

Plasmid antibiotic resistance gene occurrence diversity analysis

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Load and prepare data for phyloseq

Create counts matrix

```
# Load counts
agr_genes_occurrence <- read.csv("plasmid_agr_occurrence.csv", header = TRUE,
                                skipNul = TRUE, sep = "\t", as.is = TRUE)

#agr_genes_occurrence
#str(agr_genes_occurrence)
#row.names(agr_genes_occurrence)
#class(agr_genes_occurrence)
colnames(agr_genes_occurrence)

##      [1] "ARG"      "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(agr_genes_occurrence))
#dim(agr_genes_occurrence)
#agr_genes_occurrence$phage

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

```

#head(counts)
#head(colnames(counts))

# delete/remove the newly created "phage" column:
counts <- counts[ , -grep("ARG", 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 C116 C118 C119 C123 C124
## AADA      0      0      0      0      0      0      0      0      0      0      0      0
## ABES      0      0      0      0      0      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      1      0      0      0
## ADES      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      2      0      0      0      0      0      0      0      0      0      0
##
##      C134 C135 C136 C137 C140 C142 C146 C147 C148 C15 C152IIP C18 C19
## AADA      0      0      0      0      0      0      0      0      0      0      0      0
## ABES      0      0      0      0      1      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      0      0      0      0
## ADES      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0
##
##      C20 C21 C23 C24 C26 C28 C30 C32 C33 C34old C35 C40 C44 C46 C47
## AADA      2      0      0      0      0      0      0      0      0      0      0      0
## ABES      1      0      0      0      0      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      0      0      0      0
## ADES      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0
##
##      C48 C49 C5 C51 C54 C59 C65 C68 C69 C7 C70 C72 C74 C75 C76II C80
## AADA      0      0      0      0      0      0      0      0      0      0      0      0
## ABES      0      0      0      0      0      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      1      0      0      0      0      0
## ADES      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0
##
##      C82 C85 C86 C87 C88 C89 C9 C90 C95 C96 C98 P100 P103 P104IIP P105
## AADA      0      0      0      0      0      0      0      0      1      0      0      0
## ABES      0      0      0      0      0      1      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      0      0      0      0
## ADES      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0
##
##      P107 P10old P114 P115 P116 P118 P119 P11old P12 P120 P14 P15 P16
## AADA      0      0      0      0      0      0      0      0      0      0      0      0
## ABES      0      0      0      0      0      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      0      0      0      0

```

```

## ADES      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0      0      0
##
##          P17 P18 P19 P20 P24 P26 P28 P31 P34 P37 P38 P4  P42 P43 P45 P46
## AADA      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ABES      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ADES      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0      0      0
##
##          P47 P48 P5  P50 P51 P52 P53 P56 P57 P58 P59 P60 P61 P62P P63 P66
## AADA      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ABES      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      1      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ADES      0      0      0      0      0      0      0      0      1      1      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0      0      0
##
##          P67 P68 P69 P70 P71 P72 P73 P74 P77 P79 P8  P83 P85 P87 P88 P9  P94
## AADA      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ABES      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ACT       0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ADEC      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## ADES      0      0      0      0      0      0      0      0      0      0      0      0      0      0
## APH(3''')-III 0      0      0      0      0      0      0      0      0      0      0      0      0      0
##
##          P95II P99
## AADA      0      0
## ABES      0      0
## ACT       0      0
## ADEC      0      0
## ADES      0      0
## APH(3''')-III 0      0

```

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

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

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

```
## [1] 87 136
```

```
#otu.table
```

```
#get otu.table ARGs name from the index
```

```
rownames(otu.table)
```

```

## [1] "AADA"
## [2] "ABES"
## [3] "ACT"
## [4] "ADEC"
## [5] "ADES"
## [6] "APH(3''')-III"
## [7] "APH(3''')-I"
## [8] "APH(6)-I"
## [9] "BACA"
## [10] "BCRA"
## [11] "BLAI"
## [12] "CATA"
## [13] "CATD"
## [14] "CATQ"

```

```

## [15] "CATU"
## [16] "CAT_CHLORAMPHENICOL_ACETYLTRANSFERASE"
## [17] "CFXA6"
## [18] "CHLORAMPHENICOL_EXPORTER"
## [19] "CLASS_A"
## [20] "CLBA"
## [21] "CMLA"
## [22] "COB(I)ALAMIN_ADENOSYLTRANSFERASE"
## [23] "DFRA12"
## [24] "DFRA17"
## [25] "DFRA21"
## [26] "DFRA3"
## [27] "DFRA5"
## [28] "DNA-BINDING_PROTEIN_H-NS"
## [29] "EDEQ"
## [30] "EFRA"
## [31] "EFRB"
## [32] "EMRB-QACA_FAMILY_MAJOR_FACILITATOR_TRANSPORTER"
## [33] "ERM(42)"
## [34] "ERMB"
## [35] "ERMF"
## [36] "ESCHERICHIA_COLI_MIPA"
## [37] "GADW"
## [38] "KASUGAMYCIN_RESISTANCE_PROTEIN_KSGA"
## [39] "KDPE"
## [40] "LLMA_23S_RIBOSOMAL_RNA_METHYLTRANSFERASE"
## [41] "LMRD"
## [42] "LNUA"
## [43] "LRFA"
## [44] "LSA"
## [45] "MARA"
## [46] "MARR"
## [47] "MCR-3"
## [48] "MECI"
## [49] "MEFA"
## [50] "MEPA"
## [51] "MEXL"
## [52] "MTRA"
## [53] "MULTIDRUG_ABC_TRANSPORTER"
## [54] "NMCR"
## [55] "OMP36"
## [56] "OMPR"
## [57] "OPRA"
## [58] "PATA"
## [59] "PMRF"
## [60] "RAMA"
## [61] "ROSA"
## [62] "ROSB"
## [63] "SDIA"
## [64] "SUL1"
## [65] "SUL3"
## [66] "TEM"
## [67] "TET34"
## [68] "TET35"

```

