

# Periodontitis

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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

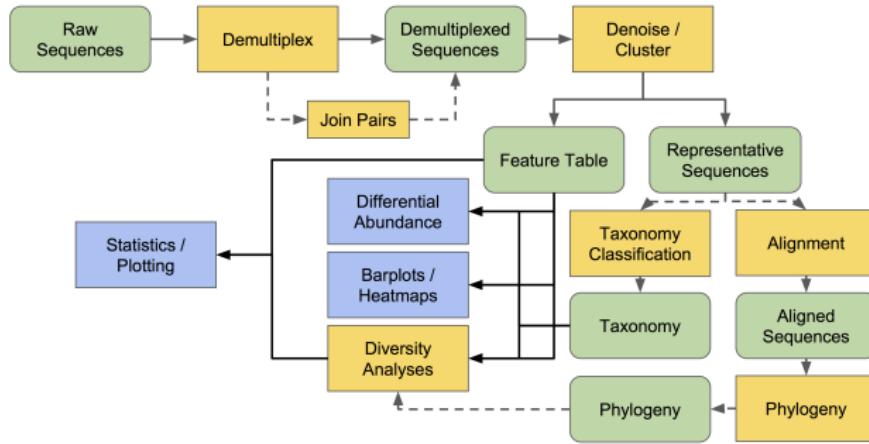


Figure: QIIME2 Workflow (Bolyen et al., 2019, 2018)

# Denoising techniques

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

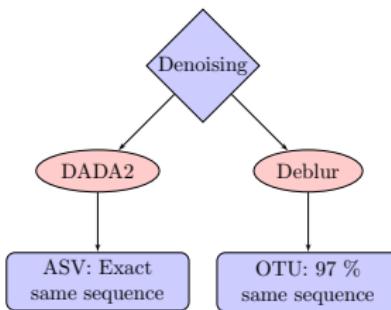


Figure: Denoising Techniques

# Taxonomy Classification

- Greengenes (GG) (DeSantis et al., 2006)
- SILVA (Pruesse et al., 2007)
- Human Oral Microbiome Database (HOMD) (Chen et al., 2010)

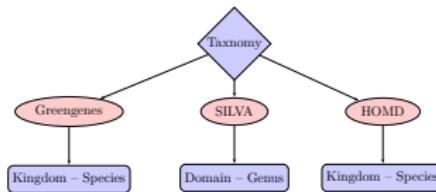


Figure: Taxonomy Classification

"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.

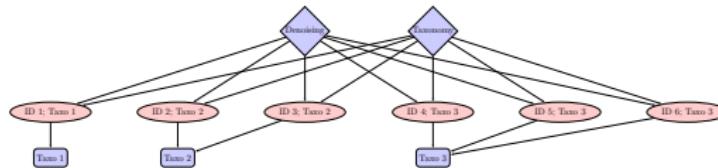


Figure: Example Diagram for Merging Denosing and Taxonomy Classification

# 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**

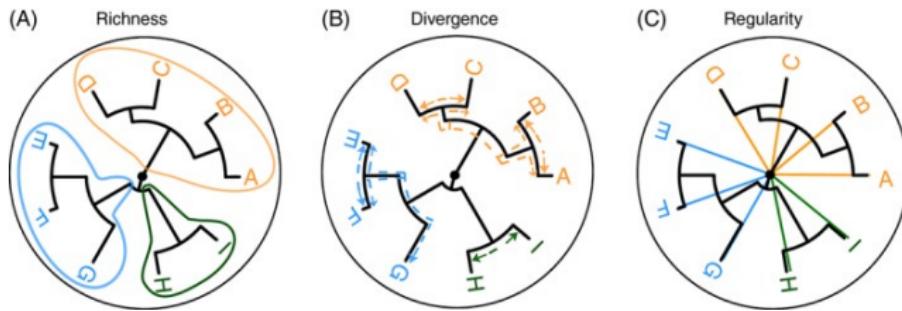


Figure: Three Dimensions of Phylogenetic Information (Tucker et al., 2017)

# Alpha-diversity

- Evenness: a measurement of diversity in different type at community (Pielou, 1966)
- Faith's Phylogenetic Diversity: a qualitative measurement of community richness which prioritizes for species conservation, incorporates with taxic diversity (Faith, 1992)
- Observed Features: a number of observed taxa
- Shannon's diversity index: a significant aspect of community richness (Shannon, 1948)

# Beta-diversity

- Bray-Curtis distance: a quantitative measurement of dissimilarity among communities (Sørensen, 1948)
- Jaccard distance: a measurement of local distribution among communities (Jaccard, 1912)
- Unweighted UniFrac distance: a qualitative measurement of phylogenetic distances (McDonald et al., 2018)
- Weighted UniFrac distance: a quantitative measurement of phylogenetic distances (McDonald et al., 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)

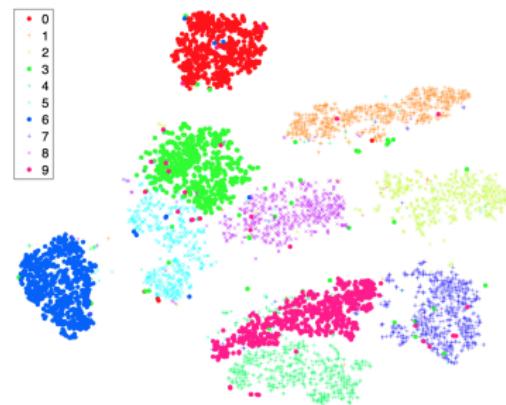


Figure: Visualization by t-SNE (Maaten & Hinton, 2008)

