

Periodontitis

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1 Introduction

1.1 Microbiome

Microbiome is consist of microbiota, the micro-organisms which live inside and on humans (Turnbaugh et al., 2007). Microbiome is also about 10^{13} micro-organisms whose which collective genome (Gill et al., 2006).

1.2 Ribosomal RNA

Ribosomal RNA (rRNA) is well-known as a key to phylogeny (Olsen & Woese, 1993).

1.3 16S rRNA Gene Sequencing

1.4 Periodontitis

Periodontitis is an inflammatory conditions which effecting periodontium, tissues which surround and support teeth. Major components of periodontitis are clinical attachment loss and bone loss (Flemmig, 1999). Previous study found risk factors of periodontitis such as smoking, diabetes, genetic factors and host response (Van Dyke & Dave, 2005).

2 Materials

2.1 16S rRNA Gene Sequencing

- 100 Healthy samples
- 50 Chronic Early Periodontitis Sample
- 50 Chronic Moderate Periodontitis Sample
- 50 Chronic Severe Periodontitis Sample

3 Methods

3.1 QIIME2 Workflow

QIIME2 is a capable, expandable and distributed microbiome analysis package with transparent analysis (Bolyen et al., 2019, 2018). A theoretic overview of QIIME2 workflow is shown as figure 2.

3.1.1 Denoising techniques

There are two denoising techniques provided by QIIME2: DADA2 (Callahan et al., 2016) and Deblur (Amir et al., 2017). Major difference between DADA2 and Deblur, as shown as figure 3, is a strategy, the strategy used to divide as different species. DADA2 uses amplicon sequence variants (ASVs), strictly divides sequences even one-base mismatch. However, Deblur uses operational taxonomic units (OTUs), considers as same sequence when sequences are 97 % or more matched.

3.1.2 Taxonomy Classification

There are three taxonomy classification databases: Greengenes (GG) (DeSantis et al., 2006), SILVA (Pruesse et al., 2007) and Human Oral Microbiome Database (HOMD) (Chen et al., 2010). Major difference among these databases is resolution. Resolution of GG and HOMD is from kingdom to species; however, resolution of SILVA is from domain to genus. Previous research have found that a higher accuracy at taxonomic levels above genus level; but accuracy drops at species level (Gihawi et al., 2019).

3.1.3 Merging Denoising and Taxonomy Classification

After denosing and taxonomy classification steps, some different IDs (ASVs or OTUs) have been identified as same taxonomy. In that case, the different IDs will be merged into one taxonomy (Figure 5).

3.1.4 Rarefaction

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

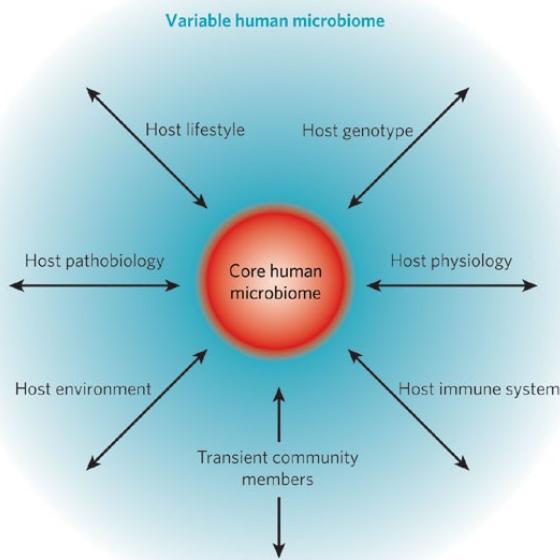


Figure 1: Concept of a Core Human Microbiome (Turnbaugh et al., 2007)

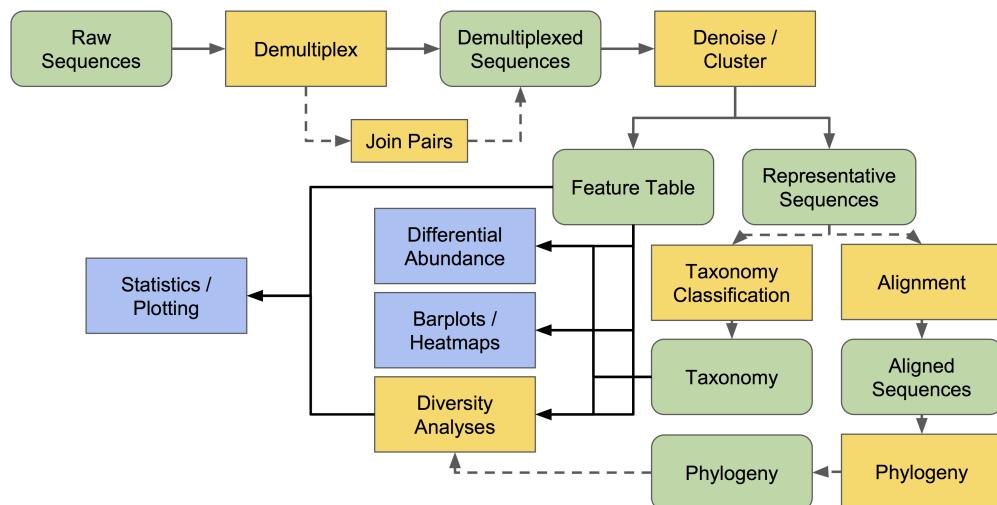


Figure 2: A Theoretic Overview of QIIME2 Workflow (Bolyen et al., 2019, 2018)

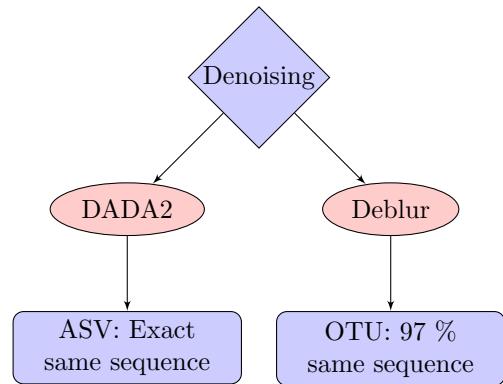


Figure 3: Denoising Techniques which provided by QIIME2

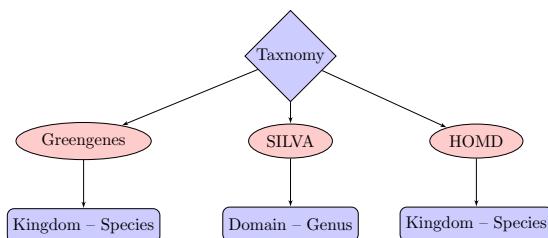


Figure 4: Taxonomy Classification which provided by QIIME2

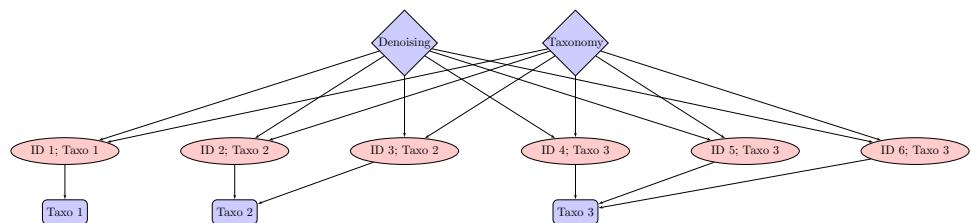


Figure 5: Example Diagram for Merging Denoising and Taxonomy Classification

3.1.5 Alpha-diversity

Alpha-diversity is a metric which shows the richness of taxa at a single community. There are four alpha-diversity indices which provided from QIIME2:

- Evenness index (Pielou, 1966).
- Faith's phylogenetic diversity (Faith PD) (Faith, 1992).
- Observed features.
- Shannon's diversity index (Shannon, 1948).

Evenness index shows a measurement of diversity in different type at community (Pielou, 1966); Faith's phylogenetic diversity, however, indicates a qualitative measurement of community richness which priorities for species conservation which incorporates with taxic diversity (Faith, 1992). Observed features, as its name, is a number of observed features in microbiome. Moreover, Shannon's diversity index means a significant aspect of community richness (Shannon, 1948).

3.1.6 Beta-diversity

Beta-diversity is a metric which indicates the taxonomic differentiation between multiple communities. There are four beta-diversity indices which provided from QIIME2:

- Bray-Curtis distance index (Sørensen, 1948).
- Jaccard distance index (Jaccard, 1912).
- Unweighted UniFrac distance index (McDonald et al., 2018).
- Weighted UniFrac distance index (McDonald et al., 2018).

Bray-Curtis distance index shows a quantitative measurement of community dissimilarity (Sørensen, 1948); Jaccard distance index, however, indicates a measurement of local distribution among communities. UniFrac distance indices reveal measurements of phylogenetic distances (McDonald et al., 2018). Difference between unweighted UniFrac distance index and weighted UniFrac distance index is a qualitative and a quantitative, respectively.

3.1.7 ANCOM

ANCOM (Analysis of composition of microbiomes) can be used for analyzing the composition of microbiome in multiple populations (Mandal et al., 2015). Example ANCOM volcano plot is shows as figure 6. In figure 6, two metrics are clearly shown: clr and W. clr stands for centered log ratio, and W is a count of the number of sub-hypothesis which have passed for given species.

3.2 Python Packages

3.2.1 Pandas

Pandas is a Python package of rich data structures and tools for analyzing with structured data sets (McKinney et al., 2011).

3.2.2 Scikit-learn

Scikit-learn grants state-of-the-art implementation of many machine learning algorithms, while controlling an easy-to-use interface tightly integrated the Python code (Pedregosa et al., 2011).

3.2.3 Matplotlib

Matplotlib is a Python graphics package which used for application development, interactive scripting and publication quality image generation (Barrett, Hunter, Miller, Hsu, & Greenfield, 2005). Matplotlib, also, is designed to create simple plots with a few commands (Hunter, 2007).

3.2.4 Seaborn

Seaborn is a Python data visualization package which based on matplotlib, allows a high-level interface for displaying engaging and descriptive statistical graphics (Waskom & the seaborn development team, 2020).

3.3 t-SNE

t-SNE (t-distributed stochastic neighbor embedding) reveals high-dimensional data a location in two-dimensional map (Maaten & Hinton, 2008). Figure 7 is example of t-SNE with hand-writing digits (Maaten & Hinton, 2008). In figure 7, all 10 digits are grouped into 10 groups clearly; some hand-writings, however, are classified into wrong groups due to their similar shapes, such as 0 and 6.

3.4 Classification

In machine learning, Classification is one of supervised learning which identifies a class of a new observation, depends on given information which consist of training observations and their classes.

In this study, classification will be carried out as figure 8; and the third step in figure 8 is demonstrated in minute detail as figure 9. Note that the first step in figure 8 is optional: due to tables herein-after, such as table3, show that no statistically significant differences between healthy samples and early periodontitis samples and between moderate periodontitis samples and severe periodontitis samples.

Moreover, evaluations of classification algorithm are carried out with derivations from confusion matrix (table 1):

- Accuracy (ACC) = $\frac{TP+TN}{TP+TN+FP+FN}$
- Balanced Accuracy (BA) = $\frac{TP}{2 \times (TP+FN)} + \frac{TN}{2 \times (TN+FP)}$
- Sensitivity (SEN) = $\frac{TP}{TP+FN}$
- Specificity (SPE) = $\frac{TN}{TN+FP}$
- Precision (PRE) = $\frac{TP}{TP+FP}$

3.4.1 Random Forest Classification

As figure 8, importance of features have to be derived by classifier. Random Forest classifier (Breiman, 2001) can get this information, and is used frequently by researchers. Hence, Random Forest classifier will be carried out with every class (Figure 10) or with merged classes (Figure 11).

4 Results

4.1 Quality Filter

Longer sequences have more fallen sequence quality than shorter. Thus, sequences which longer than threshold should be trimmed out due to their low quality. However, gold-standard strategy for deciding the threshold does not exist; the threshold is set as longest sequence length which have half of sequences have greater than 30 quality score. Hence, sequence quality plot is shown as figure 12; trimmed length in forward reads is 300, and trimmed length in reverse reads is 265.

4.2 Rarefaction

Sampling depth should be decided for rarefaction. Gold-standard method for determining sampling depth is minimum frequency in the samples. Hence, sampling depth with DADA2 is 3,786 (Figure 13), and sampling depth with Deblur is 7,253 (Figure 14).

4.3 Alpha-diversity

Alpha-diversity analysis with DADA2 was done: Evenness index (Table 3 and Figure 15), Faith PD (Table 4 and Figure 16), observed feature index (Table 5 and Figure 17) and Shannon's diversity index (Table 6 and Figure 18). Also, alpha-diversity analysis with DADA2 was done: Evenness index (Table 8 and Figure 19), Faith PD (Table 9 and Figure 20), observed feature index (Table 10 and Figure 21) and Shannon's diversity index (Table 11 and Figure 22). Moreover, Kruskal-Wallis tests among all groups are shown as table 2 (with DADA2) and table 7 (with Deblur).

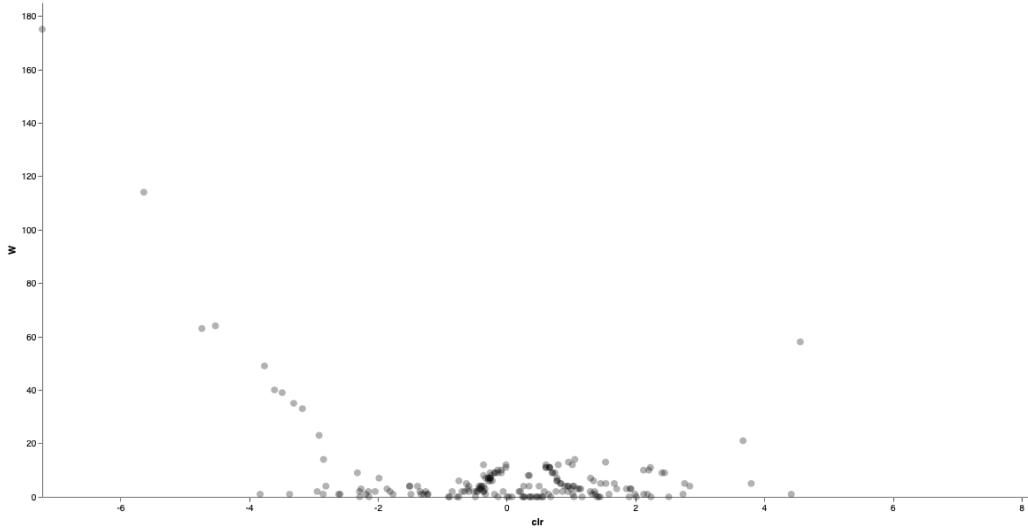


Figure 6: Example ANCOM Volcano Plot which Provided by QIIME2 (Bolyen et al., 2019, 2018)

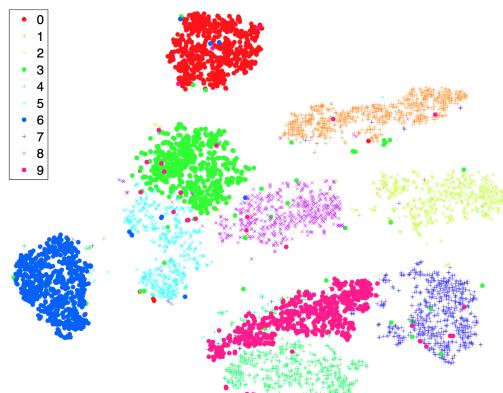


Figure 7: Visualization by t-SNE (Maaten & Hinton, 2008)

Table 1: Confusion Matrix

| | | Actual Class | |
|-----------------|----------|---------------------|---------------------|
| | | Positive | Negative |
| Predicted Class | Positive | True Positive (TP) | False Positive (FP) |
| | Negative | False Negative (FN) | True Negative (TN) |

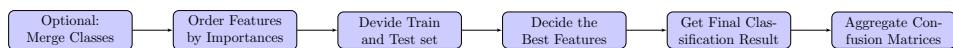


Figure 8: Workflow of Classification

Table 2: Kruskal-Wallis Tests among All Group with DADA2

| Alpha-Diversity | H | p-value |
|--------------------|--------------------|-----------------------|
| Evenness | 12.185457848605665 | 0.006774123738087294 |
| Faith PD | 33.42272318725111 | 2.6227945981005624e-7 |
| Observed Features | 21.019370066584198 | 0.0001043055436502384 |
| Shnnon's Diversity | 7.311350438247132 | 0.06260902704190516 |