```
## [69] "TET40"
## [70] "TETB(60)"
## [71] "TETM"
## [72] "TETO"
## [73] "TETP"
## [74] "TRUNCATED_PUTATIVE_RESPONSE_REGULATOR_ARLR"
## [75] "UGD"
## [76] "VANH"
## [77] "VANR"
## [78] "VANRI"
## [79] "VANS"
## [80] "VANTRL"
## [81] "VANU"
## [82] "VANY"
## [83] "VANZ"
## [84] "VATB"
## [85] "VATE"
## [86] "VGAC"
## [87] "YKKD"
```

Create dummy tax table for TAX

```
taxmat = matrix(sample(letters, 87, 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:

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

## [1] TRUE
sampladata = sample_data(sampladata)
```

Create physeq

```
physeqfinal <- phyloseq(otu.table, TAX, sampladata)
#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: [ 87 taxa and 136 samples ]
## sample_data() Sample Data: [ 136 samples by 4 sample variables ]
## tax_table() Taxonomy Table: [ 87 taxa by 7 taxonomic ranks ]
```

```
physeqfinal
```

```
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 87 taxa and 136 samples ]
## sample_data() Sample Data: [ 136 samples by 4 sample variables ]
## tax_table() Taxonomy Table: [ 87 taxa by 7 taxonomic ranks ]
```

```
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)

```
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          5  11.0  7.012975 20.78462  2.4677437  0.9943537  1.884615
## C102         7  14.5  8.064530 28.00000  0.9698548  1.9061547  6.400000
## C103         2   3.0  2.041241      NaN      NaN  0.6931472  2.000000
## C104         8  29.0 17.206154 53.69231  3.3584607  1.6661023  3.500000
## C105         2   3.0  2.041241      NaN      NaN  0.6931472  2.000000
## C107         3   4.0  2.121320  7.00000  1.1461852  0.7963116  1.814815
```

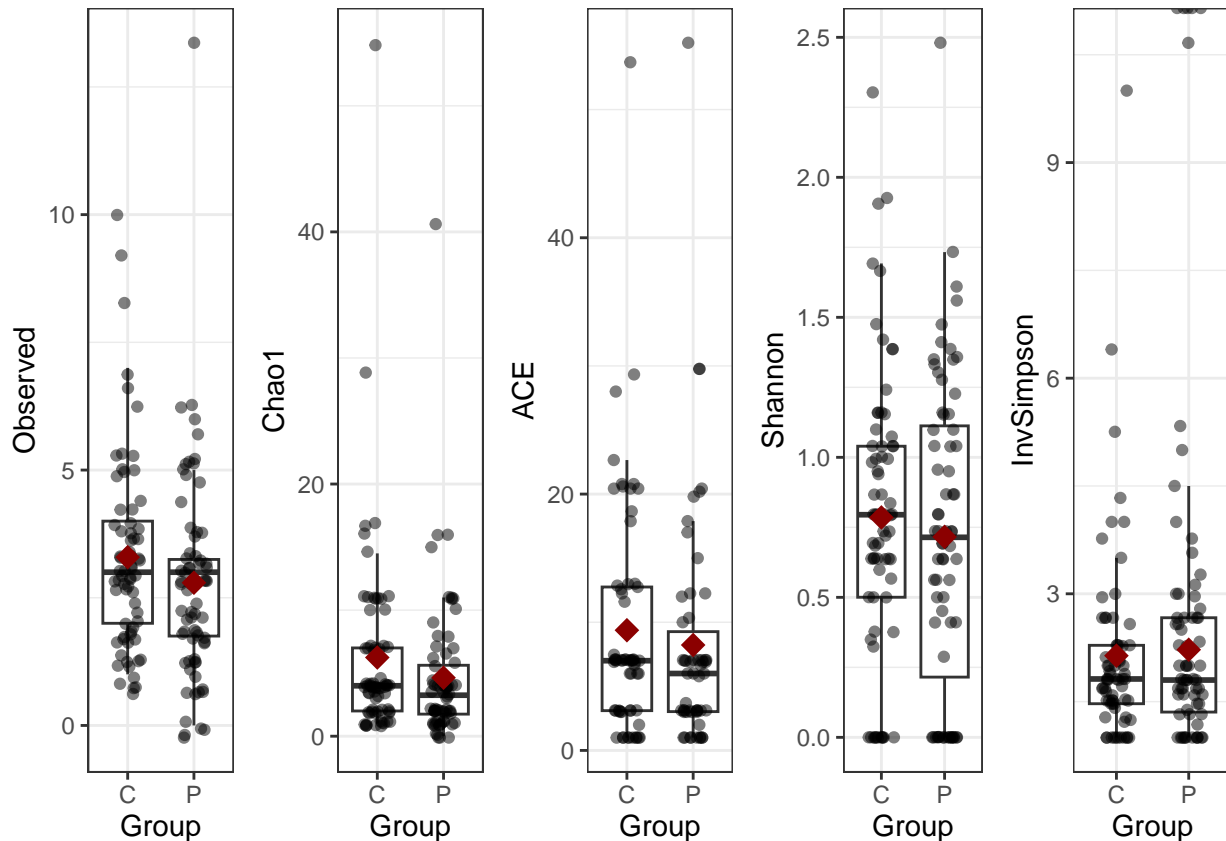
```
richness.table$Group <- sample_data(physeqfinal.2)$Group
```

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)
```



Calculate mean and standard deviation for each alpha diversity metric

```
# Function to calculate mean and SD, ignoring NaN values and handling Inf
calc_mean_sd <- function(x) {
  x_clean <- x[!is.nan(x) & is.finite(x)]

  if (length(x_clean) > 0) {
    c(mean = mean(x_clean, na.rm = TRUE),
      sd = sd(x_clean, na.rm = TRUE),
      n = length(x_clean),
      n_total = length(x),
      n_inf = sum(is.infinite(x)),
      n_nan = sum(is.nan(x)))
  } else {
    c(mean = NA, sd = NA, n = 0, n_total = length(x),
```

