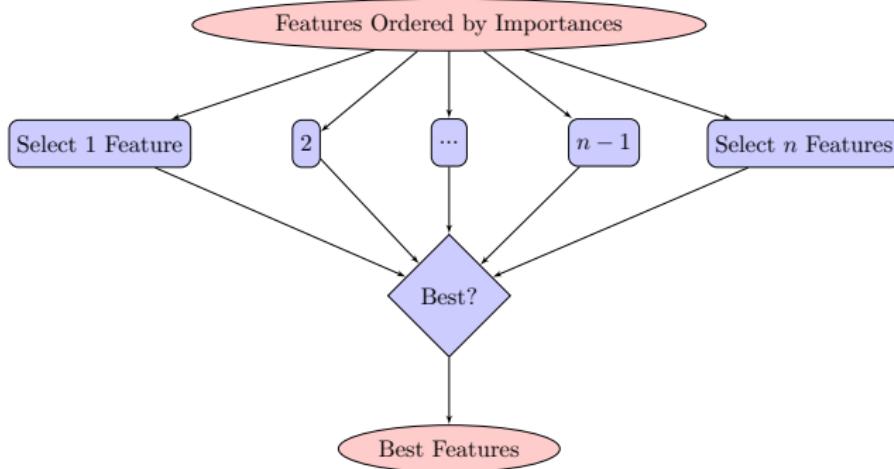
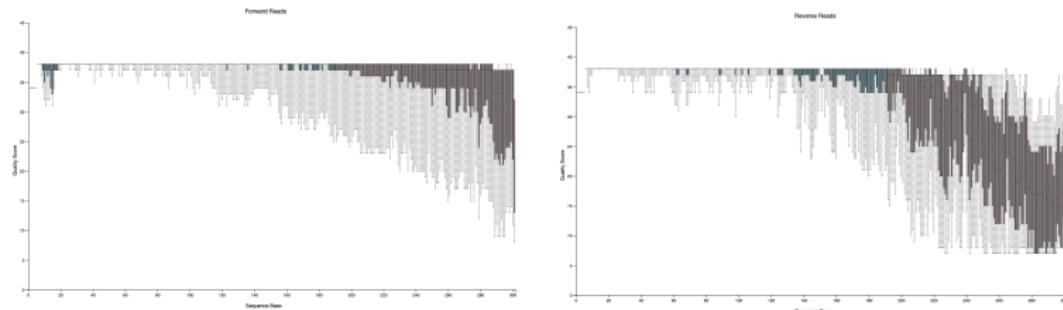


Figure: Deciding the Best Features

Results

Quality Filter



(a) Forward Reads

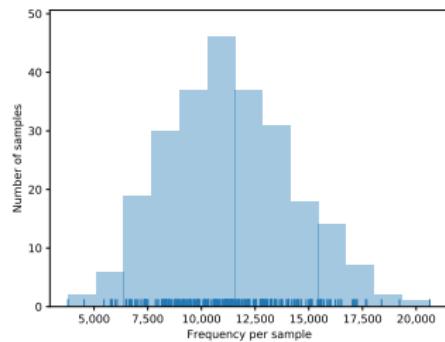
(b) Reverse Reads

Figure: Sequence Quality Plot

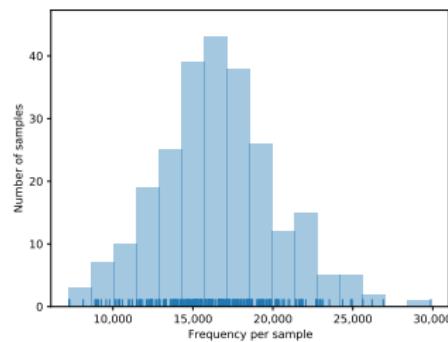
∴ Maximum Sequence Length $n_{forward} = 300$, $n_{reverse} = 265$

∴ The longest length which has sequence quality ≥ 30 at middle.

Rarefaction



(a) DADA2

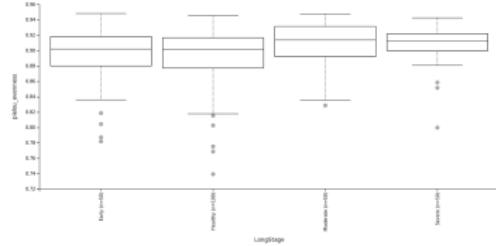


(b) Deblur

Figure: Frequency per sample

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

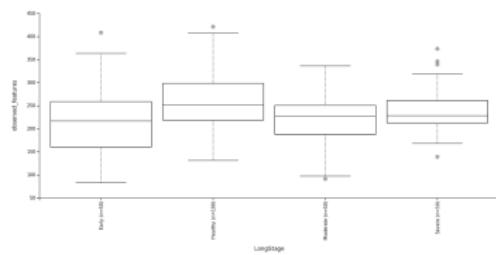
Alpha-diversity I



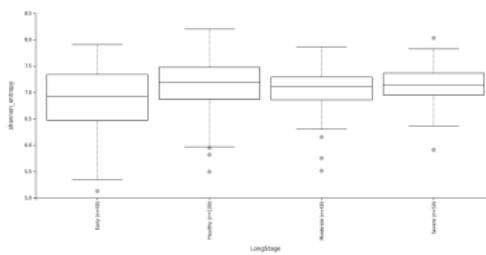
(a) Evenness ($p < 0.01$)



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



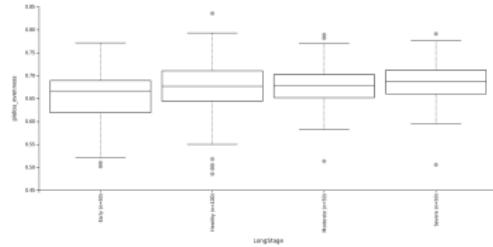
(c) Observed features ($p < 10^{-3}$)