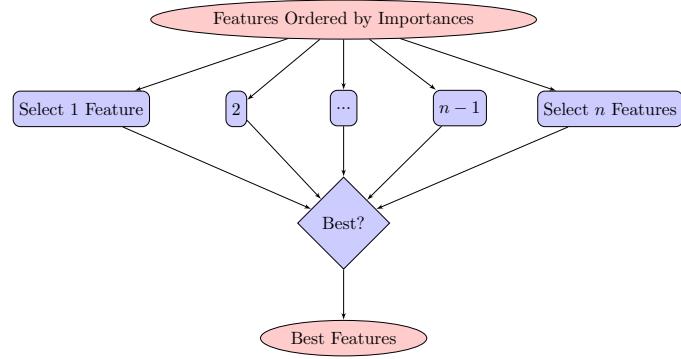


Figure 9: Deciding the Best Features

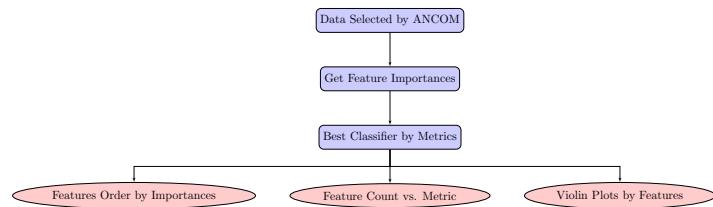


Figure 10: Random Forest Classifier Workflow

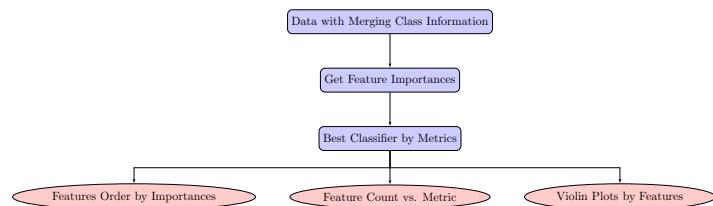


Figure 11: Random Forest Classifier Workflow with Merging

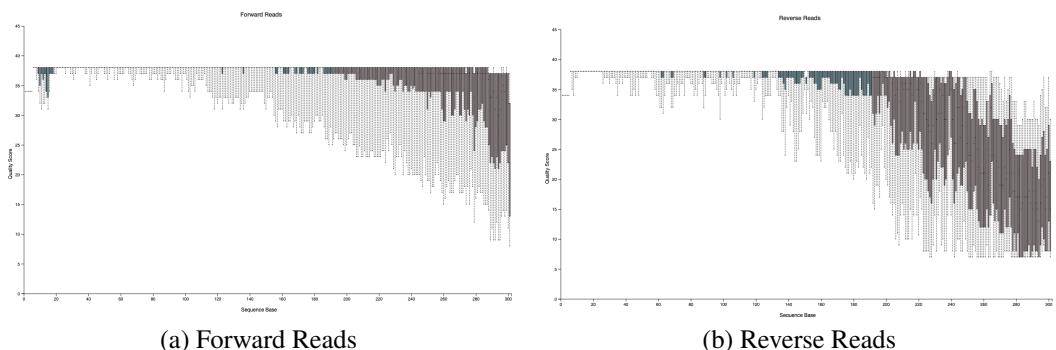


Figure 12: Sequence Quality Plot

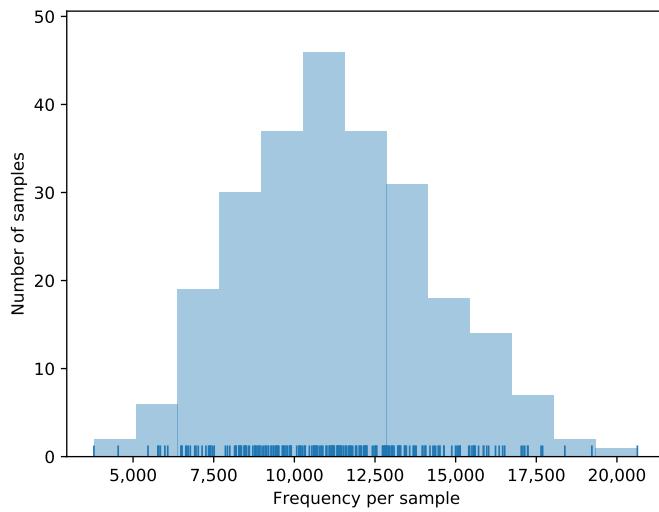


Figure 13: Frequency and Number per Sample by DADA2

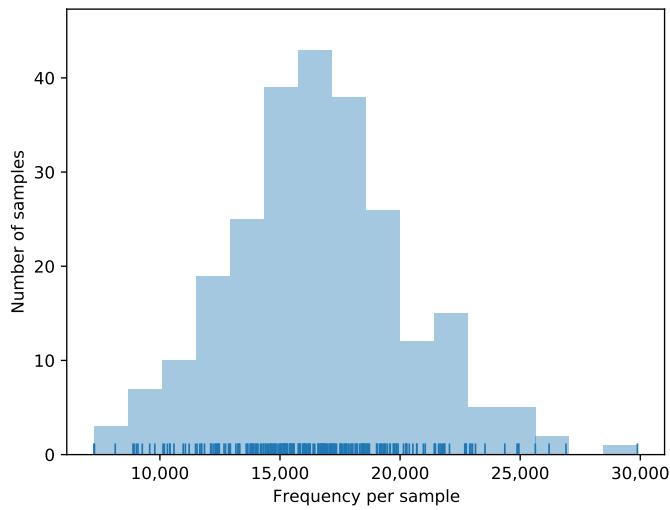


Figure 14: Frequency and Number per Sample by Deblur

Table 3: Kruskal-Wallis Tests from Evenness Index with DADA2

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|----------------------|----------------------|---------------------|
| Early (n=50) | Healthy (n=100) | 0.003576158940404639 | 0.9523141335184352 | 0.9523141335184352 |
| Early (n=50) | Moderate (n=50) | 5.112902970297 | 0.02374855135702787 | 0.03562282703554181 |
| Early (n=50) | Severe (n=50) | 5.206859405940577 | 0.022497939047433364 | 0.03562282703554181 |
| Healthy (n=100) | Moderate (n=50) | 6.591830463576116 | 0.01024477815032801 | 0.03073433445098403 |
| Healthy (n=100) | Severe (n=50) | 6.756619867549659 | 0.0093400517403089 | 0.03073433445098403 |
| Moderate (n=50) | Severe (n=50) | 0.01216633663364064 | 0.9121705706341857 | 0.9523141335184352 |

Table 4: Kruskal-Wallis Tests from Faith PD Index with DADA2

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|--------------------|-----------------------|-----------------------|
| Early (n=50) | Healthy (n=100) | 0.3434543046357703 | 0.557842085850555 | 0.557842085850555 |
| Early (n=50) | Moderate (n=50) | 7.833790099009889 | 0.005127846488653557 | 0.0076917697329803355 |
| Early (n=50) | Severe (n=50) | 19.832839603960394 | 8.451807369366e-06 | 2.5355422108098e-05 |
| Healthy (n=100) | Moderate (n=50) | 8.964254304635801 | 0.0027531304578610103 | 0.005506260915722021 |
| Healthy (n=100) | Severe (n=50) | 24.32056688741727 | 8.156352492752821e-07 | 4.893811495651693e-06 |
| Moderate (n=50) | Severe (n=50) | 5.461592079207946 | 0.019438927334967618 | 0.02332671280196114 |

Table 5: Kruskal-Wallis Tests from Observed Features Index with DADA2

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|---------------------|-----------------------|-----------------------|
| Early (n=50) | Healthy (n=100) | 9.559750209810552 | 0.001988901703187571 | 0.005966705109562713 |
| Early (n=50) | Moderate (n=50) | 0.01069480203811357 | 0.9176330712208788 | 0.9176330712208788 |
| Early (n=50) | Severe (n=50) | 1.8918489487993617 | 0.1689935259025544 | 0.20279223108306527 |
| Healthy (n=100) | Moderate (n=50) | 16.280824652808626 | 5.461383546704547e-05 | 0.0003276830128022728 |
| Healthy (n=100) | Severe (n=50) | 6.9139163882453465 | 0.008552745576573654 | 0.017105491153147308 |
| Moderate (n=50) | Severe (n=50) | 2.1161415616917054 | 0.145753334857958 | 0.20279223108306527 |

Table 6: Kruskal-Wallis Tests from Shannon's Diversity Index with DADA2

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|---------------------|----------------------|---------------------|
| Early (n=50) | Healthy (n=100) | 5.291586754966886 | 0.021428686619934936 | 0.11394854365524665 |
| Early (n=50) | Moderate (n=50) | 1.3095920792079028 | 0.2524685249140654 | 0.3029622298968785 |
| Early (n=50) | Severe (n=50) | 4.305790099009869 | 0.037982847885082216 | 0.11394854365524665 |
| Healthy (n=100) | Moderate (n=50) | 2.223194701986756 | 0.13595148461788642 | 0.27190296923577284 |
| Healthy (n=100) | Severe (n=50) | 0.06109668874171348 | 0.8047709009969876 | 0.8047709009969876 |
| Moderate (n=50) | Severe (n=50) | 1.3573544554455452 | 0.2439965042398798 | 0.3029622298968785 |

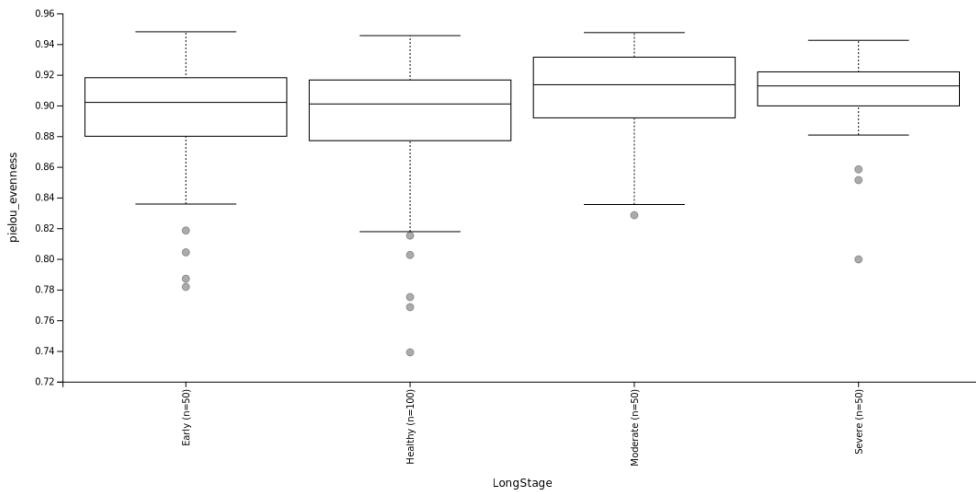


Figure 15: Evenness Index from DADA2

Table 7: Kruskal-Wallis Tests among All Group with Deblur

| Alpha-Diversity | H | p-value |
|--------------------|--------------------|-------------------------|
| Evenness | 9.242885737051779 | 0.026229960554059864 |
| Faith PD | 87.83605864541846 | 6.386769940789011e-19 |
| Observed Features | 59.59138364929631 | 7.186872791755095e-13 |
| Shnnon's Diversity | 24.823351075697246 | 0.000016810908296023026 |

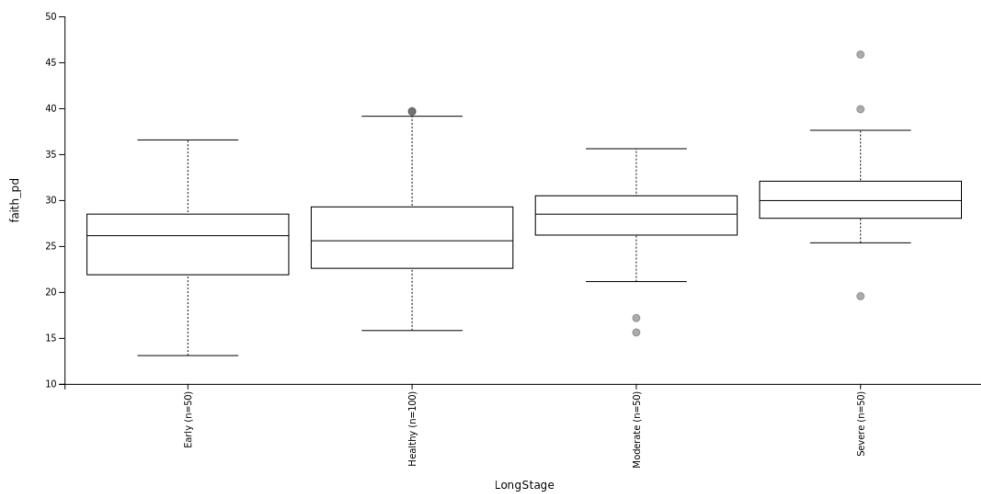


Figure 16: Faith PD Index from DADA2

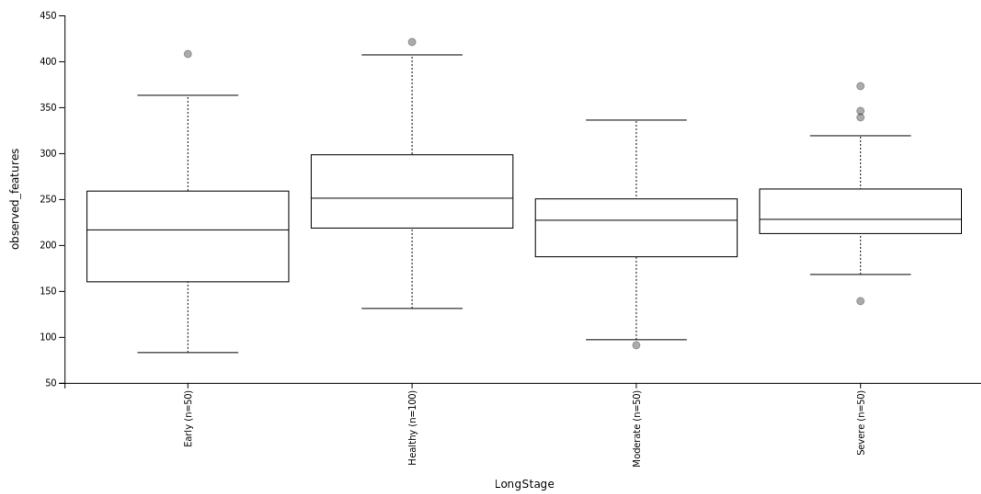


Figure 17: Observed Features Index from DADA2

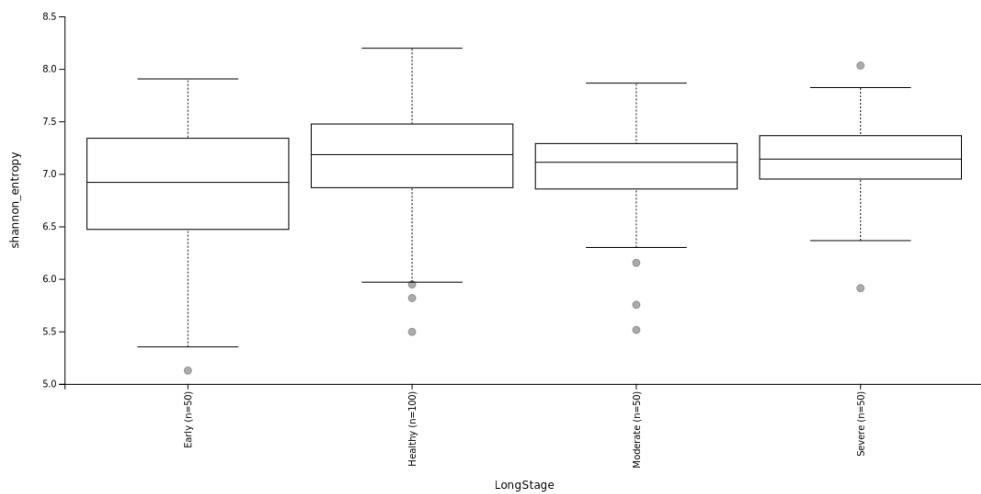


Figure 18: Shannon's Diversity Index from DADA2

Table 8: Kruskal-Wallis Tests from Evenness Index with Deblur

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|--------------------|----------------------|----------------------|
| Early (n=50) | Healthy (n=100) | 2.884386754966897 | 0.0894420544121846 | 0.15829564582637523 |
| Early (n=50) | Moderate (n=50) | 4.392047524752456 | 0.03610692636685824 | 0.10832077910057474 |
| Early (n=50) | Severe (n=50) | 8.828245544554477 | 0.002966034055389358 | 0.017796204332336148 |
| Healthy (n=100) | Moderate (n=50) | 0.6168317880794802 | 0.43222705558822094 | 0.43597874518665736 |
| Healthy (n=100) | Severe (n=50) | 2.6199099337748066 | 0.1055304305509168 | 0.15829564582637523 |
| Moderate (n=50) | Severe (n=50) | 0.6068435643564385 | 0.43597874518665736 | 0.43597874518665736 |

Table 9: Kruskal-Wallis Tests from Faith PD Index with Deblur

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|----------------------|-----------------------|------------------------|
| Early (n=50) | Healthy (n=100) | 2.7110304635762077 | 0.09965659889456922 | 0.11958791867348306 |
| Early (n=50) | Moderate (n=50) | 26.80400792079206 | 2.251698564500841e-07 | 3.3775478467512613e-07 |
| Early (n=50) | Severe (n=50) | 29.06252673267329 | 7.007948881210323e-08 | 1.4015897762420645e-07 |
| Healthy (n=100) | Moderate (n=50) | 51.153949668874134 | 8.539868055189094e-13 | 2.5619604165567283e-12 |
| Healthy (n=100) | Severe (n=50) | 54.86883178807949 | 1.288482355374052e-13 | 7.730894132244311e-13 |
| Moderate (n=50) | Severe (n=50) | 0.005750495049483106 | 0.9395527422741722 | 0.9395527422741722 |

Table 10: Kruskal-Wallis Tests from Observed Features Index with Deblur

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|----------------------|------------------------|------------------------|
| Early (n=50) | Healthy (n=100) | 0.4675226919952207 | 0.49412905906624816 | 0.5929548708794977 |
| Early (n=50) | Moderate (n=50) | 18.684815977243918 | 1.542055834477253e-05 | 2.31308375171588e-05 |
| Early (n=50) | Severe (n=50) | 20.703272962949605 | 5.362426456004328e-06 | 1.0724852912008657e-05 |
| Healthy (n=100) | Moderate (n=50) | 35.26606516292951 | 2.875998708064018e-09 | 8.627996124192055e-09 |
| Healthy (n=100) | Severe (n=50) | 37.015293460828644 | 1.1720632904898772e-09 | 7.032379742939263e-09 |
| Moderate (n=50) | Severe (n=50) | 0.003849966992737873 | 0.9505245257136643 | 0.9505245257136643 |

Table 11: Kruskal-Wallis Tests from Shannon's Diversity Index with Deblur

| Group 1 | Group 2 | H | p-value | q-value |
|-----------------|-----------------|---------------------|------------------------|-----------------------|
| Early (n=50) | Healthy (n=100) | 0.3867973509933357 | 0.5339876723058008 | 0.6407852067669609 |
| Early (n=50) | Moderate (n=50) | 10.767968316831627 | 0.0010327180791227218 | 0.0020654361582454436 |
| Early (n=50) | Severe (n=50) | 14.428562376237608 | 0.00014557751137778065 | 0.000627545643904027 |
| Healthy (n=100) | Moderate (n=50) | 10.172185430463571 | 0.0014257517732722547 | 0.002138627659908382 |
| Healthy (n=100) | Severe (n=50) | 13.746754966887409 | 0.0002091818813013423 | 0.000627545643904027 |
| Moderate (n=50) | Severe (n=50) | 0.15987326732670226 | 0.6892732232396639 | 0.6892732232396639 |

