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Fecal microbiota of pregnant mice in a divergent selection experiment

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Session 55
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Introduction

Microbiota → Animal health, productivity and well-being

Essential roles in:

- ✓ Digestion
- ✓ Immunity
- ✓ Metabolism

Homogeneity

- Animal welfare
- Survival
- Productivity
- Robustness

Introduction

Divergent selection experiment for birth weight environmental variability

- High variability line
- Low variability line

Introduction

Low variability line

- ✓ Higher litter size
- ✓ Reproductive efficiency and fertility
- ✓ Survival
- ✓ Stressors: Changes in temperature or feed restriction

Objetives

To study if animals selected divergently for birth weight environmental variability have different microbiota composition.

Could genetic selection affect the microbial profiles?

Materials

Data

Stool samples:

77 pregnant females from 4 generations of selection (22,23,24,25)

- -34 High variability line
- -43 Low variability line

DNA Extraction

QIAmp PowerFecal Pro Kit / QIAGEN

Amplification and Sequencing of V4 region 16S rRNA

Illumina Miseq

Bioinformatic analyses

R version 4.2.2 → phyloseq, dada2, vegan, ggplot2

Methods

Diversity Quantitative measure of the variety or number of different species

Alpha diversity: mean diversity of samples from each line.

✓ Richness: count of the number of species in a sample.

✓ Simpson index: How evenly distributed are the abundances of species in each line.

$$S_D = 1 - \sum_{i=1}^{R} p_i^2$$

p_i is proportion of species i

b is the base, usually e

S is number of species (richness)

Greenberg, J. H. (1956)

✓ Shannon-Wienner: combines richness and evenness.

$$H' = -\sum_{i=1}^{R} p_i \log_e(p_i)$$

p_i is proportion of species i

b is the base, usually e

S is number of species (richness)

Margalef, R. (1958)

Methods

Beta diversity: diversity among samples.

- ✓ Dissimilarity: compositional dissimilarity between two samples, based on the counts of amplicon secuence variants (ASVs) in each sample.
- ✓ Bray-Curtis Dissimilarity matrix

$$BC_{AB} = \frac{\sum_i |a_i - b_i|}{\sum_i (a_i + b_i)}$$

Where:

- $|a_i - b_i|$ is the absolute difference in abundance of ASV i between samples A and B.
- $\sum_i (a_i + b_i)$ is the sum of the total ASV counts in both samples.

Bray, R. J. & J. T. Curtis. (1957)

