

Metagenome Analysis of Preterm Birth

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Overview

1 Introduction

2 Materials

3 Methods

4 Results

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1. Introduction

Microbiome

- Microbiota: the microorganisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome: 10^{13} to 10^{14} microorganisms whose collective genome (Gill et al., 2006)



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

rRNA

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

Preterm Birth (PTB)

PTB:

- ① PTB < 37 GW (Gestational week)
- ② Normal ≥ 37 GW

Detailed PTB:

- ① Early PTB < 34 GW
- ② 34 GW \leq Late PTB < 37 GW
- ③ Normal ≥ 37 GW

(J. Tucker & McGuire, 2004; Voronkov, Solonovych, Liashenko, & Revenko, 2018)

2. Materials

16S rRNA Sequencing

16S rRNA sequencing is the *reference method* for bacterial taxonomy & identification (Mignard & Flandrois, 2006)

Three main reasons (Janda & Abbott, 2007):

- 16S rRNA exists in almost all bacteria
- Functions of the 16S rRNA has not changed over evolution.
- 16S rRNA is large enough for bioinformatics

Data Composition

- JBNU/Helixco data
 - First data
 - Second data
 - Stool data

Table: Sample Information

Data	Participants	Samples	Remarks
First	24	107	-
Second	35	288	-
Third	10	106	-
Stool	63	126	Stool

3. Methods

QIIME 2 Workflow

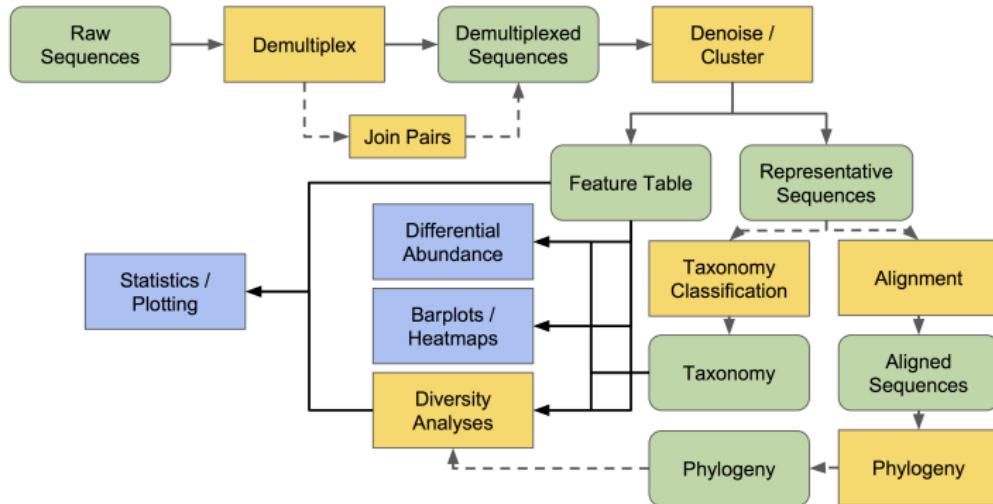


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal, Van Treuren, White, Eggesbø, et al., 2015; McDonald et al., 2012)

4. Results

4. Results

4.1. Data Processing with Qiime

Filtering with Quality Score

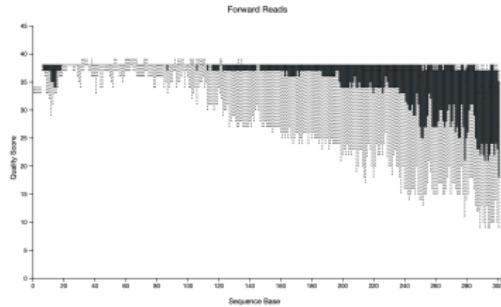
Drawback between:

- Longer sequence read
- Higher quality value

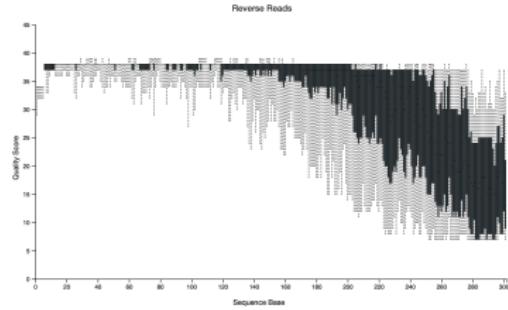
∴ Select the maximum length n where:

$$\begin{aligned} \forall n_i \in \{n_k | \text{MedianQualityScore} \geq 30\} \\ \exists! n \in \{n_i\} : n \geq n_i \end{aligned} \tag{1}$$

Quality Score from JBU/Helixco Data



(a) Forward



(b) Reverse

Figure: Quality Score Plot

- $\ell_{Forward} = 300$
- $\ell_{Reverse} = 245$

Denoising Techniques

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

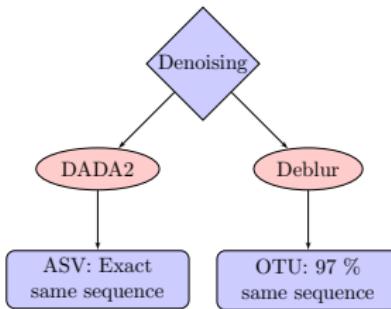


Figure: Denoising Algorithms

Taxonomy Classification

- Greengenes (GG) (DeSantis et al., 2006)
- SILVA (Pruesse et al., 2007; Quast et al., 2012)
- Human Oral Microbiome Database (HOMD) (Chen et al., 2010)

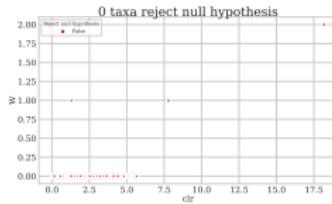
"A **higher** performance at taxonomic levels above *genus level*;
but performance appears to **drop** at *species level*" (Gihawi et al., 2019)

4. Results

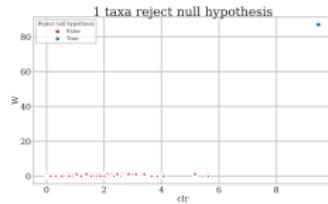
4.2. Abundance Test with ANCOM

- Analysis with composition of microbiome (Mandal, Van Treuren, White, Eggesbø, et al., 2015)
- ANCOM detects significantly abundant taxa, while maintain high statistical power
- Find taxa that can divide each classes

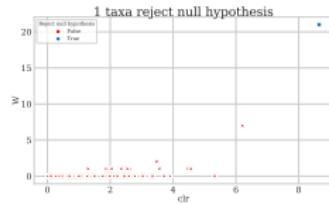
ANCOM with Detail PTB



(a) 1-day



(b) 3-day



(c) 5-day

Figure: ANCOM for Detail PTB from Neonatal Mouth with DADA2+HOMD

4. Results

4.3. Abundance Test with LefSe

- Linear discriminant analysis Effect Size (Segata et al., 2011)
- LefSe finds the features likely to explicate differences between groups

