

Task 2: Microbiome Analysis and Protein Structure Prediction

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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", "devtoo"))
      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

```
## class: TreeSummarizedExperiment
## dim: 557 23
## metadata(0):
## assays(1): counts
## rownames(557): 53818a706e38c1584f139d2f90fb8df
##   062b090c083845612e0ed7dc400c9106 ... f96524240d0c7e6c19bc0e3f7eb068a9
##   bd7ffe913695618020ca3db344280988
## rowData names(8): kingdom phylum ... species Confidence
## colnames(23): AN10 AN22 ... CTRLB CTRLRC
## 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

## class: TreeSummarizedExperiment
## dim: 557 23
## metadata(0):
## assays(1): counts
## rownames(557): 53818a706e38c1584f139d2f90fb8df
##   062b090c083845612e0ed7dc400c9106 ... f96524240d0c7e6c19bc0e3f7eb068a9
##   bd7ffe913695618020ca3db344280988
## rowData names(8): kingdom phylum ... species Confidence
## colnames(23): AN10 AN22 ... CTRLB CTRLRC
## 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

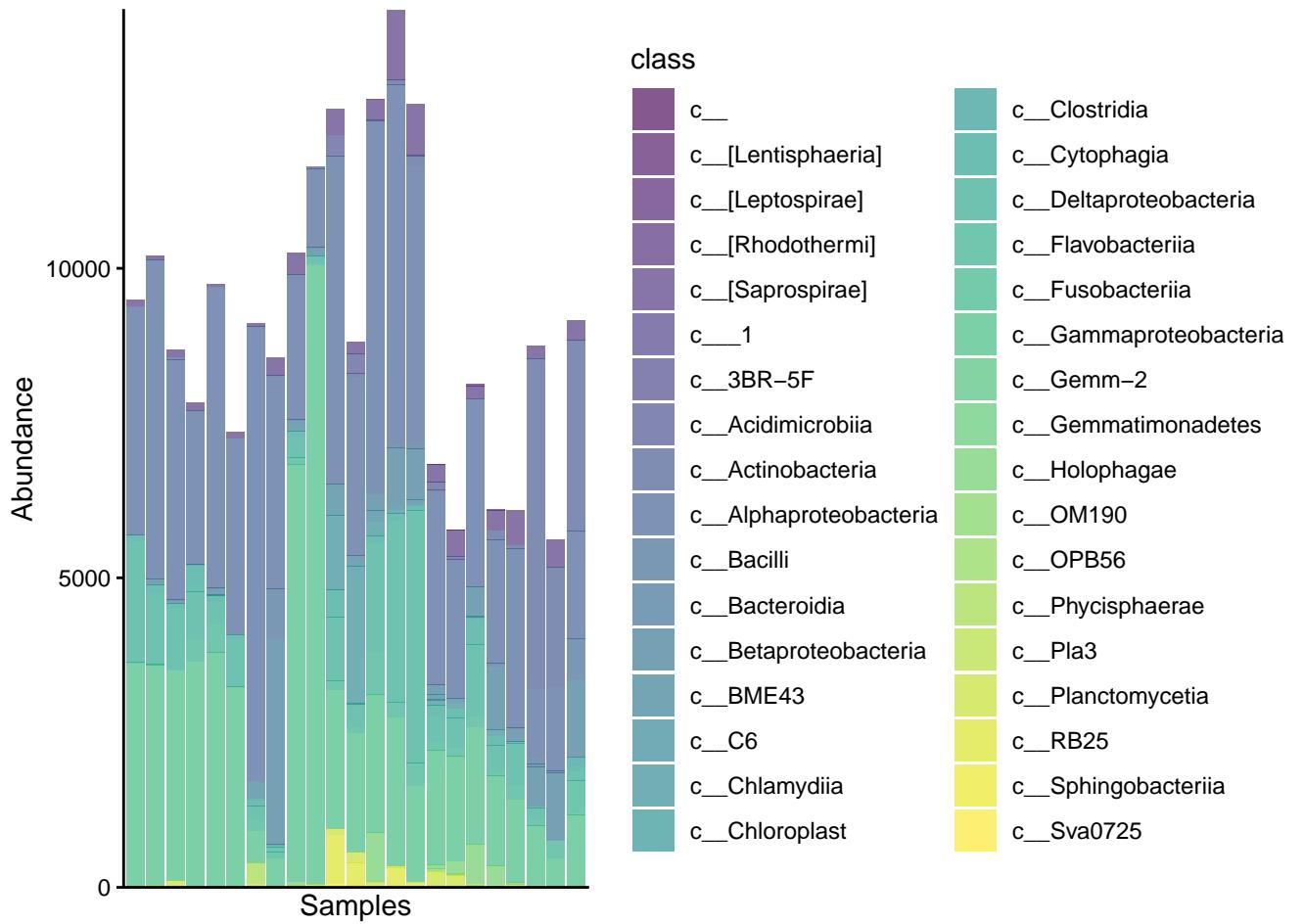
```

