Periodontitis

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Overview

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)

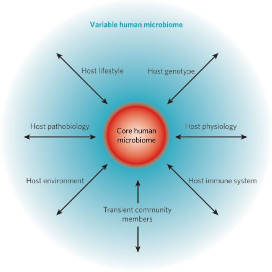


Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

rRNA

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

Periodontitis (Periodontal disease)

- 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

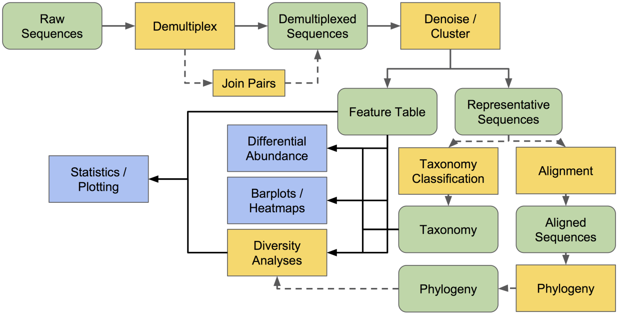


Figure: QIIME2 Workflow (Bolyen et al., 2019, 2018)

Denoising techniques

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

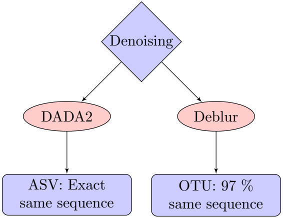


Figure: Denoising Techniques

Taxonomy Classification

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

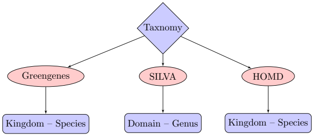


Figure: Taxonomy Classification

"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.

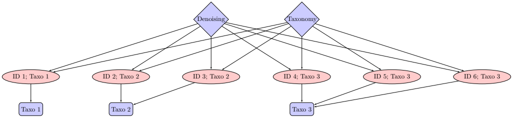


Figure: Example Diagram for Merging Denosing and Taxonomy Classification

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**

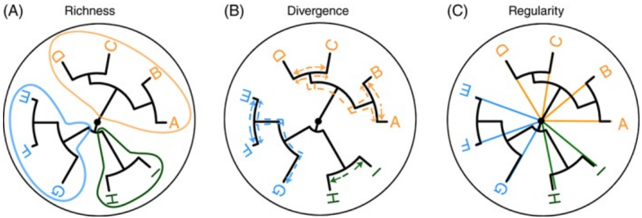


Figure: Three Dimensions of Phylogenetic Information (Tucker et al., 2017)

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 priorities 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

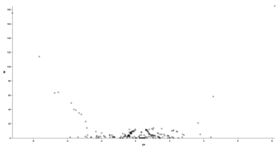


Figure: Example ANCOM Volcano Plot (Bolyen et al., 2019, 2018)

- 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)

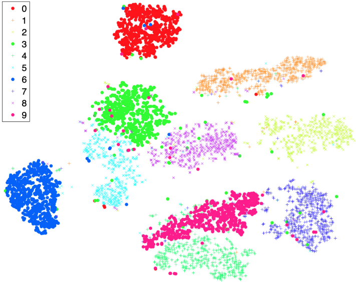


Figure: Visualization by t-SNE (Maaten & Hinton, 2008)

Classification I

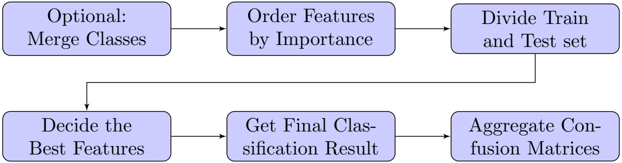


Figure: Workflow of Classification

Classification Metrics:

- Accuracy
- Balanced Accuracy
- Sensitivity

Classification II

- Specificity
- Precision

Classification III

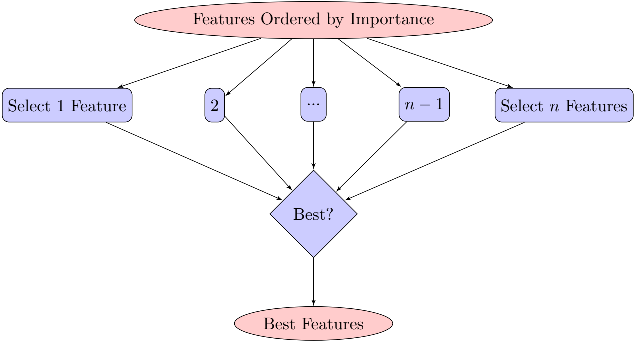
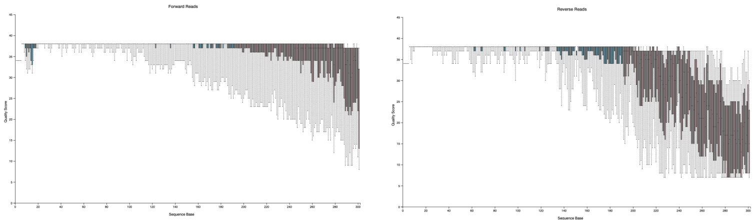


Figure: Deciding the Best Features

Results

Quality Filter



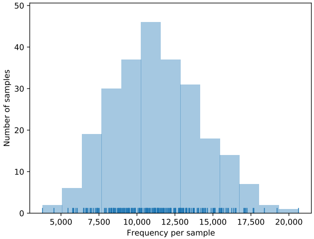
(a) Forward Reads (b) Reverse Reads

Figure: Sequence Quality Plot

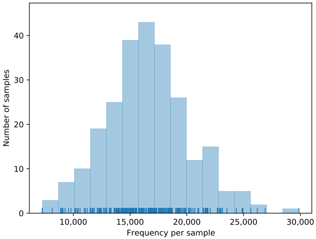
$\therefore$ Maximum Sequence Length $n_{forword} = 300$, $n_{reverse} = 265$

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

Rarefaction



(a) DADA2

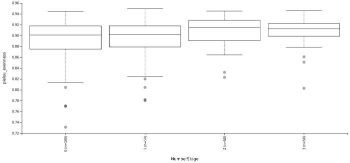


(b) Deblur

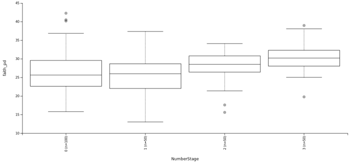
Figure: Frequency per sample

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

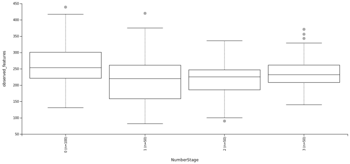
Alpha-diversity I



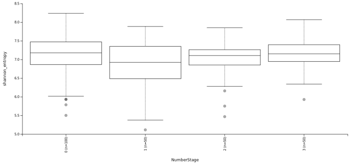
(a) Evenness ($p < 0.01$)



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



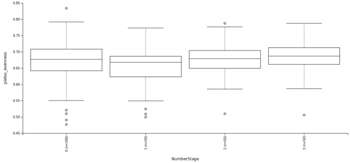
(c) Observed features ($p < 10^{-3}$)



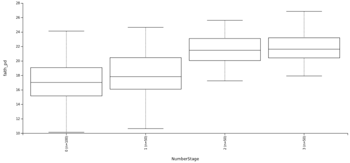
(d) Shannon's diversity ($p > 0.05$)

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

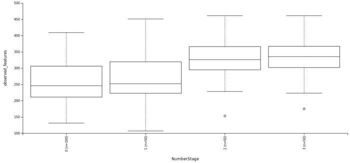
Alpha-diversity II



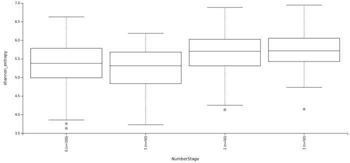
(a) Evenness ($p < 0.05$)