LefSe with Detail PTB

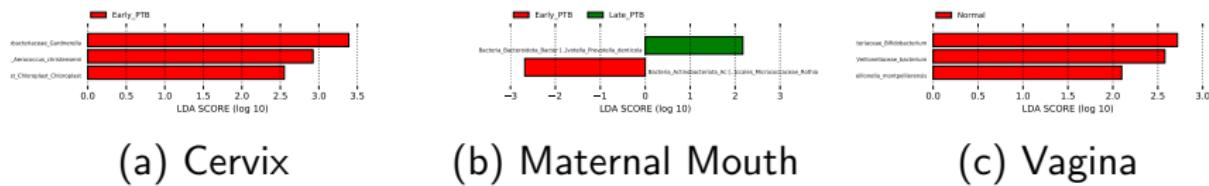


Figure: LefSe for Detail PTB with Deblur + Silva

4. Results

4.4. Taxonomy Overview

4. Results

4.4. Taxonomy Overview

4.4.1. Abundance

Abundance Distribution

Abundance

- Minimum: 0
- Mean: 1.8
- Median: 0.0
- Maximum: 8848.0

Abundance without Zero

- Minimum: 1.0
- Mean: 189.6
- Median: 28.0
- Maximum: 8848.0

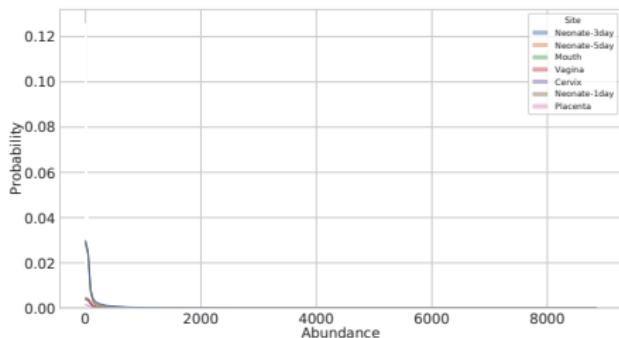


Figure: Abundance distribution

Clustermap with Abundance

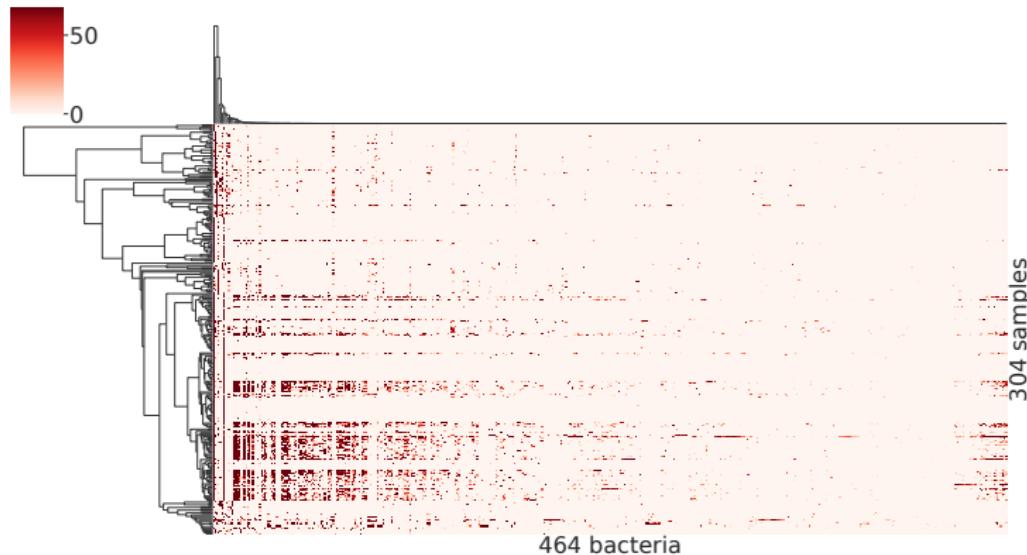
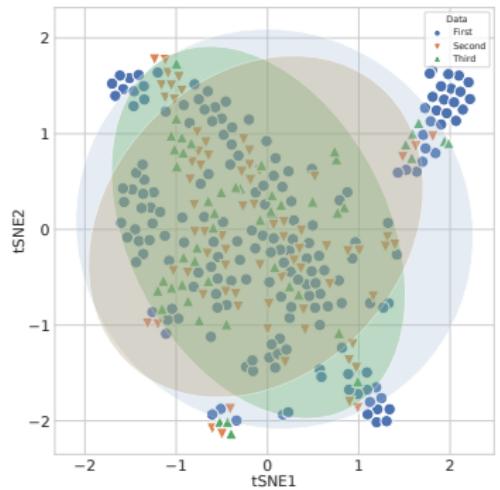
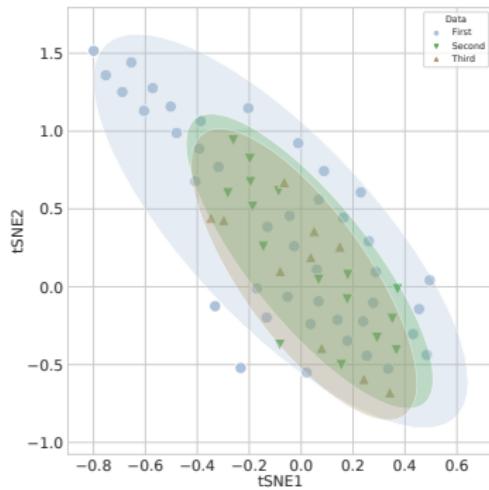


