Plasmid Alpha and Beta Diversity Using de novo Binary Data

Ilhan Cem Duru

2024-08-02

Load and prepare data for phyloseq

Create counts matrix

```
# Load counts
plasmid_binary <- read.csv("all_results_cluster_simp_pivot.tsv", header = TRUE,
                      skipNul = TRUE, sep = "\t", as.is = TRUE)
#plasmid_binary
#str(plasmid_binary)
#row.names(plasmid_binary)
#class(plasmid_binary)
colnames(plasmid_binary)
```

```
##   [1] "Plasmid" "C1"      "C102"    "C103"    "C104"    "C105"    "C107"
##   [8] "C111"    "C114"    "C116"    "C118"    "C119"    "C123"    "C124"
##  [15] "C134"    "C135"    "C136"    "C137"    "C140"    "C142"    "C146"
##  [22] "C147"    "C148"    "C15"     "C152IIP" "C18"     "C19"     "C20"
##  [29] "C21"     "C23"     "C24"     "C26"     "C28"     "C30"     "C32"
##  [36] "C33"     "C34old"  "C35"     "C40"     "C44"     "C46"     "C47"
##  [43] "C48"     "C49"     "C5"      "C51"     "C54"     "C59"     "C65"
##  [50] "C68"     "C69"     "C7"      "C70"     "C72"     "C74"     "C75"
##  [57] "C76II"   "C80"     "C82"     "C85"     "C86"     "C87"     "C88"
##  [64] "C89"     "C9"      "C90"     "C95"     "C96"     "C98"     "P100"
##  [71] "P103"    "P104IIP" "P105"    "P107"    "P10old"  "P114"    "P115"
##  [78] "P116"    "P118"    "P119"    "P11old"  "P12"     "P120"    "P14"
##  [85] "P15"     "P16"     "P17"     "P18"     "P19"     "P20"     "P24"
##  [92] "P26"     "P28"     "P31"     "P34"     "P37"     "P38"     "P4"
##  [99] "P42"     "P43"     "P45"     "P46"     "P47"     "P48"     "P5"
## [106] "P50"     "P51"     "P52"     "P53"     "P56"     "P57"     "P58"
## [113] "P59"     "P60"     "P61"     "P62P"    "P63"     "P66"     "P67"
## [120] "P68"     "P69"     "P70"     "P71"     "P72"     "P73"     "P74"
## [127] "P77"     "P79"     "P8"      "P83"     "P85"     "P87"     "P88"
## [134] "P9"      "P94"     "P95II"   "P99"
```

```
#head(colnames(plasmid_binary))
#dim(plasmid_binary)
#plasmid_binary$phage

## Put this in `matrix` class for `phyloseq`'s `otu table`, and create it:
counts <- as.matrix(sapply(plasmid_binary, as.numeric))
#head(counts)
#head(colnames(counts))
rownames(counts) <- plasmid_binary$Plasmid
```

```r
#head(counts)
#head(colnames(counts))

# delete/remove the newly created "phage" column:
counts <- counts[ , -grep("Plasmid", colnames(counts))]
#head(counts)
#head(colnames(counts))
#dim(counts)

otu.table <- otu_table(counts, taxa_are_rows = TRUE)
head(otu.table)
```

```
## OTU Table:          [6 taxa and 136 samples]
##                      taxa are rows
##                                          C1 C102 C103 C104 C105 C107 C111 C114
## C1-NODE_10182_length_6088_cov_3.171059    1    0    0    0    0    0    0    0
## C1-NODE_10195_length_6078_cov_3.637722    1    0    0    0    0    0    0    0
## C1-NODE_10214_length_6069_cov_3.628700    1    0    0    0    0    0    0    0
## C1-NODE_10275_length_6034_cov_20.391370   1    0    0    0    0    0    0    0
## C1-NODE_10759_length_5749_cov_4.134528    1    0    0    0    0    0    0    0
## C1-NODE_10936_length_5650_cov_3.782306    1    0    0    0    0    0    0    0
##                                         C116 C118 C119 C123 C124 C134 C135 C136
## C1-NODE_10182_length_6088_cov_3.171059     0    0    0    0    0    0    0    0
## C1-NODE_10195_length_6078_cov_3.637722     0    0    0    0    0    0    0    0
## C1-NODE_10214_length_6069_cov_3.628700     0    0    0    0    0    0    0    0
## C1-NODE_10275_length_6034_cov_20.391370    0    0    0    0    0    0    0    0
## C1-NODE_10759_length_5749_cov_4.134528     0    0    0    0    0    0    0    0
## C1-NODE_10936_length_5650_cov_3.782306     0    0    0    0    0    0    0    0
##                                         C137 C140 C142 C146 C147 C148 C15
## C1-NODE_10182_length_6088_cov_3.171059     0    0    0    0    0    0   0
## C1-NODE_10195_length_6078_cov_3.637722     0    0    0    0    0    0   0
## C1-NODE_10214_length_6069_cov_3.628700     0    0    0    0    0    0   0
## C1-NODE_10275_length_6034_cov_20.391370    0    0    0    0    0    0   0
## C1-NODE_10759_length_5749_cov_4.134528     0    0    0    0    0    0   0
## C1-NODE_10936_length_5650_cov_3.782306     0    0    0    0    0    0   0
##                                         C152IIP C18 C19 C20 C21 C23 C24 C26 C28
## C1-NODE_10182_length_6088_cov_3.171059        0   0   0   0   0   0   0   0   0
## C1-NODE_10195_length_6078_cov_3.637722        0   0   0   0   0   0   0   0   0
## C1-NODE_10214_length_6069_cov_3.628700        0   0   0   0   0   0   0   0   0
## C1-NODE_10275_length_6034_cov_20.391370       0   0   0   0   0   0   0   0   0
## C1-NODE_10759_length_5749_cov_4.134528        0   0   0   0   0   0   0   0   0
## C1-NODE_10936_length_5650_cov_3.782306        0   0   0   0   0   0   0   0   0
##                                         C30 C32 C33 C34old C35 C40 C44 C46 C47
## C1-NODE_10182_length_6088_cov_3.171059    0   0   0      0   0   0   0   0   0
## C1-NODE_10195_length_6078_cov_3.637722    0   0   0      0   0   0   0   0   0
## C1-NODE_10214_length_6069_cov_3.628700    0   0   0      0   0   0   0   0   0
## C1-NODE_10275_length_6034_cov_20.391370   0   0   0      0   0   0   0   0   0
## C1-NODE_10759_length_5749_cov_4.134528    0   0   0      0   0   0   0   0   0
## C1-NODE_10936_length_5650_cov_3.782306    0   0   0      0   0   0   0   0   0
##                                         C48 C49 C5 C51 C54 C59 C65 C68 C69 C7
## C1-NODE_10182_length_6088_cov_3.171059    0   0  0   0   0   0   0   0   0  0
## C1-NODE_10195_length_6078_cov_3.637722    0   0  0   0   0   0   0   0   0  0
## C1-NODE_10214_length_6069_cov_3.628700    0   0  0   0   0   0   0   0   0  0
## C1-NODE_10275_length_6034_cov_20.391370   0   0  0   0   0   0   0   0   0  0
```

```
## C1-NODE_10759_length_5749_cov_4.134528    0   0  0   0   0   0   0   0   0  0
## C1-NODE_10936_length_5650_cov_3.782306    0   0  0   0   0   0   0   0   0  0
##                                        C70 C72 C74 C75 C76II C80 C82 C85 C86
## C1-NODE_10182_length_6088_cov_3.171059    0   0   0   0     0   0   0   0   0
## C1-NODE_10195_length_6078_cov_3.637722    0   0   0   0     0   0   0   0   0
## C1-NODE_10214_length_6069_cov_3.628700    0   0   0   0     0   0   0   0   0
## C1-NODE_10275_length_6034_cov_20.391370   0   0   0   0     0   0   0   0   0
## C1-NODE_10759_length_5749_cov_4.134528    0   0   0   0     0   0   0   0   0
## C1-NODE_10936_length_5650_cov_3.782306    0   0   0   0     0   0   0   0   0
##                                        C87 C88 C89 C9 C90 C95 C96 C98 P100
## C1-NODE_10182_length_6088_cov_3.171059    0   0   0  0   0   0   0   0    0
## C1-NODE_10195_length_6078_cov_3.637722    0   0   0  0   0   0   0   0    0
## C1-NODE_10214_length_6069_cov_3.628700    0   0   0  0   0   0   0   0    0
## C1-NODE_10275_length_6034_cov_20.391370   0   0   0  0   0   0   0   0    0
## C1-NODE_10759_length_5749_cov_4.134528    0   0   0  0   0   0   0   0    0
## C1-NODE_10936_length_5650_cov_3.782306    0   0   0  0   0   0   0   0    0
##                                        P103 P104IIP P105 P107 P10old P114 P115
## C1-NODE_10182_length_6088_cov_3.171059    0       0    0    0      0    0    0
## C1-NODE_10195_length_6078_cov_3.637722    0       0    0    0      0    0    0
## C1-NODE_10214_length_6069_cov_3.628700    0       0    0    0      0    0    0
## C1-NODE_10275_length_6034_cov_20.391370   0       0    0    0      0    0    0
## C1-NODE_10759_length_5749_cov_4.134528    0       0    0    0      0    0    0
## C1-NODE_10936_length_5650_cov_3.782306    0       0    0    0      0    0    0
##                                        P116 P118 P119 P11old P12 P120 P14 P15
## C1-NODE_10182_length_6088_cov_3.171059    0    0    0       0   0    0   0   0
## C1-NODE_10195_length_6078_cov_3.637722    0    0    0       0   0    0   0   0
## C1-NODE_10214_length_6069_cov_3.628700    0    0    0       0   0    0   0   0
## C1-NODE_10275_length_6034_cov_20.391370   0    0    0       0   0    0   0   0
## C1-NODE_10759_length_5749_cov_4.134528    0    0    0       0   0    0   0   0
## C1-NODE_10936_length_5650_cov_3.782306    0    0    0       0   0    0   0   0
##                                        P16 P17 P18 P19 P20 P24 P26 P28 P31 P34
## C1-NODE_10182_length_6088_cov_3.171059   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10195_length_6078_cov_3.637722   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10214_length_6069_cov_3.628700   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10275_length_6034_cov_20.391370  0   0   0   0   0   0   0   0   0   0
## C1-NODE_10759_length_5749_cov_4.134528   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10936_length_5650_cov_3.782306   0   0   0   0   0   0   0   0   0   0
##                                        P37 P38 P4 P42 P43 P45 P46 P47 P48 P5
## C1-NODE_10182_length_6088_cov_3.171059   0   0  0   0   0   0   0   0   0  0
## C1-NODE_10195_length_6078_cov_3.637722   0   0  0   0   0   0   0   0   0  0
## C1-NODE_10214_length_6069_cov_3.628700   0   0  0   0   0   0   0   0   0  0
## C1-NODE_10275_length_6034_cov_20.391370  0   0  0   0   0   0   0   0   0  0
## C1-NODE_10759_length_5749_cov_4.134528   0   0  0   0   0   0   0   0   0  0
## C1-NODE_10936_length_5650_cov_3.782306   0   0  0   0   0   0   0   0   0  0
##                                        P50 P51 P52 P53 P56 P57 P58 P59 P60 P61
## C1-NODE_10182_length_6088_cov_3.171059   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10195_length_6078_cov_3.637722   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10214_length_6069_cov_3.628700   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10275_length_6034_cov_20.391370  0   0   0   0   0   0   0   0   0   0
## C1-NODE_10759_length_5749_cov_4.134528   0   0   0   0   0   0   0   0   0   0
## C1-NODE_10936_length_5650_cov_3.782306   0   0   0   0   0   0   0   0   0   0
##                                        P62P P63 P66 P67 P68 P69 P70 P71 P72
## C1-NODE_10182_length_6088_cov_3.171059    0   0   0   0   0   0   0   0   0
## C1-NODE_10195_length_6078_cov_3.637722    0   0   0   0   0   0   0   0   0
```

```
## C1-NODE_10214_length_6069_cov_3.628700     0   0   0   0   0   0   0   0   0
## C1-NODE_10275_length_6034_cov_20.391370    0   0   0   0   0   0   0   0   0
## C1-NODE_10759_length_5749_cov_4.134528     0   0   0   0   0   0   0   0   0
## C1-NODE_10936_length_5650_cov_3.782306     0   0   0   0   0   0   0   0   0
##                                          P73 P74 P77 P79 P8 P83 P85 P87 P88 P9
## C1-NODE_10182_length_6088_cov_3.171059     0   0   0   0  0   0   0   0   0  0
## C1-NODE_10195_length_6078_cov_3.637722     0   0   0   0  0   0   0   0   0  0
## C1-NODE_10214_length_6069_cov_3.628700     0   0   0   0  0   0   0   0   0  0
## C1-NODE_10275_length_6034_cov_20.391370    0   0   0   0  0   0   0   0   0  0
## C1-NODE_10759_length_5749_cov_4.134528     0   0   0   0  0   0   0   0   0  0
## C1-NODE_10936_length_5650_cov_3.782306     0   0   0   0  0   0   0   0   0  0
##                                          P94 P95II P99
## C1-NODE_10182_length_6088_cov_3.171059     0     0   0
## C1-NODE_10195_length_6078_cov_3.637722     0     0   0
## C1-NODE_10214_length_6069_cov_3.628700     0     0   0
## C1-NODE_10275_length_6034_cov_20.391370    0     0   0
## C1-NODE_10759_length_5749_cov_4.134528     0     0   0
## C1-NODE_10936_length_5650_cov_3.782306     0     0   0
```

```
head(colnames(otu.table))
```

```
## [1] "C1"   "C102" "C103" "C104" "C105" "C107"
```

```
dim(otu.table)
```

```
## [1] 29096   136
```

```
#otu.table
```