1.3 Then we plot a basic abundance plot:

```

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

```



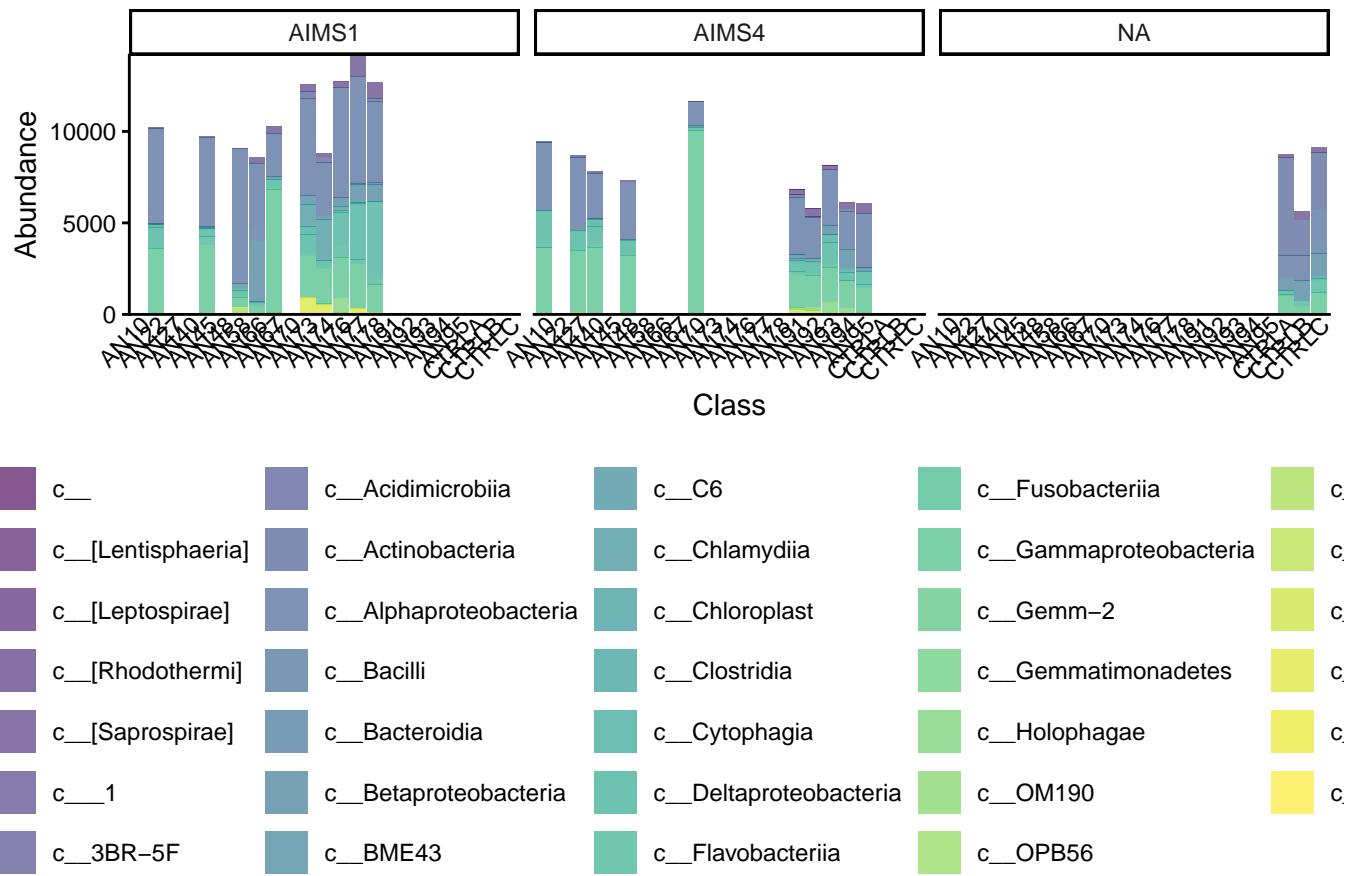
The basic plot, while informative, is challenging to interpret due to:

- 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)
```

Bacterial Abundance at Class Level



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 separate 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:
```

```

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

| | gini |
|------|-----------|
| AN10 | 0.9757104 |
| AN22 | 0.9601692 |
| AN27 | 0.9458373 |
| AN40 | 0.9623399 |
| AN45 | 0.9580389 |
| AN48 | 0.9726627 |

```

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

```

```

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

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:

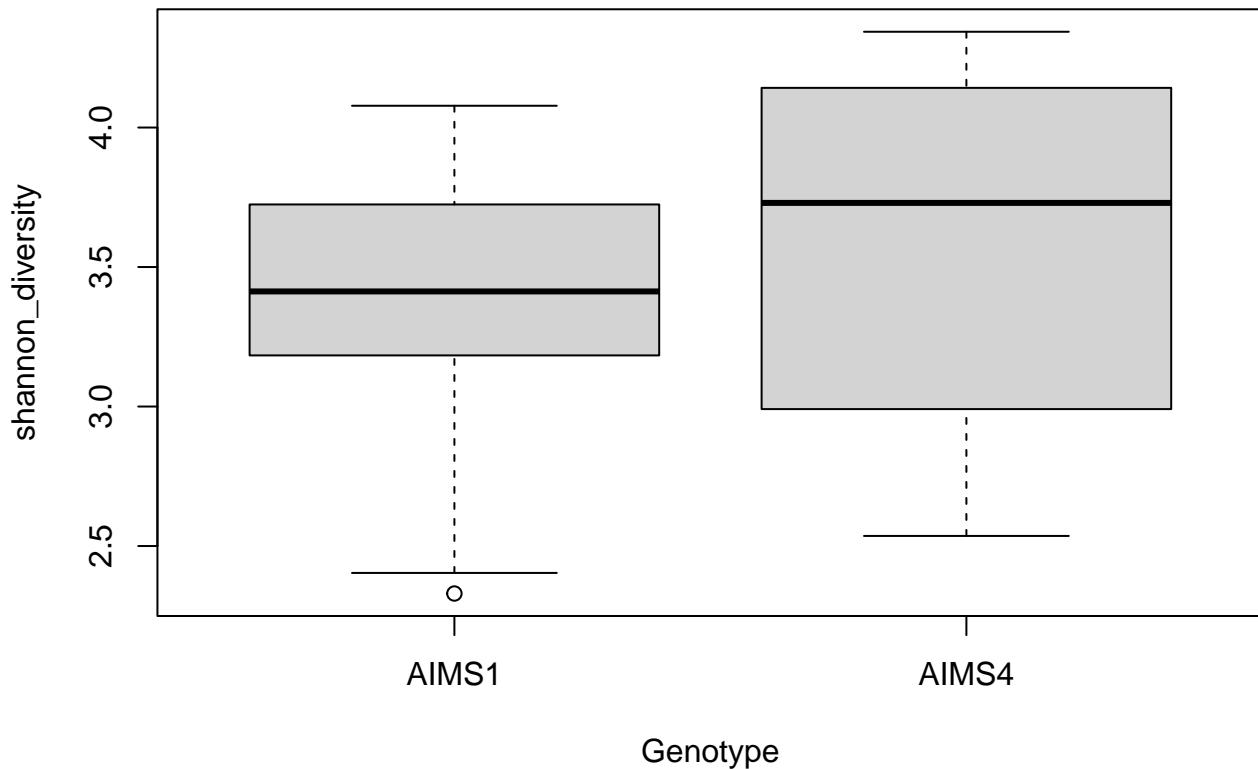
2.2 Statistical Testing

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")

