# QIIME 2 analysis on 16S RNA paired sequences for Scheel et al. 2022.

## Importing

qiime tools import --type 'SampleData[PairedEndSequencesWithQuality]' --input-path […] --input-format CasavaOneEightSingleLanePerSampleDirFmt --output-path demux-paired-end.qza

## Demultiplexing

qiime cutadapt trim-paired --i-demultiplexed-sequences demux-paired-end.qza --p-cores 16 --p-front-f CCTAYGGGRBGCASCAG --p-adapter-f ATTAGATACCCNNGTAGTCC --p-front-r GGACTACNNGGGTATCTAAT --p-adapter-r CTGSTGCVYCCCRTAGG --p-minimum-length 238 --p-discard-untrimmed --o-trimmed-sequences demux-paired-end-trimmed.qza --verbose

qiime demux summarize --i-data demux-paired-end-trimmed.qza --o-visualization demux-paired-end-trimmed.qzv

* Select for denoising

## Denoising until final feature table and frequencies

qiime dada2 denoise-paired --i-demultiplexed-seqs demux-paired-end-trimmed.qza --p-trunc-len-f 243 --p-trunc-len-r 200 --p-trim-left-r 4 --p-n-threads 15 --output-dir DADA2\_denoising\_output --verbose

qiime metadata tabulate --m-input-file DADA2\_denoising\_output/denoising\_stats.qza --o-visualization DADA2\_denoising\_output/denoising\_stats.qzv

qiime feature-table summarize --i-table DADA2\_denoising\_output/table.qza --o-visualization DADA2\_denoising\_output/table.qzv

qiime feature-table tabulate-seqs --i-data DADA2\_denoising\_output/representative\_sequences.qza --o-visualization DADA2\_denoising\_output/rep\_seqs.qzv

## Assigning Taxonomy

qiime feature-classifier classify-sklearn --i-classifier /Databases/QIIME2\_Databases/silva-138-99-nb-classifier.qza --i-reads DADA2\_denoising\_output/representative\_sequences.qza --output-dir classified\_sequences --verbose

qiime metadata tabulate --m-input-file classified\_sequences/classification.qza --o-visualization classified\_sequences/taxonomy.qzv

qiime alignment mafft --i-sequences DADA2\_denoising\_output/representative\_sequences.qza --o-alignment phylogeny/aligned-rep-seqs.qza --verbose

qiime alignment mask --i-alignment phylogeny/aligned-rep-seqs.qza --o-masked-alignment phylogeny/masked-aligned-rep-seqs.qza --verbose

qiime phylogeny fasttree --i-alignment phylogeny/masked-aligned-rep-seqs.qza --o-tree phylogeny/fasttree-tree.qza

qiime phylogeny midpoint-root --i-tree phylogeny/fasttree-tree.qza --o-rooted-tree phylogeny/fasttree-tree-rooted.qza

qiime tools export --input-path DADA2\_denoising\_output/table.qza --output-path export/table

biom convert -i export/table/feature-table.biom -o export/table/table.tsv --to-tsv

qiime tools export --input-path DADA2\_denoising\_output/representative\_sequences.qza --output-path export/rep-seqs.fasta

qiime tools export --input-path classified\_sequences/classification.qza --output-path export/taxonomy

qiime tools export --input-path phylogeny/fasttree-tree.qza --output-path export/exported-tree

### Alpha diversity

mkdir diversity

qiime diversity alpha-phylogenetic --i-table DADA2\_denoising\_output/table.qza --i-phylogeny phylogeny/fasttree-tree-rooted.qza --p-metric faith\_pd --o-alpha-diversity diversity/alpha\_faithpd.qza

qiime diversity alpha --i-table DADA2\_denoising\_output/table.qza --p-metric simpson --o-alpha-diversity diversity/alpha\_shannon.qza

### alpha correlation (numeric metadata)

qiime diversity alpha-correlation --i-alpha-diversity diversity/alpha\_shannon.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --p-method pearson --o-visualization diversity/alpha\_correlation\_shannon.qzv

### Alpha group significance (categorical data)

qiime diversity alpha-group-significance --i-alpha-diversity diversity/alpha\_shannon.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --o-visualization diversity/alpha\_groupsignificance\_shannon.qzv

