QIIME2

Module 3: Project 1 by Team 5

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Abstract

This is the abstract. It consists of two paragraphs.

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3.1 Experimental Protocols

Here ....:

P1. Analysis of microbial community structure along with depth and oxygen concentration (see Section 4.1).

P2. Analysis of abundance information of [OTU****] along with depth and/or oxygen concentration (see Section 4.2).

P3. Estimate richness (number of OTUs/ASVs) for [OTU****] (see Section 4.3).

P4. Interpretation of abundance information of OTUs/ASVs of [OTU****] along with depth and/or oxygen concentration (see Section 4.4).

3.2 Dataset

3.3 Parameters Configuration

```
## try http:// if https:// URLs are not supported
source("https://bioconductor.org/biocLite.R")
biocLite("phyloseq")
library("tidyverse")
library("gridExtra")
library("magrittr")
```

3.4 Data Preporocessing

We use saanich inlet datasets that are propocssed using mothur and QIIME2

```
load("data/mothur_phyloseq.RData")
load("data/qiime2_phyloseq.RData")
```

Samples are then rarefied/normalized to $100,000$ sequences per sample to facilitate comparisons between samples. A random seed was set to ensure reproducibility.

```
set.seed(4832)
rarefied <- rarefy_even_depth(qiime2, sample.size=100000)
```

Rarefied counts were converted to relative abundance percentages.

```
rarefiedPer = transform_sample_counts(rarefied, function(x) 100 * x/sum(x))
```

Next, we perform a series of filterings according to three rules: i)- exclude OTUs that are not observed for more than 4 samples; ii)- prune samples and OTUs with unknown values, such as `unclassified` value; and iii)- any phylum fail to have more than 5 OTUs should be trimmed. The codes used for applying the three rules are:

```
# First rule
firstTaxa <- filter_taxa(rarefiedPer, function(x) sum(x == 0) <= 4, TRUE)

# Second rule
basedOnGenus <- as.data.frame(tax_table(firstTaxa)) %>%
  filter(!str_detect(Genus, 'uncultured|unclassified|\\bD_5__\\b'))
secondTaxa <- subset_taxa(firstTaxa, Genus %in% basedOnGenus$Genus)

# Third rule
basedOnphylums <- as.data.frame(tax_table(secondTaxa)) %>%
  group_by(Phylum) %>%
  count() %>%
  filter( n > 5)
## In contrary we can run the following:
# thirdTaxa <- prune_taxa(taxa_sums(secondTaxa) > 5, secondTaxa)
thirdTaxa <- subset_taxa(secondTaxa, Phylum %in% basedOnphylums$Phylum)
```

