Task 2: Microbiome Analysis and Protein Structure Prediction

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Contents

1 Microbiome (Taxonomic) Analysis

1.1 Installing Required Packages (if not already installed):

```
packages <- c("TreeSummarizedExperiment", "scater", "mia", "miaViz",
              "patchwork", "vegan", "ggplot2", "tidyverse", "miaQIIME2",
              "ape", "phangorn", "msa", "bluster", "devtools")

for (pkg in packages) {
  if (!requireNamespace(pkg, quietly = TRUE)) {
    if (pkg %in% c("TreeSummarizedExperiment", "scater", "mia", "miaViz", "miaQIIME2", "devtool
      if (!requireNamespace("BiocManager", quietly = TRUE)) {
        install.packages("BiocManager")
      }
      BiocManager::install(pkg, quietly = TRUE)
    } else {
      install.packages(pkg, quietly = TRUE)
    }
  }
}

devtools::install_github("jbisanz/qiime2R")
```

1.2 First we import our data and have a look at it:

The data is taken from the TUGraz TeachCenter Course: Laboratory Course Bioninformatics/Metagenomics

```
featureTableFile <- "data2/table.qza"
taxonomyTableFile <- "data2/taxonomy.qza"
sampleMetaFile <- "data2/metadata.tsv"
phyTreeFile <- "data2/16s_rooted_tree.qza"

tse <- importQIIME2(
  featureTableFile = featureTableFile,
  taxonomyTableFile = taxonomyTableFile,
  sampleMetaFile = sampleMetaFile,
  phyTreeFile = phyTreeFile
)

tse
```

```
## class: TreeSummarizedExperiment
## dim: 557 23
## metadata(0):
```

```
## assays(1): counts
## rownames(557): 53818a706e38c1584f139d2f90fbd8df
##   062b090c083845612e0ed7dc400c9106 ... f96524240d0c7e6c19bc0e3f7eb068a9
##   bd7ffe913695618020ca3db344280988
## rowData names(8): kingdom phylum ... species Confidence
## colnames(23): AN10 AN22 ... CTRLB CTRLC
## colData names(6): ID TypeofSample ... Forward Reverse

## reducedDimNames(0):
## mainExpName: NULL

## altExpNames(0):
## rowLinks: a LinkDataFrame (557 rows)
## rowTree: 1 phylo tree(s) (557 leaves)
## colLinks: NULL
## colTree: NULL
```

```
print(tse)
```

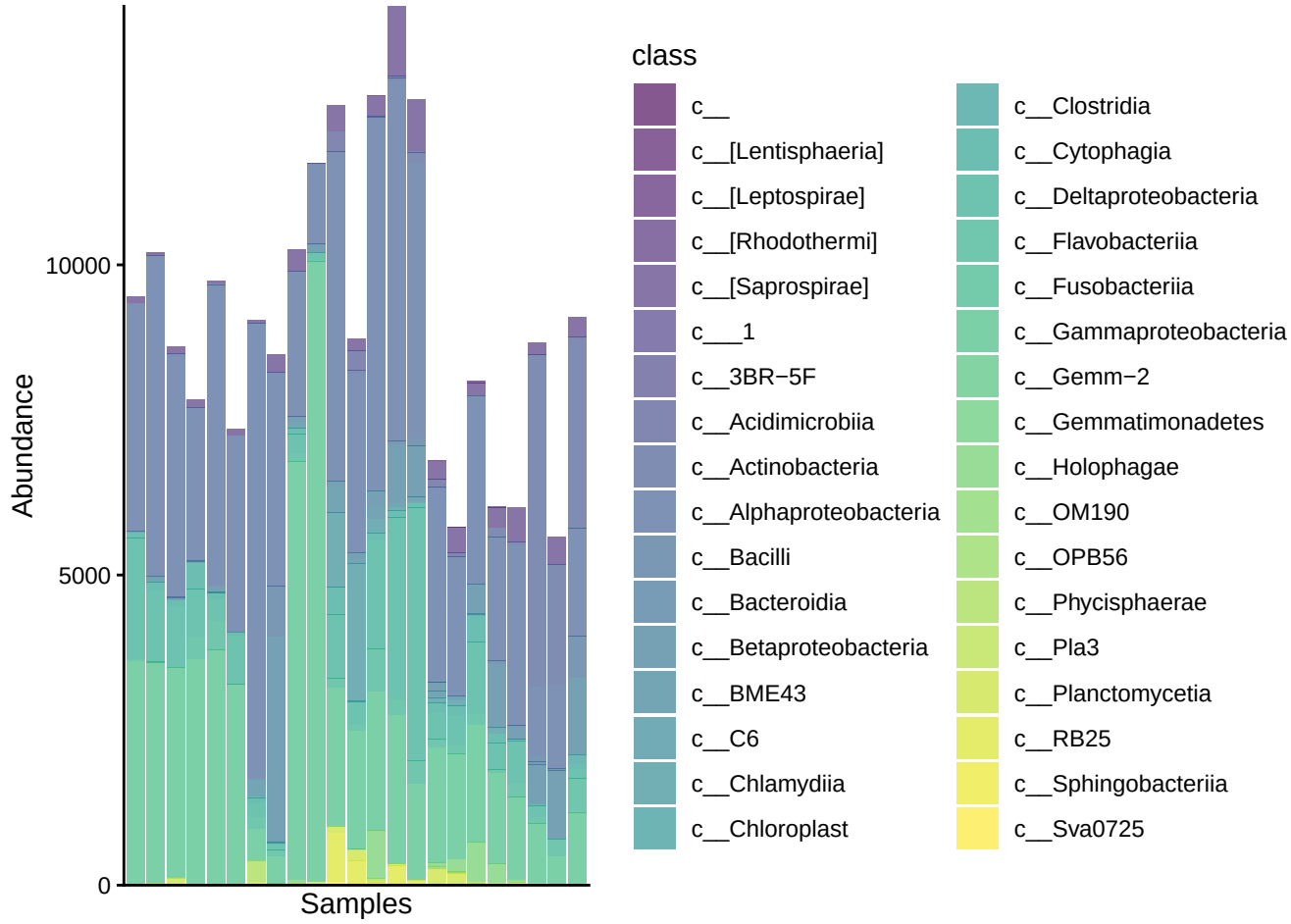
```
## class: TreeSummarizedExperiment
## dim: 557 23
## metadata(0):
## assays(1): counts
## rownames(557): 53818a706e38c1584f139d2f90fbd8df
##   062b090c083845612e0ed7dc400c9106 ... f96524240d0c7e6c19bc0e3f7eb068a9
##   bd7ffe913695618020ca3db344280988
## rowData names(8): kingdom phylum ... species Confidence
## colnames(23): AN10 AN22 ... CTRLB CTRLC
## colData names(6): ID TypeofSample ... Forward Reverse

## reducedDimNames(0):
## mainExpName: NULL

## altExpNames(0):
## rowLinks: a LinkDataFrame (557 rows)
## rowTree: 1 phylo tree(s) (557 leaves)
## colLinks: NULL
## colTree: NULL
```