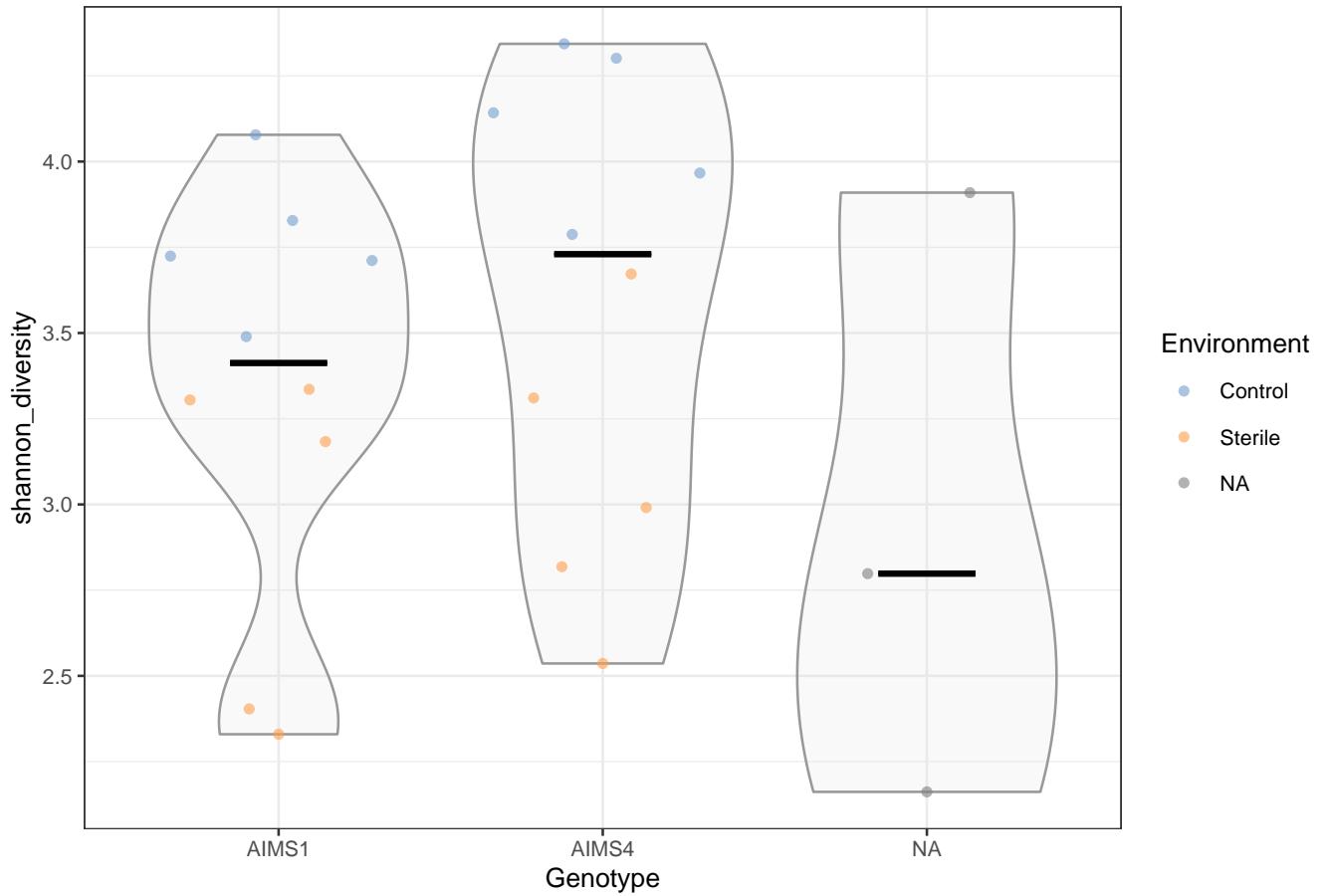
plot(shannon_diversity ~ Genotype, col_dat)
```



```
#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)
```

Shannon Diversity by 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:

```

# 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

# 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:

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

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")
```

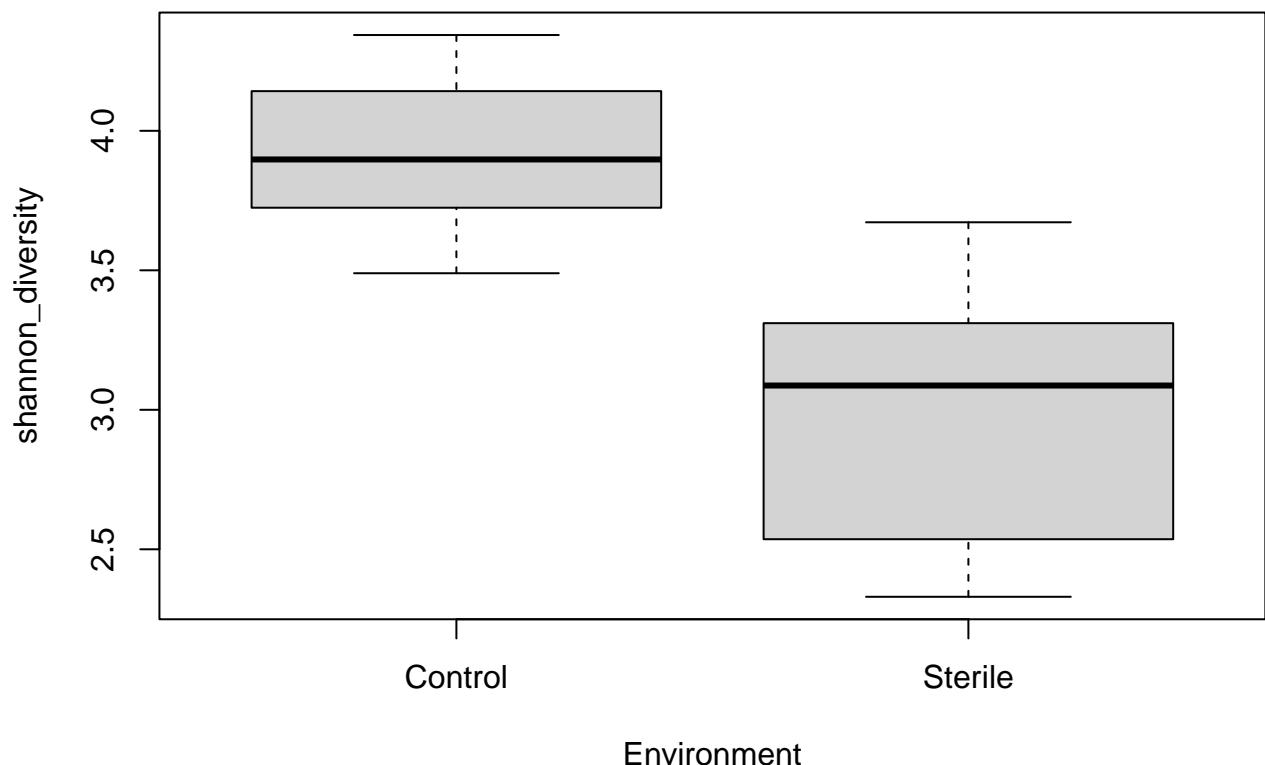
```
## Difference: 0.248
```

2.3 Environmental significance