Create dummy tax table for TAX

```
taxmat = matrix(sample(letters, 29096, replace = TRUE),
                nrow = nrow(otu.table), ncol = 7)
rownames(taxmat) <- rownames(otu.table)
colnames(taxmat) <- c("Domain", "Phylum", "Class", "Order",
                      "Family", "Genus", "Species")
#taxmat
TAX = tax_table(taxmat)
```

Import the metadata:

```
sampledata <- as.data.frame(read.csv
                            (file = "pd_meta_with_ffq_and_scfa_only_oursamples_3variables_ordered.csv",
                            header = TRUE, sep = ",", row.names = 1))
#rownames(sampledata)
#colnames(otu.table)
identical(rownames(sampledata), colnames(otu.table))
```

```
## [1] TRUE
```

```
sampledata = sample_data(sampledata)
```

Create physeq

```r
physeqfinal <- phyloseq(otu.table,TAX, sampledata)
#physeqfinal
#sample_data(physeqfinal)
#summary(sample_data(physeqfinal))

physeqfinal.2 <- subset_taxa(physeqfinal, taxa_sums(physeqfinal) >0)
#head(sort(taxa_sums(physeqfinal.2), decreasing = FALSE))

physeqfinal.2
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 29096 taxa and 136 samples ]
## sample_data() Sample Data:       [ 136 samples by 4 sample variables ]
## tax_table()   Taxonomy Table:    [ 29096 taxa by 7 taxonomic ranks ]
```

```r
physeqfinal
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 29096 taxa and 136 samples ]
## sample_data() Sample Data:       [ 136 samples by 4 sample variables ]
## tax_table()   Taxonomy Table:    [ 29096 taxa by 7 taxonomic ranks ]
```

```r
summary(sample_data(physeqfinal.2))
```

```
##     Group              gender          age_at_stool_collection      BMI
##  Length:136         Length:136         Min.   :51.00           Min.   :17.51
##  Class :character   Class :character   1st Qu.:61.00           1st Qu.:24.13
##  Mode  :character   Mode  :character   Median :65.00           Median :26.31
##                                        Mean   :64.98           Mean   :26.69
##                                        3rd Qu.:69.00           3rd Qu.:28.62
##                                        Max.   :78.00           Max.   :37.87
##                                                                NA's   :13
```

Alpha Diversity for Group (C vs P)