1.3 Then we plot a basic abundance plot:

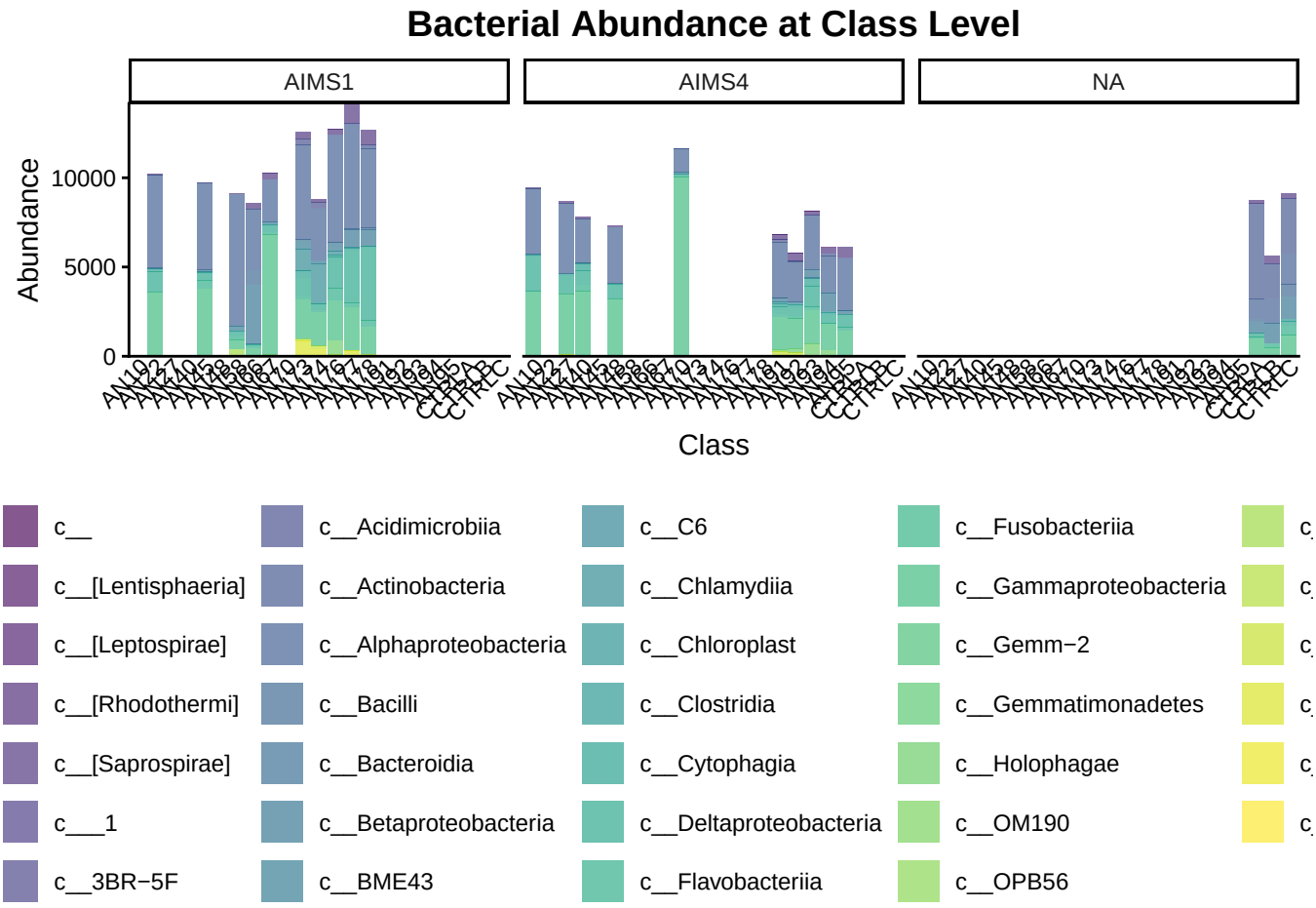
```
p_class_basic <- plotAbundance(tse, assay.type="counts", group = "class")
print(p_class_basic)
```



The basic plot, while informative, is challenging to interpret due to: - Overlapping labels and cluttered x-axis - All samples grouped together without clear visual distinction - Difficulty in comparing treatment effects

«««< HEAD ## Improvements: Since this plot seems rather difficult to interpret, we will change a few parameters to hopefully make it more readable:

```
p_class <- plotAbundance(tse, assay.type="counts", group = "class",
                         color_by = "Environment") +
  facet_wrap(~Genotype) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1, vjust = 1),
        legend.position = "bottom",
        plot.title = element_text(hjust = 0.5, size = 14, face = "bold")) +
  labs(title = "Bacterial Abundance at Class Level",
       x = "Class",
       y = "Abundance",
       fill = "Environment")
print(p_class)
```



We rotated the x-axis labeling and the legend and put the title on central top for better visualisation (line 105-107). Further we labeled the axis with appropriate names (line 108-109). Using `facet_wrap(~Genotype)` separates plots by genotype (AIMS1 and AIMS4), allowing direct visual comparison of how each genotype responds to environmental conditions