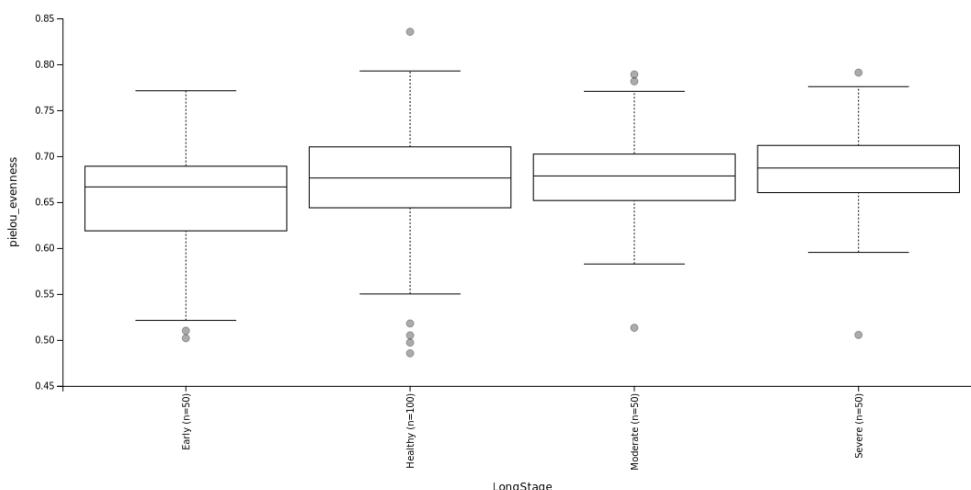


Figure 19: Evenness Index from Deblur

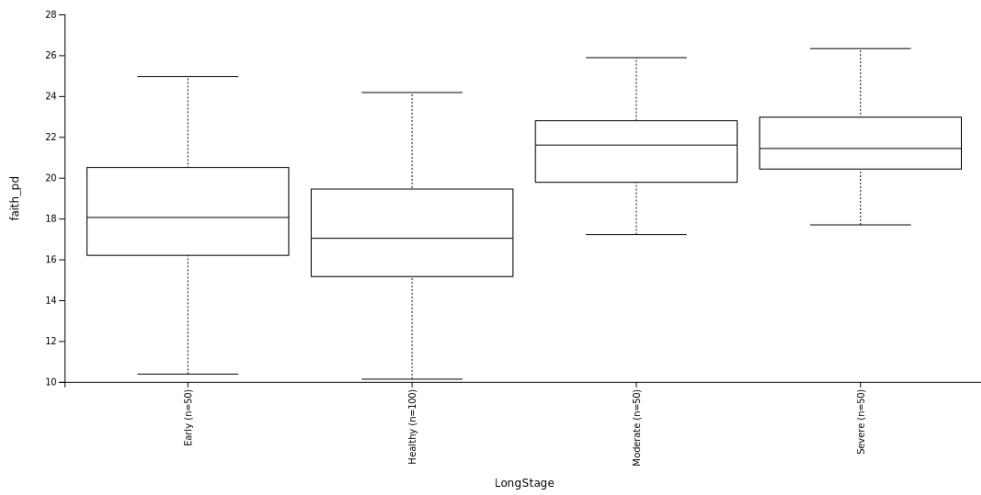


Figure 20: Faith PD Index from Deblur

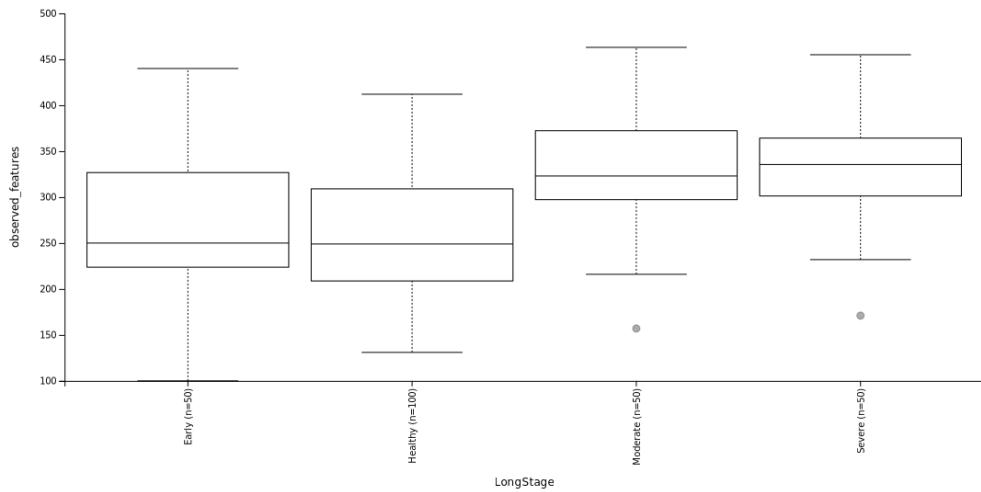


Figure 21: Observed Features Index from Deblur

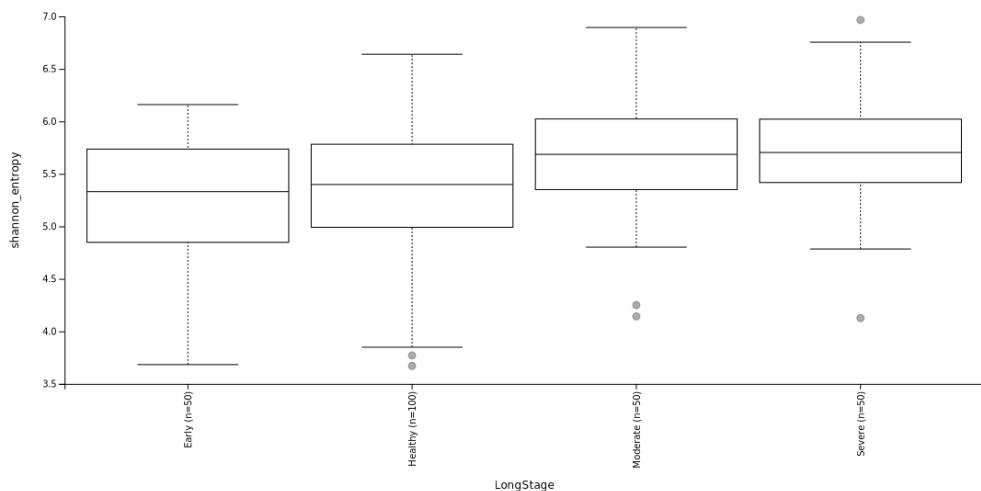


Figure 22: Shannon's Diversity Index from Deblur

4.4 Beta-diversity

Beta-diversity analysis with DADA2 was done: Bray-Curtis distance (Table 12 and Figure 24), Jaccard distance (Table 13 and Figure 26), unweighted UniFrac distance (Table 14 and Figure 28) and weighted UniFrac distance (Table 15 and Figure 28). Also, beta-diversity analysis with Deblur was done: Bray-Curtis distance (Table 16 and Figure 32), Jaccard distance (Table 17 and Figure 34), unweighted UniFrac distance (Table 18 and Figure 36) and weighted UniFrac distance (Table 19 and Figure 36).

4.5 ANCOM

Statistically significant different taxa and volcano plots by ANCOM were derived: DADA2 and GG (Table 20 and Figure 39), DADA2 and SILVA (Table 21 and Figure 40), DADA2 and HOMD (Table 22 and Figure 41), Deblur and GG (Table 23 and Figure 42), Deblur and SILVA (Table 24 and Figure 43) and Deblur and HOMD (Table 25 and Figure 44).

4.6 t-SNE Plot with Whole Microbiome

As mentioned herein-before, t-SNE is a technique which reduce multi-dimensional data into two-dimension. Whole microbiome data are multi-dimensional data, which have *circa* 600 columns, so the data should be reduced their dimension for readability. Hence, by the grace of t-SNE, the microbiome data have been deflated their dimension: 328 taxa from DADA2 and GG (Figure 45), 633 taxa from DADA2 and SILVA (Figure 46), 425 taxa from DADA2 and HOMD (Figure 47), 232 taxa from Deblur and GG (Figure 48), 414 taxa from Deblur and SILVA (Figure 49) and 235 taxa from Deblur and HOMD (Figure 50).

4.7 t-SNE Plot with ANCOM Selected Microbiome Data

As whole microbiome data, ANCOM selected microbiome data are also multi-dimensional data, even though their columns are selected by ANCOM. Hence, with t-SNE, ANCOM selected microbiome data have also been deflated their dimension: 15 taxa (as Table 20) from DADA2 and GG (Figure 51), 23 taxa (as Table 21) from DADA2 and SILVA (Figure 52), 20 taxa (as Table 22) from DADA2 and HOMD (Figure 53), 27 taxa (as Table 23) from Deblur and GG (Figure 48), 20 taxa (as Table 24) from Deblur and SILVA (Figure 55) and 28 taxa (as Table 25) from Deblur and HOMD (Figure 56).

4.8 Random Forest Classifier with Every Class

As figure 3 and figure 4, there are six combinations. Thus, classification algorithm is carried out on these six combinations (Figure 10). Among these six combinations, Deblur and HOMD pair gives the best result in terms of balanced accuracy. Features which are ordered by importance are in table 26. Also, five metrics by feature count are shown as figure 57; then, the highest value of balanced accuracy is 0.778 with using 13 taxa. Moreover, violin plots from the two most important taxa, *Porphyromonas gingivalis* and *Actinomyces*, are in figure 58.

4.9 Random Forest Classifier with Merging (Healthy+Early) Classes

As figure 3 and figure 4, there are six combinations. However, there is no statistically significant difference between Healthy and Early classes. Thus, classification algorithm is carried out on these six combinations with merging Healthy and Early classes. Among these six combinations, DADA2 and HOMD pair gives the best result in terms of balanced accuracy. Features which are ordered by importance are in table 27. Also, five metrics by feature count are shown as figure 59; then, the highest value of balanced accuracy is 0.938 with using 11 taxa. Moreover, violin plots from the two most important taxa, *Porphyromonas gingivalis* and *Actinomyces*, are in figure 60.

4.10 Random Forest Classifier with Merging (Moderate+Severe) Classes

As figure 3 and figure 4, there are six combinations. However, there is no statistically significant difference between Moderate and Severe classes. Thus, classification algorithm is carried out on these six combinations with merging Moderate and Severe classes. Among these six combinations, Deblur and HOMD pair gives the best result in terms of balanced accuracy. Features which are ordered by importance are in table 28. Also, five metrics by feature count are shown as figure 61; then, the highest value of balanced accuracy is 0.777 with using 28 taxa. Moreover, violin plots from the two most important taxa, *Porphyromonas gingivalis* and *Actinomyces*, are in figure 62.

Table 12: Bray-Curtis Distance Index with DADA2

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|---------|
| Early | Healthy | 150 | 999 | 1.8288671026193992 | 0.004 | 0.0048 |
| Early | Moderate | 100 | 999 | 2.4738348324475568 | 0.001 | 0.0015 |
| Early | Severe | 100 | 999 | 3.3691960533567005 | 0.001 | 0.0015 |
| Healthy | Moderate | 150 | 999 | 5.602936565444328 | 0.001 | 0.0015 |
| Healthy | Severe | 150 | 999 | 6.325447306476738 | 0.001 | 0.0015 |
| Moderate | Severe | 100 | 999 | 1.1018815494184453 | 0.219 | 0.219 |

Table 13: Jaccard Distance Index with DADA2

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|---------|
| Early | Healthy | 150 | 999 | 1.5875955458962276 | 0.001 | 0.0012 |
| Early | Moderate | 100 | 999 | 1.7486415070626309 | 0.001 | 0.0012 |
| Early | Severe | 100 | 999 | 1.8371794988000507 | 0.001 | 0.0012 |
| Healthy | Moderate | 150 | 999 | 3.9547515710373635 | 0.001 | 0.0012 |
| Healthy | Severe | 150 | 999 | 3.8380356039546784 | 0.001 | 0.0012 |
| Moderate | Severe | 100 | 999 | 0.9700395015774723 | 0.62 | 0.62 |

Table 14: Unweighted UniFrac Distance Index with DADA2

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|---------|
| Early | Healthy | 150 | 999 | 2.414078271406213 | 0.002 | 0.0024 |
| Early | Moderate | 100 | 999 | 4.941256726696032 | 0.001 | 0.0015 |
| Early | Severe | 100 | 999 | 6.184322196061149 | 0.001 | 0.0015 |
| Healthy | Moderate | 150 | 999 | 12.484494695636283 | 0.001 | 0.0015 |
| Healthy | Severe | 150 | 999 | 13.432593034368626 | 0.001 | 0.0015 |
| Moderate | Severe | 100 | 999 | 1.2428267228930112 | 0.084 | 0.084 |

Table 15: Weighted UniFrac Distance Index with DADA2

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|----------------------|
| Early | Healthy | 150 | 999 | 2.6584441800971716 | 0.019 | 0.022799999999999997 |
| Early | Moderate | 100 | 999 | 8.702906307484113 | 0.001 | 0.0015 |
| Early | Severe | 100 | 999 | 14.068214366598513 | 0.001 | 0.0015 |
| Healthy | Moderate | 150 | 999 | 22.059259782524673 | 0.001 | 0.0015 |
| Healthy | Severe | 150 | 999 | 31.310013450629775 | 0.001 | 0.0015 |
| Moderate | Severe | 100 | 999 | 1.7543213081828324 | 0.115 | 0.115 |

Table 16: Bray-Curtis Distance Index with Deblur

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|----------------------|
| Early | Healthy | 150 | 999 | 1.7634974220433302 | 0.019 | 0.022799999999999997 |
| Early | Moderate | 100 | 999 | 3.203442604434298 | 0.001 | 0.0015 |
| Early | Severe | 100 | 999 | 4.192790849454974 | 0.001 | 0.0015 |
| Healthy | Moderate | 150 | 999 | 6.953487468508356 | 0.001 | 0.0015 |
| Healthy | Severe | 150 | 999 | 7.5433379986347155 | 0.001 | 0.0015 |
| Moderate | Severe | 100 | 999 | 1.0959020597220626 | 0.313 | 0.313 |

Table 17: Jaccard Distance Index with Deblur

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|---------|
| Early | Healthy | 150 | 999 | 1.3701039884255466 | 0.001 | 0.0012 |
| Early | Moderate | 100 | 999 | 2.198029993855521 | 0.001 | 0.0012 |
| Early | Severe | 100 | 999 | 2.237738583770674 | 0.001 | 0.0012 |
| Healthy | Moderate | 150 | 999 | 4.528432929980079 | 0.001 | 0.0012 |
| Healthy | Severe | 150 | 999 | 4.374635292015638 | 0.001 | 0.0012 |
| Moderate | Severe | 100 | 999 | 1.0036296853126103 | 0.429 | 0.429 |

