## **PCoA**

Here we make beta diversity PCoA plots. Make any character vectors in to factors for plotting purposes.

```
metadata <- metadata %>%
    mutate(Ring = factor(Ring,
        levels = c("Ring_1",
            "Ring_3",
            "Ring_8",
            "Ring_4",
            "Ring_7",
            "Ring_12"),
        labels = c("Pen 1",
            "Pen 3",
            "Pen 8".
            "Pen 4",
            "Pen 7",
            "Pen 12")),
        Type = factor(Type),
        Shelter = factor(Shelter),
        Cycle = factor(Cycle))
```

Bray-curtis PCoA plot.

```
VFLA_bray_curtis$data$Vectors %>%
    select(SampleID,
        PC1, PC2) %>%
   left_join(metadata,
        by = c(SampleID = "SampleID")) %>%
   filter(Shelter1 !=
        "Growth") %>%
   ggplot(aes(x = PC1,
        y = PC2, color = Type)) +
   geom_point(alpha = 0.7) +
   theme_bw() + theme(legend.position = "bottom",
   legend.margin = margin(t = -10),
   panel.grid.major = element_blank(),
   panel.grid.minor = element_blank(),
   panel.spacing = unit(0.8,
        "lines"), text = element_text(size = 13)) +
    scale_color_manual(values = c("purple2",
        "springgreen3",
        "deepskyblue1",
        "gold")) + facet_grid(Cycle ~
   Ring + Shelter1)
# ggsave('PCoA_VFLA_braycurtis_ringcycle_b_v5.tiff',
# dpi = 300)
```

Distance matrix created in R rather than Qiime2

```
VFLA_transposed <- t(VFLA_table)
VFLA_bray <- vegdist(VFLA_transposed,
    method = "bray")

metadata2 <- metadata

rownames(metadata2) <- metadata2$SampleID
metadata2$SampleID <- NULL

permanova_result <- adonis2(VFLA_bray ~
    Shelter1 + Cycle1 +
        Type1, data = metadata2,
    by = "terms", permutations = 10000)

print(permanova_result)</pre>
```