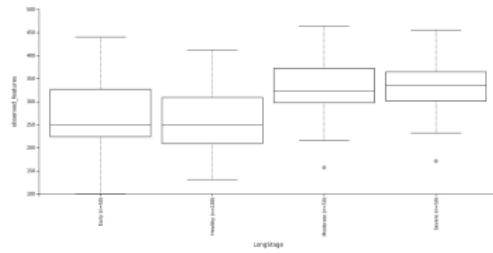
(d) Shannon's diversity ($p > 0.05$)

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

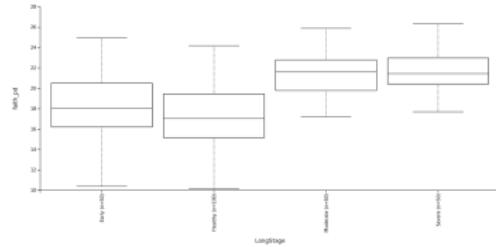
Alpha-diversity II



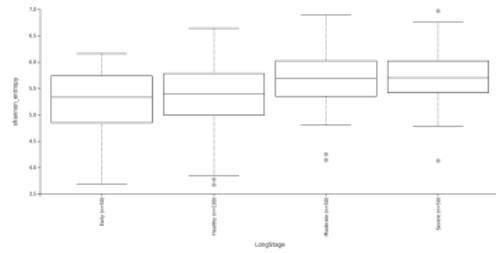
(a) Evenness ($p < 0.05$)



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



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



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

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

Beta-diversity I

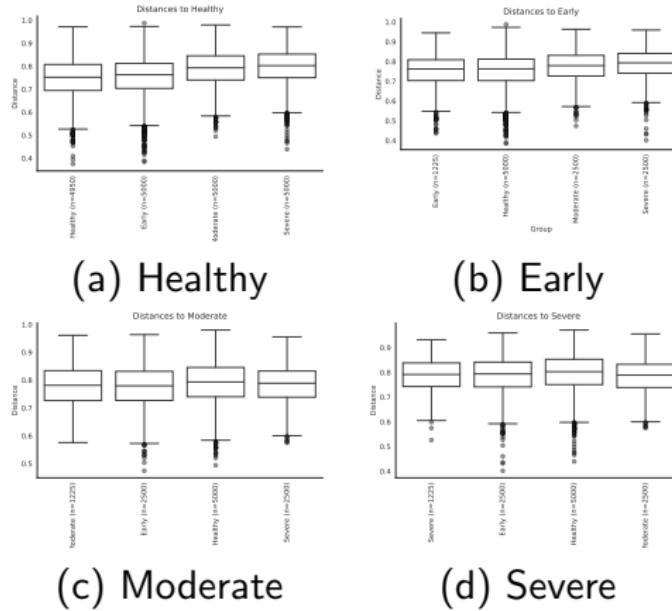


Figure: Bray-Curtis Distance with DADA2

Beta-diversity II

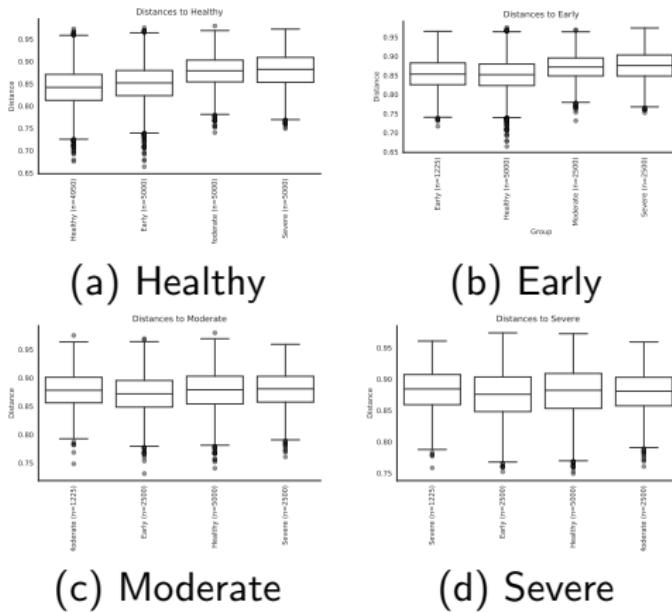


Figure: Jaccard Distance with DADA2

Beta-diversity III

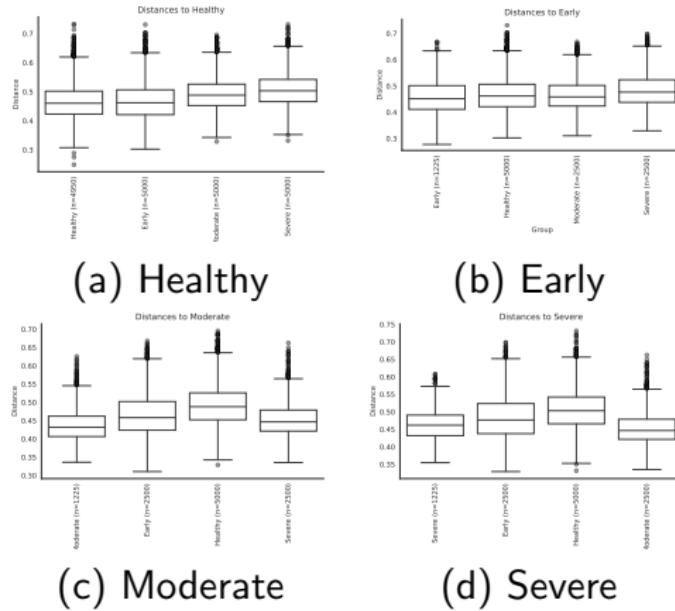
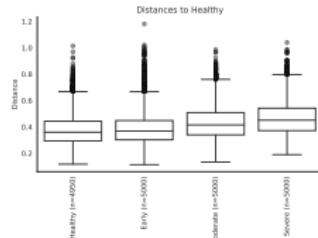
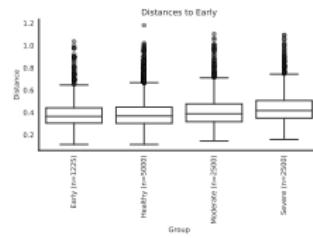