Methods

- ✓ Clustering: Calinski-Harabasz criterion

$$\frac{\mathrm{SSB}/(k-1)}{\mathrm{SSW}/(n-k)}$$

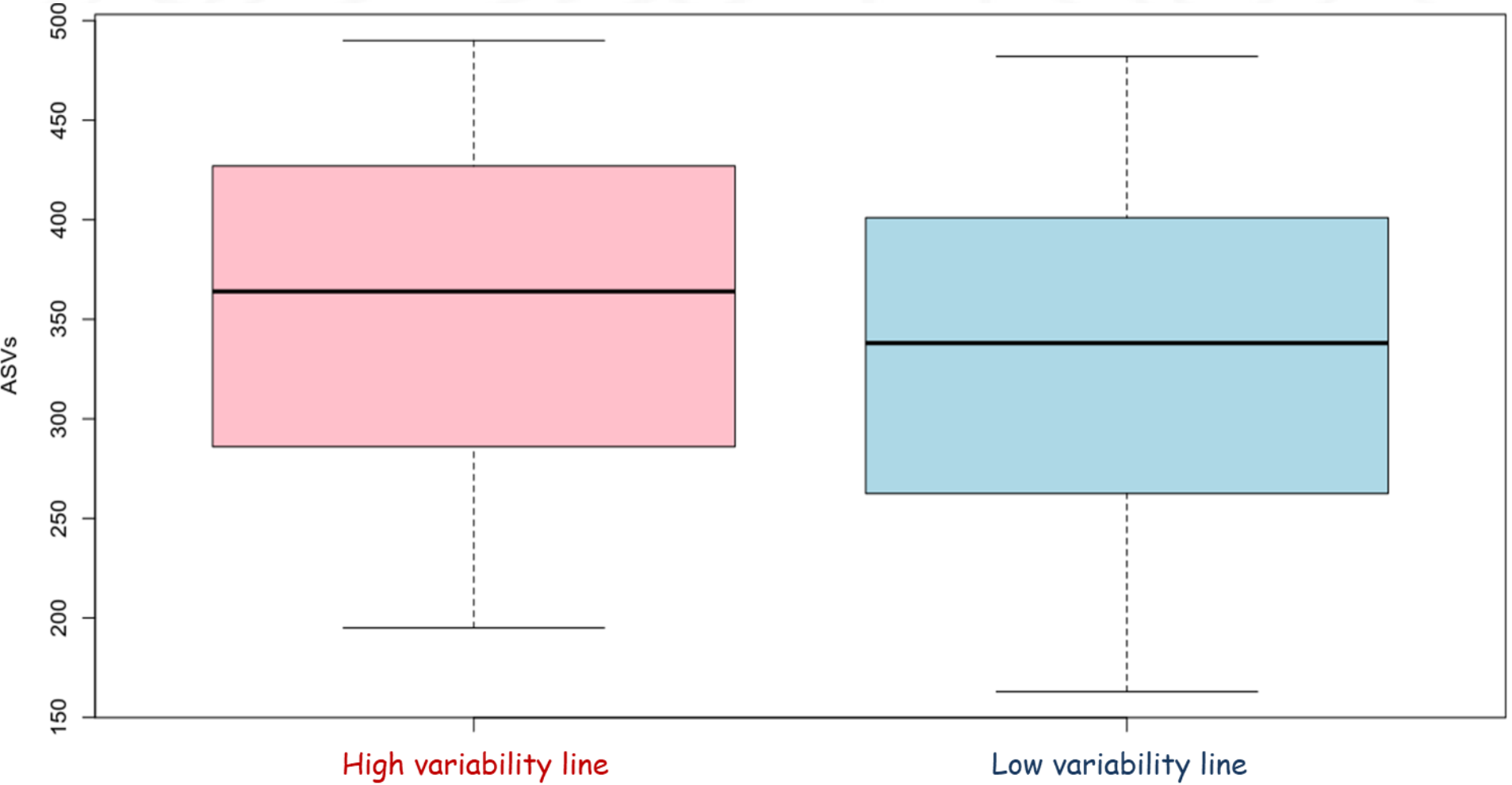
- SSW \& SSB within cluster and between cluster sums of squares
- k is number of clusters, n is number of observations

Calinski, T. and Harabasz, J. (1974)

- ✓ Ordination: Principal Coordinates Analysis (PCoA).
- ✓ PERMANOVA (Permutational Multivariate Analysis of Variance) differences between groups.
- ✓ Genus abundances in both lines.