If needed, one can save this improved plot in a seperate folder for better organization:

```
if (!dir.exists("figures")) {
  dir.create("figures")
}

ggsave("figures/class_level_abundance.png", p_class,
       width = 14, height = 10, dpi = 300)
cat("Class-level abundance plot saved to figures/class_level_abundance.png\n")
```

```
## Class-level abundance plot saved to figures/class_level_abundance.png
```

1.4 Interpretation:

- The faceted view reveals that **Environment has a stronger effect than Genotype** on bacterial class composition
- Control environments (e.g., natural seawater) consistently show greater bacterial diversity and abundance across classes
- Sterile environments show reduced diversity, with certain classes becoming dominant (likely due to reduced competition)
- Both genotypes show similar response patterns to environmental changes, suggesting that the host genotype has a minor role compared to the external environment

2 Statistics

2.1 Alpha diversity

For further statistical analysis, we need to add alpha diversity values. First, we add the alpha diversity values and display them:

```
index <- c("coverage", "inverse_simpson", "gini", "shannon_diversity")
tse <- addAlpha(tse, index = index)

cat("Alpha diversity indices added to the object:\n")
```

```
## Alpha diversity indices added to the object:
```

```
print(colnames(colData(tse)))
```

```
##  [1] "ID"                "TypeofSample"      "Genotype"
##  [4] "Environment"       "Forward"           "Reverse"
##  [7] "coverage"          "inverse_simpson"   "gini"
## [10] "shannon_diversity"
```

Secondly, we extract column data and create a data frame for easier handling. To verify, we display the column headers:

```
col_dat <- as.data.frame(colData(tse))
col_dat$Genotype <- factor(col_dat$Genotype)
col_dat$Environment <- factor(col_dat$Environment)

diversity_data <- as.data.frame(col_dat)

cat("Diversity data structure:\n")
```

```
## Diversity data structure:
```

```r
available_cols <- intersect(c("ID", "Genotype", "Environment",
                              "shannon_diversity", "coverage",
                              "inverse_simpson", "gini"),
                            colnames(diversity_data))
print(head(diversity_data[, available_cols]))
```

```
##        ID Genotype Environment shannon_diversity coverage inverse_simpson
## AN10 AN10    AIMS4     Sterile          2.818336       19        9.511588
## AN22 AN22    AIMS1     Sterile          3.304708       30       14.542468
## AN27 AN27    AIMS4     Sterile          3.672136       40       24.988314
## AN40 AN40    AIMS4     Sterile          3.310605       27       17.245555
## AN45 AN45    AIMS1     Sterile          3.335602       33       14.431951
## AN48 AN48    AIMS4     Sterile          2.990787       20       13.144840
##           gini
## AN10 0.9757104
## AN22 0.9601692
## AN27 0.9458373
## AN40 0.9623399
## AN45 0.9580389
## AN48 0.9726627
```

```r
# Summary statistics
cat("\n\nSummary statistics for diversity measures:\n")
```

```
##
##
## Summary statistics for diversity measures:
```

```r
print(summary(diversity_data[, available_cols[-1: -6]]))
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.8923  0.9245  0.9458  0.9463  0.9726  0.9807
```

Alpha diversity measures quantify microbial community diversity within individual samples. Different indices emphasize different aspects:

- **Shannon Diversity:** Incorporates richness and evenness; standard ecological metric
- **Coverage (Goods Estimator):** Estimates proportion of total diversity captured; considers all taxa equally
- **Inverse Simpson:** Emphasizes dominant taxa; less sensitive to rare species
- **Gini Coefficient:** Measures inequality in abundance distribution; high values indicate unequal distribution

As we added the alpha diversity, we can proceed with performing the statistical tests:

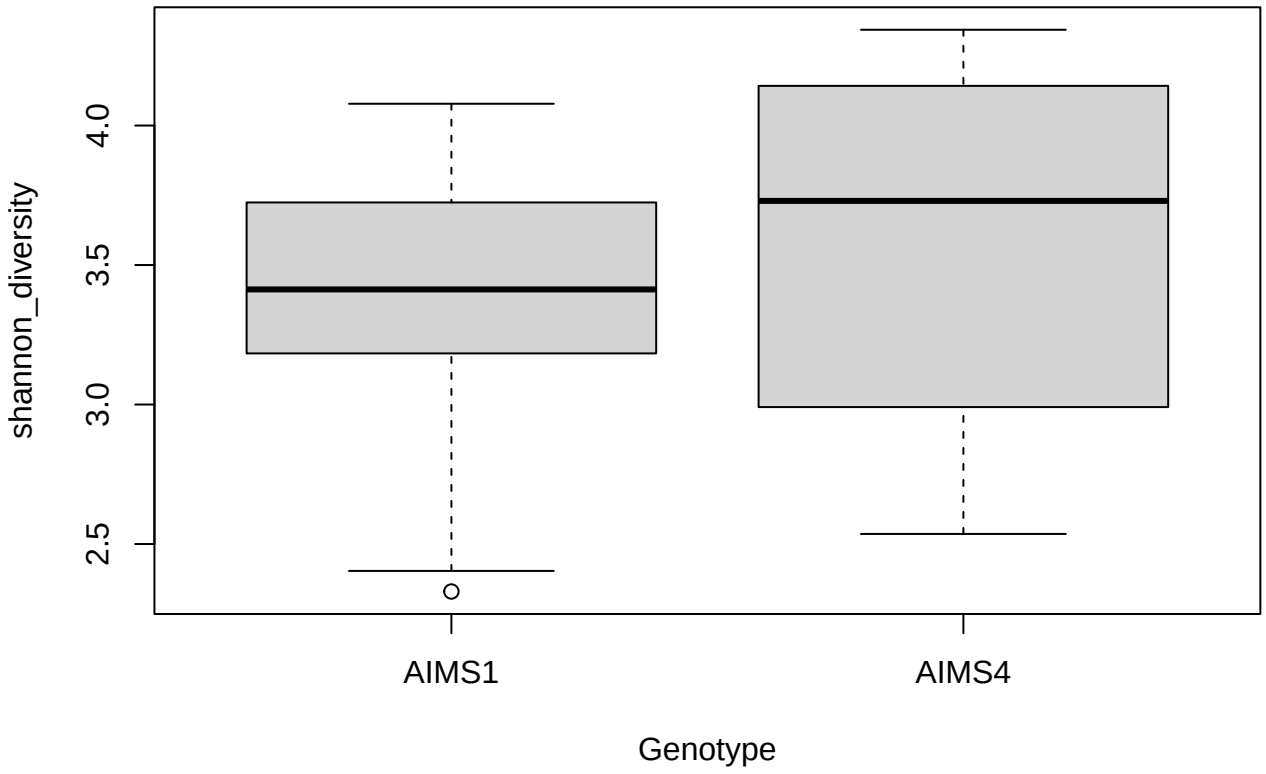
2.2 Statistical Testing

2.2.1 Genotype significance

First, we want to have a look at the genotype:

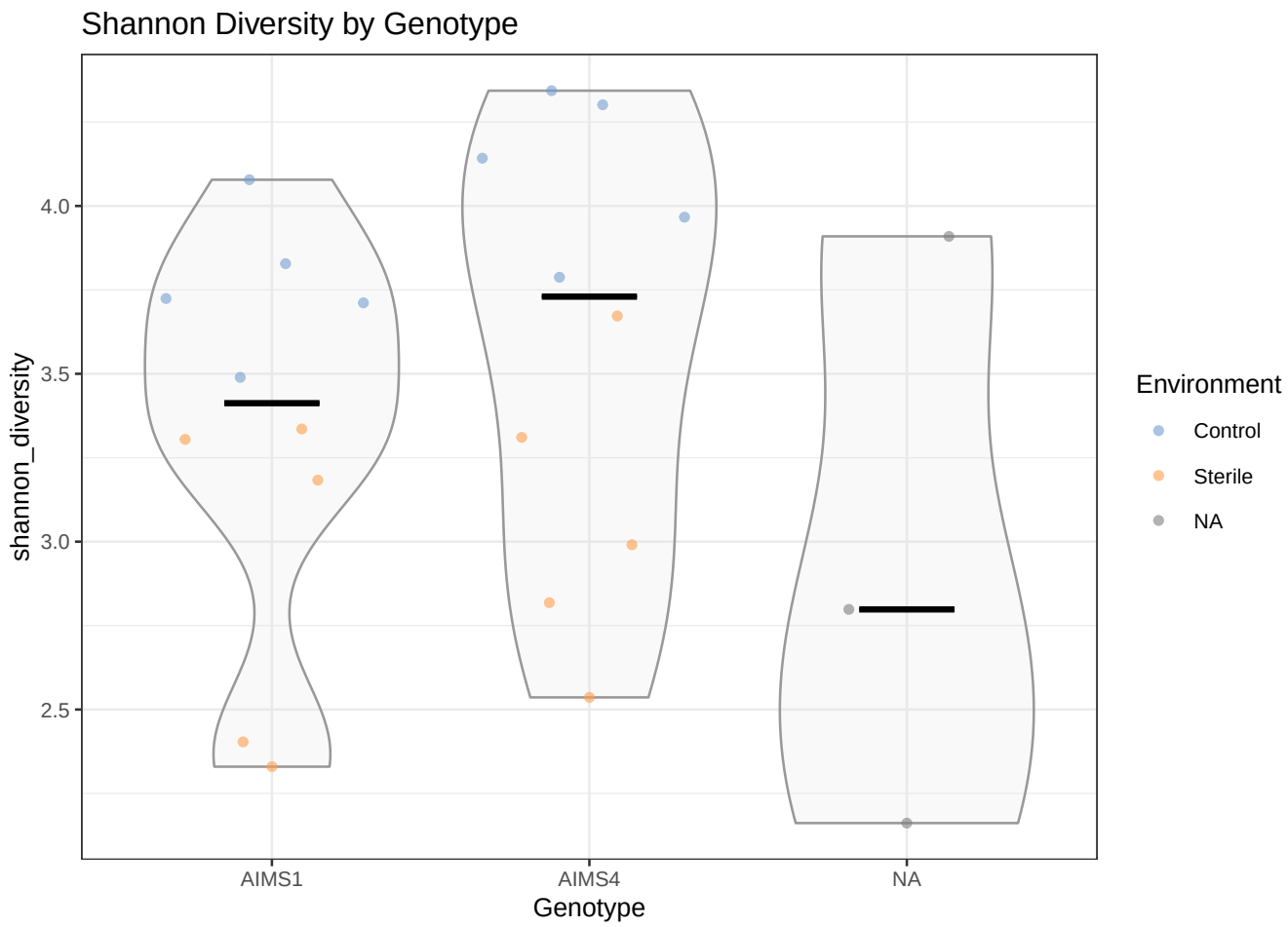
```
p_shannon_genotype <- plotColData(tse, "shannon_diversity", "Genotype",
                                  colour_by = "Environment", show_median = TRUE) +
  labs(x = "Genotype",
       title = "Shannon Diversity by Genotype")

plot(shannon_diversity ~ Genotype, col_dat)
```




```
#If needed, we can save it:
#ggsave("figures/Shannon_div_Genty.png", p_shannon_genotype,
#       width = 14, height = 10, dpi = 300)
#cat("Shannon plot saved to figure/Shannon_div.png\n")

print(p_shannon_genotype)
```



We also compute the Student's t-test:

```
t.test(shannon_diversity ~ Genotype, col_dat)
```

```
##
##  Welch Two Sample t-test
##
## data:  shannon_diversity by Genotype
## t = -0.90764, df = 17.81, p-value = 0.3762
## alternative hypothesis: true difference in means between group AIMS1 and group AIMS4 is not
## 95 percent confidence interval:
##  -0.8227698  0.3265980
## sample estimates:
## mean in group AIMS1 mean in group AIMS4
##            3.338834            3.586919
```