```

    n_inf = sum(is.infinite(x)), n_nan = sum(is.nan(x)))
  }
}

# Calculate statistics for each index by group
richness_stats <- richness.table %>%
  group_by(Group) %>%
  summarise(
    across(c(Observed, Chao1, ACE, Shannon, InvSimpson),
      list(Mean = ~calc_mean_sd(.)[1],
           SD = ~calc_mean_sd(.)[2],
           N = ~calc_mean_sd(.)[3],
           N_Total = ~calc_mean_sd(.)[4],
           N_Inf = ~calc_mean_sd(.)[5],
           N_NaN = ~calc_mean_sd(.)[6]))
  )

## group_by: one grouping variable (Group)
## summarise: now 2 rows and 31 columns, ungrouped

# Print the results
print(richness_stats)

## # A tibble: 2 x 31
##   Group Observed_Mean Observed_SD Observed_N Observed_N_Total Observed_N_Inf
##   <chr>      <dbl>      <dbl>      <dbl>      <dbl>      <dbl>
## 1 C          3.29        1.89        68          68          0
## 2 P          2.79        2.03        68          68          0
## # i 25 more variables: Observed_N_NaN <dbl>, Chao1_Mean <dbl>, Chao1_SD <dbl>,
## #   Chao1_N <dbl>, Chao1_N_Total <dbl>, Chao1_N_Inf <dbl>, Chao1_N_NaN <dbl>,
## #   ACE_Mean <dbl>, ACE_SD <dbl>, ACE_N <dbl>, ACE_N_Total <dbl>,
## #   ACE_N_Inf <dbl>, ACE_N_NaN <dbl>, Shannon_Mean <dbl>, Shannon_SD <dbl>,
## #   Shannon_N <dbl>, Shannon_N_Total <dbl>, Shannon_N_Inf <dbl>,
## #   Shannon_N_NaN <dbl>, InvSimpson_Mean <dbl>, InvSimpson_SD <dbl>,
## #   InvSimpson_N <dbl>, InvSimpson_N_Total <dbl>, InvSimpson_N_Inf <dbl>, ...
# write the results to a CSV file
write.csv(richness_stats, "group_richness_statistics_detailed.csv", row.names = FALSE)

```

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 = 2708, p-value = 0.07896
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
## -1.333564e-06 1.000055e+00
## sample estimates:
## difference in location
## 5.362855e-05

```


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 = 2746.5, p-value = 0.05615  
## alternative hypothesis: true location shift is not equal to 0  
## 95 percent confidence interval:  
## -4.367662e-05 2.000039e+00  
## sample estimates:  
## difference in location  
## 0.9999652
```

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 = 1870.5, p-value = 0.2101  
## alternative hypothesis: true location shift is not equal to 0  
## 95 percent confidence interval:  
## -0.04767301 3.65368448  
## sample estimates:  
## difference in location  
## 0.1893541
```

InvSimpson Wilcoxon rank sum test (Group)

```
richness.table$InvSimpson[which(is.infinite(richness.table$InvSimpson))] <- NA  
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 = 2061, p-value = 0.7099  
## alternative hypothesis: true location shift is not equal to 0  
## 95 percent confidence interval:  
## -0.3294410 0.2004612  
## sample estimates:  
## difference in location  
## -4.424907e-05
```

Shannon Wilcoxon rank sum test (Group)

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

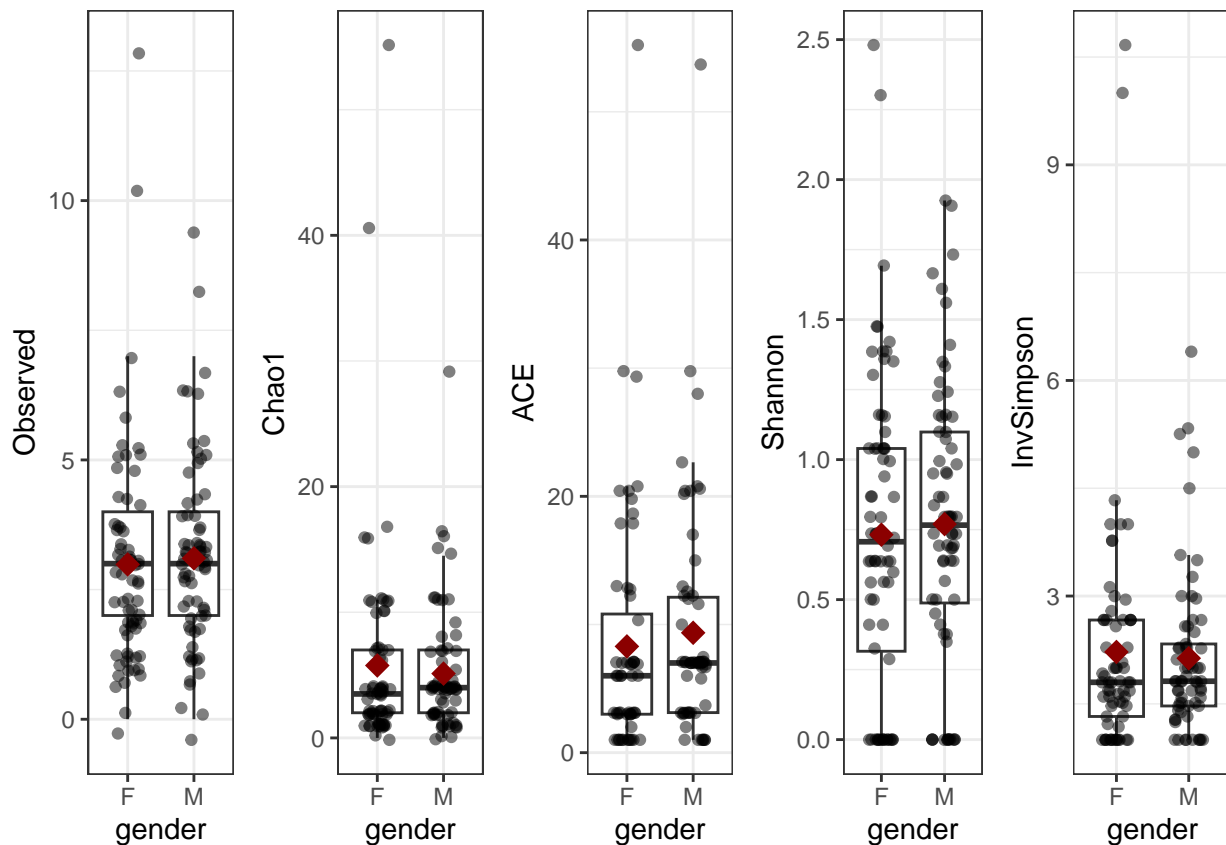
```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: richness.table$Shannon by sample_data(physeqfinal.2)$Group  
## W = 2483.5, p-value = 0.4546  
## alternative hypothesis: true location shift is not equal to 0  
## 95 percent confidence interval:  
## -0.08874981 0.24338387  
## sample estimates:  
## difference in location  
## 0.03455073
```

