**Qiime2 script for-**

Inter-colony comparisons of gut microbiome composition from lab reared Eastern subterranean termites (Blattodea: [Rhinotermitidae](https://www.google.com/search?sxsrf=AOaemvJdfd3WZs_sku9Q6pqbiRqc6Tn9kQ:1636649987698&q=Rhinotermitidae&stick=H4sIAAAAAAAAAONgVuLSz9U3MDcrTymwXMTKH5SRmZdfklqUm1mSmZKYCgBLsFyDIAAAAA&sa=X&ved=2ahUKEwi-gY2p5JD0AhVKmWoFHSsrCBYQmxMoAHoFCI8BEAI))

**QIIME2 loadings**

module load bioinfo

module load Qiime/2-2020.11

module list

**Making manifest file**

module load bioinfo

module load mothur

mothur "#make.file(inputdir=., type=gz, prefix=manifest)"

nano manifest.files

module load Qiime/2-2020.11

qiime tools import \

--type 'SampleData[PairedEndSequencesWithQuality]' \

--input-path manifest.files \

--input-format PairedEndFastqManifestPhred33V2 \

--output-path demux-paired-end.qza

**To visualise**

qiime demux summarize \

--i-data demux-paired-end.qza \

--o-visualization demux-paired-end.qzv

**Denoising**

qiime dada2 denoise-paired \

--i-demultiplexed-seqs demux-paired-end.qza \

--p-trim-left-f 15 \

--p-trim-left-r 20 \

--p-trunc-len-f 275 \

--p-trunc-len-r 215 \

--o-table table.qza \

--o-representative-sequences rep-seqs.qza \

--o-denoising-stats denoising-stats.qza

qiime feature-table summarize \

--i-table table.qza \

--o-visualization table.qzv \

--m-sample-metadata-file termite-meta

qiime feature-table tabulate-seqs \

--i-data rep-seqs.qza \

--o-visualization rep-seqs.qzv

qiime metadata tabulate \

--m-input-file denoising-stats.qza \

--o-visualization denoising-stats.qzv

### FeatureTable and FeatureData summaries

qiime feature-table summarize \

--i-table table.qza \

--o-visualization table.qzv \

--m-sample-metadata-file termite-meta

qiime feature-table tabulate-seqs \

--i-data rep-seqs.qza \

--o-visualization rep-seqs.qzv

### Tree for phylogenetic diversity analyses

qiime phylogeny align-to-tree-mafft-fasttree \

--i-sequences rep-seqs.qza \

--o-alignment aligned-rep-seqs.qza \

--o-masked-alignment masked-aligned-rep-seqs.qza \

--o-tree unrooted-tree.qza \

--o-rooted-tree rooted-tree.qza

### Alpha rarefaction plotting

qiime diversity alpha-rarefaction \

--i-table table.qza \

--i-phylogeny rooted-tree.qza \

--p-max-depth 46500 \

--m-metadata-file termite-meta \

--o-visualization alpha-rarefaction-46500.qzv

### Alpha and beta diversity analysis

qiime diversity core-metrics-phylogenetic \

--i-phylogeny rooted-tree.qza \

--i-table table.qza \

--p-sampling-depth 46500 \

--m-metadata-file termite-meta \

--output-dir core-metrics-results

**#Alpha diversities**

qiime diversity alpha-group-significance \

--i-alpha-diversity core-metrics-results/faith\_pd\_vector.qza \

--m-metadata-file termite-meta \

--o-visualization core-metrics-results/faith-pd-group-significance.qzv

qiime diversity alpha-group-significance \

--i-alpha-diversity core-metrics-results/evenness\_vector.qza \

--m-metadata-file termite-meta \

--o-visualization core-metrics-results/evenness-group-significance.qzv

qiime diversity alpha-group-significance \

--i-alpha-diversity core-metrics-results/shannon\_vector.qza \

--m-metadata-file termite-meta \

--o-visualization core-metrics-results/shannon-group-significance.qzv

qiime diversity alpha-group-significance \

--i-alpha-diversity core-metrics-results/observed\_features\_vector.qza \

--m-metadata-file termite-meta \

--o-visualization core-metrics-results/observed\_features-group-significance.qzv

**# Beta diversities**

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/unweighted\_unifrac\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/unweighted-unifrac-time-significance.qzv \

--p-method permdisp

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/unweighted\_unifrac\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/permanova-unweighted-unifrac-time-significance.qzv \

--p-pairwise

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/weighted\_unifrac\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/permanova-weighted-unifrac-time-significance.qzv \

--p-pairwise

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/weighted\_unifrac\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/permdisp-weighted-unifrac-time-significance.qzv \

