Bioinformatics procedure

Our data analysis followed the Qiime2 “moving pictures” tutorial

Importing demultiplexed sequences to qiime2:

qiime demux emp-single **\**

--i-seqs emp-single-end-sequences.qza **\**

--m-barcodes-file sample-metadata.tsv **\**

--m-barcodes-column barcode-sequence **\**

--o-per-sample-sequences demux.qza **\**

--o-error-correction-details demux-details.qza

**Output artifacts:**

* demux-details.qza
* demux.qza

Summarize the demultiplexing results

qiime demux summarize **\**

--i-data demux.qza **\**

--o-visualization demux.qzv

**Output visualizations:**

* demux.qzv

to view run command: qiime tools view demux.qzv

Sequence quality control and feature table construction

qiime dada2 denoise-single **\**

--i-demultiplexed-seqs demux.qza **\**

--p-trim-left 0 **\**

--p-trunc-len 120 **\**

--o-representative-sequences rep-seqs-dada2.qza **\**

--o-table table-dada2.qza **\**

--o-denoising-stats stats-dada2.qza

**Output artifacts:**

* stats-dada2.qza
* table-dada2.qza
* rep-seqs-dada2.qza
* qiime metadata tabulate **\**
* --m-input-file stats-dada2.qza **\**
* --o-visualization stats-dada2.qzv

**Output visualizations:**

* stats-dada2.qzv
* mv rep-seqs-dada2.qza rep-seqs.qza
* mv table-dada2.qza table.qza

**Output artifacts:**

* rep-seqs.qza
* table.qza

FeatureTable and FeatureData summaries

qiime feature-table summarize **\**

--i-table table.qza **\**

--o-visualization table.qzv **\**

--m-sample-metadata-file sample-metadata.tsv

qiime feature-table tabulate-seqs **\**

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

--o-visualization rep-seqs.qzv

**Output visualizations:**

* table.qzv
* rep-seqs.qzv

## Generate 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

**Output artifacts:**

* aligned-rep-seqs.qza
* masked-aligned-rep-seqs.qza
* rooted-tree.qza
* unrooted-tree.qza

## Alpha and beta diversity analysis

qiime diversity core-metrics-phylogenetic **\**

--i-phylogeny rooted-tree.qza **\**

--i-table table.qza **\**

--p-sampling-depth 400 **\**

--m-metadata-file sample-metadata.tsv **\**

--output-dir core-metrics-results

**Output artifacts:**

* core-metrics-results/faith\_pd\_vector.qza
* core-metrics-results/unweighted\_unifrac\_distance\_matrix.qza
* core-metrics-results/bray\_curtis\_pcoa\_results.qza
* core-metrics-results/shannon\_vector.qza
* core-metrics-results/rarefied\_table.qza
* core-metrics-results/weighted\_unifrac\_distance\_matrix.qza
* core-metrics-results/jaccard\_pcoa\_results.qza
* core-metrics-results/weighted\_unifrac\_pcoa\_results.qza
* core-metrics-results/observed\_features\_vector.qza
* core-metrics-results/jaccard\_distance\_matrix.qza
* core-metrics-results/evenness\_vector.qza
* core-metrics-results/bray\_curtis\_distance\_matrix.qza
* core-metrics-results/unweighted\_unifrac\_pcoa\_results.qza

**Output visualizations:**

* core-metrics-results/unweighted\_unifrac\_emperor.qzv
* core-metrics-results/jaccard\_emperor.qzv
* core-metrics-results/bray\_curtis\_emperor.qzv
* core-metrics-results/weighted\_unifrac\_emperor.qzv
* qiime diversity alpha-group-significance **\**
* --i-alpha-diversity core-metrics-results/faith\_pd\_vector.qza **\**
* --m-metadata-file sample-metadata.tsv **\**
* --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 sample-metadata.tsv **\**
* --o-visualization core-metrics-results/evenness-group-significance.qzv

**Output visualizations:**

* core-metrics-results/faith-pd-group-significance.qzv
* core-metrics-results/evenness-group-significance.qzv
* qiime diversity beta-group-significance **\**
* --i-distance-matrix core-metrics-results/unweighted\_unifrac\_distance\_matrix.qza **\**
* --m-metadata-file sample-metadata.tsv **\**
* --m-metadata-column body-site **\**
* --o-visualization core-metrics-results/unweighted-unifrac-body-site-significance.qzv **\**
* --p-pairwise
* qiime diversity beta-group-significance **\**
* --i-distance-matrix core-metrics-results/unweighted\_unifrac\_distance\_matrix.qza **\**
* --m-metadata-file sample-metadata.tsv **\**
* --m-metadata-column subject **\**
* --o-visualization core-metrics-results/unweighted-unifrac-subject-group-significance.qzv **\**
* --p-pairwise

**Output visualizations:**

* core-metrics-results/unweighted-unifrac-body-site-significance.qzv
* core-metrics-results/unweighted-unifrac-subject-group-significance.qzv
* qiime emperor plot **\**
* --i-pcoa core-metrics-results/unweighted\_unifrac\_pcoa\_results.qza **\**
* --m-metadata-file sample-metadata.tsv **\**
* --p-custom-axes days-since-experiment-start **\**
* --o-visualization core-metrics-results/unweighted-unifrac-emperor-days-since-experiment-start.qzv
* qiime emperor plot **\**
* --i-pcoa core-metrics-results/bray\_curtis\_pcoa\_results.qza **\**
* --m-metadata-file sample-metadata.tsv **\**
* --p-custom-axes days-since-experiment-start **\**
* --o-visualization core-metrics-results/bray-curtis-emperor-days-since-experiment-start.qzv

**Output visualizations:**

* core-metrics-results/bray-curtis-emperor-days-since-experiment-start.qzv
* core-metrics-results/unweighted-unifrac-emperor-days-since-experiment-start.qzv

## Alpha rarefaction plotting

qiime diversity alpha-rarefaction **\**

--i-table table.qza **\**

--i-phylogeny rooted-tree.qza **\**

--p-max-depth 4000 **\**

--m-metadata-file sample-metadata.tsv **\**

--o-visualization alpha-rarefaction.qzv

**Output visualizations:**

* alpha-rarefaction.qzv

## Taxonomic analysis

iime feature-classifier classify-sklearn **\**

--i-classifier silva-138-99-nb-classifier.qza **\**

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

--o-classification taxonomy.qza

qiime metadata tabulate **\**

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

--o-visualization taxonomy.qzv

**Output artifacts:**

* taxonomy.qza
* silva-138-99-nb-classifier.qza

**Output visualizations:**

* taxonomy.qzv

qiime taxa barplot **\**

--i-table table.qza **\**

--i-taxonomy taxonomy.qza **\**

--m-metadata-file sample-metadata.tsv **\**

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

**Output visualizations:**

* taxa-bar-plots.qzv