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)
```



Calculate mean and standard deviation for each alpha diversity metric

```
# Function to calculate mean and SD, ignoring NaN values
calc_mean_sd <- function(x) {
  x_clean <- x[!is.nan(x)]
  if (length(x_clean) > 0) {
    c(mean = mean(x_clean, na.rm = TRUE),
      sd = sd(x_clean, na.rm = TRUE))
  } else {
    c(mean = NA, sd = NA)
  }
}

# Calculate mean and standard deviation for each index by group, ignoring NaN values
richness_stats <- richness.table %>%
  group_by(gender) %>%
  summarise(
    across(c(Observed, Chao1, ACE, Shannon, InvSimpson),
      list(Mean = ~calc_mean_sd(.)[1],
           SD = ~calc_mean_sd(.)[2]))
  )
```

```
## group_by: one grouping variable (gender)
## summarise: now 2 rows and 11 columns, ungrouped
```

```
# Print the results
print(richness_stats)
```

```
## # A tibble: 2 x 11
##   gender Observed_Mean Observed_SD Chao1_Mean Chao1_SD ACE_Mean ACE_SD
##   <chr>      <dbl>      <dbl>      <dbl>      <dbl>      <dbl> <dbl>
## 1 F          2.99        2.15        5.76        8.51        8.29  9.67
## 2 M          3.10        1.79        5.12        4.88        9.35  9.04
## # i 4 more variables: Shannon_Mean <dbl>, Shannon_SD <dbl>,
## #   InvSimpson_Mean <dbl>, InvSimpson_SD <dbl>
# write the results to a CSV file
write.csv(richness_stats, "gender_richness_statistics.csv", row.names = FALSE)
```

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 = 2110, p-value = 0.3708
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
## -9.999408e-01 1.609902e-05
## sample estimates:
## difference in location
## -4.99234e-06
```

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 = 2096.5, p-value = 0.3441
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
## -1.000042e+00 6.483986e-05
## sample estimates:
## difference in location
## -6.31708e-05
```

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 = 1339.5, p-value = 0.07997
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
## -3.8889146819 0.0000038722
## sample estimates:
## difference in location
## -1.00005
```

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 = 2062.5, p-value = 0.7049
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
## -0.3177253 0.2117986
## sample estimates:
## difference in location
## -1.75061e-05
```

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 = 2168.5, p-value = 0.5317
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
## -0.2200162 0.1005600
## sample estimates:
## difference in location
## -0.002531018
```

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,

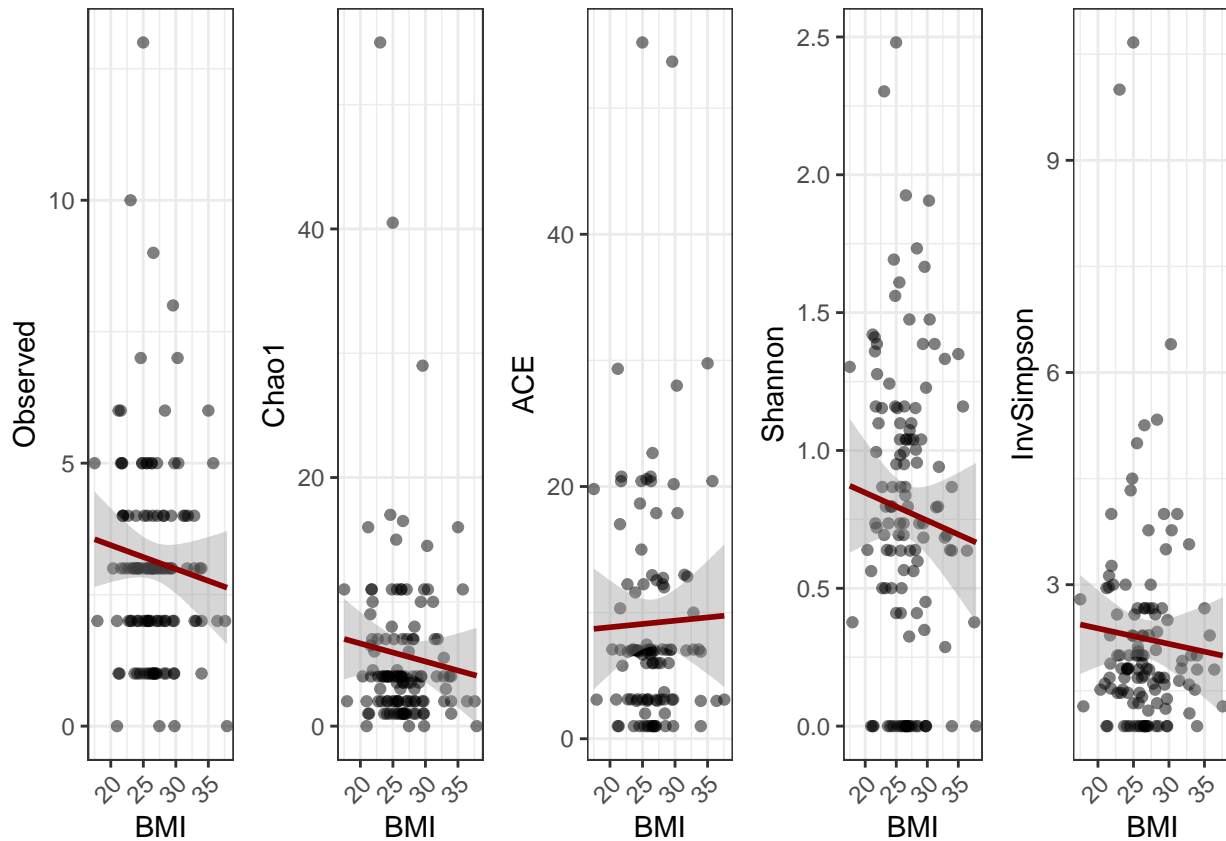
  ggplot(richness.table, aes(x = BMI, y = Shannon)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "BMI", y = "Shannon") +
    scale_x_continuous(breaks = seq(20, 35, by = 5)) +
    common_theme,