--p-method permdisp

**##Bray Curtis**

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/bray\_curtis\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/permanova-bray\_curtis-time-significance.qzv \

--p-pairwise

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/bray\_curtis\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/permdisp-bray\_curtis-time-significance.qzv \

--p-method permdisp

**##Jaccard**

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/jaccard\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/permanova-jaccard-time-significance.qzv \

--p-pairwise

qiime diversity beta-group-significance \

--i-distance-matrix core-metrics-results/jaccard\_distance\_matrix.qza \

--m-metadata-file termite-meta \

--m-metadata-column Colonies \

--o-visualization core-metrics-results/permdisp-jaccard-time-significance.qzv \

--p-method permdisp

**To train the classifier**

$ wget https://www.arb-silva.de/fileadmin/silva\_databases/qiime/Silva\_132\_release.zip

$ unzip Silva\_132\_release.zip

$ cp SILVA\_132\_QIIME\_release/taxonomy/16S\_only/99/taxonomy\_7\_levels.txt .

$ cp SILVA\_132\_QIIME\_release/rep\_set/rep\_set\_16S\_only/99/silva\_132\_99\_16S.fna .

$ qiime tools import \

--type 'FeatureData[Taxonomy]' \

--input-format HeaderlessTSVTaxonomyFormat \

--input-path taxonomy\_7\_levels.txt \

--output-path ref-taxonomy.qza

 $ qiime tools import \

--type 'FeatureData[Sequence]' \

--input-path silva\_132\_99\_16S.fna \

--output-path silva\_132\_99\_16S.qza

 $ qiime feature-classifier extract-reads \

--i-sequences silva\_132\_99\_16S.qza \

--p-f-primer CCTACGGGAGGCAGCAG \

--p-r-primer GGACTACHVGGGTWTCTAAT \

--p-min-length 250  \

--p-max-length 500 \

--o-reads ref-seqs-v3-4.qza

$ qiime feature-classifier fit-classifier-naive-bayes \

--i-reference-reads ref-seqs-v3-4.qza \

--i-reference-taxonomy ref-taxonomy.qza \

--o-classifier classifier-v3-4.qza

### Taxonomic analysis

qiime feature-classifier classify-sklearn **\**

--i-classifier classifier-v3-4.qza **\**

--i-reads rep-seqs.qza **\**

--o-classification termite-taxonomy.qza

qiime metadata tabulate **\**

--m-input-file termite-taxonomy.qza **\**

--o-visualization termite-taxonomy.qzv

qiime taxa barplot \

--i-table table.qza \

--i-taxonomy termite-taxonomy.qza \

--m-metadata-file termite-meta \

--o-visualization termite-taxa-bar-plots.qzv

### Differential abundance testing with ANCOM

qiime feature-table filter-samples \

--i-table table.qza \

--p-min-frequency 46500 \

--o-filtered-table temp.qza

qiime feature-table filter-features \

--i-table temp.qza \

--p-min-samples 1 \

--o-filtered-table temp2.qza

qiime feature-table filter-features \

--i-table temp2.qza \

--p-min-frequency 8 \

--o-filtered-table temp3.qza

mkdir ANCOM

*# Collapse table at phylum level*

qiime taxa collapse \

--i-table temp3.qza \

--i-taxonomy termite-taxonomy.qza \

--p-level 2 \

--o-collapsed-table ANCOM/feature\_table\_for\_ANCOM-2.qza

*# Collapse table at genus level*

qiime taxa collapse \

--i-table temp3.qza \

--i-taxonomy termite-taxonomy.qza \

--p-level 6 \

--o-collapsed-table ANCOM/feature\_table\_for\_ANCOM-6.qza

cd ANCOM

#For genus

*# Add pseudocount*

qiime composition add-pseudocount \

--i-table feature\_table\_for\_ANCOM-6.qza \

--o-composition-table ANCOM\_ready\_table-6.qza

qiime composition ancom \

--i-table ANCOM\_ready\_table-6.qza \

--m-metadata-file ../termite-meta \

--m-metadata-column Colonies \

--o-visualization ancom\_group\_results-6.qzv \

--verbose

#For phylum

*# Add pseudocount*

qiime composition add-pseudocount \

--i-table feature\_table\_for\_ANCOM-2.qza \

--o-composition-table ANCOM\_ready\_table-2.qza

qiime composition ancom \

--i-table ANCOM\_ready\_table-2.qza \

--m-metadata-file ../termite-meta \

--m-metadata-column Colonies \

--o-visualization ancom\_group\_results-2.qzv \

--verbose