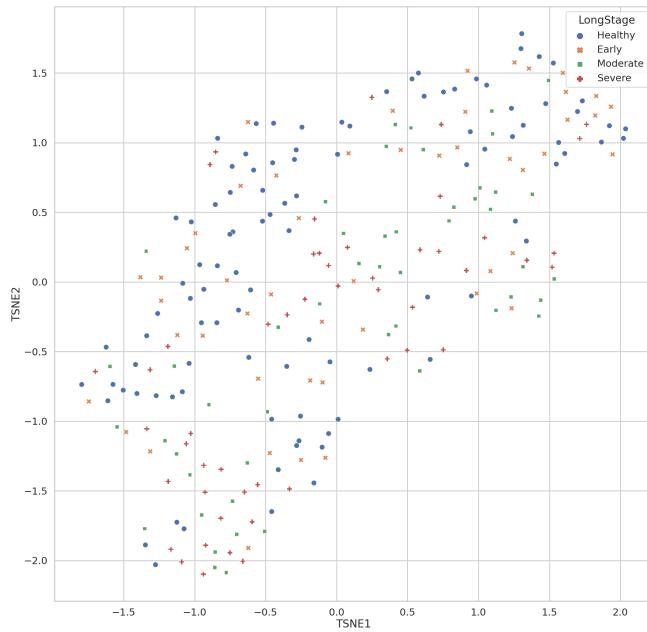


Figure 23: t-SNE Plot from Bray-Curtis Distance Index with DADA2

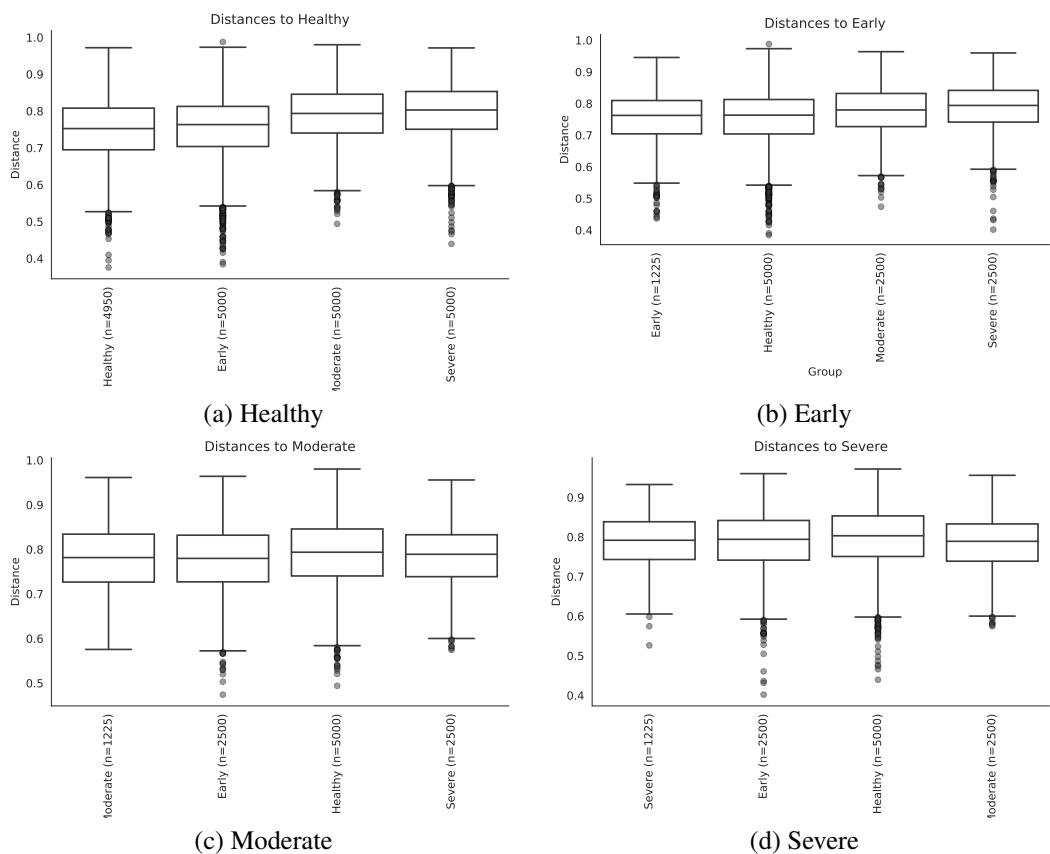


Figure 24: Bray-Curtis Distance Index with DADA2

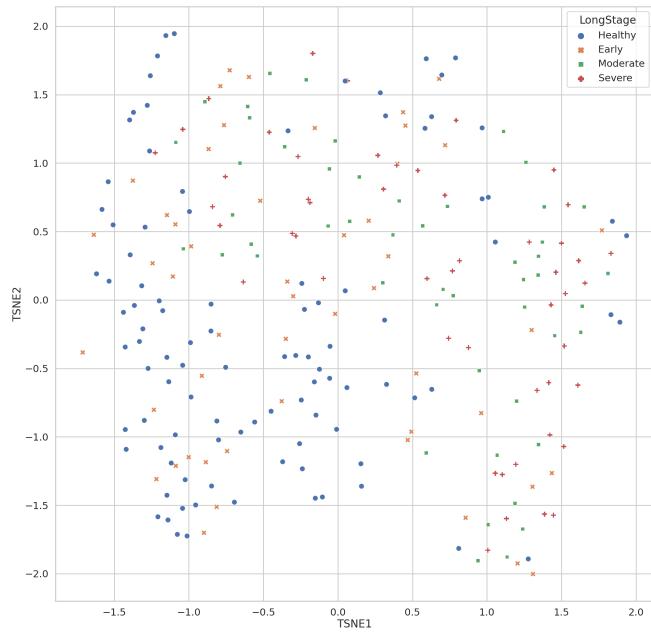


Figure 25: t-SNE Plot from Jaccard Distance Index with DADA2

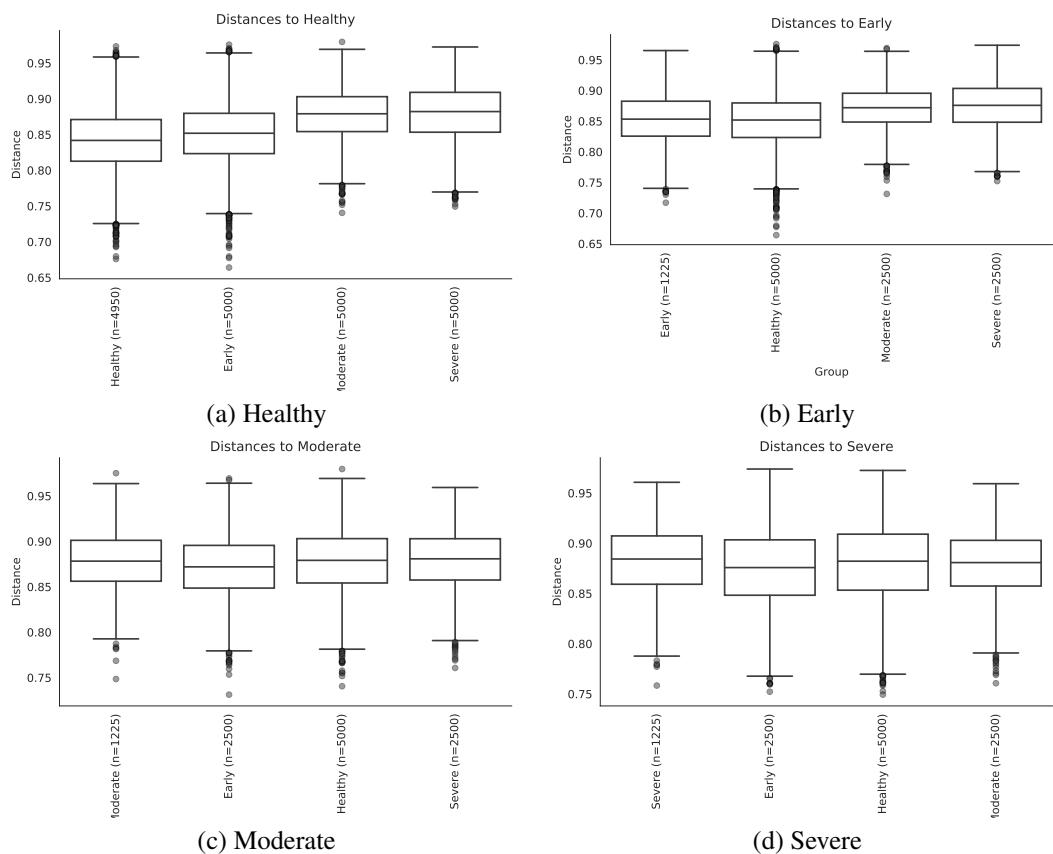


Figure 26: Jaccard Distance Index with DADA2

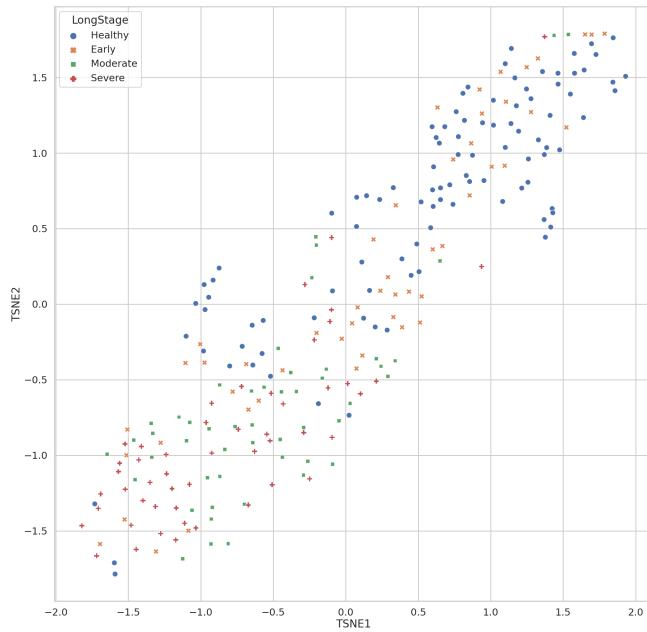


Figure 27: t-SNE Plot from Unweighted UniFrac Distance Index with DADA2

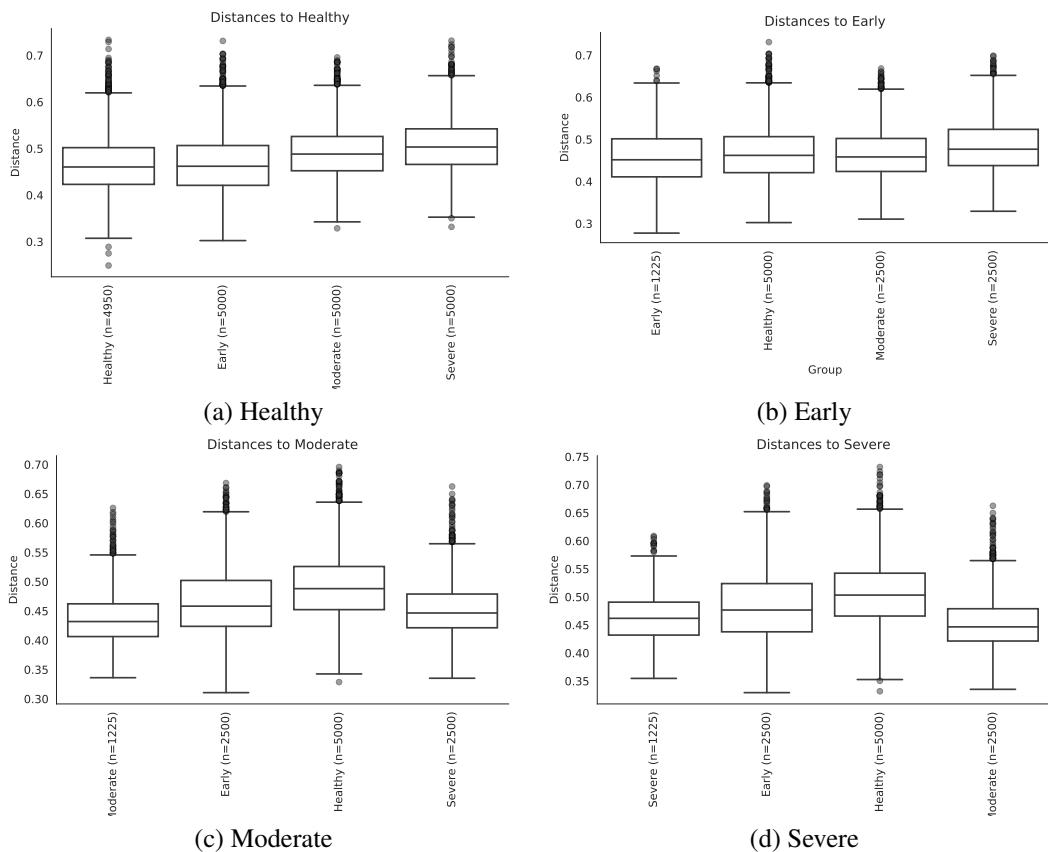


Figure 28: Unweighted UniFrac Distance Index with DADA2

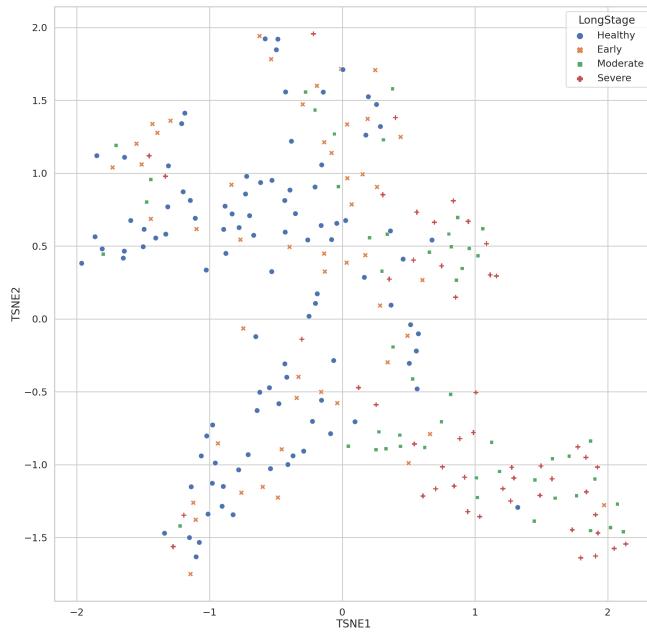


Figure 29: t-SNE Plot from Weighted UniFrac Distance Index with DADA2

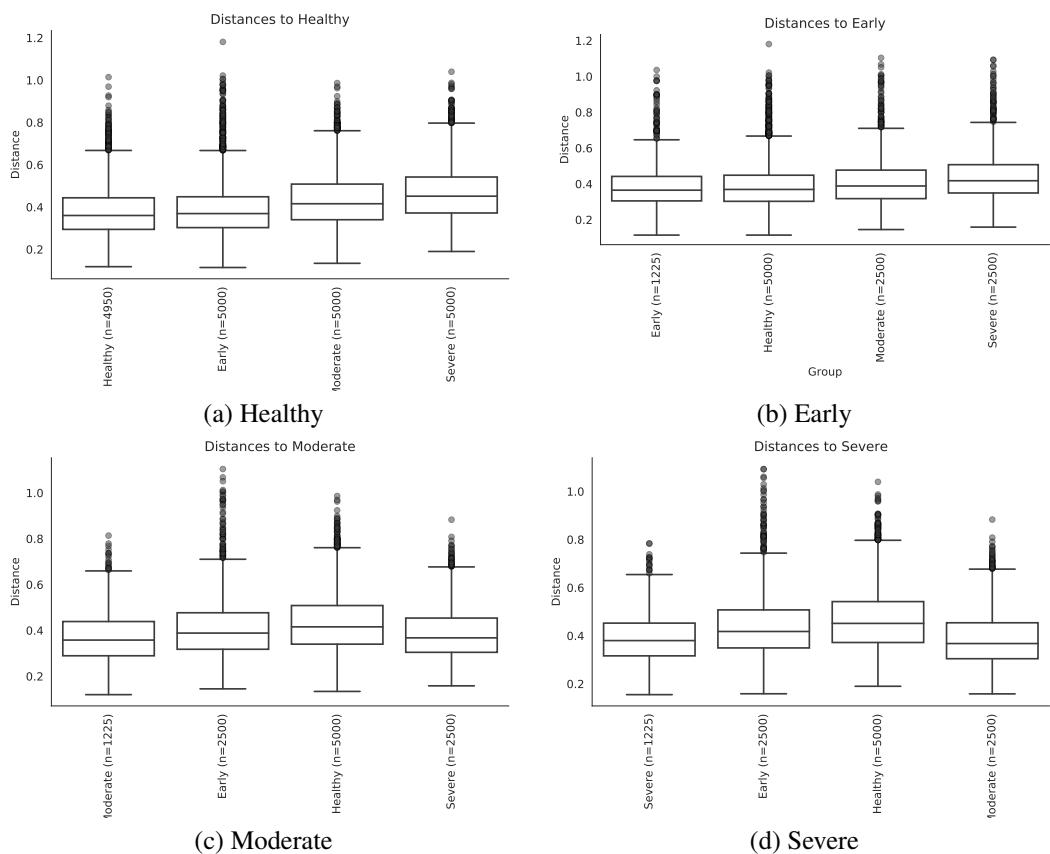


Figure 30: Weighted UniFrac Distance Index with DADA2

Table 18: Unweighted UniFrac Distance Index with Deblur

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|---------|
| Early | Healthy | 150 | 999 | 2.709074154153053 | 0.003 | 0.0036 |
| Early | Moderate | 100 | 999 | 7.547240014264336 | 0.001 | 0.0015 |
| Early | Severe | 100 | 999 | 7.772239667697252 | 0.001 | 0.0015 |
| Healthy | Moderate | 150 | 999 | 19.48285778321118 | 0.001 | 0.0015 |
| Healthy | Severe | 150 | 999 | 20.254907535032658 | 0.001 | 0.0015 |
| Moderate | Severe | 100 | 999 | 1.061788954262309 | 0.34 | 0.34 |

Table 19: Weighted UniFrac Distance Index with Deblur

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|----------|----------|-------------|--------------|--------------------|---------|---------|
| Early | Healthy | 150 | 999 | 2.0087857905677193 | 0.088 | 0.088 |
| Early | Moderate | 100 | 999 | 5.981646579135783 | 0.002 | 0.003 |
| Early | Severe | 100 | 999 | 16.572566883582837 | 0.001 | 0.002 |
| Healthy | Moderate | 150 | 999 | 9.494764618252377 | 0.001 | 0.002 |
| Healthy | Severe | 150 | 999 | 20.338834647304648 | 0.001 | 0.002 |
| Moderate | Severe | 100 | 999 | 5.026218407543304 | 0.003 | 0.0036 |