Figure: Unweighted Unifrac Distance with DADA2

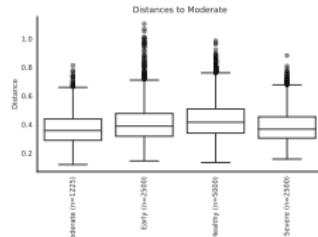
Beta-diversity IV



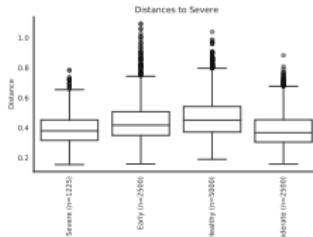
(a) Healthy



(b) Early



(c) Moderate



(d) Severe

Figure: Weighted Unifrac Distance with DADA2

Beta-diversity V

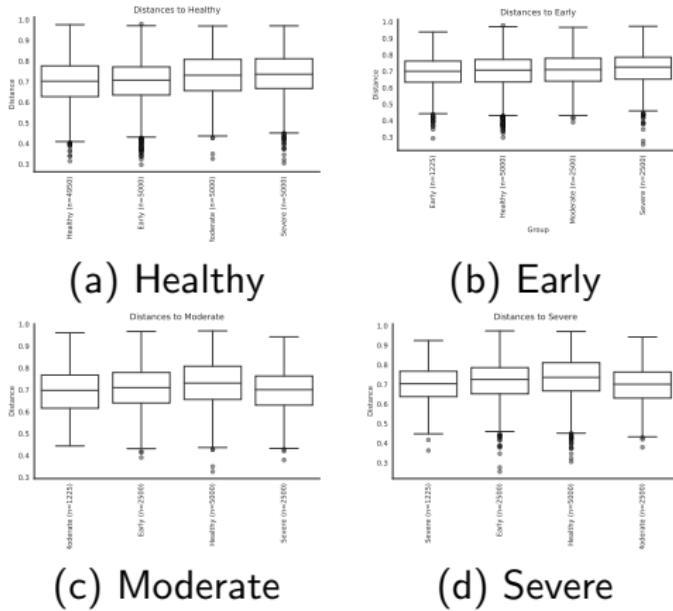


Figure: Bray-Curtis Distance with Deblur

Beta-diversity VI

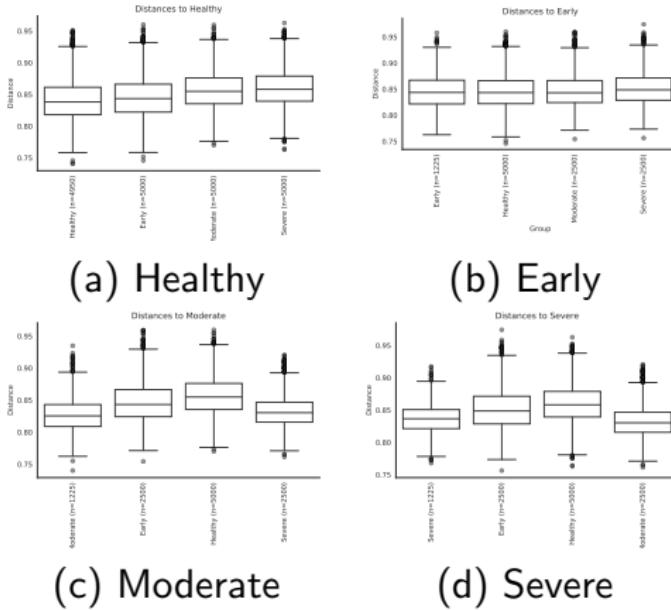


Figure: Jaccard Distance with Deblur

Beta-diversity VII

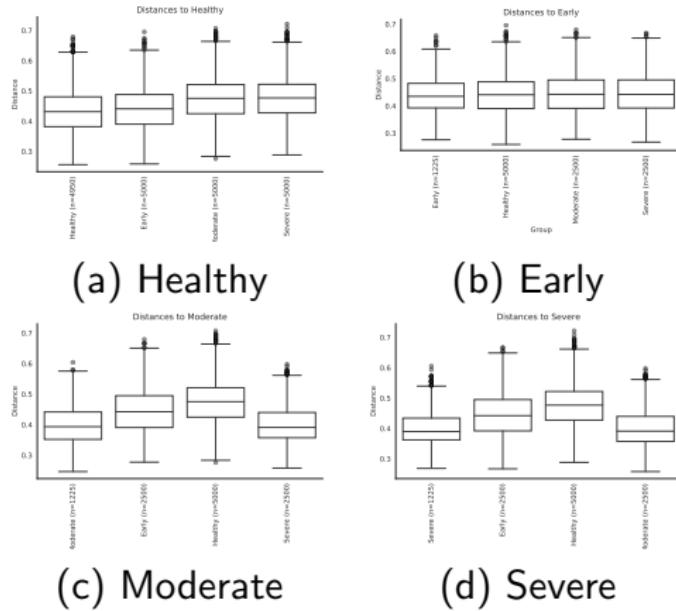


Figure: Unweighted Unifrac Distance with Deblur

Beta-diversity VIII

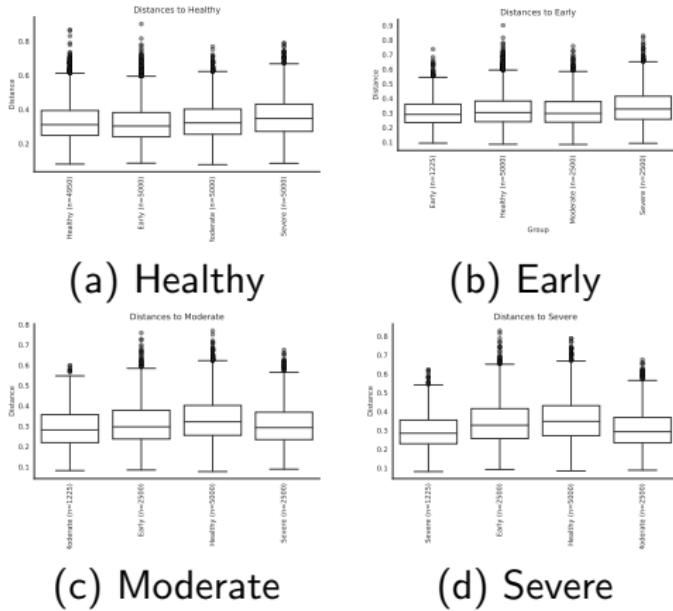


Figure: Weighted Unifrac Distance with Deblur