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

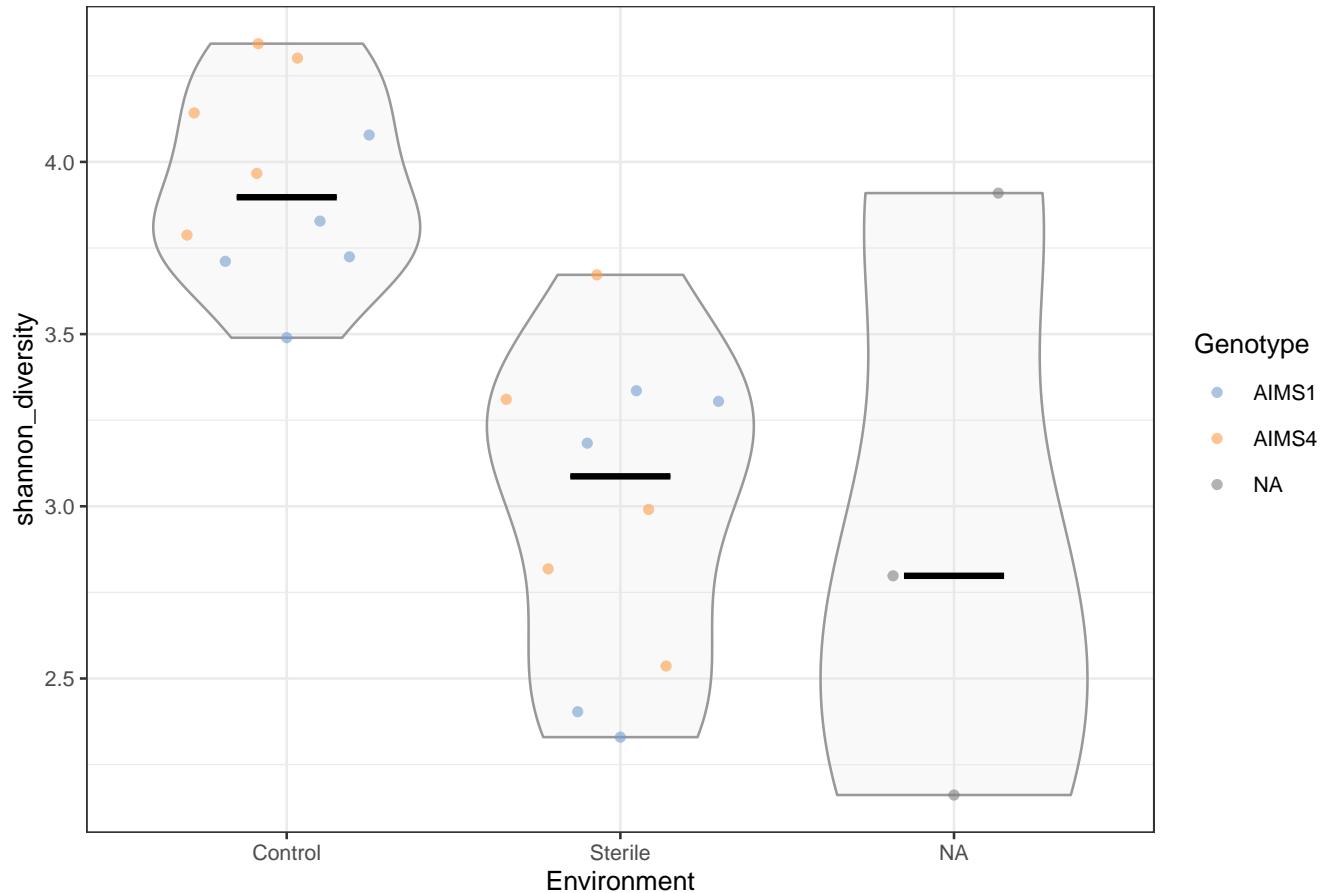
```
p_shannon_environment <- plotColData(tse, "shannon_diversity", "Environment",
                                         colour_by = "Genotype", show_median = TRUE) +
  labs(x = "Environment",
       title = "Shannon Diversity by Environment")

