# Periodontitis

Jaewoong Lee Seunghoon Kim Semin Lee

2020-12-09

## **Contents**

1	Intr		5
	1.1	Microbiome	5
	1.2	Ribosomal RNA	5
	1.3	16S rRNA Gene Sequencing	5
	1.4		5
2	Mat	terials	5
	2.1	16S rRNA Gene Sequencing	5
3	Met	hods	5
	3.1	QIIME2 Workflow	5
			5
			5
			5
			5
			8
			8
			8
	3.2		8
			8
		3.2.2 Scikit-learn	8
		3.2.3 Matplotlib	8
		3.2.4 Seaborn	8
	3.3	t-SNE	9
	3.4		9
4	Rest	ults	9
	4.1	Quality Filter	9
	4.2		9
	4.3		9
	4.4	r	9
		• • • • • • • • • • • • • • • • • • • •	
	4.5		
	4.6	t-SNE Plot with Whole Microbiome	
	4.7	t-SNE Plot with ANCOM Selected Microbiome Data	
	4.8	Random Forest Classifier with Every Class	
		4.8.1 DADA2 + Greengenes	
		4.8.2 DADA2 + SILVA	2
		4.8.3 Deblur + Greengenes	2
		4.8.4 Deblur + SILVA	2
	4.9	Random Forest Classifier with Merging (Healthy+Early) Classes	2
		, and the same of	
5	Disc	cussion 22	2
	5.1	Alpha-diversity	2
	5.2	Beta-diversity	2
	5.3	t-SNE Plot	
	5.4	Random Forest Classifier with Every Class	
	J. <b>⊤</b>	Random Forest Classifici with Every Class	_
6	Refe	erences 2	2
U	ICIC	Li chees	_
T :	ist a	of Tables	
1	ist t	of Tubics	
	1	Confusion Matrix	Λ
	2	Kruskal-Wallis Tests among All Group with DADA2	
	3	Kruskal-Wallis Tests from Evenness Index with DADA2	
	4	Kruskal-Wallis Tests from Faith PD Index with DADA2	
	5	Kruskal-Wallis Tests from Observed Features Index with DADA2	
	6	Kruskal-Wallis Tests from Shannon's Diversity Index with DADA2	2
	7	Kruskal-Wallis Tests among All Group with Deblur	2
	8	Kruskal-Wallis Tests from Evenness Index with Deblur	4

9	Kruskal-Wallis Tests from Faith PD Index with Deblur	14
10	Kruskal-Wallis Tests from Observed Features Index with Deblur	14
11	Kruskal-Wallis Tests from Shannon's Diversity Index with Deblur	14
12	Bray-Curtis Distance Index with DADA2	16
13	Jaccard Distance Index with DADA2	16
14	Unweighted UniFrac Distance Index with DADA2	16
15	Weighted UniFrac Distance Index with DADA2	16
		19
16	Bray-Curtis Distance Index with Deblur	
17	Jaccard Distance Index with Deblur	19
18	Unweighted UniFrac Distance Index with Deblur	19
19	Weighted UniFrac Distance Index with Deblur	19
20	ANCOM Significant Taxa with DADA2 and Greengenes	23
21	ANCOM Significant Taxa with DADA2 and SILVA	24
22	ANCOM Significant Taxa with Deblur and Greengenes	26
23	ANCOM Significant Taxa with DADA2 and SILVA	27
24	Taxa with DADA2 and Greengenes Ordered by Random Forest	32
25	Taxa with DADA2 and SILVA Ordered by Random Forest	33
26	Taxa with Deblur and Greengenes Ordered by Random Forest	35
27	Taxa with Deblur and SILVA Ordered by Random Forest	36
21	Taxa with Debita and SillyA Ordered by Random Forest	30
List o	of Figures	
LIST 0		
1	Concept of a Core Human Microbiome (Turnbaugh et al., 2007)	6
2	A Theoretic Overview of QIIME2 Workflow (Bolyen et al., 2019, 2018)	6
3	Denoising Techniques which provided by QIIME2	7
4		7
	Taxonomy Classification which provided by QIIME2	7
5	Example Diagram for Merging Denoising and Taxonomy Classification	
6	Example ANCOM Volcano Plot which Provided by QIIME2 (Bolyen et al., 2019, 2018)	10
7	Visualization by t-SNE (Maaten & Hinton, 2008)	10
8	Workflow of Classification	10
9	Deciding the Best Features	11
10	Sequence Quality Plot	11
11	Frequency and Number per Sample by DADA2	11
12	Frequency and Number per Sample by Deblur	12
13	Evenness Index from DADA2	13
14	Faith PD Index from DADA2	13
15	Observed Features Index from DADA2	
16	Shannon's Diversity Index from DADA2	14
17	Evenness Index from Deblur	15
18	Faith PD Index from Deblur	15
19	Observed Features Index from Deblur	15
20	Shannon's Diversity Index from Deblur	16
21	Bray-Curtis Distance Index with DADA2	17
22	Jaccard Distance Index with DADA2	17
23	Unweighted Unifrac Distance Index with DADA2	18
24	Weighted Unifrac Distance Index with DADA2	18
25	Bray-Curtis Distance Index with Deblur	20
26	Jaccard Distance Index with Deblur	20
27	Unweighted Unifrac Distance Index with Deblur	21
28	Weighted Unifrac Distance Index with Deblur	21
29	ANCOM Volcano Plot with DADA2 and Greengenes	23
30	ANCOM Volcano Plot with DADA2 and SILVA	25
31	ANCOM Volcano Plot with Deblur and Greengenes	25
32	ANCOM Volcano Plot with Deblur and SILVA	25
33	t-SNE Plot with Whole Microbiome from DADA2 and Greengenes	28
34	t-SNE Plot with Whole Microbiome from DADA2 and SILVA	28
35	t-SNE Plot with Whole Microbiome from Deblur and Greengenes	29
36	t-SNE Plot with Whole Microbiome from Deblur and SILVA	29
37	t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and Greengenes	30
38	t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and SILVA	30

39	t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and Greengenes	31
40	t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and SILVA	31
41	Feature Importances with DADA2 and Greengenes	32
42	Feature Importances with DADA2 and SILVA	34
43	Feature Importances with Deblur and Greengenes	34
44	Feature Importances with Deblur and SILVA	38

## 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 two taxonomy classification databases which provided by QIIME2: Greengenes (GG) (DeSantis et al., 2006) and SILVA (Pruesse et al., 2007). Major difference between Greengenes and SILVA is resolution. Resolution of Greengenes is from kingdom to species; however, resolution of SILVA is from domain to genus. Note 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).