(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

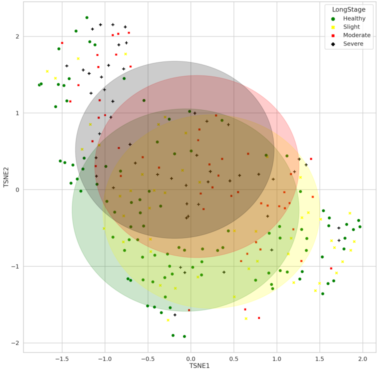


Figure: Bray-Curtis Distance with DADA2

Beta-diversity II

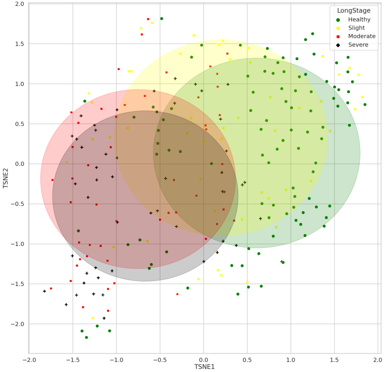


Figure: Jaccard Distance with DADA2

Beta-diversity III

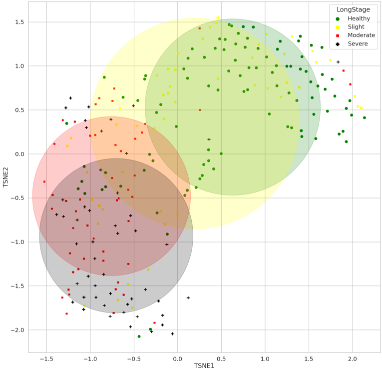


Figure: Unweighted Unifrac Distance with DADA2

Beta-diversity IV

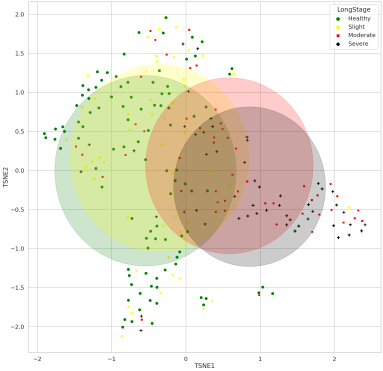


Figure: Weighted Unifrac Distance with DADA2

Beta-diversity V

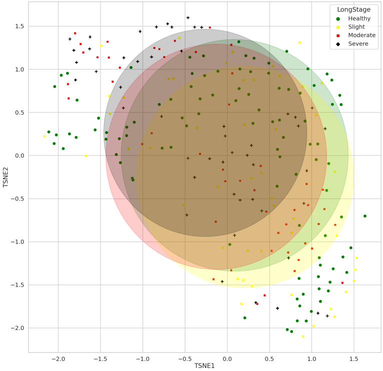


Figure: Bray-Curtis Distance with Deblur

Beta-diversity VI

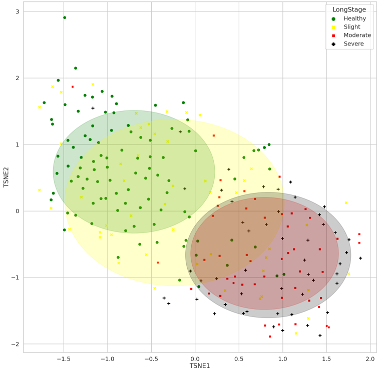


Figure: Jaccard Distance with Deblur

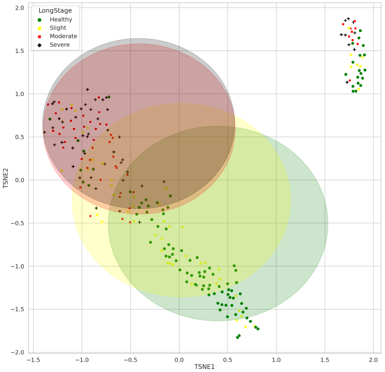


Figure: Unweighted Unifrac Distance with Deblur

Beta-diversity VIII

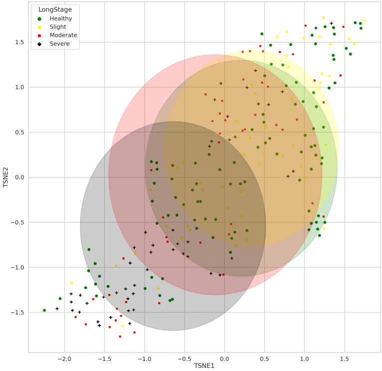
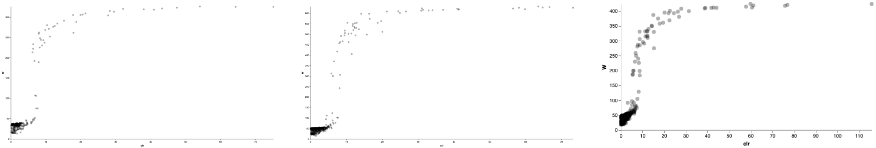


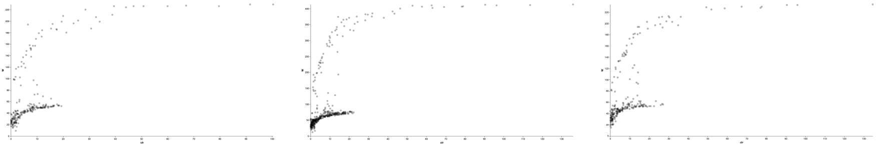
