

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).

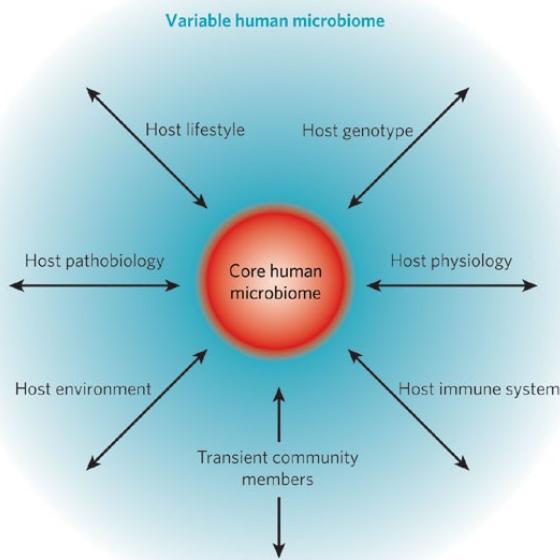


Figure 1: Concept of a Core Human Microbiome (Turnbaugh et al., 2007)

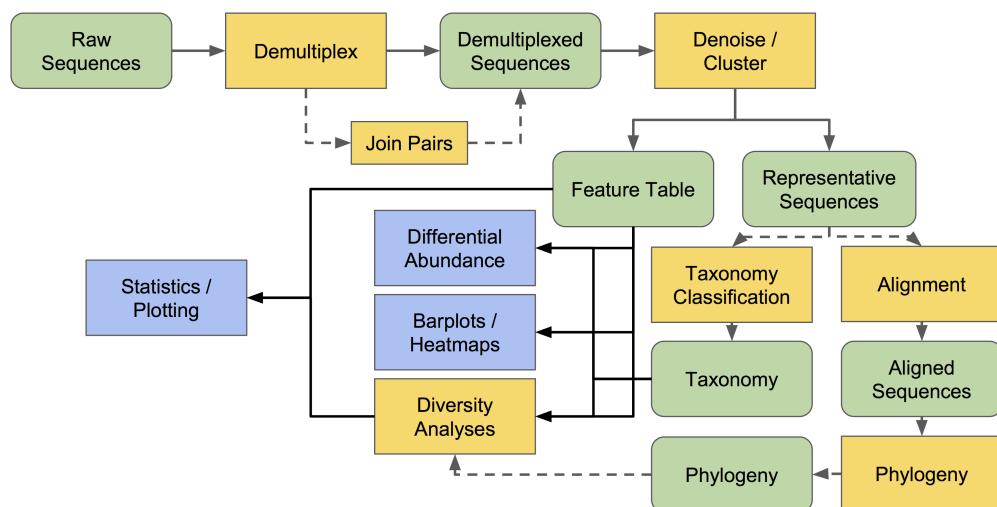


Figure 2: A Theoretic Overview of QIIME2 Workflow (Bolyen et al., 2019, 2018)

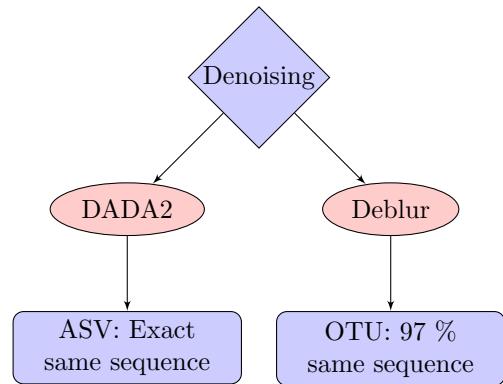


Figure 3: Denoising Techniques which provided by QIIME2

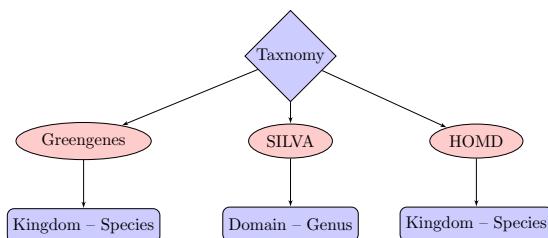


Figure 4: Taxonomy Classification which provided by QIIME2

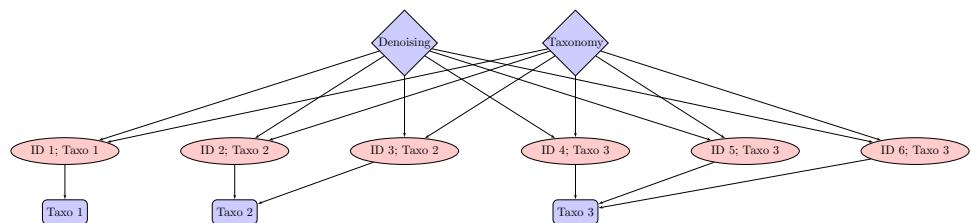


Figure 5: Example Diagram for Merging Denoising and Taxonomy Classification

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).

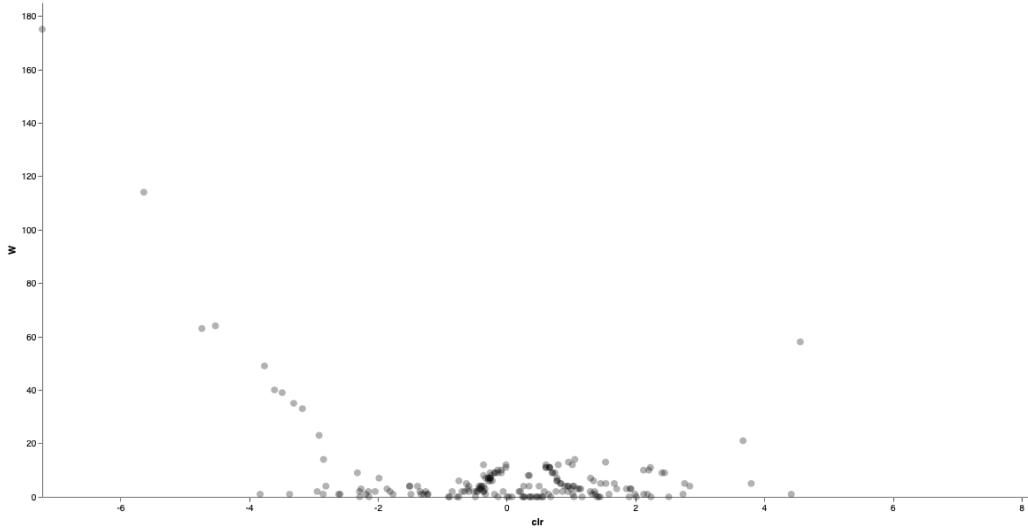


Figure 6: Example ANCOM Volcano Plot which Provided by QIIME2 (Bolyen et al., 2019, 2018)

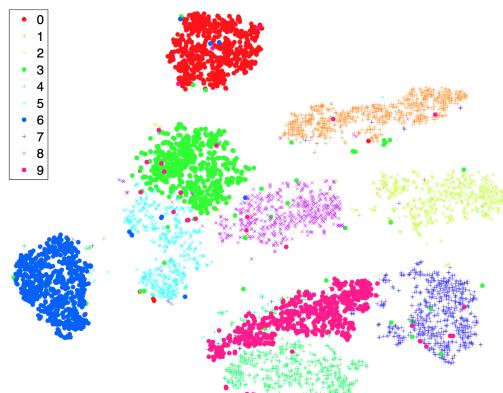


Figure 7: Visualization by t-SNE (Maaten & Hinton, 2008)