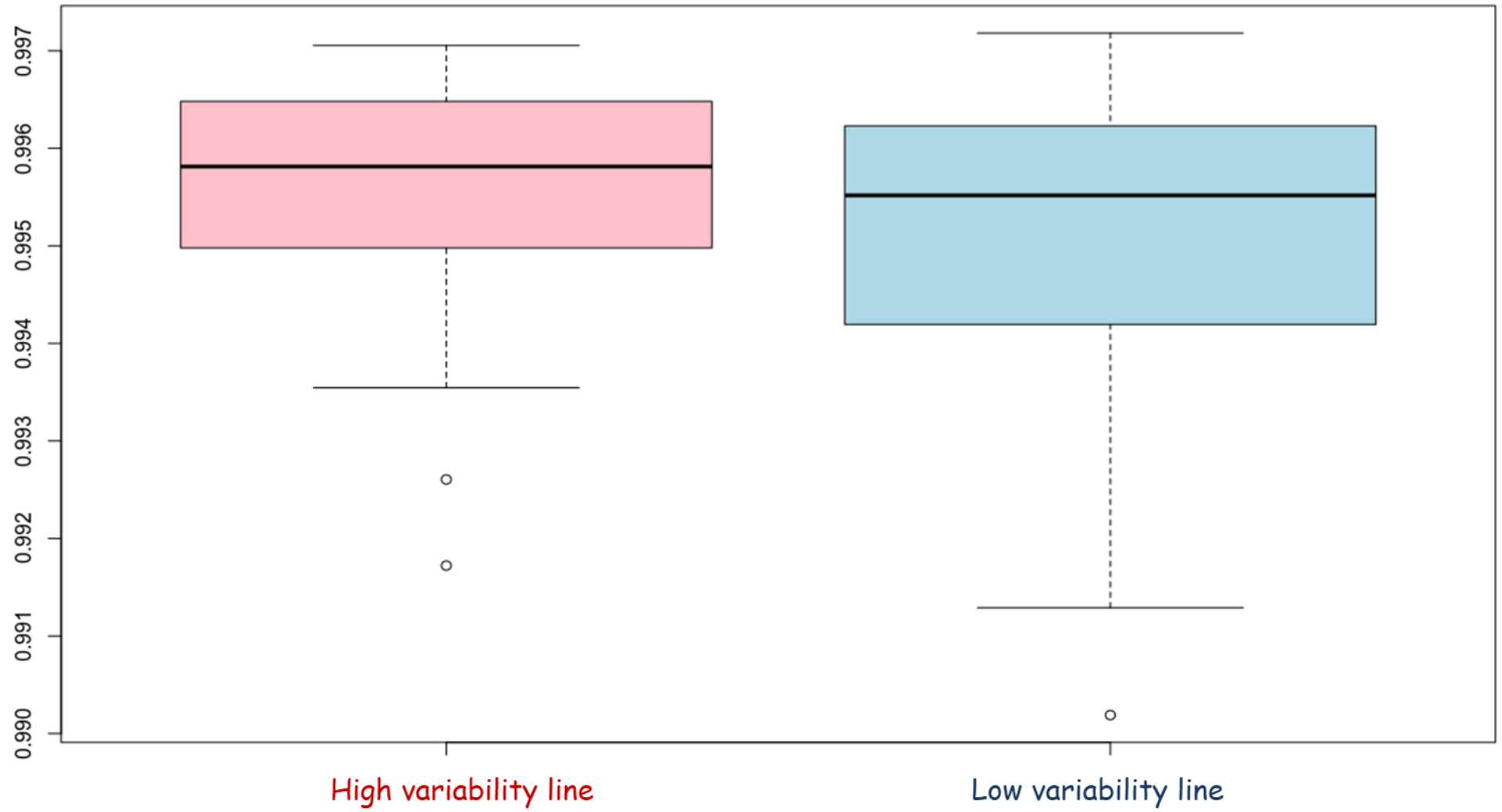
Results

Richness



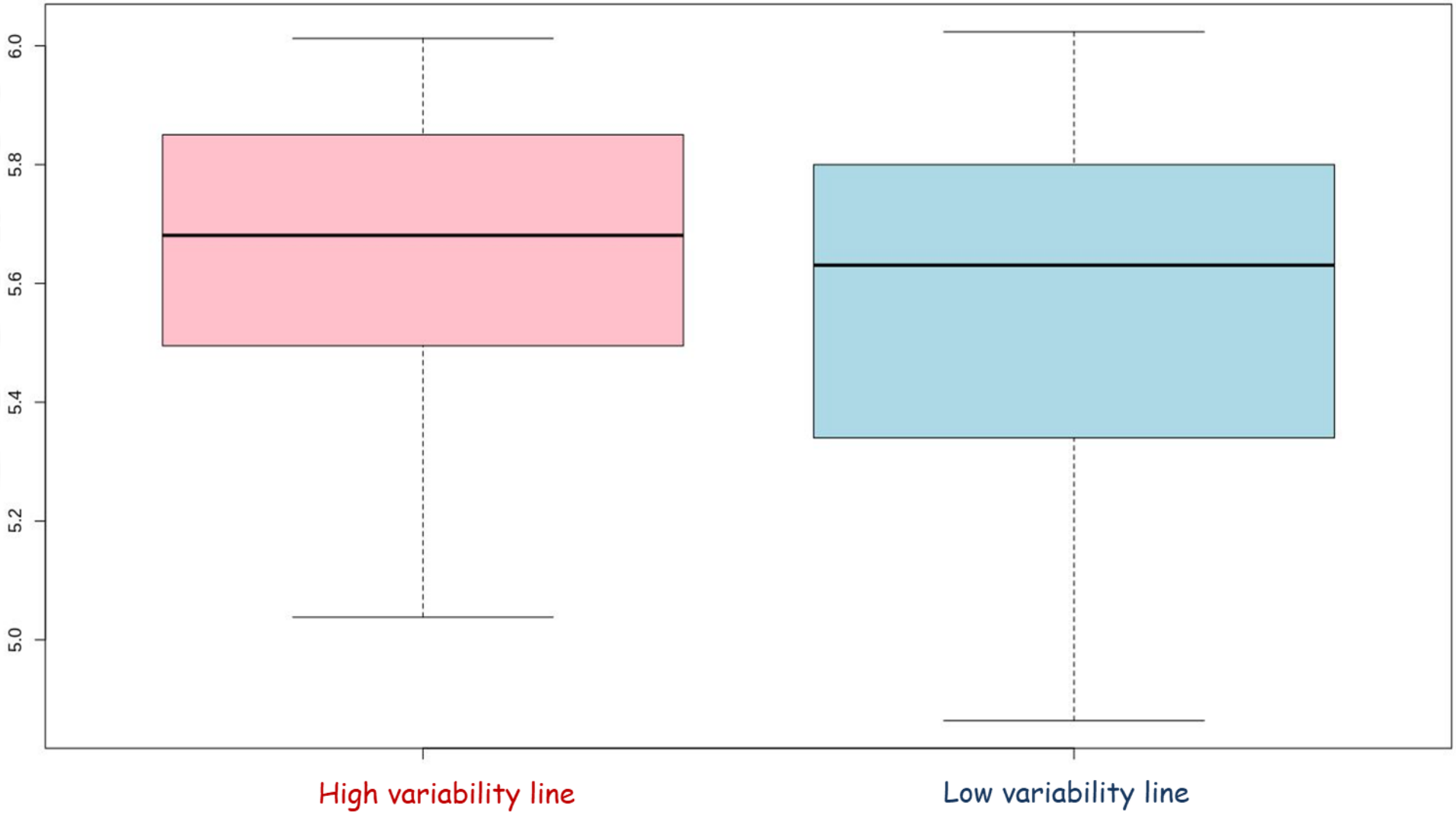