Figure: Weighted Unifrac Distance with Deblur

ANCOM



(a) GG (15 Taxa) (b) SILVA (23 Taxa) (c) HOMD (20 Taxa)

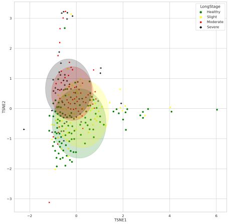
Figure: ANCOM Volcano Plot with DADA2



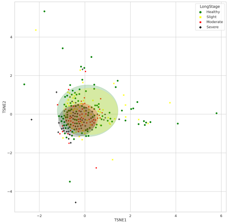
(a) GG (27 Taxa) (b) SILVA (20 Taxa) (c) HOMD (28 Taxa)

Figure: ANCOM Volcano Plot with Deblur

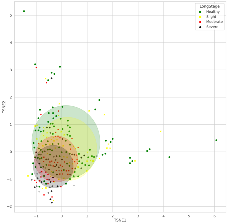
t-SNE with Whole Microbiome I



(a) GG (328 Taxa)



(b) SILVA (633 Taxa)



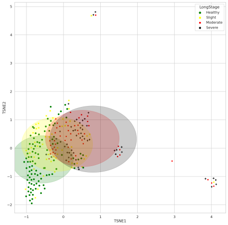
(c) HOMD (425 Taxa)

Figure: t-SNE Plot with Whole Microbiome from DADA2

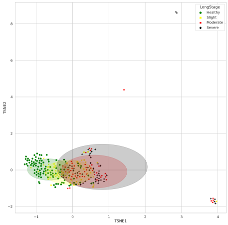
(a) GG (232 Taxa) (b) SILVA (414 Taxa) (c) HOMD (235 Taxa)

Figure: t-SNE Plot with Whole Microbiome from Deblur

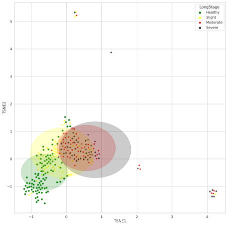
t-SNE with ANCOM Selected I



(a) GG (15 Taxa)



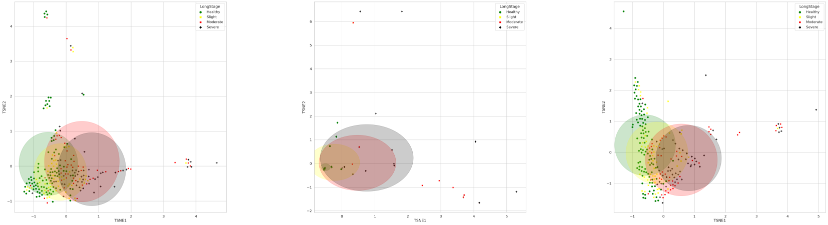
(b) SILVA (23 Taxa)