  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 = 335408, p-value = 0.37
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
## -0.08152781
```

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 = 336421, p-value = 0.3511
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
```

```
## -0.08479362
```

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 = 183511, p-value = 0.9383  
## alternative hypothesis: true rho is not equal to 0  
## sample estimates:  
## rho  
## -0.007726253
```

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 = 292555, p-value = 0.6524  
## alternative hypothesis: true rho is not equal to 0  
## sample estimates:  
## rho  
## -0.04171462
```

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 = 332576, p-value = 0.4262  
## alternative hypothesis: true rho is not equal to 0  
## sample estimates:  
## rho  
## -0.07239689
```

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,

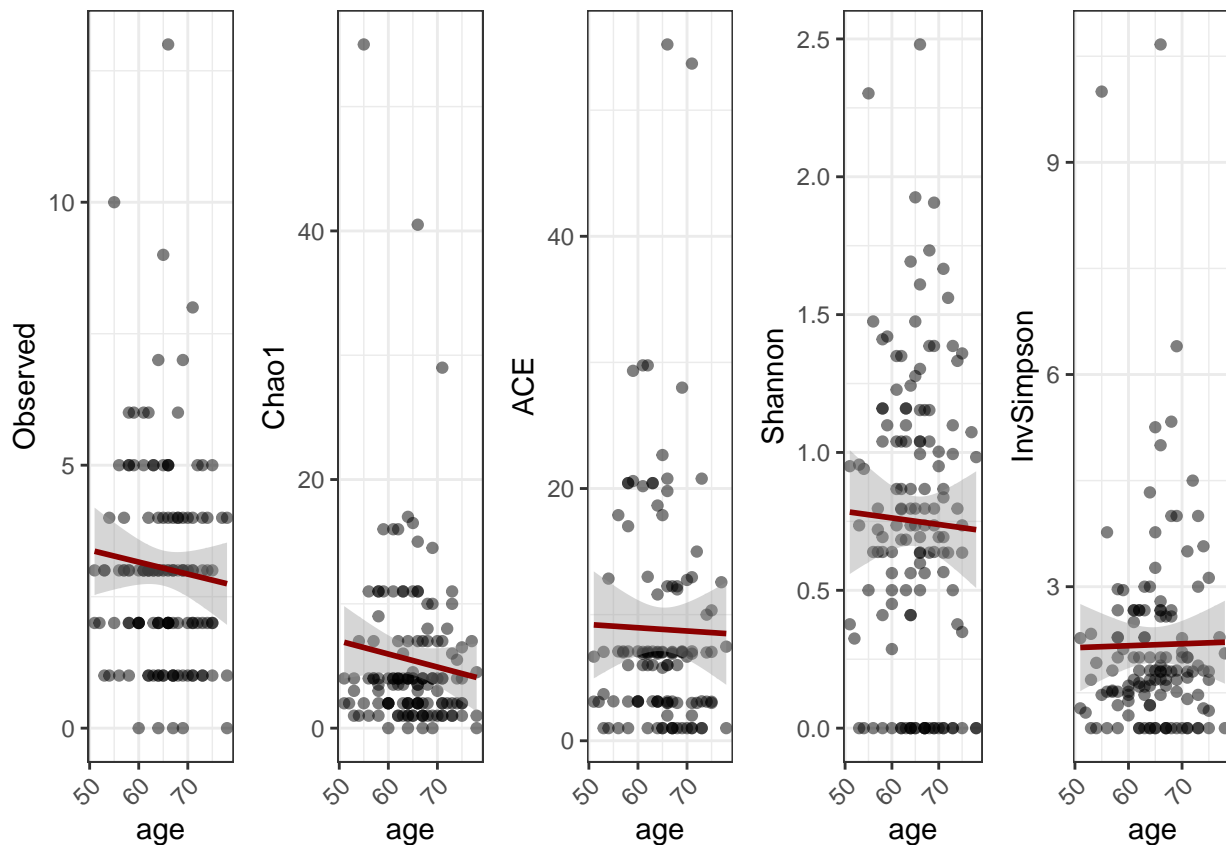
  ggplot(richness.table, aes(x = age, y = Shannon)) +
    geom_point(alpha = 0.5) +
    geom_smooth(method = "lm", color = "darkred") +
    labs(x = "age", y = "Shannon") +
    scale_x_continuous(breaks = seq(50, 80, by = 10)) +
    common_theme,

  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 = 450411, p-value = 0.3893
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
## -0.07440267
```