Results

Simpson index



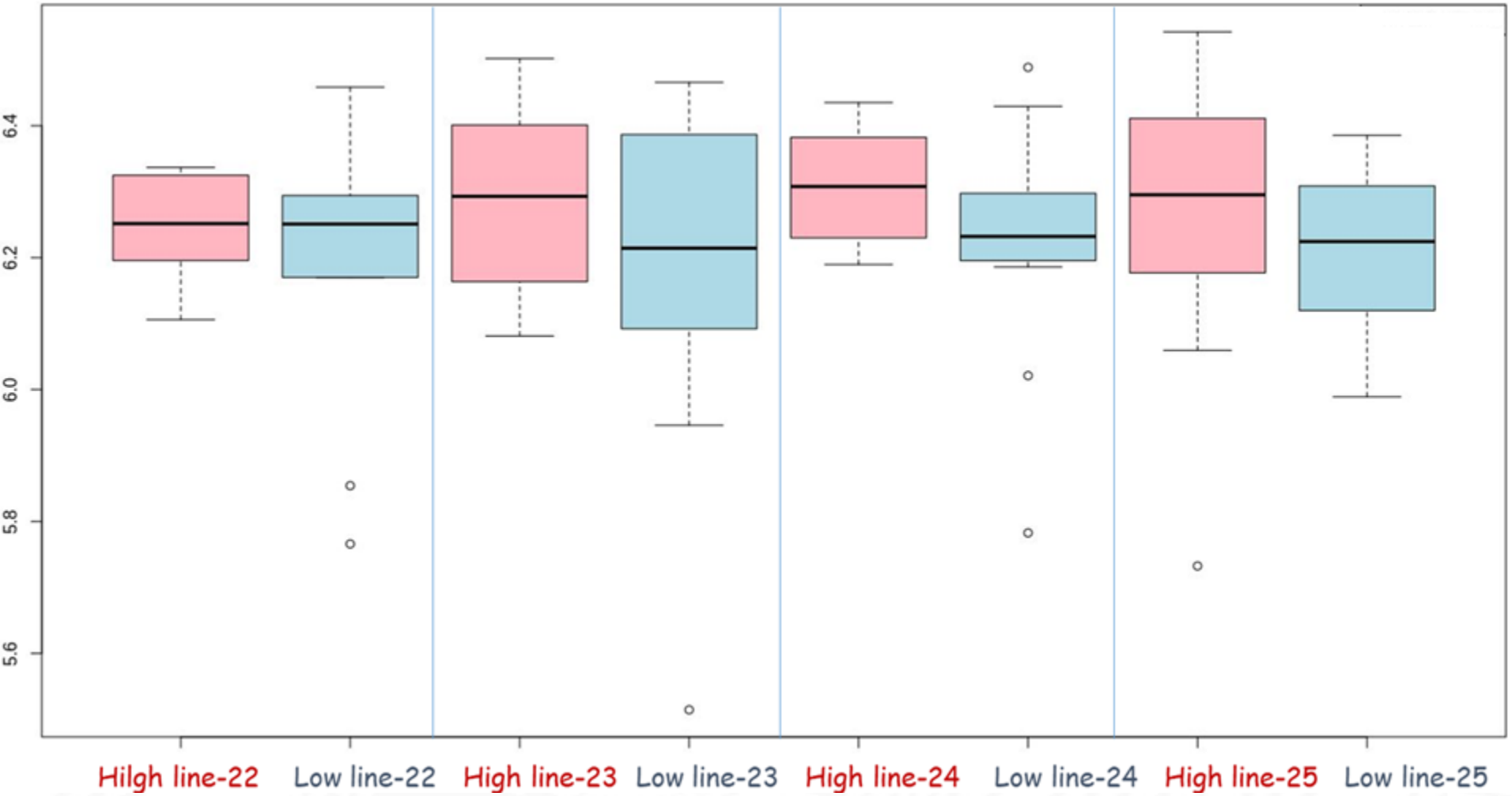
Results