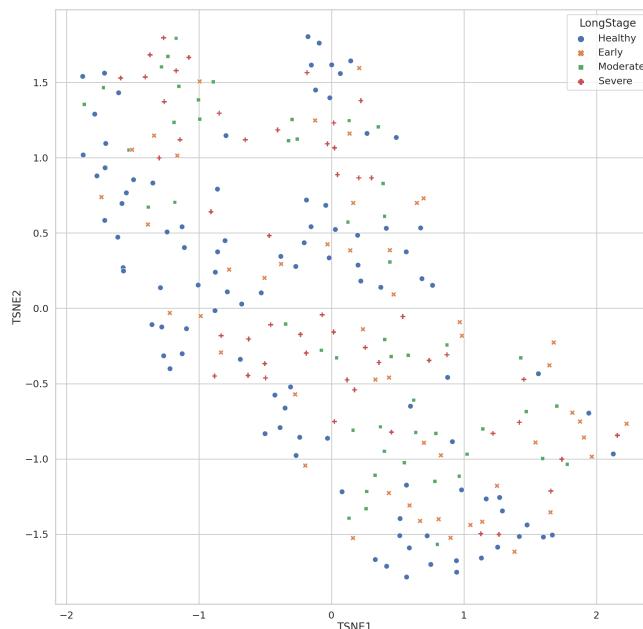


Figure 31: t-SNE Plot from Bray-Curtis Distance Index with Deblur

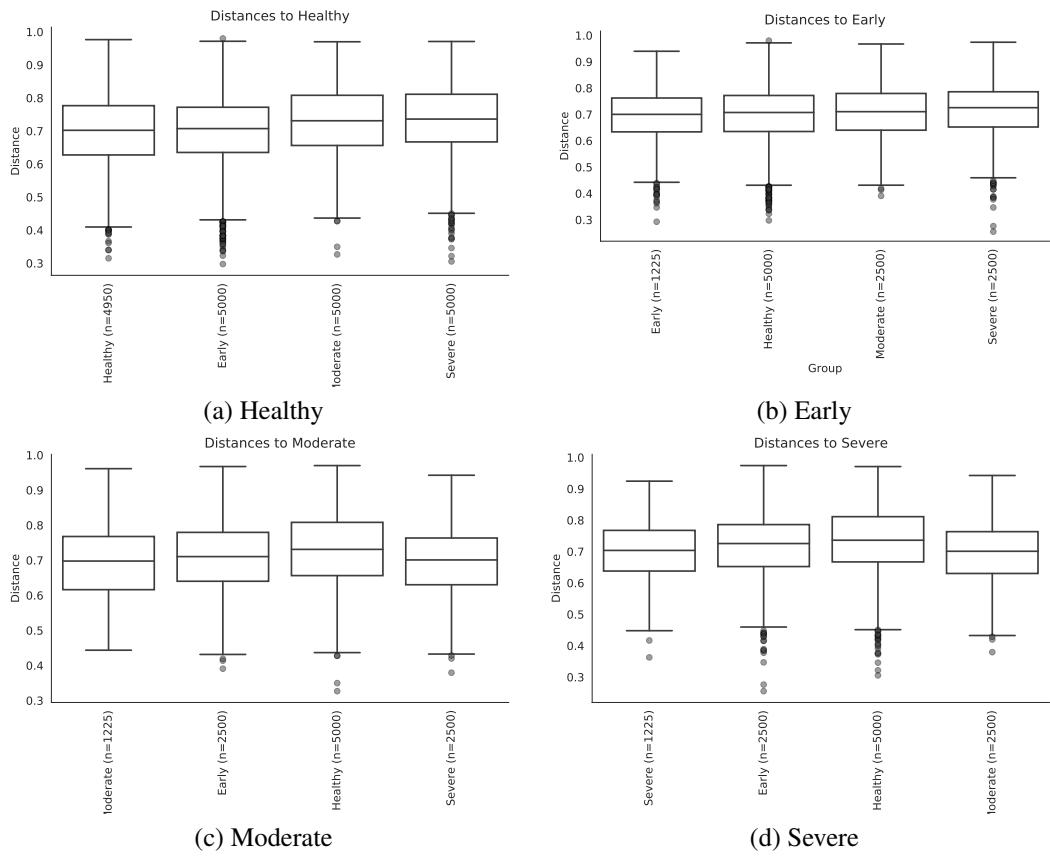


Figure 32: Bray-Curtis Distance Index with Deblur

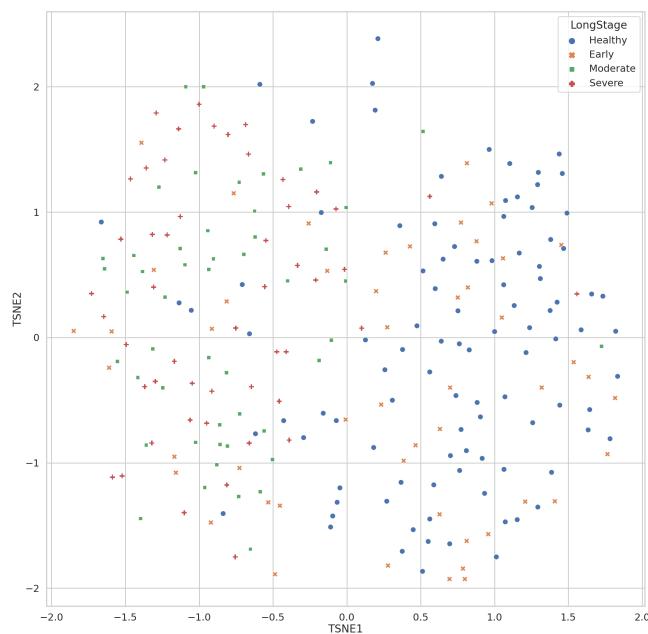


Figure 33: t-SNE Plot from Jaccard Distance Index with Deblur

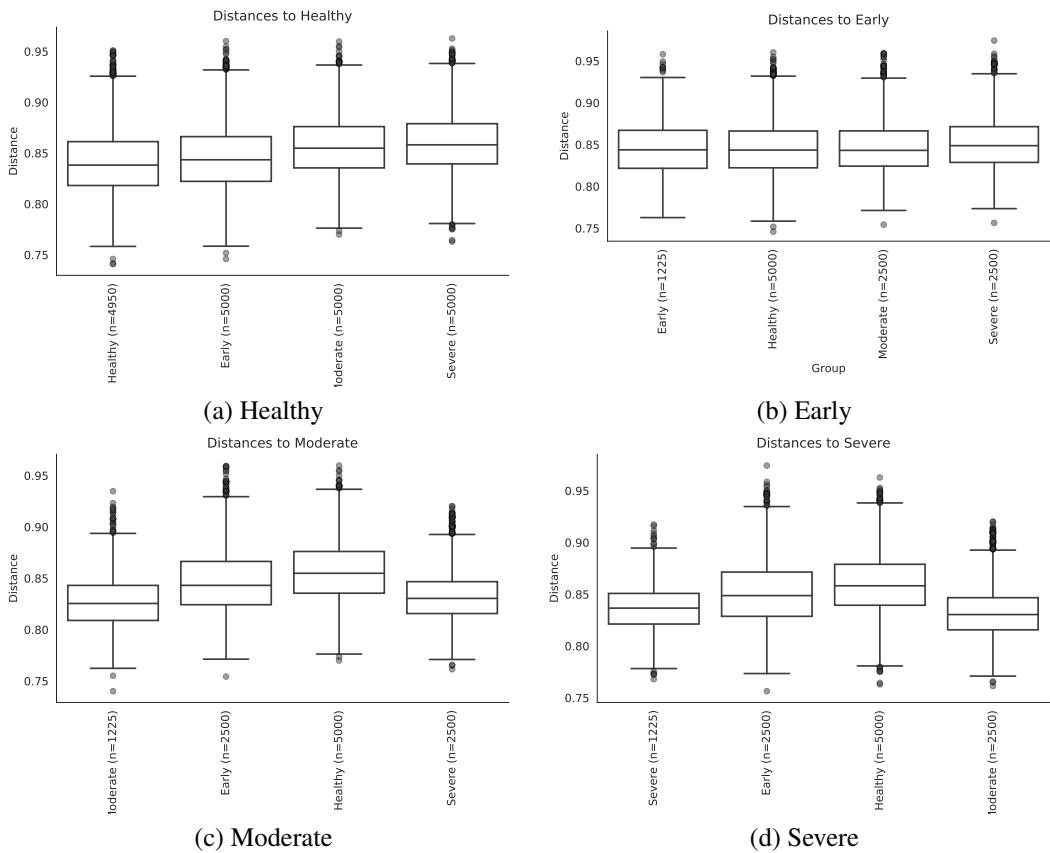


Figure 34: Jaccard Distance Index with Deblur

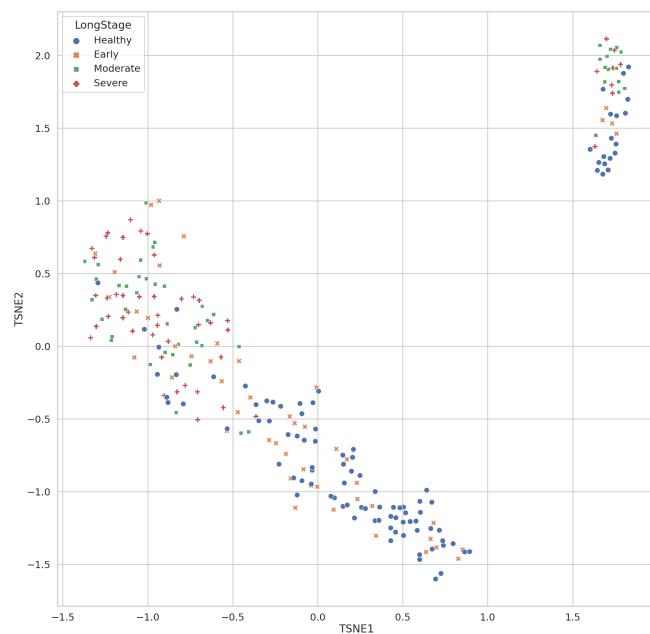


Figure 35: t-SNE Plot from Unweighted UniFrac Distance Index with Deblur

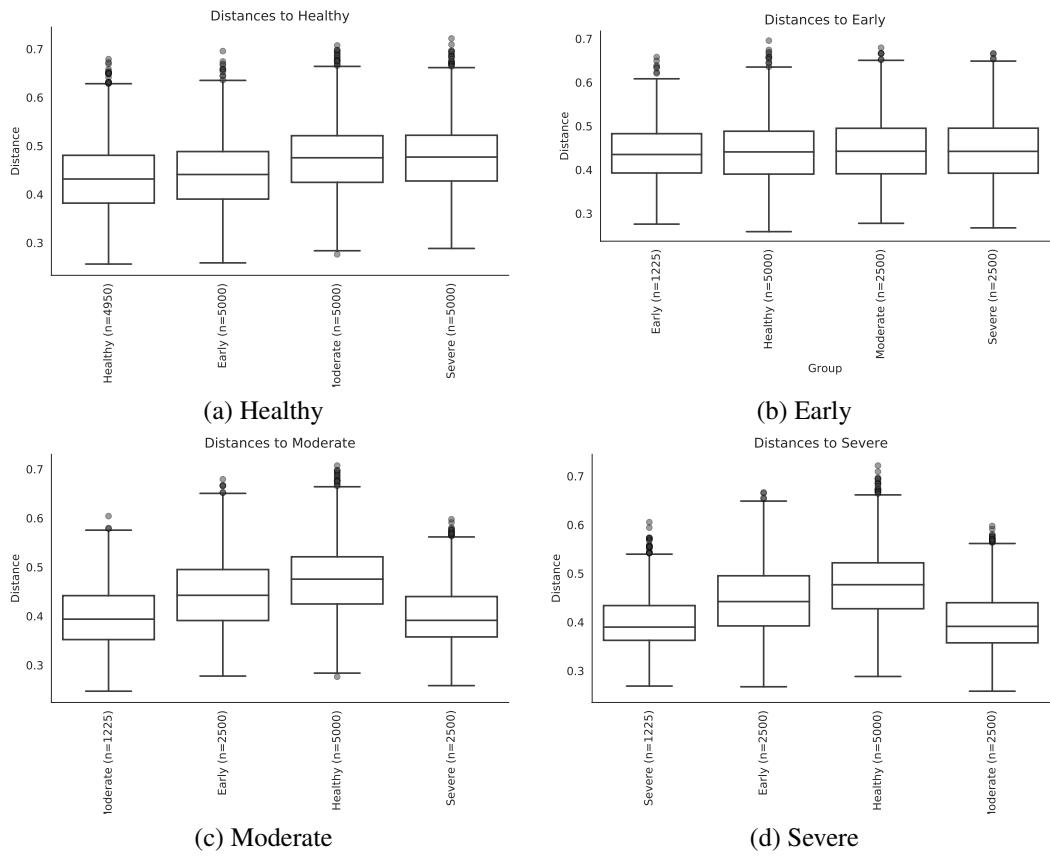


Figure 36: Unweighted UniFrac Distance Index with Deblur

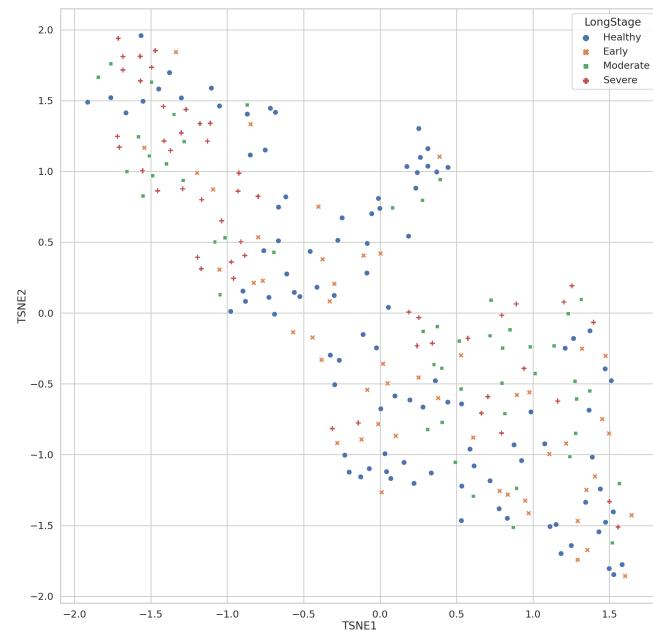


Figure 37: t-SNE Plot from Weighted UniFrac Distance Index with Deblur

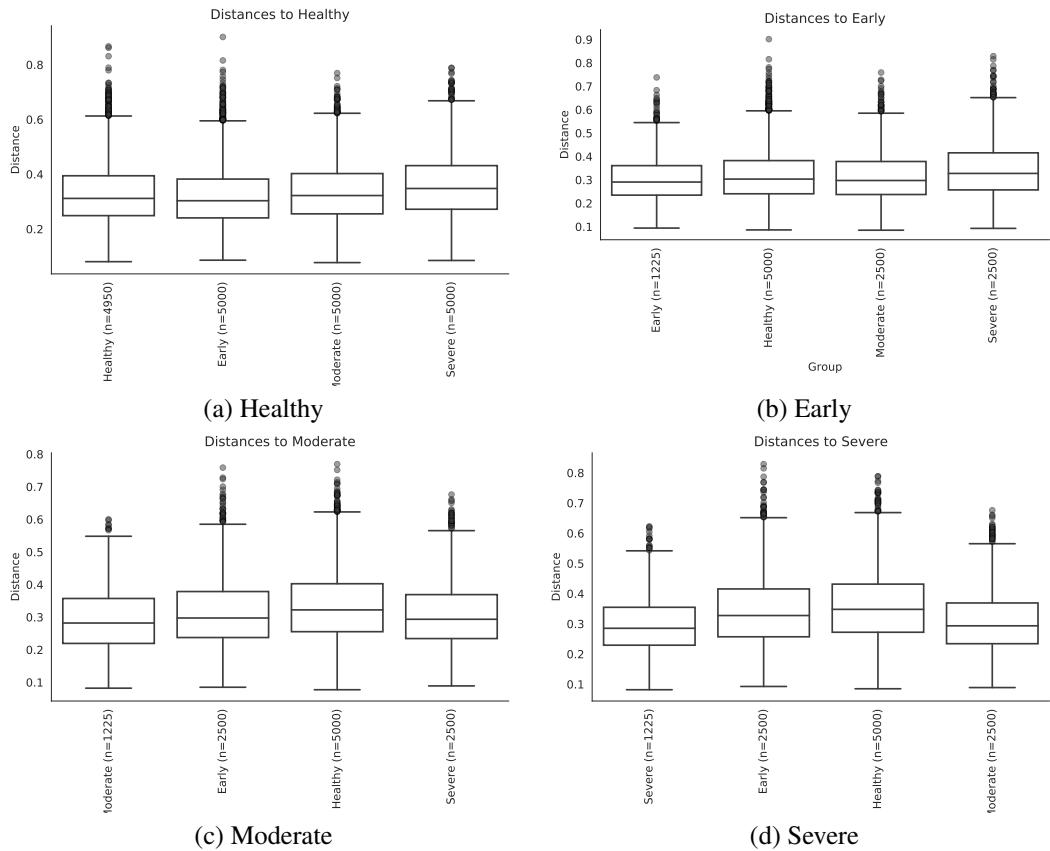


Figure 38: Weighted UniFrac Distance Index with Deblur

Table 20: ANCOM Significant Taxa with DADA2 and GG

| | | W | Reject null hypothesis | | | |
|----------|----------------|-----------------------|------------------------|-------------------------|-----|------|
| Bacteria | Actinobacteria | Actinobacteria | Actinomycetales | Actinomycetaceae | 326 | True |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Peptostreptococcaceae | 325 | True |
| Bacteria | Spirochaetes | Spirochaetes | Spirochaetales | Spirochaetaceae | 325 | True |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Prevotellaceae | 323 | True |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | 321 | True |
| Bacteria | Synergistetes | Synergistia | Synergistales | Dethiosulfovibrionaceae | 319 | True |
| Bacteria | Tenericutes | Mollicutes | Mycoplasmatales | Mycoplasmataceae | 318 | True |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | 315 | True |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | 313 | True |
| Bacteria | Actinobacteria | Actinobacteria | Actinomycetales | Corynebacteriaceae | 309 | True |
| Bacteria | Firmicutes | Clostridia | Clostridiales | [Mogibacteriaceae] | 306 | True |
| Bacteria | Proteobacteria | Epsilonproteobacteria | Campylobacterales | Campylobacteraceae | 305 | True |
| Bacteria | Firmicutes | Clostridia | Clostridiales | [Acidaminobacteraceae] | 304 | True |

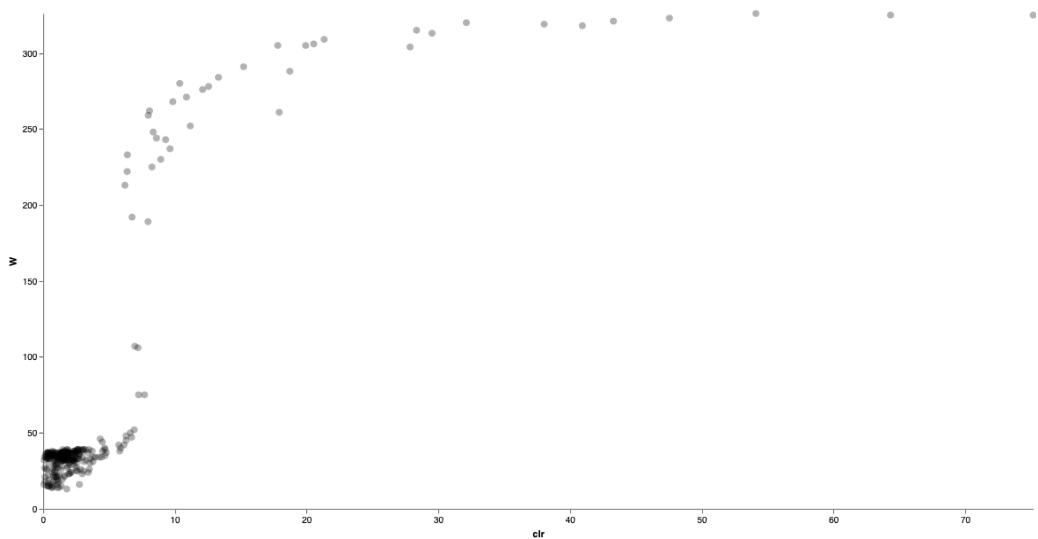


Figure 39: ANCOM Volcano Plot with DADA2 and GG

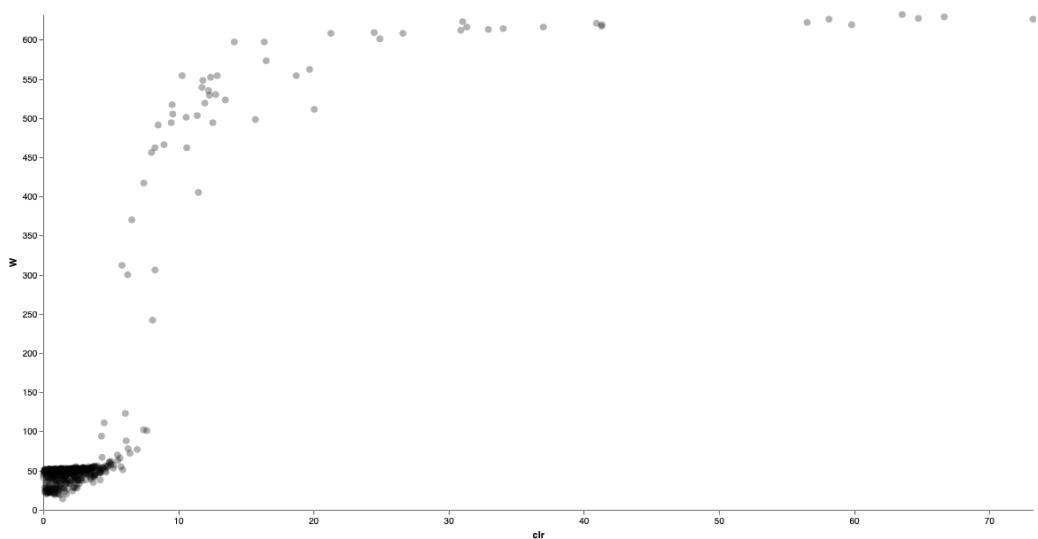


Figure 40: ANCOM Volcano Plot with DADA2 and HOMD

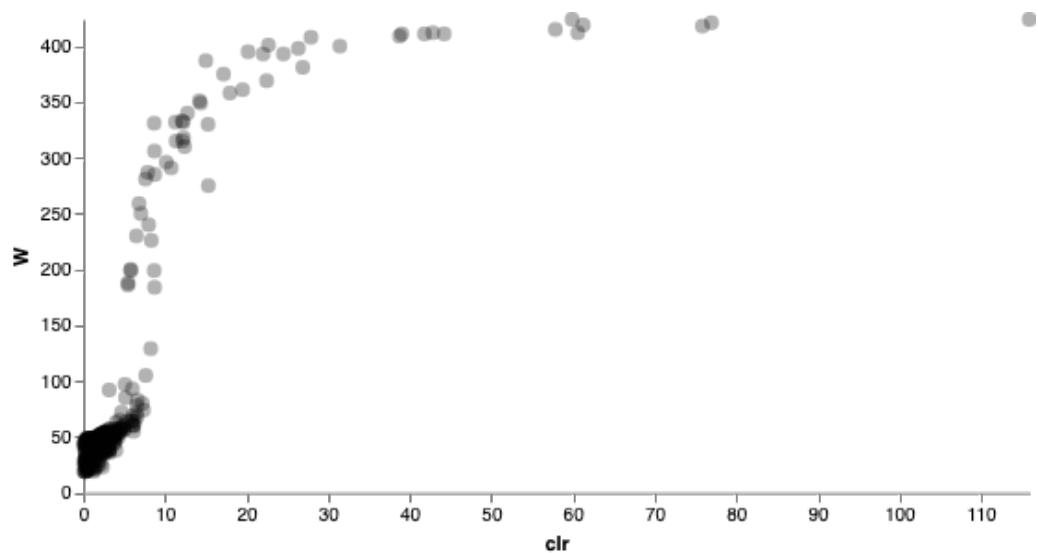


Figure 41: ANCOM Volcano Plot with DADA2 and SILVA

Table 21: ANCOM Significant Taxa with DADA2 and SILVA

| | W | Reject null hypothesis |
|---|-----|------------------------|
| Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 632 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas gingivalis | 629 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Filifactor Filifactor alocis | 627 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Prevotella Prevotella intermedia | 626 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema denticola | 626 | True |
| Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Schaalialia odontolytica | 623 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tannerella Tannerella forsythia | 622 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema medium | 621 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae [Eubacterium] nodatum group [Eubacterium] nodatum | 619 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema uncultured bacterium | 619 | True |
| Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae Mycoplasma Metamyoplasma faecium | 617 | True |
| Bacteria Synergistota Synergistia Synergistales Synergistaceae Fretibacterium | 616 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema | 616 | True |
| Bacteria Firmicutes Clostridia Lachnospirales Defluvitaleaceae Defluvitaleaceae UCG-011 Lachnospiraceae bacterium | 614 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas | 613 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae [Eubacterium] brachy group [Eubacterium] brachy | 612 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae | 609 | True |
| Bacteria Actinobacteriota Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium Corynebacterium durum | 608 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae [Eubacterium] saphenum group Eubacterium saphenum | 608 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema maltophilum | 601 | True |
| Bacteria Campylobacterota Campylobacteria Campylobacterales Campylobacteraceae Campylobacter Campylobacter showae | 597 | True |
| Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Actinomyces graevenitzii | 597 | True |
| Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribacterium | 573 | True |

Table 22: ANCOM Significant Taxa with DADA2 and HOMD

| | | W | Reject null hypothesis |
|--|-----|------|------------------------|
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae | 424 | True | |
| Porphyromonas gingivalis | | | |
| Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 424 | True | |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Filifactor alocis | 421 | True | |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia | 419 | True | |
| Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema putidum | 418 | True | |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella forsythia | 415 | True | |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas sp. HMT 285 | 412 | True | |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcaceae [XI][G-6] nodatum | 412 | True | |
| Bacteria Synergistetes Synergistia Synergistales Synergistaceae Fretibacterium | 411 | True | |
| Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma faicum | 411 | True | |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 304 | 411 | True | |
| Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae [XIV] Lachnospiraceae [G-8] bacterium HMT 500 | 409 | True | |
| Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema | 408 | True | |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 526 | 401 | True | |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcaceae [XI][G-9] brachy | 400 | True | |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcaceae [XI][G-5] saphenum | 398 | True | |
| Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales Campylobacteraceae Campylobacter showae | 395 | True | |
| Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 260 | 393 | True | |
| Bacteria Actinobacteria Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium durum | 393 | True | |
| Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces graevenitzii | 387 | True | |