Figure: Clutermap with Abundance

t-SNE with Abundance I



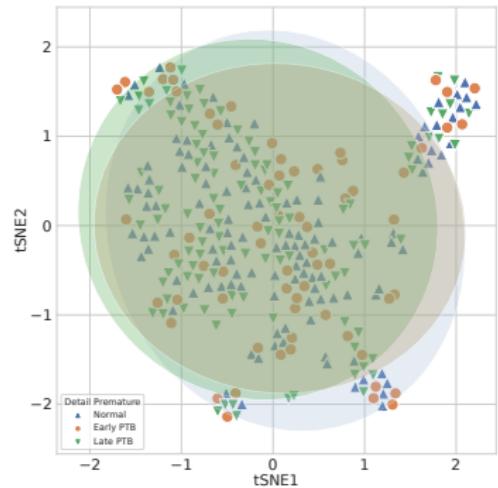
(a) All



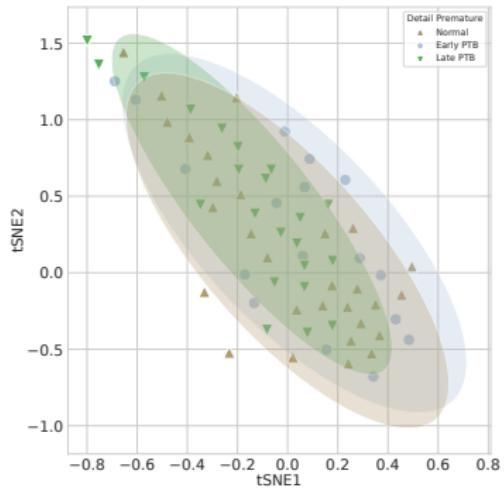
(b) Mother Mouth

Figure: t-SNE plot of Abundance with Data Batch

t-SNE with Abundance II



(a) All



(b) Mother Mouth

Figure: t-SNE plot of Abundance with PTB

4. Results

4.4. Taxonomy Overview

4.4.2. Proportion

Proportion Distribution

Proportion

- Minimum: 0.0
- Mean: 0.00008
- Median: 0.0
- Maximum: 0.793

Proportion without Zero

- Minimum: 0.00002
- Mean: 0.00008
- Median: 0.00153
- Maximum: 0.793

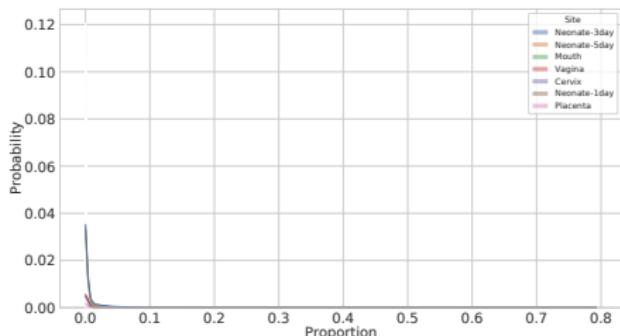


Figure: Proportion distribution

Clustermap with Proportion

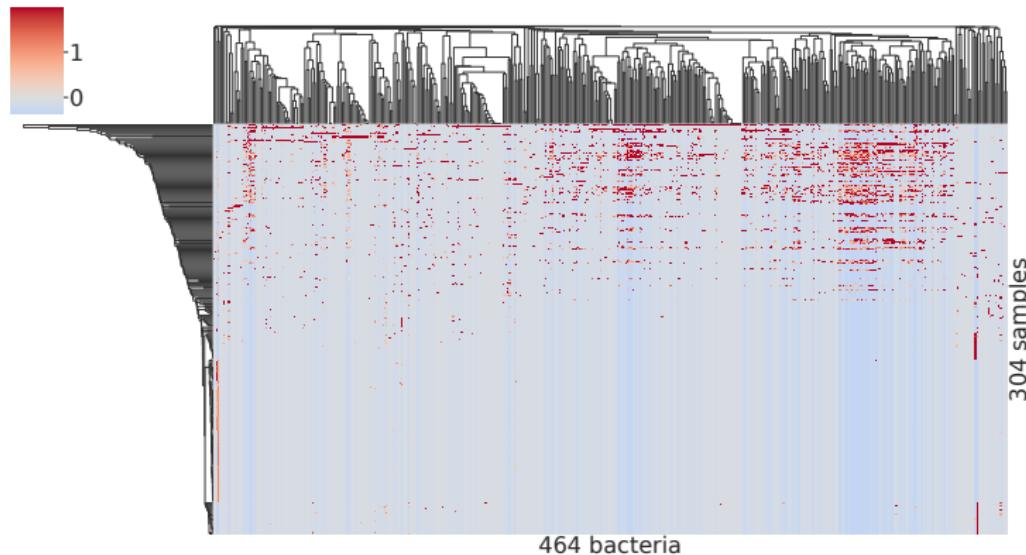
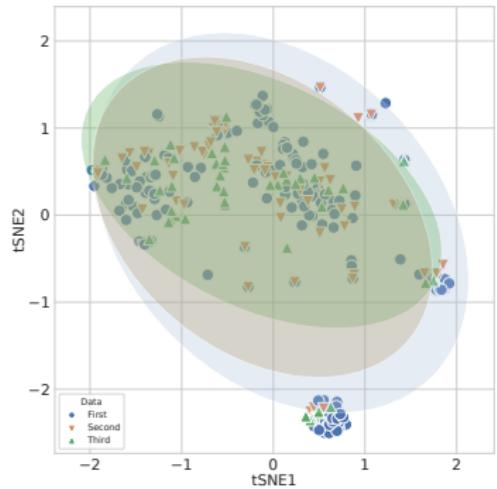
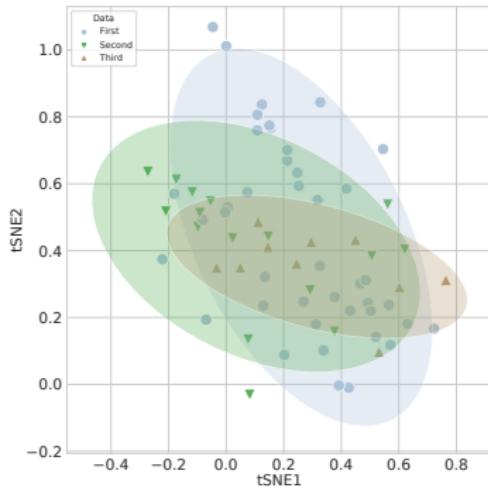


Figure: Clustermap with Proportion

t-SNE with Proportion I



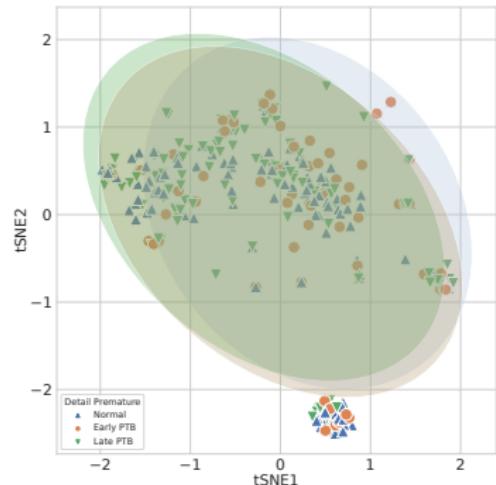
(a) All



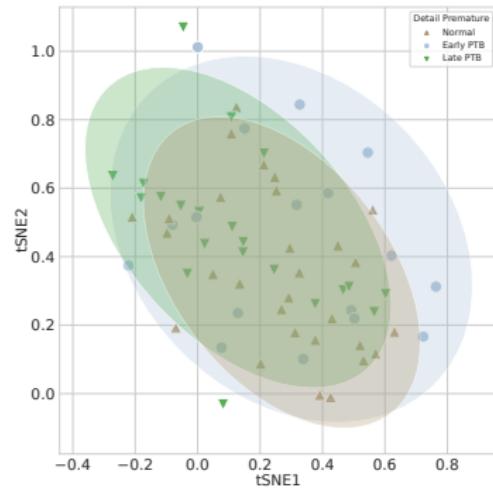
(b) Mother Mouth

Figure: t-SNE plot of Proportion with Data Batch

t-SNE with Proportion II



(a) All



(b) Mother Mouth

Figure: t-SNE plot of Proportion with PTB

4. Results

4.5. Diversity Index

Diversity Index

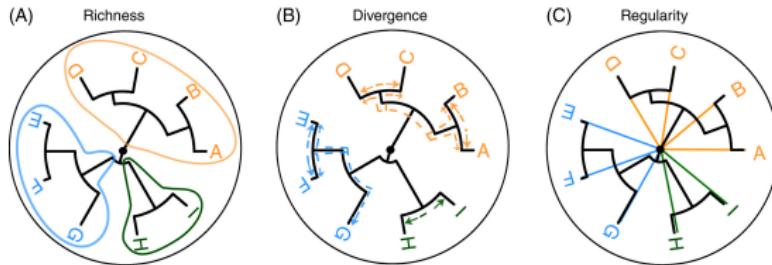


Figure: Three dimensions of phylogenetic information (C. M. Tucker et al., 2017)

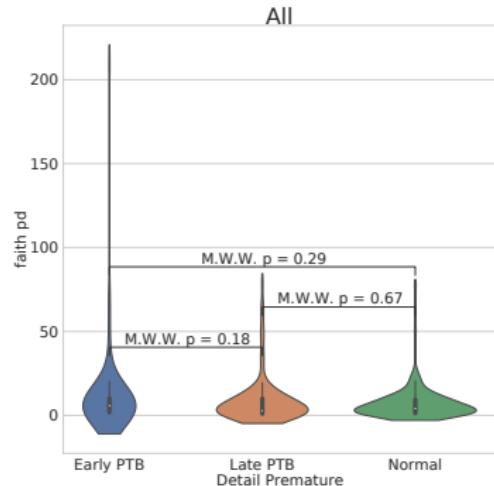
- A quantitative measure that shows richness, divergence, and regularity (C. M. Tucker et al., 2017)
- Alpha diversity: the richness of taxa **at a single community**
- Beta diversity: taxonomy differentiation **between communities**