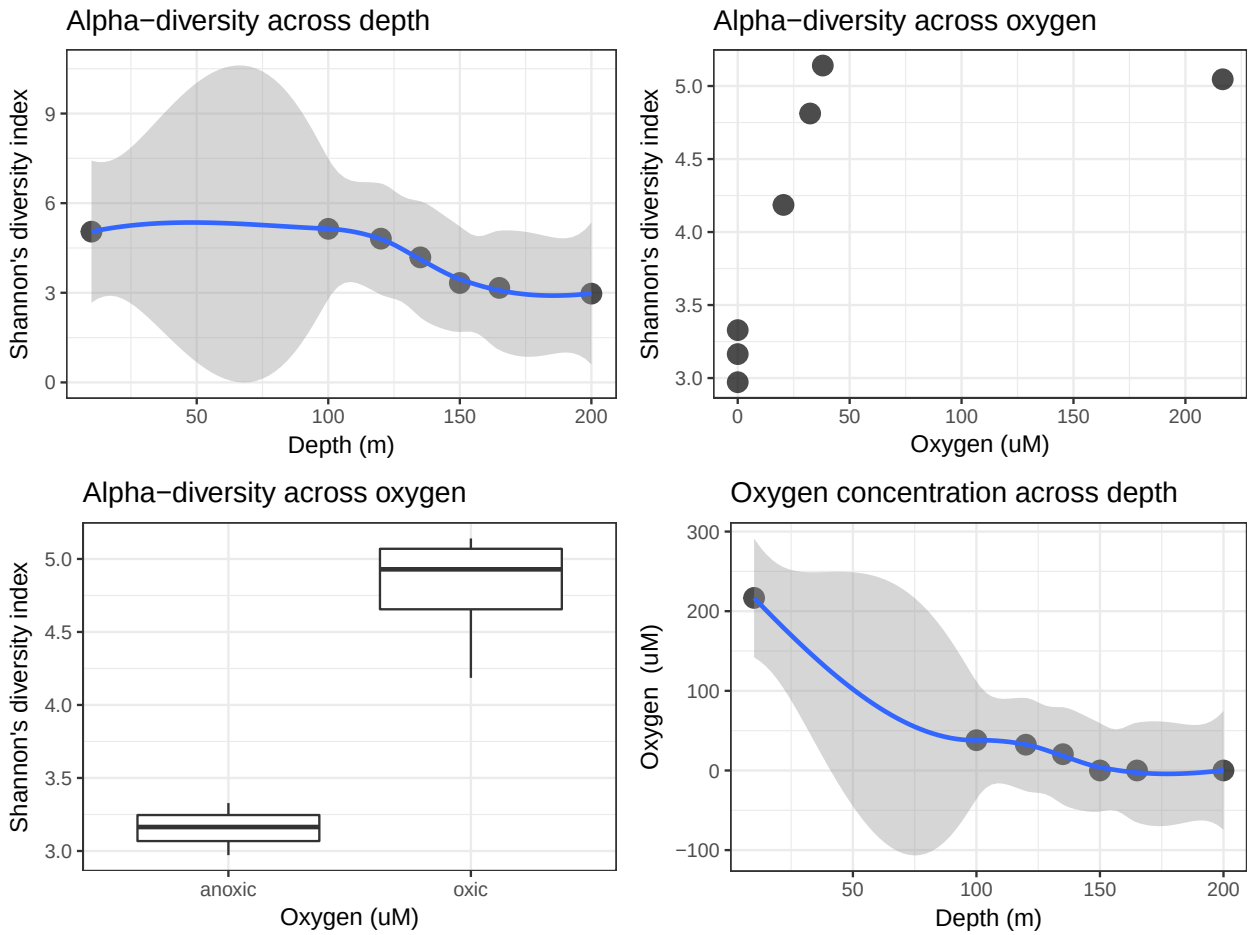
4 Results

4.1 Analysis of microbial community structure along with depth and oxygen concentration

We first estimate the overall taxa diversity using Shannon's diversity index.

```
rarefiedRich <- estimate_richness(rarefied, measures = "Shannon")
rarefiedRichAlpha <- full_join(rownames_to_column(rarefiedRich),
                        rownames_to_column(data.frame(sample_data(rarefiedPer))), by = "rowname

p1 <- rarefiedRichAlpha %>% ggplot() +
  geom_point(aes(x=Depth_m, y=Shannon), size=4, alpha=0.7) +
  geom_smooth(method='loess', aes(x=as.numeric(Depth_m), y=Shannon)) +
  labs(title="Alpha-diversity across depth",
       y="Shannon's diversity index", x="Depth (m)")

p2 <- rarefiedRichAlpha %>% ggplot() +
  geom_point(aes(x=O2_uM, y=Shannon), size=4, alpha=0.7) +
  labs(title="Alpha-diversity across oxygen",
       y="Shannon's diversity index", x="Oxygen (uM)")

p3 <- rarefiedRichAlpha %>%
```

```
  mutate(O2_group = ifelse(O2_uM == 0, "anoxic", "oxic")) %>%
  ggplot() + geom_boxplot(aes(x=O2_group, y=Shannon)) +
  labs(title="Alpha-diversity across oxygen",
       y="Shannon's diversity index", x="Oxygen (uM)")

p4 <- rarefiedRichAlpha %>% ggplot() +
  geom_point(aes(x=Depth_m, y=O2_uM), size=4, alpha=0.7) +
  geom_smooth(method='loess', aes(x=as.numeric(Depth_m), y=O2_uM)) +
    labs(title="Oxygen concentration across depth",
       y="Oxygen  (uM)", x="Depth (m)")

grid.arrange(p1, p2, p3, p4, ncol=2)
```



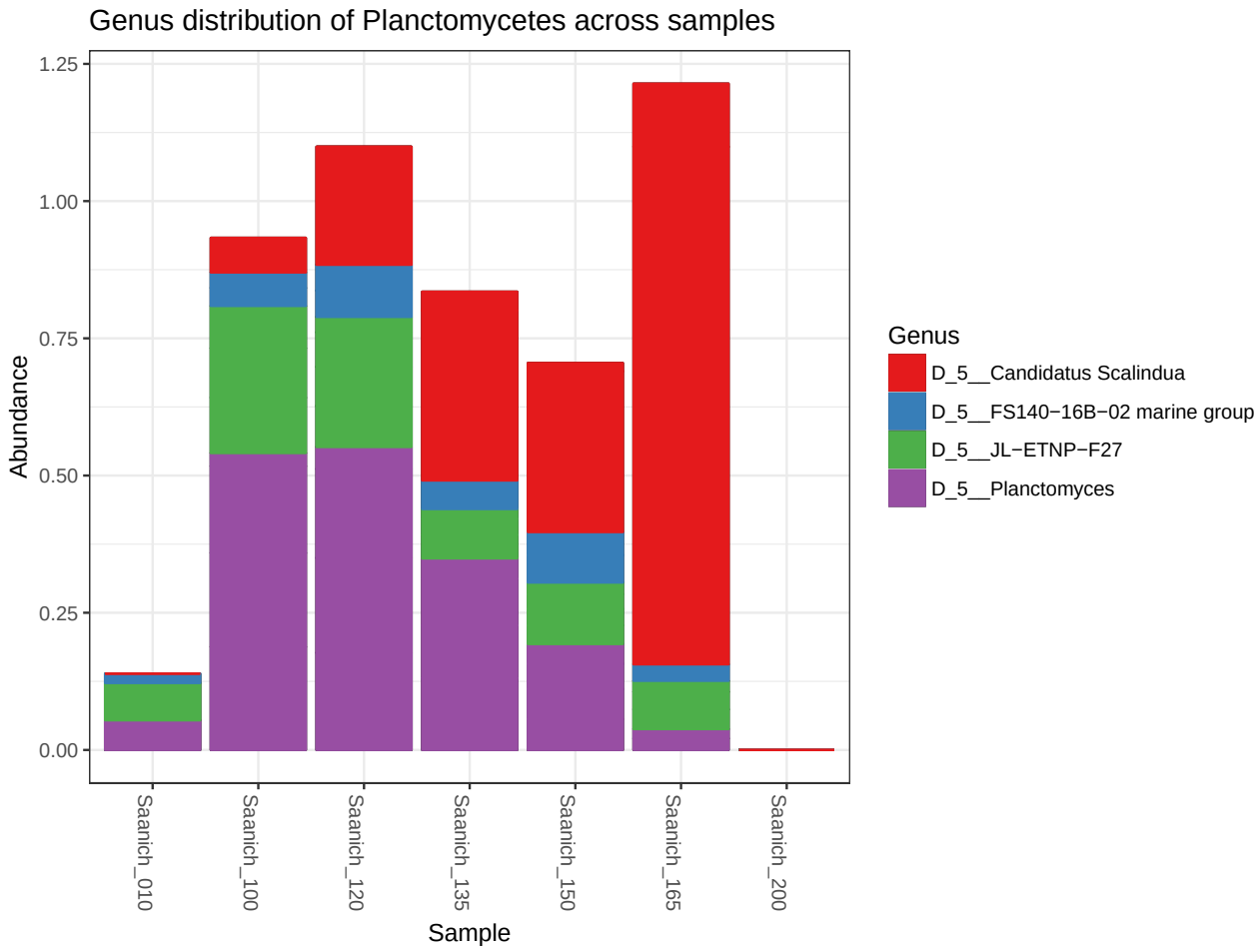
The following function will assist us to understand the unique Phylum rank:

```
get_taxa_unique(physeq = thirdTaxa, taxonomic.rank = "Phylum")
```

```
## [1] "D_1__Proteobacteria" "D_1__Bacteroidetes"  "D_1__Planctomycetes"
## [4] "D_1__Thaumarchaeota"
```