Shannon index



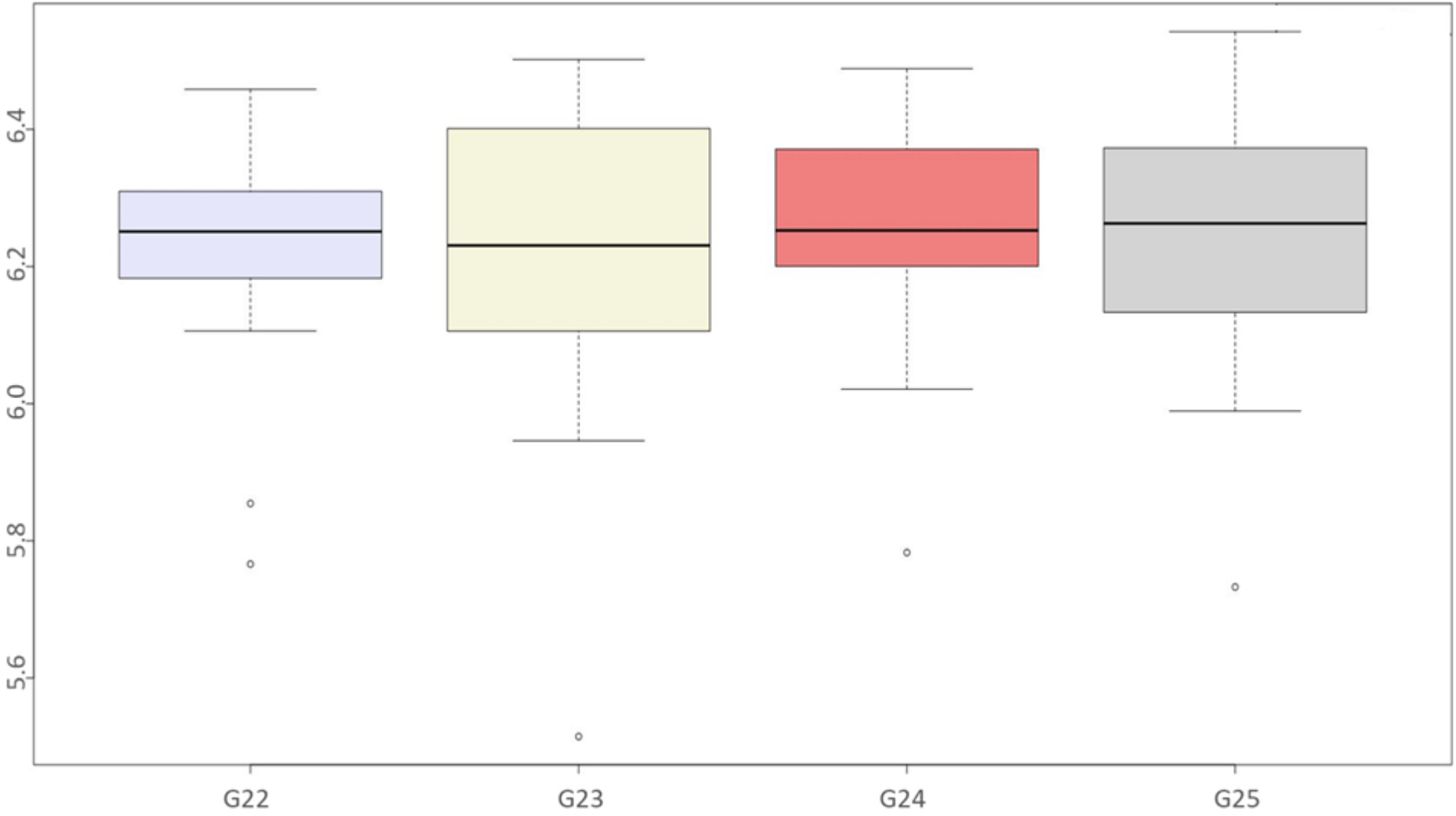
Results

Shannon index per generation and line