4. Results

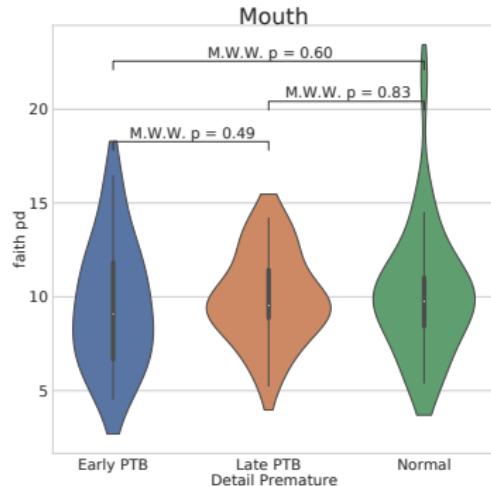
4.5. Diversity Index

4.5.1. Alpha-diversity

Violin Plot with Alpha-diversity I



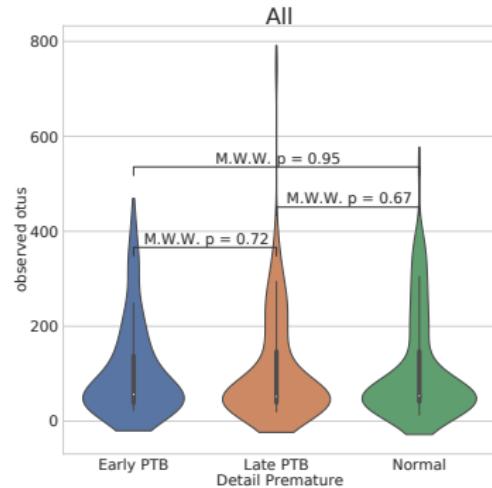
(a) All



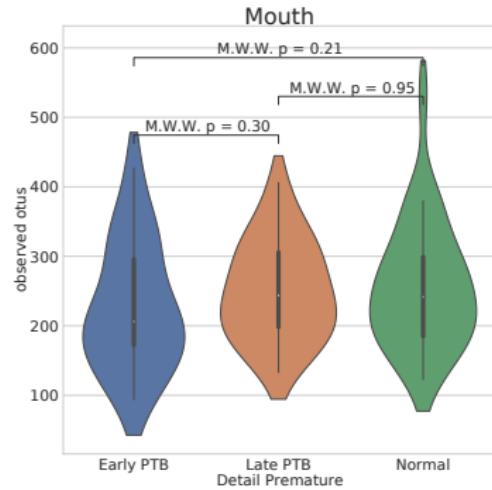
(b) Mother Mouth

Figure: Detail premature & Faith's PD

Violin Plot with Alpha-diversity II



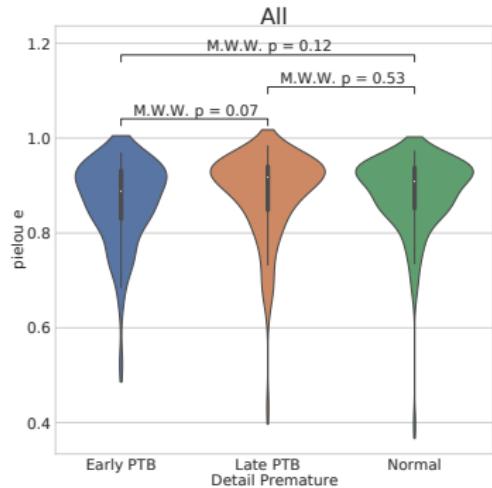
(a) All



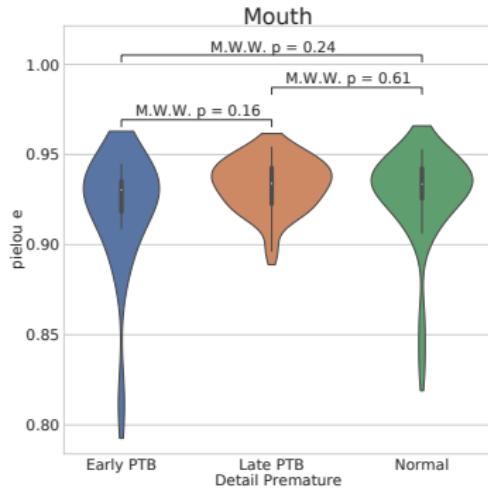
(b) Mother Mouth

Figure: Detail premature & Observed OTUs

Violin Plot with Alpha-diversity III



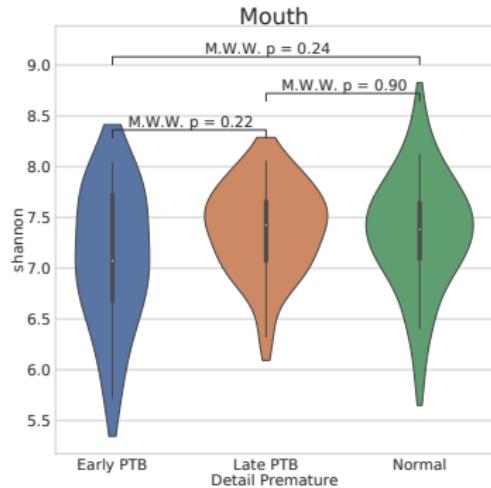
(a) All



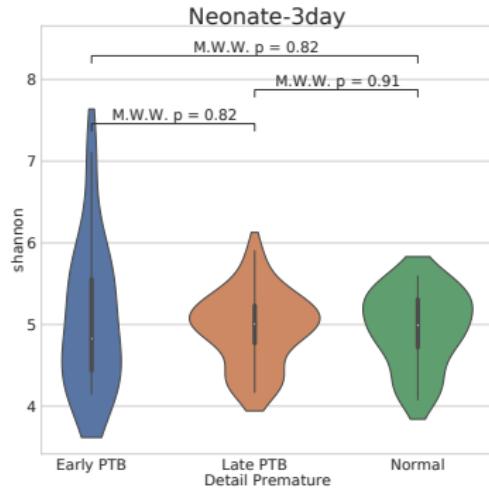
(b) Mother Mouth

Figure: Detail premature & Pielou Evenness

Violin Plot with Alpha-diversity IV



(a) All



(b) Mother Mouth

Figure: Detail premature & Shannon Entropy

4. Results

4.5. Diversity Index

4.5.2. Beta-diversity

Cluster map with Beta-diversity I

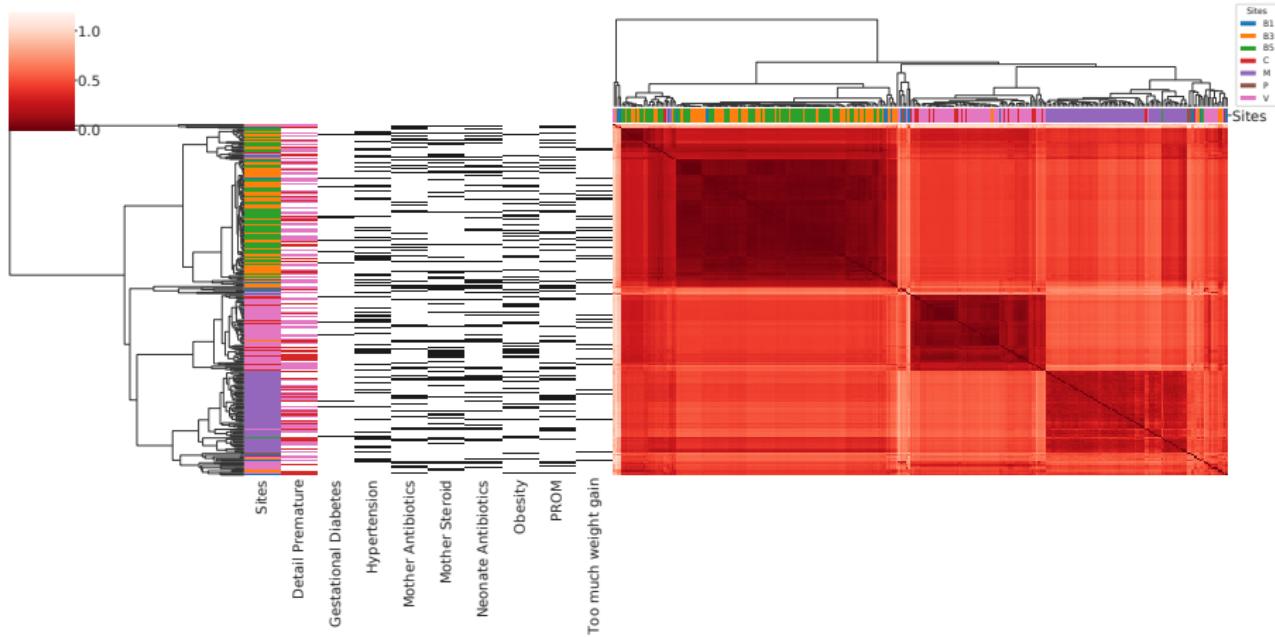
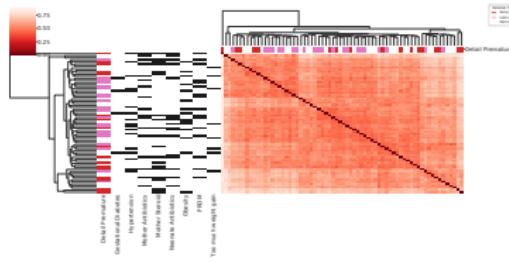
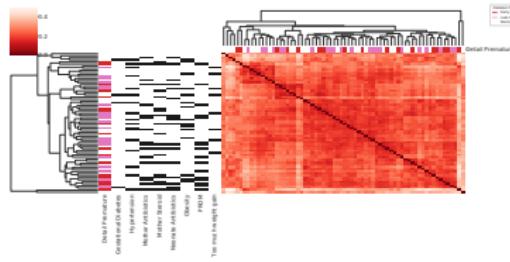