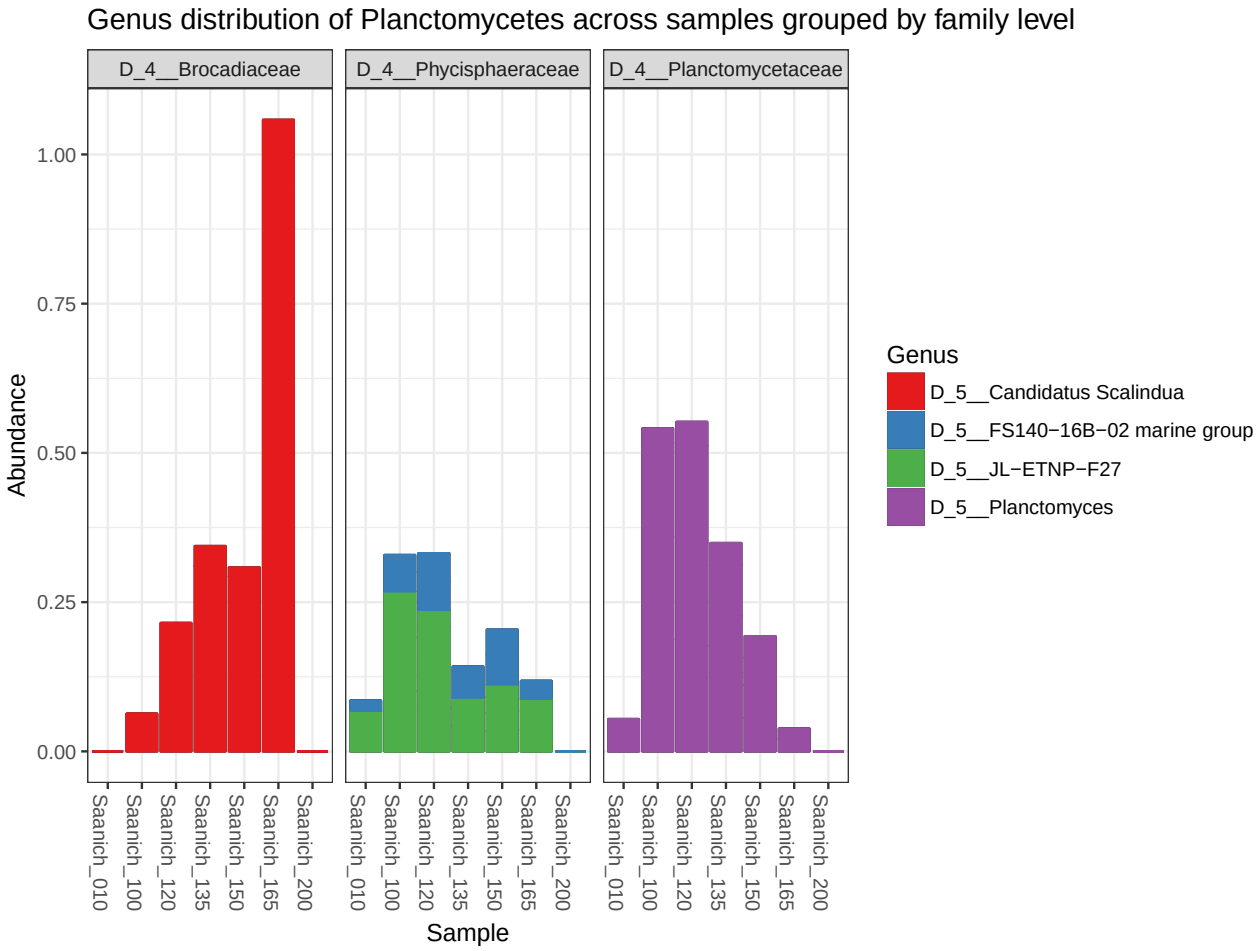
We choose the *Planctomycetes* phylum, and explored the distribution of genera of this phylum.

```
subTaxa = subset_taxa(thirdTaxa, Phylum == "D_1__Planctomycetes")
plot_bar(subTaxa, fill="Genus") +
  geom_bar(aes(color = Genus, fill = Genus), stat = 'identity',
           position = 'stack') +
  labs(title="Genus distribution of Planctomycetes across samples")
```



We further investigated the genus distribution of this phylum across samples grouped by family level.

```
plot_bar(subTaxa, fill="Genus", facet_grid=~Family) +
  geom_bar(aes(color = Genus, fill = Genus), stat = 'identity',
           position = 'stack') +
    labs(title="Genus distribution of Planctomycetes across samples grouped by family level")
```