Table 1: Confusion Matrix

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

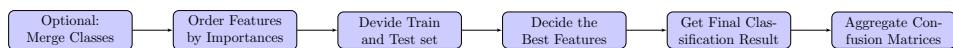


Figure 8: Workflow of Classification

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

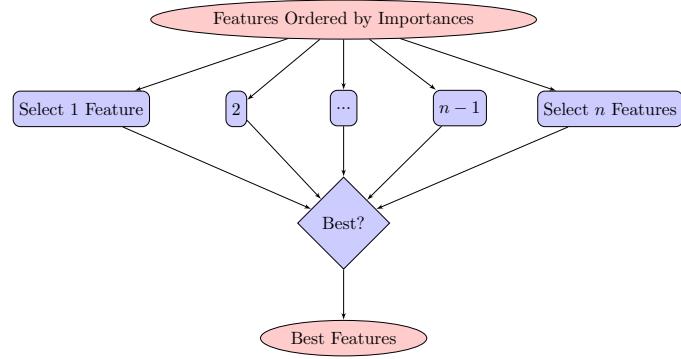


Figure 9: Deciding the Best Features

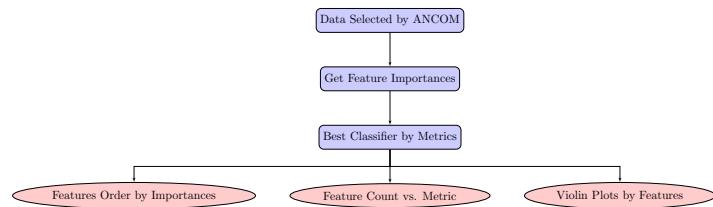


Figure 10: Random Forest Classifier Workflow

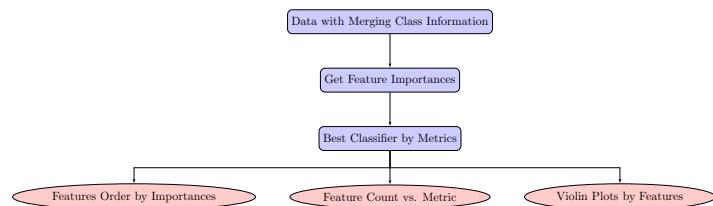


Figure 11: Random Forest Classifier Workflow with Merging

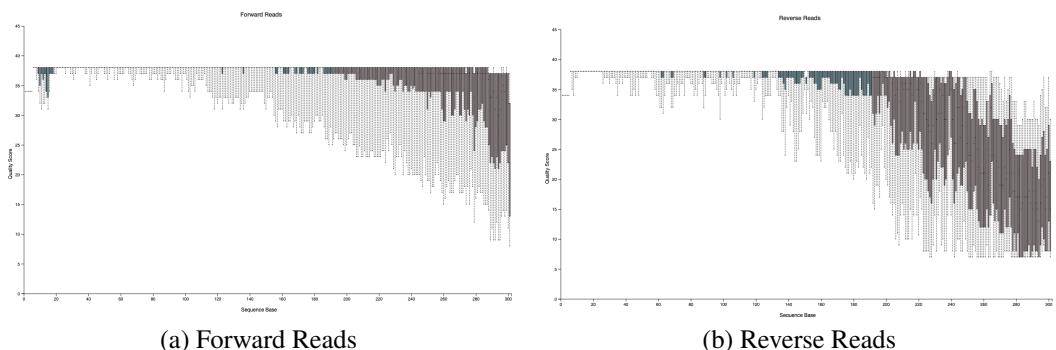


Figure 12: Sequence Quality Plot

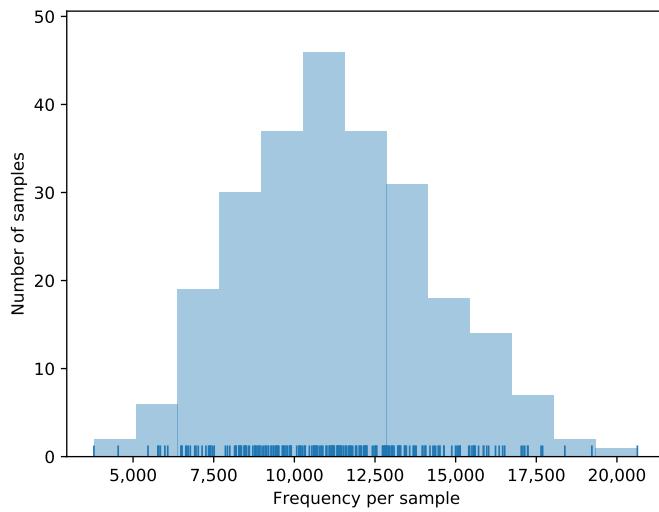


Figure 13: Frequency and Number per Sample by DADA2

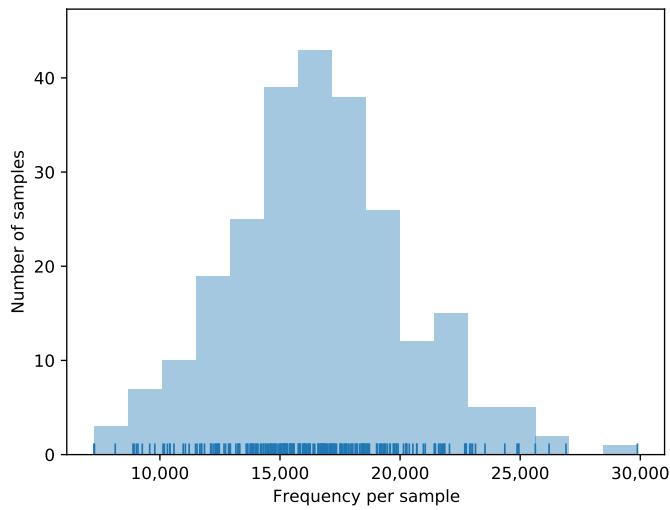


Figure 14: Frequency and Number per Sample by Deblur

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

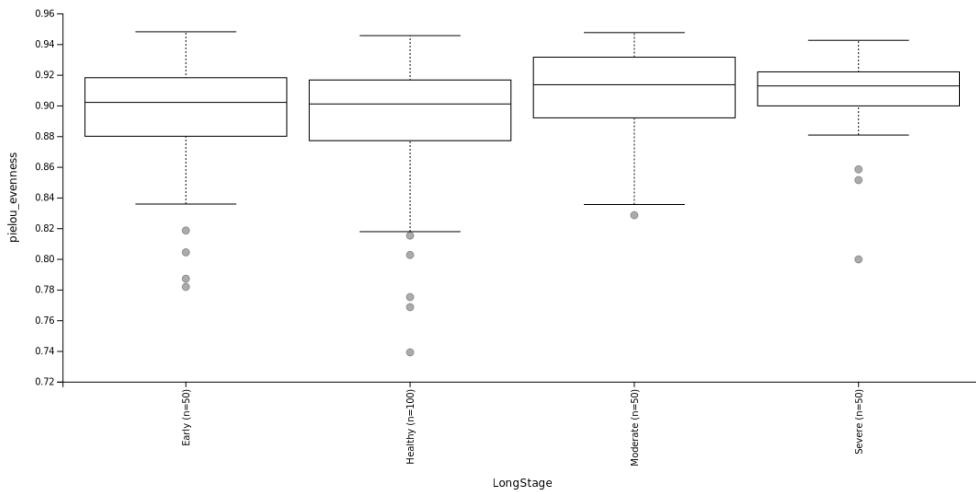


Figure 15: Evenness Index from DADA2

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

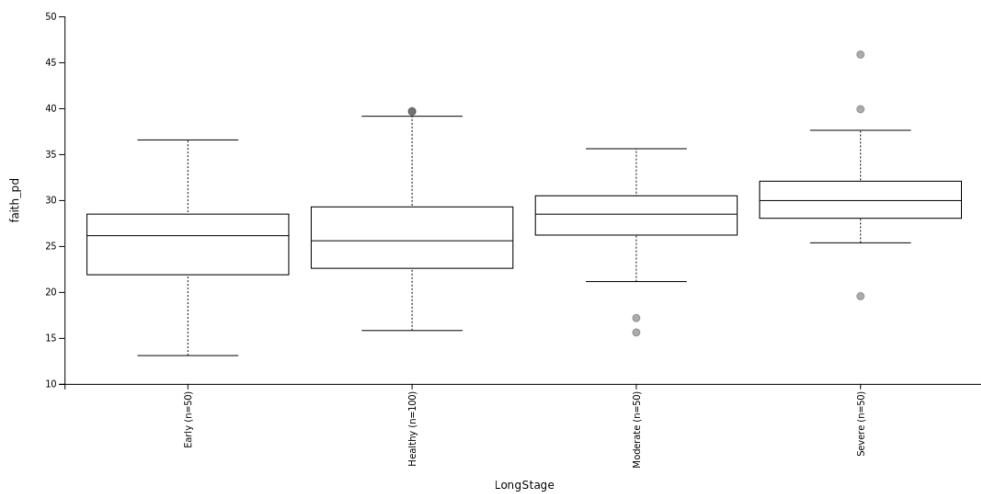


Figure 16: Faith PD Index from DADA2

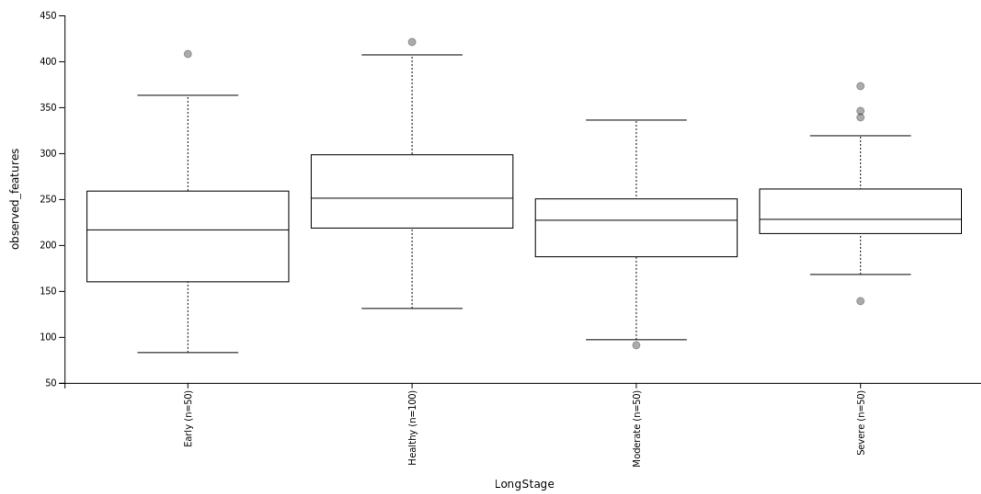


Figure 17: Observed Features Index from DADA2

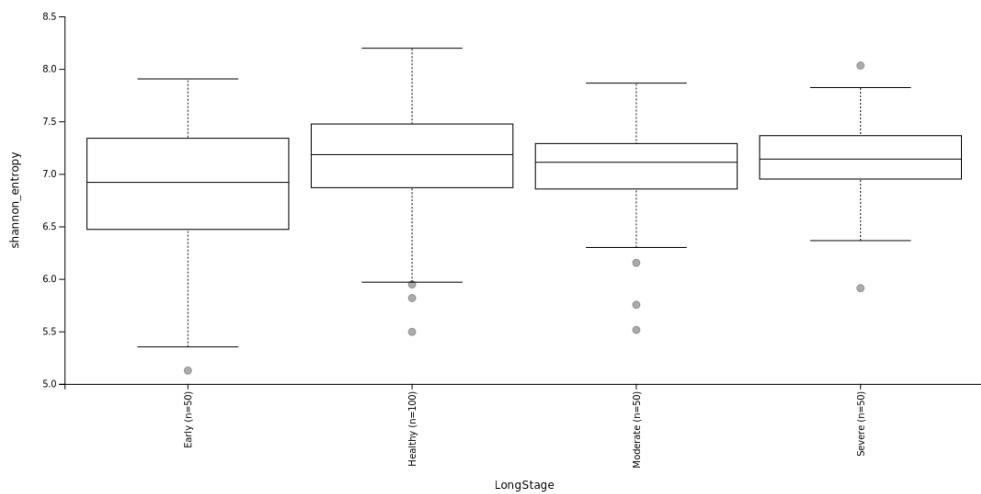


Figure 18: Shannon's Diversity Index from DADA2

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

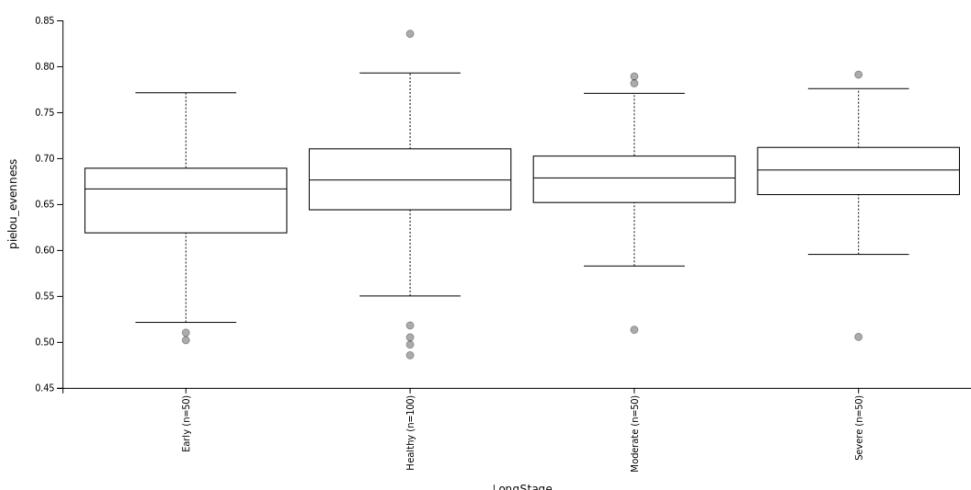


Figure 19: Evenness Index from Deblur

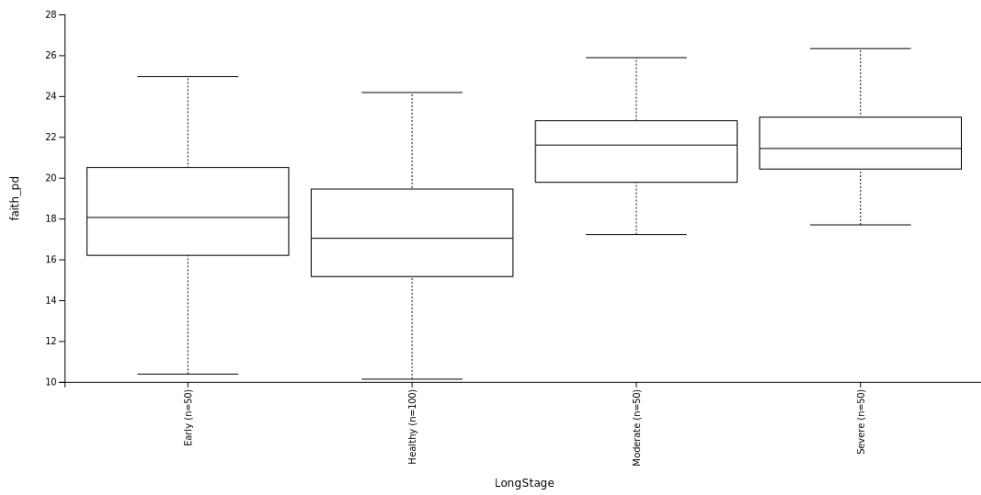


Figure 20: Faith PD Index from Deblur

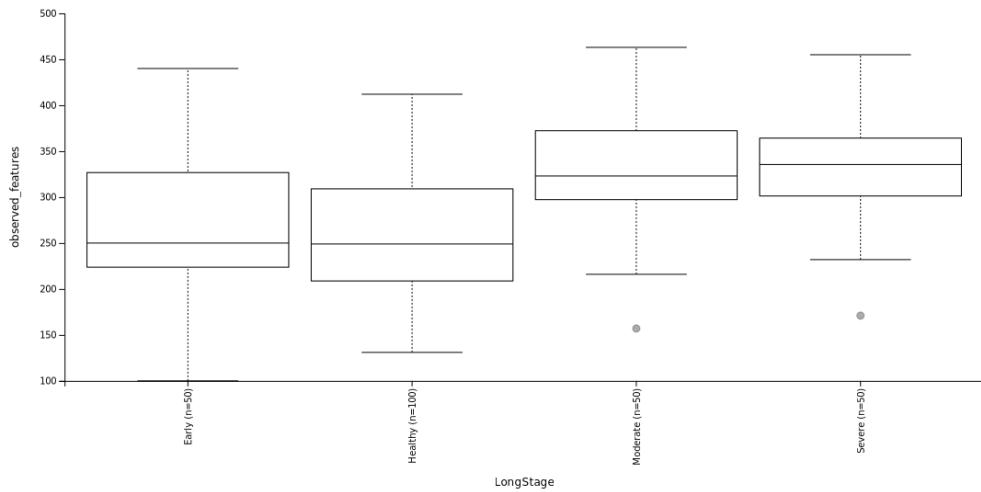


Figure 21: Observed Features Index from Deblur

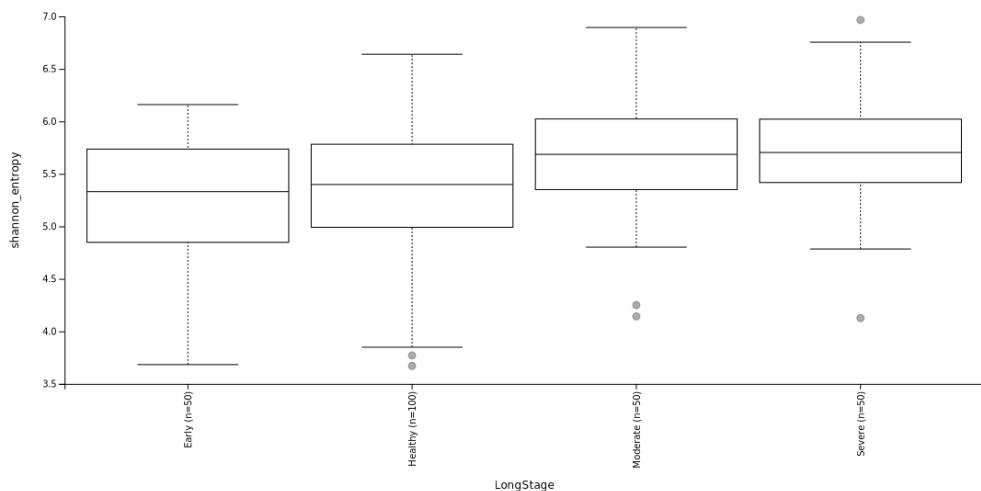


Figure 22: Shannon's Diversity Index from Deblur

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). Features are ordered by importance in every combination (Table 26, Table 27, Table 28, Table 29, Table 30 and Table 31); metrics changed by feature count in every combination are displayed (Figure 57, Figure 59, Figure 61, Figure 63, Figure 65 and Figure 61); and violin plots from the most two important features in every class are shown (Figure 58, Figure 60, Figure 62, Figure 64, Figure 66 and Figure 68).

Among these six combinations, Deblur and HOMD pair gives the best result in terms of balanced accuracy. Features which are ordered by importance are table 31. Also, five metrics by feature count are shown as figure 67; then, the highest value of balanced accuracy is 0.778 with using 13 taxa. Moreover, violin plots from two most important taxa, *Porphyromonas gingivalis* and (b) *Actinomyces*, are in figure 68.

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

As figures 3 and 4, there are six combinations. However, there is no significant difference between Healthy and Early classes. Thus, classification algorithm is carried out on these six combinations with merging Healthy and Early classes. In every combination, results were derived (Figure 11): Features which are ordered by importance (Table 32, Table 33, Table 34, Table 32, Table 36 and Table 37), metrics changed by feature count (Figure 69, Figure 71, Figure 73, Figure 75, Figure 77 and Figure 79) and violin plots from the most two important features (Figure 70, Figure 72, Figure 74, Figure 76, Figure 78 and Figure 80).