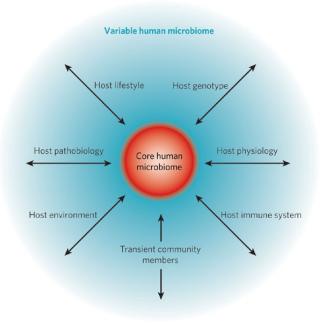


Figure 1: Concept of a Core Human Microbiome (Turnbaugh et al., 2007)



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



Figure 3: Denoising Techniques which provided by QIIME2

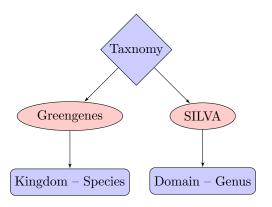


Figure 4: Taxonomy Classification which provided by QIIME2

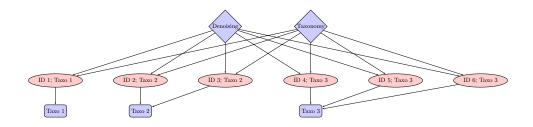


Figure 5: Example Diagram for Merging Denoising and Taxonomy Classification

#### 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.
- Faith's phylogenetic diversity (Faith PD).
- Observed features.
- · Shannon's diversity index.

Shannon's diversity index shows a quantitative measure of community richness; Observed features, however, is a qualitative measure of community richness. Faith's phylogenetic diversity index indicates a qualitative measure of community richness which assimilates phylogenetic relationship among features. Finally, evenness index, as its name, shows a measure of community evenness.

## 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.
- · Jaccard distance.
- Unweighted UniFrac distance.
- Weighted UniFrac distance.

Bray-Curtis distance shows a quantitative of community dissimilarity; Jaccard distance, however, indicates a qualitative measure of community dissimilarity. UniFrac distances reveal a measure of community dissimilarity which consolidates phylogenetic relationship among features. Difference between unweighted UniFrac distance and weighted UniFrac distance 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, in this study, followed classification algorithms are used:

• RandomForest Classification Algorithm (Breiman, 2001; Pedregosa et al., 2011)

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) =  $\frac{TP}{TP+FP}$

## 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 10; 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 3786 (Figure 11), and sampling depth with Deblur is 7253 (Figure 12).

## 4.3 Alpha-diversity

Alpha-diversity analysis with DADA2 was done: Evenness index (Table 3 and Figure 13), Faith PD (Table 4 and Figure 14), observed feature index (Table 5 and Figure 15) and Shannon's diversity index (Table 6 and Figure 16). Also, alpha-diversity analysis with DADA2 was done: Evenness index (Table 8 and Figure 17), Faith PD (Table 9 and Figure 18), observed feature index (Table 10 and Figure 19) and Shannon's diversity index (Table 11 and Figure 20). Moreover, Kruskal-Wallis tests among all groups are shown as table 2 (with DADA2) and table 7 (with Deblur).

## 4.4 Beta-diversity

Beta-diversity analysis with DADA2 was done: Bray-Curtis distance (Table 12 and Figure 21), Jaccard distance (Table 13 and Figure 22), unweighted UniFrac distance (Table 14 and Figure 23) and weighted UniFrac distance (Table 15 and Figure 23). Also, beta-diversity analysis with Deblur was done: Bray-Curtis distance (Table 16 and Figure 25), Jaccard distance (Table 17 and Figure 26), unweighted UniFrac distance (Table 18 and Figure 27) and weighted UniFrac distance (Table 19 and Figure 27).

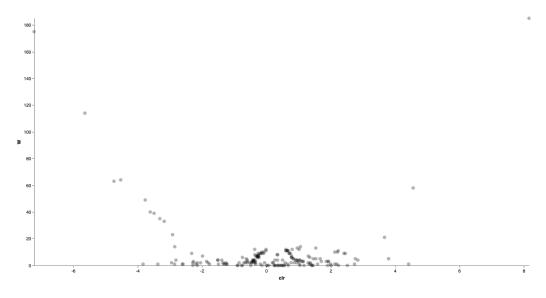


Figure 6: Example ANCOM Volcano Plot which Provided by QIIME2 (Bolyen et al., 2019, 2018)

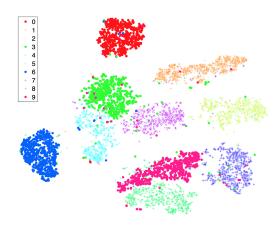


Figure 7: Visualization by t-SNE (Maaten & Hinton, 2008)

Optional:	Order Features	Devide Train	Decide the	Get Final Classification Result	Aggregate Con-
Merge Classes	by Importances	and Test set	Best Features		fusion Matrices

Figure 8: Workflow of Classification

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

Table 2. Kraskai Wallis Tests alliong 7th Group with Dr Dr 2					
Alpha-Diversity	Н	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			

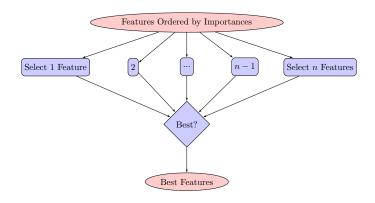


Figure 9: Deciding the Best Features

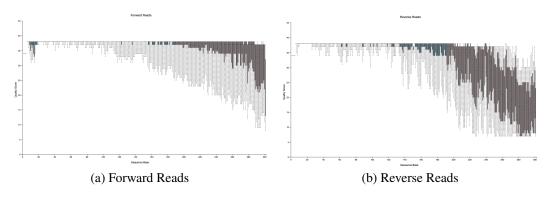


Figure 10: Sequence Quality Plot

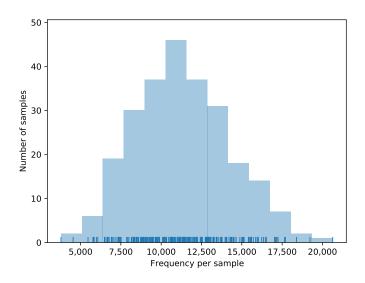


Figure 11: Frequency and Number per Sample by DADA2

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

Group 1	Group 2	Н	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

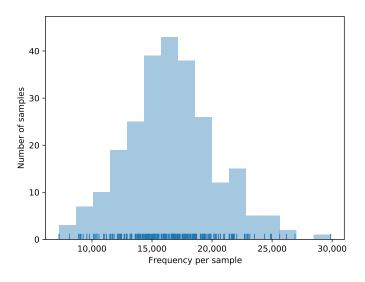


Figure 12: Frequency and Number per Sample by Deblur

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

Group 1	Group 2	Н	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	Н	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	Н	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