ANCOM I

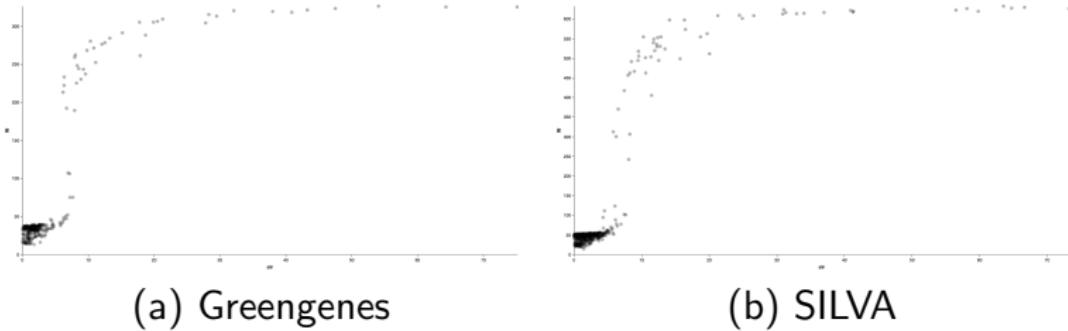
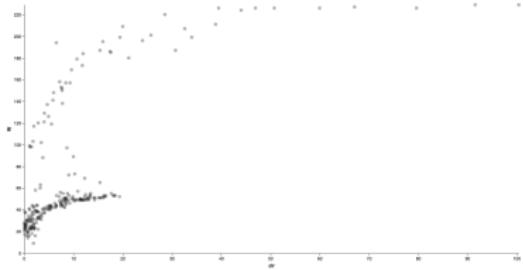
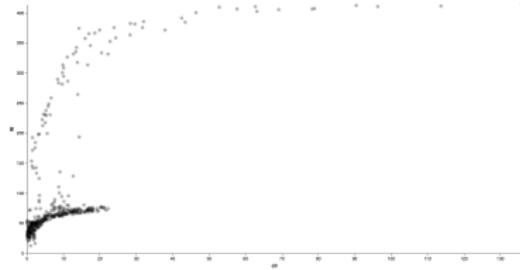


Figure: ANCOM Volcano Plot with DADA2

ANCOM II



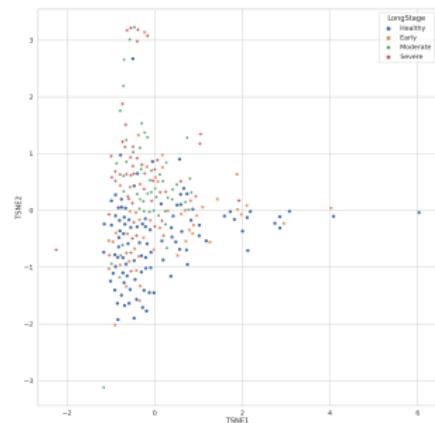
(a) Greengenes



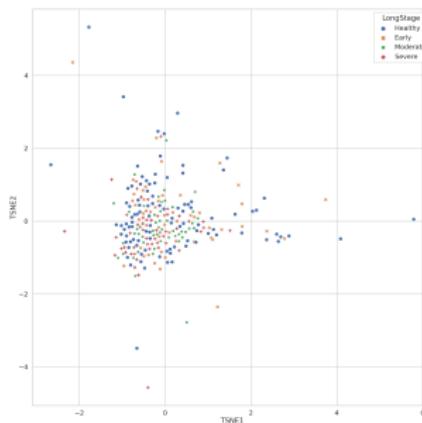
(b) SILVA

Figure: ANCOM Volcano Plot with Deblur

t-SNE with Whole Microbiome I



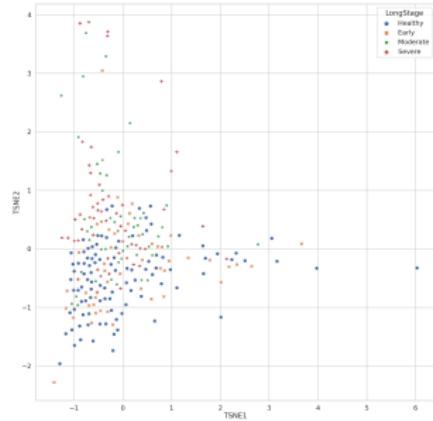
(a) Greengenes (328 Taxa)



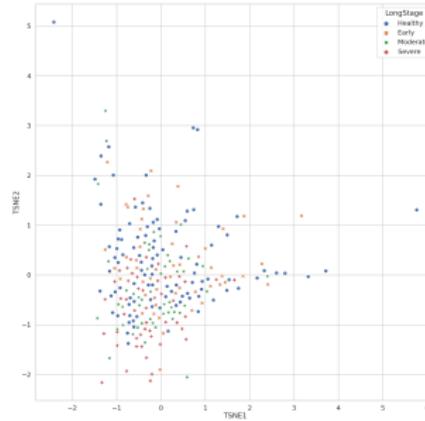
(b) SILVA (633 Taxa)

Figure: t-SNE Plot with Whole Microbiome from DADA2

t-SNE with Whole Microbiome II



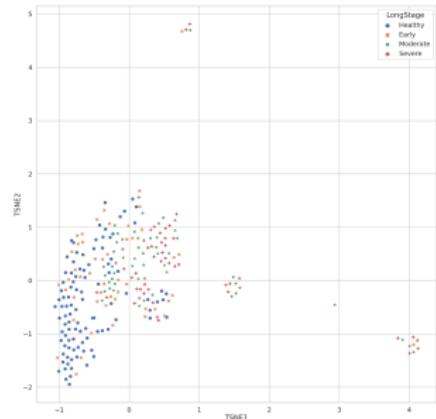
(a) Greengenes (232 Taxa)