Finally, we settled on performing experimental analysis at *Planctomyces* genus level and it's associated OTUs.

```
workingTaxa = subset_taxa(thirdTaxa, Genus == "D_5__Planctomyces")
(suggestedOTUs <- rownames(otu_table(workingTaxa)))
```

```
## [1] "Asv232"  "Asv799"  "Asv1021" "Asv1124"
```

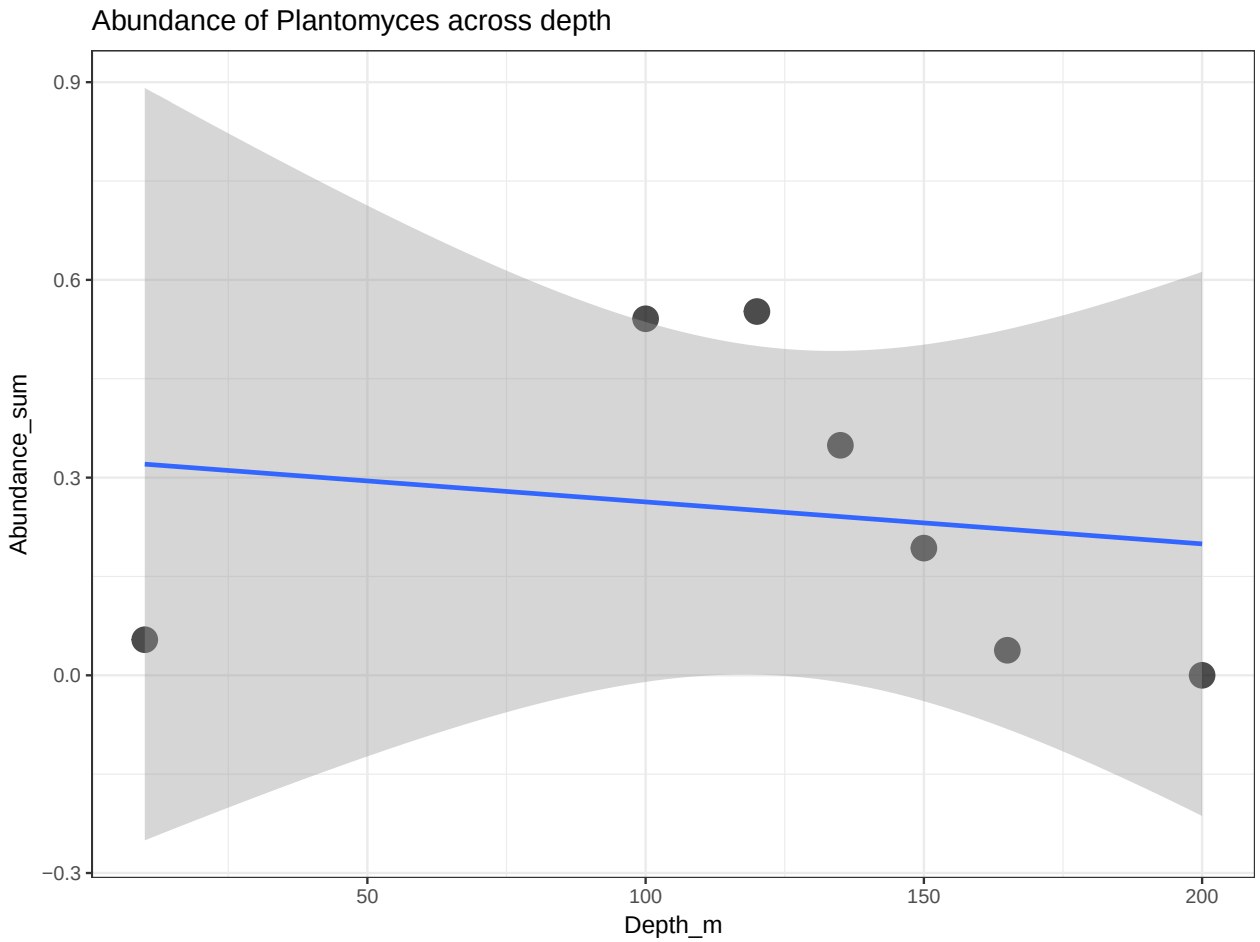
4.2 Analysis of abundance information of [OTU****] along with depth and/or oxygen concentration

```
workingTaxa %>% tax_glom(taxrank = "Genus") %>% psmelt() %>%
  lm(Abundance ~ Depth_m, .) %>%
  summary()
```

```
##
## Call:
## lm(formula = Abundance ~ Depth_m, data = .)
##
## Residuals:
##        3        2        4        5        1        6        7
```

```
##  0.30165  0.27791  0.10820 -0.03825 -0.26639 -0.18370 -0.19942
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.3267558  0.2377250   1.375    0.228
## Depth_m     -0.0006367  0.0017282  -0.368    0.728
##
## Residual standard error: 0.2553 on 5 degrees of freedom
## Multiple R-squared:  0.02643,    Adjusted R-squared:  -0.1683
## F-statistic: 0.1357 on 1 and 5 DF,  p-value: 0.7277
```