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

Alpha-Diversity	Н	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

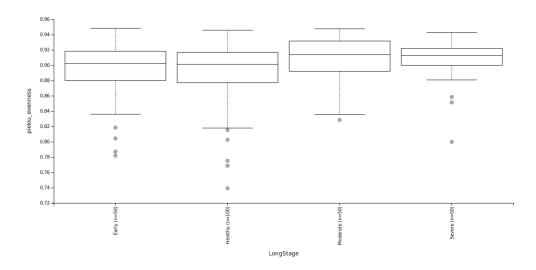


Figure 13: Evenness Index from DADA2

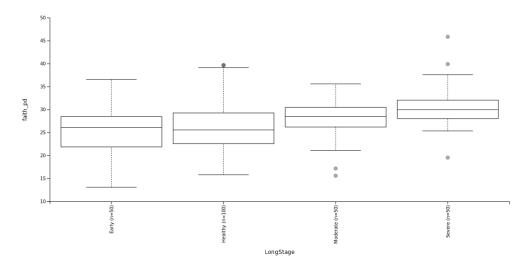


Figure 14: Faith PD Index from DADA2

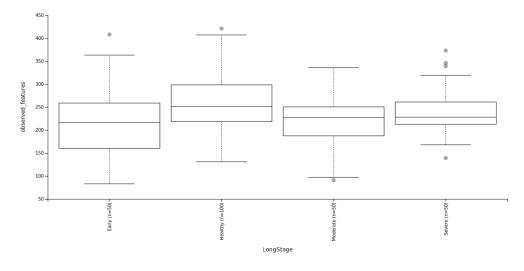


Figure 15: Observed Features Index from DADA2

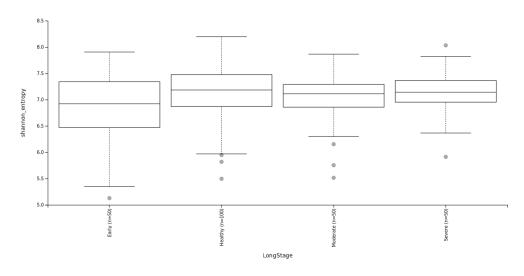


Figure 16: Shannon's Diversity Index from DADA2

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

Group 1	Group 2	Н	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	Н	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	Н	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	Н	p-value	q-value
Early (n=50)	Healthy (n=100)	0.38679735099333357	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

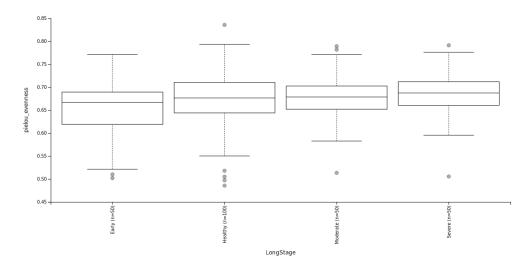


Figure 17: Evenness Index from Deblur

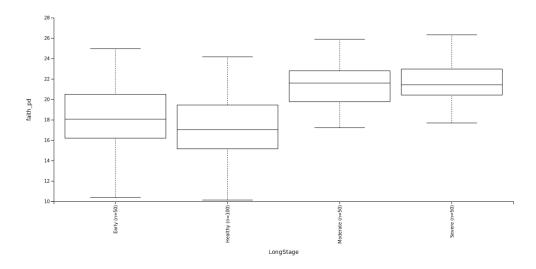


Figure 18: Faith PD Index from Deblur

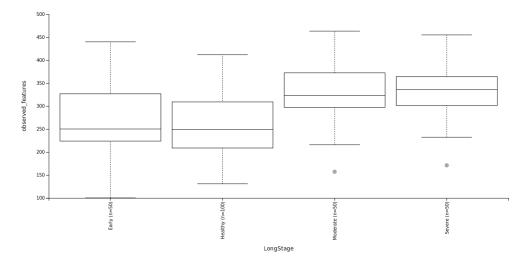


Figure 19: Observed Features Index from Deblur

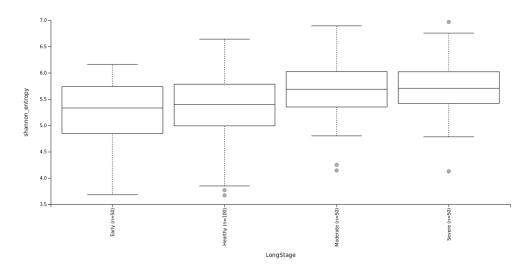


Figure 20: Shannon's Diversity Index from Deblur

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

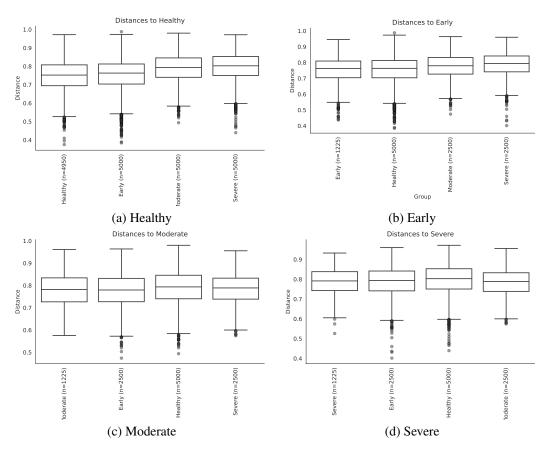


Figure 21: Bray-Curtis Distance Index with DADA2

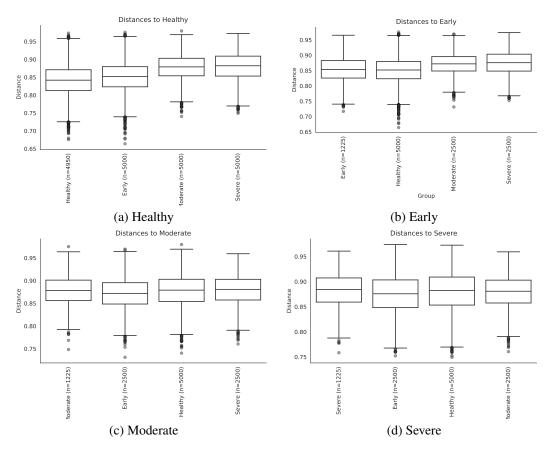


Figure 22: Jaccard Distance Index with DADA2



Figure 23: Unweighted Unifrac Distance Index with DADA2

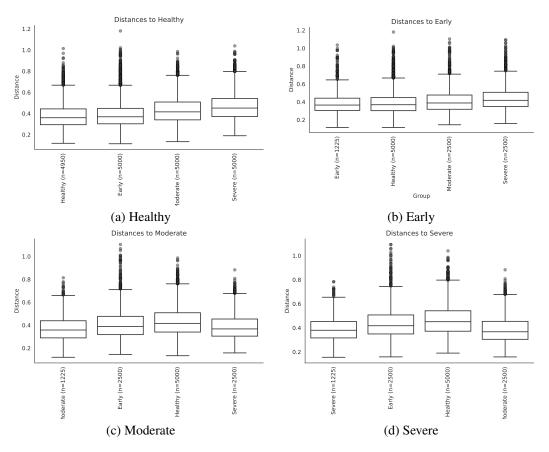


Figure 24: Weighted Unifrac Distance Index with DADA2

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	0.02279999999999997
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

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

	Table 19. Weighted Chiri the Distance mack with Debia								
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			