# Classification I



Figure: Workflow of Classification

## Classification Metrics:

- Accuracy
- Balanced Accuracy
- Sensitivity
- Specificity
- Precision

# Classification II

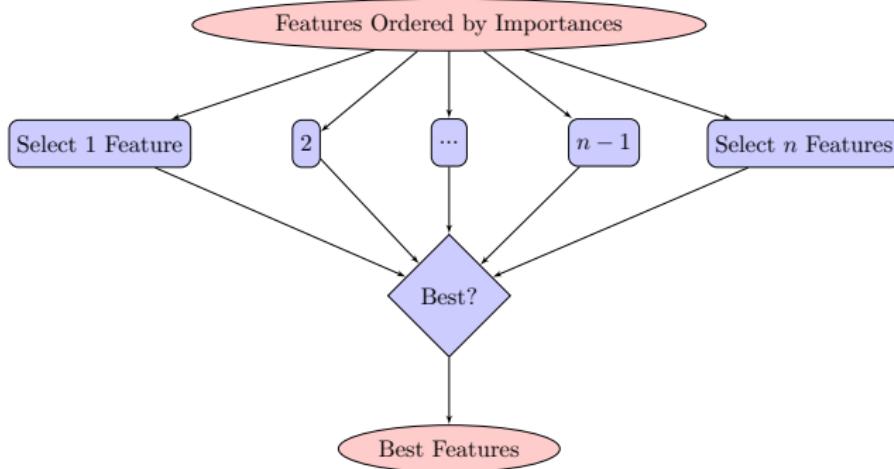
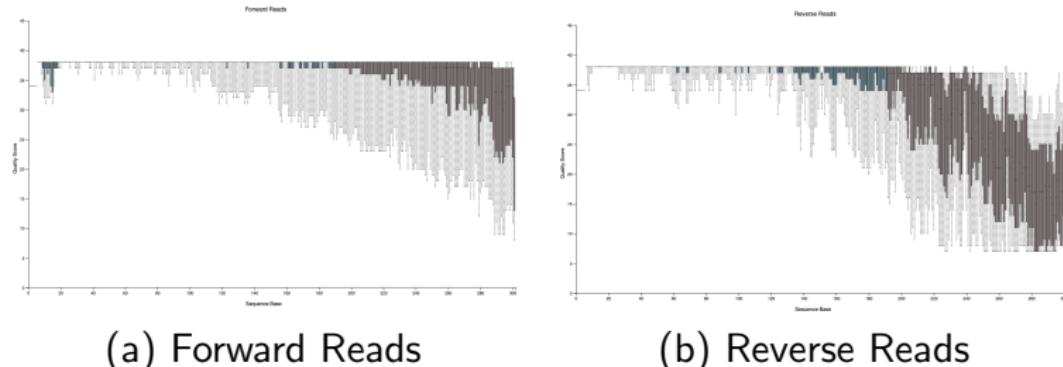


Figure: Deciding the Best Features

# Results

# Quality Filter



(a) Forward Reads

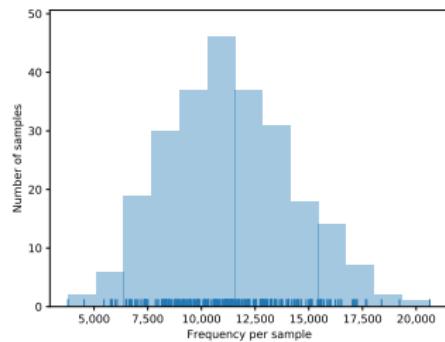
(b) Reverse Reads

Figure: Sequence Quality Plot

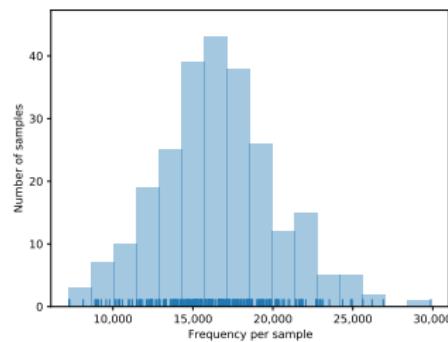
∴ Maximum Sequence Length  $n_{forward} = 300$ ,  $n_{reverse} = 265$

∴ The longest length which has sequence quality  $\geq 30$  at middle.

# Rarefaction



(a) DADA2

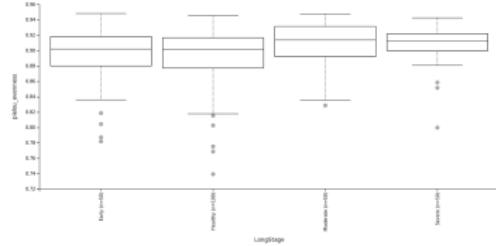


(b) Deblur

Figure: Frequency per sample

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

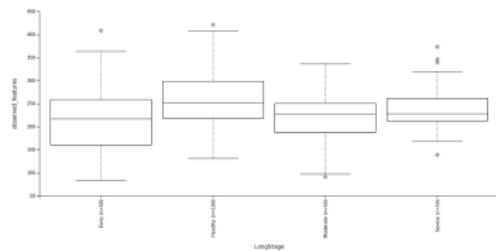
# Alpha-diversity I



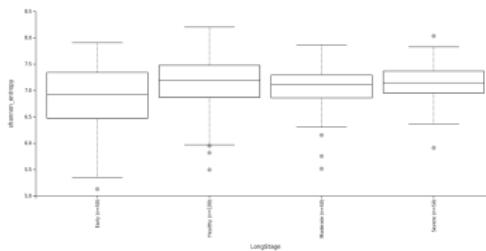
(a) Evenness ( $p < 0.01$ )



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



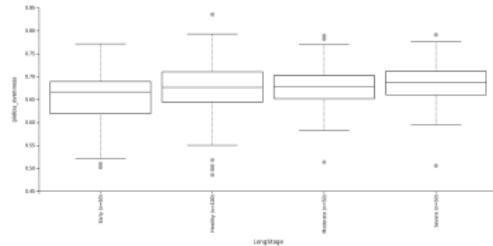
(c) Observed features ( $p < 10^{-3}$ )



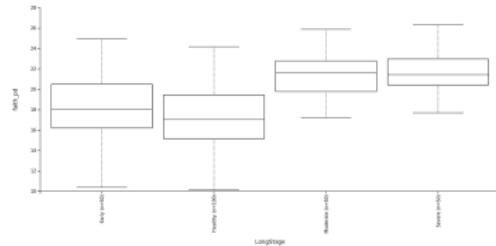
(d) Shannon's diversity ( $p > 0.05$ )

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

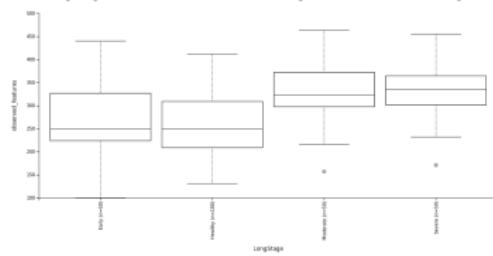
# Alpha-diversity II



(a) Evenness ( $p < 0.05$ )