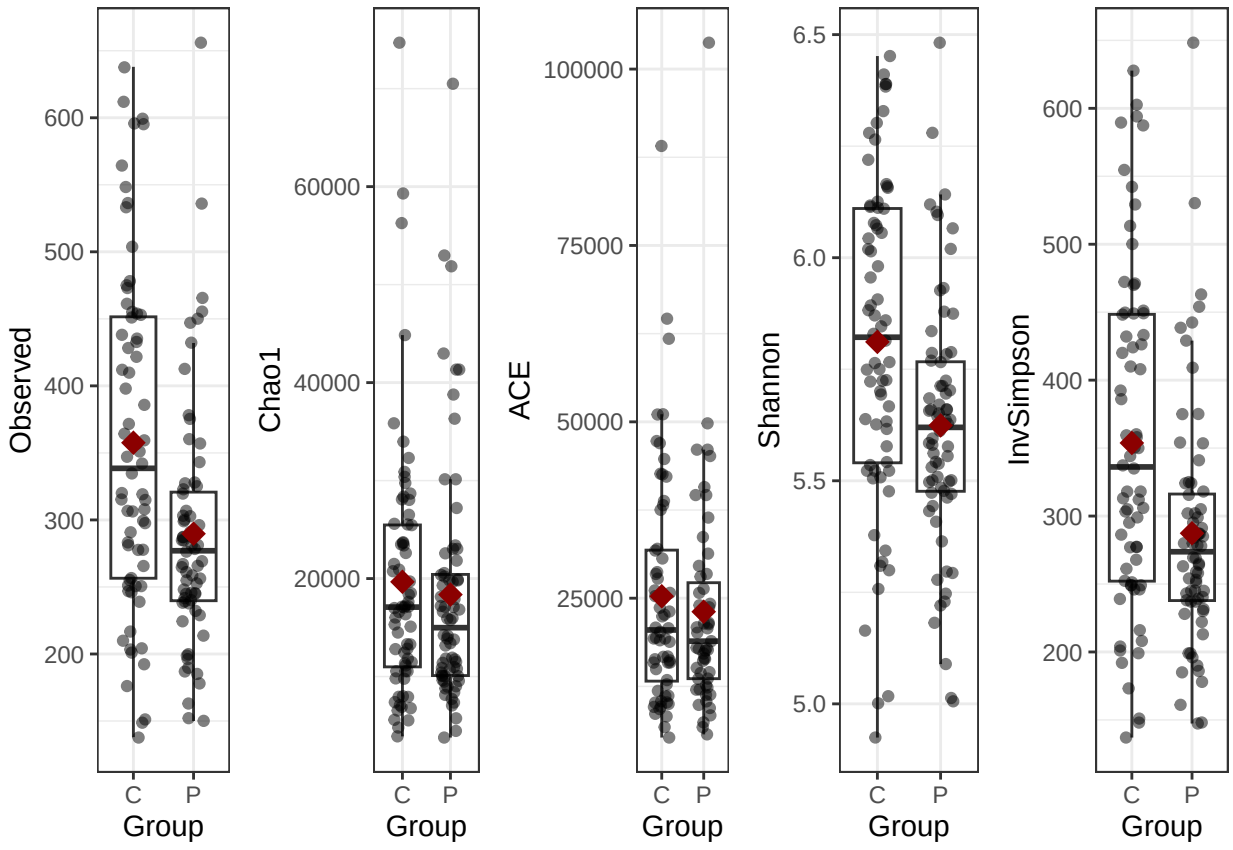
```r
richness.table <- estimate_richness(physeqfinal.2, split = TRUE,
                                    measures = c("Observed", "Chao1",
                                    "ACE", "Shannon", "InvSimpson"))

head(richness.table)
```

```
##      Observed   Chao1  se.chao1      ACE   se.ACE  Shannon InvSimpson
## C1        410 28086.0 13332.893 42230.00 1.413345 6.014294   408.0385
## C102      192 18528.0  2653.502      NaN      NaN 5.257495   192.0000
## C103      204 20910.0  2906.561      NaN      NaN 5.318120   204.0000
## C104      536 35847.5 15595.515 42563.42 2.630661 6.279640   529.2604
## C105      257  5528.0  2075.259  6733.40 2.233336 5.541889   252.3676
## C107      335 56280.0  6122.355      NaN      NaN 5.814131   335.0000
```

```r
richness.table$Group <- sample_data(physeqfinal.2)$Group
```

Plot Alpha Diversity

```
theme_set(theme_bw())

grid.arrange(
  ggplot(richness.table, aes(x = Group, y = Observed)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = Group, y = Chao1)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = Group, y = ACE)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = Group, y = Shannon)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = Group, y = InvSimpson)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  nrow = 1)
```



Observed Richness Wilcoxon rank sum test (Group)

```
wilcox.test(richness.table$Observed ~ sample_data(physeqfinal.2)$Group,
            conf.level = 0.95, conf.int = TRUE)
```

```
##
```

```
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$Observed by sample_data(physeqfinal.2)$Group
## W = 3090.5, p-value = 0.0007085
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##   23.00004 101.00003
## sample estimates:
## difference in location
##               60.99996
```

CHAO1 Wilcoxon rank sum test (Group)

```
wilcox.test(richness.table$Chao1 ~ sample_data(physeqfinal.2)$Group,
          conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$Chao1 by sample_data(physeqfinal.2)$Group
## W = 2524.5, p-value = 0.3562
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -1693  4664
## sample estimates:
## difference in location
##               1357.992
```

ACE Wilcoxon rank sum test (Group)

```
wilcox.test(richness.table$ACE ~ sample_data(physeqfinal.2)$Group,
          conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$ACE by sample_data(physeqfinal.2)$Group
## W = 1749.5, p-value = 0.5794
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -2648.000  5501.083
## sample estimates:
## difference in location
##               1349.771
```

InvSimpson Wilcoxon rank sum test (Group)

```
wilcox.test(richness.table$InvSimpson ~ sample_data(physeqfinal.2)$Group,
            conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$InvSimpson by sample_data(physeqfinal.2)$Group
```

```
## W = 3082.5, p-value = 0.0008043
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  22.93046 99.45676
## sample estimates:
## difference in location
##               59.97253
```

Shannon Wilcoxon rank sum test (Group)

```
wilcox.test(richness.table$Shannon ~ sample_data(physeqfinal.2)$Group,
            conf.level = 0.95, conf.int = TRUE)
```

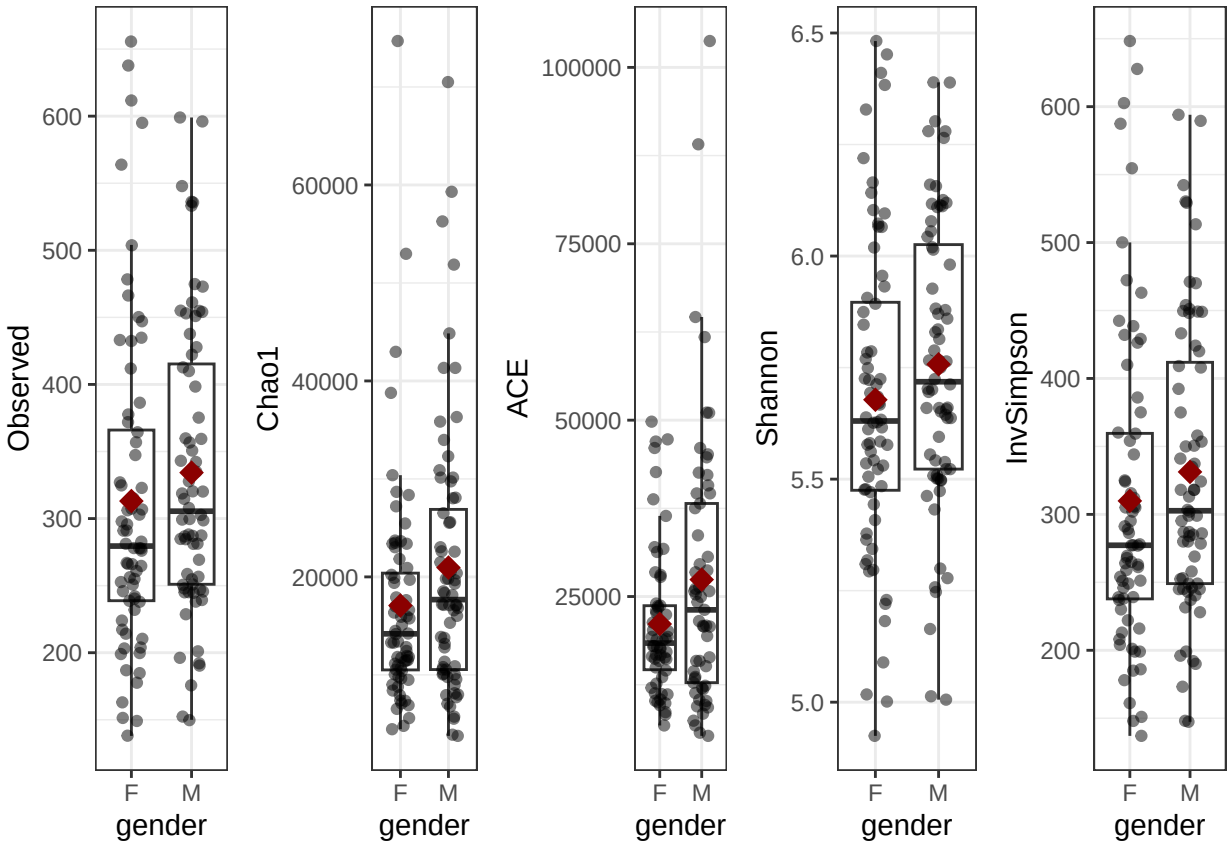
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$Shannon by sample_data(physeqfinal.2)$Group
## W = 3086.5, p-value = 0.0007551
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  0.08128253 0.32375666
## sample estimates:
## difference in location
##              0.2047963
```

Alpha Diversity for Gender (F vs M)