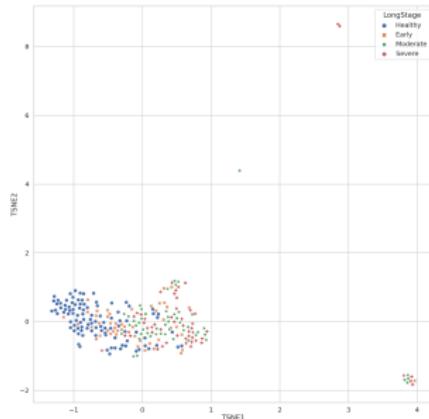
(b) SILVA (414 Taxa)

Figure: t-SNE Plot with Whole Microbiome from Deblur

t-SNE with ANCOM Selected I



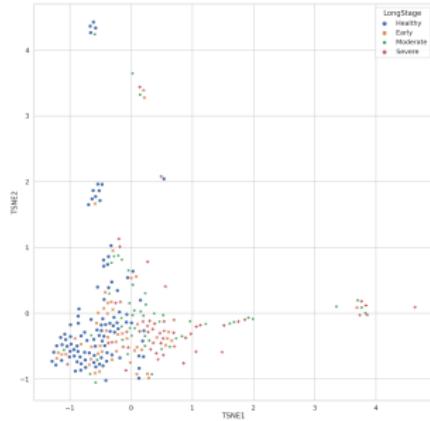
(a) Greengenes (15 Taxa)



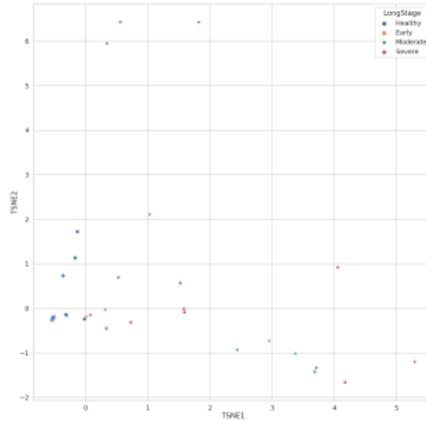
(b) SILVA (23 Taxa)

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

Random Forest Classifier I

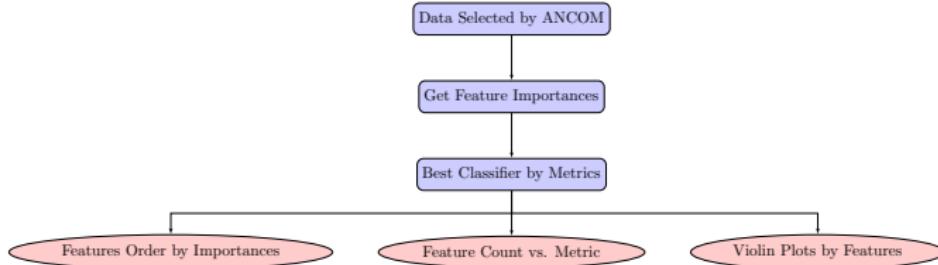


Figure: Random Forest Classifier Workflow

DADA2 + SILVA gives the best result with balanced accuracy.

Random Forest Classifier II

Table: Features Order by Importances

| Order | Taxonomy (Genus [Species]) |
|-------|--|
| 0 | <i>Actinomyces</i> |
| 1 | <i>Actinomyces Schaalia-odontolytica</i> |
| 2 | <i>Prevotella Prevotella-intermedia</i> |
| 3 | <i>Filifactor Filifactor-alocis</i> |
| 4 | <i>Oribacterium</i> |
| 5 | <i>Tannerella Tannerella-forsythia</i> |