Figure: Cluster map with Weighted UniFrac distance index for DADA2

Cluster map with Beta-diversity II



(a) Unweighted UniFrac



(b) Weighted UniFrac

Figure: Clustermap of Abundance in Maternal Mouth

4. Results

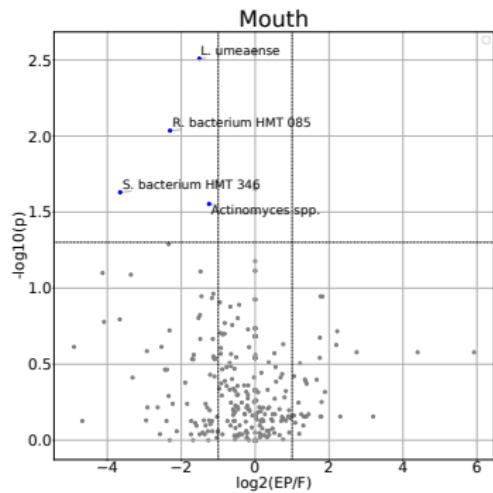
4.6. Taxonomy Analyses

4. Results

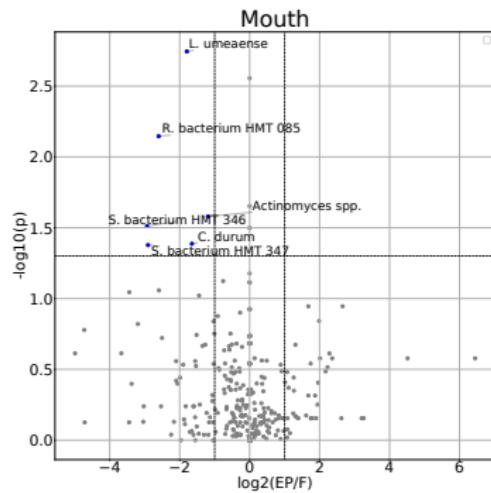
4.6. Taxonomy Analyses

4.6.1. Differentially Abundant Taxa

Volcano plot



(a) Abundance



(b) Proportion

Figure: Differentially abundant taxa in Maternal Mouth

Differentially Abundant Taxa I

L. umeaense



4. Results

4.6. Taxonomy Analyses

4.6.2. Correlation with Clinical Data

Correlation between Taxonomy & Clinical data I

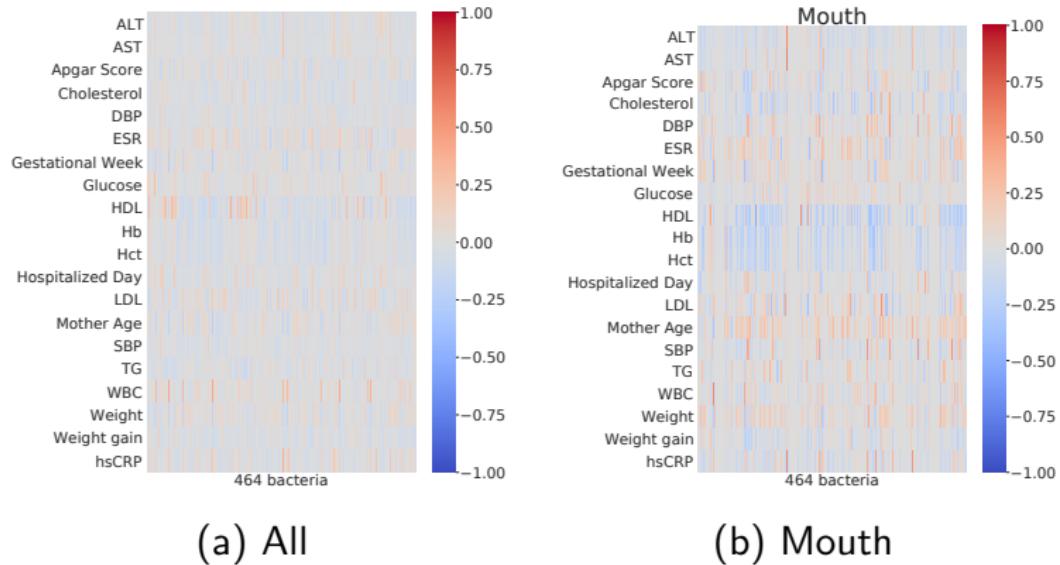


Figure: Pearson correlation on Taxonomy Abundance

Correlation between Taxonomy & Clinical data II

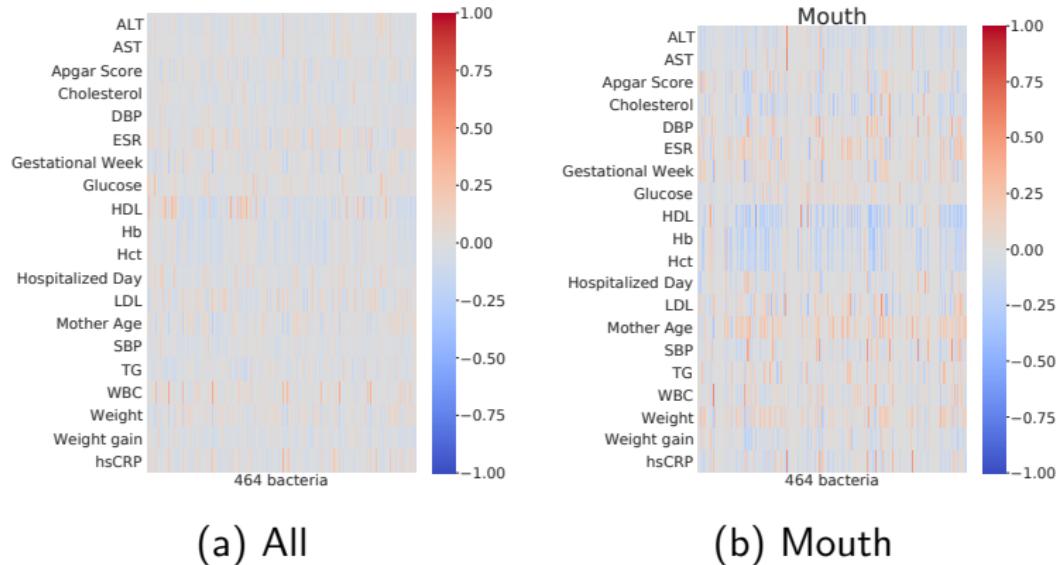