```
richness.table$gender <- sample_data(physeqfinal.2)$gender
```

Plot Alpha Diversity for Gender (F vs M)

```
theme_set(theme_bw())

grid.arrange(
  ggplot(richness.table, aes(x = gender, y = Observed)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = gender, y = Chao1)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = gender, y = ACE)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = gender, y = Shannon)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  ggplot(richness.table, aes(x = gender, y = InvSimpson)) +
    geom_boxplot(outlier.shape = NA) + geom_jitter(width = 0.2, alpha =.5) +
    stat_summary(fun = mean, color = "darkred", geom = "point", shape = 18, size = 4),
  nrow = 1)
```



Observed Richness Wilcoxon rank sum test (gender)

```
wilcox.test(richness.table$Observed ~ sample_data(physeqfinal.2)$gender,
            conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$Observed by sample_data(physeqfinal.2)$gender
## W = 1960.5, p-value = 0.1266
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -58.999938   7.999976
## sample estimates:
## difference in location
##              -25.00002
```

CHAO1 Wilcoxon rank sum test (gender)

```
wilcox.test(richness.table$Chao1 ~ sample_data(physeqfinal.2)$gender,
          conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$Chao1 by sample_data(physeqfinal.2)$gender
```

```
## W = 1918.5, p-value = 0.08718
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -6133.867   369.500
## sample estimates:
## difference in location
##               -2753.91
```

ACE Wilcoxon rank sum test (gender)

```
wilcox.test(richness.table$ACE ~ sample_data(physeqfinal.2)$gender,
          conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$ACE by sample_data(physeqfinal.2)$gender
## W = 1417.5, p-value = 0.1887
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -8120.625  1617.865
## sample estimates:
## difference in location
##              -3048.683
```

InvSimpson Wilcoxon rank sum test (gender)

```
wilcox.test(richness.table$InvSimpson ~ sample_data(physeqfinal.2)$gender,
            conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$InvSimpson by sample_data(physeqfinal.2)$gender
## W = 1962.5, p-value = 0.1288
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -58.397175   6.950941
## sample estimates:
## difference in location
##              -25.75633
```

Shannon Wilcoxon rank sum test (gender)

```
wilcox.test(richness.table$Shannon ~ sample_data(physeqfinal.2)$gender,
            conf.level = 0.95, conf.int = TRUE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  richness.table$Shannon by sample_data(physeqfinal.2)$gender
## W = 1964.5, p-value = 0.131
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
```

```
##  -0.20358297  0.02492071
## sample estimates:
## difference in location
##            -0.08627405
```

Alpha Diversity for BMI (Continuous variable)

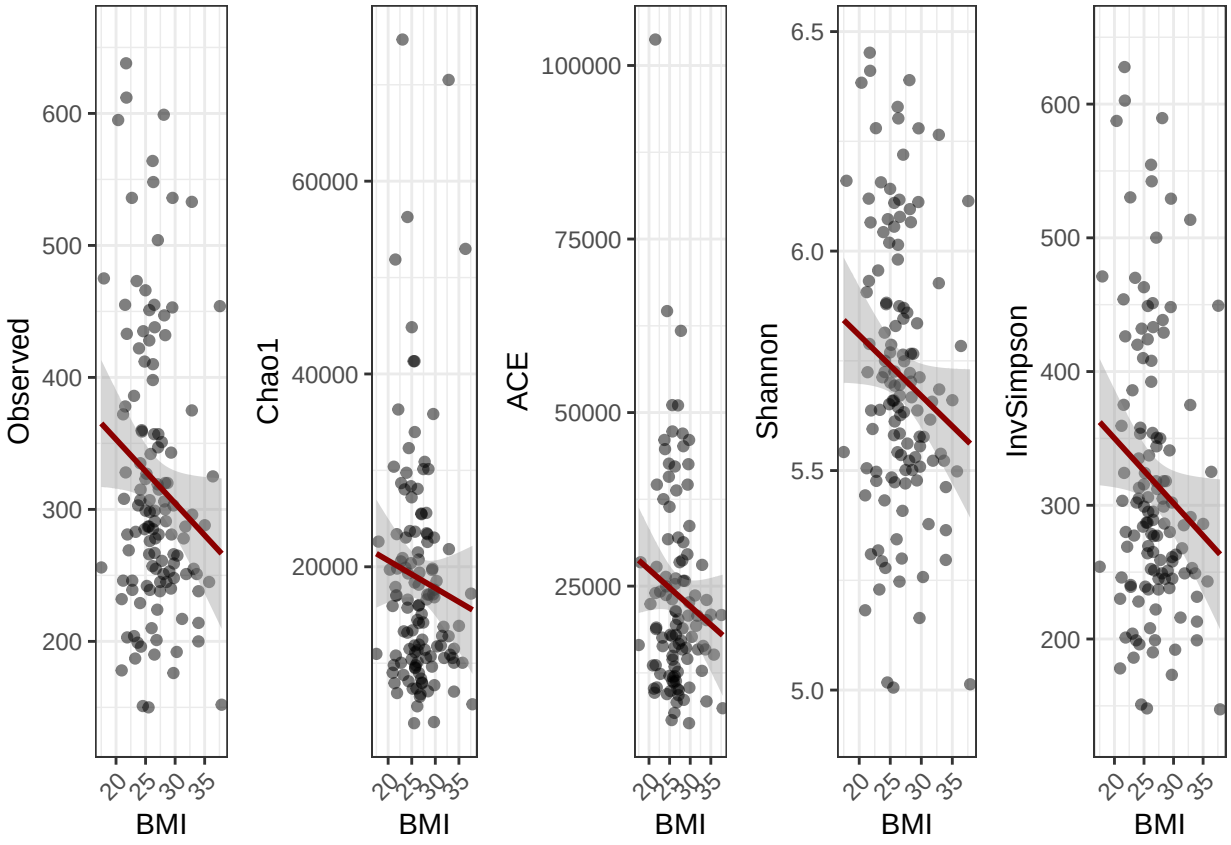
```
richness.table$BMI <- sample_data(physeqfinal.2)$BMI
```

Plot Alpha Diversity for BMI (Continuous variable)