Random Forest Classifier III

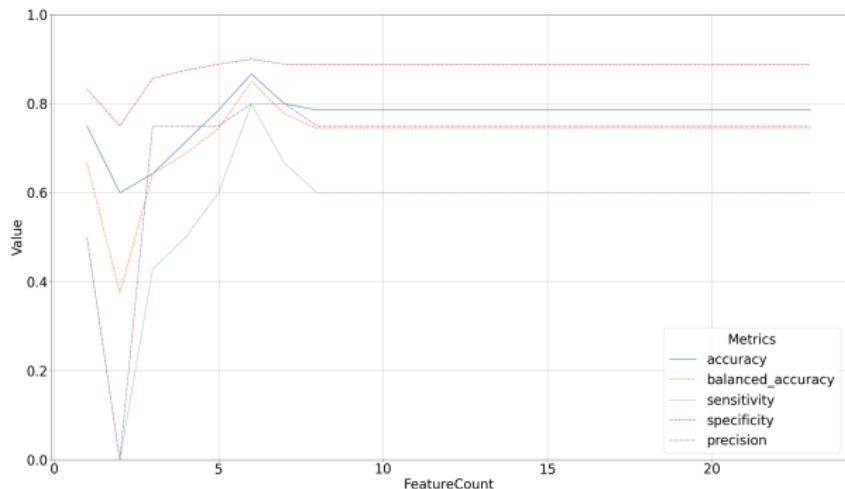


Figure: Metrics by Feature Count

Random Forest Classifier IV

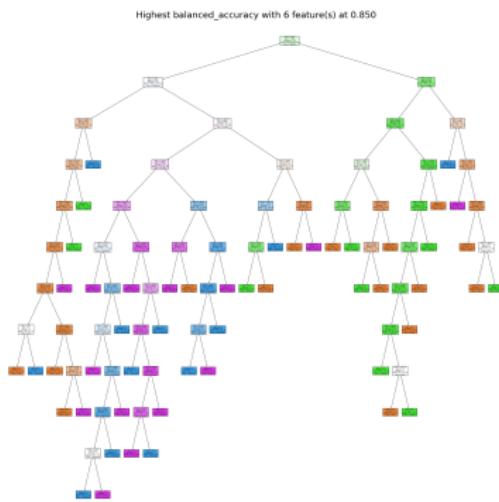
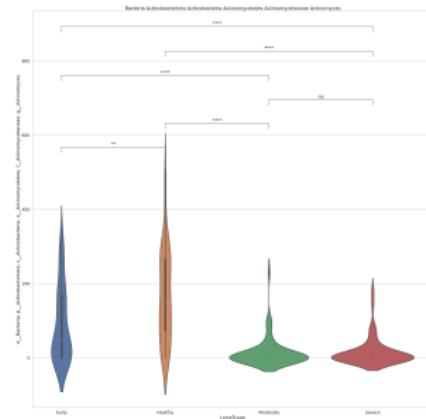
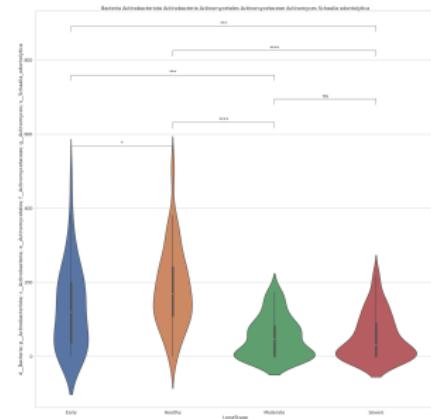


Figure: Random Forest Tree Plot

Random Forest Classifier V



(a) *Actinomyces*



(b) *Actinomyces Schaalia-odontolytica*

Figure: Violin Plot by Features

Random Forest Classifier – Merge (Healthy+Early) I

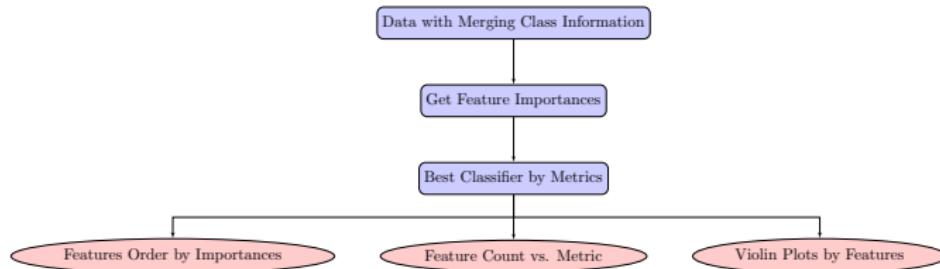


Figure: Random Forest Classifier with Merging Workflow

DADA2 + GG gives the best result with balanced accuracy.

Random Forest Classifier – Merge (Healthy+Early) II

Table: Features Order by Importances

| Order | Taxonomy (Genus [Species]) |
|-------|------------------------------|
| 0 | <i>Actinomyces</i> |
| 1 | <i>Filifactor</i> |
| 2 | <i>Prevotella intermedia</i> |

Random Forest Classifier – Merge (Healthy+Early) III

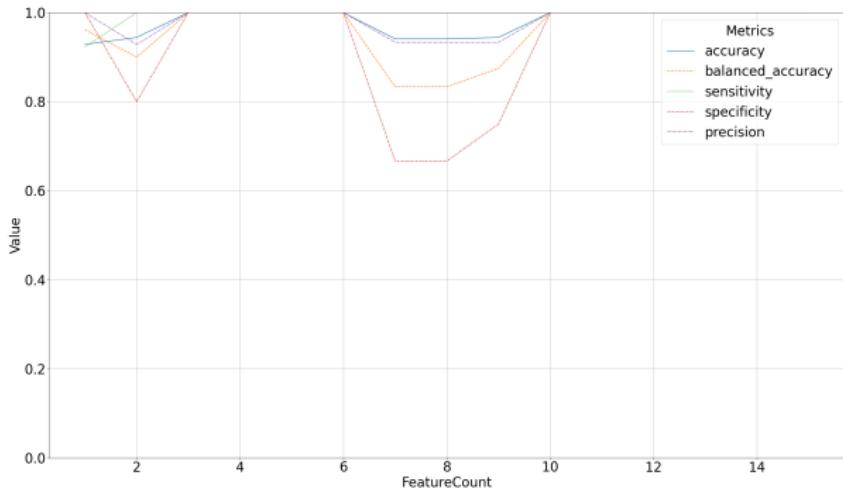


Figure: Metrics by Feature Count

Random Forest Classifier – Merge (Healthy+Early) IV

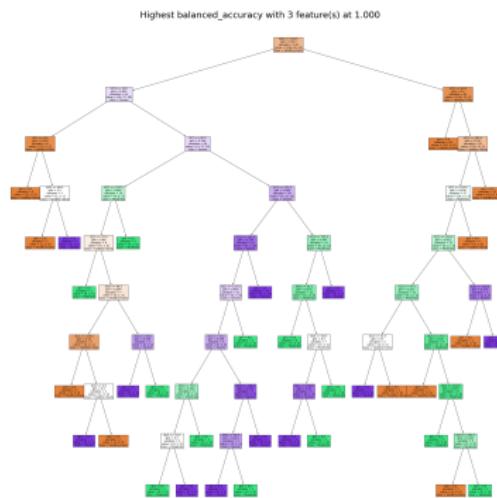
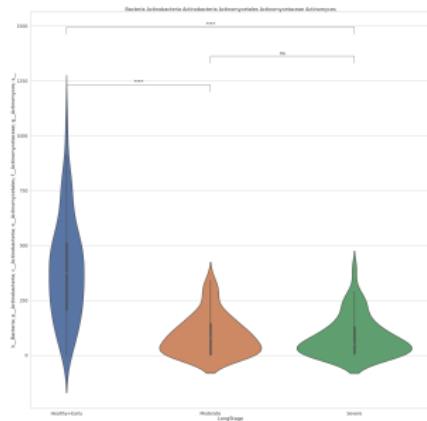
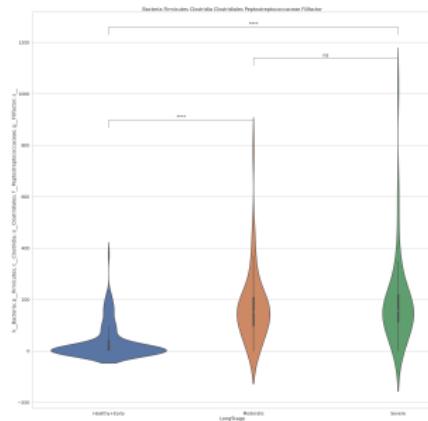