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



(c) Observed features ( $p < 10^{-12}$ ) (d) Shannon's diversity ( $p < 10^{-4}$ )

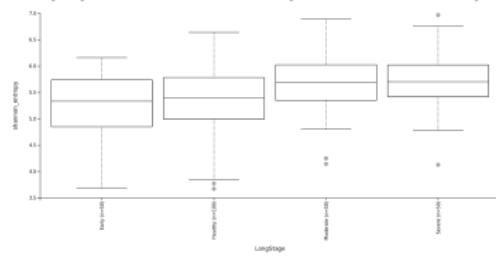


Figure: Alpha Diversity from Deblur with Kruskal-Wallis among All Groups

# Beta-diversity I

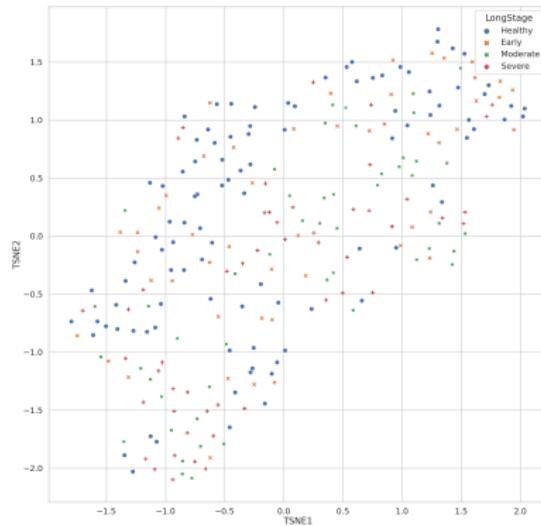


Figure: Bray-Curtis Distance with DADA2

# Beta-diversity II

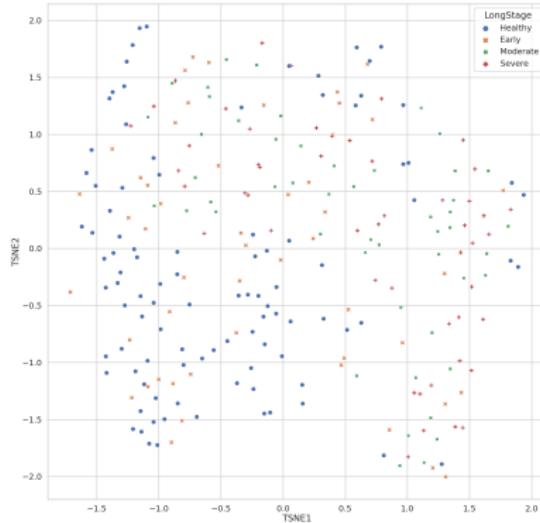


Figure: Jaccard Distance with DADA2

# Beta-diversity III

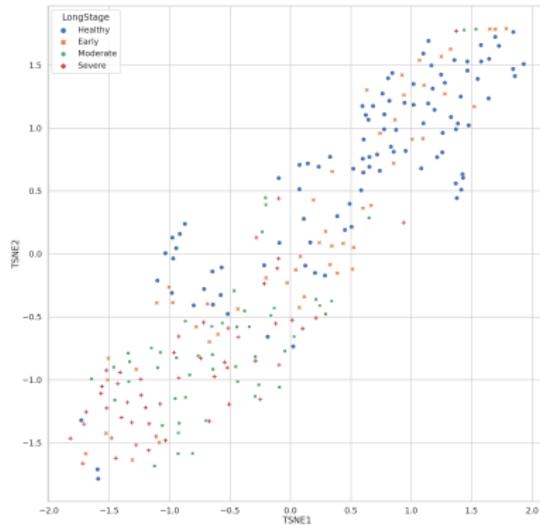


Figure: Unweighted Unifrac Distance with DADA2

# Beta-diversity IV

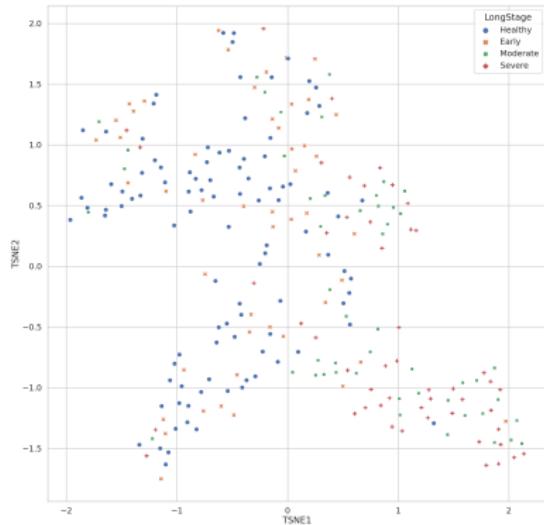


Figure: Weighted Unifrac Distance with DADA2

# Beta-diversity V

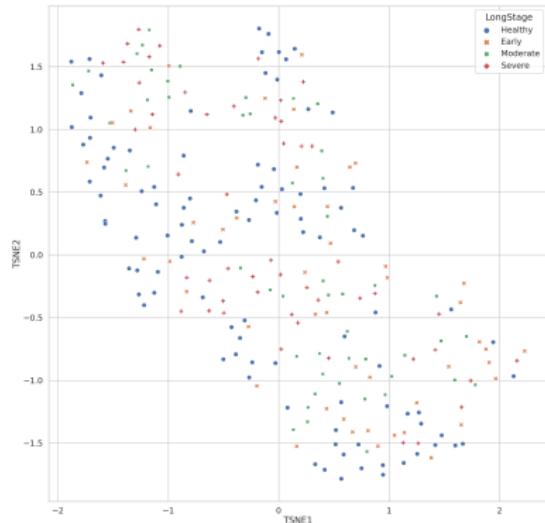


Figure: Bray-Curtis Distance with Deblur