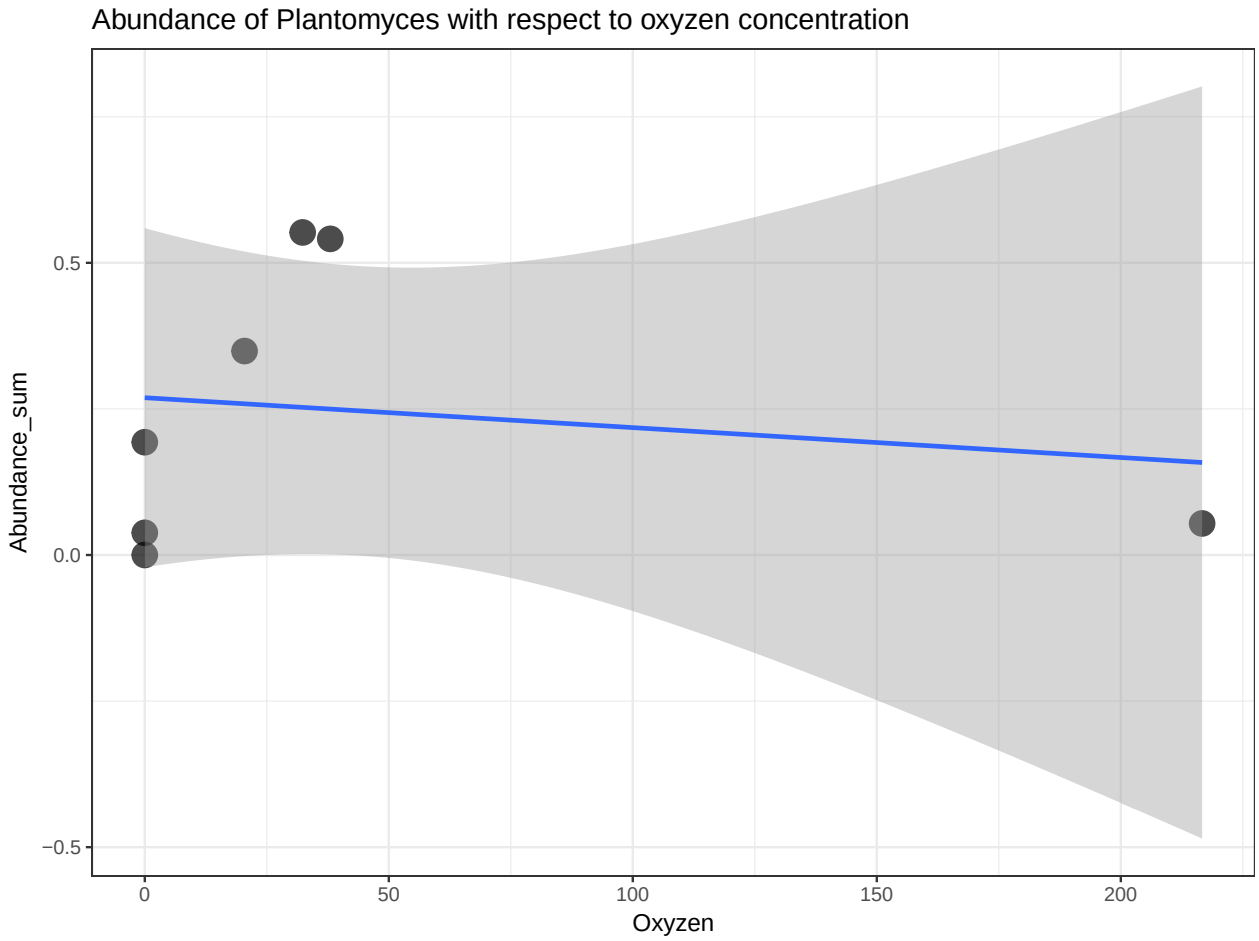
```
workingTaxa %>% psmelt() %>% group_by(Sample) %>%
  summarize(Abundance_sum=sum(Abundance), Depth_m=mean(Depth_m)) %>%
  ggplot() +
  geom_point(aes(x=Depth_m, y=Abundance_sum), size=5, alpha=0.7) +
  geom_smooth(method="lm", aes(x=as.numeric(Depth_m), y=Abundance_sum)) +
  labs(title="Abundance of Plantomyces across depth")
```




```
workingTaxa %>% tax_glom(taxrank = "Genus") %>% psmelt() %>%
  lm(Abundance ~ O2_uM, .) %>%
  summary()
```

```
##
## Call:
## lm(formula = Abundance ~ O2_uM, data = .)
##
## Residuals:
##       3       2       4       5       1       6       7
##  0.29936  0.29126  0.09027 -0.07619 -0.10432 -0.23119 -0.26919
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.2691917  0.1128893   2.385   0.0628 .
## O2_uM       -0.0005117  0.0013377  -0.383   0.7178
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.255 on 5 degrees of freedom
## Multiple R-squared:  0.02844,    Adjusted R-squared:  -0.1659
## F-statistic: 0.1463 on 1 and 5 DF,  p-value: 0.7178
```