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 = 448422, p-value = 0.4203
## alternative hypothesis: true rho is not equal to 0
```

```
## sample estimates:
##      rho
## -0.06965858
```

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 = 276443, p-value = 0.3352
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
## -0.09067628
```

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 = 357338, p-value = 0.6
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
## 0.04623493
```

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 = 428387, p-value = 0.8005
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##      rho
## -0.0218662
```

Distance-based multivariate analysis / beta diversity

CLR transformation followed by Euclidian Distance (a.k.a. Aitchinson Distance)

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

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

```
set.seed(1337)
adonis.Res.clr <- adonis2(physeqfinal.2.clr.eucl_dist ~
                        sample_data(physeqfinal.2.clr)$Group,
                        by = "margin",
                        perm = 10000,
                        na.action = na.exclude,
                        parallel = 10)

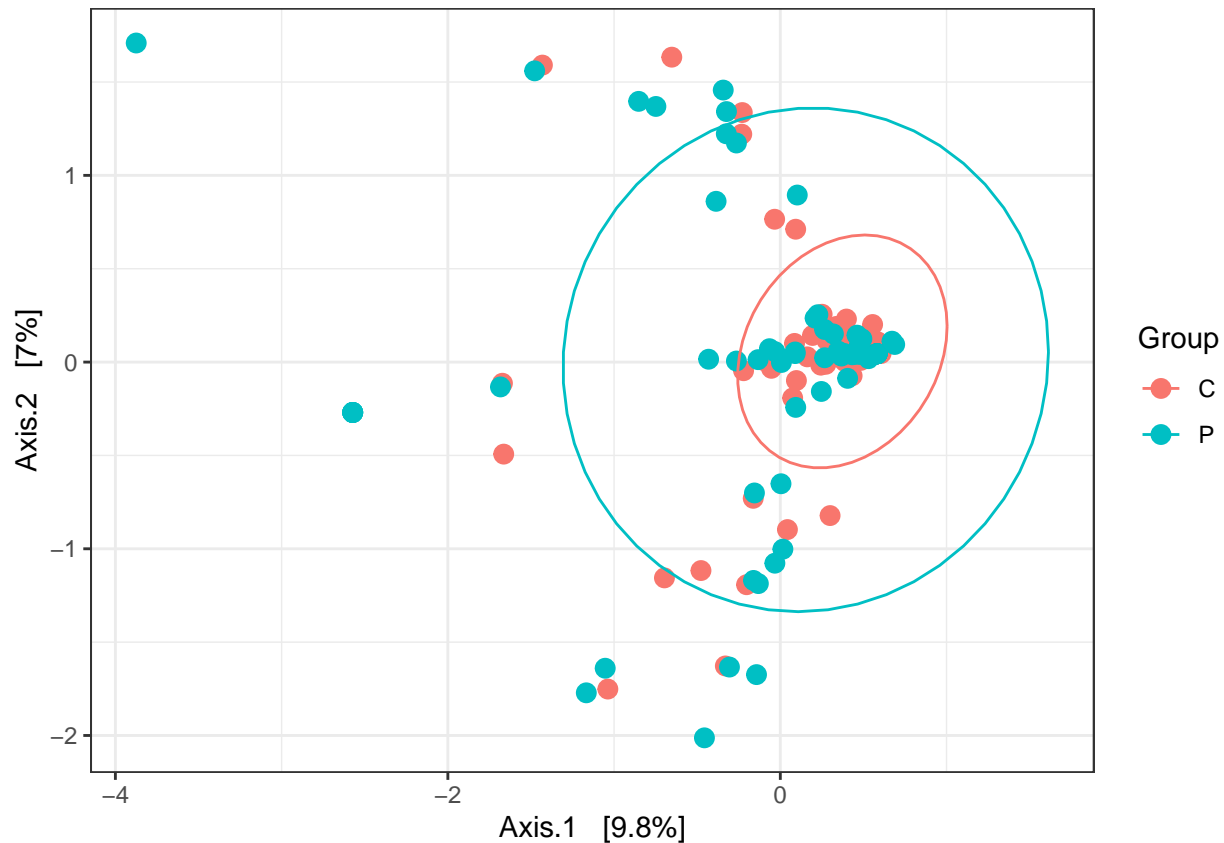
adonis.Res.clr

## Permutation test for adonis under reduced model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.eucl_dist ~ sample_data(physeqfinal.2.clr)$Group, permutations =
##
##           Df SumOfSqs      R2      F Pr(>F)
## sample_data(physeqfinal.2.clr)$Group    1      8.05 0.00919 1.2429 0.1408
## Residual                               134   868.03 0.99081
## Total                                   135   876.08 1.00000
```

Principal Coordinates Analysis (PCoA) for Group

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
                   distance = "euclidean", binary = FALSE, 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

```
set.seed(1337)
adonis.Res.clr <- adonis2(physeqfinal.2.clr.eucl_dist ~
  sample_data(physeqfinal.2.clr)$gender,
  by = "margin",
  perm = 10000,
  na.action = na.exclude,
  parallel = 10)
adonis.Res.clr
```

```
## Permutation test for adonis under reduced model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.eucl_dist ~ sample_data(physeqfinal.2.clr)$gender, permutations = 10000)
##
```

	Df	SumOfSqs	R2	F	Pr(>F)
sample_data(physeqfinal.2.clr)\$gender	1	5.74	0.00655	0.8839	0.6572
Residual	134	870.34	0.99345		
Total	135	876.08	1.00000		