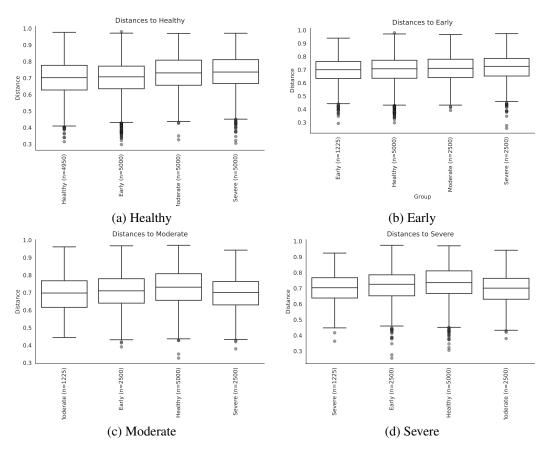


Figure 25: Bray-Curtis Distance Index with Deblur

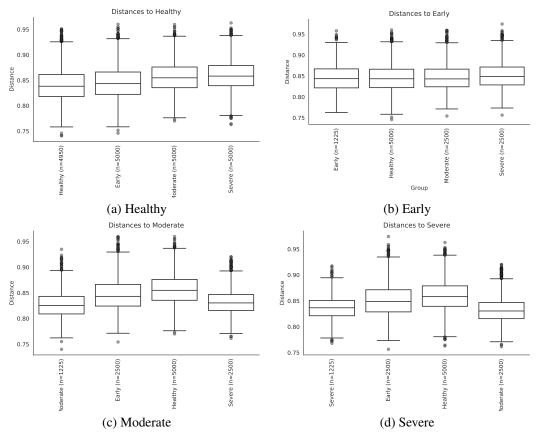


Figure 26: Jaccard Distance Index with Deblur

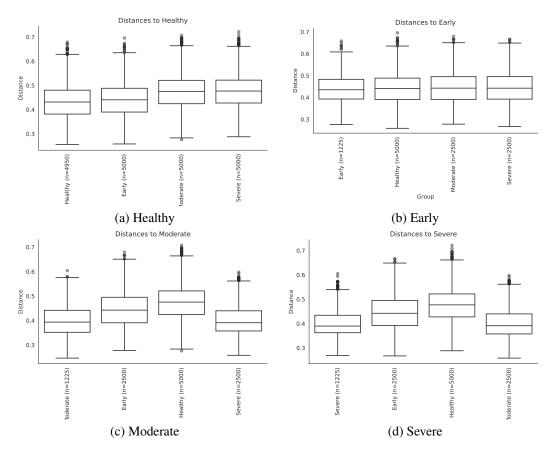


Figure 27: Unweighted Unifrac Distance Index with Deblur

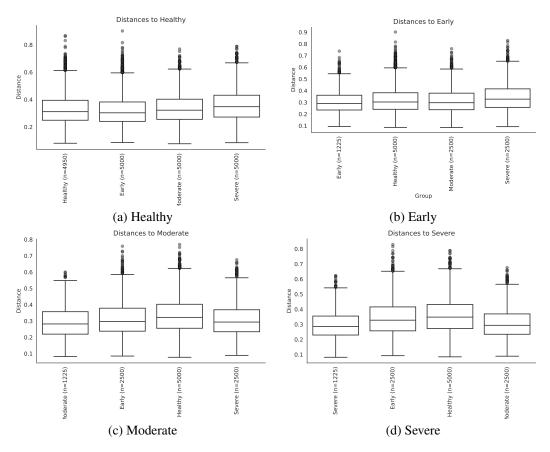


Figure 28: Weighted Unifrac Distance Index with Deblur

## 4.5 ANCOM

Statistically significant different taxa and volcano plots by ANCOM were derived: DADA2 and Greengenes (Table 20 and Figure 29), DADA2 and SILVA (Table 21 and Figure 30), Deblur and Greengenes (Table 22 and Figure 31) and Deblur and SILVA (Table 23 and Figure 32).

#### 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 Greengenes (Figure 33), 633 taxa from DADA2 and SILVA (Figure 34), 232 taxa from Deblur and Greengenes (Figure 35) and 414 taxa from Deblur and SILVA (Figure 36).

## 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 Greengenes (Figure 37), 23 taxa (as Table 21) from DADA2 and SILVA (Figure 38), 27 taxa (as Table 22) from Deblur and Greengenes (Figure 35) and 20 taxa (as Table 23) from Deblur and SILVA (Figure 40).

## 4.8 Random Forest Classifier with Every Class

- 4.8.1 DADA2 + Greengenes
- 4.8.2 DADA2 + SILVA
- 4.8.3 Deblur + Greengenes
- 4.8.4 Deblur + SILVA
- 4.9 Random Forest Classifier with Merging (Healthy+Early) Classes
- 5 Discussion
- 5.1 Alpha-diversity
- 5.2 Beta-diversity
- 5.3 t-SNE Plot
- 5.4 Random Forest Classifier with Every Class

## 6 References

- Amir, A., McDonald, D., Navas-Molina, J. A., Kopylova, E., Morton, J. T., Xu, Z. Z., ... others (2017). Deblur rapidly resolves single-nucleotide community sequence patterns. *MSystems*, 2(2).
- Barrett, P., Hunter, J., Miller, J. T., Hsu, J.-C., & Greenfield, P. (2005). matplotlib–a portable python plotting package. In *Astronomical data analysis software and systems xiv* (Vol. 347, p. 91).
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A., ... others (2018). *Qiime 2: Reproducible, interactive, scalable, and extensible microbiome data science* (Tech. Rep.). PeerJ Preprints.
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... others (2019). Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. *Nature biotechnology*, *37*(8), 852–857.
- Breiman, L. (2001). Random forests. Machine learning, 45(1), 5-32.
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). Dada2: high-resolution sample inference from illumina amplicon data. *Nature methods*, *13*(7), 581–583.
- DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., ... Andersen, G. L. (2006). Greengenes, a chimera-checked 16s rrna gene database and workbench compatible with arb. *Applied and environmental microbiology*, 72(7), 5069–5072.
- Flemmig, T. F. (1999). Periodontitis. Annals of Periodontology, 4(1), 32–37.