Figure: Pearson correlation on Taxonomy Proportion

4. Results

4.7. Machine Learning

ML algorithm comparison

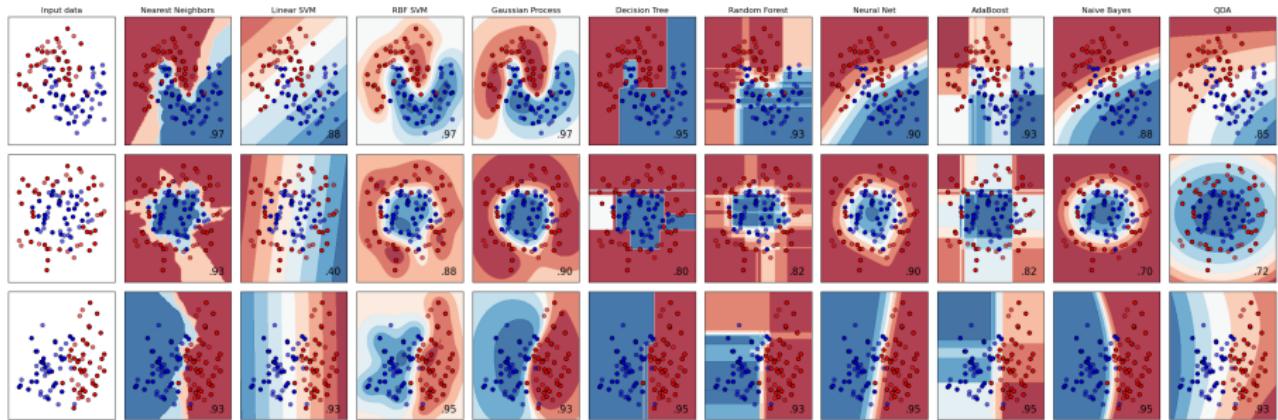


Figure: Classification Comparison (Pedregosa et al., 2011)

Oversampling

Naive Random Oversampling

- ① Randomly pick a sample from under-represented class.
- ② Duplicate it.
- ③ Repeat until all classes are even.

4. Results

4.7. Machine Learning

4.7.1. Random Forest Classifier on Abundance

Random Forest with (Early vs. Late vs. Full) I

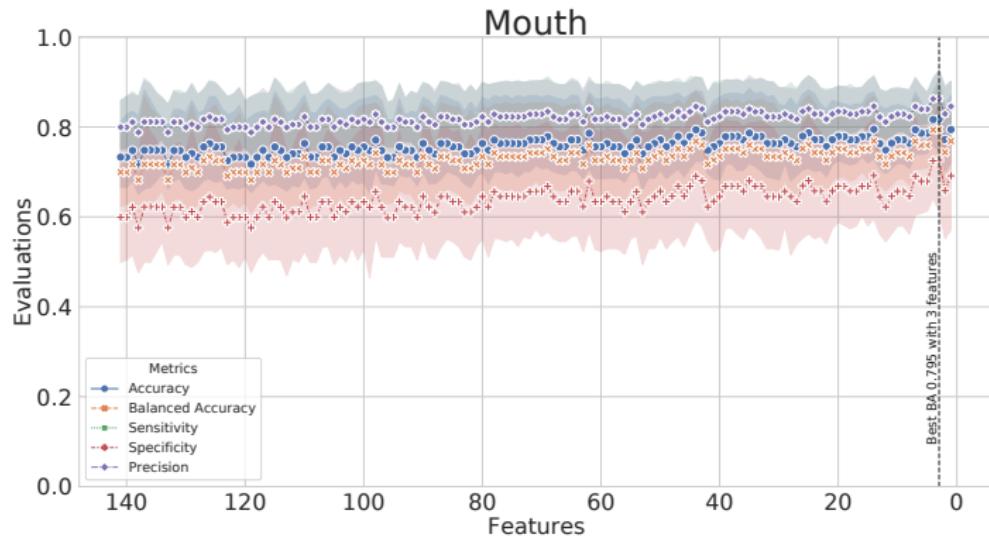


Figure: RF evaluations with feature counts

Random Forest with (Early vs. Late vs. Full) II

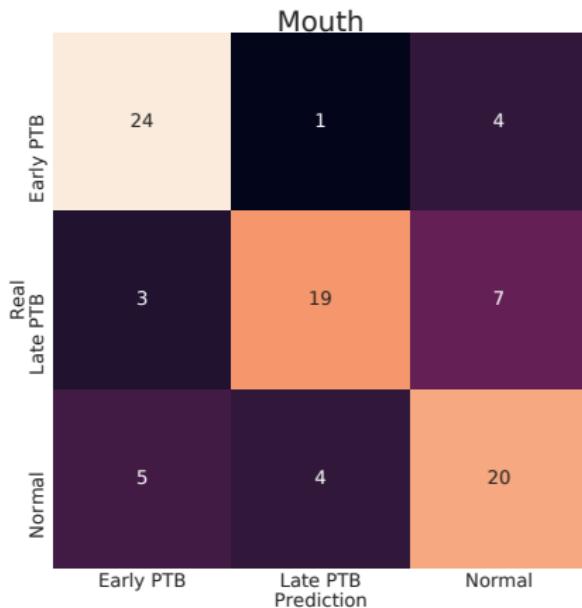


Figure: RF confusion matrix

Random Forest with (Early vs. Late vs. Full) III

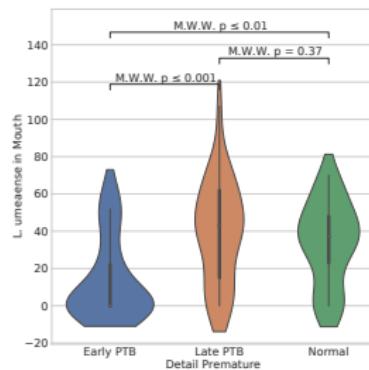
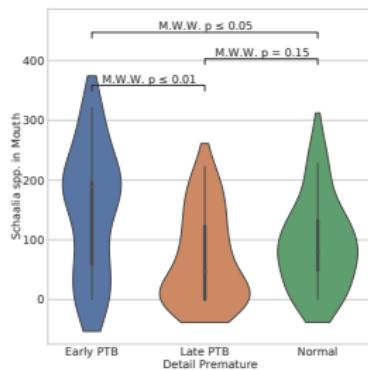
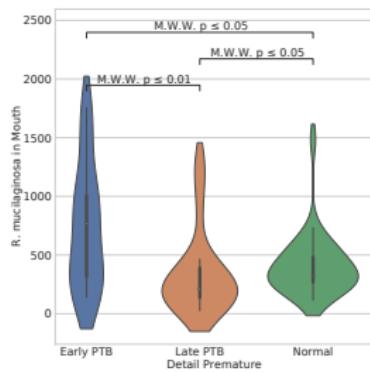


