**Impact of gut bacteria in presoldier initiation in termite**

Supplemental material 1

Qiime code

**To read the sequences in qiime 2**

qiime tools import \

--type 'SampleData[PairedEndSequencesWithQuality]' \

--input-path my\_project.files \

--input-format PairedEndFastqManifestPhred33V2 \

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

**To visualize summary of number of sequences obtained per sample**

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 6 \

--p-trim-left-r 6 \

--p-trunc-len-f 300 \

--p-trunc-len-r 240 \

--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

### Generating a 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 and beta diversity analysis

qiime diversity core-metrics-phylogenetic \

--i-phylogeny rooted-tree.qza \

--i-table table.qza \

--p-sampling-depth 1570 \

--m-metadata-file termite.meta \

--output-dir core-metrics-results

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

**Beta diversity**

qiime diversity beta-group-significance \

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

--m-metadata-file termite.meta \

--m-metadata-column treatment \

--o-visualization core-metrics-results/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 treatment \

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

--p-pairwise

### Alpha rarefaction plotting

qiime diversity alpha-rarefaction \

--i-table table.qza \

--i-phylogeny rooted-tree.qza \

--p-max-depth 5000 \

--m-metadata-file termite.meta \

--o-visualization alpha-rarefaction-5000.qzv

### Creating own classifier

$ 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 200 \

--p-max-length 500 \

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

**Training of the classifier**

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

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

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

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

## **Test the classifier (with your re-seqs.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 composition add-pseudocount \

--i-table table.qza \

--o-composition-table comp-table.qza

qiime composition ancom \

--i-table comp-table.qza \

--m-metadata-file termite.meta \

--m-metadata-column treatment \

--o-visualization ancom-treatment.qzv