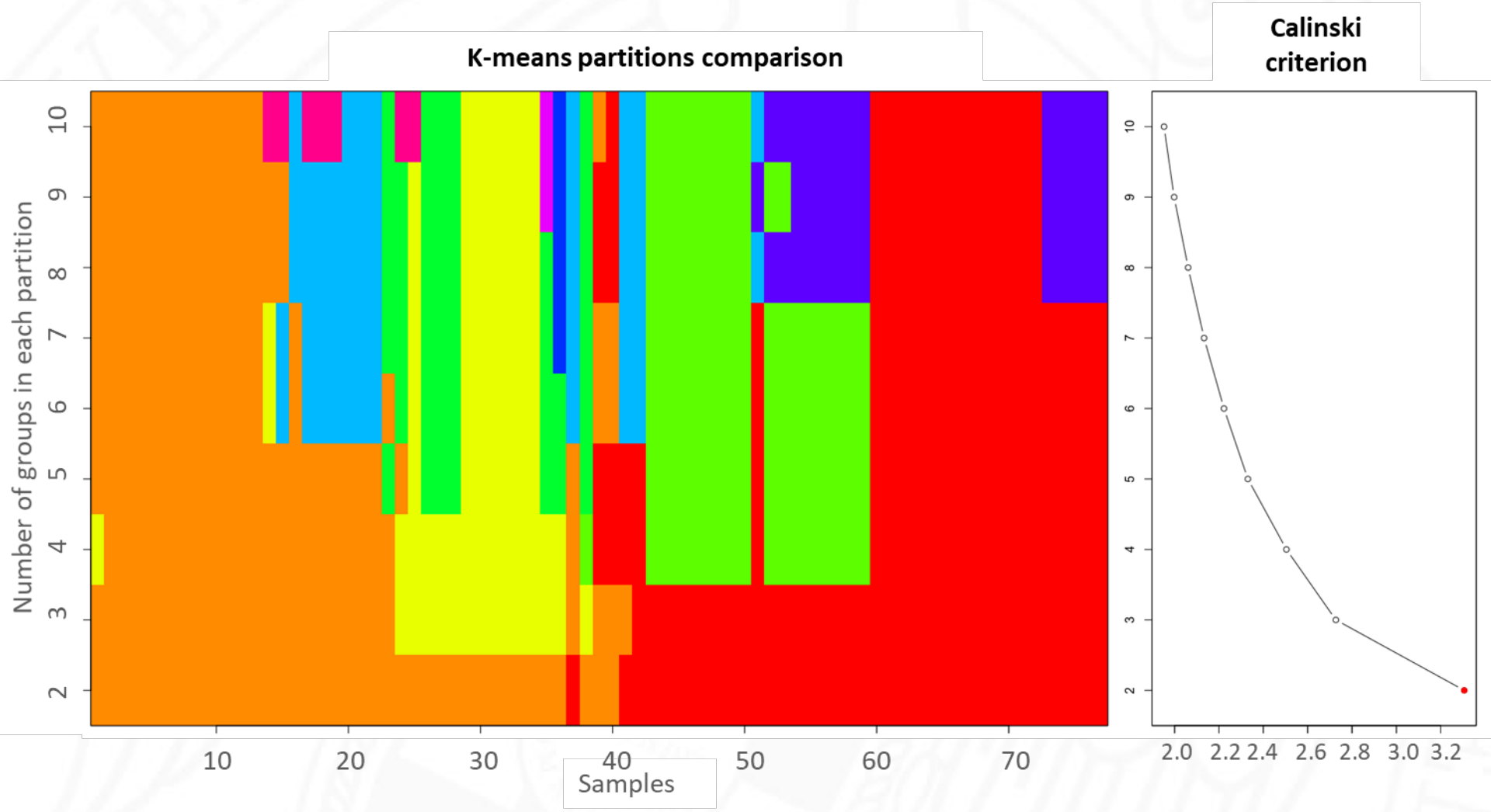
Results

Shannon index per generation.