Table 20: ANCOM Significant Taxa with DADA2 and Greengenes

Table 20. AIVCOM Significant Taxa with DADA2 and Greengenes						
	W	Reject null hypothesis				
Bacteria Actinobacteria Actinomycetales Actinomyc-	326	True				
etaceae Actinomyces						
Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Fil-	325	True				
ifactor						
Bacteria Spirochaetes Spirochaetaes Spirochaetaceae	325	True				
Treponema						
Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Pre-	323	True				
votella intermedia						
Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	321	True				
Porphyromonas endodontalis						
Bacteria Spirochaetes Spirochaetales Spirochaetaceae	320	True				
Treponema amylovorum						
Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibri-	319	True				
onaceae TG5						
Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae	318	True				
Mycoplasma						
Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	315	True				
Tannerella						
Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	313	True				
Porphyromonas						
Bacteria Actinobacteria Actinobacteria Actinomycetales Corynebacte-	309	True				
riaceae Corynebacterium durum						
Bacteria Bacteroidetes Bacteroidia Bacteroidales	306	True				
Bacteria Firmicutes Clostridia Clostridiales [Mogibacteriaceae]	305	True				
Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales	305	True				
Campylobacteraceae Campylobacter						
Bacteria Firmicutes Clostridia Clostridiales [Acidaminobacteraceae]	304	True				

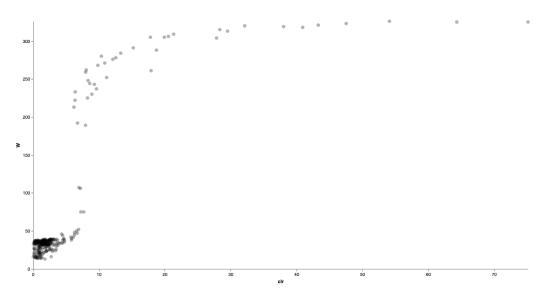


Figure 29: ANCOM Volcano Plot with DADA2 and Greengenes

Table 21: ANCOM Significant Taxa with DADA2 and SILVA

	W	Reject null hypothesis
Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	632	True
etaceae Actinomyces		
Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	629	True
Porphyromonas Porphyromonas gingivalis		
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Pep-	627	True
tostreptococcaceae Filifactor Filifactor alocis		
Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Pre-	626	True
votella Prevotella intermedia		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	626	True
Treponema Treponema denticola		
Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	623	True
etaceae Actinomyces Schaalia odontolytica		
Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tan-	622	True
nerella Tannerella forsythia		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	621	True
Treponema Treponema medium		
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	619	True
Anaerovoracaceae [Eubacterium] nodatum group [Eubacterium]		
nodatum		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	619	True
Treponema uncultured bacterium		
Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae My-	617	True
coplasma Metamycoplasma faucium		
Bacteria Synergistota Synergistia Synergistales Synergistaceae	616	True
Fretibacterium		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	616	True
Treponema	010	1100
Bacteria Firmicutes Clostridia Lachnospirales Defluviitaleaceae Deflu-	614	True
viitaleaceae UCG-011 Lachnospiraceae bacterium	01.	1100
Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	613	True
Porphyromonas	013	1140
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	612	True
Anaerovoracaceae [Eubacterium] brachy group [Eubacterium] brachy	012	Truc
Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	609	True
Bacteria Actinobacteriota Actinobacteria Corynebacteriales	608	True
•	008	True
Corynebacteriaceae Corynebacterium Corynebacterium durum	608	True
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Anaerovoracaceae [Eubacterium] saphenum group Eubacterium	000	1146
- 1 0 1		
saphenum	601	Т
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	601	True
Treponema Treponema maltophilum	507	Tr.
Bacteria Campilobacterota Campylobacteria Campylobacterales	597	True
Campylobacteraceae Campylobacter Campylobacter showae	505	T.
Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	597	True
etaceae Actinomyces Actinomyces graevenitzii	550	<b></b>
Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribac-	573	True
erium		

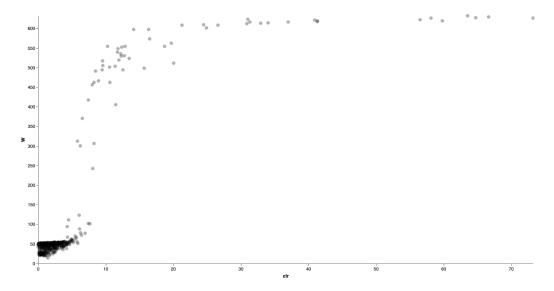


Figure 30: ANCOM Volcano Plot with DADA2 and SILVA

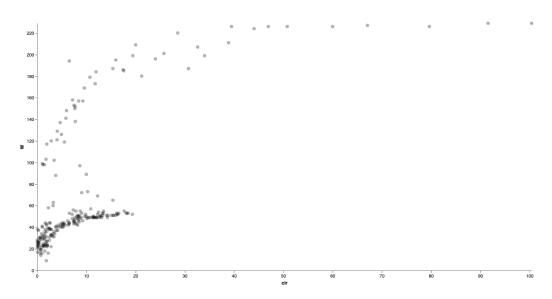


Figure 31: ANCOM Volcano Plot with Deblur and Greengenes

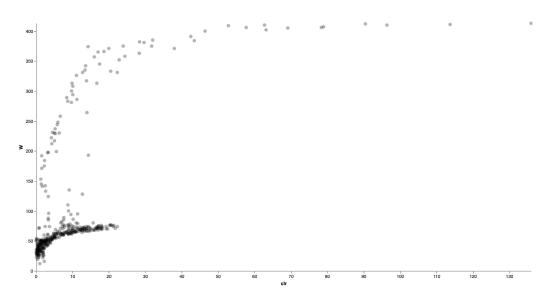


Figure 32: ANCOM Volcano Plot with Deblur and SILVA

Table 22: ANCOM Significant Taxa with Deblur and Greengenes

Table 22: ANCOM Significant Taxa with Deblur and		
	W	Reject null hypothesis
Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Fil-	229	True
ifactor		
Bacteria Spirochaetes Spirochaetaes Spirochaetaceae	229	True
Treponema		
Bacteria Spirochaetes Spirochaetaes Spirochaetaceae	227	True
Treponema amylovorum		
Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Pre-	226	True
votella intermedia		
Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae	226	True
Mycoplasma		
Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	226	True
Porphyromonas endodontalis		
Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	226	True
Tannerella	220	Truc
Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibri-	226	True
onaceae TG5	220	Truc
Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	224	True
Porphyromonas	224	True
* *	220	Terro
Bacteria Bacteroidetes Bacteroidia Bacteroidales	220	True
Bacteria Firmicutes Clostridia Clostridiales [Mogibacteriaceae]	211	True
Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Pep-	209	True
tostreptococcus	205	<b></b>
Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desul-	207	True
fobulbaceae Desulfobulbus		_
Bacteria Spirochaetes Spirochaetales Spirochaetaceae	201	True
Treponema socranskii		
Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales	199	True
Campylobacteraceae Campylobacter		
Bacteria Firmicutes Clostridia Clostridiales [Acidaminobacteraceae]	199	True
Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurel-	196	True
laceae Haemophilus parainfluenzae		
Bacteria Firmicutes Clostridia Clostridiales [Tissierellaceae] Parvi-	195	True
monas		
Bacteria Proteobacteria Betaproteobacteria Neisseriales Neisseriaceae	194	True
Neisseria subflava		
Bacteria Firmicutes Clostridia Clostridiales [Mogibacteriaceae]	187	True
Mogibacterium		
Bacteria Actinobacteria Actinomycetales Actinomyc-	187	True
etaceae Actinomyces		
Bacteria Firmicutes Clostridia Clostridiales [Tissierellaceae]	186	True
Bacteria Actinobacteria Actinobacteria Actinomycetales	185	True
Bacteria Firmicutes Clostridia Clostridiales	184	True
Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae Oribac-	180	True
terium	100	TIUC
	170	Tmio
Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae	179	True
Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Pre-	173	True
votella nanceiensis		