# Beta-diversity VI

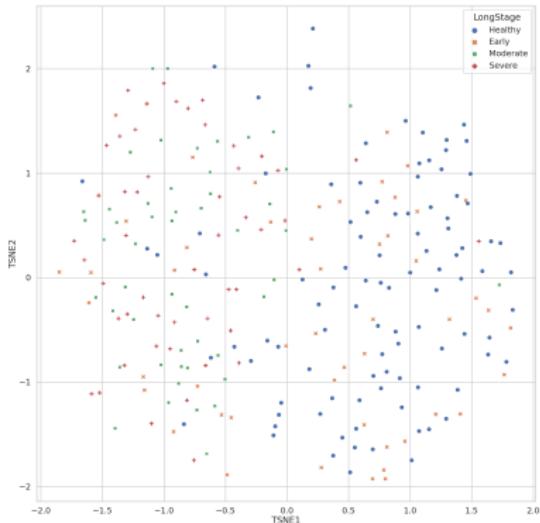


Figure: Jaccard Distance with Deblur

# Beta-diversity VII

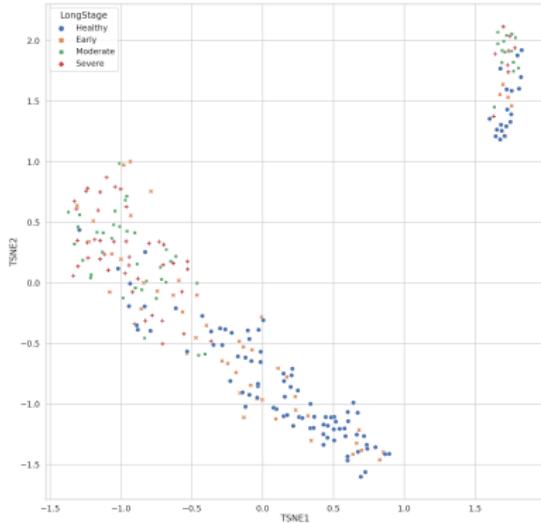


Figure: Unweighted Unifrac Distance with Deblur

# Beta-diversity VIII

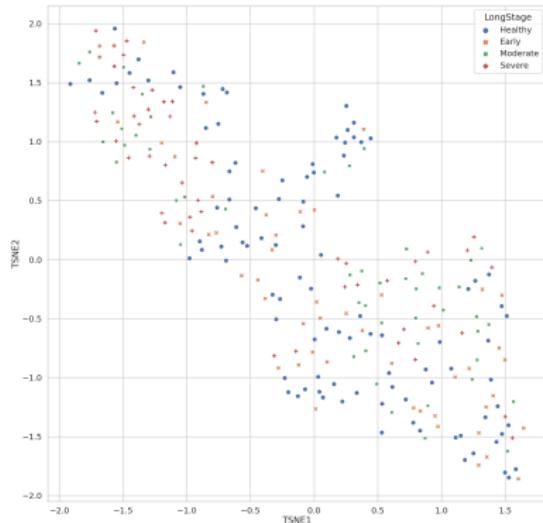


Figure: Weighted Unifrac Distance with Deblur

# ANCOM



Figure: ANCOM Volcano Plot with DADA2

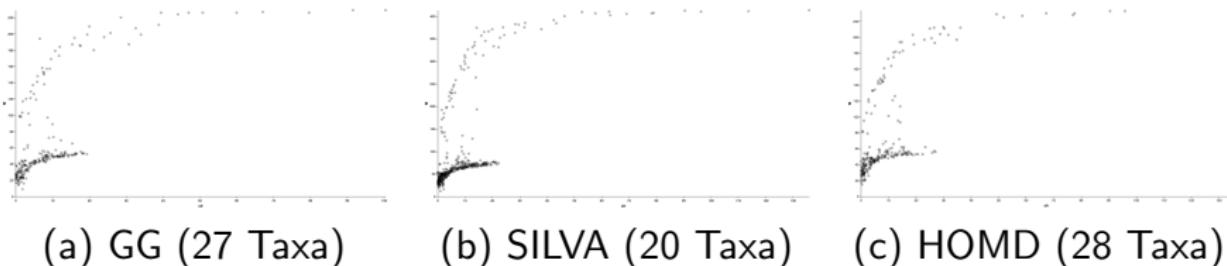
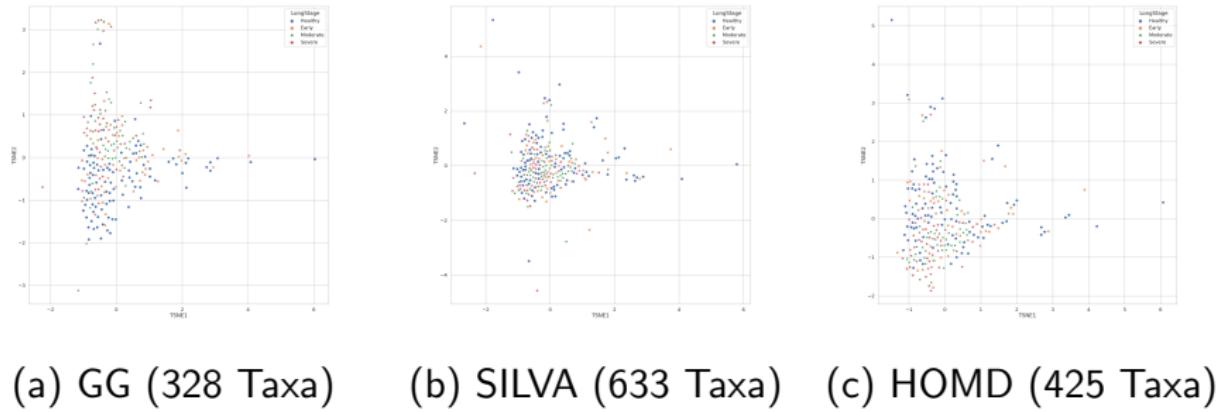


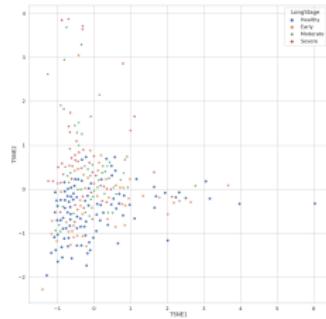
Figure: ANCOM Volcano Plot with Deblur

# t-SNE with Whole Microbiome I

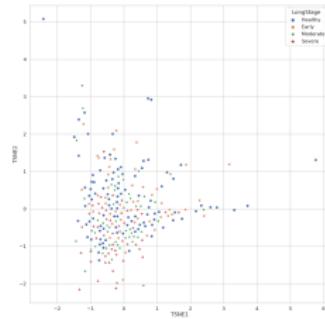


**Figure:** t-SNE Plot with Whole Microbiome from DADA2

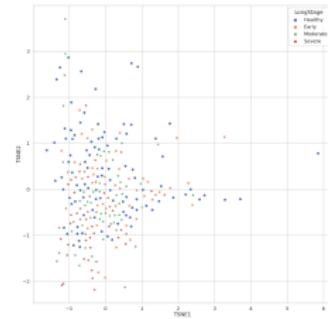
# t-SNE with Whole Microbiome II



(a) GG (232 Taxa)