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

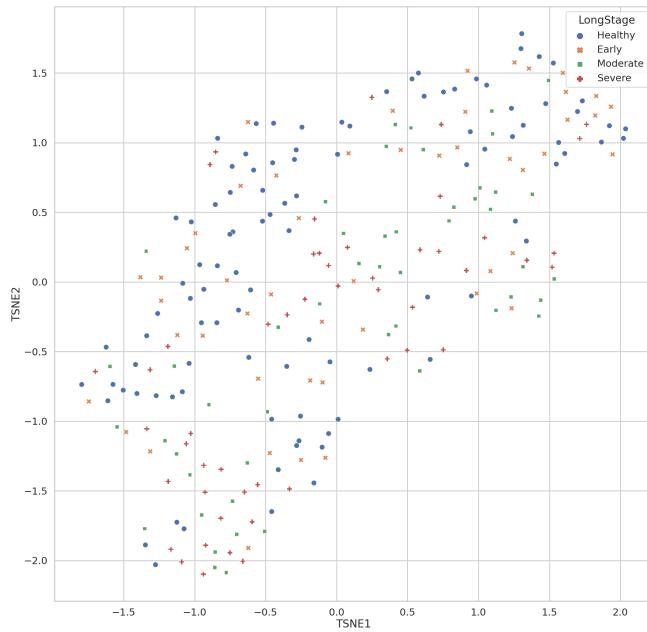


Figure 23: t-SNE Plot from Bray-Curtis Distance Index with DADA2

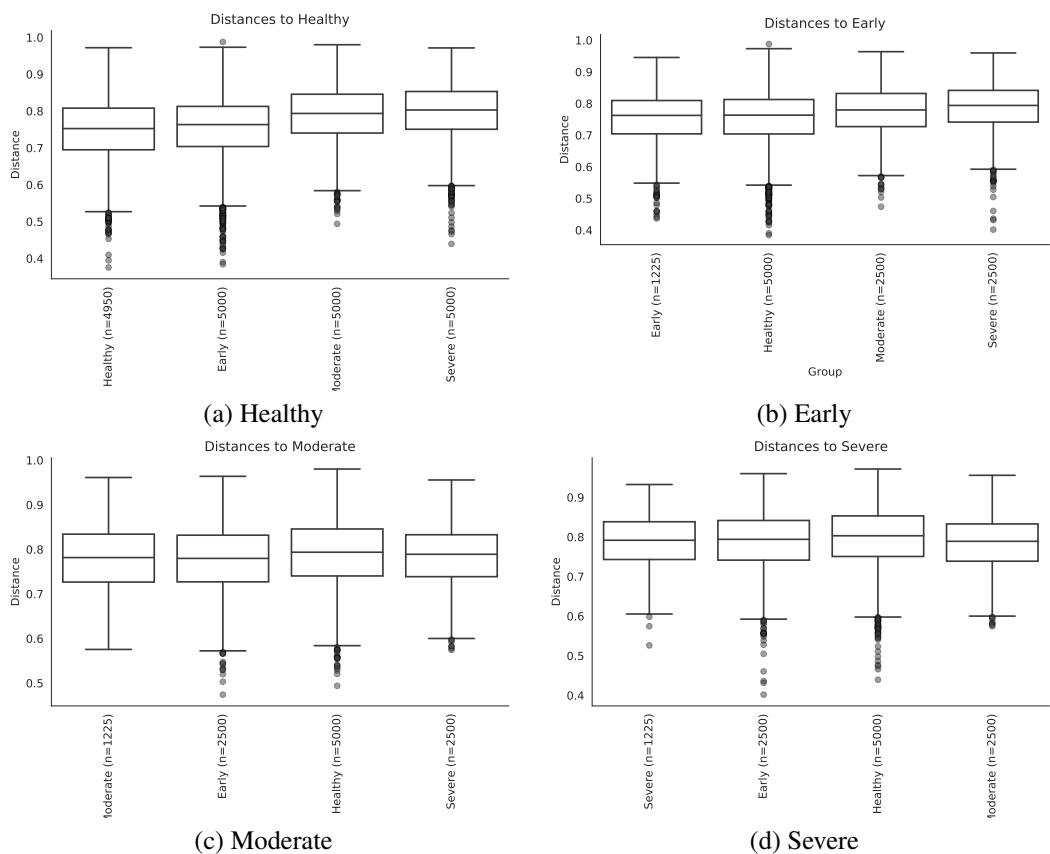


Figure 24: Bray-Curtis Distance Index with DADA2

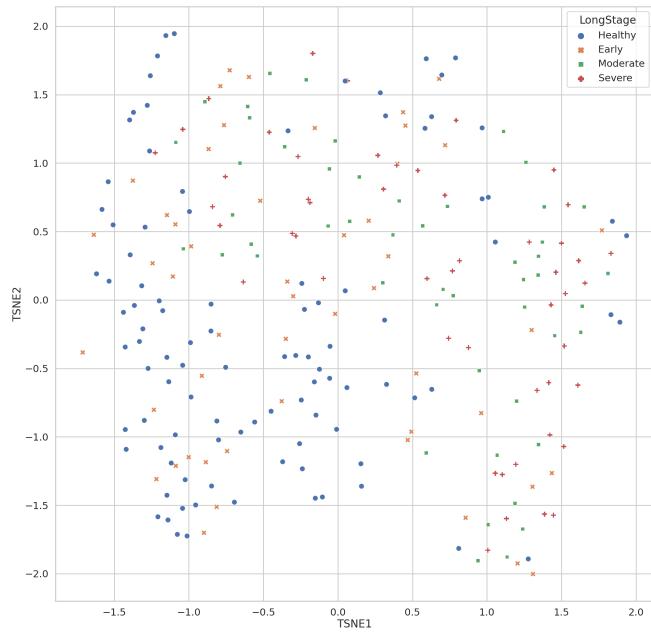


Figure 25: t-SNE Plot from Jaccard Distance Index with DADA2

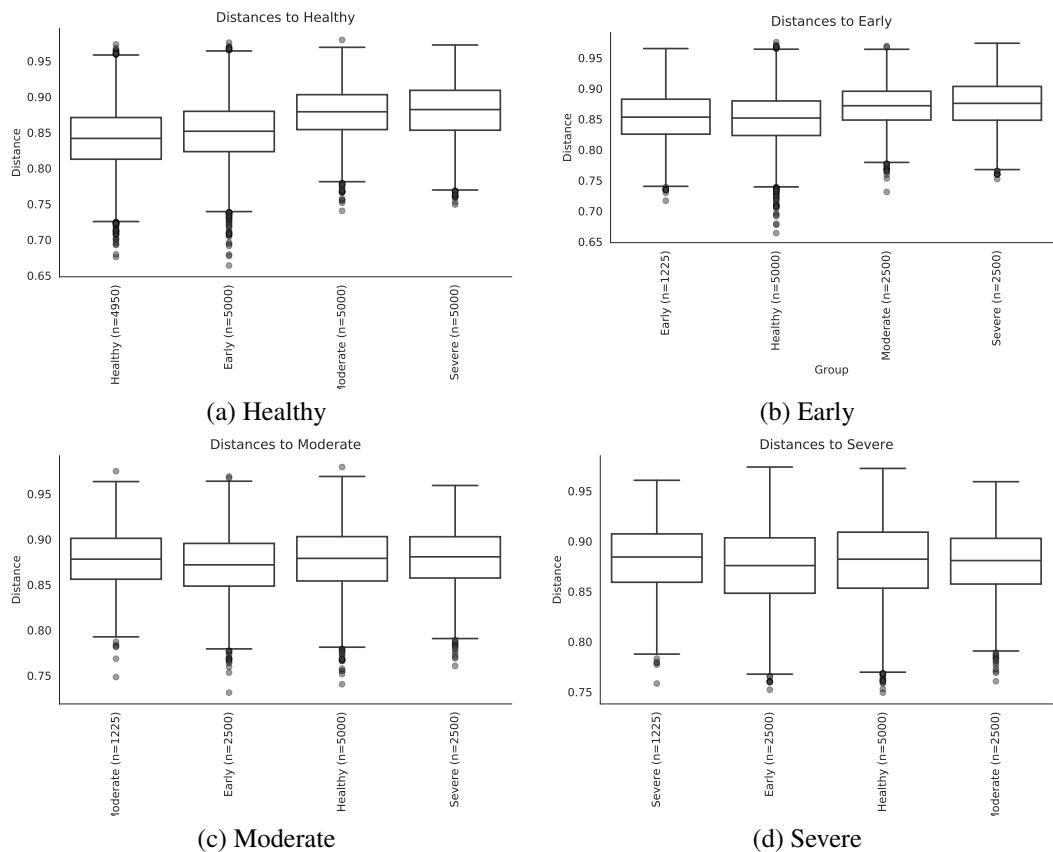


Figure 26: Jaccard Distance Index with DADA2

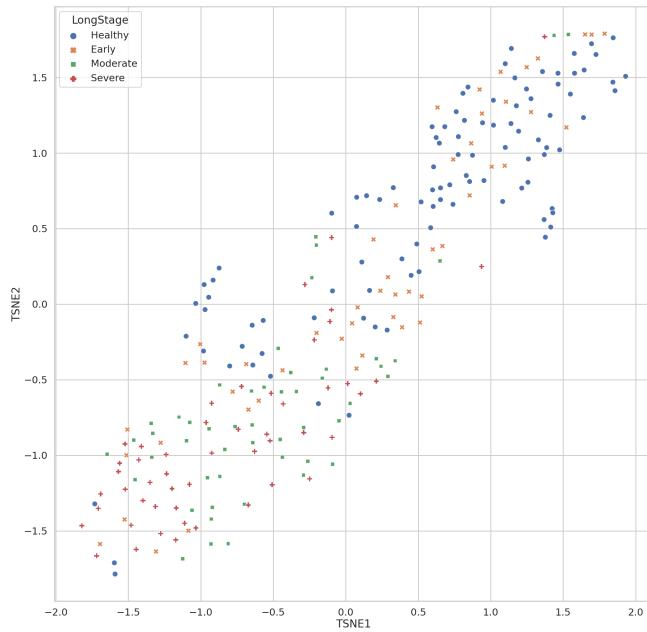


Figure 27: t-SNE Plot from Unweighted UniFrac Distance Index with DADA2

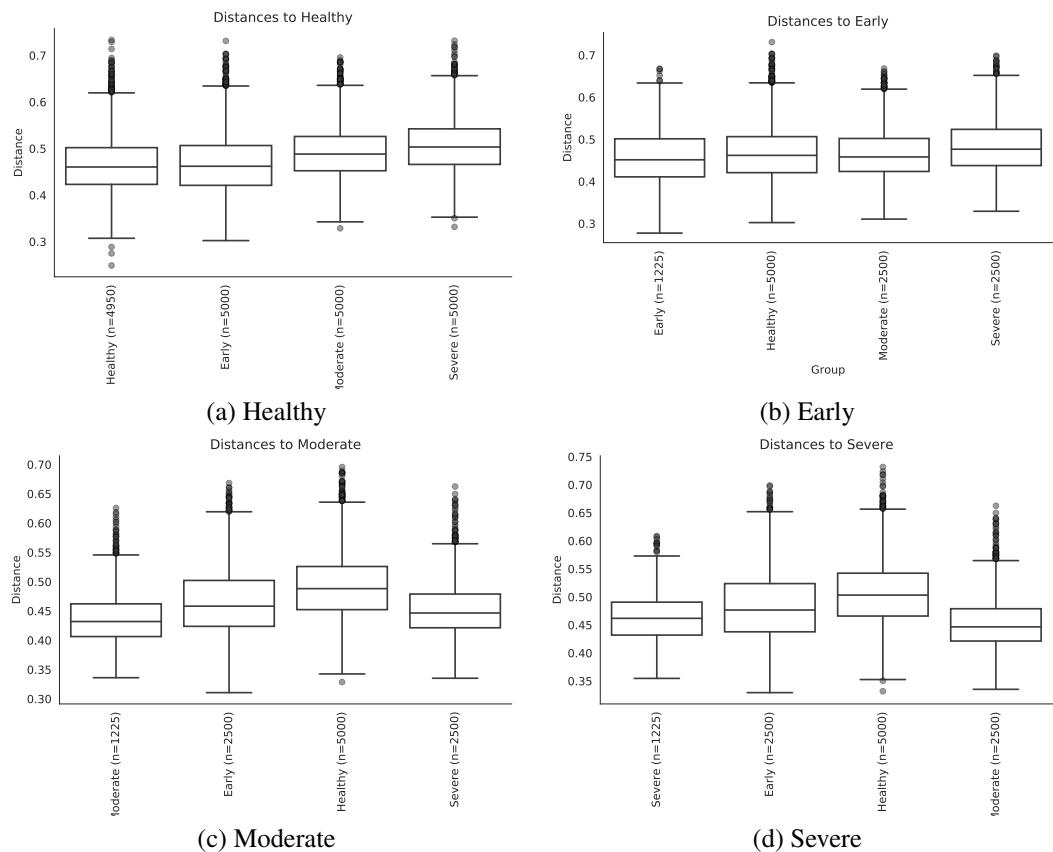


Figure 28: Unweighted UniFrac Distance Index with DADA2

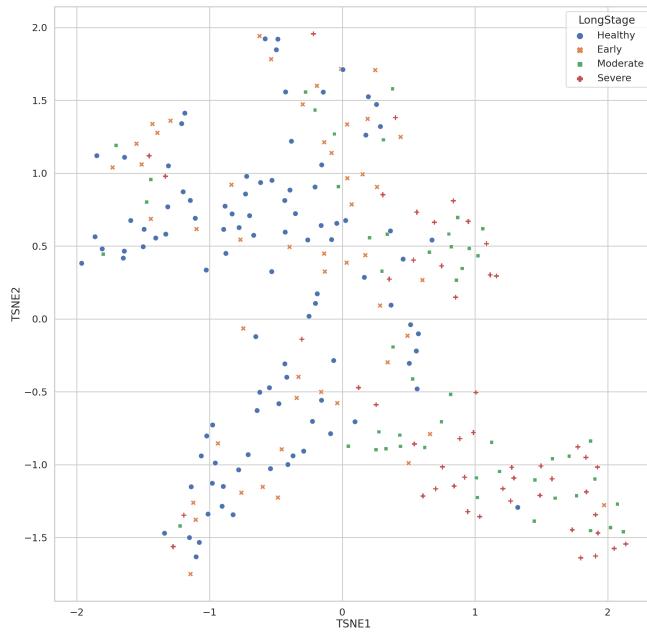


Figure 29: t-SNE Plot from Weighted UniFrac Distance Index with DADA2

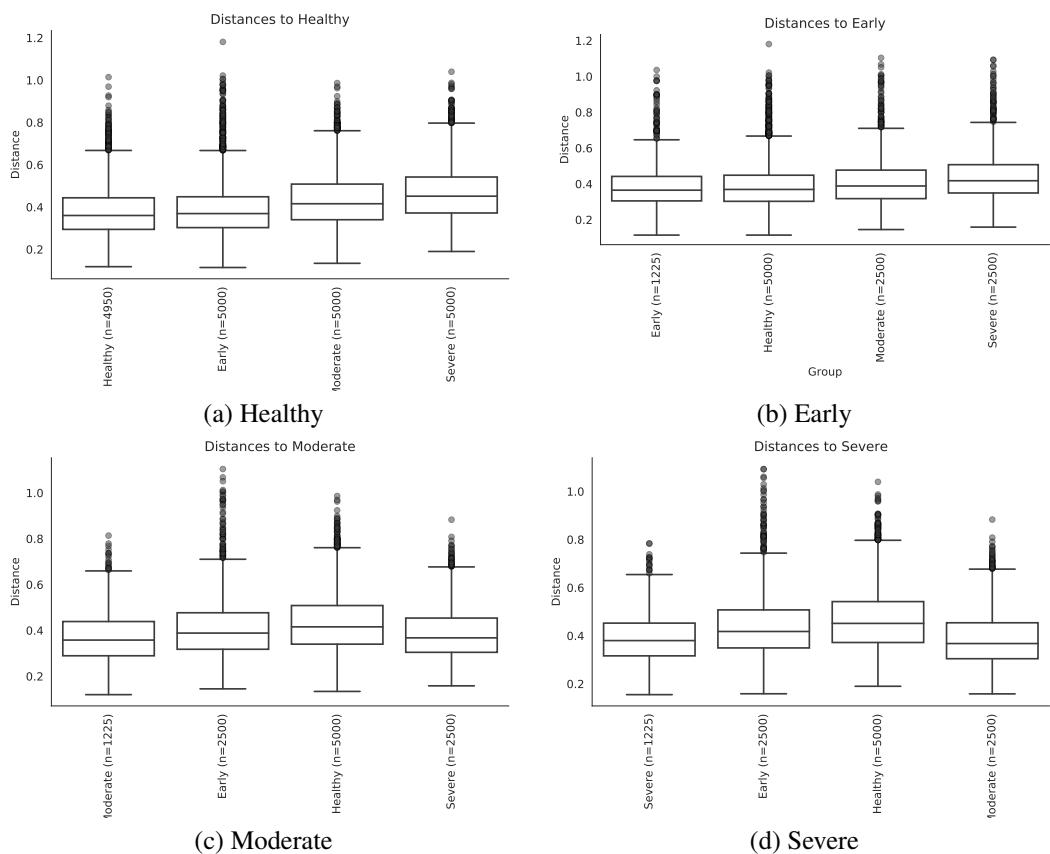


Figure 30: Weighted UniFrac Distance Index with DADA2

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

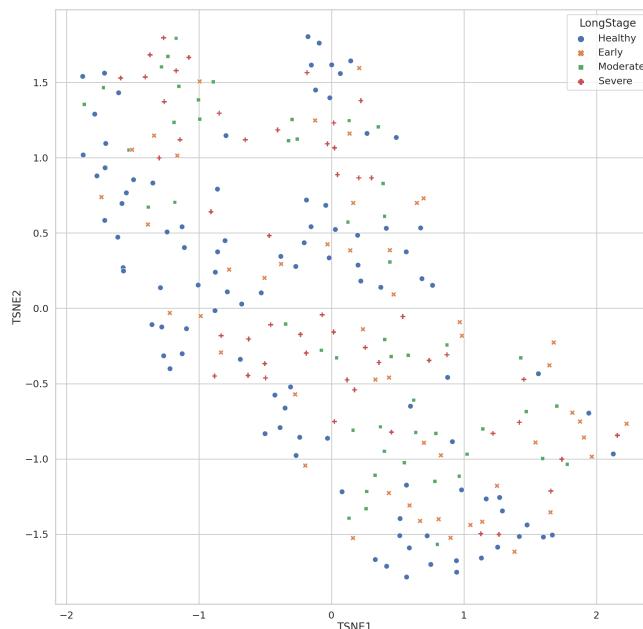


Figure 31: t-SNE Plot from Bray-Curtis Distance Index with Deblur

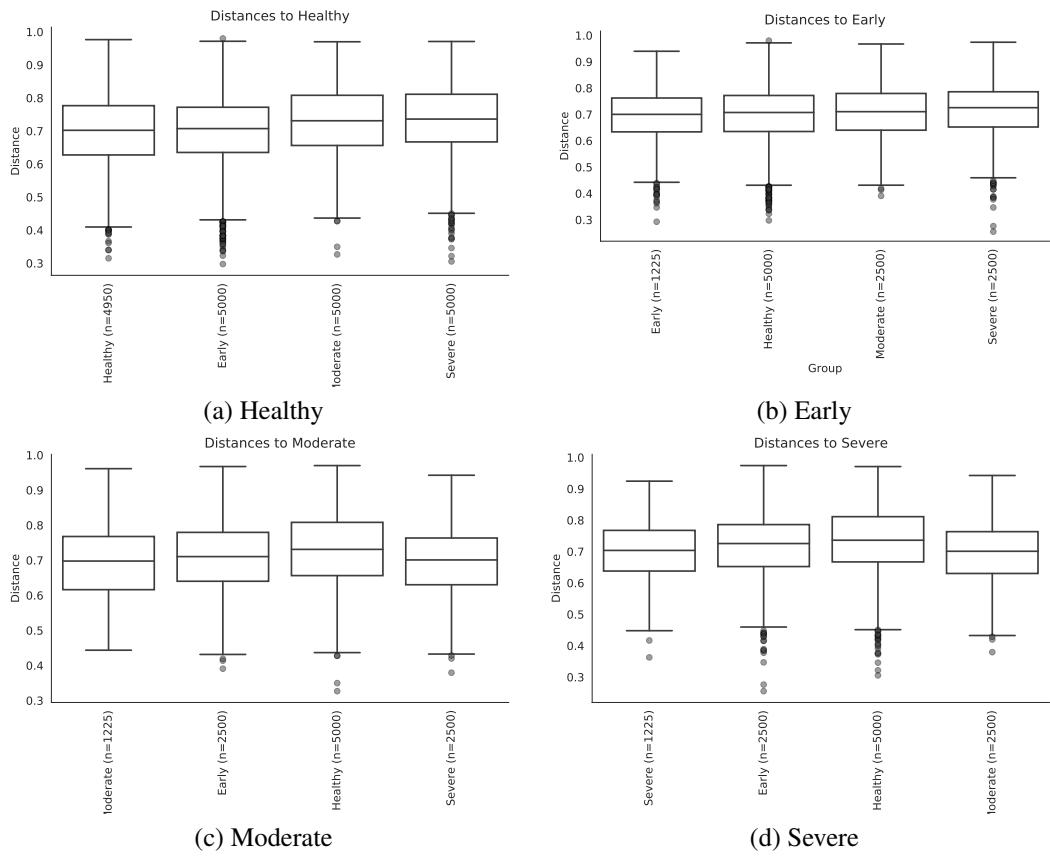


Figure 32: Bray-Curtis Distance Index with Deblur