```
common_theme <- theme(
  axis.text.x = element_text(angle = 45, hjust = 1),
)

grid.arrange(
  ggplot(richness.table, aes(x = BMI, y = Observed)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "BMI", y = "Observed") +
    scale_x_continuous(breaks = seq(20, 35, by = 5)) +  # Adjust range as needed
    common_theme,

  ggplot(richness.table, aes(x = BMI, y = Chao1)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "BMI", y = "Chao1") +
    scale_x_continuous(breaks = seq(20, 35, by = 5)) +
    common_theme,

  ggplot(richness.table, aes(x = BMI, y = ACE)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "BMI", y = "ACE") +
    scale_x_continuous(breaks = seq(20, 35, by = 5)) +
    common_theme,

  ggplot(richness.table, aes(x = BMI, y = Shannon)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "BMI", y = "Shannon") +
    scale_x_continuous(breaks = seq(20, 35, by = 5)) +
    common_theme,

  ggplot(richness.table, aes(x = BMI, y = InvSimpson)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "BMI", y = "InvSimpson") +
    scale_x_continuous(breaks = seq(20, 35, by = 5)) +
    common_theme,

  nrow = 1
```

```
)
```

```
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
```



Observed Richness Spearman rank sum test (BMI)

```
cor.test(richness.table$Observed, sample_data(physeqfinal.2)$BMI, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$Observed and sample_data(physeqfinal.2)$BMI
## S = 357951, p-value = 0.08855
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## -0.1542187
```

CHAO1 Richness Spearman rank sum test (BMI)

```
cor.test(richness.table$Chao1, sample_data(physeqfinal.2)$BMI, method = "spearman")
```

```
##
```

```
##  Spearman's rank correlation rho
##
## data:  richness.table$Chao1 and sample_data(physeqfinal.2)$BMI
## S = 357269, p-value = 0.09324
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## -0.1520187
```

ACE Richness Spearman rank sum test (BMI)

```
cor.test(richness.table$ACE, sample_data(physeqfinal.2)$BMI, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$ACE and sample_data(physeqfinal.2)$BMI
## S = 197939, p-value = 0.573
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## -0.0558989
```

InvSimpson Richness Spearman rank sum test (BMI)

```
cor.test(richness.table$InvSimpson, sample_data(physeqfinal.2)$BMI, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$InvSimpson and sample_data(physeqfinal.2)$BMI
## S = 358359, p-value = 0.08583
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## -0.1555335
```

Shannon Richness Spearman rank sum test (BMI)

```
cor.test(richness.table$Shannon, sample_data(physeqfinal.2)$BMI, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$Shannon and sample_data(physeqfinal.2)$BMI
## S = 358100, p-value = 0.08755
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## -0.1546983
```

Alpha Diversity for Age (Continuous variable)

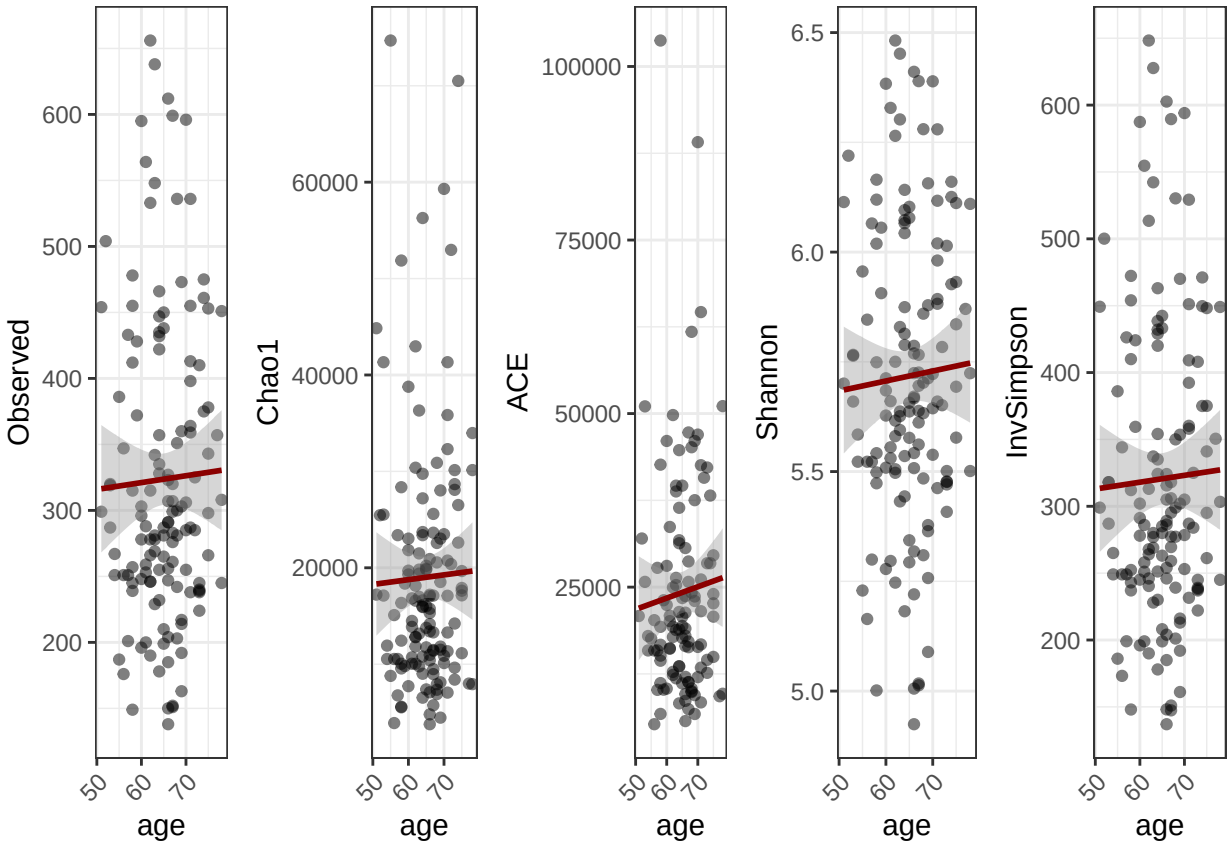
```
richness.table$age <- sample_data(physeqfinal.2)$age_at_stool_collection
```

Plot Alpha Diversity for Age (Continuous variable)

```
common_theme <- theme(
  axis.text.x = element_text(angle = 45, hjust = 1),
)

grid.arrange(
  ggplot(richness.table, aes(x = age, y = Observed)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "age", y = "Observed") +
    scale_x_continuous(breaks = seq(50, 80, by = 10)) +  # Adjust range as needed
    common_theme,

  ggplot(richness.table, aes(x = age, y = Chao1)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "age", y = "Chao1") +
    scale_x_continuous(breaks = seq(50, 80, by = 10)) +
    common_theme,

  ggplot(richness.table, aes(x = age, y = ACE)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "age", y = "ACE") +
    scale_x_continuous(breaks = seq(50, 80, by = 10)) +
    common_theme,

  ggplot(richness.table, aes(x = age, y = Shannon)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "age", y = "Shannon") +
    scale_x_continuous(breaks = seq(50, 80, by = 10)) +
    common_theme,

  ggplot(richness.table, aes(x = age, y = InvSimpson)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "age", y = "InvSimpson") +
    scale_x_continuous(breaks = seq(50, 80, by = 10)) +
    common_theme,

  nrow = 1
)
```

```
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
```

```
## `geom_smooth()` using formula = 'y ~ x'
```



Observed Richness Spearman rank sum test (age)

```
cor.test(richness.table$Observed,
         sample_data(physeqfinal.2)$age_at_stool_collection, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$Observed and sample_data(physeqfinal.2)$age_at_stool_collection
## S = 399673, p-value = 0.5899
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## 0.04662687
```

CHAO1 Richness Spearman rank sum test (age)

```
cor.test(richness.table$Chao1,
         sample_data(physeqfinal.2)$age_at_stool_collection, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$Chao1 and sample_data(physeqfinal.2)$age_at_stool_collection
## S = 391793, p-value = 0.4492
```

```
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.06542371
```

ACE Richness Spearman rank sum test (age)

```
cor.test(richness.table$ACE,
         sample_data(physeqfinal.2)$age_at_stool_collection, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$ACE and sample_data(physeqfinal.2)$age_at_stool_collection
## S = 237101, p-value = 0.4932
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.06454271
```

InvSimpson Richness Spearman rank sum test (age)