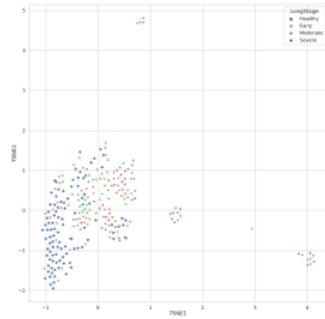
(b) SILVA (414 Taxa)



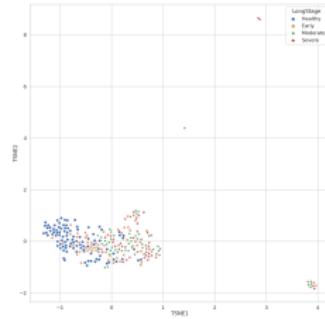
(c) HOMD (235 Taxa)

Figure: t-SNE Plot with Whole Microbiome from Deblur

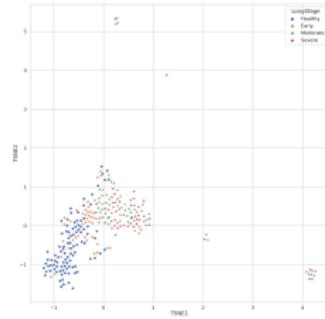
# t-SNE with ANCOM Selected I



(a) GG (15 Taxa)



(b) SILVA (23 Taxa)



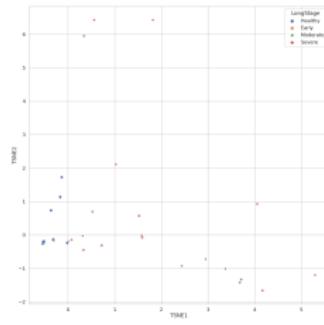
(c) HOMD (20 Taxa)

**Figure:** t-SNE Plot with ANCOM Selected from DADA2

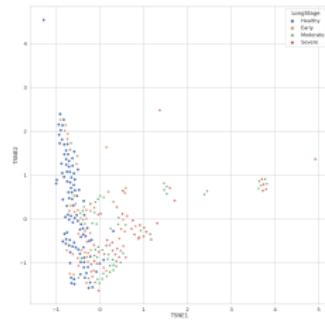
# t-SNE with ANCOM Selected II



(a) GG (27 Taxa)



(b) SILVA (20 Taxa)



(c) HOMD (28 Taxa)

Figure: t-SNE Plot with ANCOM Selected from Deblur

# Random Forest Classifier – Every Classes I

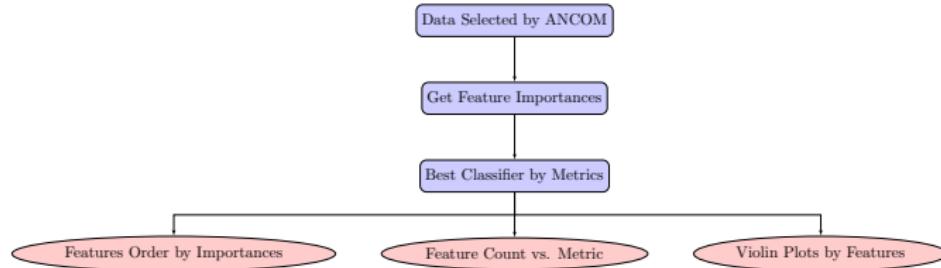


Figure: Random Forest Classifier Workflow

**Deblur + HOMD** gives the best result with balanced accuracy (0.778 with 13 taxa).

# Random Forest Classifier – Every Classes II

Table: Features Order by Importances

Order	Taxonomy (Genus [Species])
0	<i>Porphyromonas gingivalis</i>
1	<i>Actinomyces</i>
2	<i>Pasteurellaceae</i>
3	<i>Veillonella denticariosi</i>
4	<i>Oribacterium sinus</i>
5	<i>Peptostreptococcus anaerobius</i>
6	<i>Prevotella nanceiensis</i>
7	<i>Treponema</i>
8	<i>Parvimonas sp. HMT 393</i>
9	<i>Filifactor alocis</i>
...	...

# Random Forest Classifier – Every Classes III

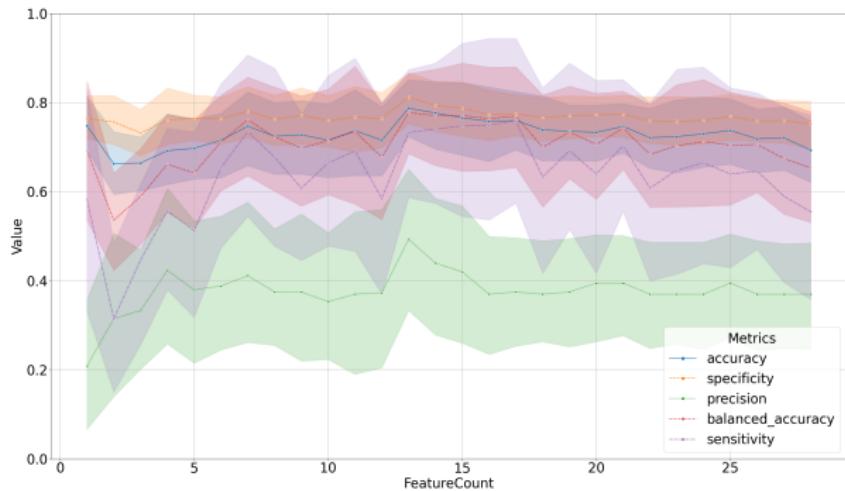
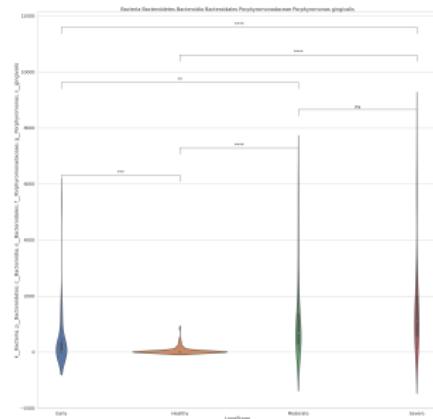
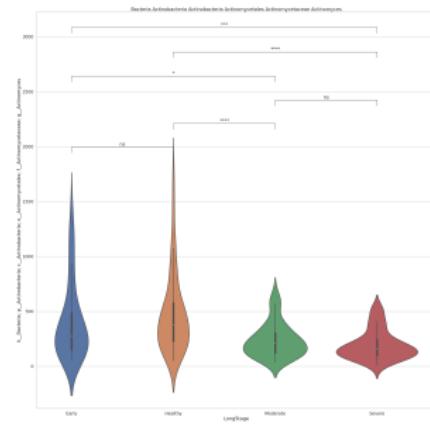