Table 23: ANCOM Significant Taxa with Deblur and GG

| | W | Reject null hypothesis |
|---|-----|------------------------|
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Filifactor | 229 | True |
| Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema | 229 | True |
| Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema amylovorum | 227 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia | 226 | True |
| Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma | 226 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas endodontalis | 226 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella | 226 | True |
| Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibrionaceae TG5 | 226 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas | 224 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales | 220 | True |
| Bacteria Firmicutes Clostridia Clostridiales [Mogibacteriaceae] | 211 | True |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Peptostreptococcus | 209 | True |
| Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desulfovulbaceae Desulfobulbus | 207 | True |
| Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema socranskii | 201 | True |
| Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales Campylobacteraceae Campylobacter | 199 | True |
| Bacteria Firmicutes Clostridia Clostridiales [Acidaminobacteraceae] | 199 | True |
| Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae Haemophilus parainfluenzae | 196 | True |
| Bacteria Firmicutes Clostridia Clostridiales [Tissierellaceae] Parvimonas | 195 | True |
| Bacteria Proteobacteria Betaproteobacteria Neisseriales Neisseriaceae Neisseria subflava | 194 | True |
| Bacteria Firmicutes Clostridia Clostridiales [Mogibacteriaceae] Mogibacterium | 187 | True |
| Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 187 | True |
| Bacteria Firmicutes Clostridia Clostridiales [Tissierellaceae] | 186 | True |
| Bacteria Actinobacteria Actinobacteria Actinomycetales | 185 | True |
| Bacteria Firmicutes Clostridia Clostridiales | 184 | True |
| Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae Oribacterium | 180 | True |
| Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae | 179 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella nanceiensis | 173 | True |

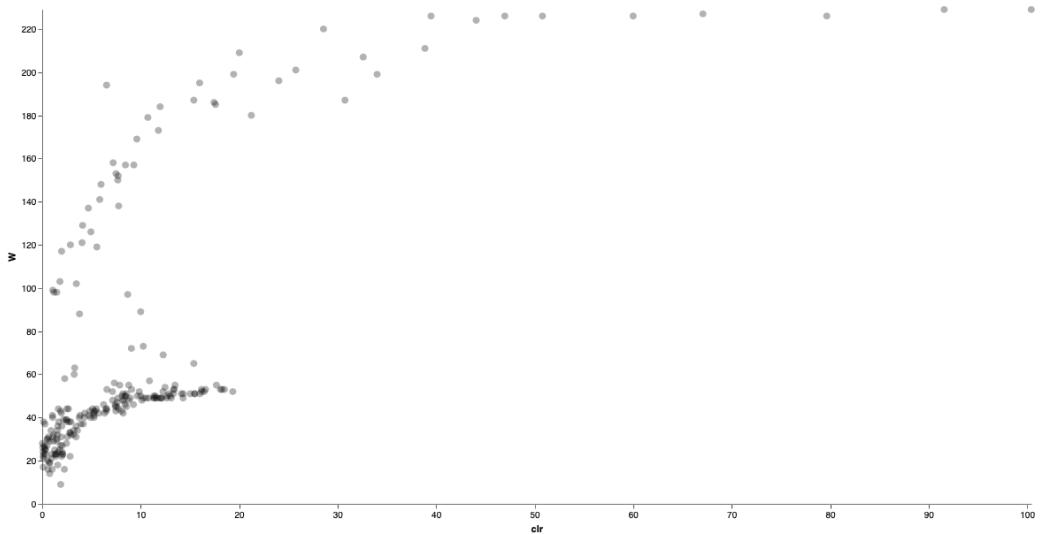


Figure 42: ANCOM Volcano Plot with Deblur and GG

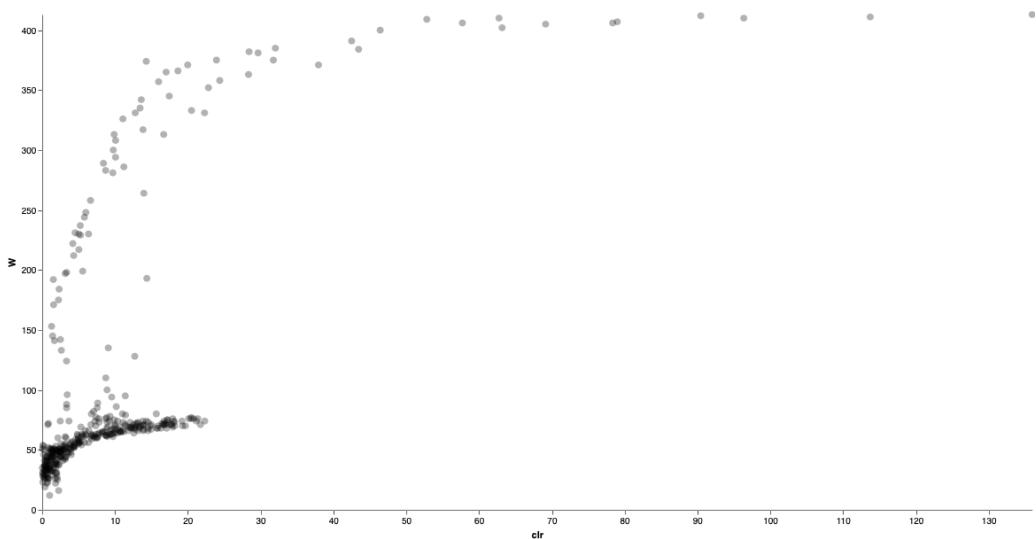


Figure 43: ANCOM Volcano Plot with Deblur and SILVA

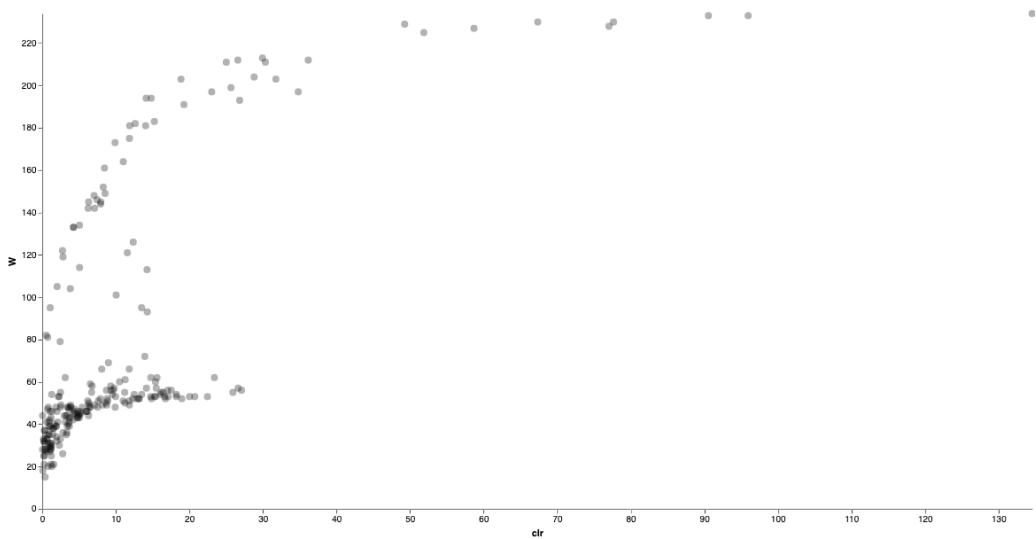


Figure 44: ANCOM Volcano Plot with Deblur and HOMD

Table 24: ANCOM Significant Taxa with Deblur and SILVA

| | W | Reject null hypothesis |
|---|-----|------------------------|
| Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 632 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas gingivalis | 629 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Filifactor Filifactor alocis | 627 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Prevotella Prevotella intermedia | 626 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema denticola | 626 | True |
| Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Schaalialia odontolytica | 623 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tannerella Tannerella forsythia | 622 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema medium | 621 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae [Eubacterium] nodatum group [Eubacterium] nodatum | 619 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema uncultured bacterium | 619 | True |
| Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae Mycoplasma Metamyoplasma faecium | 617 | True |
| Bacteria Synergistota Synergistia Synergistales Synergistaceae Fretibacterium | 616 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema | 616 | True |
| Bacteria Firmicutes Clostridia Lachnospirales Defluvitaleaceae Defluvitaleaceae UCG-011 Lachnospiraceae bacterium | 614 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas | 613 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae [Eubacterium] brachy group [Eubacterium] brachy | 612 | True |
| Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae | 609 | True |
| Bacteria Actinobacteriota Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium Corynebacterium durum | 608 | True |
| Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae [Eubacterium] saphenum group Eubacterium saphenum | 608 | True |
| Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema maltophilum | 601 | True |
| Bacteria Campylobacterota Campylobacteria Campylobacteriales Campylobacteraceae Campylobacter Campylobacter showae | 597 | True |
| Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Actinomyces graevenitzii | 597 | True |
| Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribacterium | 573 | True |

Table 25: ANCOM Significant Taxa with Deblur and HOMD

| | W | Reject null hypothesis |
|---|-----|------------------------|
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae | 234 | True |
| Porphyromonas gingivalis | | |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] | 233 | True |
| Filifactor alocis | | |
| Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema | 233 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae | 230 | True |
| Tannerella forsythia | | |
| Bacteria Synergistetes Synergistia Synergistales Synergistaceae Fretibacterium | 230 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae | 229 | True |
| Porphyromonas sp. HMT 285 | | |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcaceae [XI][G-6] nodatum | 228 | True |
| Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma | 227 | True |
| Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae [XIV] Lachnospiraceae [G-8] bacterium HMT 500 | 225 | True |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcaceae [XI][G-5] saphenum | 213 | True |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcaceae [XI][G-9] brachy | 212 | True |
| Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 260 | 212 | True |
| Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desulfovulbaceae Desulfobulbus sp. HMT 041 | 211 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella dentalis | 211 | True |
| Bacteria Bacteroidetes Bacteroidetes [C-1] Bacteroidetes [O-1] Bacteroidetes [F-1] Bacteroidetes [G-3] | 204 | True |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcus anaerobius | 203 | True |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Peptostreptococcaceae [XI][G-4] bacterium HMT 369 | 203 | True |
| Bacteria Firmicutes Clostridia Clostridiales Clostridiales [F-1] Clostridiales [F-1][G-1] bacterium HMT 093 | 199 | True |
| Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae [XIV] Oribacterium sinus | 197 | True |
| Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 197 | True |
| Bacteria Firmicutes Clostridia Clostridiales Peptoniphilaceae Parvimonas sp. HMT 393 | 194 | True |
| Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella nanceiensis | 194 | True |
| Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae | 193 | True |
| Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae [XIV] Johnsonella sp. HMT 166 | 191 | True |
| Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 258 | 183 | True |
| Bacteria Firmicutes Negativicutes Veillonellales Veillonellaceae Veilonella denticariosi | 182 | True |
| Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae [XIV] Catonella sp. HMT 164 | 181 | True |
| Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae [XI] Mogibacterium | 181 | True |

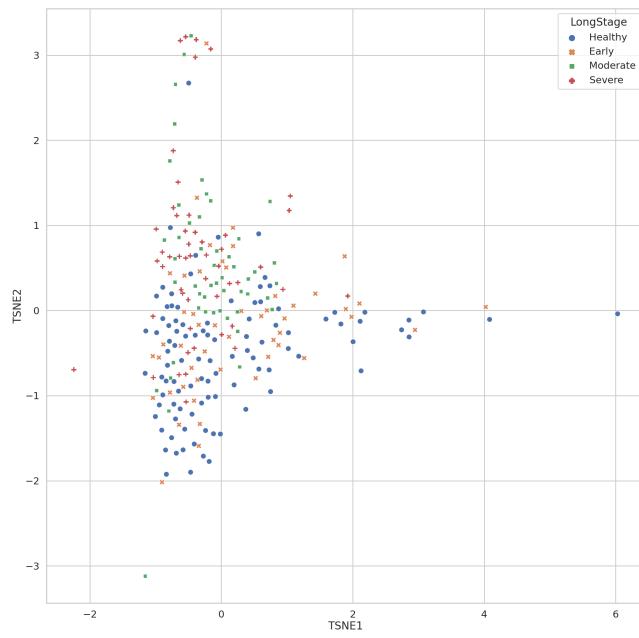


Figure 45: t-SNE Plot with Whole Microbiome from DADA2 and GG (328 taxa)

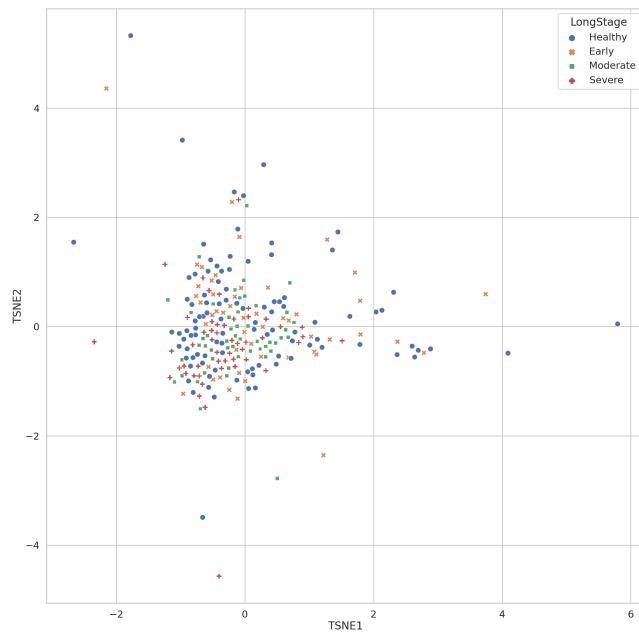


Figure 46: t-SNE Plot with Whole Microbiome from DADA2 and SILVA (633 taxa)

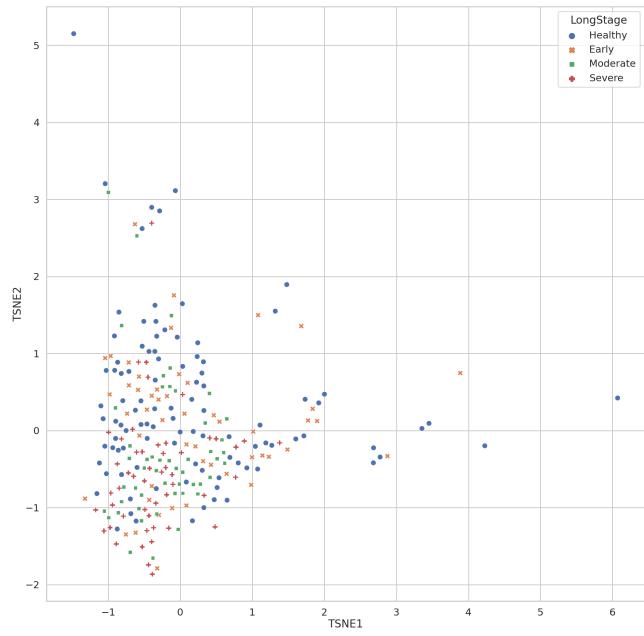


Figure 47: t-SNE Plot with Whole Microbiome from DADA2 and HOMD (425 taxa)

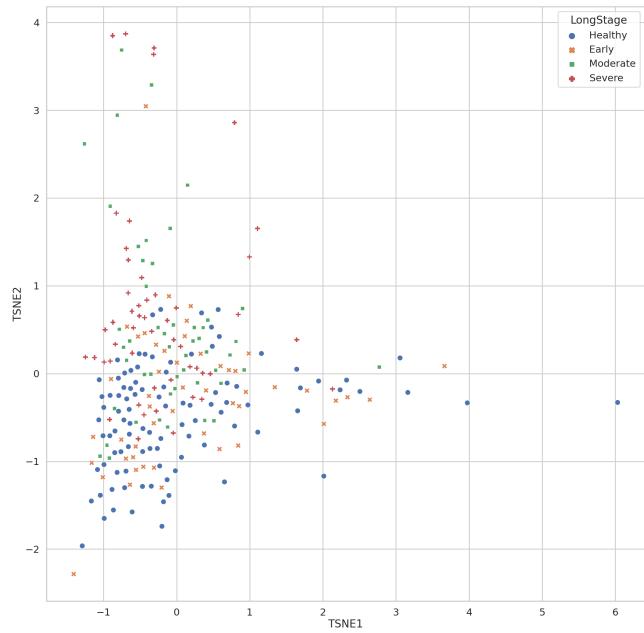


Figure 48: t-SNE Plot with Whole Microbiome from Deblur and GG (232 taxa)

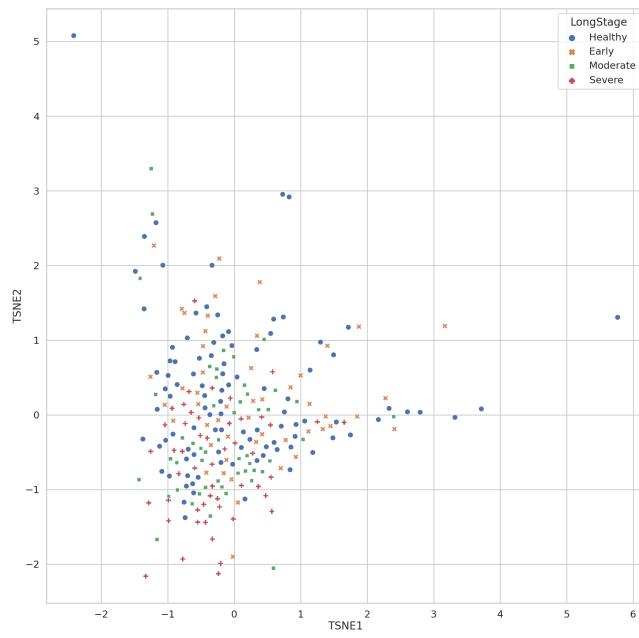


Figure 49: t-SNE Plot with Whole Microbiome from Deblur and SILVA (414 taxa)

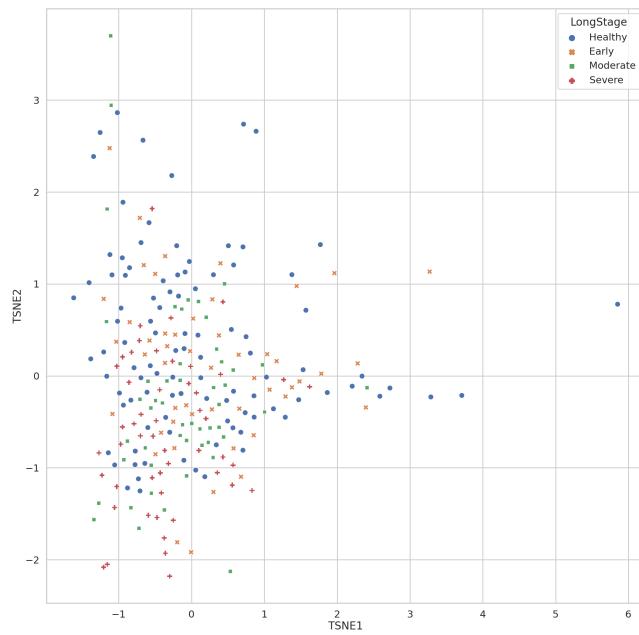


Figure 50: t-SNE Plot with Whole Microbiome from Deblur and HOMD (235 taxa)