Table 23: ANCOM Significant Taxa with DADA2 and SILVA

	W	Reject null hypothesis
Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	632	True
etaceae Actinomyces		
Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	629	True
Porphyromonas Porphyromonas gingivalis		
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Pep-	627	True
tostreptococcaceae Filifactor Filifactor alocis		
Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Pre-	626	True
votella Prevotella intermedia		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	626	True
Treponema Treponema denticola		
Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	623	True
etaceae Actinomyces Schaalia odontolytica		
Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tan-	622	True
nerella Tannerella forsythia		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	621	True
Treponema Treponema medium		
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	619	True
Anaerovoracaceae [Eubacterium] nodatum group [Eubacterium]		
nodatum		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	619	True
Treponema uncultured bacterium		
Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae My-	617	True
coplasma Metamycoplasma faucium		
Bacteria Synergistota Synergistia Synergistales Synergistaceae	616	True
Fretibacterium		
Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	616	True
Treponema	010	1100
Bacteria Firmicutes Clostridia Lachnospirales Defluviitaleaceae Deflu-	614	True
viitaleaceae UCG-011 Lachnospiraceae bacterium	011	1140
Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	613	True
Porphyromonas	015	1140
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	612	True
Anaerovoracaceae [Eubacterium] brachy group [Eubacterium] brachy	012	Truc
Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	609	True
Bacteria Actinobacteriota Actinobacteria Corynebacteriales	608	True
Corynebacteriaceae Corynebacterium Corynebacterium durum	000	Truc
· · · · · · · · · · · · · · · · · · ·	608	True
Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	008	irue
Anaerovoracaceae [Eubacterium] saphenum group Eubacterium		
saphenum	601	Т
Bacteria Spirochaetota Spirochaeta Spirochaetales Spirochaetaceae	601	True
Treponema Treponema maltophilum	507	<b>T</b> D
Bacteria Campilobacterota Campylobacteria Campylobacterales	597	True
Campylobacteraceae Campylobacter Campylobacter showae	<b>505</b>	<b></b>
Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	597	True
etaceae Actinomyces Actinomyces graevenitzii	<b></b> -	
Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribac-	573	True
terium		

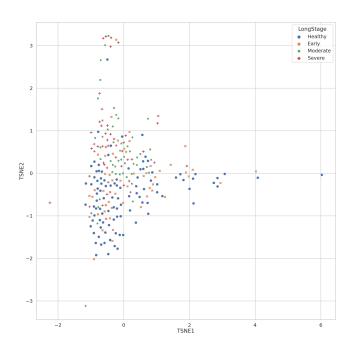


Figure 33: t-SNE Plot with Whole Microbiome from DADA2 and Greengenes

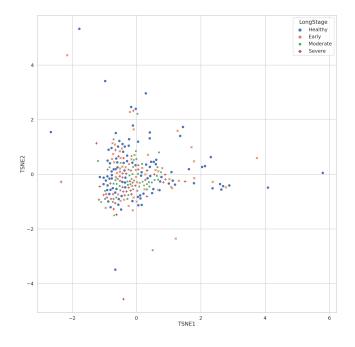


Figure 34: t-SNE Plot with Whole Microbiome from DADA2 and SILVA

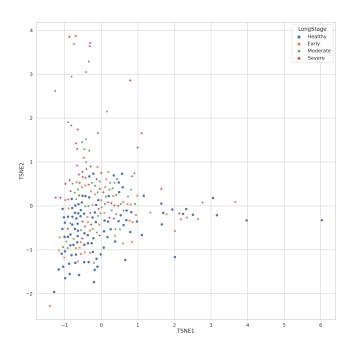


Figure 35: t-SNE Plot with Whole Microbiome from Deblur and Greengenes

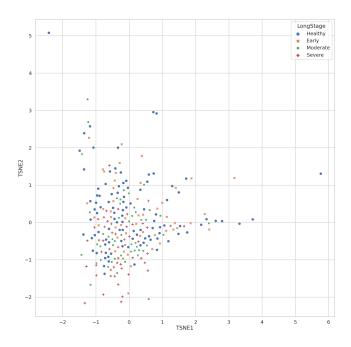


Figure 36: t-SNE Plot with Whole Microbiome from Deblur and SILVA

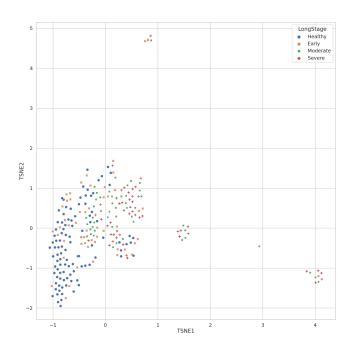


Figure 37: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and Greengenes

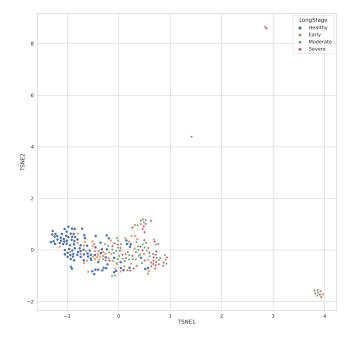


Figure 38: t-SNE Plot with ANCOM Selected Microbiome Data from DADA2 and SILVA

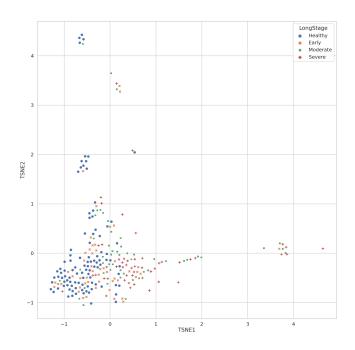


Figure 39: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and Greengenes

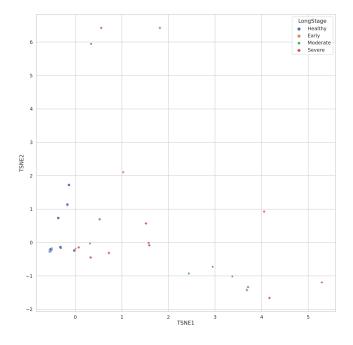


Figure 40: t-SNE Plot with ANCOM Selected Microbiome Data from Deblur and SILVA

Table 24: Taxa with DADA2 and Greengenes Ordered by Random Forest

Order	Taxonomy Classification	Importances
0	Bacteria Actinobacteria Actinomycetales Actinomyc-	0.2897668387897927
	etaceae Actinomyces	
1	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Fil-	0.1493288396019592
	ifactor	
2	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Pre-	0.07273019878053422
2	votella intermedia	0.05225255446642020
3	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	0.07237355446643938
4	Porphyromonas	0.050720055254220606
4	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	0.050739855254238686
5	Porphyromonas endodontalis Bacteria Spirochaetes Spirochaetales Spirochaetaceae	0.049447217415646096
3	Treponema amylovorum	0.043447217413040030
6	Bacteria Spirochaetes Spirochaetales Spirochaetaceae	0.046058702915828996
O	Treponema	0.040036702713626770
7	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae	0.044589335747511734
8	Bacteria Bacteroidetes Bacteroidia Bacteroidales	0.03896215615382719
9	Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales	0.038672108530872294
	Campylobacteraceae Campylobacter	***************************************
10	Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibrionaceae TG5	0.03538306656428921
11	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	0.03488113476890314
1.1	Tannerella	0.034001134/0090314
12	Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae	0.03140761957044326
12	Mycoplasma  Mycoplasma  Mycoplasmatates Mycoplasmatatecae  Mycoplasma	0.03140701737044320
13	Bacteria Actinobacteria Actinobacteria Actinomycetales Corynebacte-	0.028628460674878065
	riaceae Corynebacterium durum	
14	Bacteria Firmicutes Clostridia Clostridiales Acidaminobacteraceae	0.01703091076483563