Figure: Metrics by Feature Count

Random Forest Classifier – Every Classes IV



(a) *Porphyromonas gingivalis*



(b) *Actinomyces*

Figure: Violin Plot by Features

# Random Forest Classifier – Merge (Healthy+Early) I

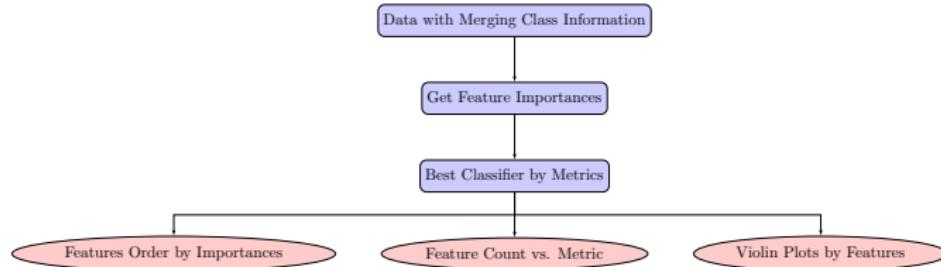


Figure: Random Forest Classifier with Merging Workflow

**DADA2 + HOMD** gives the best result with balanced accuracy (0.938 with 11 taxa).

# Random Forest Classifier – Merge (Healthy+Early) II

Table: Features Order by Importances

Order	Taxonomy (Genus [Species])
0	<i>Porphyromonas gingivalis</i>
1	<i>Actinomyces</i>
2	<i>Prevotella intermedia</i>
3	<i>Filifactor alocis</i>
4	<i>Treponema sp. HMT 260</i>
5	<i>Campylobacter showae</i>
6	<i>Porphyromonas sp. HMT 285</i>
7	<i>Peptostreptococcaceae XIG-6 nodatum</i>
8	<i>Prevotella sp. HMT 304</i>
9	<i>Actinomyces graevenitzii</i>
10	<i>Tannerella forsythia</i>

# Random Forest Classifier – Merge (Healthy+Early) III

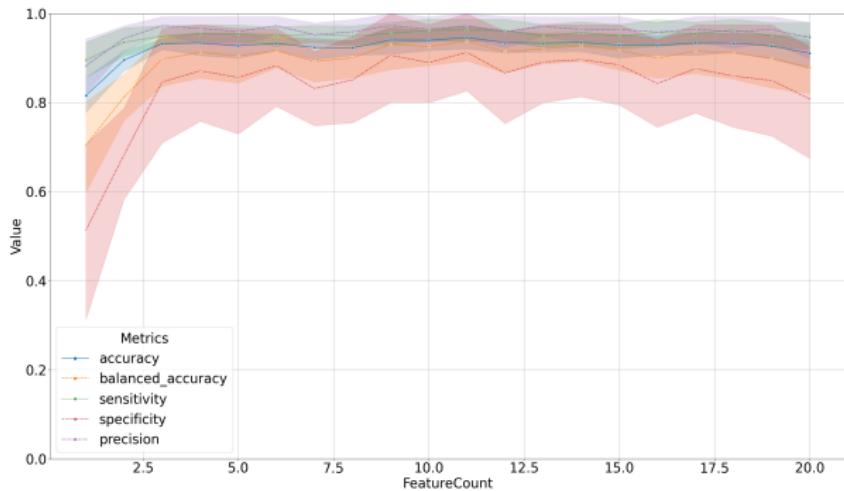
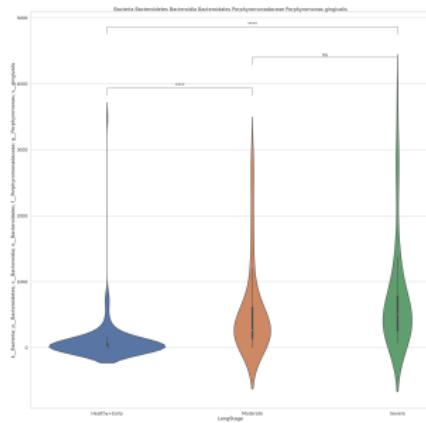
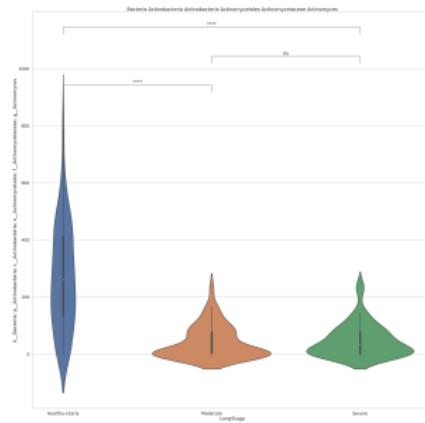