```
cor.test(richness.table$InvSimpson,
         sample_data(physeqfinal.2)$age_at_stool_collection, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$InvSimpson and sample_data(physeqfinal.2)$age_at_stool_collection
## S = 400239, p-value = 0.6007
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.04527809
```

Shannon Richness Spearman rank sum test (age)

```
cor.test(richness.table$Shannon,
         sample_data(physeqfinal.2)$age_at_stool_collection, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  richness.table$Shannon and sample_data(physeqfinal.2)$age_at_stool_collection
## S = 399416, p-value = 0.585
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.04723938
```

Distance-based multivariate analysis / beta diversity

CLR transformation followed by Jaccard distance calculation (Binary Data)

```
physeqfinal.2.clr <- microbiome::transform(physeqfinal.2, transform = "clr", target = "OTU",
                                           shift = 0, scale = 1)
physeqfinal.2.clr.jaccard_dist <- vegdist(t(as.data.frame(as.matrix(otu_table(physeqfinal.2.clr)))),
                                          method = "jaccard", binary = TRUE)

sample_data(physeqfinal.2.clr)$Group <- factor(sample_data(physeqfinal.2.clr)$Group,
                                               levels=c("C" , "P"),
                                               ordered = FALSE)
sample_data(physeqfinal.2.clr)$gender <- factor(sample_data(physeqfinal.2.clr)$gender,
                                               levels=c("F" , "M"),
                                               ordered = FALSE)
```

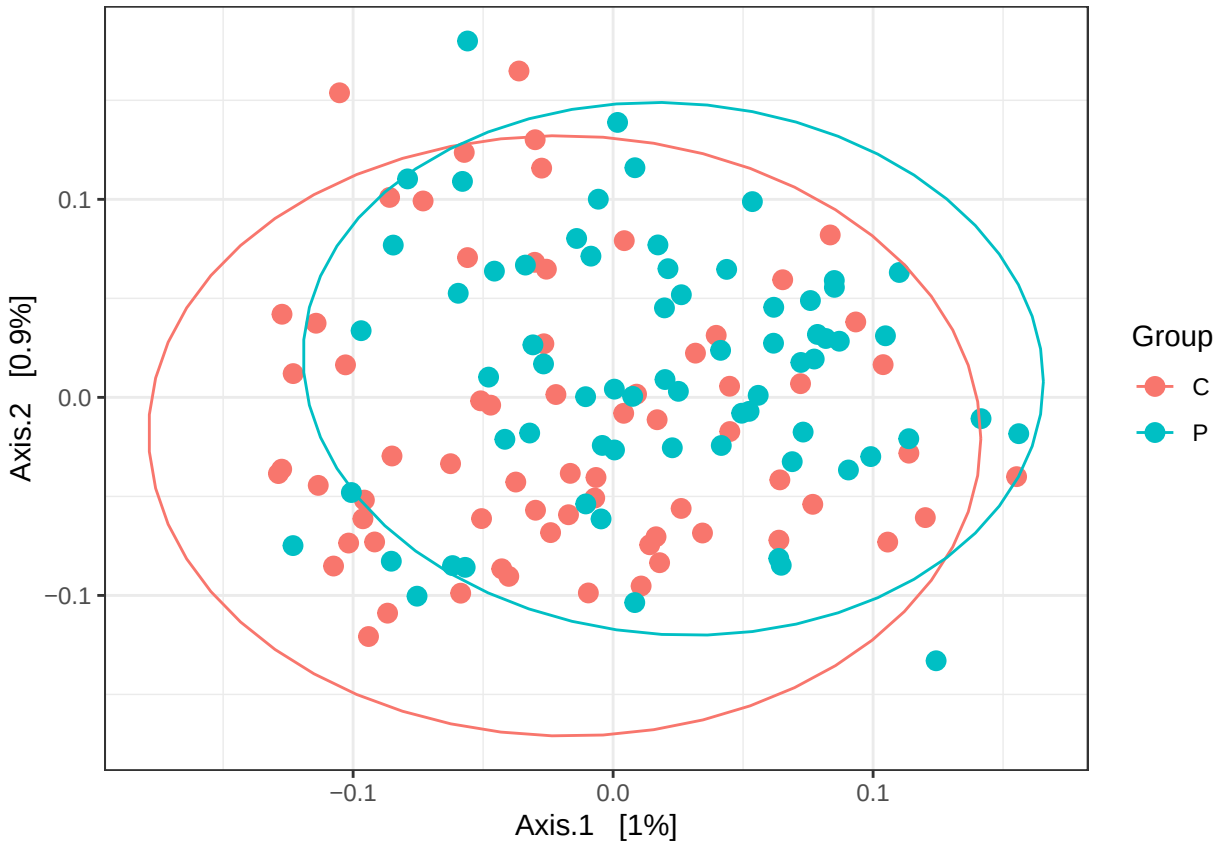
Run the statistics for group variable

```
adonis.Res.clr <- adonis2(physeqfinal.2.clr.jaccard_dist ~
                          sample_data(physeqfinal.2.clr)$Group,
                          perm = 10000,
                          na.action = na.exclude,
                          parallel = 10)

adonis.Res.clr
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.jaccard_dist ~ sample_data(physeqfinal.2.clr)$Group, permutations
##                                       Df SumOfSqs      R2      F Pr(>F)
## sample_data(physeqfinal.2.clr)$Group   1    0.514 0.00778 1.0507  3e-04 ***
## Residual                             134   65.490 0.99222
## Total                                135   66.004 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Principal Coordinates Analysis (PCoA) for Group

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
                    distance = "jaccard", binary = TRUE, autotransform = FALSE,
                    trymax = 1000, maxit = 10000, sratmax = 0.999999,
                    previous.best, parallel = 10)

plot_ordination(physeqfinal.2.clr, ord_clr,
                type = "Samples", color = "Group") + geom_point(size = 3) +
  stat_ellipse(level = 0.95)
```



Run the statistics for gender variable

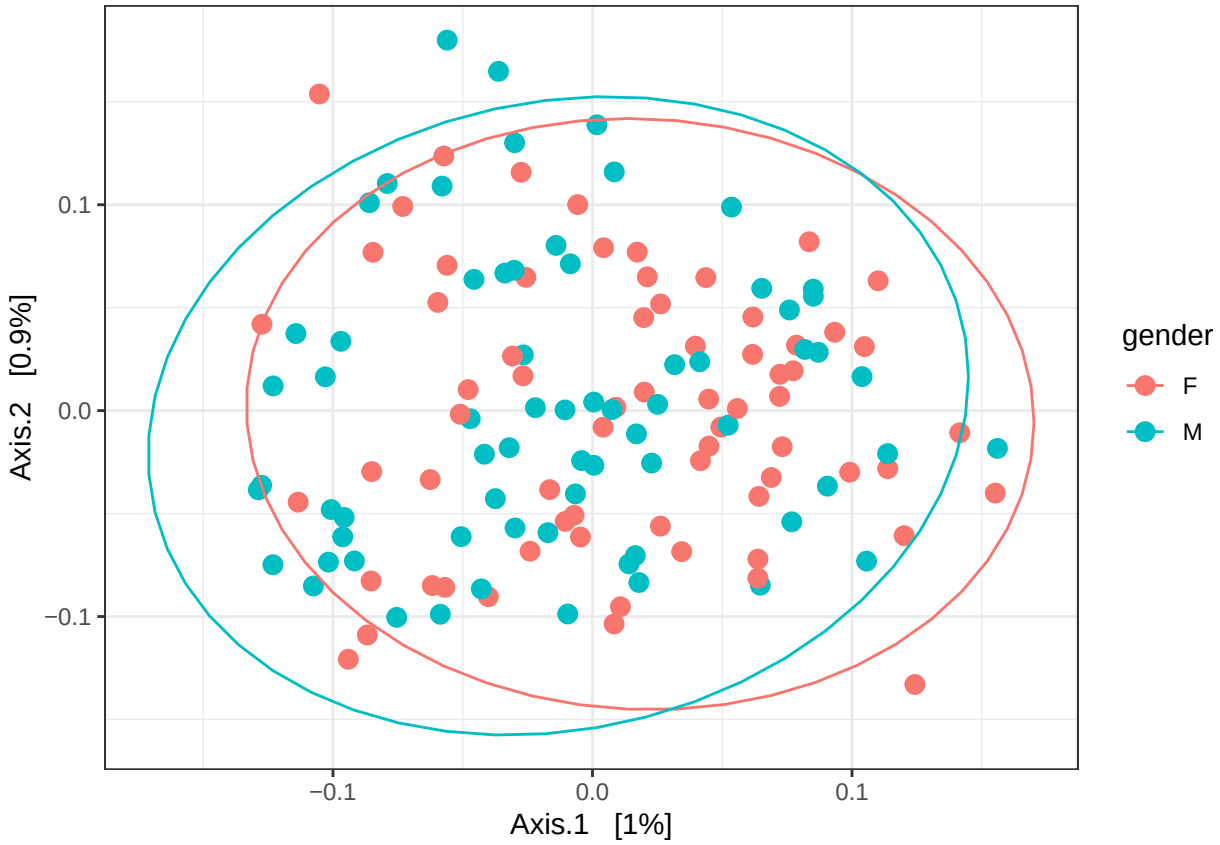
```
adonis.Res.clr <- adonis2(physeqfinal.2.clr.jaccard_dist ~
                          sample_data(physeqfinal.2.clr)$gender,
                          perm = 10000,
                          na.action = na.exclude,
                          parallel = 10)
adonis.Res.clr
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.jaccard_dist ~ sample_data(physeqfinal.2.clr)$gender, permutatio
##                                       Df SumOfSqs      R2      F Pr(>F)
## sample_data(physeqfinal.2.clr)$gender   1    0.494 0.00748 1.0101 0.1834
## Residual                              134   65.510 0.99252
## Total                                 135   66.004 1.00000
```

Principal Coordinates Analysis (PCoA) for Gender

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
                    distance = "jaccard", binary = TRUE, autotransform = FALSE,
                    trymax = 1000, maxit = 10000, sratmax = 0.999999,
                    previous.best, parallel = 10)
```

```
plot_ordination(physeqfinal.2.clr, ord_clr,
                type = "Samples", color = "gender") + geom_point(size = 3) +
  stat_ellipse(level = 0.95)
```