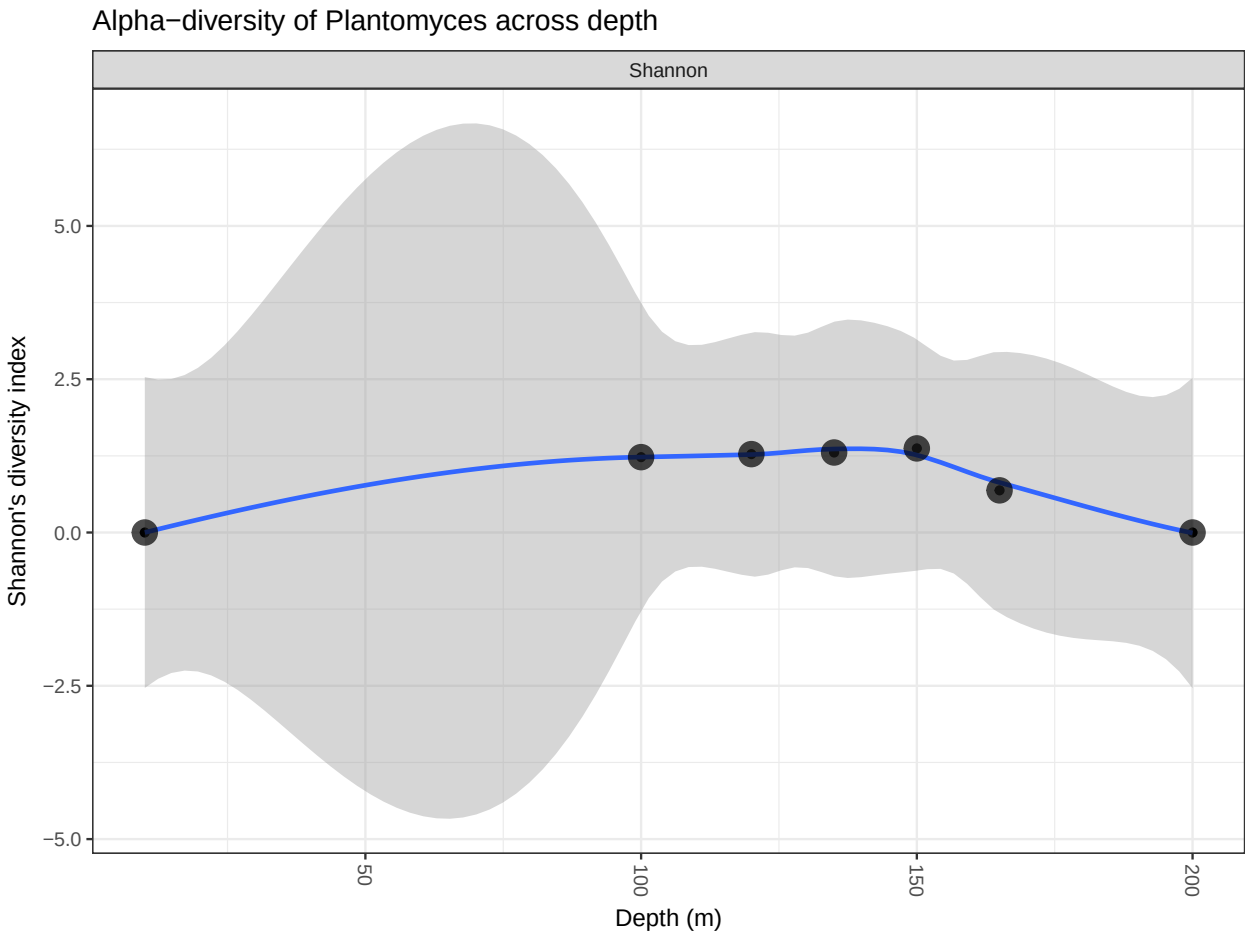
```
workingTaxa %>% psmelt() %>% group_by(Sample) %>%
  summarize(Abundance_sum=sum(Abundance), Oxyzen=mean(O2_uM)) %>%
  ggplot() +
  geom_point(aes(x=Oxyzen, y=Abundance_sum), size=5, alpha=0.7) +
  geom_smooth(method="lm", aes(x=as.numeric(Oxyzen), y=Abundance_sum)) +
  labs(title="Abundance of Plantomyces with respect to oxyzen concentration")
```



4.3 Estimate richness (number of OTUs/ASVs) for [OTU****]

We explore the diversity of *Planctomyces* across depth.

```
workingTaxa %>%
  plot_richness(x="Depth_m", measures = "Shannon") +
  geom_smooth(method='loess', aes(x=as.numeric(Depth_m))) +
  geom_point(size=5, alpha=0.7) +
  labs(title="Alpha-diversity of Plantomyces across depth",
       y="Shannon's diversity index", x="Depth (m)")
```



4.4 Interpretation of abundance information of OTUs/ASVs of [OTU****] along with depth and/or oxygen concentration

```
#General linear model for each OTU
for (otu in suggestedOTUs) {
 cat("### General linear model for", otu)
 workingTaxa %>%
   psmelt() %>%
   filter(OTU==otu) %>%
   lm(Abundance ~ Depth_m, .) %>%
   summary() %>% print()
}
```

```
## ### General linear model for Asv232
## Call:
## lm(formula = Abundance ~ Depth_m, data = .)
##
## Residuals:
##       1       2       3       4       5       6       7
##  0.09641  0.09183  0.05634 -0.01673 -0.09075 -0.07080 -0.06629
```