Figure: RF most important taxa

Random Forest with (Early vs. Late + Full) I

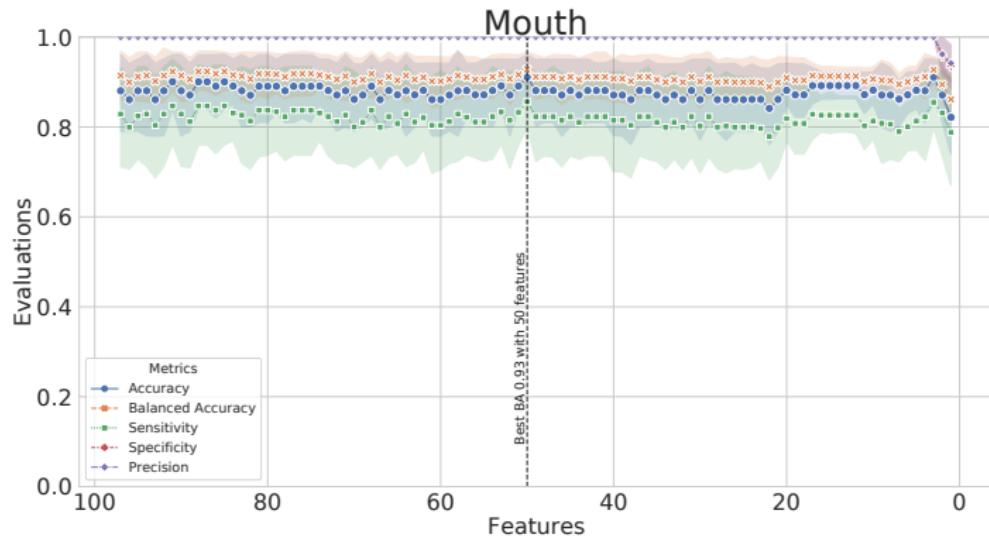


Figure: RF evaluations with feature counts

Random Forest with (Early vs. Late + Full) II

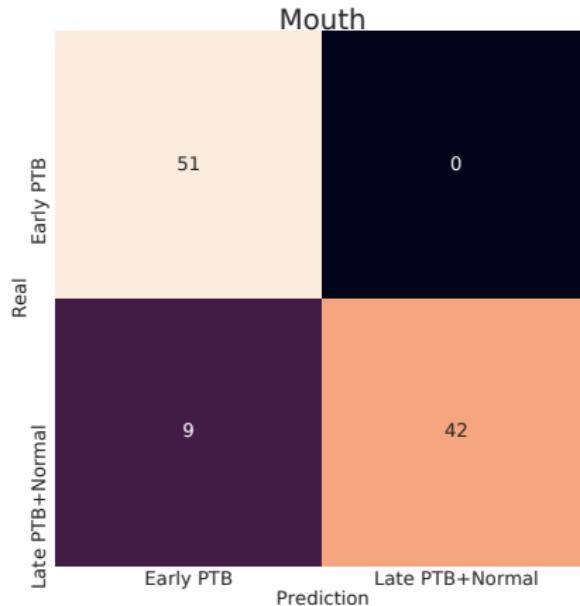


Figure: RF confusion matrix

Random Forest with (Early vs. Late + Full) III

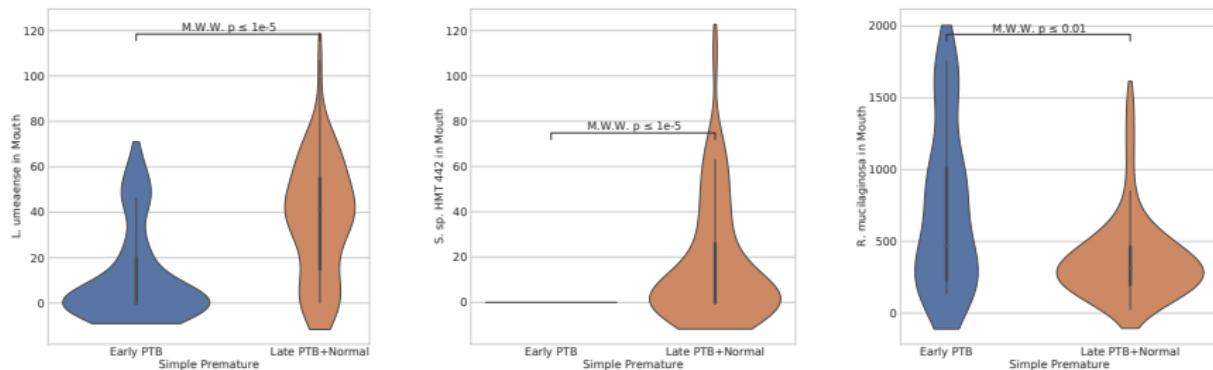


Figure: RF most important taxa

4. Results

4.7. Machine Learning

4.7.2. Random Forest Classifier on Proportion

Random Forest with (Early vs. Late vs. Full) I

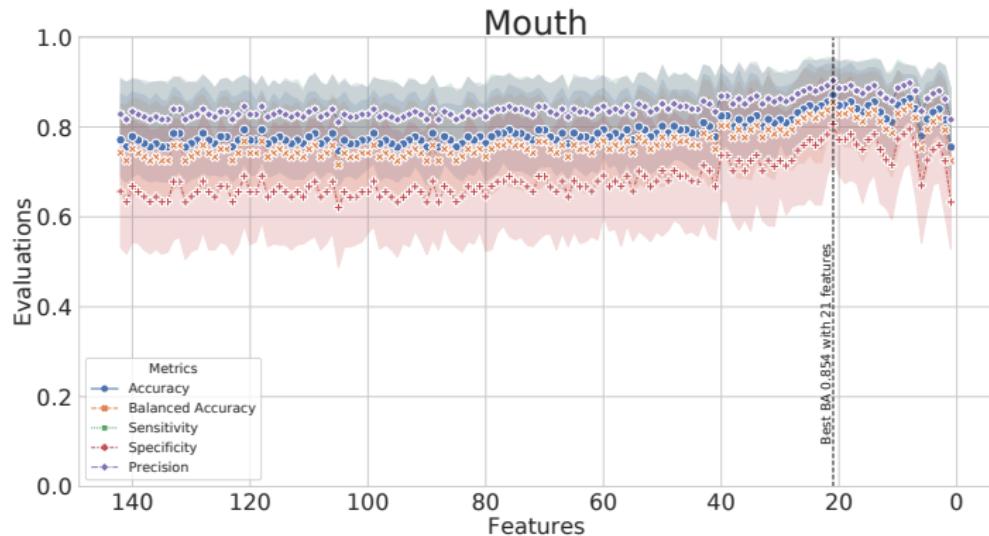


Figure: RF evaluations with feature counts

Random Forest with (Early vs. Late vs. Full) II