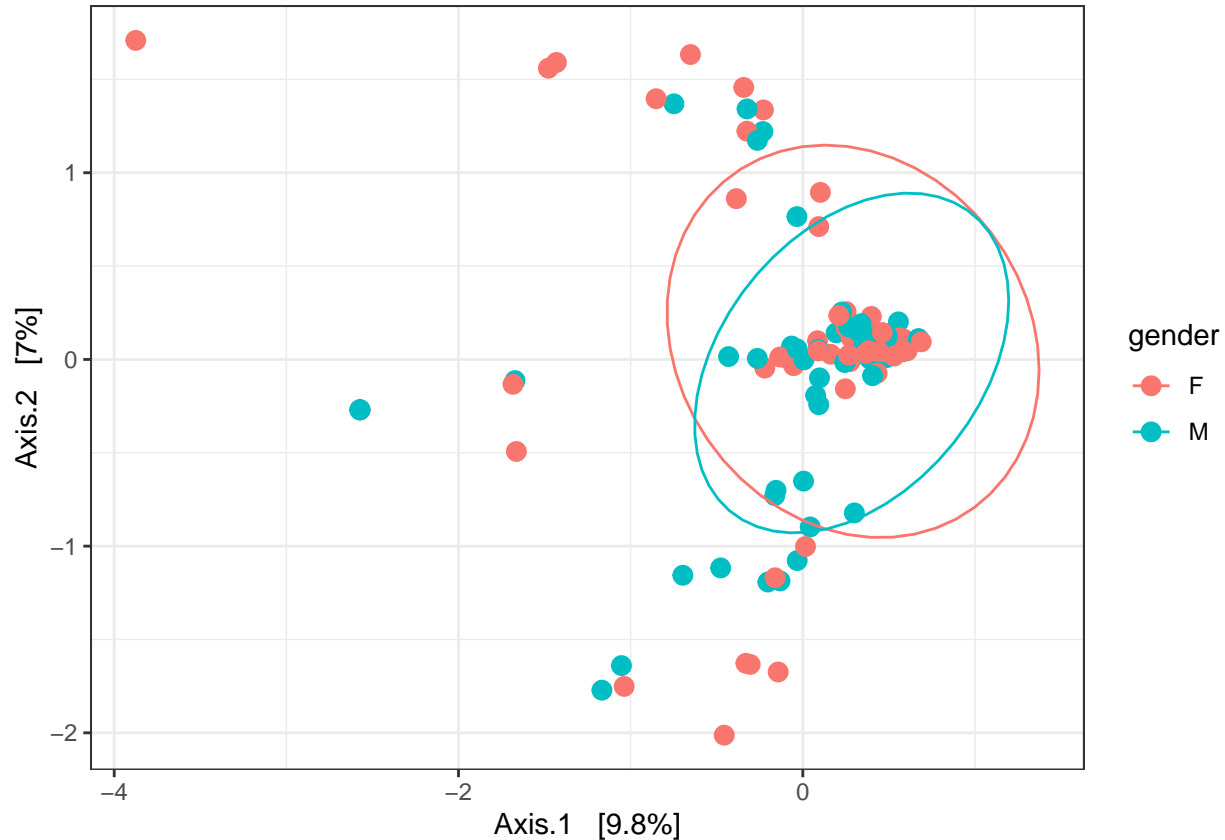
Principal Coordinates Analysis (PCoA) for Gender

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
  distance = "euclidean", binary = FALSE, 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

```

set.seed(1337)
adonis.Res.clr <- adonis2(physeqfinal.2.clr.eucl_dist ~
  sample_data(physeqfinal.2.clr)$BMI,
  by = "margin",
  perm = 10000,
  na.action = na.exclude,
  parallel = 10)

adonis.Res.clr

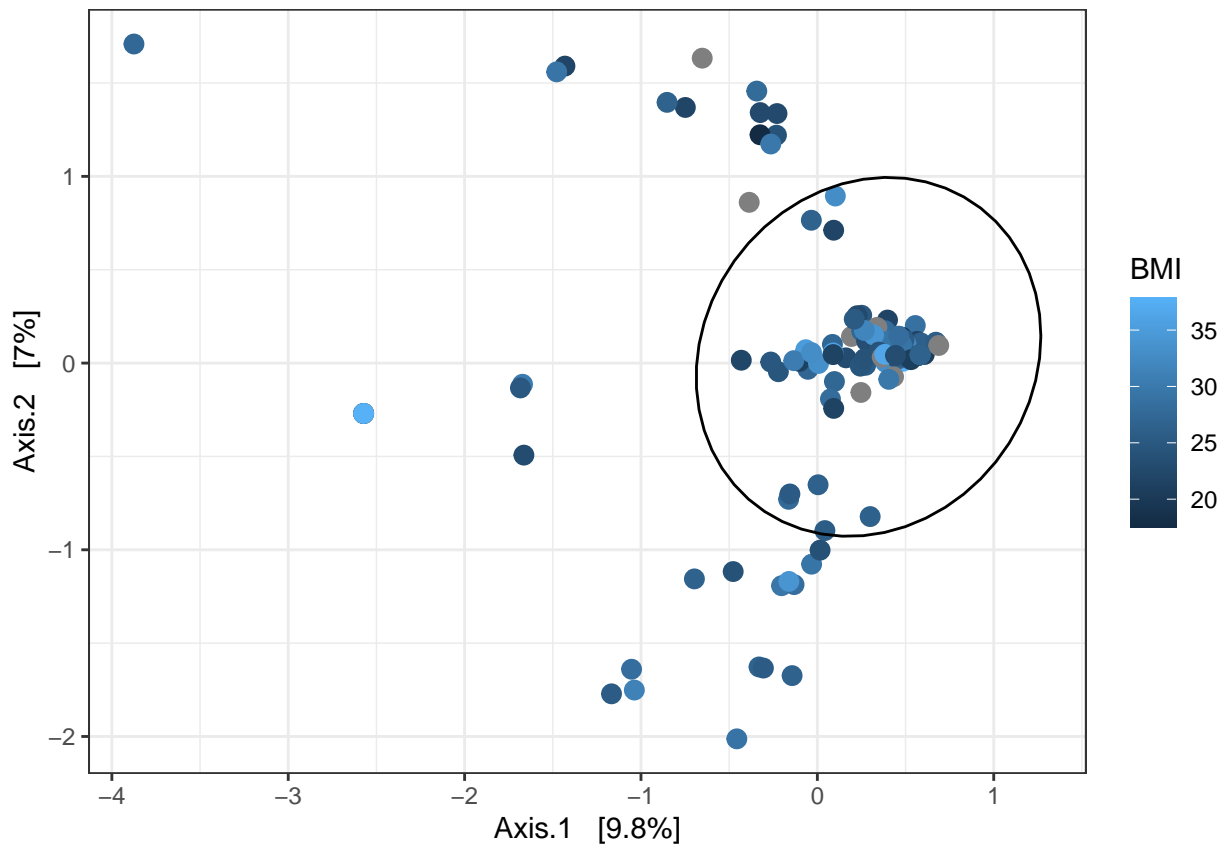
## Permutation test for adonis under reduced model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.eucl_dist ~ sample_data(physeqfinal.2.clr)$BMI, permutations = 10000)
##               Df SumOfSqs      R2      F Pr(>F)
## sample_data(physeqfinal.2.clr)$BMI  1      6.14 0.00752 0.9173 0.5916