Figure: Random Forest Tree Plot

Random Forest Classifier – Merge (Healthy+Early) V



(a) *Actinomyces*



(b) *Filifactor*

Figure: Violin Plot by Features

Random Forest Classifier – Merge (Moderate + Severe) I

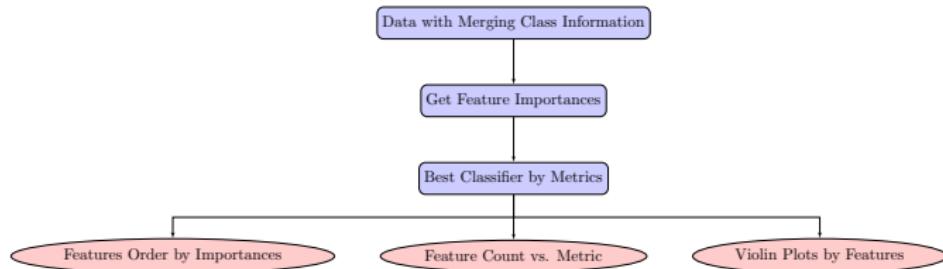


Figure: Random Forest Classifier with Merging Workflow

DADA2 + GG gives the best result with balanced accuracy.

Random Forest Classifier – Merge (Moderate + Severe) II

Table: Features Order by Importances

| Order | Taxonomy (Genus [Species]) |
|-------|------------------------------|
| 0 | <i>Actinomyces</i> |
| 1 | <i>Filifactor</i> |
| 2 | <i>Prevotella intermedia</i> |

Random Forest Classifier – Merge (Moderate + Severe) III

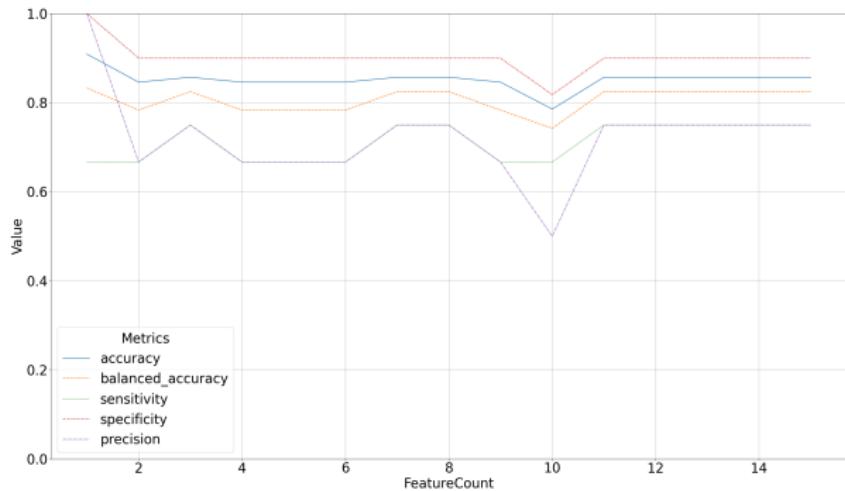


Figure: Metrics by Feature Count

Random Forest Classifier – Merge (Moderate + Severe) IV

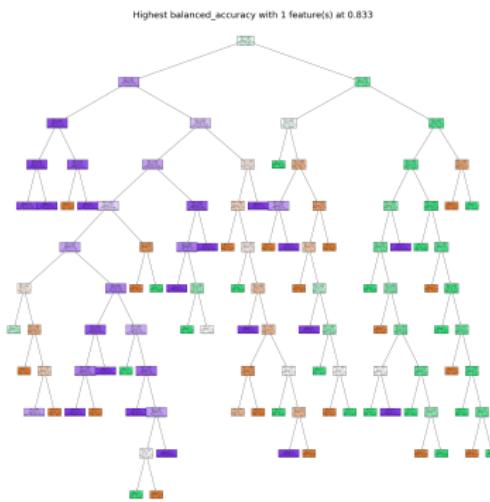
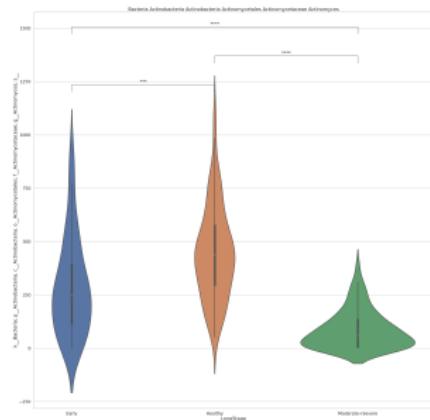
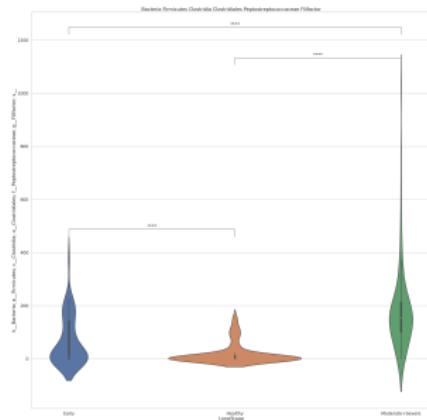


Figure: Random Forest Tree Plot

Random Forest Classifier – Merge (Moderate + Severe) V



(a) *Actinomyces*



(b) *Filifactor*

Figure: Violin Plot by Features

Random Forest Classifier – Merge (H + E) & (M + S) I

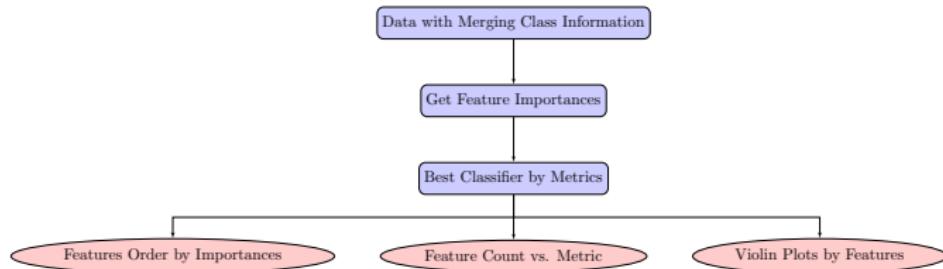


Figure: Random Forest Classifier with Merging Workflow

Deblur + SILVA gives the best result with balanced accuracy.