(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

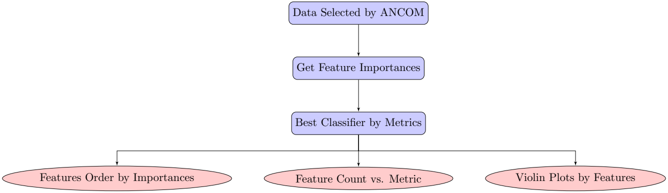


Figure: Random Forest Classifier Workflow

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	*Porphyromonas gingivalis*
1	*Actinomyces*
2	*Pasteurellaceae*
3	*Veillonella denticariosi*
4	*Oribacterium sinus*
5	*Peptostreptococcus anaerobius*
6	*Prevotella nanceiensis*
7	*Treponema*
8	*Parvimonas sp. HMT 393*
9	*Filifactor alocis*
...	...

Random Forest Classifier – Every Classes III

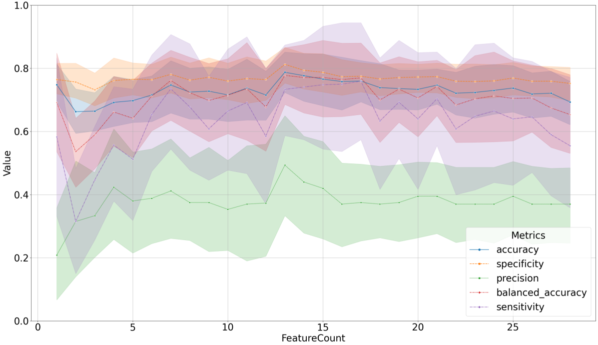
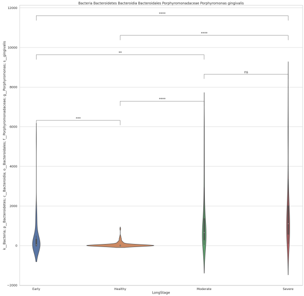
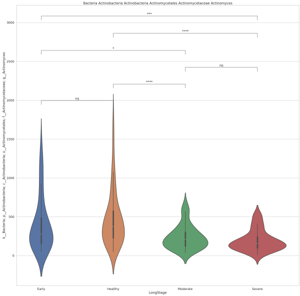


Figure: Metrics by Feature Count

Random Forest Classifier – Every Classes IV



(a) *Porphyromonas gingivalis*



(b) *Actinomyces*

Figure: Violin Plot by Features

Random Forest Classifier – Merge (Healthy+Early) I

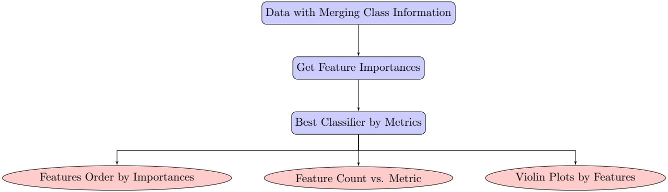


Figure: Random Forest Classifier with Merging Workflow

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	*Porphyromonas gingivalis*
1	*Actinomyces*
2	*Prevotella intermedia*
3	*Filifactor alocis*
4	*Treponema sp. HMT 260*
5	*Campylobacter showae*
6	*Porphyromonas sp. HMT 285*
7	*Peptostreptococcaceae XIG-6 nodatum*
8	*Prevotella sp. HMT 304*
9	*Actinomyces graevenitzii*
10	*Tannerella forsythia*