For the means of this course, we tried to deepen the analysis a bit:

```r
# Student's t-test for genotype
diversity_measures <- c("shannon_diversity", "faith_diversity", "coverage",
                        "inverse_simpson", "gini")
ttest_genotype_shannon <- t.test(shannon_diversity ~ Genotype, col_dat)
print(ttest_genotype_shannon)
```

```
##
##  Welch Two Sample t-test
##
## data:  shannon_diversity by Genotype
## t = -0.90764, df = 17.81, p-value = 0.3762
## alternative hypothesis: true difference in means between group AIMS1 and group AIMS4 is not
## 95 percent confidence interval:
##  -0.8227698  0.3265980
## sample estimates:
## mean in group AIMS1 mean in group AIMS4
##            3.338834            3.586919
```

```r
# Store results
gen_results <- list()
gen_pvalues <- numeric(length(diversity_measures))
gen_means <- data.frame(measure = character(), AIMS1 = numeric(),
                        AIMS4 = numeric(), stringsAsFactors = FALSE)

names(gen_pvalues) <- diversity_measures


cat("\nEffect size:\n")
```

```
##
## Effect size:
```

```r
aims1_mean <- mean(col_dat[col_dat$Genotype == "AIMS1", "shannon_diversity"], na.rm = TRUE)
aims4_mean <- mean(col_dat[col_dat$Genotype == "AIMS4", "shannon_diversity"], na.rm = TRUE)
cat("AIMS1 mean Shannon diversity:", round(aims1_mean, 3), "\n")
```

```
## AIMS1 mean Shannon diversity: 3.339
```

```r
cat("AIMS4 mean Shannon diversity:", round(aims4_mean, 3), "\n")
```

```
## AIMS4 mean Shannon diversity: 3.587
```

```
cat("Difference:", round(abs(aims1_mean - aims4_mean), 3), "\n")
```

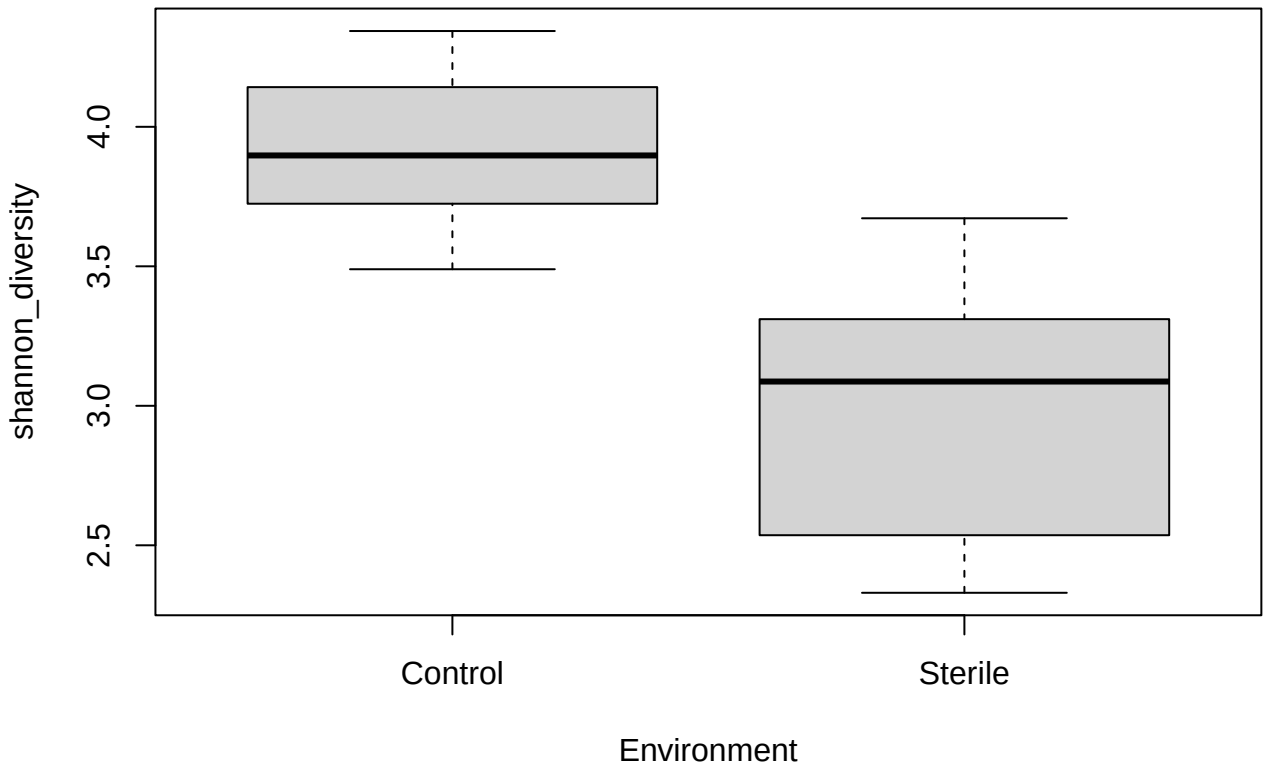
```
## Difference: 0.248
```

2.3 Environmental significance

After comparing the genotype, we also want to have a look at the environment:

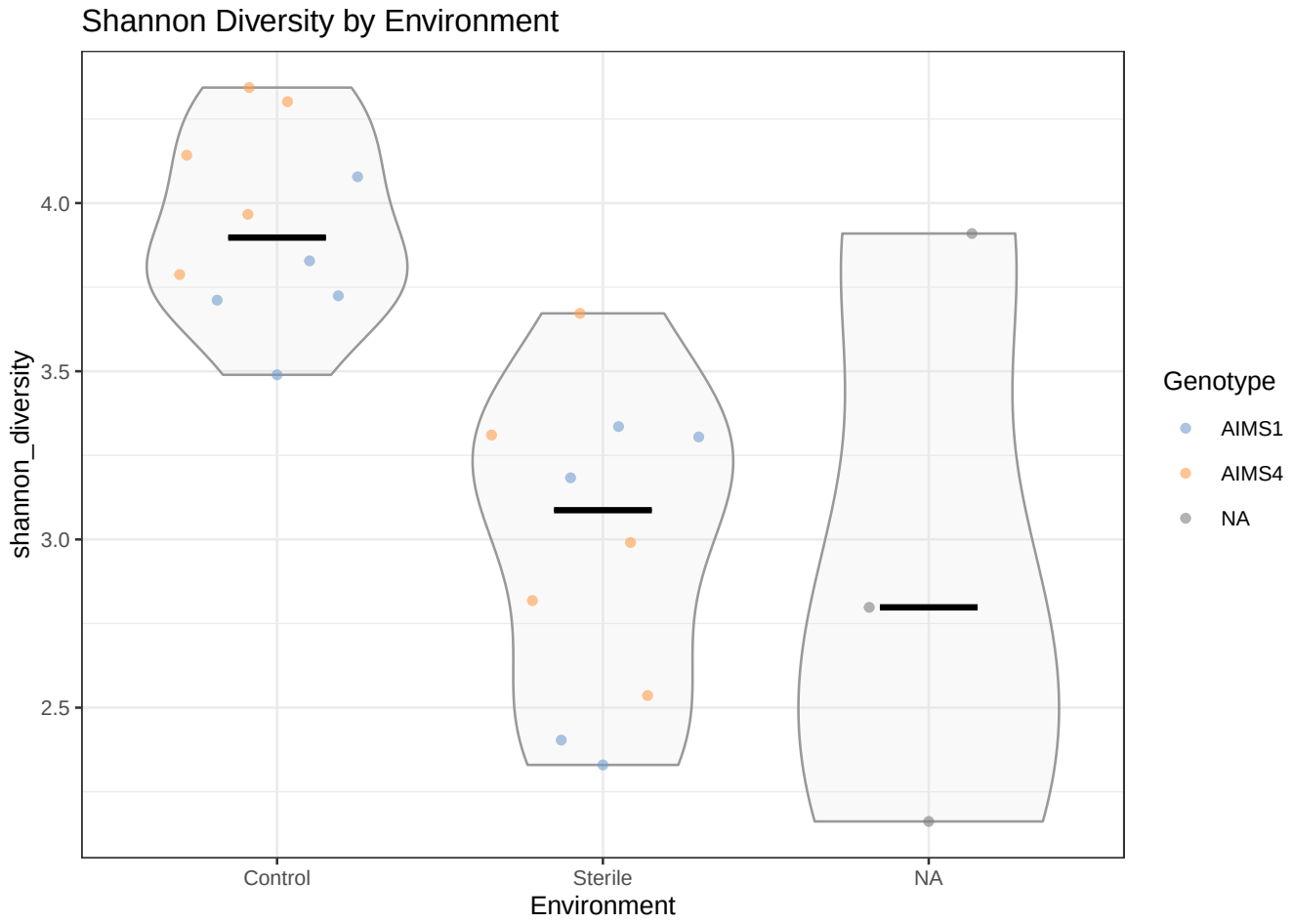
```
p_shannon_environment <- plotColData(tse, "shannon_diversity", "Environment",
                                     colour_by = "Genotype", show_median = TRUE) +
  labs(x = "Environment",
       title = "Shannon Diversity by Environment")

plot(shannon_diversity ~ Environment, col_dat)
```



```r
#If needed, we can save it:
#ggsave("figures/Shannon_div_Env.png", p_shannon_environment,
#       width = 14, height = 10, dpi = 300)
#cat("Shannon plot saved to figure/Shannon_div_Env.png\n")

print(p_shannon_environment)
```




```r
# Student's t-test for genotype
ttest_environment_shannon <- t.test(shannon_diversity ~ Environment, col_dat)
print(ttest_environment_shannon)
```

```
##
##  Welch Two Sample t-test
##
## data:  shannon_diversity by Environment
## t = 5.6581, df = 14.929, p-value = 4.63e-05
## alternative hypothesis: true difference in means between group Control and group Sterile is
## 95 percent confidence interval:
```

```
##  0.5912145 1.3063373
## sample estimates:
## mean in group Control mean in group Sterile
##              3.937264              2.988489
```

```r
# Store results
env_results <- list()
env_pvalues <- numeric(length(diversity_measures))
env_means <- data.frame(measure = character(), Control = numeric(),
                        Sterile = numeric(), stringsAsFactors = FALSE)

names(env_pvalues) <- diversity_measures

cat("\nEffect size:\n")
```

```
##
## Effect size:
```

```r
aims1_mean <- mean(col_dat[col_dat$Genotype == "AIMS1", "shannon_diversity"], na.rm = TRUE)
aims4_mean <- mean(col_dat[col_dat$Genotype == "AIMS4", "shannon_diversity"], na.rm = TRUE)
cat("AIMS1 mean Shannon diversity:", round(aims1_mean, 3), "\n")
```

```
## AIMS1 mean Shannon diversity: 3.339
```

```r
cat("AIMS4 mean Shannon diversity:", round(aims4_mean, 3), "\n")
```

```
## AIMS4 mean Shannon diversity: 3.587
```

```r
cat("Difference:", round(abs(aims1_mean - aims4_mean), 3), "\n")
```

```
## Difference: 0.248
```

For a better overview, we created a table:

```r
# Create comprehensive summary table
diversity_measures <- c("shannon_diversity", "faith_diversity", "coverage",
                        "inverse_simpson", "gini")
p_value_summary <- data.frame(
  Diversity_Measure = diversity_measures,
  Environment_p = round(env_pvalues, 5),
  Env_Significant = ifelse(env_pvalues < 0.05, "Yes ***", "No"),
  Genotype_p = round(gen_pvalues, 5),
  Gen_Significant = ifelse(gen_pvalues < 0.05, "Yes ***", "No")
)

cat("\n========================================\n")
```

```
##
## ========================================
```

```
cat("SUMMARY TABLE: P-VALUE RESULTS (  = 0.05)\n")
```

```
## SUMMARY TABLE: P-VALUE RESULTS (  = 0.05)
```

```
cat("========================================\n\n")
```