plot(shannon_diversity ~ Environment, col_dat)
```



```
#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)
```

Shannon Diversity by Environment



```
# 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

# 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:

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

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

```

For a better overview, we created a table:

```

# 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 Effects"))

```

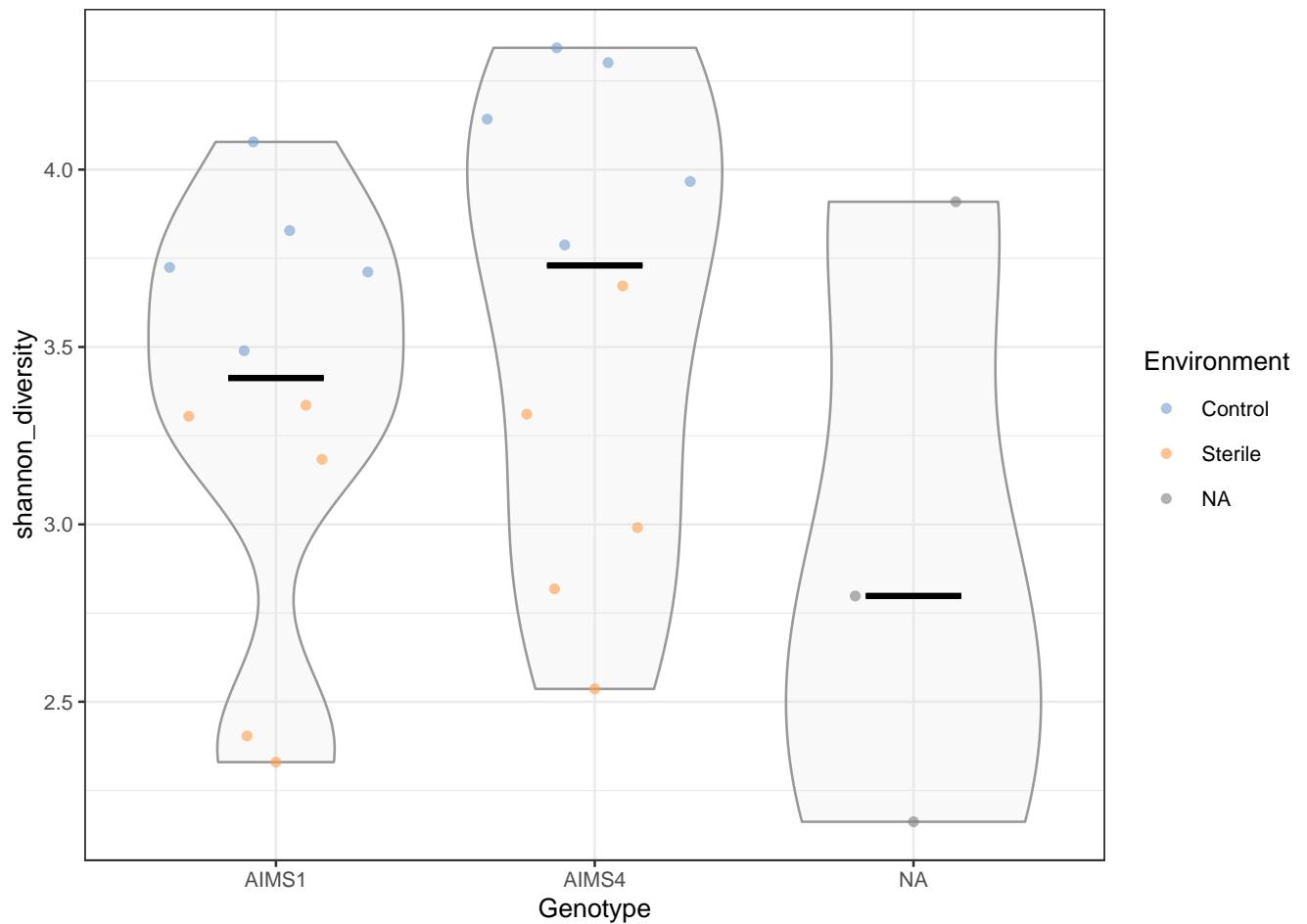
| | Diversity_Measure | Environment_p | Env_Significant | Genotype_p | Gen_Significant |
|-------------------|-------------------|---------------|-----------------|------------|-----------------|
| shannon_diversity | shannon_diversity | 0 | Yes *** | 0 | Yes *** |
| faith_diversity | faith_diversity | 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:

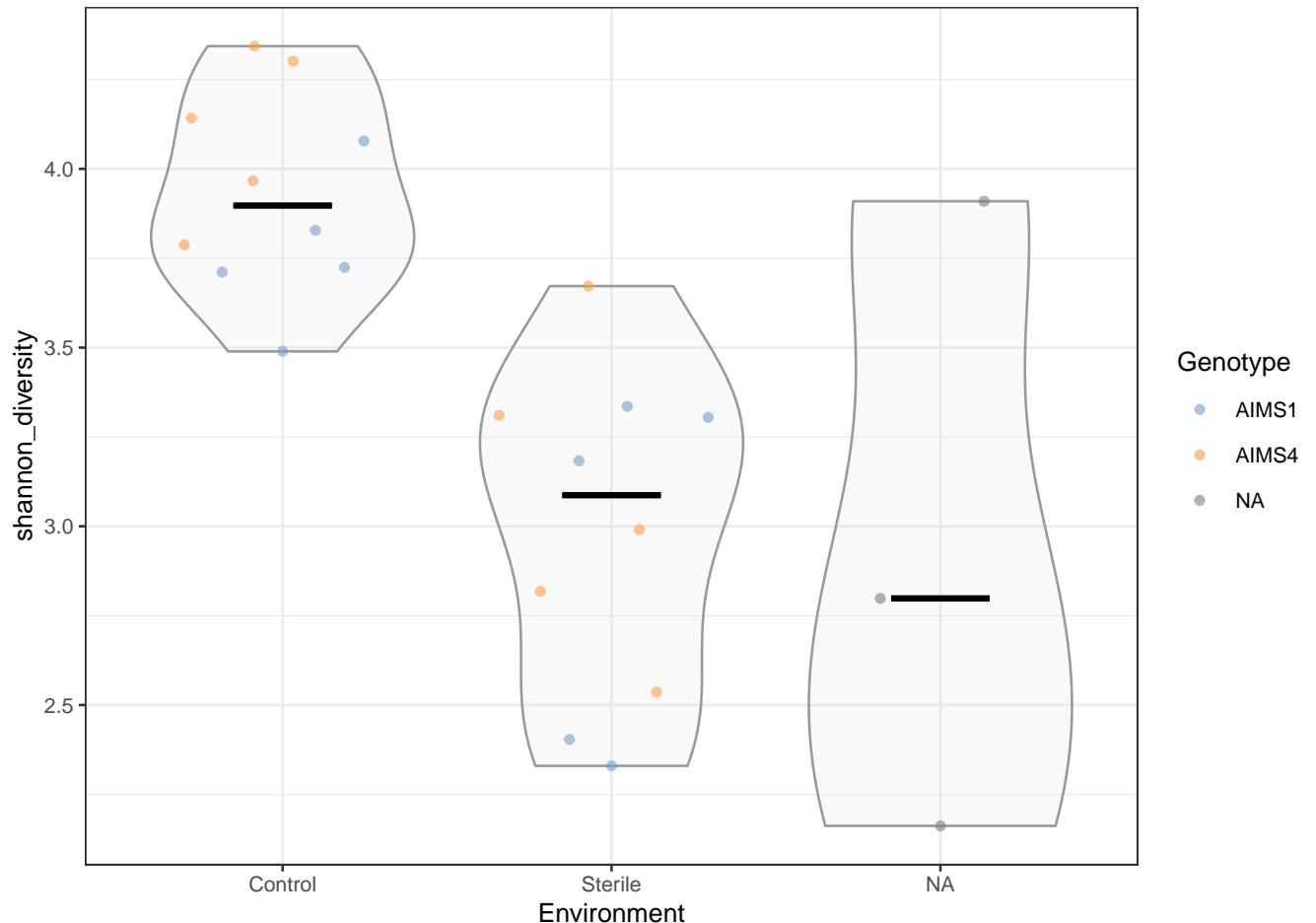
```

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:

- Bodenhofer, Ulrich, Enrico Bonatesta, Christoph Horejs-Kainrath, and Sepp Hochreiter. 2015. “Msa: An r Package for Multiple Sequence Alignment.” *Bioinformatics* 31 (24): 3997–99. <https://doi.org/10.1093/bioinformatics/btv494>.
- Bonatesta, Enrico, Christoph Kainrath, and Ulrich Bodenhofer. 2025. *Msa: Multiple Sequence Alignment*. <https://github.com/UBod/msa>.
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- Huang, Ruizhu. 2025. *TreeSummarizedExperiment: A S4 Class for Data with Tree Structures*.
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- McCarthy, Davis J., Kieran R. Campbell, Aaron T. L. Lun, and Quin F. Willis. 2017. “Scater: Pre-Processing, Quality Control, Normalisation and Visualisation of Single-Cell RNA-Seq Data in R.” *Bioinformatics* 33: 1179–86. <https://doi.org/10.1093/bioinformatics/btw777>.
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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>.
- . 2023. *Tidyverse: Easily Install and Load the Tidyverse*. <https://tidyverse.tidyverse.org>.

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>.