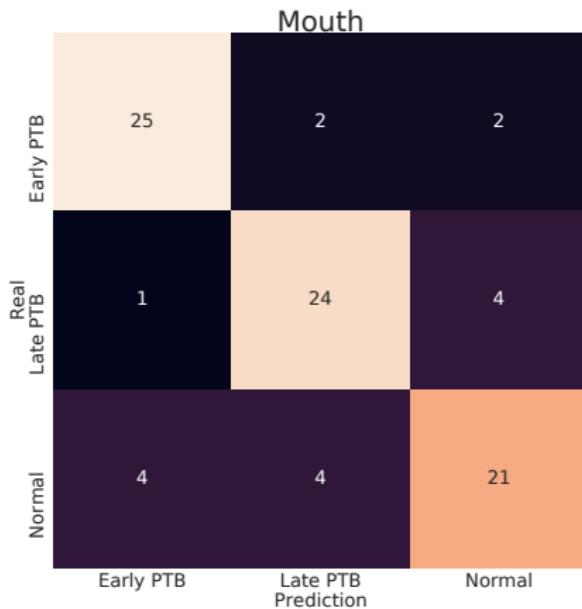


Figure: RF confusion matrix

Random Forest with (Early vs. Late vs. Full) III

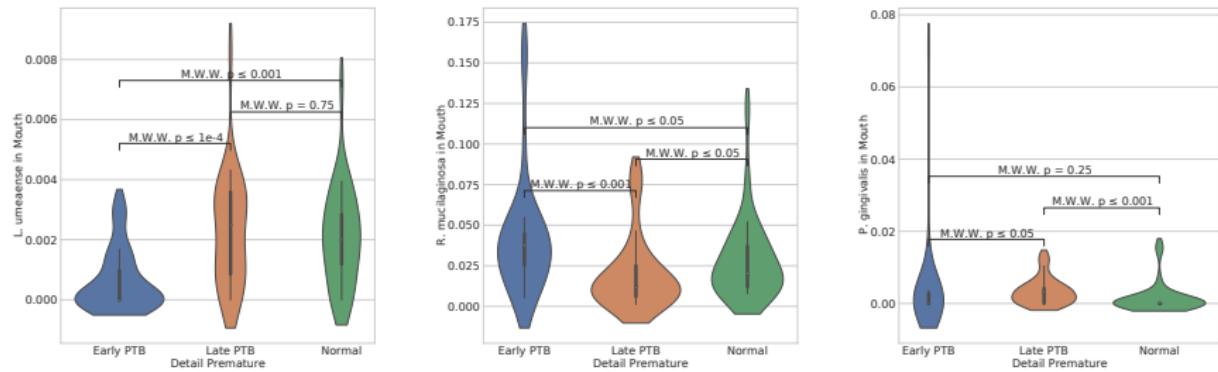


Figure: RF most important taxa

Random Forest with (Early vs. Late + Full) I

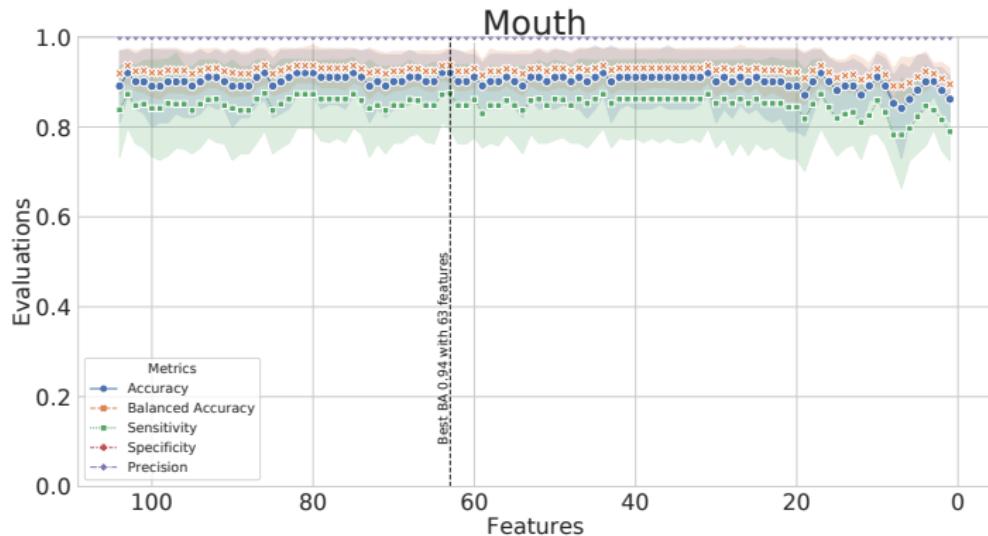


Figure: RF evaluations with feature counts

Random Forest with (Early vs. Late + Full) II

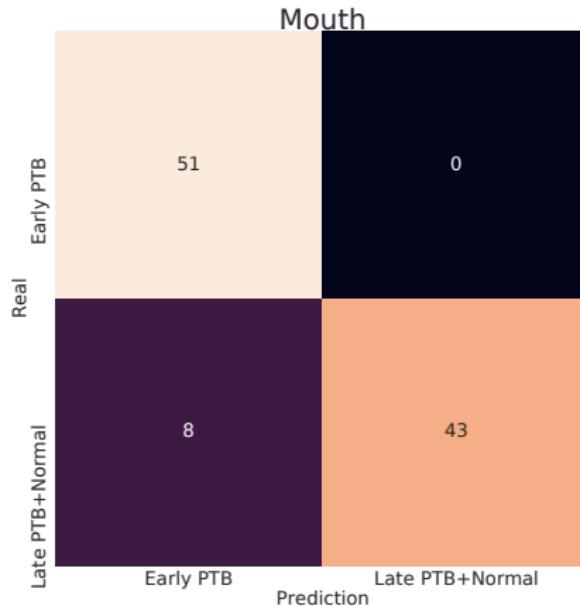


Figure: RF confusion matrix

Random Forest with (Early vs. Late + Full) III

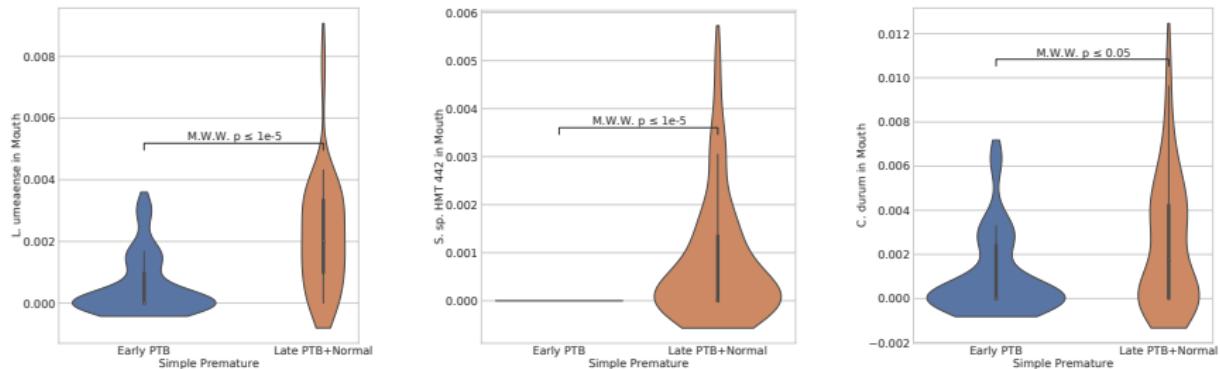


Figure: RF most important taxa

5. Discussion

6. References

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