Random Forest Classifier – Merge (Healthy+Early) III

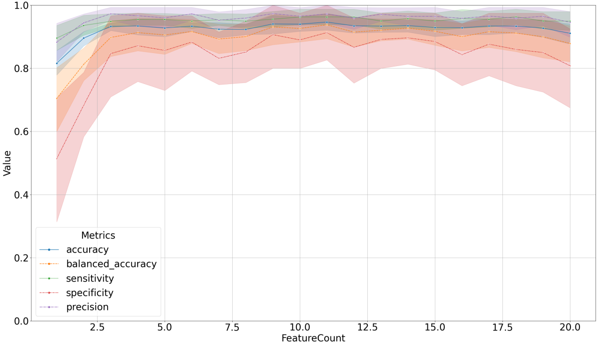
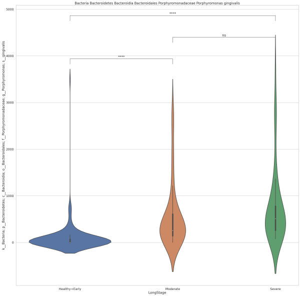
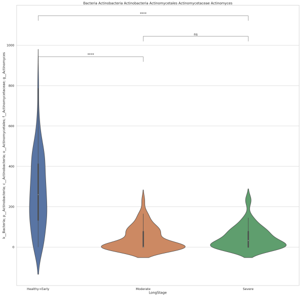


Figure: Metrics by Feature Count

Random Forest Classifier – Merge (Healthy+Early) IV



(a) *Porphyromonas gingivalis*



(b) *Actinomyces*

Figure: Violin Plot by Features

Random Forest Classifier – Merge (Moderate + Severe) I

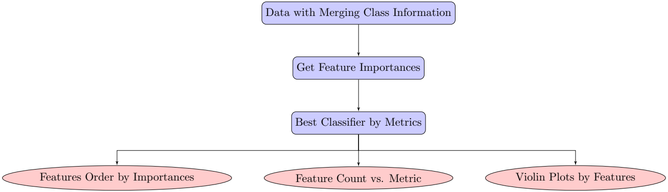


Figure: Random Forest Classifier with Merging Workflow

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	*Porphyromonas gingivalis*
1	*Actinomyces*
2	*Pasteurellaceae*
3	*Veillonella denticariosi*
4	*Oribacterium sinus*
5	*Peptostreptococcus anaerobius*
6	*Treponema*
7	*Prevotella nanceiensis*
8	*Porphyromonas sp. HMT 285*
9	*Parvimonas sp. HMT 393*
...	...

Random Forest Classifier – Merge (Moderate + Severe) III

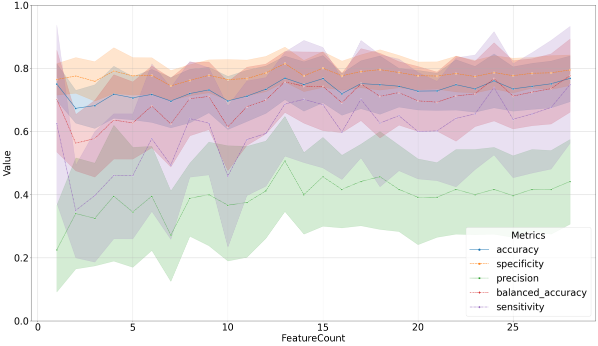
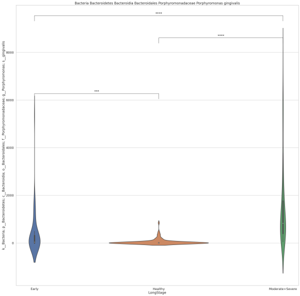
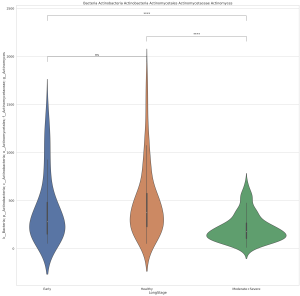


Figure: Metrics by Feature Count

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

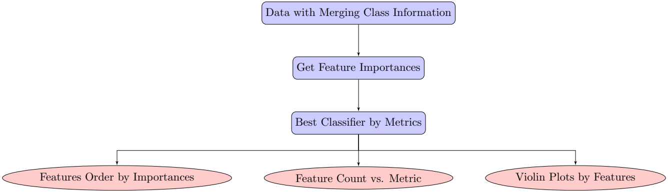


Figure: Random Forest Classifier with Merging Workflow

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	*Actinomyces*
1	*Treponema denticola*
2	*Porphyromonas gingivalis*
3	*Prevotella intermedia*
4	*Filifactor alocis*
5	*Schaalia odontolytica*
6	*Tannerella forsythia*
7	*Eubacterium nodatum*
8	*Corynebacterium durum*
9	*Oribacterium*
...	...