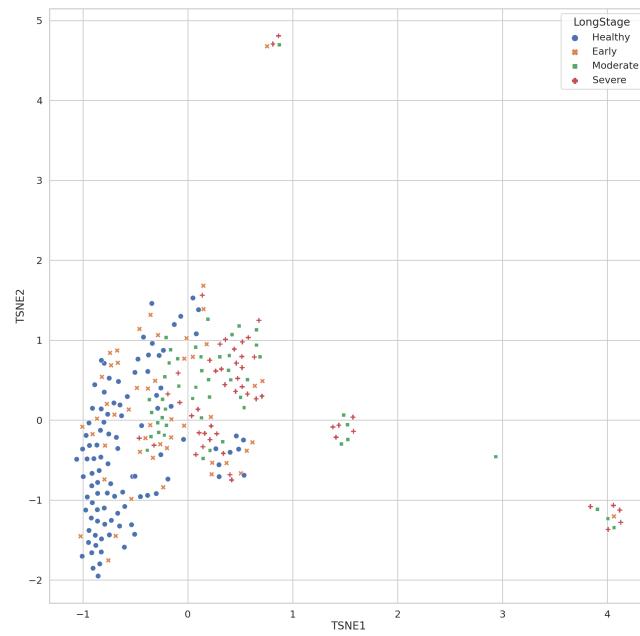


Figure 51: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and GG (15 taxa)

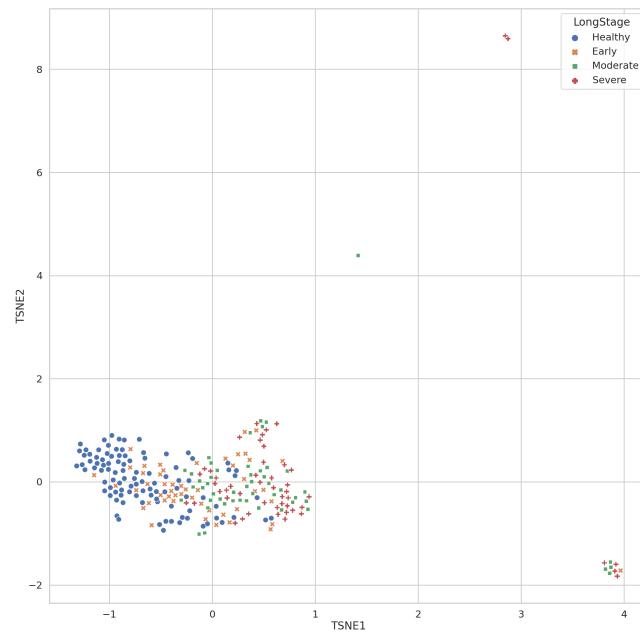


Figure 52: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and SILVA (23 taxa)

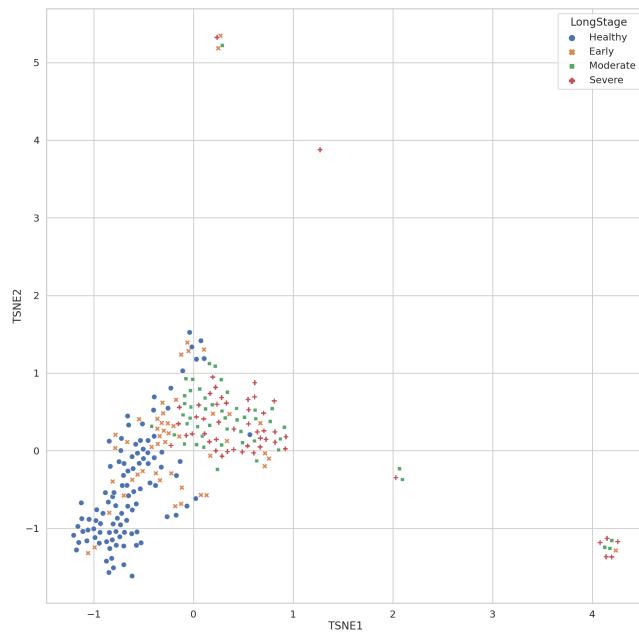


Figure 53: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and HOMD (20 taxa)

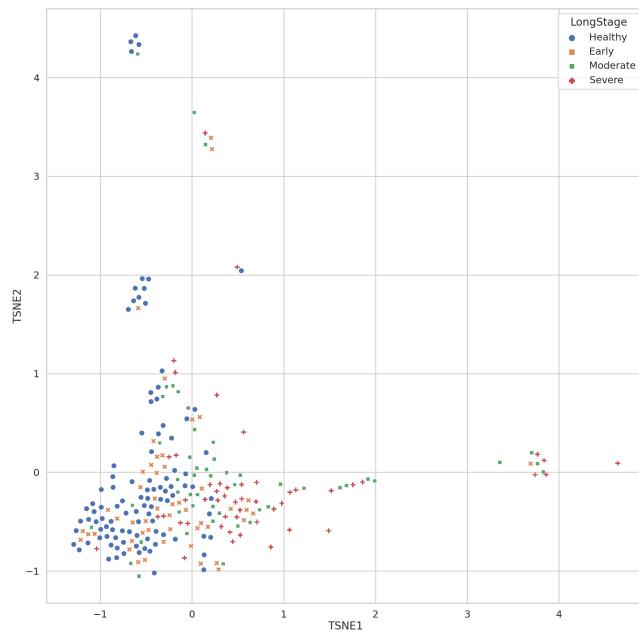


Figure 54: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and GG (27 taxa)

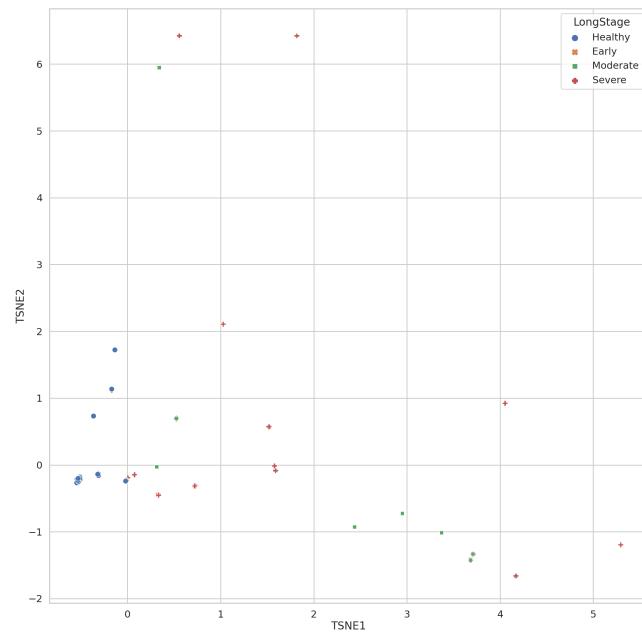


Figure 55: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and SILVA (20 taxa)

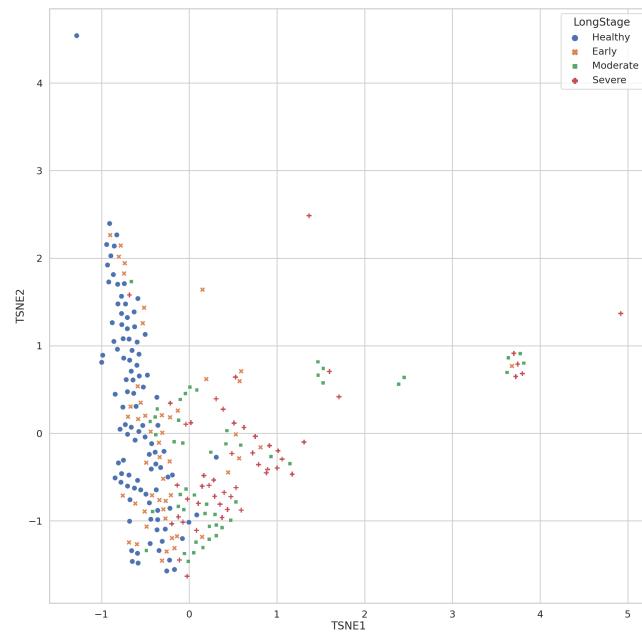


Figure 56: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and HOMD (28 taxa)

Table 26: Taxa with Deblur and HOMD Ordered by Random Forest

| Order | Taxonomy Classification | Importances |
|-------|---|-----------------------|
| 0 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas gingivalis | 0.2855642402730913 |
| 1 | Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 0.08351318031178692 |
| 2 | Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae | 0.059350781911881166 |
| 3 | Bacteria Firmicutes Negativicutes Veillonellales Veillonellaceae Veilonella denticariosi | 0.057871723793766865 |
| 4 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Oribacterium sinus | 0.047358553418271984 |
| 5 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcus anaerobius | 0.042675610482295 |
| 6 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella nanceiensis | 0.029233765358459122 |
| 7 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema | 0.02859828627467567 |
| 8 | Bacteria Firmicutes Clostridia Clostridiales Peptoniphilaceae Parvimonas sp. HMT 393 | 0.028457010101267423 |
| 9 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Filifactor alocis | 0.027837761835260755 |
| 10 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas sp. HMT 285 | 0.026641811829482817 |
| 11 | Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma | 0.024424656992519996 |
| 12 | Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desulfovulbaceae Desulfobulbus sp. HMT 041 | 0.023536845838453724 |
| 13 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Mogibacterium | 0.021340516173708687 |
| 14 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 260 | 0.02095790927625119 |
| 15 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Catonella sp. HMT 164 | 0.020180690978135088 |
| 16 | Bacteria Synergistetes Synergistia Synergistales Synergistaceae Fretibacterium | 0.02009509852741801 |
| 17 | Bacteria Bacteroidetes Bacteroides C-1 Bacteroidetes O-1 Bacteroidetes F-1 Bacteroidetes G-3 | 0.01968216985850785 |
| 18 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-6 nodatum | 0.019647822582083987 |
| 19 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-9 brachy | 0.018425192429676936 |
| 20 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella forsythia | 0.01816201283157638 |
| 21 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Lachnospiraceae G-8 bacterium HMT 500 | 0.016666550803754784 |
| 22 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella dentalis | 0.014244514636384266 |
| 23 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-5 saphenum | 0.011086499520111613 |
| 24 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 258 | 0.009166997621775088 |
| 25 | Bacteria Firmicutes Clostridia Clostridiales Clostridiales F-1 Clostridiales F-1G-1 bacterium HMT 093 | 0.00894020078417408 |
| 26 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-4 bacterium HMT 369 | 0.008838253430012495 |
| 27 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Johnsonella sp. HMT 166 | 0.0075013421252168085 |

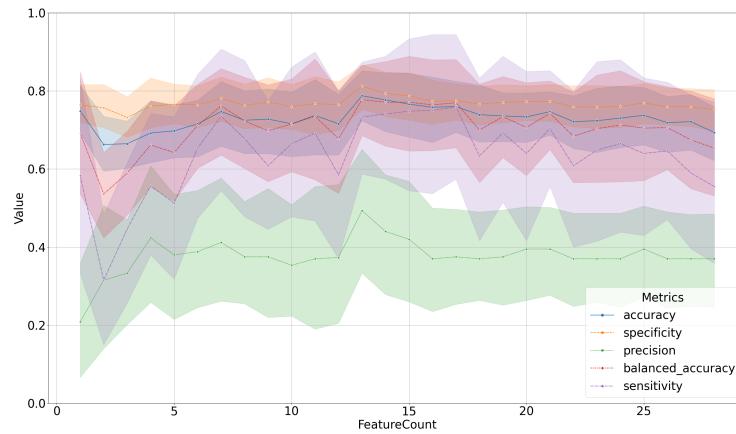
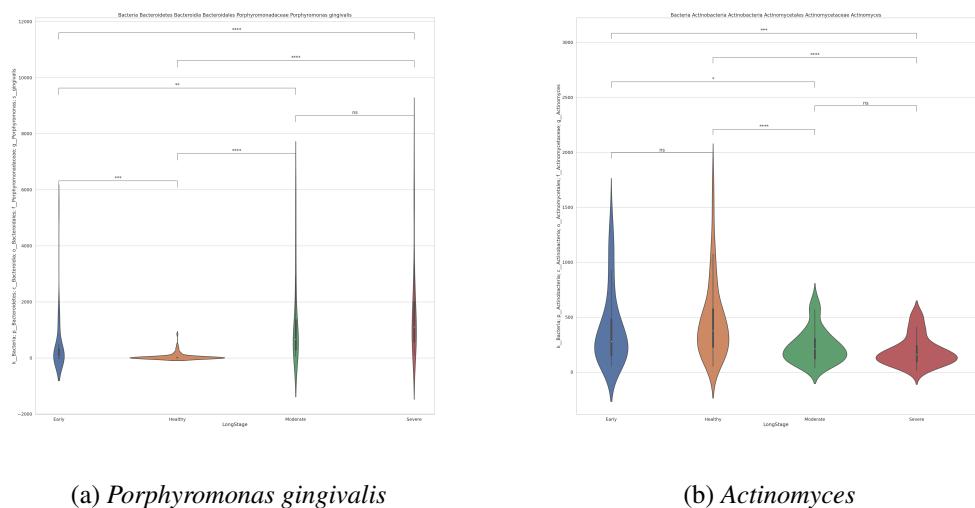


Figure 57: Metrics by Feature Count with Deblur and HOMD



(a) *Porphyromonas gingivalis*

(b) *Actinomyces*

Figure 58: Most and Second Most Important Features with Deblur and HOMD

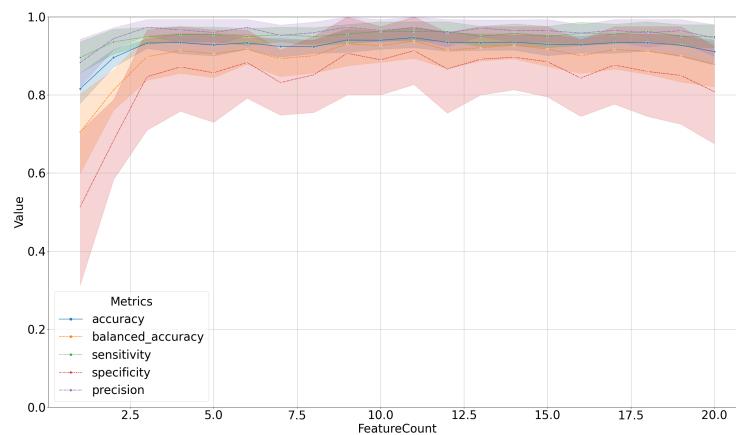


Figure 59: Metrics by Feature Count with DADA2 and HOMD for Merging (Healthy+Early) Classes

Table 27: Taxa with DADA2 and HOMD Ordered by Random Forest for Merging (Healthy+Early) Classes

| Order | Taxonomy Classification | Importances |
|-------|--|----------------------|
| 0 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas gingivalis | 0.3492215094954864 |
| 1 | Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 0.2053680101280024 |
| 2 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia | 0.046910132999688776 |
| 3 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Filifactor alocis | 0.04537783279841328 |
| 4 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 260 | 0.03995303090221915 |
| 5 | Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales Campylobacteraceae Campylobacter showae | 0.036126247249477515 |
| 6 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas sp. HMT 285 | 0.030505434425849725 |
| 7 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-6 nodatum | 0.02574268888684369 |
| 8 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 304 | 0.025653960507079653 |
| 9 | Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces graevenitzii | 0.021694782458252272 |
| 10 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella forsythia | 0.02139528465263749 |
| 11 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-9 brachy | 0.019849874772476486 |
| 12 | Bacteria Actinobacteria Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium durum | 0.01925997572442972 |
| 13 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema putidum | 0.019089810584091933 |
| 14 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema | 0.018026952071605436 |
| 15 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 526 | 0.01773296746323165 |
| 16 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-5 saphenum | 0.017477689904220505 |
| 17 | Bacteria Synergistetes Synergistia Synergistales Synergistaceae Fretibacterium | 0.016853985437158086 |
| 18 | Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma faecium | 0.014880940813824687 |
| 19 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Lachnospiraceae G-8 bacterium HMT 500 | 0.008878888725011094 |

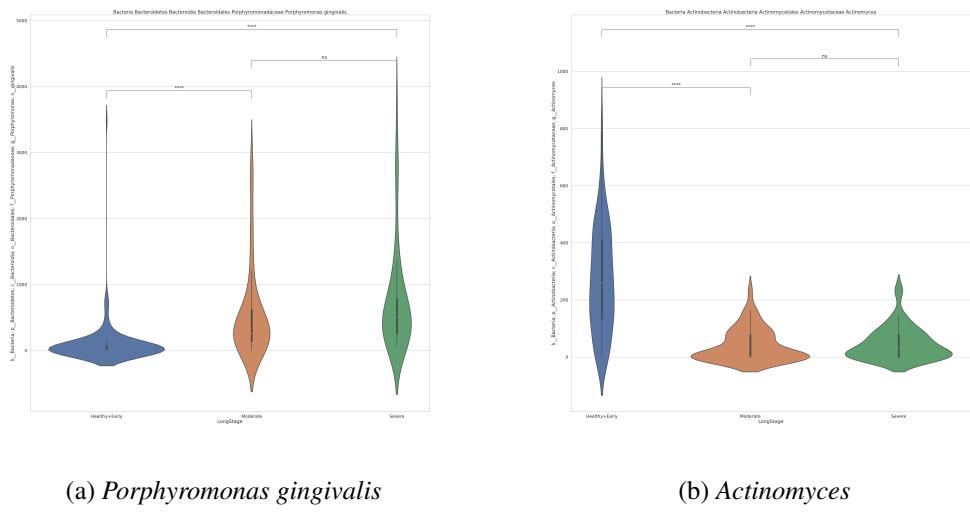


Figure 60: Most and Second Most Important Features with DADA2 and HOMD for Merging (Healthy+Early) Classes

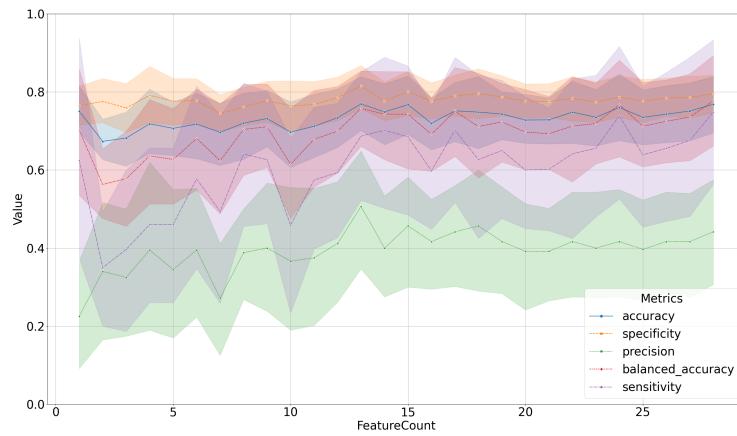


Figure 61: Metrics by Feature Count with Deblur and HOMD for Merging (Moderate+Severe) Classes

