Tourmaline Python Notebook

Package Imports

In [1]:
```
from qiime2 import Artifact
from qiime2 import Visualization
import pandas as pd
import matplotlib.pyplot as plt
import seaborn as sns
%matplotlib inline
```

Variables

In [2]:
```
method = 'dada2-pe'
filtering = 'unfiltered'
adiv_metric = 'observed_features'
bdiv_metric = 'unweighted_unifrac'
factor1='filter_size'
factor2='region'
```

Paths

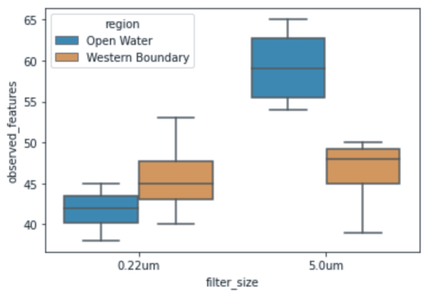
In [3]:
```
inputs = {
  'metadata': '../00-data/metadata.tsv',
  'taxonomy': '../02-output-%s-%s/01-taxonomy/taxonomy.qza' % (method, filtering),
  'repseq_prop': '../02-output-%s-%s/02-alignment-tree/repseqs_properties.tsv' % (method, filtering),
  'adiv_vector': '../02-output-%s-%s/03-alpha-diversity/%s_vector.qza' % (method, filtering, adiv_metric),
  'repseqs_viz': '../02-output-%s-%s/00-table-repseqs/repseqs.qzv' % (method, filtering),
  'table_viz': '../02-output-%s-%s/00-table-repseqs/table.qzv' % (method, filtering),
  'taxonomy_viz': '../02-output-%s-%s/01-taxonomy/taxonomy.qzv' % (method, filtering),
  'taxa_bar': '../02-output-%s-%s/01-taxonomy/taxa_barplot.qzv' % (method, filtering),
  'rooted_tree': '../02-output-%s-%s/02-alignment-tree/rooted_tree.qzv' % (method, filtering),
  'adiv_grpsig': '../02-output-%s-%s/03-alpha-diversity/%s_group_significance.qzv' % (method, filtering, adiv_metric),
  'bdiv_emperor': '../02-output-%s-%s/04-beta-diversity/%s_emperor.qzv' % (method, filtering, bdiv_metric),
  'bdiv_grpsig': '../02-output-%s-%s/04-beta-diversity/%s_group_significance.qzv' % (method, filtering, bdiv_metric)
}
```

Static Plots

Alpha-diversity boxplots

In [9]:
```
adiv_vector = Artifact.load(inputs['adiv_vector'])
df_adiv = adiv_vector.view(pd.Series)
df_adiv_md = pd.merge(df_md, df_adiv, left_index=True, right_index=True)
sns.boxplot(data=df_adiv_md, y=adiv_metric, x=factor1, hue=factor2)
```