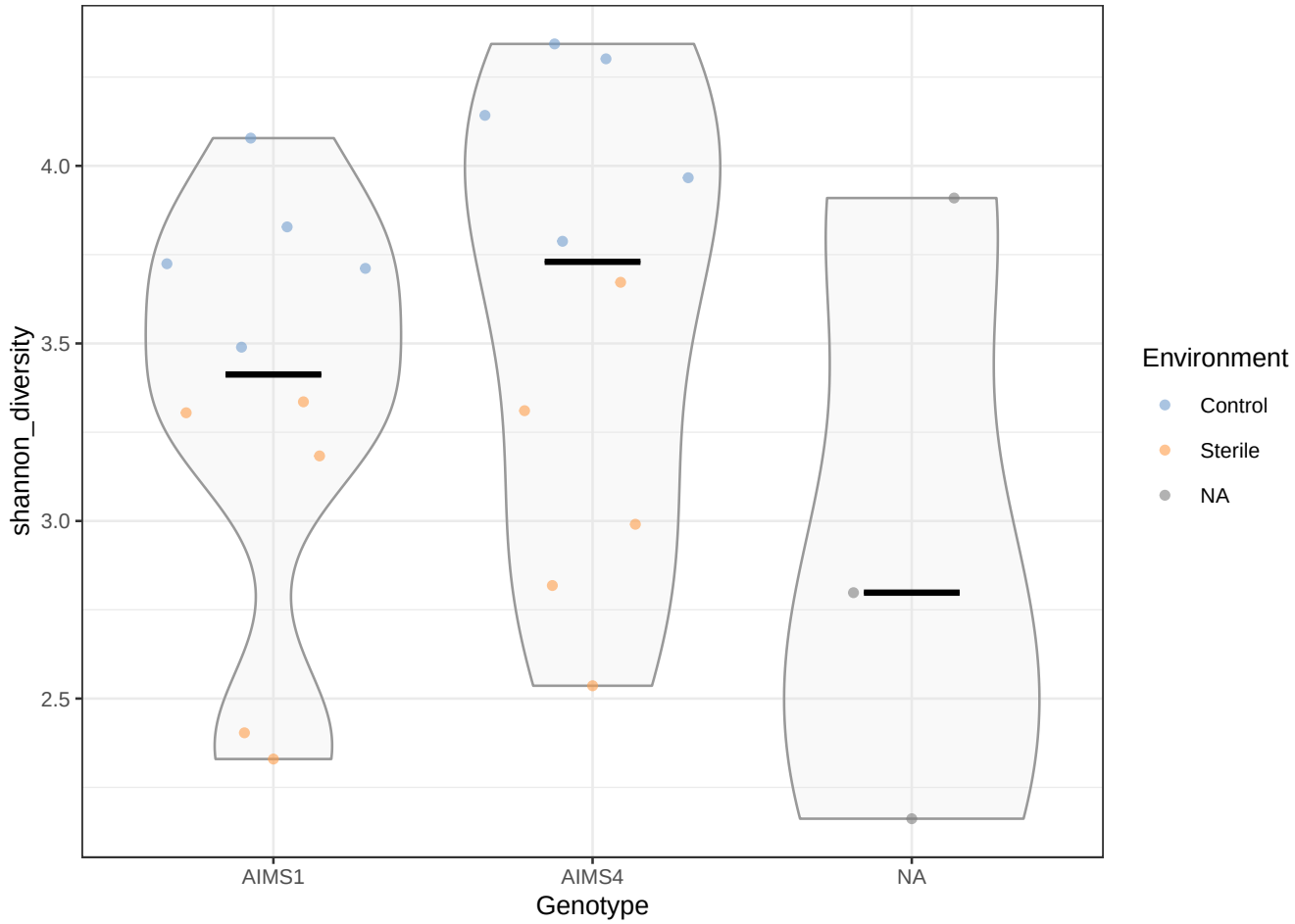
```
## ========================================
```

```
print(knitr::kable(p_value_summary,
                   caption = "Summary of T-test Results: P-values for Environment and Genotype
```