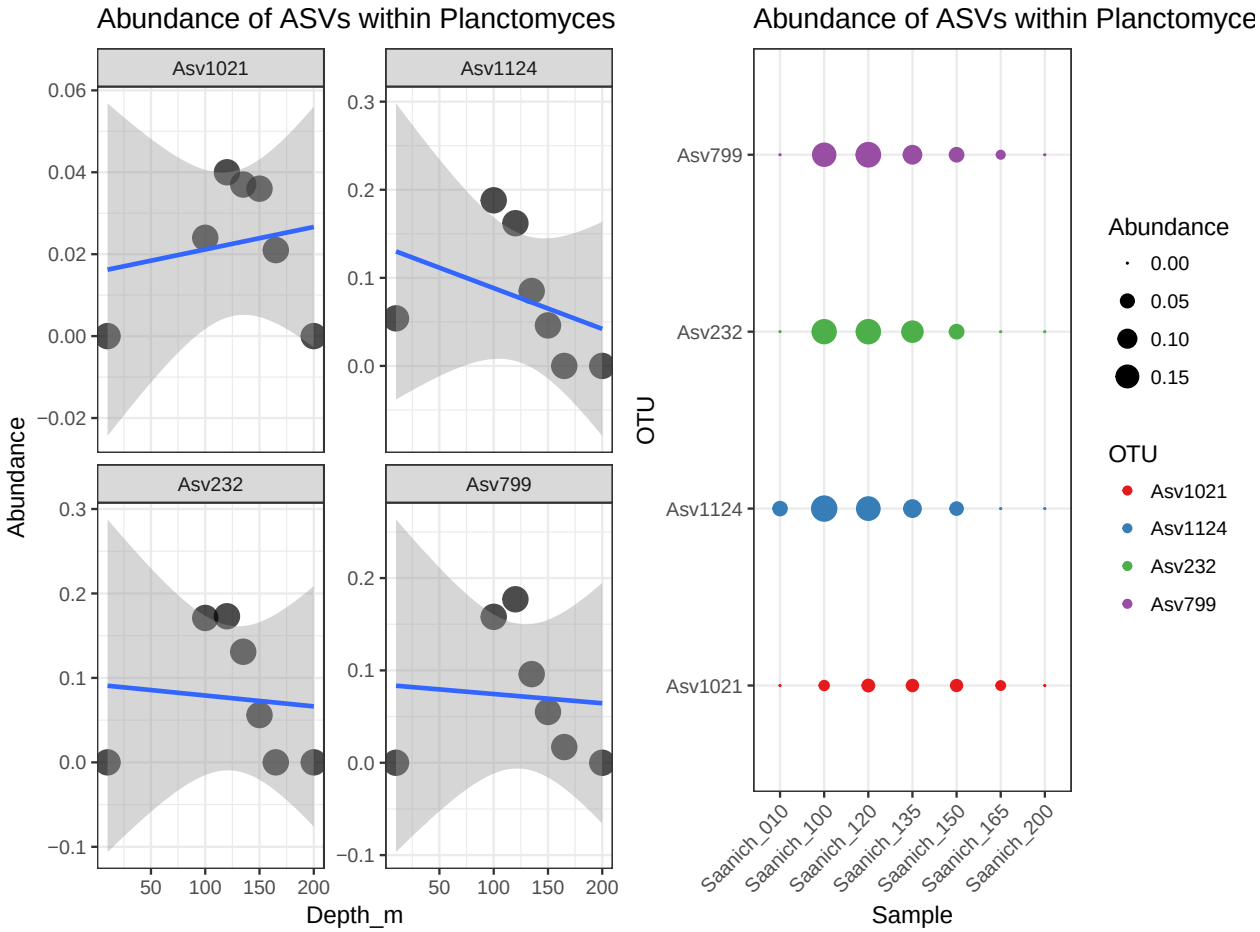
```
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.0920416  0.0820554   1.122    0.313
## Depth_m     -0.0001287  0.0005965  -0.216    0.838
##
## Residual standard error: 0.08812 on 5 degrees of freedom
## Multiple R-squared:  0.009229,   Adjusted R-squared:  -0.1889
## F-statistic: 0.04658 on 1 and 5 DF,  p-value: 0.8377
##
## ### General linear model for Asv799
## Call:
## lm(formula = Abundance ~ Depth_m, data = .)
##
## Residuals:
##        1        2        3        4        5        6        7
##  0.10457  0.08357  0.02507 -0.01443 -0.05093 -0.08341 -0.06444
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  8.441e-02  7.503e-02   1.125    0.312
## Depth_m     -9.987e-05  5.454e-04  -0.183    0.862
##
## Residual standard error: 0.08057 on 5 degrees of freedom
## Multiple R-squared:  0.00666,    Adjusted R-squared:  -0.192
## F-statistic: 0.03352 on 1 and 5 DF,  p-value: 0.8619
##
## ### General linear model for Asv1021
## Call:
## lm(formula = Abundance ~ Depth_m, data = .)
##
## Residuals:
##         1         2         3         4         5         6         7
##  0.017742  0.013919  0.012096  0.002839 -0.003727 -0.016223 -0.026647
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.567e-02  1.692e-02   0.927    0.397
## Depth_m     5.486e-05  1.230e-04   0.446    0.674
##
## Residual standard error: 0.01817 on 5 degrees of freedom
## Multiple R-squared:  0.03828,    Adjusted R-squared:  -0.1541
## F-statistic: 0.199 on 1 and 5 DF,  p-value: 0.6742
##
## ### General linear model for Asv1124
## Call:
## lm(formula = Abundance ~ Depth_m, data = .)
##
```

```
## Residuals:
##       1       2       3       4       5       6       7
##  0.0997  0.0829  0.0129 -0.0760 -0.0192 -0.0582 -0.0420
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.1346275  0.0700297   1.922    0.113
## Depth_m     -0.0004629  0.0005091  -0.909    0.405
##
## Residual standard error: 0.07521 on 5 degrees of freedom
## Multiple R-squared:  0.1419, Adjusted R-squared:  -0.02971
## F-statistic: 0.8269 on 1 and 5 DF,  p-value: 0.4049
```