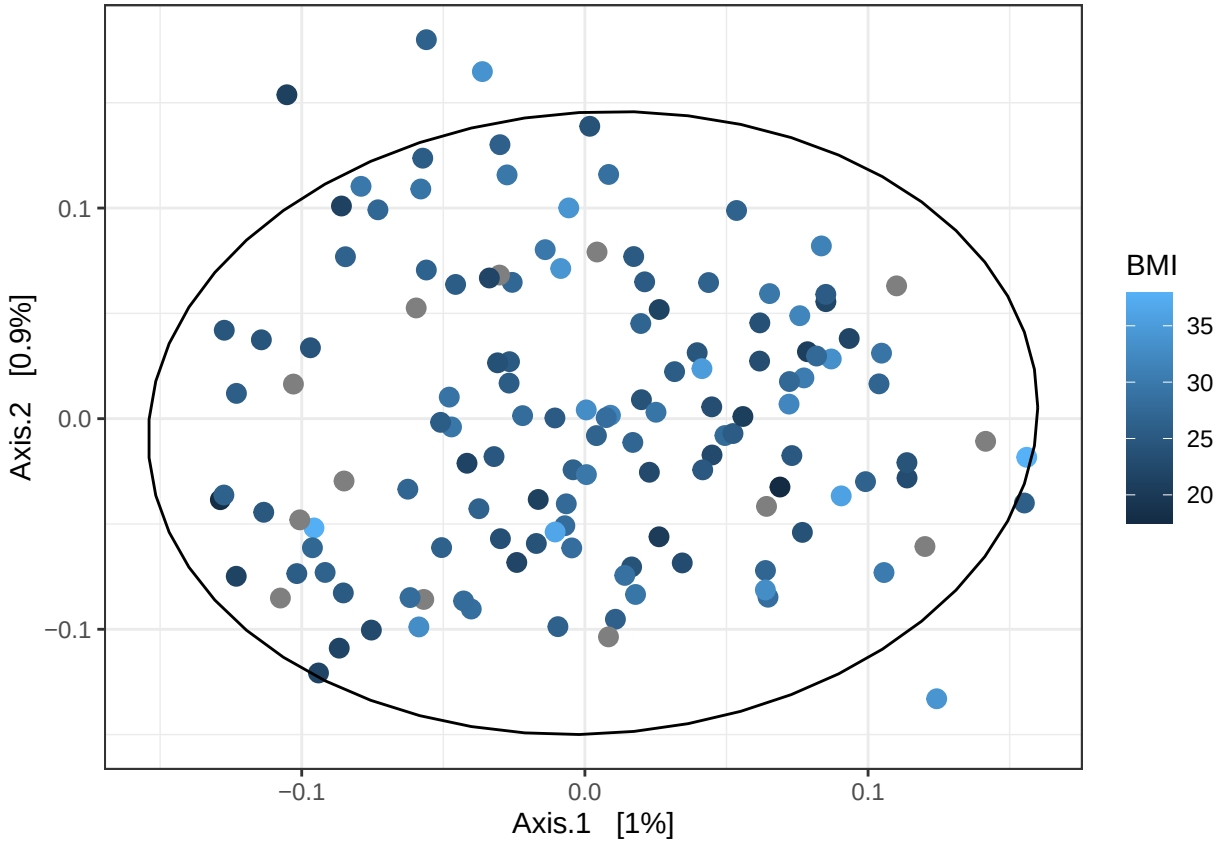
Run the statistics for BMI variable

```
adonis.Res.clr <- adonis2(physeqfinal.2.clr.jaccard_dist ~
                          sample_data(physeqfinal.2.clr)$BMI,
                          perm = 10000,
                          na.action = na.exclude,
                          parallel = 10)

adonis.Res.clr
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.jaccard_dist ~ sample_data(physeqfinal.2.clr)$BMI, permutations =
##                                    Df SumOfSqs      R2      F Pr(>F)
## sample_data(physeqfinal.2.clr)$BMI   1    0.488 0.00818 0.9981 0.5553
## Residual                           121   59.146 0.99182
## Total                              122   59.634 1.00000
```

Principal Coordinates Analysis (PCoA) for BMI

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
                    distance = "jaccard", binary = TRUE, autotransform = FALSE,
                    trymax = 1000, maxit = 10000, sratmax = 0.999999,
                    previous.best, parallel = 10)
plot_ordination(physeqfinal.2.clr, ord_clr,
                type = "Samples", color = "BMI") + geom_point(size = 3) +
  stat_ellipse(level = 0.95)
```



Run the statistics for Age variable

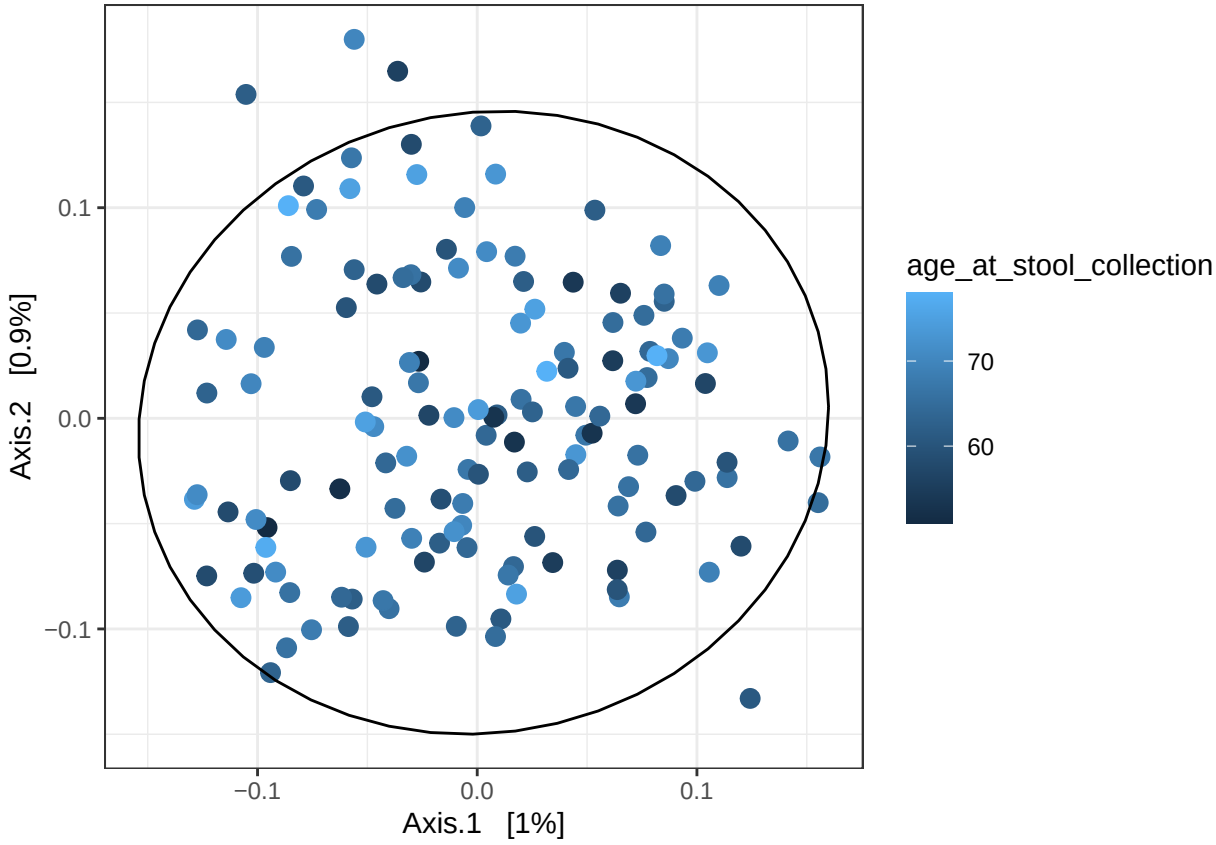
```
adonis.Res.clr <- adonis2(physeqfinal.2.clr.jaccard_dist ~
                          sample_data(physeqfinal.2.clr)$age_at_stool_collection,
                          perm = 10000,
                          na.action = na.exclude,
                          parallel = 10)

