

## 3 Results

### 3.1 Analysis of microbial community structure along with depth and oxygen concentration

Using Shannon's diversity index (SDI), which considered both the species abundances and the total number of distinct species in its diversity estimation, an attempt was made to understand the compositional complexity of a microbial community across samples from Saanich Inlet. Fig. 1(a) and 2(a) depicts the change in SDI across depth for Mothur and QIIME2 datasets. It was observed that SDI values peak at depths 10m and 100m before monotonically decreasing and reaching the minimum value at 200m. The SDI values were maximal when the microbes were evenly distributed. The results indicated in Fig. 3 supports this claim since an uneven distribution of phylum was observed at 200m more than at 10m or 100m depths. Analyses showed that oxygen level was slowly decreasing with increased depth of Saanich Inlet as shown in Fig. 1(d). The SDI was higher in the oxic part of the ocean and was much lower in the anoxic part of Saanich Inlet as presented in Fig. 1(b) and 2(b). Therefore, alpha diversity was higher at high oxygen concentration levels at low depth and lower at low oxygen concentration at high depth.

```
rarefiedMRich <- estimate_richness(rarefiedM, measures = "Shannon")
rarefiedMRichAlpha <- full_join(rownames_to_column(rarefiedMRich),
  rownames_to_column(data.frame(sample_data(rarefiedMPer))),
  by = "rowname")

p1 <- rarefiedMRichAlpha %>% 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 <- rarefiedMRichAlpha %>% ggplot() + geom_point(aes(x = O2_uM,
  y = Shannon), size = 4, alpha = 0.7) + geom_smooth(method = "loess",
  aes(x = as.numeric(O2_uM), y = Shannon)) + labs(title = "Alpha-diversity across oxygen",
  y = "Shannon's diversity index", x = "Oxygen (uM)")

p3 <- rarefiedMRichAlpha %>% 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 <- rarefiedMRichAlpha %>% 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)")

figureM <- ggarrange(p1, p2, p3, p4, ncol = 2, nrow = 2, labels = c("(a)",
  "(b)", "(c)", "(d)"))
annotate_figure(figureM, bottom = text_grob("Figure 1: Mothur",
  face = "bold", size = 12))
```