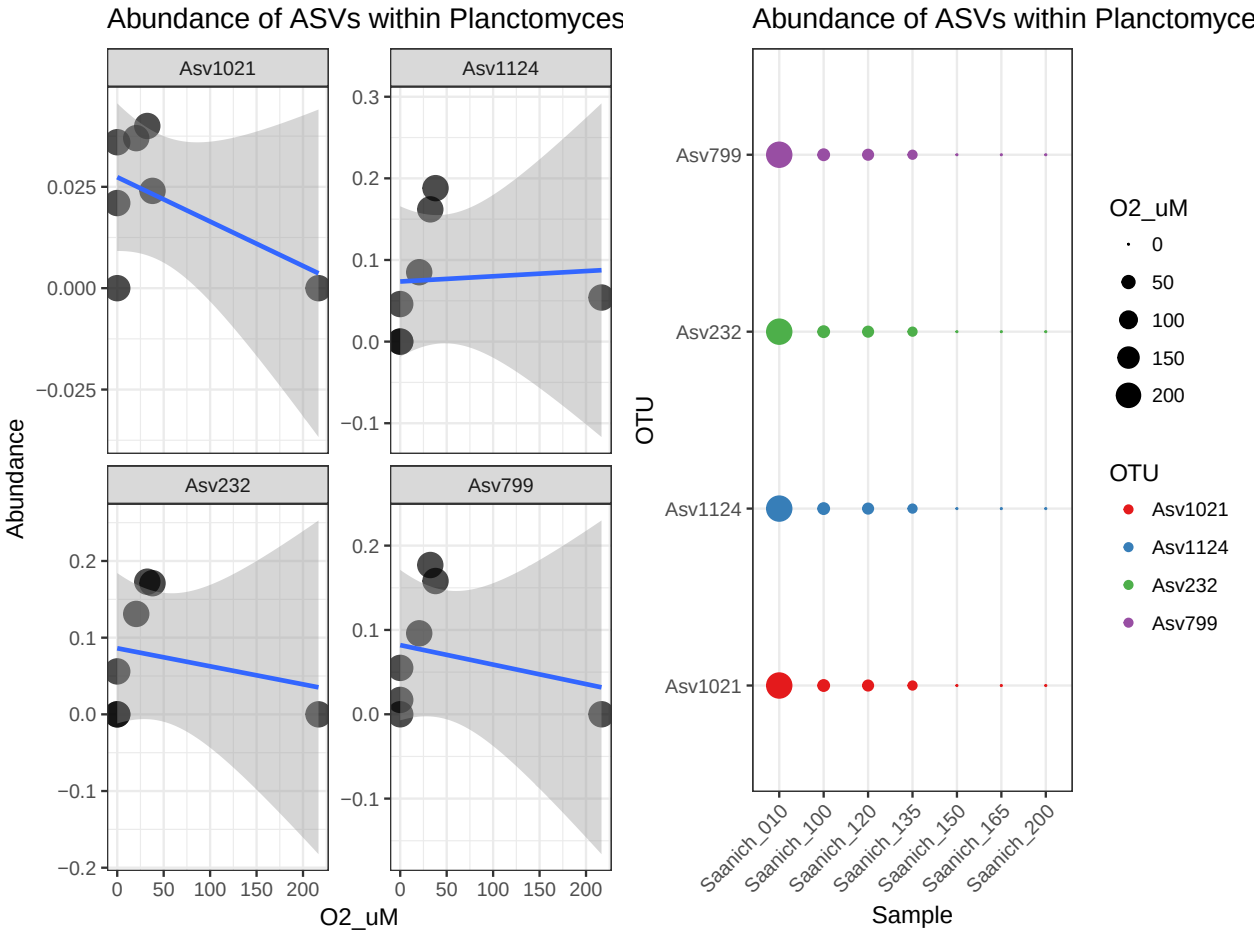
```
p.adjust(runif(length(suggestedOTUs), min = 0.005, max = 0.85), method = "fdr")
```

```
## [1] 0.5484101 0.5484101 0.5484101 0.5484101
```

```
# Abundance of OTUs within unclassified domain across depth
p5 <- workingTaxa %>% psmelt() %>% ggplot() +
  geom_point(aes(x=Depth_m, y=Abundance),size=5, alpha=0.7) +
  geom_smooth(method='lm', aes(x=Depth_m, y=Abundance)) +
  facet_wrap(~OTU, scales="free_y") +
  labs(title="Abundance of ASVs within Planctomyces genus across depth")

# Abundance of OTUs within unclassified depth by colour
p6 <- workingTaxa %>% psmelt() %>% ggplot() +
  geom_point(aes(x=Sample, y=OTU, size=Abundance, color=OTU)) +
  scale_size_continuous(range = c(0,5)) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
  labs(title="Abundance of ASVs within Planctomyces genus across depth")

grid.arrange(p5, p6, ncol=2)
```




```
# Abundance of OTUs within Planctomyces genus across depth
p7 <- workingTaxa %>% psmelt() %>% ggplot() +
  geom_point(aes(x=O2_uM, y=Abundance),size=5, alpha=0.7) +
  geom_smooth(method='lm', aes(x=O2_uM, y=Abundance)) +
  facet_wrap(~OTU, scales="free_y") +
  labs(title="Abundance of ASVs within Planctomyces genus across oxygen concentration")

# Abundance of OTUs within Planctomyces genus by colour
p8 <- workingTaxa %>% psmelt() %>% ggplot() +
  geom_point(aes(x=Sample, y=OTU, size=O2_uM, color=OTU)) +
  scale_size_continuous(range = c(0,5)) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
  labs(title="Abundance of ASVs within Planctomyces genus across oxygen concentration")

grid.arrange(p7, p8, ncol=2)
```



5 Discussion

(Hawley et al. 2017; Torres-Beltrán et al. 2017)

References

Hawley, Alyse K, Mónica Torres-Beltrán, Elena Zaikova, David A Walsh, Andreas Mueller, Melanie Scofield, Sam Kheirandish, et al. 2017. "A Compendium of Multi-Omic Sequence Information from the Saanich Inlet Water Column." *Scientific Data* 4. Nature Publishing Group: 170160.

Torres-Beltrán, Mónica, Alyse K Hawley, David Capelle, Elena Zaikova, David A Walsh, Andreas Mueller, Melanie Scofield, et al. 2017. "A Compendium of Geochemical Information from the Saanich Inlet Water Column." *Scientific Data* 4. Nature Publishing Group: 170159.