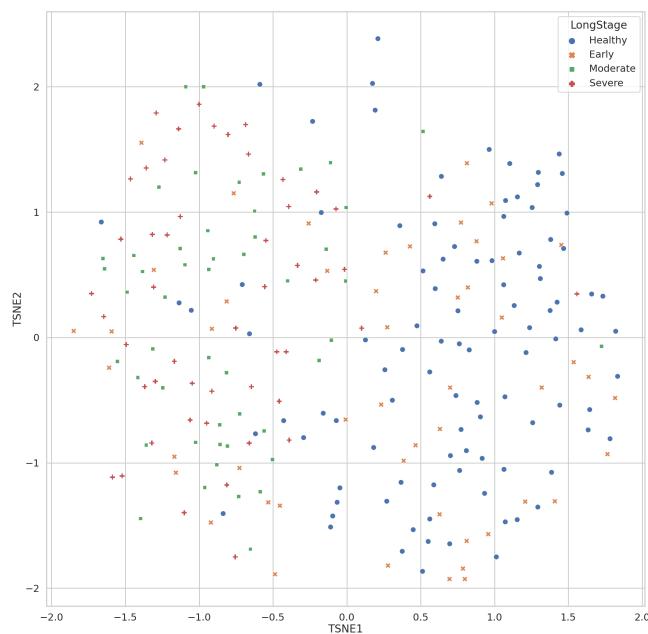


Figure 33: t-SNE Plot from Jaccard Distance Index with Deblur

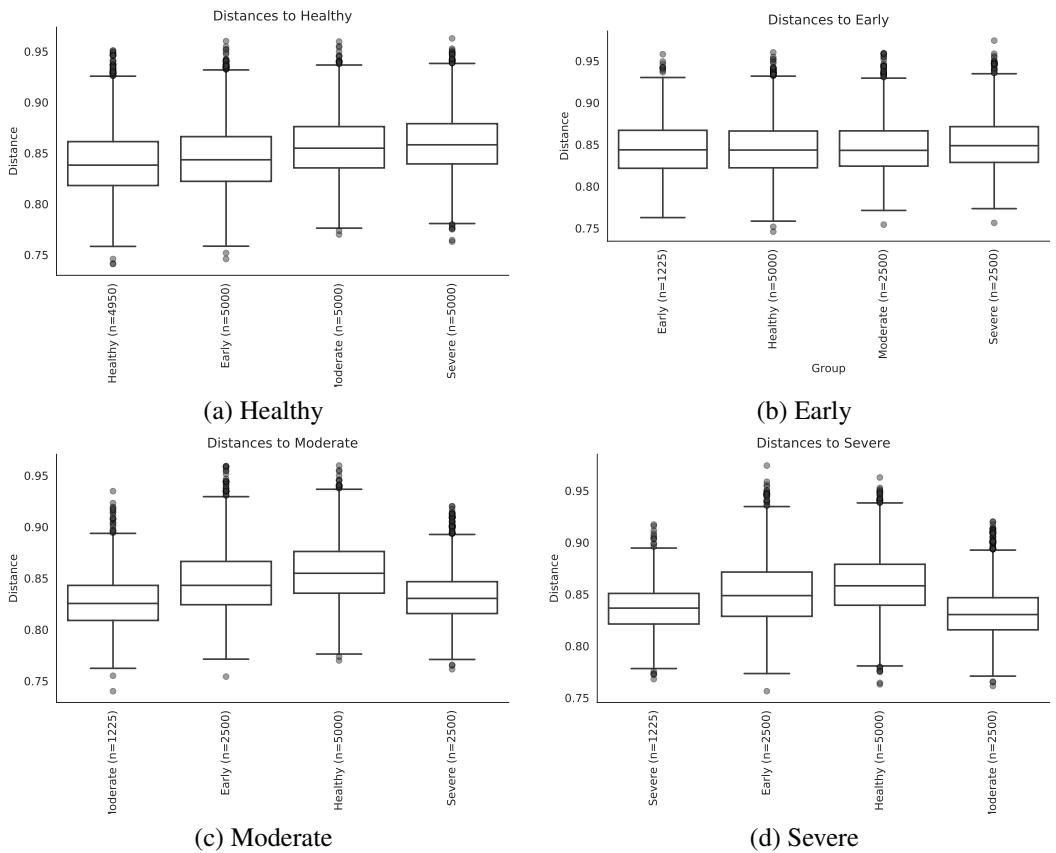


Figure 34: Jaccard Distance Index with Deblur

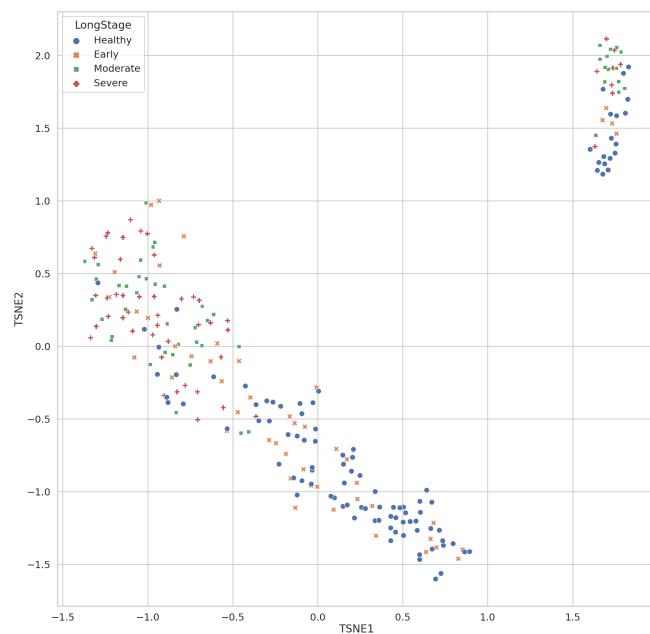


Figure 35: t-SNE Plot from Unweighted UniFrac Distance Index with Deblur

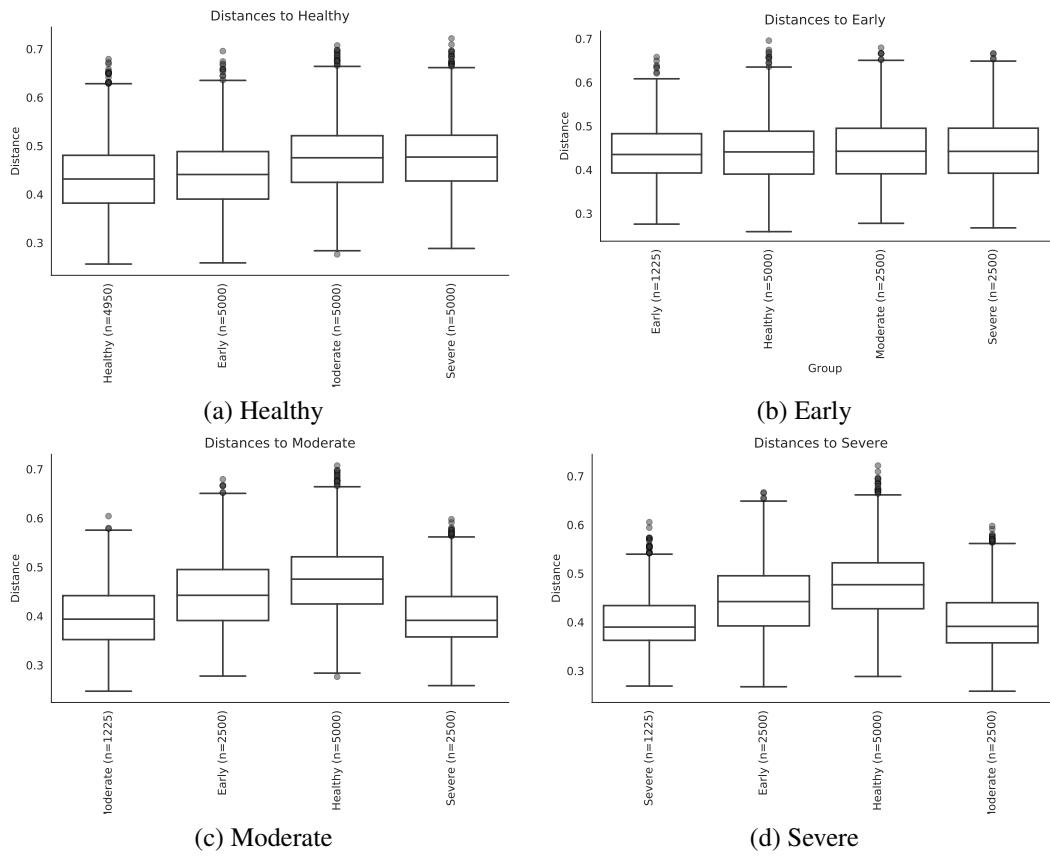


Figure 36: Unweighted UniFrac Distance Index with Deblur

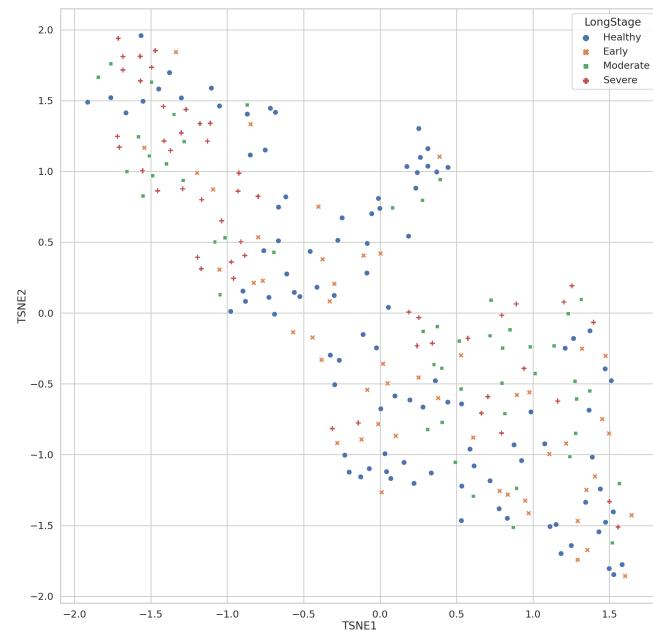


Figure 37: t-SNE Plot from Weighted UniFrac Distance Index with Deblur

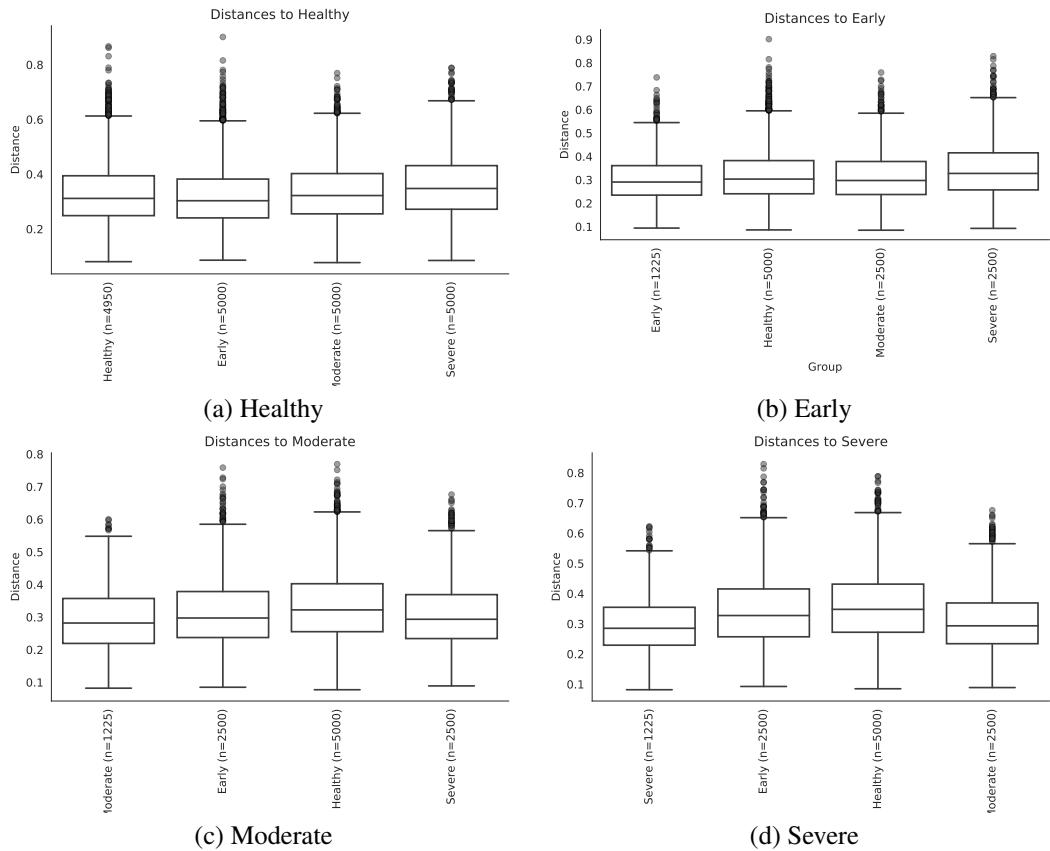


Figure 38: Weighted UniFrac Distance Index with Deblur

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

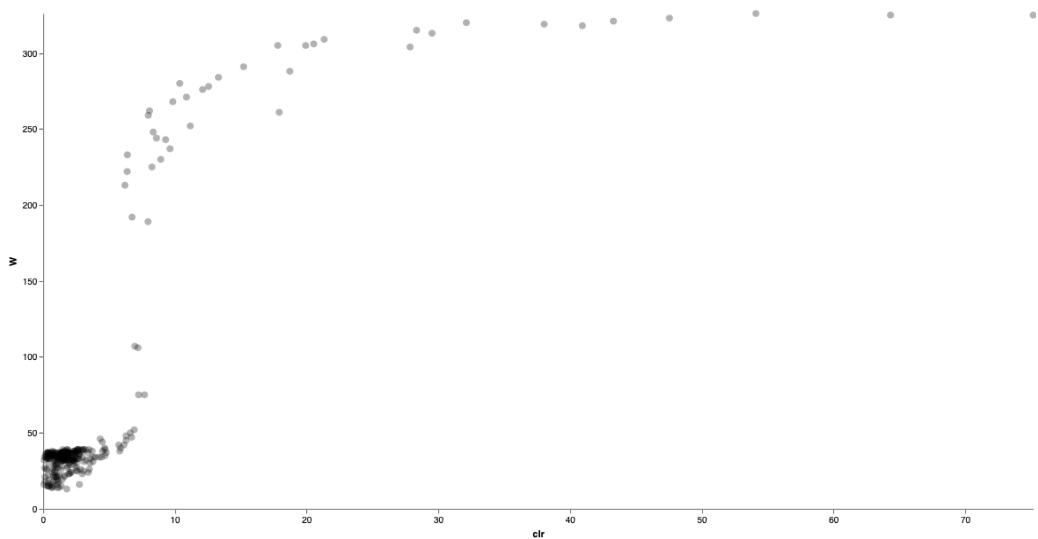


Figure 39: ANCOM Volcano Plot with DADA2 and GG

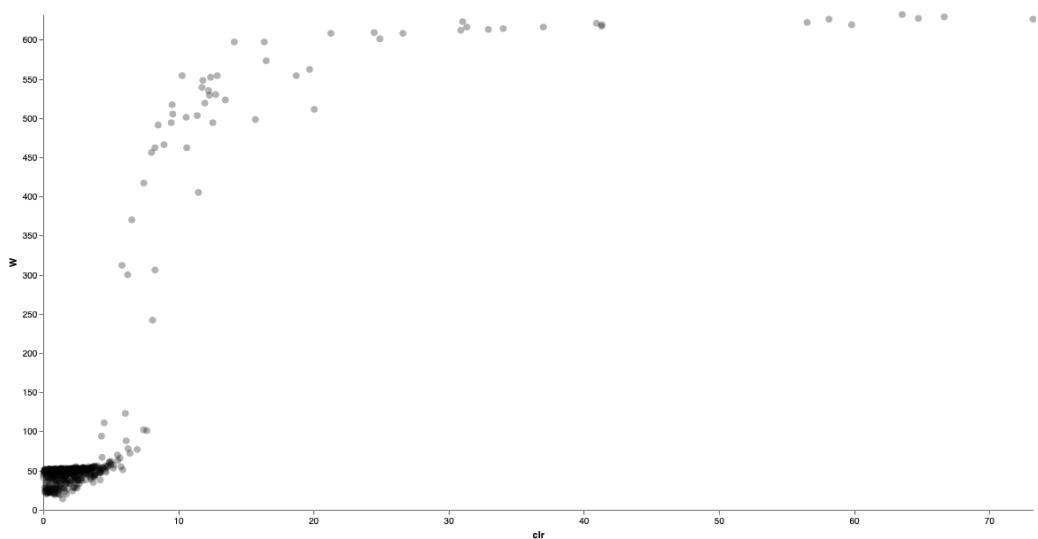


Figure 40: ANCOM Volcano Plot with DADA2 and HOMD

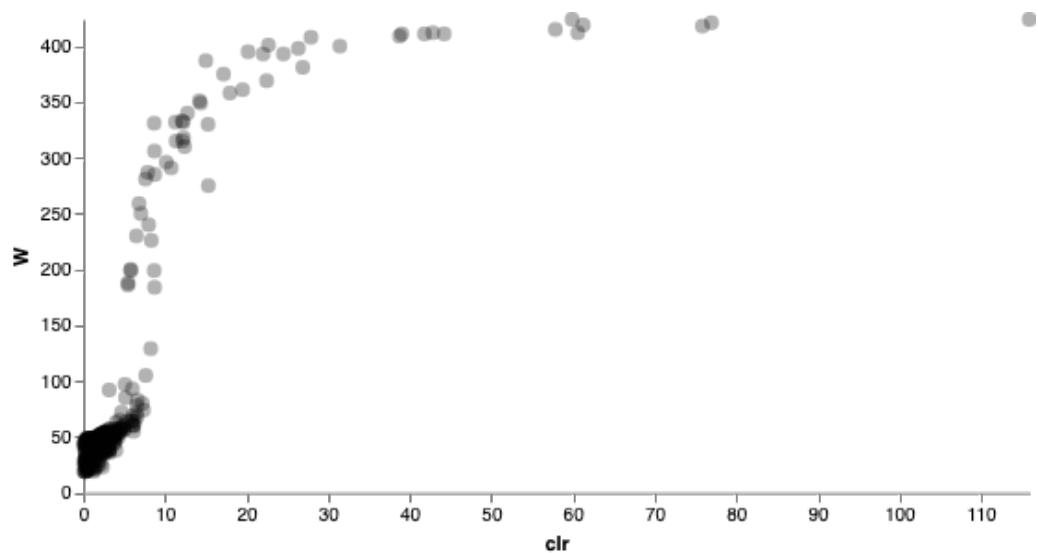


Figure 41: ANCOM Volcano Plot with DADA2 and SILVA

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 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 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