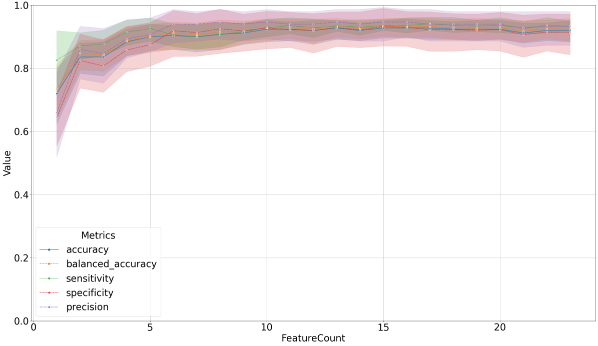
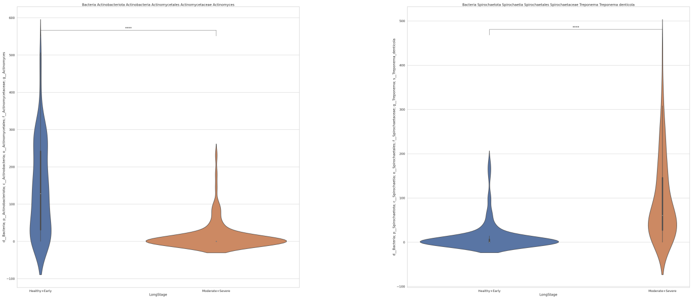


Figure: Metrics by Feature Count

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

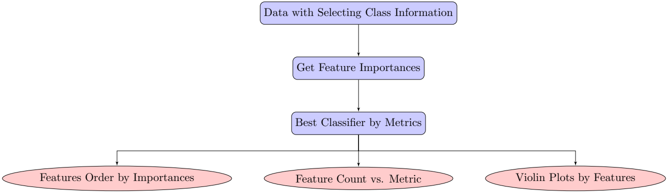


Figure: Random Forest Classifier with Merging Workflow

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	*Actinomyces*
1	*Porphyromonas gingivalis*
2	*Actinomyces graevenitzii*
3	*Filifactor alocis*

Random Forest Classifier – Only (H + E) III

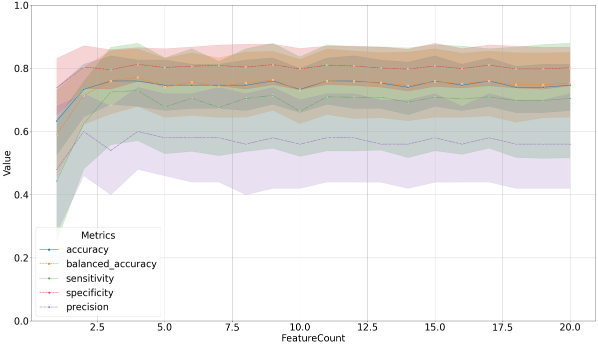
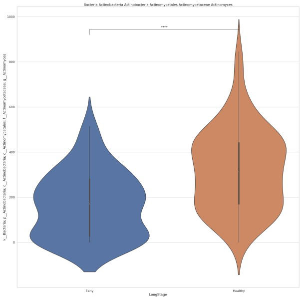
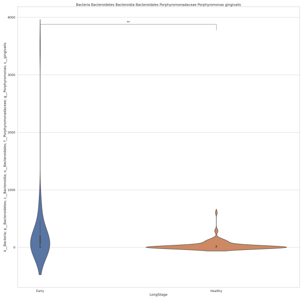


Figure: Metrics by Feature Count



(a) *Actinomyces*



(b) *Porphyromonas gingivalis*

Figure: Violin Plot by Features

Discussion

Porphyromonas Genus

Actinomyces Genus

Treponema Genus

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