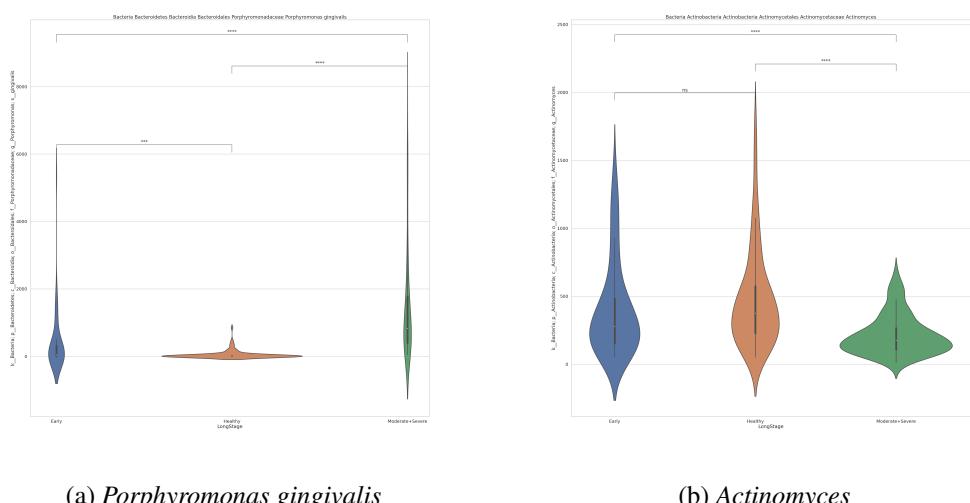


Figure 62: Most and Second Most Important Features with Deblur and HOMD for Merging (Moderate+Severe) Classes

Table 28: Taxa with Deblur and HOMD Ordered by Random Forest for Merging (Moderate+Severe) Classes

| Order | Taxonomy Classification | Importances |
|-------|--|-----------------------|
| 0 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae <i>Porphyromonas gingivalis</i> | 0.4144686252638225 |
| 1 | Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae <i>Actinomyces</i> | 0.08873724892929263 |
| 2 | Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae | 0.05872096059326508 |
| 3 | Bacteria Firmicutes Negativicutes Veillonellales Veillonellaceae <i>Veillonella denticariosi</i> | 0.0549195919147654 |
| 4 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV <i>Oribacterium sinus</i> | 0.04875410567003475 |
| 5 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI <i>Peptostreptococcus anaerobius</i> | 0.03859001648845735 |
| 6 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae <i>Treponema</i> | 0.03343455221088604 |
| 7 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae <i>Prevotella nanceiensis</i> | 0.029135391759886588 |
| 8 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae <i>Porphyromonas sp. HMT 285</i> | 0.02341829862659222 |
| 9 | Bacteria Firmicutes Clostridia Clostridiales Peptoniphilaceae <i>Parvimonas sp. HMT 393</i> | 0.022358291951558314 |
| 10 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI <i>Peptostreptococcaceae XIG-6 nodatum</i> | 0.019543904306518294 |
| 11 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI <i>Filifactor alocis</i> | 0.018425404323942896 |
| 12 | Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desulfovulbaceae <i>Desulfobulbus sp. HMT 041</i> | 0.018419168652741125 |
| 13 | Bacteria Synergistetes Synergistia Synergistales Synergistaceae <i>Fretibacterium</i> | 0.017107142536152323 |
| 14 | Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae <i>Mycoplasma</i> | 0.01487882742536733 |
| 15 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV <i>Catonella sp. HMT 164</i> | 0.013850705225041114 |
| 16 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI <i>Mogibacterium</i> | 0.01232424594626922 |
| 17 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI <i>Peptostreptococcaceae XIG-9 brachy</i> | 0.01130947752210783 |
| 18 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae <i>Tannerella forsythia</i> | 0.01096361242183682 |
| 19 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV <i>Lachnospiraceae G-8 bacterium HMT 500</i> | 0.0103642993062346 |
| 20 | Bacteria Bacteroidetes Bacteroidetes C-1 Bacteroidetes O-1 Bacteroidetes F-1 Bacteroidetes G-3 | 0.008213542625615187 |
| 21 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae <i>Prevotella dentalis</i> | 0.007866054386698889 |
| 22 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae <i>Treponema sp. HMT 260</i> | 0.00602082301326922 |
| 23 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI <i>Peptostreptococcaceae XIG-5 saphenum</i> | 0.005679967474816547 |
| 24 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI <i>Peptostreptococcaceae XIG-4 bacterium HMT 369</i> | 0.003860710789744734 |
| 25 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV <i>Johnsonella sp. HMT 166</i> | 0.003213540498893907 |
| 26 | Bacteria Firmicutes Clostridia Clostridiales Clostridiales F-1 Clostridiales F-1G-1 bacterium HMT 093 | 0.0027569916822530816 |
| 27 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae <i>Treponema sp. HMT 258</i> | 0.0026644984539360215 |

4.11 Random Forest Classifier with Merging (Healthy+Early) & (Moderate+Severe) Classes

As figure 3 and figure 4, there are six combinations. However, there are not statistically significant difference between (Healthy and Early) classes and between (Moderate and Severe) classes. Thus, classification algorithm is carried out on these six combinations with merging (Healthy and Early) classes and (Moderate and Severe) classes. Among these six combinations, DADA2 and SILVA pair gives the best result in terms of balanced accuracy. Features which are ordered by importance are in talbe 29. Also, five metrics by feature count are shown as figure 63; then, the highest value of balanced accuracy is 0.934 with using 15 taxa. Moreover, violin plots from the two most important taxa, *Porphyromonas gingivalis* and *Treponema denticola*, are in figure 64.

4.12 Random Forest Classifier with Healthy Class and Early Class Only

5 Discussion

5.1 Alpha-diversity

Alpha-diversity indices among all groups from DADA2 are in table 2. Shannon's diversity index in DADA2, though, has marginally significant p-value; the other indices have strongly significant p-values. Additionally, there are no statistically significant differences between (Healthy and Early) classes and (Moderate and Severe) classes with evenness index from DADA2 (Table 3 and Figure 15). Also, there is no statistically significant difference between (Healthy and Early) classes with Faith's phylogenetic diversity index from DADA2 (Table 4 and Figure 16). Moreover, there are no statistically significant differences between (Early and Moderate) classes, (Early and Severe) classes and (Moderate and Severe) classes with observed feature index from DADA2 (Table 5 and Figure 17). Furthermore, there are no statistically significant differences between (Healthy and Moderate) classes, (Healthy and Severe) classes, (Early and Moderate) classes and (Moderate and Severe) classes from Shannon's diversity index from DADA2 (Table 6 and Figure 18).

Alpha-diversity indices among all groups from Deblur are in table 7. Every index have strongly significant p-values. Additionally, there are no statistically significant differences between (Healthy and Early) classes, (Healthy and Moderate) classes, (Healthy and Severe) classes and (Moderate and Severe) classes with evenness index from Deblur (Table 8 and Figure 19). Also, there are no statistically significant differences between (Healthy and Early) classes and (Moderate and Severe) classes with Faith's phylogenetic diversity index from Deblur (Table 9 and Figure 20). Moreover, there are no statistically significant differences between (Healthy and Early) classes and (Moderate and Severe) classes with observed features index from Deblur (Table 10 and Figure 21). Furthermore, there are no statistically significant differences between (Healthy and Early) classes and (Moderate and Severe) classes with Shannon's diversity index from Deblur (Table 11 and Figure 22).

Merging similar classes could elevate classification metrics; while merging must result mere primitive classification than without merging classes. Accordingly, deciding merged classes should be rigorous and resolutely reasoned. In result, many pairs of classes should be merged as value of alpha-diversity indices, for instance (Healthy and Early) classes, (Healthy and Severe) classes and (Moderate and Severe) classes. Despite alpha-diversity indices show no significant differences, some pairs of classes have to refuse to be merged in two major reasons. First, merging those classes is fallacious. For example, (Healthy and Severe) classes, without loss of generality. Healthy class and Severe class does not adjoin each other, in terms of clinical stage. Second, even those classes are adjacent each other, some pairs of classes have not enough results to merge. For instance, null hypothesis from (Early and Moderate) classes is only sustained by Shannon's diversity index (Table 6), so merging Early class and Moderate class cannot be reasoned. Hence, two pairs of classes will be merged in classification: (Healthy and Early) classes and (Moderate and Severe) classes.

5.2 Beta-diversity

From data with DADA2, all beta-diversity distance index, includes Bray-Curtis distance index (Table 12, Figure 23 and Figure 24), Jaccard distance index (Table 13, Figure 25 and Figure 26), unweighted UniFrac distance index (Table 14, Figure 27 and Figure 28) and weighted UniFrac distance index (Table 15, Figure 29 and Figure 30), show statistically significant differences in every pair of classes, except (Moderate and Severe) classes.

Bray-Curtis distance index with Deblur has no statistically significant differences between (Healthy and Early) classes and (Moderate and Severe) classes (Table 16, Figure 31 and Figure 32). Moreover, Jaccard distance index with Deblur has no statistically significant difference between (Moderate and Severe) classes (Table 17, Figure 33 and Figure 34). Additionally, unweighted UniFrac distance index with Deblur has no statistically significant difference between (Moderate and Severe) classes (Table 18, Figure 35 and Figure 36). Furthermore, weighted UniFrac distance index with Deblur has no statistically significant difference between (Healthy and Early) classes (Table 38, Figure 37 and Figure 38).

As results of alpha-diversity indices, (Healthy and Early) classes and (Moderate and Severe) classes will be merged. Mercifully, alternative fact does totally not sustained by beta-divesity indices. Hence, (Healthy and Early) classes and (Moderate and Severe) classes, as mentioned herein-before, will be merged in classification.

Table 29: Taxa with DADA2 and SILVA Ordered by Random Forest for Merging (Healthy+Early) & (Moderate+Severe) Classes

| Order | Taxonomy Classification | Importances |
|-------|---|----------------------|
| 0 | Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 0.17687976430182906 |
| 1 | Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema denticola | 0.14012712311139505 |
| 2 | Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas gingivalis | 0.1289166118021612 |
| 3 | Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Prevotella Prevotella intermedia | 0.10096005217548329 |
| 4 | Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Filifactor Filifactor alocis | 0.08532065341737015 |
| 5 | Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Schaalialia odontolytica | 0.07617988203012623 |
| 6 | Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tannerella Tannerella forsythia | 0.04257924432451972 |
| 7 | Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium nodatum group Eubacterium nodatum | 0.0394033280870367 |
| 8 | Bacteria Actinobacteriota Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium Corynebacterium durum | 0.03888694653838536 |
| 9 | Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribacterium | 0.02874633485322674 |
| 10 | Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas | 0.025953291699330285 |
| 11 | Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema uncultured bacterium | 0.019969001887543223 |
| 12 | Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Actinomyces graevenitzii | 0.01669413939956822 |
| 13 | Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium saphenum group Eubacterium saphenum | 0.015478476262645296 |
| 14 | Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae Mycoplasma Metamycoplasma faicum | 0.012969704487821905 |
| 15 | Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema medium | 0.011723998306095005 |
| 16 | Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium brachy group Eubacterium brachy | 0.010018818489078716 |
| 17 | Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae | 0.007674062157826236 |
| 18 | Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema maltophilum | 0.005390066368971191 |
| 19 | Bacteria Synergistota Synergistia Synergistales Synergistaceae Fretibacterium | 0.004413474258744408 |
| 20 | Bacteria Campylobacterota Campylobacteria Campylobacteriales Campylobacteraceae Campylobacter Campylobacter showae | 0.00425722837145789 |
| 21 | Bacteria Firmicutes Clostridia Lachnospirales Defluvitaleaceae Defluvitaleaceae UCG-011 Lachnospiraceae bacterium | 0.003786126928675039 |
| 22 | Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema | 0.00367167074070895 |

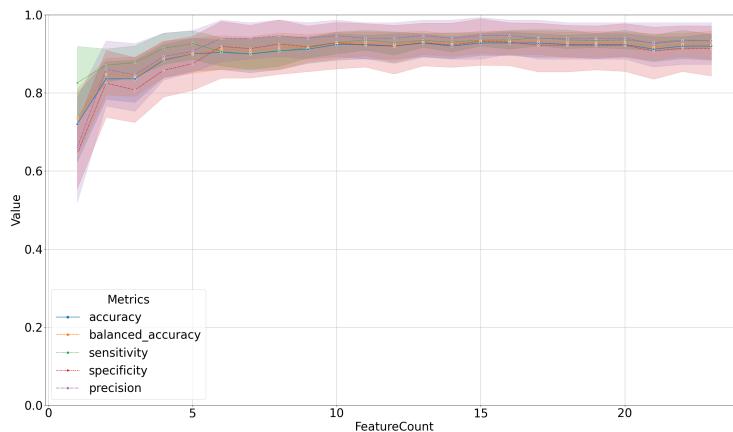


Figure 63: Metrics by Feature Count with DADA2 and SILVA for Merging (Healthy+Early) & (Moderate+Severe) Classes

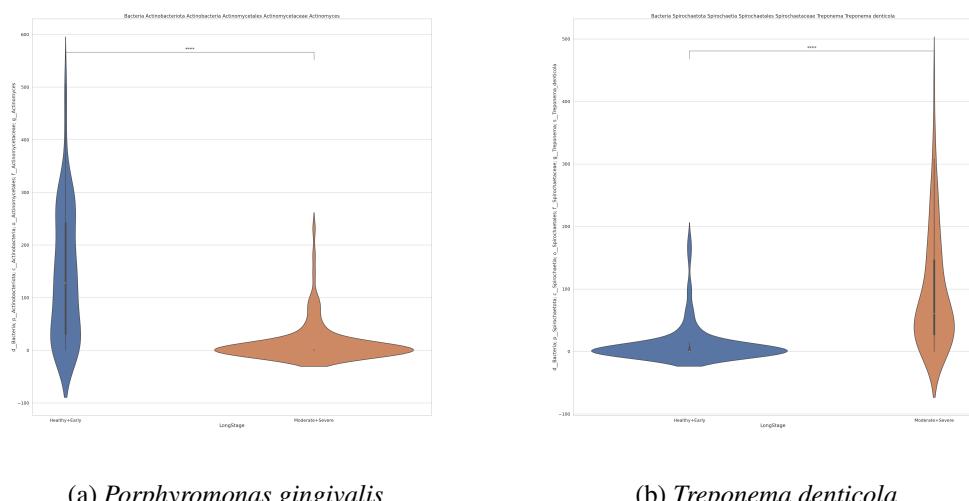


Figure 64: Most and Second Most Important Features with DADA2 and HOMD for Merging (Healthy+Early) & (Moderate+Severe) Classes

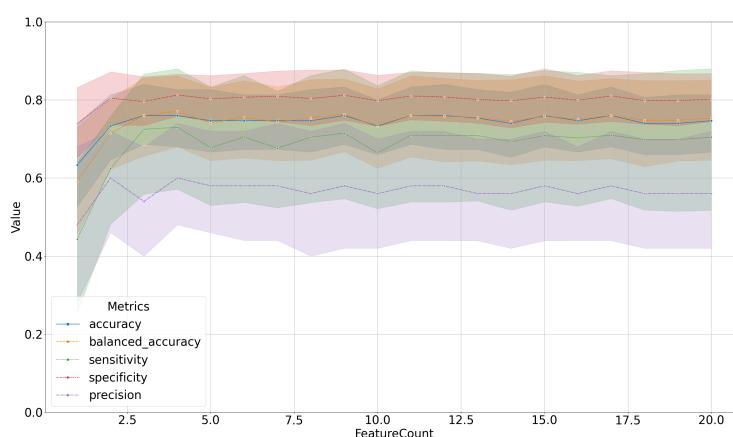


Figure 65: Metrics by Feature Count with DADA2 and HOMD for Healthy Class and Early Class Only

Table 30: Taxa with DADA2 and HOMD Ordered by Random Forest for Healthy Class and Early Class Only

| Order | Taxonomy Classification | Importances |
|-------|--|-----------------------|
| 0 | Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces | 0.3800329614200073 |
| 1 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas gingivalis | 0.11599155442053782 |
| 2 | Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces graevenitzii | 0.08377015225545748 |
| 3 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Filifactor alocis | 0.07355851820527473 |
| 4 | Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Lachnospiraceae G-8 bacterium HMT 500 | 0.055862102028897874 |
| 5 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas sp. HMT 285 | 0.051266737996019496 |
| 6 | Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales Campylobacteraceae Campylobacter showae | 0.03768573586643332 |
| 7 | Bacteria Actinobacteria Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium durum | 0.032556906788312044 |
| 8 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia | 0.02510972430269449 |
| 9 | Bacteria Synergistetes Synergistia Synergistales Synergistaceae Fretibacterium | 0.020454891486853158 |
| 10 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella forsythia | 0.02023382279668738 |
| 11 | Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma faicum | 0.019175163953451355 |
| 12 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema | 0.01699482819483286 |
| 13 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-9 brachy | 0.01474114962345347 |
| 14 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema putidum | 0.012552567408042328 |
| 15 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 526 | 0.009883103591414436 |
| 16 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-6 nodatum | 0.009487308947517453 |
| 17 | Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 260 | 0.008833841116352032 |
| 18 | Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-5 saphenum | 0.008254892599607861 |
| 19 | Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 304 | 0.0035540369981530828 |

5.3 t-SNE Plot

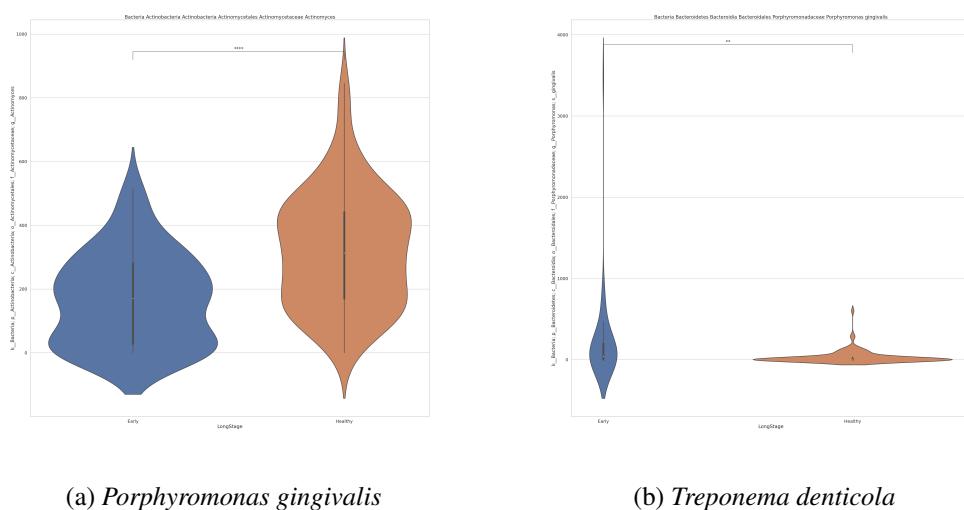
Overall distribution of taxa from each sample can be realized by t-SNE plot. If each class is evenly distributed on t-SNE plot, then the data might be difficult for classifying. In this manner, t-SNE plots with whole microbiome (Figure 45, Figure 46, Figure 47, Figure 48, Figure 49 and Figure 50) are more evenly distributed, whereas t-SNE plots with ANCOM selected microbiome data (Figure 51, Figure 52, Figure 53, Figure 54, Figure 55 and Figure 56) are biased by classes. *Id est*, data with ANCOM selected microbiome could result better in classifying. Hence, ANCOM selected taxa will be used in classification.

5.4 Random Forest Classifier

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(a) *Porphyromonas gingivalis*

(b) *Treponema denticola*

Figure 66: Most and Second Most Important Features with DADA2 and HOMD for Healthy Class and Early Class Only