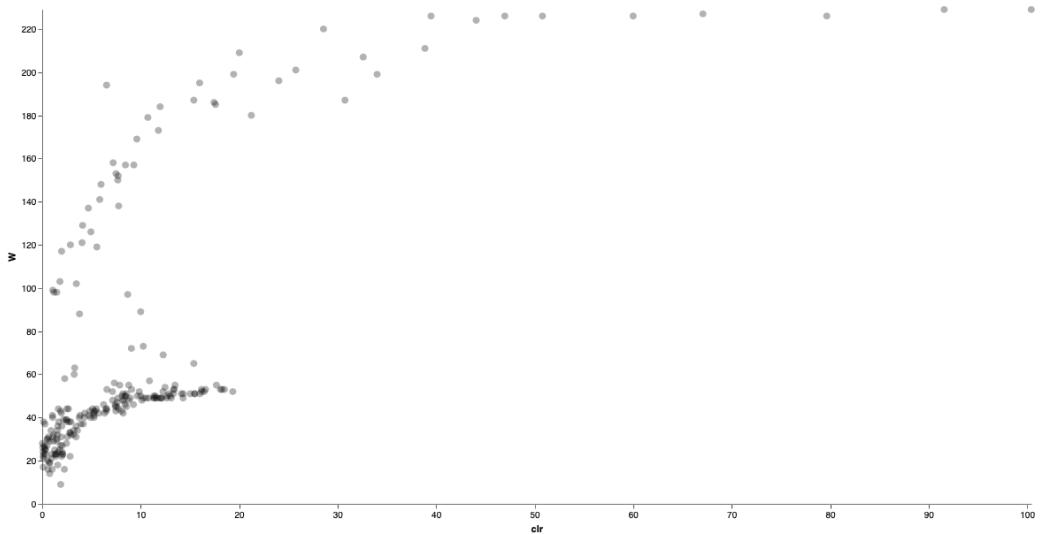


Figure 42: ANCOM Volcano Plot with Deblur and GG

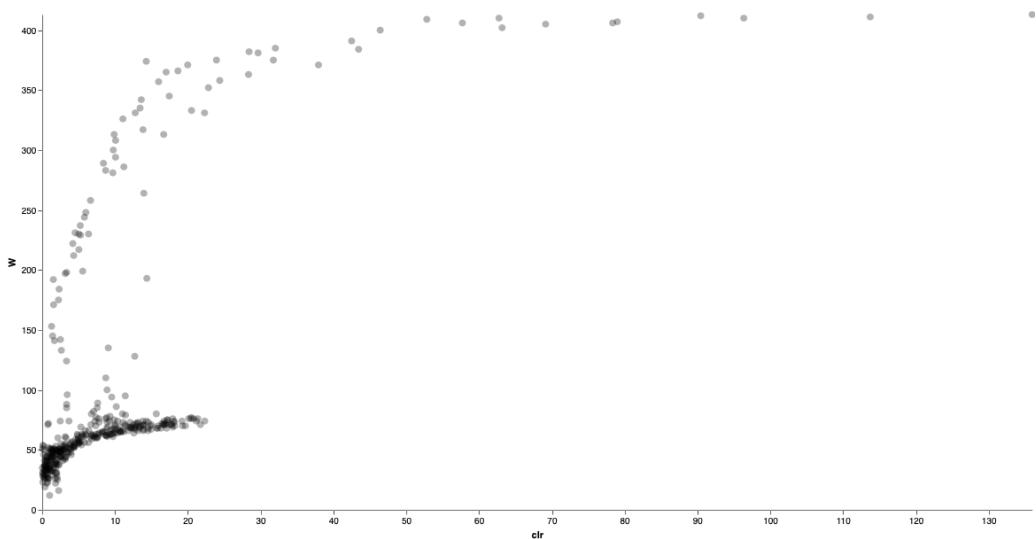


Figure 43: ANCOM Volcano Plot with Deblur and SILVA

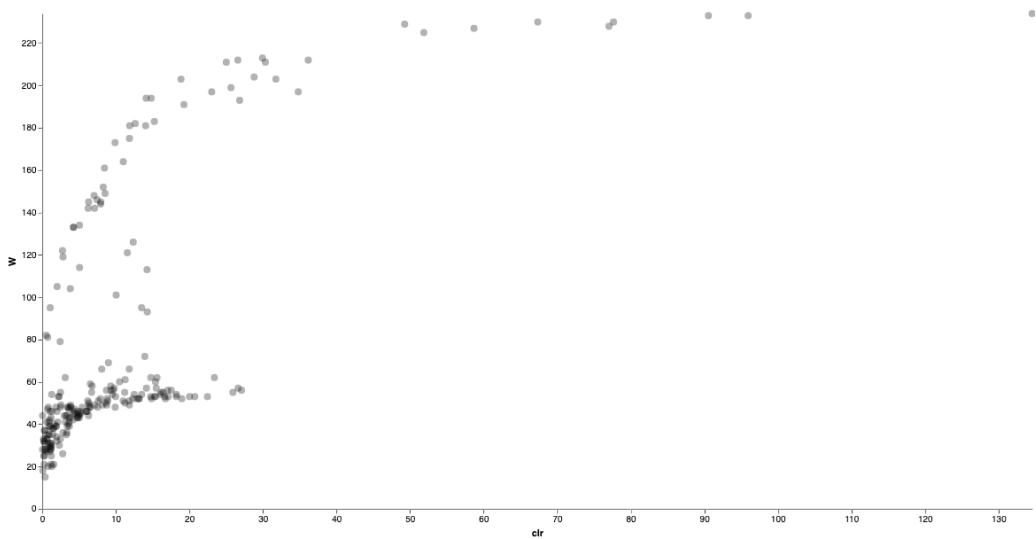


Figure 44: ANCOM Volcano Plot with Deblur and HOMD

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

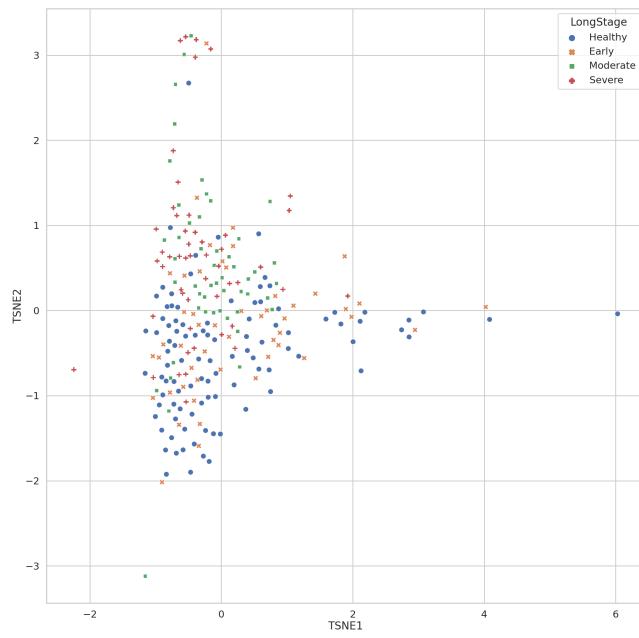


Figure 45: t-SNE Plot with Whole Microbiome from DADA2 and GG (328 taxa)

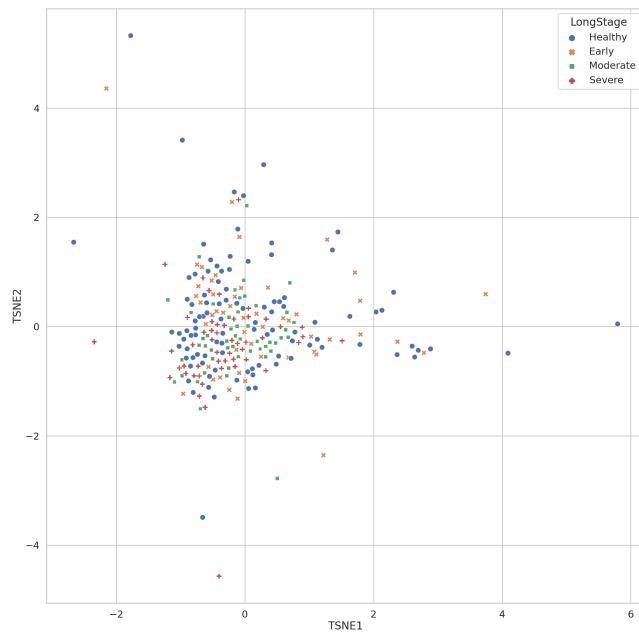


Figure 46: t-SNE Plot with Whole Microbiome from DADA2 and SILVA (633 taxa)

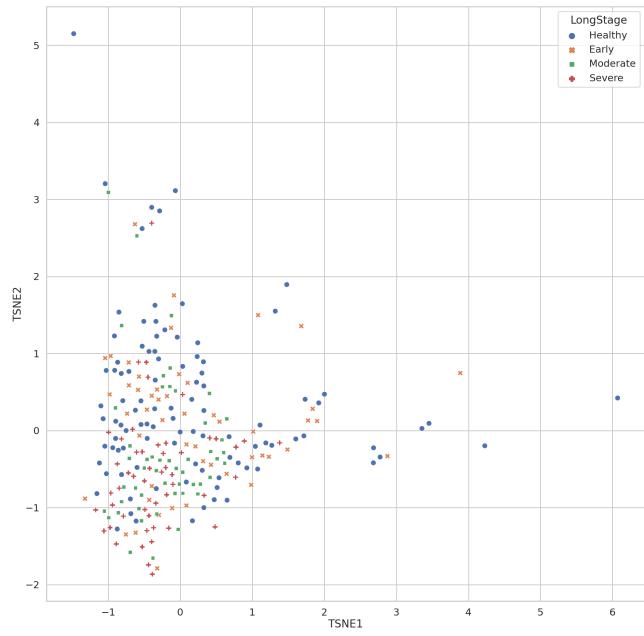


Figure 47: t-SNE Plot with Whole Microbiome from DADA2 and HOMD (425 taxa)

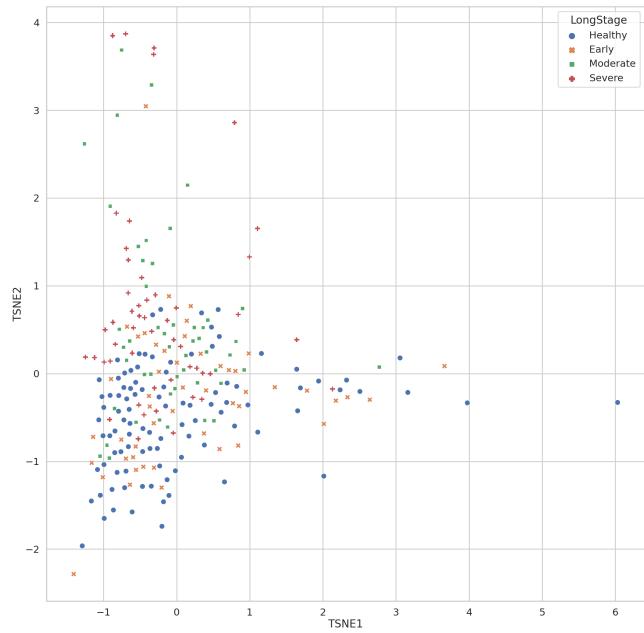


Figure 48: t-SNE Plot with Whole Microbiome from Deblur and GG (232 taxa)

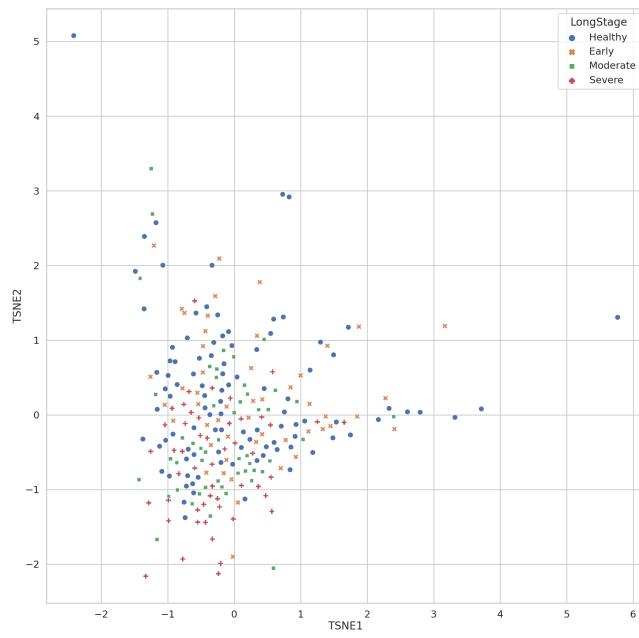


Figure 49: t-SNE Plot with Whole Microbiome from Deblur and SILVA (414 taxa)

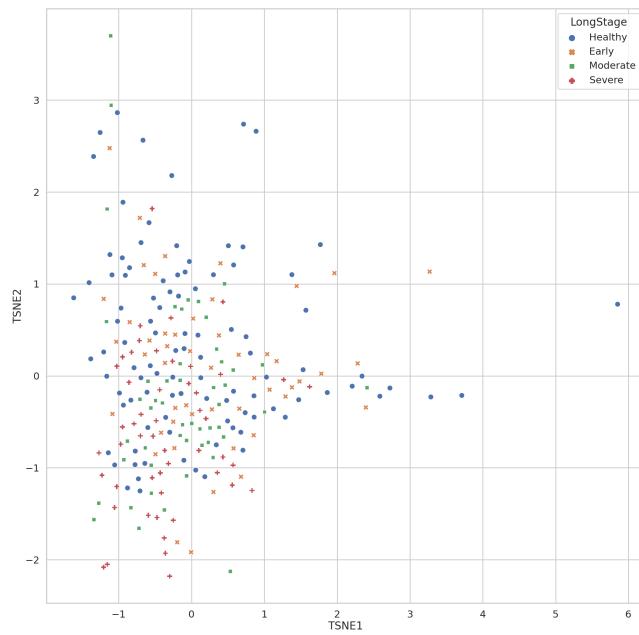


Figure 50: t-SNE Plot with Whole Microbiome from Deblur and HOMD (235 taxa)

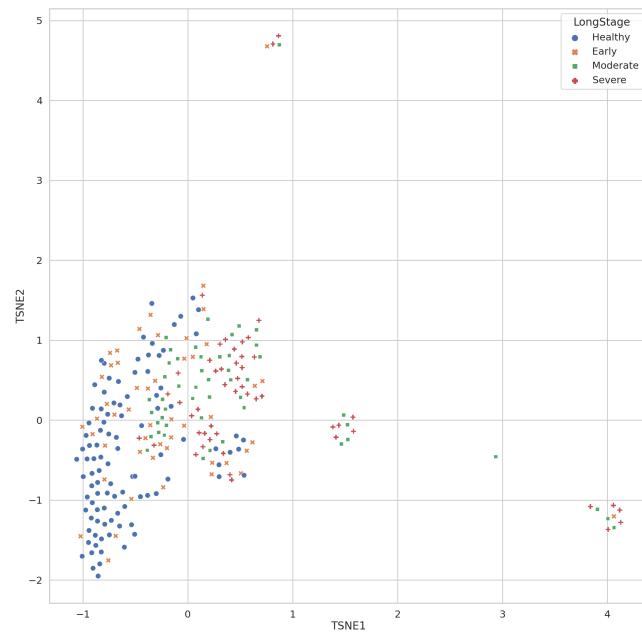


Figure 51: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and GG (15 taxa)

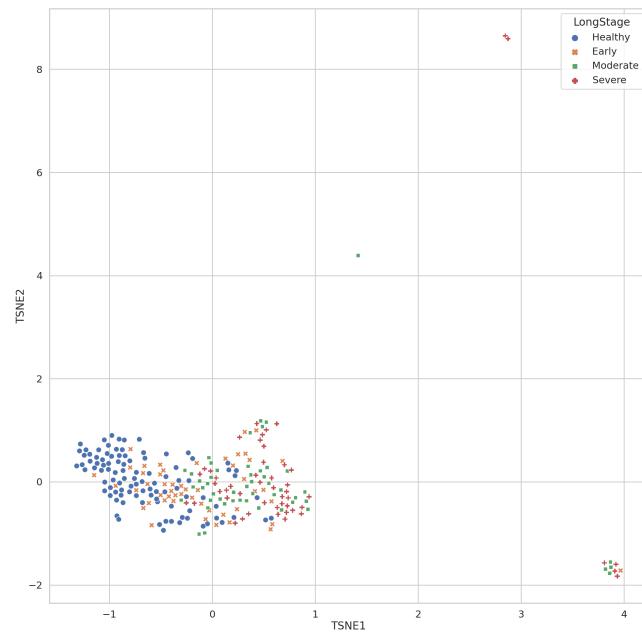


Figure 52: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and SILVA (23 taxa)

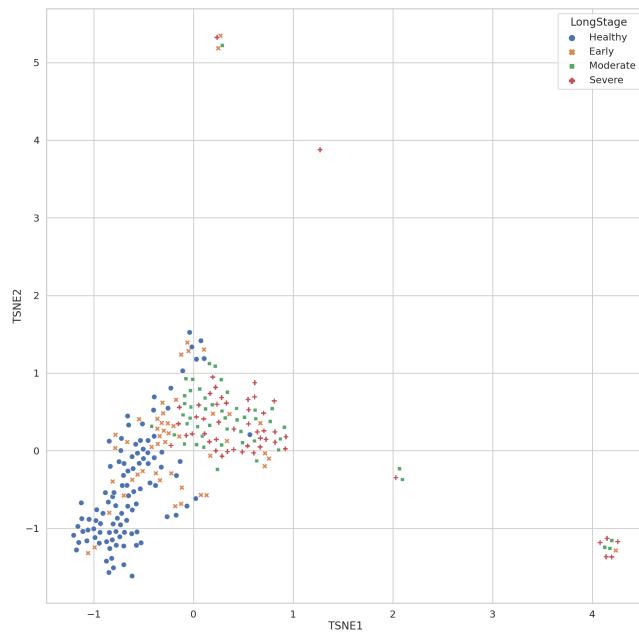


Figure 53: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and HOMD (20 taxa)

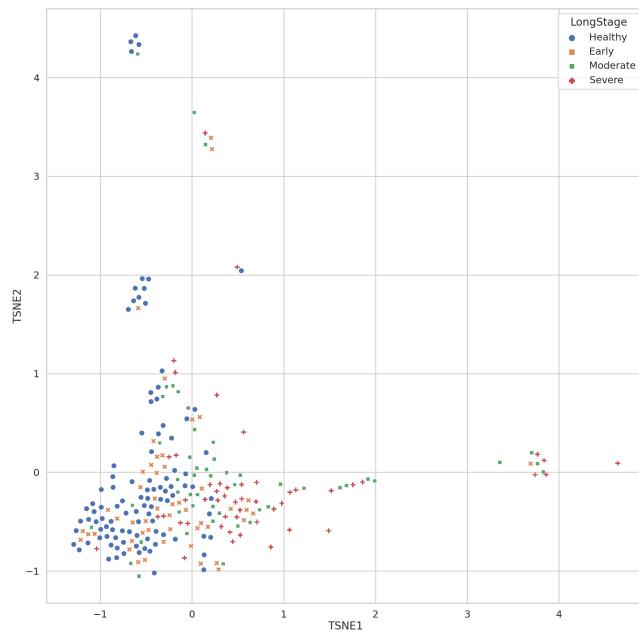


Figure 54: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and GG (27 taxa)