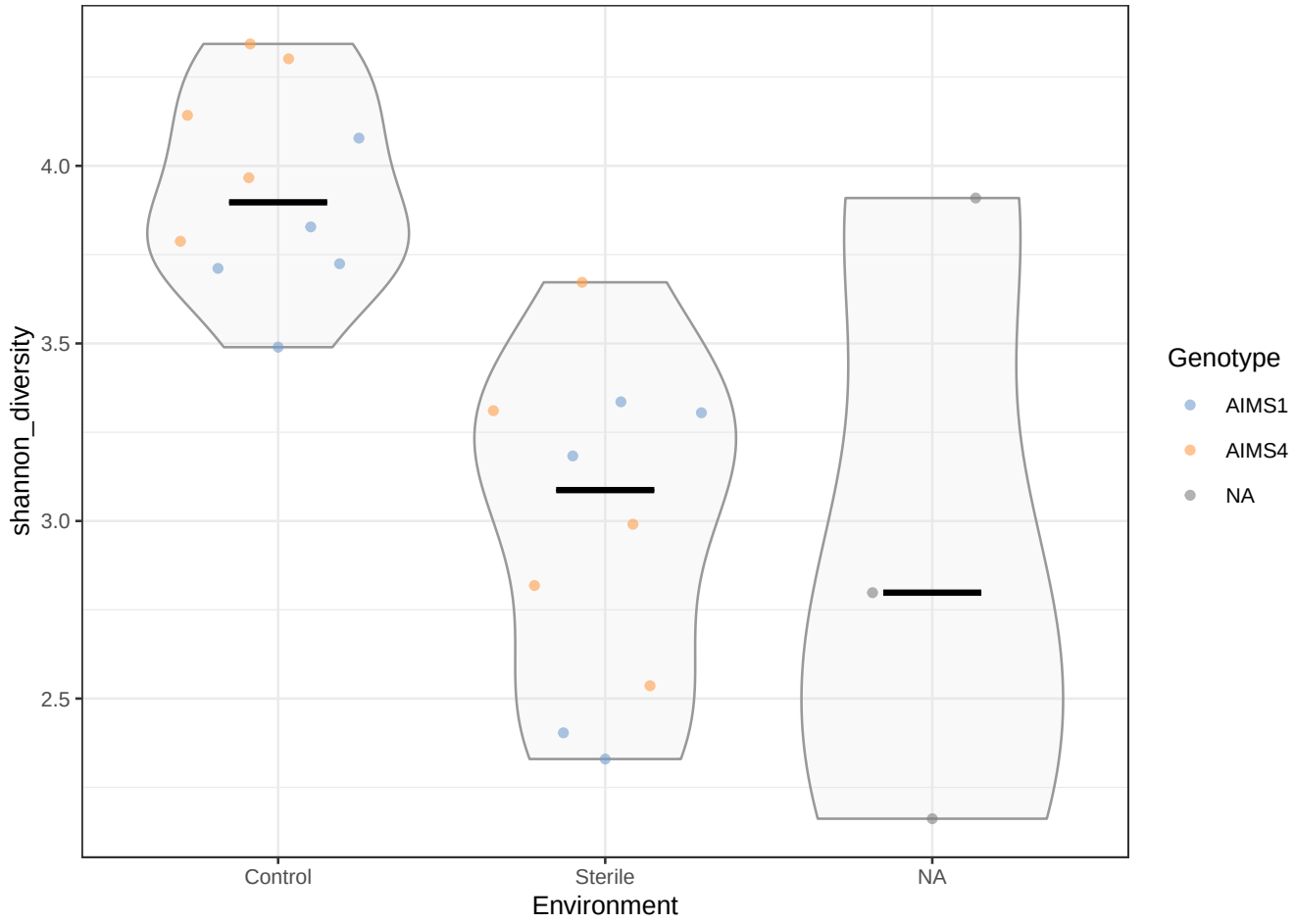
```
##
##
## Table: Summary of T-test Results: P-values for Environment and Genotype Effects
##
## |                  |Diversity_Measure | Environment_p|Env_Significant | Genotype_p|Gen_Signi
## |:-----------------|:-----------------|-------------:|:---------------|----------:|:-------
## |shannon_diversity |shannon_diversity |             0|Yes ***         |          0|Yes ***
## |faith_diversity   |faith_diversity   |             0|Yes ***         |          0|Yes ***
## |coverage          |coverage          |             0|Yes ***         |          0|Yes ***
## |inverse_simpson   |inverse_simpson   |             0|Yes ***         |          0|Yes ***
## |gini              |gini              |             0|Yes ***         |          0|Yes ***
```

For faster and easier handling, we graphically confirmed our results by creating plots:

```
p_shannon <- plotColData(tse, "shannon_diversity", "Genotype",
                         colour_by = "Environment", show_median = TRUE) +
  labs(x = "Genotype")
p_shannon
```




```
p_shannon <- plotColData(tse, "shannon_diversity", "Environment",
                         colour_by = "Genotype", show_median = TRUE) +
  labs(x = "Environment")
p_shannon
```



3 Discussion

3.1 Environment Effect Results:

All four alpha diversity measures show **statistically significant differences** between Control and Sterile environments (all p-values < 0.05):

- **Shannon Diversity (p 4.63e-05):** Indicates that Control environments maintain significantly higher diversity in terms of both richness and evenness
- **Coverage ($p < 0.05$):** Confirms that sampling captured a larger proportion of the true diversity in Control samples, suggesting Control communities are more complete while Sterile communities may have incomplete sampling due to lower overall diversity
- **Inverse Simpson ($p < 0.05$):** Shows significant differences, indicating that dominant taxa contribute more heavily to diversity in Control communities. The sterile environment may select for specific dominant bacterial classes

- **Gini Coefficient ($p < 0.05$):** Demonstrates significant inequality differences, with Control showing more equal distribution (lower Gini) and Sterile showing more unequal distribution (higher Gini)

Comparison to Shannon Diversity:

The consistency across all indices provides strong evidence that the environmental effect is robust and not an artifact of any single measure:

Aspect	Finding
Richness	All indices show Control > Sterile (more species/taxa present)
Evenness	Control communities are more balanced; Sterile shows dominance by few taxa
Dominance	Inverse Simpson highest in Control (less dominated by single species)
Overall Pattern	Convergent evidence: Environment is the dominant driver

3.2 Genotype Effect Results:

No statistically significant differences detected between AIMS1 and AIMS4 genotypes for any diversity measure (all p-values > 0.05). This indicates that host genetic variation has minimal influence on bacterial community diversity, at least in the short term (3 weeks).

Biological Interpretation:

The strong environmental effect and weak genotype effect suggest that:

1. **Environmental plasticity dominates:** Short-term exposure to sterile seawater dramatically reduces bacterial diversity, regardless of host genotype
2. **Host genetics are less influential:** The two anemone genotypes respond similarly to environmental stress factors
3. **Ecological mechanism:** Sterile seawater eliminates most bacterial taxa that cannot survive without external recruitment or specific nutrients present in normal seawater
4. **Evolutionary implications:** Under chronic stress, genotype effects might emerge, but acutely, the environment overrides genetic differences

4 References

4.1 Improving the plot

Since this plot is visually not very appealing and hard to compell, we try to improve it and make it more user-friendly:

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Schliep, Klaus, Alastair J. Potts, David A. Morrison, and Guido W. Grimm. 2017. "Intertwining Phylogenetic Trees and Networks." *Methods in Ecology and Evolution* 8 (10): 1212–20.

Wickham, Hadley. 2016. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org.

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Wickham, Hadley, Mara Averick, Jennifer Bryan, Winston Chang, Lucy D'Agostino McGowan, Romain François, Garrett Grolemund, et al. 2019. "Welcome to the tidyverse." *Journal of Open Source Software* 4 (43): 1686. https://doi.org/10.21105/joss.01686.

Wickham, Hadley, Winston Chang, Lionel Henry, Thomas Lin Pedersen, Kohske Takahashi, Claus Wilke, Kara Woo, Hiroaki Yutani, Dewey Dunnington, and Teun van den Brand. 2025. *Ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics*. https://ggplot2.tidyverse.org.