```

```
## Residual          121    810.08 0.99248
## Total             122    816.22 1.00000
```

Principal Coordinates Analysis (PCoA) for BMI

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
                    distance = "euclidean", binary = FALSE, 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

```
set.seed(1337)
adonis.Res.clr <- adonis2(physeqfinal.2.clr.eucl_dist ~
  sample_data(physeqfinal.2.clr)$age_at_stool_collection,
  by = "margin",
  perm = 10000,
  na.action = na.exclude,
  parallel = 10)

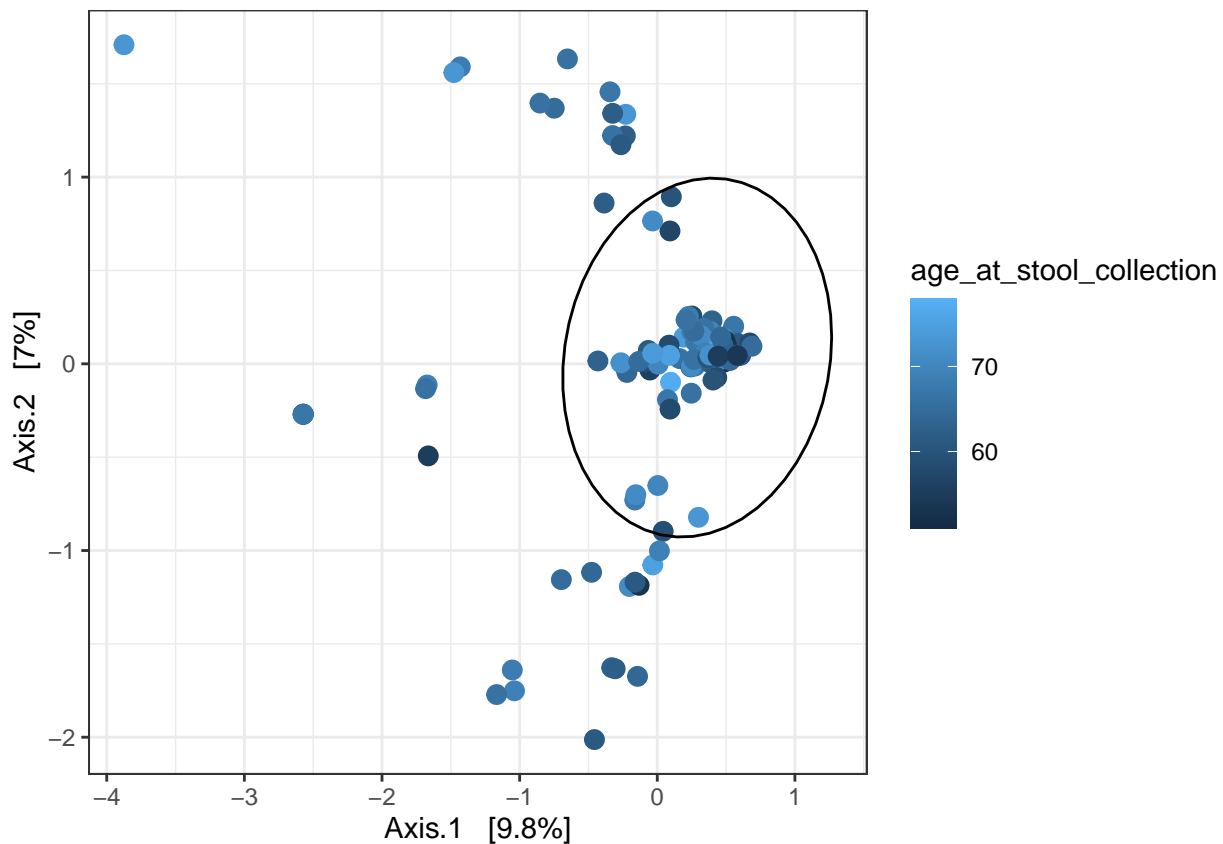
adonis.Res.clr
```

```
## Permutation test for adonis under reduced model
## Marginal effects of terms
```

```
## Permutation: free
## Number of permutations: 10000
##
## adonis2(formula = physeqfinal.2.clr.eucl_dist ~ sample_data(physeqfinal.2.clr)$age_at_stool_collection,
##
##           Df SumOfSqs      R2
## sample_data(physeqfinal.2.clr)$age_at_stool_collection 1      7.06 0.00806
## Residual      134    869.02 0.99194
## Total        135    876.08 1.00000
##
##           F Pr(>F)
## sample_data(physeqfinal.2.clr)$age_at_stool_collection 1.0893 0.3113
## Residual
## Total
```

Principal Coordinates Analysis (PCoA) for Age

```
ord_clr <- ordinate(physeqfinal.2.clr, method = "PCoA",
                    distance = "euclidean", binary = FALSE, 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/libopenblas-p-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
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## [13] htmttools_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	