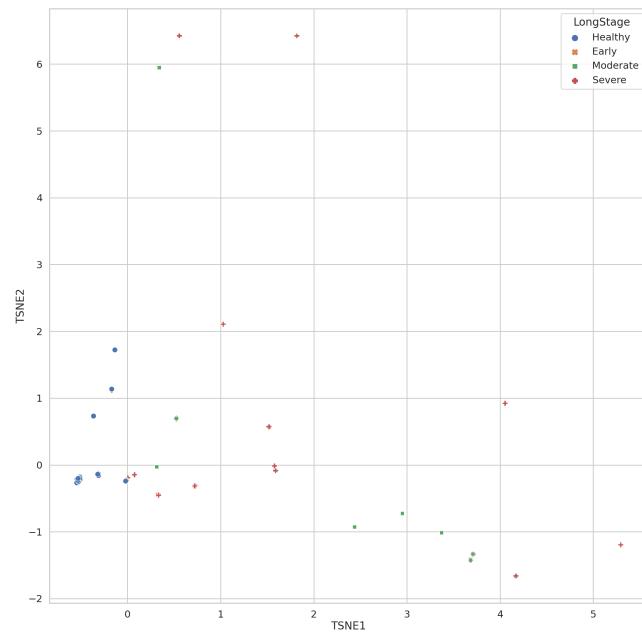


Figure 55: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and SILVA (20 taxa)

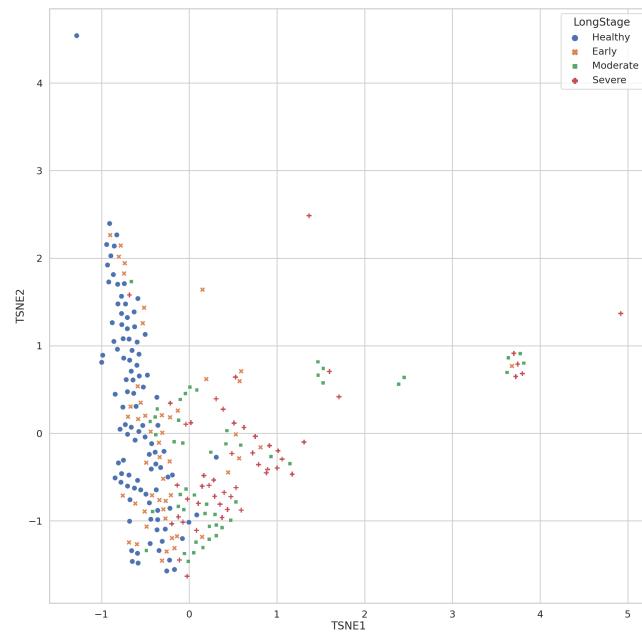


Figure 56: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and HOMD (28 taxa)

Table 26: Taxa with DADA2 and GG Ordered by Random Forest

Order	Taxonomy Classification	Importances
0	Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.2897668387897927
1	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Filifactor	0.1493288396019592
2	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia	0.07273019878053422
3	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas	0.07237355446643938
4	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas endodontalis	0.050739855254238686
5	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema amylovorum	0.049447217415646096
6	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema	0.046058702915828996
7	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae	0.044589335747511734
8	Bacteria Bacteroidetes Bacteroidia Bacteroidales	0.03896215615382719
9	Bacteria Proteobacteria Epsilonproteobacteria Campylobacteriales Campylobacteraceae Campylobacter	0.038672108530872294
10	Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibrionaceae TG5	0.03538306656428921
11	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella	0.03488113476890314
12	Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma	0.03140761957044326
13	Bacteria Actinobacteria Actinobacteria Actinomycetales Corynebacteriaceae Corynebacterium durum	0.028628460674878065
14	Bacteria Firmicutes Clostridia Clostridiales Acidaminobacteraceae	0.01703091076483563

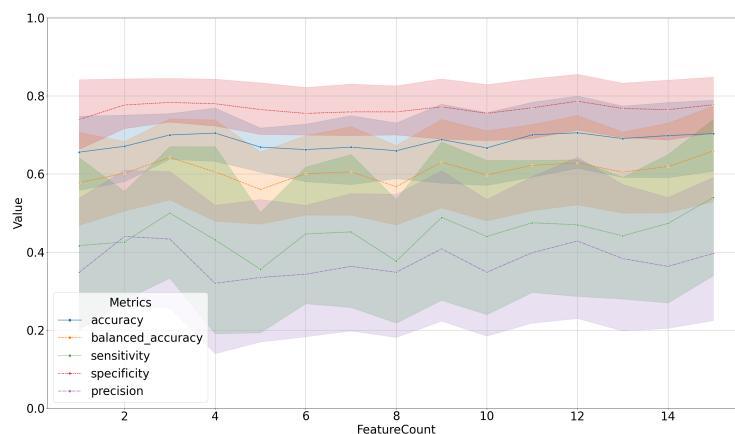


Figure 57: Metrics by Feature Count with DADA2 and GG

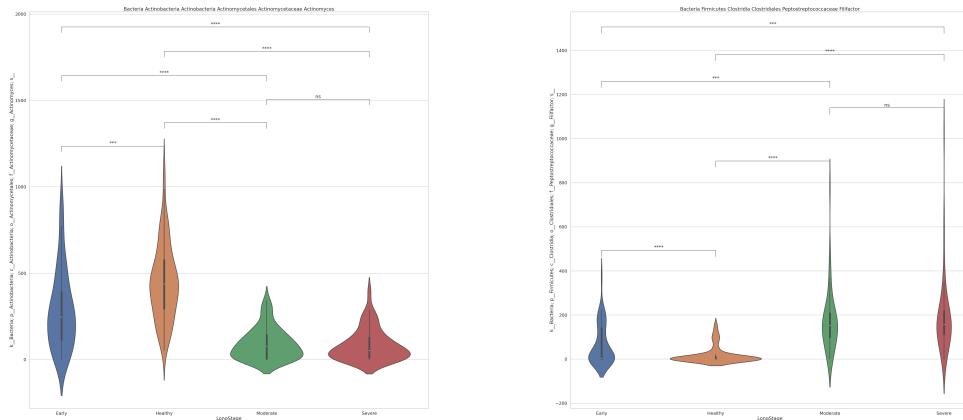


Figure 58: Most and Second Most Important Features with DADA2 and GG

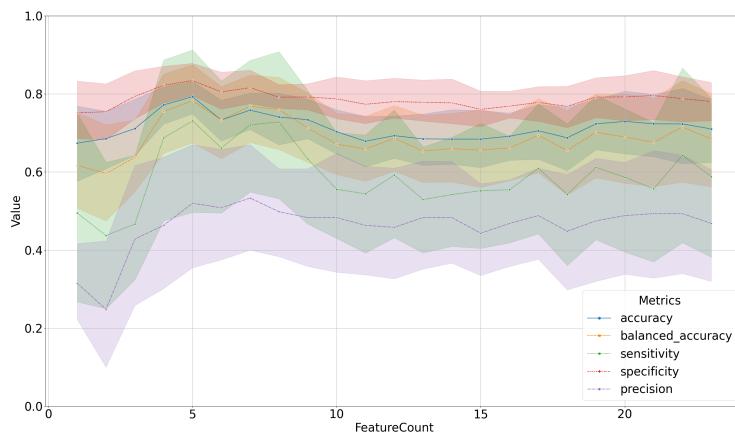


Figure 59: Metrics by Feature Count with DADA2 and SILVA

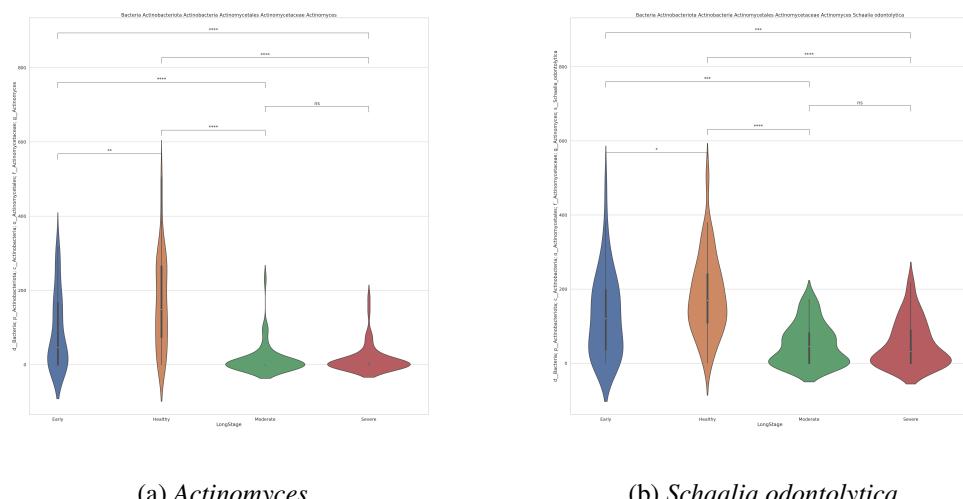


Figure 60: Most and Second Most Important Features with DADA2 and SILVA

Table 27: Taxa with DADA2 and SILVA Ordered by Random Forest

Order	Taxonomy Classification	Importances
0	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.15428126769688613
1	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Schaalia odontolytica	0.09830435718569176
2	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Prevotella Prevotella intermedia	0.07571596825743382
3	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Filifactor Filifactor alocis	0.07143963350528947
4	Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribacterium	0.05844201887575999
5	Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tannerella Tannerella forsythia	0.05805089098293928
6	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas	0.0539170951526226
7	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema denticola	0.0523022341592361
8	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas gingivalis	0.045795280266258155
9	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Actinomyces graevenitzii	0.035433257099296185
10	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema uncultured bacterium	0.0330307414299068
11	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema medium	0.029307500356325216
12	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema maltophilum	0.028176578352262113
13	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	0.027844370549456147
14	Bacteria Campylobacterota Campylobacteria Campylobacteriales Campylobacteraceae Campylobacter Campylobacter showae	0.026801724029521152
15	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium nodatum group Eubacterium nodatum	0.02594971243973025
16	Bacteria Actinobacteriota Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium Corynebacterium durum	0.022962989295219716
17	Bacteria Synergistota Synergistia Synergistales Synergistaceae Fretibacterium	0.022114274615729068
18	Bacteria Firmicutes Clostridia Lachnospirales Defluvitaleaceae Defluvitaleaceae UCG-011 Lachnospiraceae bacterium	0.0182279448380252
19	Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae Mycoplasma Metamycoplasma faecium	0.017715469601623523
20	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium brachy group Eubacterium brachy	0.016409717419062424
21	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema	0.01483146818784845
22	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium saphenum group Eubacterium saphenum	0.012945505703876552

Table 28: Taxa with DADA2 and HOMD Ordered by Random Forest

Order	Taxonomy Classification	Importances
0	Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.2563358219539378
1	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas gingivalis	0.23196557322229505
2	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Filifactor alocis	0.05939593656609779
3	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia	0.047788324527495964
4	Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces graevenitzii	0.0436241876822214
5	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas sp. HMT 285	0.04330230979636331
6	Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales Campylobacteraceae Campylobacter showae	0.041507035664934466
7	Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 260	0.035914066247796333
8	Bacteria Actinobacteria Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium durum	0.028488659124825007
9	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella forsythia	0.02755242782877868
10	Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema putidum	0.02407253635910223
11	Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema	0.023393398798141077
12	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Lachnospiraceae G-8 bacterium HMT 500	0.021534750022711564
13	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-9 brachy	0.021026493991759487
14	Bacteria Synergistetes Synergistia Synergistales Synergistaceae Fretibacterium	0.017748489962311726
15	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 526	0.01743345729576584
16	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-6 nodatum	0.016063177113771455
17	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella sp. HMT 304	0.01592053147101768
18	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-5 saphenum	0.01446869437546418
19	Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma faecium	0.012464127995209072

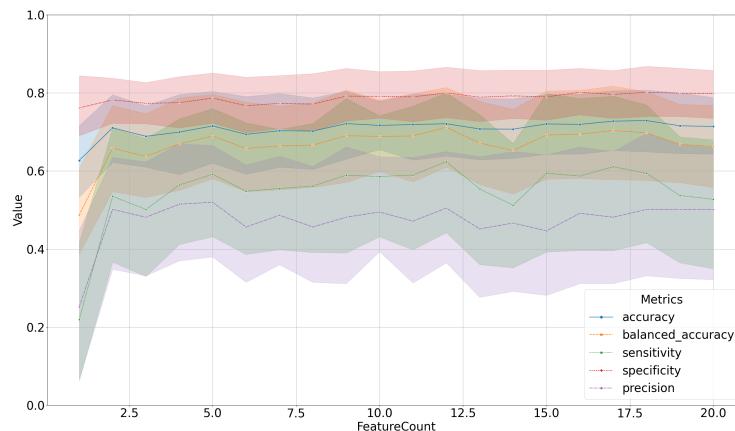
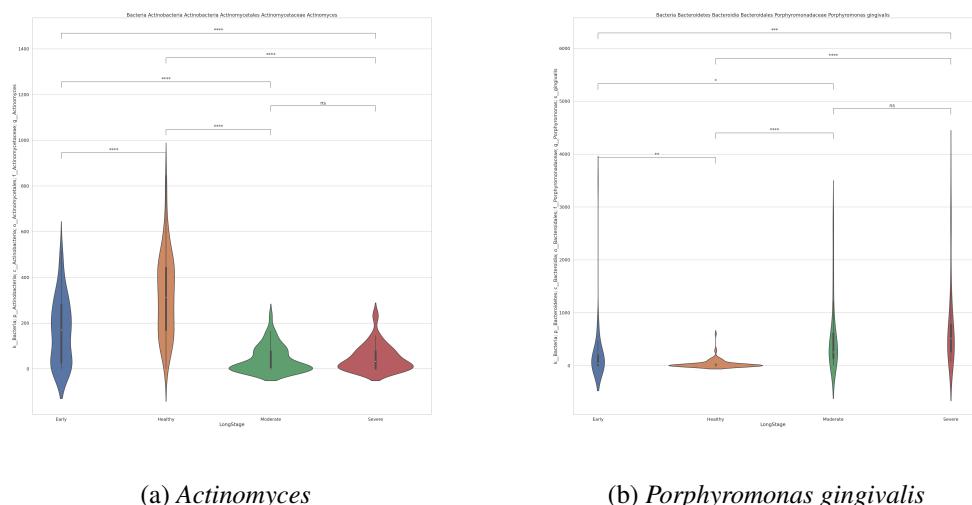


Figure 61: Metrics by Feature Count with DADA2 and HOMD



(a) *Actinomyces* (b) *Porphyromonas gingivalis*

Figure 62: Most and Second Most Important Features with DADA2 and HOMD

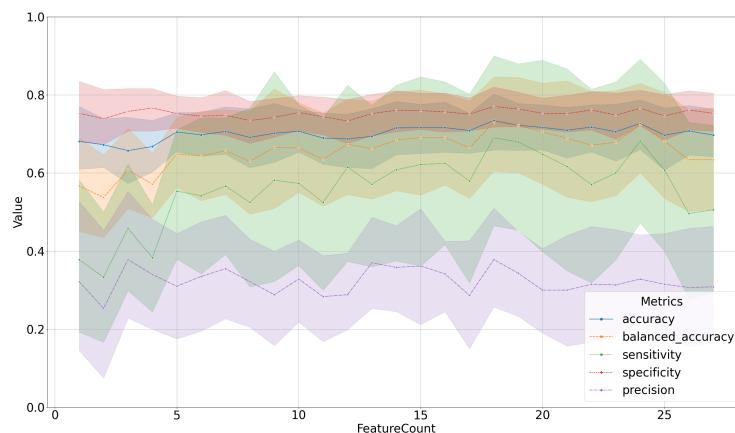
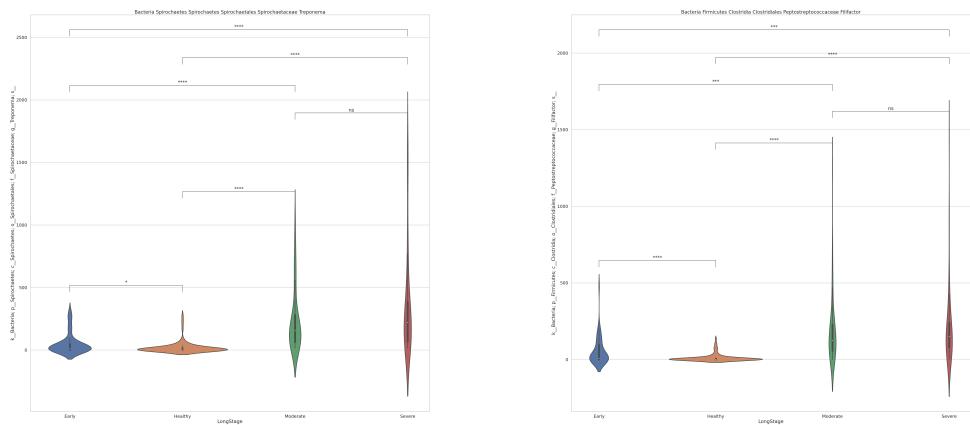