Figure: Metrics by Feature Count

# Random Forest Classifier – Merge (Healthy+Early) IV



(a) *Porphyromonas gingivalis*



(b) *Actinomyces*

Figure: Violin Plot by Features

# Random Forest Classifier – Merge (Moderate + Severe) I

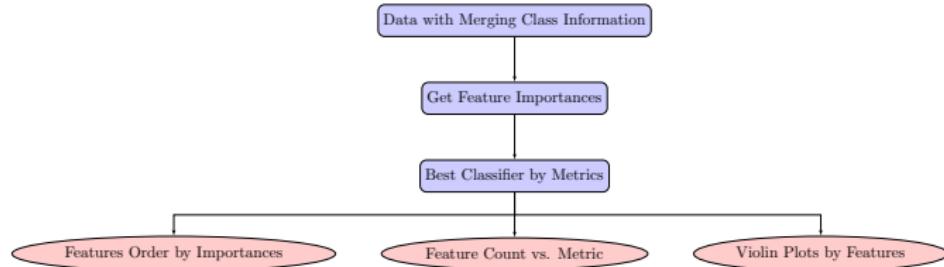


Figure: Random Forest Classifier with Merging Workflow

**Deblur + HOMD** gives the best result with balanced accuracy (0.777 with 28 taxa).

# Random Forest Classifier – Merge (Moderate + Severe) II

Table: Features Order by Importances

Order	Taxonomy (Genus [Species])
0	<i>Porphyromonas gingivalis</i>
1	<i>Actinomyces</i>
2	<i>Pasteurellaceae</i>
3	<i>Veillonella denticariosi</i>
4	<i>Oribacterium sinus</i>
5	<i>Peptostreptococcus anaerobius</i>
6	<i>Treponema</i>
7	<i>Prevotella nanceiensis</i>
8	<i>Porphyromonas sp. HMT 285</i>
9	<i>Parvimonas sp. HMT 393</i>
...	...

# Random Forest Classifier – Merge (Moderate + Severe) III

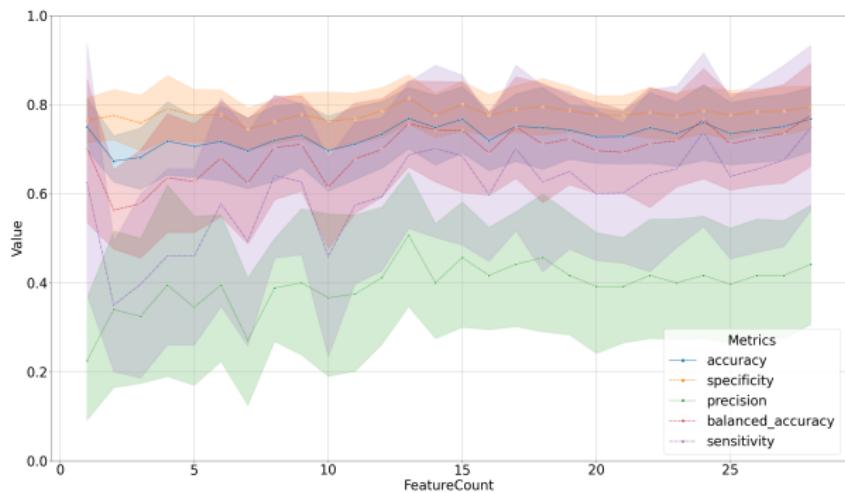
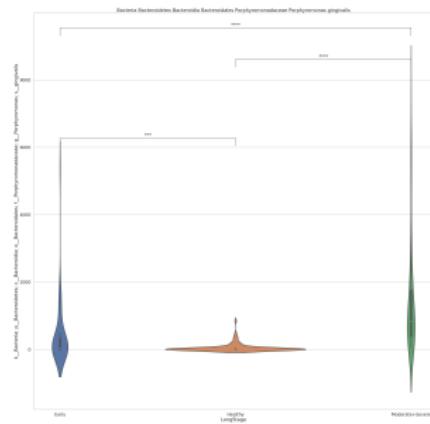
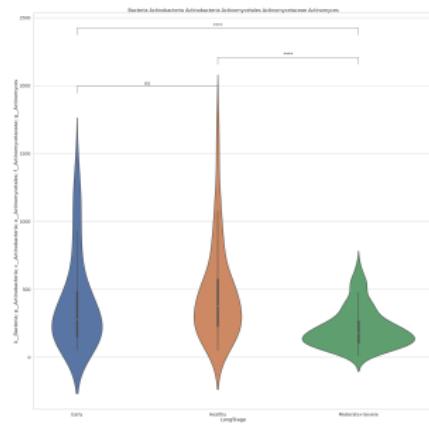


Figure: Metrics by Feature Count

# Random Forest Classifier – Merge (Moderate + Severe) IV



(a) *Porphyromonas gingivalis*



(b) *Actinomyces*

Figure: Violin Plot by Features

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

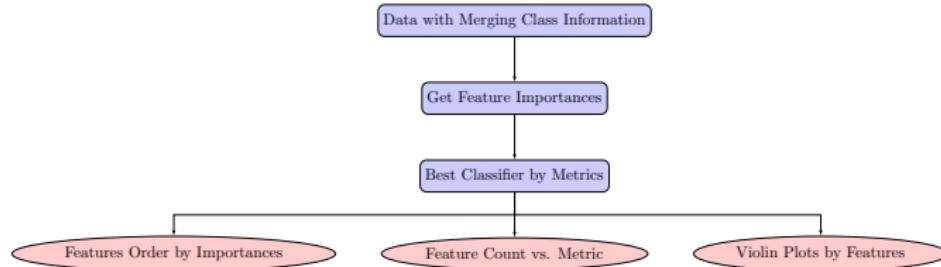


Figure: Random Forest Classifier with Merging Workflow

**DADA2 + SILVA** gives the best result with balanced accuracy (0.934 with 15 taxa).

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

Table: Features Order by Importances

Order	Taxonomy (Genus [Species])
0	<i>Actinomyces</i>
1	<i>Treponema denticola</i>
2	<i>Porphyromonas gingivalis</i>
3	<i>Prevotella intermedia</i>
4	<i>Filifactor alocis</i>
5	<i>Schaalia odontolytica</i>
6	<i>Tannerella forsythia</i>
7	<i>Eubacterium nodatum</i>
8	<i>Corynebacterium durum</i>
9	<i>Oribacterium</i>
...	...