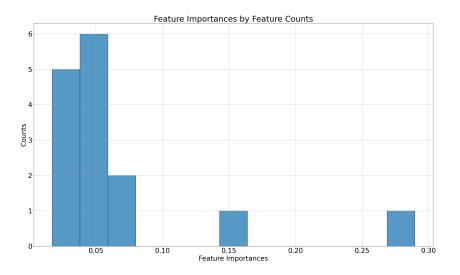


Figure 41: Feature Importances with DADA2 and Greengenes

Table 25: Taxa with DADA2 and SILVA Ordered by Random Forest		
Order	Taxonomy Classification	Importances
0	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	0.15428126769688613
	etaceae Actinomyces	
1	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	0.09830435718569176
	etaceae Actinomyces Schaalia odontolytica	
2	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Pre-	0.07571596825743382
	votella Prevotella intermedia	
3	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Pep-	0.07143963350528947
	tostreptococcaceae Filifactor Filifactor alocis	
4	Bacteria Firmicutes Clostridia Lachnospirales Lachnospiraceae Oribac-	0.05844201887575999
	terium	
5	Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tan-	0.05805089098293928
	nerella Tannerella forsythia	
6	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	0.0539170951526226
	Porphyromonas	
7	Bacteria Spirochaetota Spirochaetales Spirochaetaceae	0.0523022341592361
	Treponema Treponema denticola	
8	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	0.045795280266258155
	Porphyromonas Porphyromonas gingivalis	
9	Bacteria Actinobacteriota Actinobacteria Actinomycetales Actinomyc-	0.035433257099296185
	etaceae Actinomyces Actinomyces graevenitzii	
10	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	0.0330307414299068
	Treponema uncultured bacterium	
11	Bacteria Spirochaetota Spirochaetales Spirochaetaceae	0.029307500356325216
	Treponema Treponema medium	
12	Bacteria Spirochaetota Spirochaetales Spirochaetaceae	0.028176578352262113
10	Treponema Treponema maltophilum	0.005044050540456145
13	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	0.027844370549456147
14	Bacteria Campilobacterota Campylobacteria Campylobacterales	0.026801724029521152
1.5	Campylobacter Campylobacter showae	0.02504071242072025
15	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	0.02594971243973025
16	Anaerovoracaceae Eubacterium nodatum group Eubacterium nodatum Bacteria Actinobacteriota Actinobacteria Corynebacteriales	0.022062000205210716
16	Bacteria Actinobacteriota Actinobacteria Corynebacteriales Corynebacteriaceae Corynebacterium Corynebacterium durum	0.022962989295219716
17	Bacteria Synergistota Synergistia Synergistales Synergistaceae	0.022114274615729068
1 /	Fretibacterium	0.022114274013729000
18	Bacteria Firmicutes Clostridia Lachnospirales Defluviitaleaceae Deflu-	0.0182270448380252
10	viitaleaceae UCG-011 Lachnospiraceae bacterium	0.0102279440300232
19	Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae My-	0.017715469601623523
19	coplasma Metamycoplasma faucium	0.017713409001023323
20	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	0.016409717419062424
20	Anaerovoracaceae Eubacterium brachy group Eubacterium brachy	0.010409/1/419002424
21	Bacteria Spirochaetota Spirochaetales Spirochaetaceae	0.01483146818784845
<i>-</i> 1	Treponema	0.011031100107043
22	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	0.012945505703876552
	Anaerovoracaceae Eubacterium saphenum group Eubacterium	3.0127 10303 1030 10332
	saphenum	
	onp	