Figure 63: Metrics by Feature Count with Deblur and GG

Table 29: Taxa with Deblur and GG Ordered by Random Forest

Order	Taxonomy Classification	Importances
0	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema	0.12074758172672563
1	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Filifactor	0.10272965893419596
2	Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.06981469110924138
3	Bacteria Proteobacteria Betaproteobacteria Neisseriales Neisseriaceae Neisseria subflava	0.06455871089828909
4	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas	0.05761714025200638
5	Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae Haemophilus parainfluenzae	0.056569557742200474
6	Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibrionaceae TG5	0.04340964763638773
7	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae Oribacterium	0.04203144037349746
8	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema amylovorum	0.03958068627108471
9	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Peptostreptococcus	0.0358587598116692
10	Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma	0.03298154288049845
11	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella nanceiensis	0.029990403308766282
12	Bacteria Actinobacteria Actinobacteria Actinomycetales	0.029360128871075676
13	Bacteria Firmicutes Clostridia Clostridiales	0.027718741874274998
14	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae	0.027495169077388817
15	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas endodontalis	0.025845221004209433
16	Bacteria Firmicutes Clostridia Clostridiales Tissierellaceae	0.02409764507377042
17	Bacteria Firmicutes Clostridia Clostridiales Tissierellaceae Parvimonas	0.022165587739824334
18	Bacteria Proteobacteria Epsilonproteobacteria Campylobacteriales Campylobacteraceae Campylobacter	0.021448855670751162
19	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae Mogibacterium	0.0198296042818533
20	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae	0.01940993201994216
21	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema socranskii	0.01868361833546748
22	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia	0.016833611871175814
23	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella	0.015630651513388796
24	Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desulfovulbaceae Desulfovulbulus	0.014284111618279582
25	Bacteria Bacteroidetes Bacteroidia Bacteroidales	0.01308119809996142
26	Bacteria Firmicutes Clostridia Clostridiales Acidaminobacteraceae	0.008226102004073872



(a) *Treponema*

(b) *Filifactor*

Figure 64: Most and Second Most Important Features with Deblur and GG

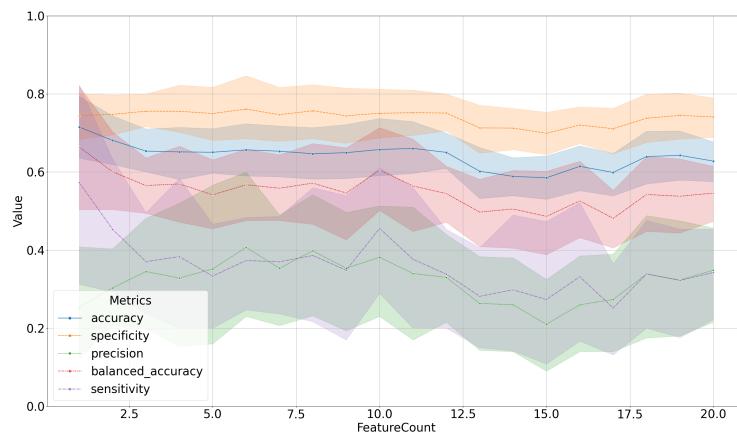
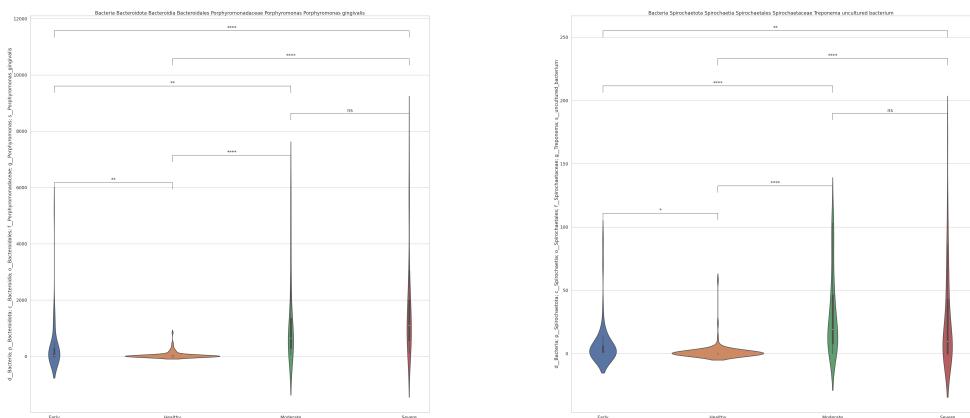


Figure 65: Metrics by Feature Count with Deblur and SILVA



(a) *Porphyromonas gingivalis*

(b) *Treponema uncultured bacterium*

Figure 66: Most and Second Most Important Features with Deblur and SILVA

Table 30: Taxa with Deblur and SILVA Ordered by Random Forest

Order	Taxonomy Classification	Importances
0	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas gingivalis	0.3054581507093521
1	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema uncultured bacterium	0.06306075553390636
2	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	0.0621211496328295
3	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Pre- votella Prevotella intermedia	0.05634637391220579
4	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema medium	0.045843324732015106
5	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema denticola	0.04104161142422072
6	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema malophilum	0.03814448314837611
7	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema	0.03749488565496832
8	Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae My- coplasma Metamycoplasma faicum	0.03746308785002029
9	Bacteria Firmicutes Bacilli Lactobacillales Streptococcaceae Strepto- coccus Streptococcus constellatus	0.03475399919186926
10	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium brachy group	0.033280774673234606
11	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Pep- tostreptococcaceae Filifactor Filifactor alocis	0.03303045116011422
12	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas endodontalis	0.0305712774697067
13	Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tan- nerella Tannerella forsythia	0.03054768095598893
14	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Pre- votella Prevotella dentalis	0.029421392615318554
15	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium nodatum group Eubacterium nodatum	0.02915547917774481
16	Bacteria Synergistota Synergistia Synergistales Synergistaceae Fretibacterium	0.028183918621745366
17	Bacteria Desulfobacterota Desulfobulbia Desulfobulbales Desulfobul- baceae Desulfobulbus	0.02447201285320467
18	Bacteria Firmicutes Clostridia Lachnospirales Defluvitaleaceae Deflu- vitaleaceae UCG-011 Lachnospiraceae bacterium	0.020836896635166788
19	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium saphenum group Eubacterium saphenum	0.018772294048011725

Table 31: 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 Desulfovulbus 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

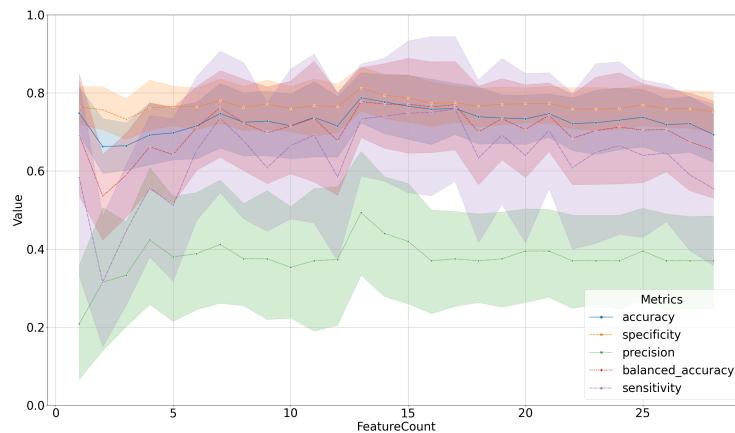


Figure 67: Metrics by Feature Count with Deblur and HOMD

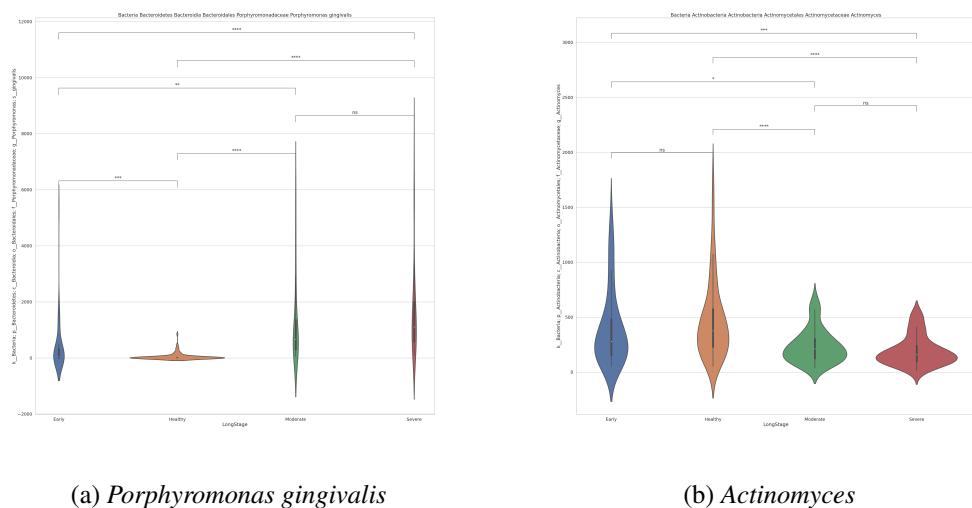


Figure 68: Most and Second Most Important Features with Deblur and HOMD

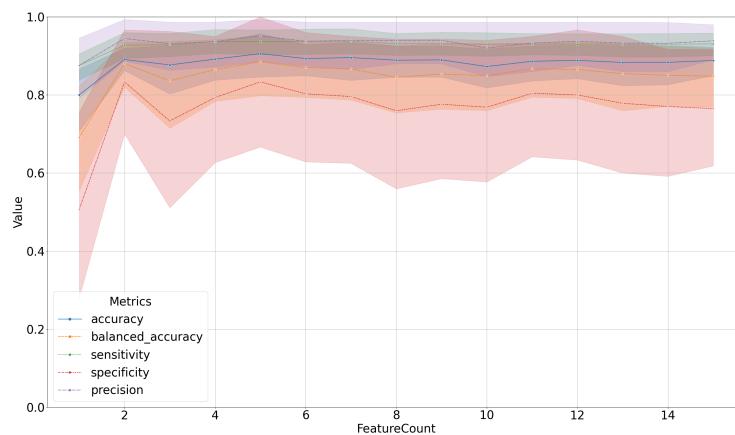
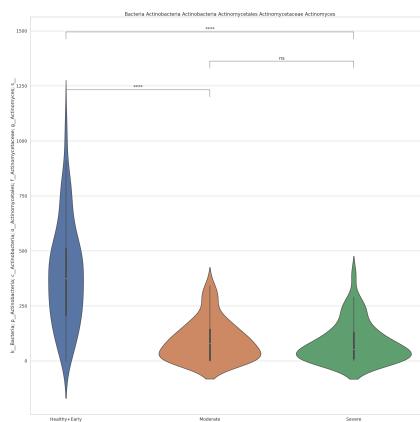


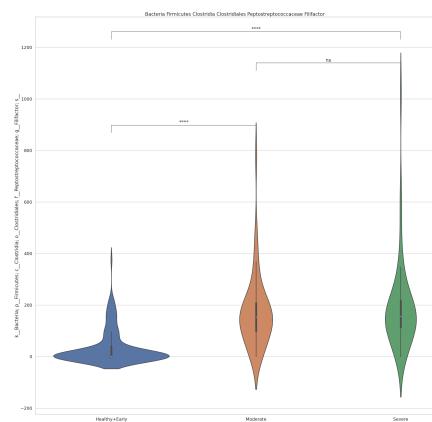
Figure 69: Metrics by Feature Count with DADA2 and GG for Merging (Healthy+Early) Classes

Table 32: Taxa with DADA2 and GG Ordered by Random Forest for Merging (Healthy+Early) Classes

Order	Taxonomy Classification	Importances
0	Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.3038342047804526
1	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Filifactor	0.17016282305143274
2	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia	0.08941031852222472
3	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas	0.07349947371345206
4	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas endodontalis	0.05341521063786635
5	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema amylovorum	0.04795905336865076
6	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema	0.0429941933317125
7	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae	0.03839388626322817
8	Bacteria Proteobacteria Epsilonproteobacteria Campylobacteriales Campylobacteraceae Campylobacter	0.034903029427182944
9	Bacteria Bacteroidetes Bacteroidia Bacteroidales	0.03235473288744429
10	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella	0.028208752894831525
11	Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma	0.025445666249280838
12	Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibrionaceae TG5	0.02143545727051063
13	Bacteria Firmicutes Clostridia Clostridiales Acidaminobacteraceae	0.020654290358461013
14	Bacteria Actinobacteria Actinobacteria Actinomycetales Corynebacteriaceae Corynebacterium durum	0.017328907243268844



(a) *Actinomyces*



(b) *Filifactor*

Figure 70: Most and Second Most Important Features with DADA2 and GG for Merging (Healthy+Early) Classes

Table 33: Taxa with DADA2 and SILVA Ordered by Random Forest for Merging (Healthy+Early) Classes

Order	Taxonomy Classification	Importances
0	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.11690547055081804
1	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas gingivalis	0.11085760166071587
2	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema denticola	0.09934825764896191
3	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Prevotella Prevotella intermedia	0.09118972601091217
4	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Schaalia odontolytica	0.06506974355992037
5	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Filifactor Filifactor alocis	0.06099767811377263
6	Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribacterium	0.04452746290372327
7	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium nodatum group Eubacterium nodatum	0.0434222896929853
8	Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tannerella Tannerella forsythia	0.042683788290101346
9	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas	0.03711935156262676
10	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema uncultured bacterium	0.031245445192002997
11	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema maltophilum	0.03105874374981756
12	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema medium	0.030139675448526946
13	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium saphenum group Eubacterium saphenum	0.024566473962687116
14	Bacteria Campylobacterota Campylobacteria Campylobacterales Campylobacteraceae Campylobacter Campylobacter showae	0.024305985196832625
15	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomycetaceae Actinomyces Actinomyces graevenitzii	0.024255370726892468
16	Bacteria Actinobacteriota Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium Corynebacterium durum	0.024240150146592164
17	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	0.02412256170468769
18	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium brachy group Eubacterium brachy	0.0203959501090319
19	Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae Mycoplasma Metamycoplasma faecium	0.018251402344480718
20	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema	0.013340627154948506
21	Bacteria Synergistota Synergistia Synergistales Synergistaceae Fretibacterium	0.011478324853494261
22	Bacteria Firmicutes Clostridia Lachnospirales Defluvitaleaceae Defluvitaleaceae UCG-011 Lachnospiraceae bacterium	0.010477919415467385

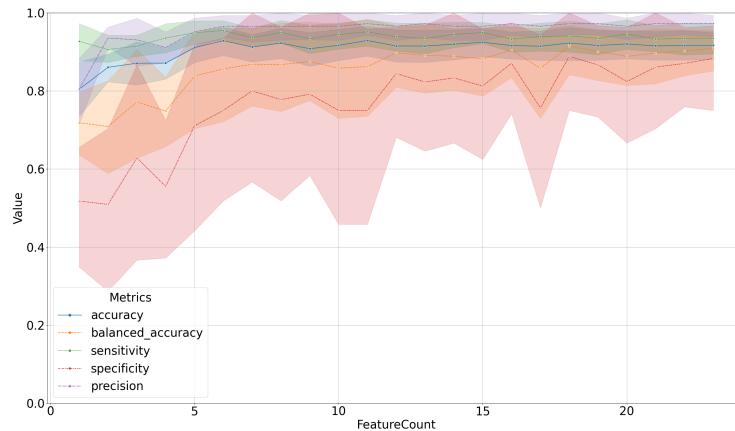


Figure 71: Metrics by Feature Count with DADA2 and SILVA for Merging (Healthy+Early) Classes

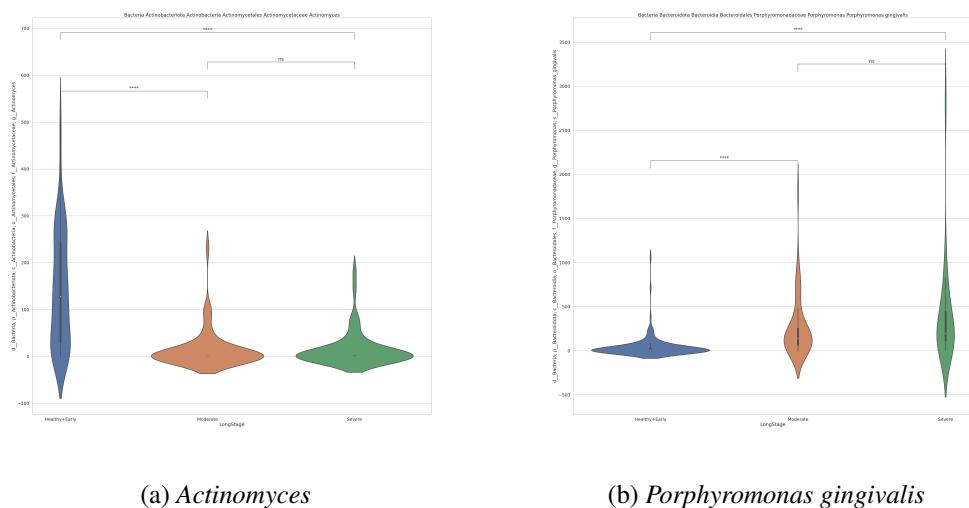


Figure 72: Most and Second Most Important Features with DADA2 and SILVA for Merging (Healthy+Early) Classes