adonis.Res.clr
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.jaccard_dist ~ sample_data(physeqfinal.2.clr)$age_at_stool_colle
##                                                    Df SumOfSqs     R2
```

```
## sample_data(physeqfinal.2.clr)$age_at_stool_collection   1    0.489 0.0074
## Residual                                                134   65.515 0.9926
## Total                                                   135   66.004 1.0000
##                                                                F Pr(>F)
## sample_data(physeqfinal.2.clr)$age_at_stool_collection 0.9992 0.5251
## Residual
## Total
```

Principal Coordinates Analysis (PCoA) for Age

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
                    distance = "jaccard", binary = TRUE, autotransform = FALSE,
                    trymax = 1000, maxit = 10000, sratmax = 0.999999,
                    previous.best, parallel = 10)
plot_ordination(physeqfinal.2.clr, ord_clr,
                type = "Samples", color = "age_at_stool_collection") + geom_point(size = 3) +
  stat_ellipse(level = 0.95)
```



Session Info for Reproducibility

```
sessionInfo()
```

```
## R version 4.4.1 (2024-06-14)
## Platform: x86_64-pc-linux-gnu
## Running under: Ubuntu 22.04.4 LTS
##
```

```
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblasp-r0.3.20.so;  LAPACK version 3.10.0
##
## locale:
##  [1] LC_CTYPE=en_GB.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_GB.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_GB.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## time zone: Europe/Helsinki
## tzcode source: system (glibc)
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] microbiome_1.26.0          fido_1.1.1
##  [3] DESeq2_1.44.0              SummarizedExperiment_1.34.0
##  [5] Biobase_2.64.0             MatrixGenerics_1.16.0
##  [7] matrixStats_1.3.0          GenomicRanges_1.56.1
##  [9] GenomeInfoDb_1.40.1        IRanges_2.38.1
## [11] S4Vectors_0.42.1           BiocGenerics_0.50.0
## [13] vegan_2.6-6.1              lattice_0.22-5
## [15] permute_0.9-7              phyloseq_1.48.0
## [17] dabestr_2023.9.12          coin_1.4-3
## [19] survival_3.7-0             ggridges_0.5.6
## [21] qqplotr_0.0.6              MatrixCorrelation_0.10.0
## [23] energy_1.7-11              corrr_0.4.4
## [25] GGally_2.2.1               patchwork_1.2.0
## [27] cowplot_1.1.3              gridExtra_2.3
## [29] kableExtra_1.4.0           magrittr_2.0.3
## [31] purrr_1.0.2                reshape2_1.4.4
## [33] tidylog_1.1.0              tidyr_1.3.1
## [35] dplyr_1.1.4                RColorBrewer_1.1-3
## [37] ggplot2_3.5.1              BiocParallel_1.38.0
## [39] knitr_1.48
##
## loaded via a namespace (and not attached):
##   [1] libcoin_1.0-10          tensorA_0.36.2.1        rstudioapi_0.16.0
##   [4] jsonlite_1.8.8          TH.data_1.1-2           modeltools_0.2-23
##   [7] farver_2.1.2            rmarkdown_2.27          zlibbioc_1.50.0
##  [10] vctrs_0.6.5             multtest_2.60.0         tinytex_0.52
##  [13] htmltools_0.5.8.1       S4Arrays_1.4.1          progress_1.2.3
##  [16] distributional_0.4.0    plotrix_3.8-4           tidybayes_3.0.6
##  [19] Rhdf5lib_1.26.0         SparseArray_1.4.8       rhdf5_2.48.0
##  [22] pracma_2.4.4            plyr_1.8.9              sandwich_3.1-0
##  [25] zoo_1.8-12              igraph_2.0.3            lifecycle_1.0.4
##  [28] iterators_1.0.14        pkgconfig_2.0.3         Matrix_1.6-5
##  [31] R6_2.5.1                fastmap_1.2.0           GenomeInfoDbData_1.2.12
##  [34] digest_0.6.36           colorspace_2.1-1        RSpectra_0.16-2
```

```
##  [37] labeling_0.4.3          fansi_1.0.6             httr_1.4.7
##  [40] abind_1.4-5             mgcv_1.9-1              compiler_4.4.1
##  [43] withr_3.0.1             doParallel_1.0.17       gsl_2.1-8
##  [46] backports_1.5.0         ggstats_0.6.0           highr_0.11
##  [49] MASS_7.3-61             DelayedArray_0.30.1     biomformat_1.32.0
##  [52] caTools_1.18.2          tools_4.4.1             ape_5.8
##  [55] qqconf_1.3.2            glue_1.7.0              nlme_3.1-165
##  [58] rhdf5filters_1.16.0     grid_4.4.1              Rtsne_0.17
##  [61] checkmate_2.3.2         cluster_2.1.6           ade4_1.7-22
##  [64] generics_0.1.3          gtable_0.3.5            data.table_1.15.4
##  [67] hms_1.1.3               xml2_1.3.6              utf8_1.2.4
##  [70] XVector_0.44.0          ggdist_3.3.2            foreach_1.5.2
##  [73] pillar_1.9.0            stringr_1.5.1           posterior_1.6.0
##  [76] robustbase_0.99-3       splines_4.4.1           tidyselect_1.2.1
##  [79] locfit_1.5-9.10         Biostrings_2.72.1       arrayhelpers_1.1-0
##  [82] svglite_2.1.3           xfun_0.46               DEoptimR_1.1-3
##  [85] stringi_1.8.4           UCSC.utils_1.0.0        yaml_2.3.10
##  [88] boot_1.3-30             evaluate_0.24.0         codetools_0.2-19
##  [91] twosamples_2.0.1        tibble_3.2.1            cli_3.6.3
##  [94] pbmcapply_1.5.1         systemfonts_1.1.0       munsell_0.5.1
##  [97] Rcpp_1.0.13             coda_0.19-4.1           svUnit_1.0.6
## [100] parallel_4.4.1          prettyunits_1.2.0       opdisDownsampling_1.0.1
## [103] bitops_1.0-8            viridisLite_0.4.2       mvtnorm_1.2-5
## [106] scales_1.3.0            crayon_1.5.3            clisymbols_1.2.0
## [109] rlang_1.1.4             multcomp_1.4-26
```