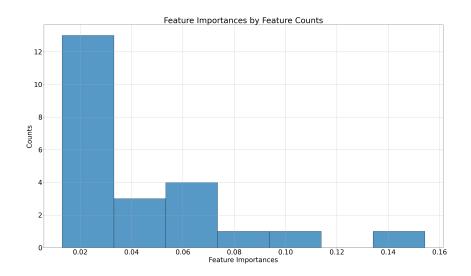


Figure 42: Feature Importances with DADA2 and SILVA

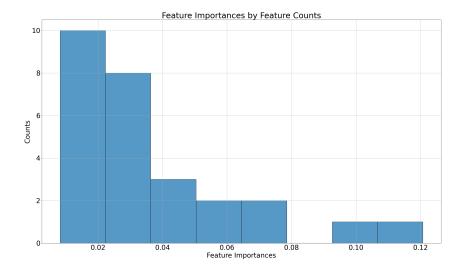


Figure 43: Feature Importances with Deblur and Greengenes

Table 26: Taxa with Deblur and Greengenes Ordered by Random Forest		
Order	Taxonomy Classification	Importances
0	Bacteria Spirochaetes Spirochaetales Spirochaetaceae	0.12074758172672563
	Treponema	
1	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Fil-	0.10272965893419596
	ifactor	
2	Bacteria Actinobacteria Actinomycetales Actinomyc-	0.06981469110924138
	etaceae Actinomyces	
3	Bacteria Proteobacteria Betaproteobacteria Neisseriales Neisseriaceae	0.06455871089828909
	Neisseria subflava	
4	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	0.05761714025200638
	Porphyromonas	
5	Bacteria Proteobacteria Gammaproteobacteria Pasteurellales Pasteurel-	0.056569557742200474
	laceae Haemophilus parainfluenzae	
6	Bacteria Synergistetes Synergistia Synergistales Dethiosulfovibri-	0.04340964763638773
	onaceae TG5	
7	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae Oribac-	0.04203144037349746
	terium	
8	Bacteria Spirochaetes Spirochaetales Spirochaetaceae	0.03958068627108471
	Treponema amylovorum	
9	Bacteria Firmicutes Clostridia Clostridiales Peptostreptococcaceae Pep-	0.0358587598116692
	tostreptococcus	
10	Bacteria Tenericutes Mollicutes Mycoplasmatales Mycoplasmataceae	0.03298154288049845
	Mycoplasma	
11	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Pre-	0.029990403308766282
10	votella nanceiensis	0.0000000000000000000000000000000000000
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	0.025845221004209433
1.6	Porphyromonas endodontalis	0.02400764507277042
16	Bacteria Firmicutes Clostridia Clostridiales Tissierellaceae	0.02409764507377042
17	Bacteria Firmicutes Clostridia Clostridiales Tissierellaceae Parvimonas	0.022165587739824334
18	Bacteria Proteobacteria Epsilonproteobacteria Campylobacterales	0.021448855670751162
19	Campylobacteraceae Campylobacter	0.0109206042818522
19	Bacteria Firmicutes Clostridia Clostridiales Mogibacteriaceae Mogibacterium	0.0198296042818533
20	C	0.01940993201994216
20	Bacteria Firmicutes Clostridia Clostridiales Lachnospiraceae	
21	Bacteria Spirochaetes Spirochaetas Spirochaetaceae Treponema socranskii	0.01868361833546748
22	Bacteria Bacteroidetes Bacteroidia Bacteroidales Prevotellaceae Pre-	0.016833611871175814
22	votella intermedia	0.0108330118/11/3814
23	Bacteria Bacteroidetes Bacteroidia Bacteroidales Porphyromonadaceae	0.015630651513388796
23	- · · · · · · · · · · · · · · · · · · ·	0.013030031313388790
24	Tannerella Bacteria Proteobacteria Deltaproteobacteria Desulfobacterales Desul-	0.014284111618279582
<b>∠</b> +	fobulbaceae Desulfobulbus	0.0172071110102/3302
25	Bacteria Bacteroidetes Bacteroidia Bacteroidales	0.01308119809996142
26	Bacteria Bacteroidaes Bacteroidaes  Bacteria Firmicutes Clostridia Clostridiales Acidaminobacteraceae	0.008226102004073872
	Dacteria i minicutes Ciostridia Ciostridiates Actualimiovactel decae	0.000220102004073072

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

Table 27: Taxa with Deblur and SILVA Ordered by Random Forest		
Order	Taxonomy Classification	Importances
0	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	0.3054581507093521
	Porphyromonas Porphyromonas gingivalis	
1	Bacteria Spirochaetota Spirochaetales Spirochaetaceae	0.06306075553390636
	Treponema uncultured bacterium	
2	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae	0.0621211496328295
3	Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Pre-	0.05634637391220579
	votella Prevotella intermedia	
4	Bacteria Spirochaetota Spirochaetales Spirochaetaceae	0.045843324732015106
	Treponema Treponema medium	
5	Bacteria Spirochaetota Spirochaetales Spirochaetaceae	0.04104161142422072
	Treponema Treponema denticola	
6	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	0.03814448314837611
	Treponema Treponema maltophilum	
7	Bacteria Spirochaetota Spirochaetia Spirochaetales Spirochaetaceae	0.03749488565496832
	Treponema	
8	Bacteria Firmicutes Bacilli Mycoplasmatales Mycoplasmataceae My-	0.03746308785002029
	coplasma Metamycoplasma faucium	
9	Bacteria Firmicutes Bacilli Lactobacillales Streptococcaceae Strepto-	0.03475399919186926
	coccus Streptococcus constellatus	
10	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	0.033280774673234606
	Anaerovoracaceae Eubacterium brachy group	0.02202045116011422
11	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales Pep-	0.03303045116011422
10	tostreptococcaceae Filifactor Filifactor alocis	0.0205712774607067
12	Bacteria Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae	0.0305712774697067
1.2	Porphyromonas Porphyromonas endodontalis	0.02054760005500002
13	Bacteria Bacteroidota Bacteroidia Bacteroidales Tannerellaceae Tan-	0.03054768095598893
14	nerella Tannerella forsythia Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae Pre-	0.029421392615318554
14	votella Prevotella dentalis	0.029421392013318334
15	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	0.02915547917774481
13	Anaerovoracaceae Eubacterium nodatum group Eubacterium nodatum	0.02913347917774401
16	Bacteria Synergistota Synergistia Synergistales Synergistaceae	0.028183918621745366
10	Fretibacterium	0.028183918021743300
17	Bacteria Desulfobacterota Desulfobulbia Desulfobulbales Desulfobul-	0.02447201285320467
17	baceae Desulfobulbus	0.02447201203320407
18	Bacteria Firmicutes Clostridia Lachnospirales Defluviitaleaceae Deflu-	0.020836896635166788
10	viitaleaceae UCG-011 Lachnospiraceae bacterium	0.020030070033100700
19	Bacteria Firmicutes Clostridia Peptostreptococcales-Tissierellales	0.018772294048011725
1)	Anaerovoracaceae Eubacterium saphenum group Eubacterium	0.010//22/4040011/23
	saphenum	
	oupriorium.	

- Gihawi, A., Rallapalli, G., Hurst, R., Cooper, C. S., Leggett, R. M., & Brewer, D. S. (2019). Sepath: benchmarking the search for pathogens in human tissue whole genome sequence data leads to template pipelines. *Genome biology*, 20(1), 1–15.
- Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., ... Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *science*, *312*(5778), 1355–1359.
- Hunter, J. D. (2007). Matplotlib: A 2d graphics environment. Computing in science & engineering, 9(3), 90–95.
- James, F. C., & Rathbun, S. (1981). Rarefaction, relative abundance, and diversity of avian communities. *The Auk*, 98(4), 785–800.
- Maaten, L. v. d., & Hinton, G. (2008). Visualizing data using t-sne. *Journal of machine learning research*, 9(Nov), 2579–2605.
- Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial ecology in health and disease*, 26(1), 27663.
- McKinney, W., et al. (2011). pandas: a foundational python library for data analysis and statistics. *Python for High Performance and Scientific Computing*, 14(9).
- Olsen, G. J., & Woese, C. R. (1993). Ribosomal rna: a key to phylogeny. The FASEB journal, 7(1), 113–123.
- Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., ... others (2011). Scikit-learn: Machine learning in python. *the Journal of machine Learning research*, 12, 2825–2830.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). Silva: a comprehensive online resource for quality checked and aligned ribosomal rna sequence data compatible with arb. *Nucleic acids research*, 35(21), 7188–7196.
- Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, 449(7164), 804–810.
- Van Dyke, T. E., & Dave, S. (2005). Risk factors for periodontitis. *Journal of the International Academy of Periodontology*, 7(1), 3.
- Waskom, M., & the seaborn development team. (2020, September). *mwaskom/seaborn*. Zenodo. Retrieved from https://doi.org/10.5281/zenodo.592845 doi: 10.5281/zenodo.592845
- Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., ... others (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, 5(1), 27.

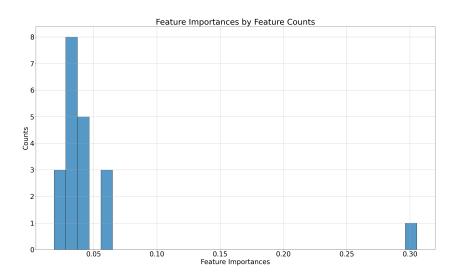


Figure 44: Feature Importances with Deblur and SILVA