Out[9]:
```
<matplotlib.axes._subplots.AxesSubplot at 0x7fca71d9ff28>
```

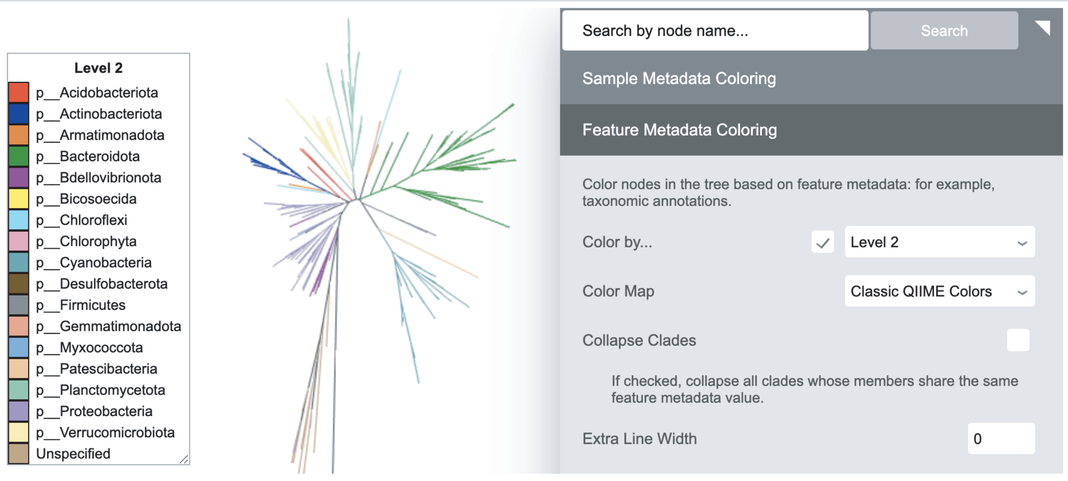


Interactive Visualizations

Rooted tree

In [12]:
```
Visualization.load(inputs['rooted_tree'])
```

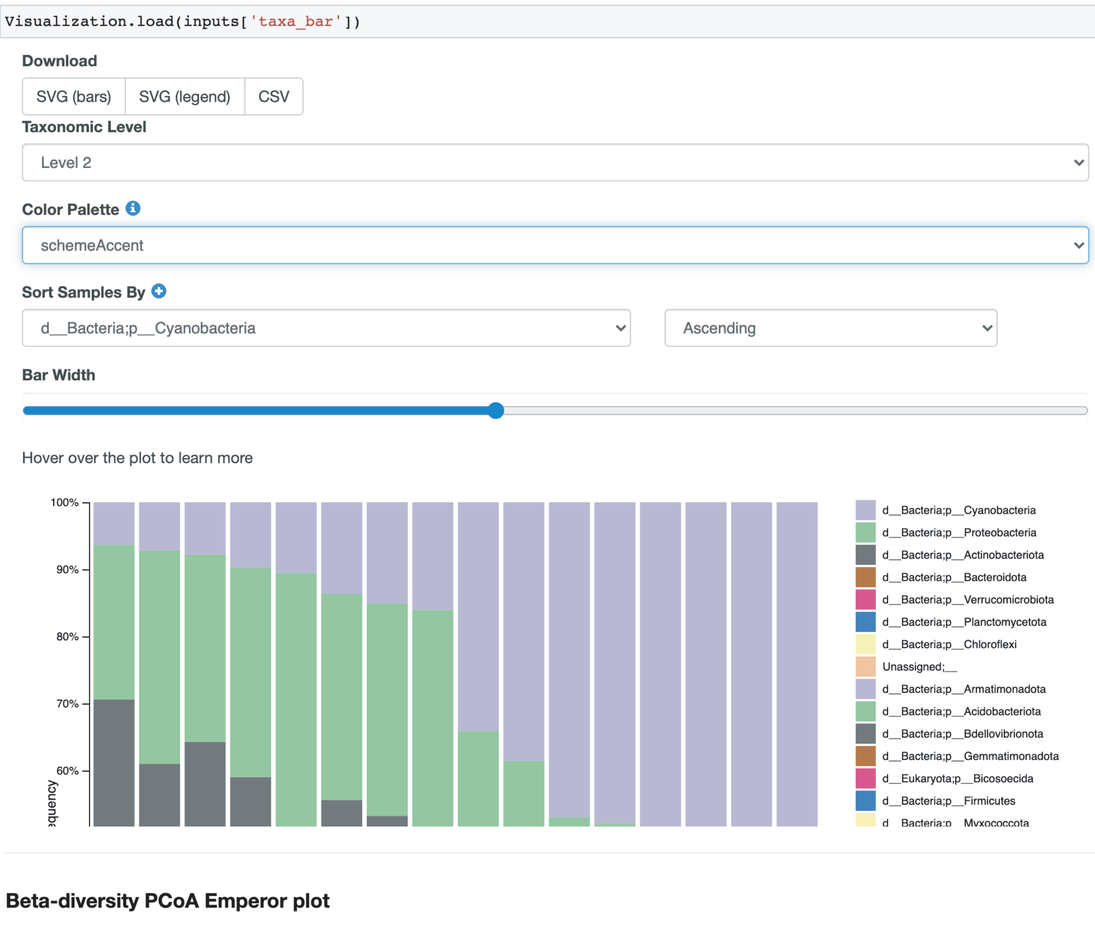
Out[12]:



Taxonomy barplot

In [14]:
```
Visualization.load(inputs['taxa_bar'])
```

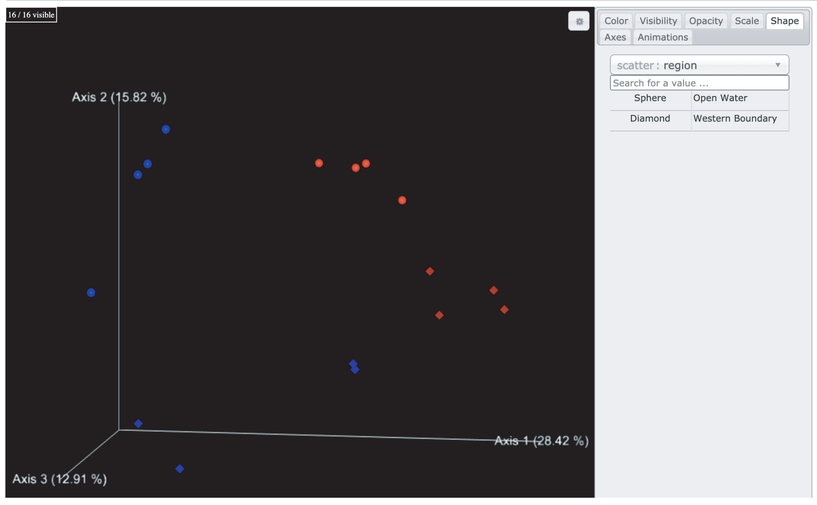
Out[14]:



Beta-diversity PCoA Emperor plot

In [16]:
```
Visualization.load(inputs['bdiv_emperor'])
```

Out[16]:



Tourmaline R Notebook

Load packages

In [1]:
```
library(qiime2R)
library(phyloseq)
library(tidyverse)
library(RColorBrewer)
library(vegan)
```

Define variables

In [2]:
```
method = "dada2-pe"
filtering = "unfiltered"
adiv_metric = "Shannon"
bdiv_metric = "bray"
factor1 = "region"
factor2 = "filter_size"
factor3 = "sample_name"
```

Read DataFrames

Representative sequences and observation table

In [3]:
```
count_table <- read_qza(file=sprintf("../02-output-%s-%s/00-table-repseqs/table.qza", method, filtering))
count_table <- count_table$data
tax_table <- read_qza(file=sprintf("../02-output-%s-%s/01-taxonomy/taxonomy.qza", method, filtering))
tax_table <- tax_table$data %>%
as_tibble() %>%
separate(Taxon, sep=";", c("Rank1", "Rank2", "Rank3", "Rank4", "Rank5", "Rank6", "Rank7")) # Taxa levels can be changed
```

Metadata

In [4]:
```
metadata_table <- read_tsv(file="../00-data/metadata.tsv")
metadata_table  <- sample_data(metadata_table)
rownames(metadata_table ) <- metadata_table$sample_name
```

Merge into phyloseq object

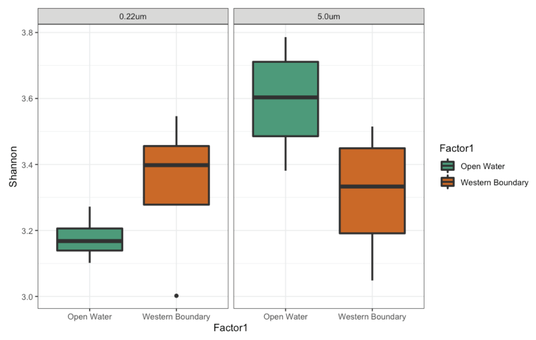
In [5]:
```
physeq <- phyloseq(otu_table(count_table, taxa_are_rows= T), tax_table(as.data.frame(tax_table) %>% column_to_rownames(

suppressMessages(physeq_rarefy <- rarefy_even_depth(physeq, sample.size = min(sample_sums(physeq)), rngseed = 714, repl
```