Random Forest Classifier – Merge (H + E) & (M + S) II

Table: Features Order by Importances

| Order | Taxonomy (Genus [Species]) |
|-------|--|
| 0 | <i>Porphyromonas gingivalis</i> |
| 1 | <i>Treponema denticola</i> |
| 2 | <i>Treponema medium</i> |
| 3 | <i>Metamycoplasmfaucium</i> |
| 4 | <i>Prevotella intermedia</i> |
| 5 | <i>Streptococcus constellatus</i> |
| 6 | <i>Eubacterium nodatum group Eubacterium nodatum</i> |
| 7 | <i>Treponema uncultured bacterium</i> |
| ... | ... |

Random Forest Classifier – Merge (H + E) & (M + S) III

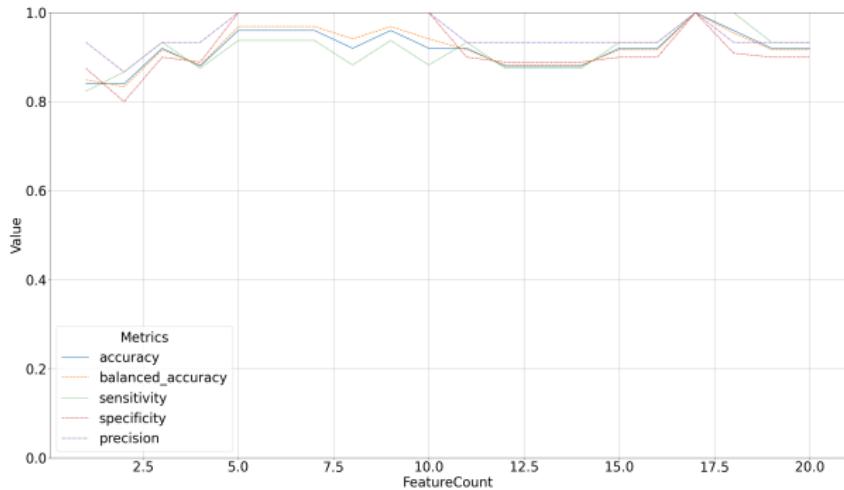


Figure: Metrics by Feature Count

Random Forest Classifier – Merge (H + E) & (M + S) IV

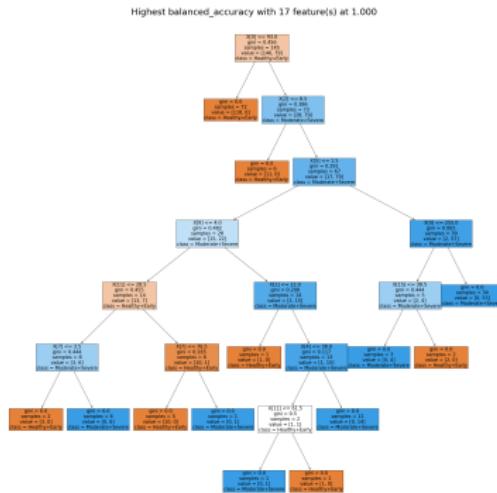
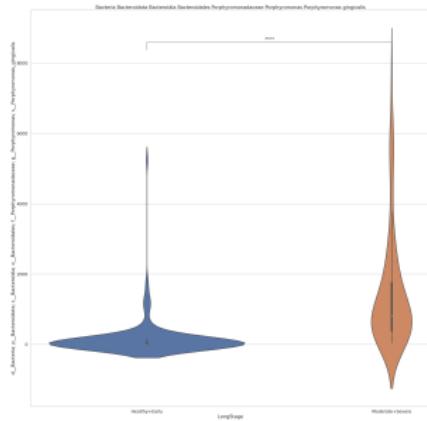
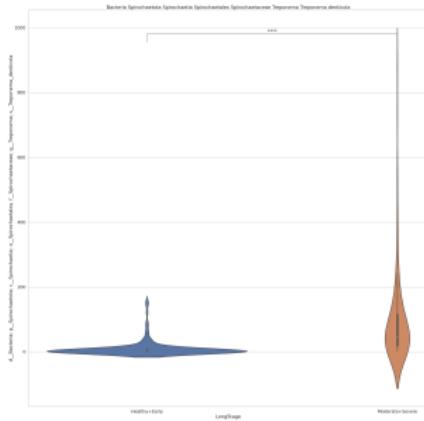


Figure: Random Forest Tree Plot

Random Forest Classifier – Merge (H + E) & (M + S) V



(a) *Porphyromonas gingivalis*



(b) *Treponema denticola*

Figure: Violin Plot by Features

Discussion

Actinomyces Genus

- *Actinomyces*: a deceptive infection of oral cavity (Thukral, Srivastav, Mathur, Barodiya, & Srivastav, 2017)
- Prevalence of *Actinomyces* spp. in patients with chronic periodontitis (Vielkind, Jentsch, Eschrich, Rodloff, & Stingu, 2015)

Filifactor Genus

- *Filifactor alocis* – a new emerging periodontal pathogen (Aruni et al., 2015)
- *In Situ* Anabolic Activity of Periodontal Pathogens *Porphyromonas gingivalis* and *Filifactor alocis* in Chronic Periodontitis (Spooner et al., 2016)

Porphyromonas Genus

- Antibody responses to *Porphyromonas gingivalis* (*P. gingivalis*) in subjects with rheumatoid arthritis and periodontitis (Mikuls et al., 2009)
- Contribution of *Porphyromonas gingivalis* lipopolysaccharide to periodontitis (Jain & Darveau, 2010)

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