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

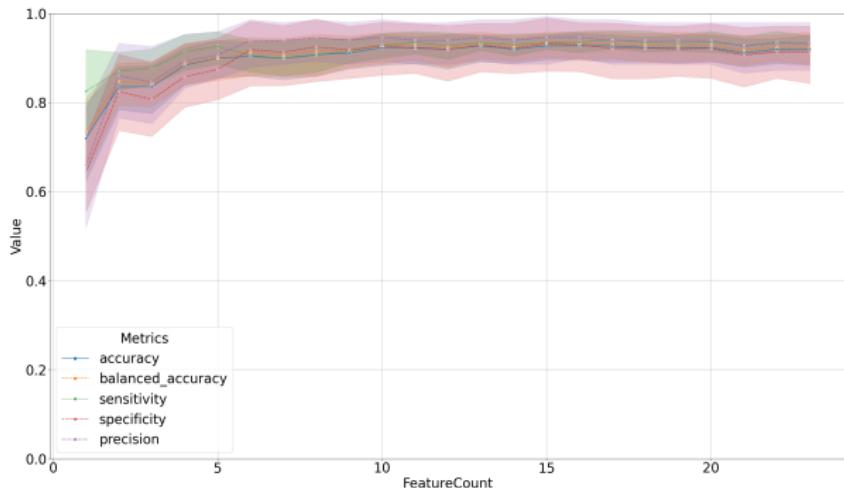
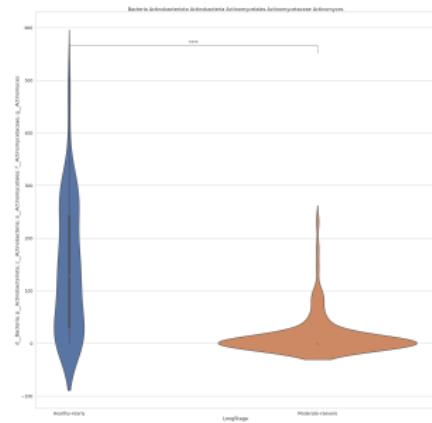
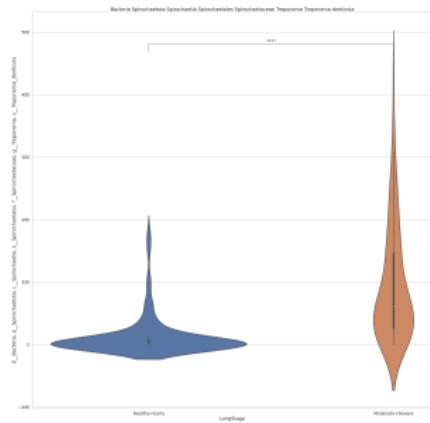


Figure: Metrics by Feature Count

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



(a) *Actinomyces*



(b) *Treponema denticola*

Figure: Violin Plot by Features

# Random Forest Classifier – Only (H + E) I

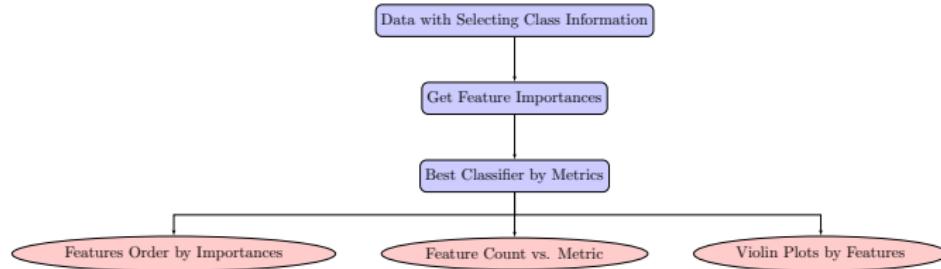


Figure: Random Forest Classifier with Merging Workflow

**DADA2 + HOMD** gives the best result with balanced accuracy (0.771 with 4 taxa).

# Random Forest Classifier – Only (H + E) II

Table: Features Order by Importances

Order	Taxonomy (Genus [Species])
0	<i>Actinomyces</i>
1	<i>Porphyromonas gingivalis</i>
2	<i>Actinomyces graevenitzii</i>
3	<i>Filifactor alocis</i>

# Random Forest Classifier – Only (H + E) III

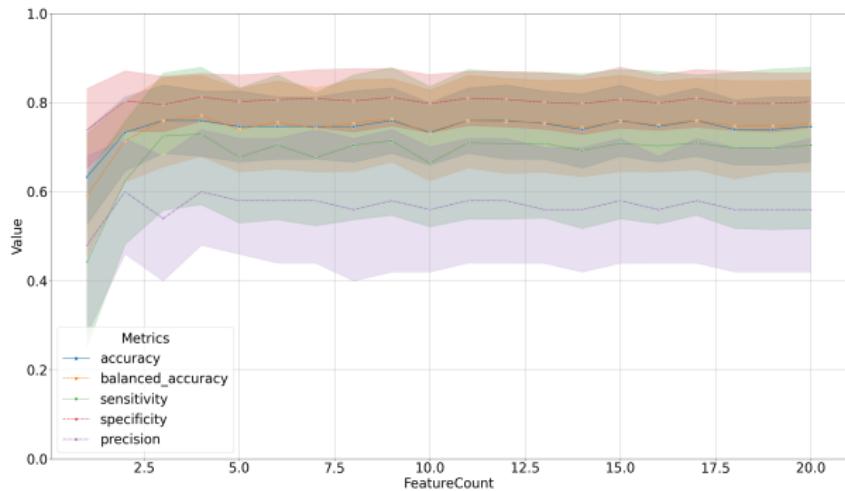
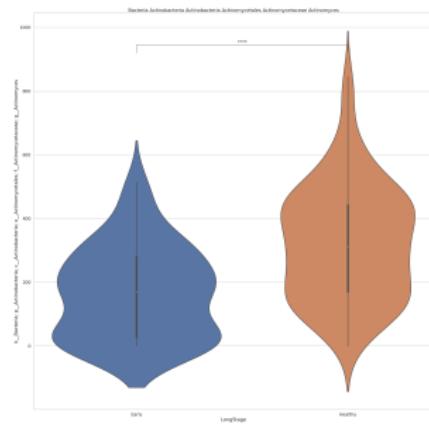
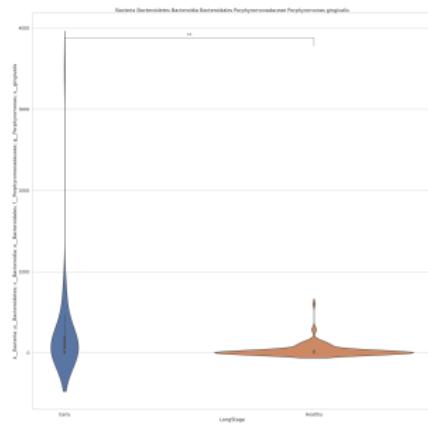


Figure: Metrics by Feature Count

# Random Forest Classifier – Only (H + E) IV



(a) *Actinomyces*



(b) *Porphyromonas gingivalis*

Figure: Violin Plot by Features

## Discussion

# *Porphyromonas* Genus

# *Actinomyces* Genus

# *Treponema* Genus

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