### Beta diversity

qiime diversity beta-phylogenetic --i-table DADA2\_denoising\_output/table.qza --i-phylogeny phylogeny/fasttree-tree-rooted.qza --p-metric unweighted\_unifrac --o-distance-matrix diversity/beta\_unw\_unifrac\_distance\_matrix.qza

qiime diversity beta --i-table DADA2\_denoising\_output/table.qza --p-metric braycurtis --o-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza

### PCoA

qiime diversity pcoa --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --o-pcoa diversity/beta\_braycurtis\_pcoa\_matrix.qza

qiime emperor plot --i-pcoa diversity/beta\_braycurtis\_pcoa\_matrix.qza --m-metadata-file sample\_info.txt --p-custom-axes age SOM H2O --o-visualization diversity/braycurtis\_pcoa\_emperor.qzv

### PCoA with deicode

PCoA using the Aitchison distance

qiime deicode rpca --i-table DADA2\_denoising\_output/table.qza --p-min-feature-count 10 --p-min-sample-count 500 --o-biplot ordination.qza --o-distance-matrix distance.qza

qiime emperor biplot --i-biplot ordination.qza --m-sample-metadata-file sample\_info\_16S\_1920\_July.txt --m-feature-metadata-file classified\_sequences/classification.qza --o-visualization biplot.qzv --p-number-of-features 8

qiime qurro loading-plot --i-table DADA2\_denoising\_output/table.qza --i-ranks ordination.qza --m-sample-metadata-file sample\_info\_16S\_1920\_July.txt --m-feature-metadata-file classified\_sequences/classification.qza --o-visualization qurro-plot.qzv

### Beta correlation (numerical metadata)

qiime diversity beta-correlation --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column SOM --p-method pearson --o-metadata-distance-matrix diversity/beta\_correlation\_SOM\_matrix.qza --p-intersect-ids --o-mantel-scatter-visualization diversity/beta\_correlation\_SOM.qzv

qiime diversity beta-correlation --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column age --p-method pearson --o-metadata-distance-matrix diversity/beta\_correlation\_14C\_matrix.qza --p-intersect-ids --o-mantel-scatter-visualization diversity/beta\_correlation\_14C.qzv

qiime diversity beta-correlation --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column H2O --p-method pearson --o-metadata-distance-matrix diversity/beta\_correlation\_H2O\_matrix.qza --p-intersect-ids --o-mantel-scatter-visualization diversity/beta\_correlation\_H2O.qzv

qiime diversity beta-correlation --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column pH --p-method pearson --o-metadata-distance-matrix diversity/beta\_correlation\_pH\_matrix.qza --p-intersect-ids --o-mantel-scatter-visualization diversity/beta\_correlation\_pH.qzv

### Beta group significance (categorical metadata)

qiime diversity beta-group-significance --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column "layer" --o-visualization diversity/beta\_groupsignificance\_layer.qzv

qiime diversity beta-group-significance --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column "horizon" --o-visualization diversity/beta\_groupsignificance\_horizon.qzv

qiime diversity beta-group-significance --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column "depth" --o-visualization diversity/beta\_groupsignificance\_depth.qzv

qiime diversity beta-group-significance --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --m-metadata-column "year" --o-visualization diversity/beta\_groupsignificance\_year.qzv

### Collapse by taxa

qiime taxa collapse --i-table DADA2\_denoising\_output/table.qza --i-taxonomy classified\_sequences/classification.qza --p-level 1 --o-collapsed-table diversity/table\_domain.qza --verbose

etc

### Taxa barplot

qiime taxa barplot --i-table DADA2\_denoising\_output/table.qza --i-taxonomy classified\_sequences/classification.qza --m-metadata-file sample\_info.txt --o-visualization diversity/taxa\_barplot.qzv

### Heatmap

qiime feature-table heatmap --i-table diversity/table\_domain.qza --o-visualization diversity/heatmap\_domain.qzv

### Adonis

qiime diversity adonis --i-distance-matrix diversity/beta\_braycurtis\_distance\_matrix.qza --m-metadata-file sample\_info\_16S\_1920\_July.txt --p-formula "depth\*year\*horizon\*layer\*SOM\*H2O\*age\*pH" --o-visualization diversity/adonis.qzv

### Export

Diversity indices:

qiime tools export --input-path diversity/alpha\_simpson.qza --output-path export/alpha\_simpson

tables:

qiime tools export --input-path diversity/table\_collapse\*.qza --output-path export/table\_collapsed

biom convert -i export/table/feature-table.biom -o export/table/table.tsv --to-tsv