Set factors and plotting parameters

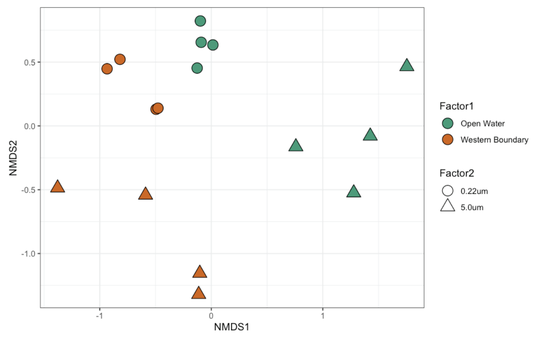
In [6]:
```
# Set factors
Factor1 = get(factor1, sample_data(physeq_rarefy))
Factor2 = get(factor2, sample_data(physeq_rarefy))
Factor3 = get(factor3, sample_data(physeq_rarefy))

# Set plotting parameters
theme = theme_bw()
point = geom_point(size = 5)
shape = scale_shape_manual(values = c(21, 24))
boxplot = geom_boxplot(lwd = 1)
color = scale_fill_brewer(palette="Dark2")
bar_color = scale_fill_brewer(palette="Paired")
stacked_bar = geom_bar(stat="identity", position = "fill")
bar_yaxis = scale_y_continuous(expand = c(0, 0), breaks=seq(0,1,0.2), limits=c(0, 1))
y_intercept = geom_hline(yintercept=0)
legend_pos = theme(legend.position="bottom")
legend_row = guides(fill=guide_legend(nrow=2))
plot_image = options(repr.plot.width=8, repr.plot.height=5)
```

Create plots

In [7]:
```
# Estimate richness
alpha <- estimate_richness(
  physeq = physeq_rarefy,
  measures=c(adiv_metric)) %>%
  cbind(Factor1, Factor2)

# Plot
ggplot(data=alpha, aes(x=Factor1, y=Shannon, fill=Factor1)) + theme +
boxplot + color + facet_grid(~ Factor2)
```



In [8]:
```
# Transform data
physeq_rel <- physeq_rarefy %>%
  transform_sample_counts(function(x) {x/sum(x)} )

# Ordinate
nmds <- ordinate(
  physeq = physeq_rel,
  method = "NMDS",
  distance = bdiv_metric) %>%
  scores() %>%
  as.data.frame() %>%
  cbind(Factor1, Factor2)

# Plot
ggplot(data=nmds, aes(x=NMDS1, y=NMDS2, fill=Factor1, shape=Factor2)) + theme +
color + point + shape + guides(fill=guide_legend(override.aes=list(shape=21)))
```



In [9]:
```
# Prepare phyloseq data for bar plots
barplot <- physeq_rarefy %>%
   tax_glom(taxrank = "Rank3") %>%                      # Agglomerate at class level, which is Rank3 in this case
   transform_sample_counts(function(x) {x/sum(x)} ) %>% # Transform to rel. abundance
   psmelt() %>%                                         # Melt to long format
   cbind(Factor1, Factor2, Factor3) %>%
   filter(Abundance > 0.05) %>%                         # Filter out low abundance taxa
   arrange(Rank3)

# Plot
ggplot(barplot, aes(x=Factor3, y=Abundance, fill=Rank3)) +
stacked_bar + bar_yaxis + y_intercept + bar_color + legend_pos +
legend_row + labs(y = "Relative Abundance") + facet_grid(~ Factor1, scales = "free_x") +
theme(axis.text.x=element_text(angle=90,vjust=0.5))
```