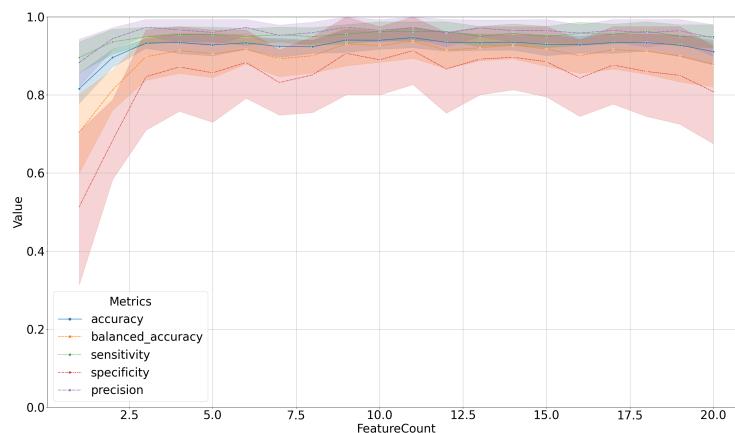
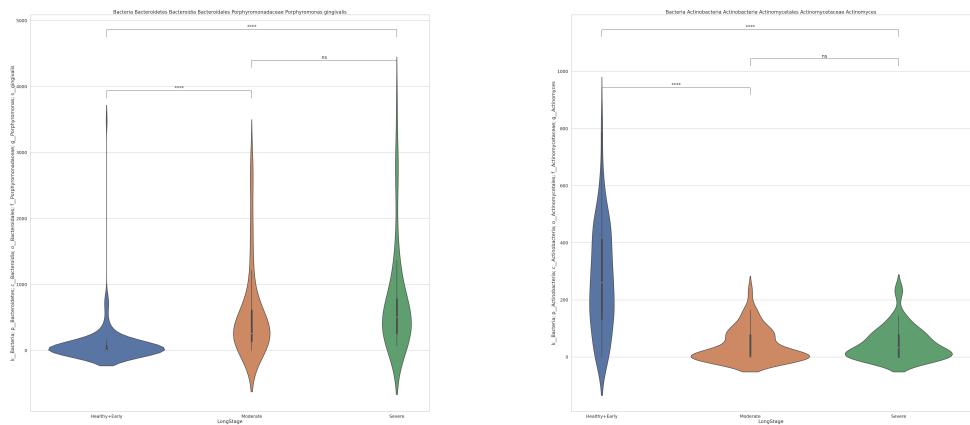


Figure 73: Metrics by Feature Count with DADA2 and HOMD for Merging (Healthy+Early) Classes

Table 34: 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 Campylobacteriales 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



(a) *Porphyromonas gingivalis*

(b) *Actinomyces*

Figure 74: Most and Second Most Important Features with DADA2 and HOMD for Merging (Healthy+Early) Classes

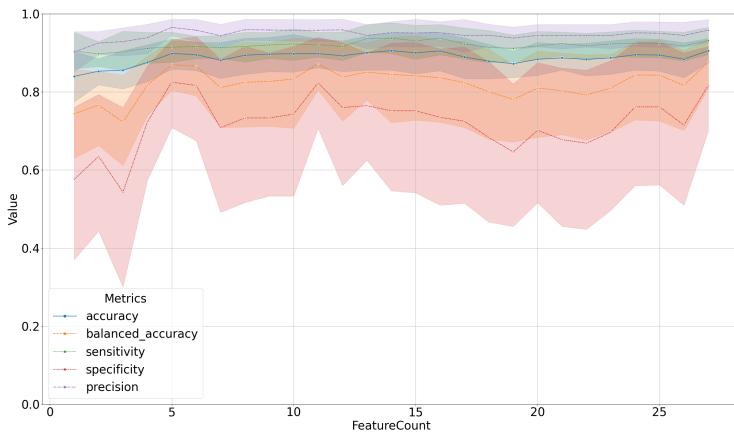
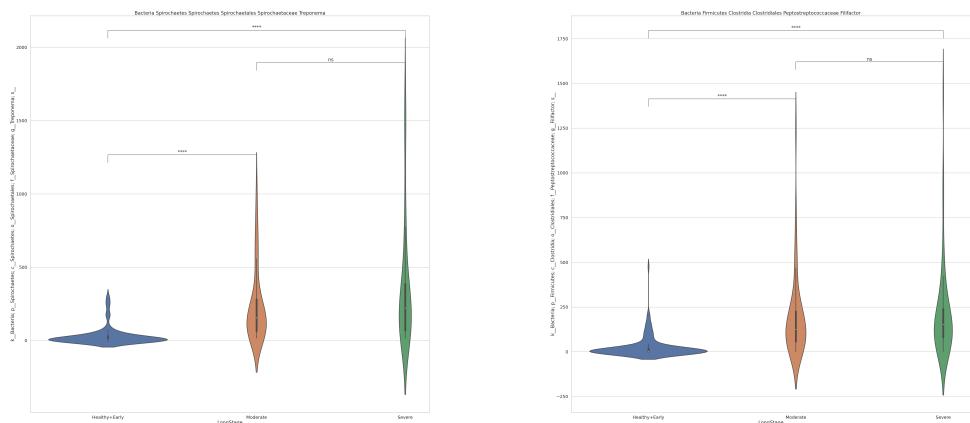


Figure 75: Metrics by Feature Count with Deblur and GG for Merging (Healthy+Early) Classes



(a) *Treponema*

(b) *Filifactor*

Figure 76: Most and Second Most Important Features with Deblur and GG for Merging (Healthy+Early) Classes

Table 35: Taxa with Deblur and GG Ordered by Random Forest for Merging (Healthy+Early) Classes

Order	Taxonomy Classification	Importances
0	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema	0.2676747647163237
1	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Filifactor	0.06195254268984466
2	Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma	0.06050720697457952
3	Bacteria Proteobacteria Betaproteobacteria Neisseriales Neisseriaceae Neisseria subflava	0.055110365785665694
4	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema amylovorum	0.05449027939654494
5	Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.04696051954215141
6	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas	0.04312015501070571
7	Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae Haemophilus parainfluenzae	0.04016167899221565
8	Bacteria Firmicutes Clostridia Clostridiales	0.03403585294135651
9	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae Oribacterium	0.033286793585524166
10	Bacteria Proteobacteria Epsilonproteobacteria Campylobacteriales Campylobacteraceae Campylobacter	0.02995663766970053
11	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae	0.028778458744828112
12	Bacteria Firmicutes Clostridia Clostridiales Tissierellaceae	0.026197588412461604
13	Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibrionaceae TG5	0.02409527218625163
14	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Peptostreptococcus	0.020151529746989166
15	Bacteria Spirochaetes Spirochaetes Spirochaetales Spirochaetaceae Treponema socranskii	0.019487588834721174
16	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas endodontalis	0.01893820175924786
17	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella intermedia	0.018428077428006798
18	Bacteria Actinobacteria Actinobacteria Actinomycetales	0.016558042384769794
19	Bacteria Firmicutes Clostridia Clostridiales Tissierellaceae Parvimonas	0.016238791562607435
20	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella nanceiensis	0.015335786046552207
21	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae Mogibacterium	0.014368475358322908
22	Bacteria Bacteroidetes Bacteroidia Bacteroidales	0.012997185164792986
23	Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desulfovibraceae Desulfovulbus	0.012939147020354157
24	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella	0.010764463650549757
25	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae	0.010300356437386188
26	Bacteria Firmicutes Clostridia Clostridiales Acidaminobacteraceae	0.007164237957545891

Table 36: Taxa with Deblur and SILVA Ordered by Random Forest for Merging (Healthy+Early) Classes

Order	Taxonomy Classification	Importances
0	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas gingivalis	0.4031949046856801
1	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema denticola	0.05939001779623957
2	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema uncultured bacterium	0.05822504150119327
3	Bacteria Firmicutes Bacilli Lactobacillales Streptococcaceae Streptococcus Streptococcus constellatus	0.042206788421850584
4	Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae Mycoplasma Metamycoplasma faucium	0.042145500506113924
5	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema medium	0.03890956670215448
6	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Prevotella Prevotella intermedia	0.03819194732725226
7	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema Treponema maltophilum	0.03647531598753172
8	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae Treponema	0.02845747673023059
9	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium nodatum group Eubacterium nodatum	0.027953589404536083
10	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium saphenum group Eubacterium saphenum	0.02688318788913423
11	Bacteria Synergistota Synergistia Synergistales Synergistaceae Fretibacterium	0.025372659385492543
12	Bacteria Firmicutes Clostridia Lachnospirales Defluvitaleaceae Defluvitaleaceae UCG-011 Lachnospiraceae bacterium	0.024723656076908643
13	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	0.024697259349598637
14	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Peptostreptococcaceae Filifactor Filifactor alocis	0.024451647845314735
15	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas Porphyromonas endodontalis	0.023151363993075897
16	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Prevotella Prevotella dentalis	0.022389445260037527
17	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae Eubacterium brachy group	0.019298131934997142
18	Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tannerella Tannerella forsythia	0.01712749680798365
19	Bacteria Desulfobacterota Desulfobulbia Desulfobulbales Desulfobulbaceae Desulfobulbus	0.016755002394674298

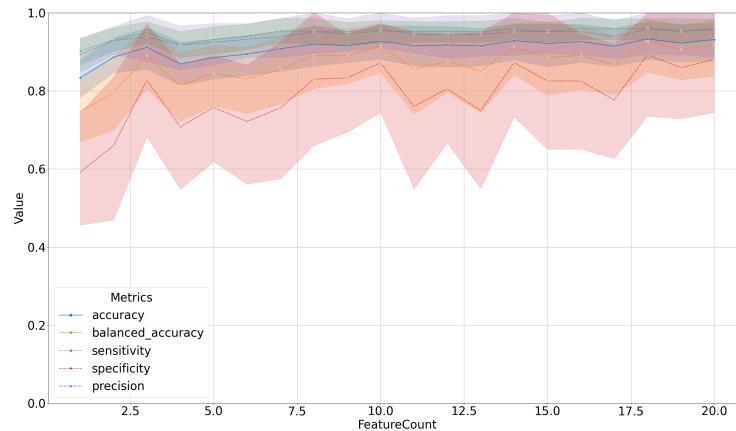


Figure 77: Metrics by Feature Count with Deblur and SILVA for Merging (Healthy+Early) Classes

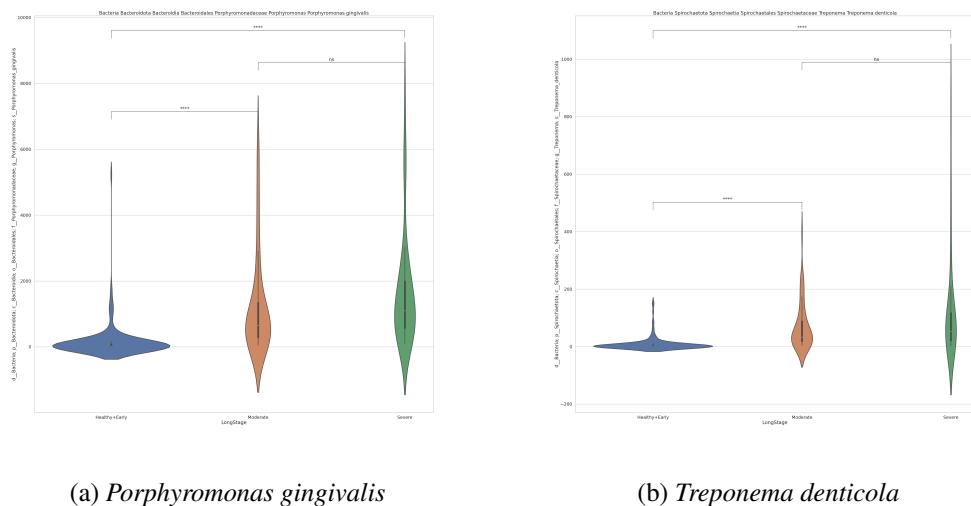


Figure 78: Most and Second Most Important Features with Deblur and SILVA for Merging (Healthy+Early) Classes

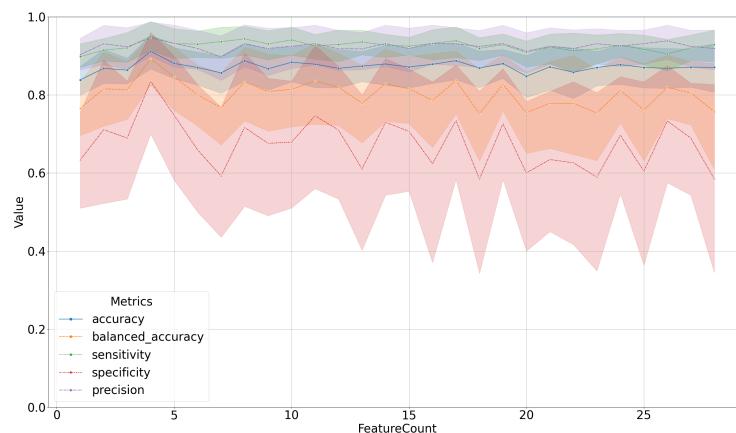


Figure 79: Metrics by Feature Count with Deblur and HOMD for Merging (Healthy+Early) Classes

Table 37: Taxa with Deblur and HOMD Ordered by Random Forest for Merging (Healthy+Early) Classes

Order	Taxonomy Classification	Importances
0	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas gingivalis	0.41125515865215273
1	Bacteria Actinobacteria Actinobacteria Actinomycetales Actinomycetaceae Actinomyces	0.06378153124993774
2	Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurellaceae	0.05196540839370839
3	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Oribacterium sinus	0.04008808122711738
4	Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema	0.03458095074116177
5	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcus anaerobius	0.03384528186576715
6	Bacteria Firmicutes Mollicutes Mycoplasmatales Mycoplasmataceae Mycoplasma	0.029796512006797487
7	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-6 nodatum	0.02397477403830387
8	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Catonella sp. HMT 164	0.021229890512071744
9	Bacteria Bacteroidetes Bacteroidetes C-1 Bacteroidetes O-1 Bacteroidetes F-1 Bacteroidetes G-3	0.02076312355179804
10	Bacteria Firmicutes Negativicutes Veillonellales Veillonellaceae Veillonella denticariosi	0.01999470850214282
11	Bacteria Synergistetes Synergistia Synergistales Synergistaceae Fretibacterium	0.019164757948883806
12	Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 260	0.019041458116433395
13	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Mogibacterium	0.0184923889371212
14	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Porphyromonas sp. HMT 285	0.01796213600159265
15	Bacteria Firmicutes Clostridia Clostridiales Peptoniphilaceae Parvimonas sp. HMT 393	0.017747508559462277
16	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Lachnospiraceae G-8 bacterium HMT 500	0.01716695716844132
17	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Filifactor alocis	0.015614729285278286
18	Bacteria Spirochaetes Spirochaetia Spirochaetales Spirochaetaceae Treponema sp. HMT 258	0.014577352393288017
19	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella nanceiensis	0.01448283214970534
20	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-5 saphenum	0.014215723158051854
21	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-9 brachy	0.014014969932268868
22	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Prevotella dentalis	0.0138073550075584
23	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae Tannerella forsythia	0.012723253885895344
24	Bacteria Firmicutes Clostridia Clostridiales Clostridiales F-1 Clostridiales F-1G-1 bacterium HMT 093	0.011779169473611241
25	Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desulfovulbaceae Desulfovulbulus sp. HMT 041	0.011386788073902964
26	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae XIV Johnsonella sp. HMT 166	0.010378295240887838
27	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae XI Peptostreptococcaceae XIG-4 bacterium HMT 369	0.006168903926657988

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

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

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.

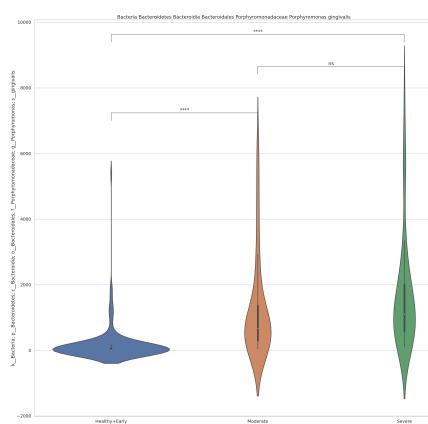
5.3 t-SNE Plot

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.

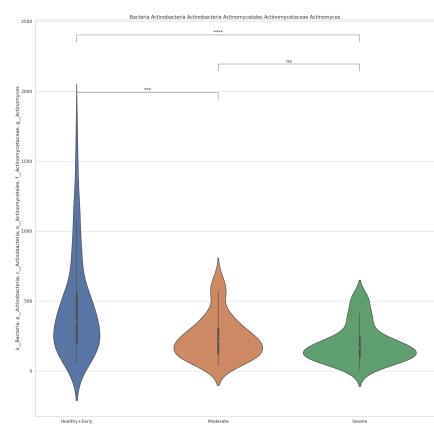
5.4 Random Forest Classifier

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(a) *Porphyromonas gingivalis*



(b) *Actinomyces*

Figure 80: Most and Second Most Important Features with Deblur and HOMD for Merging (Healthy+Early) Classes