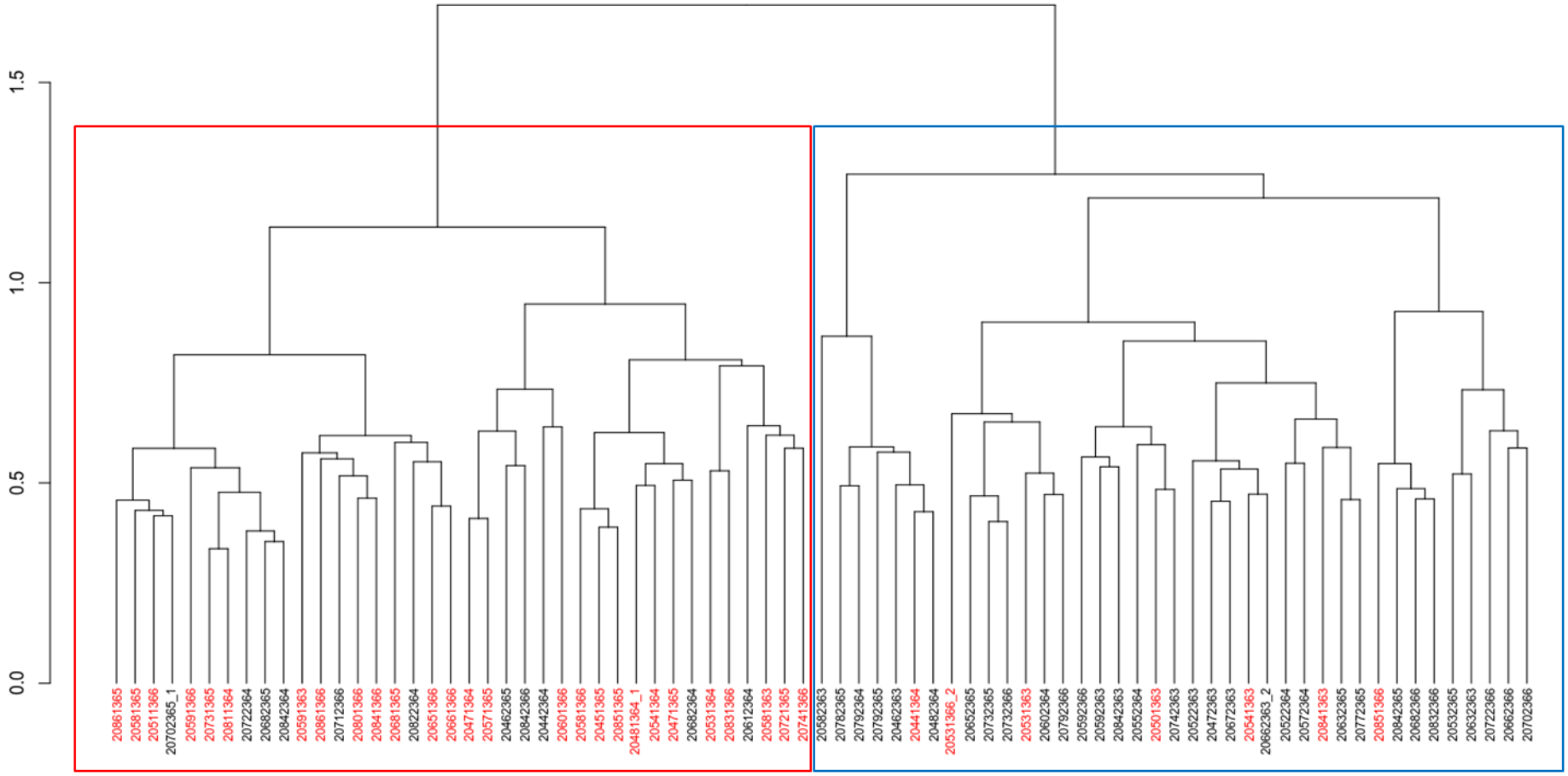
Results

Calinski-Harabasz criterion



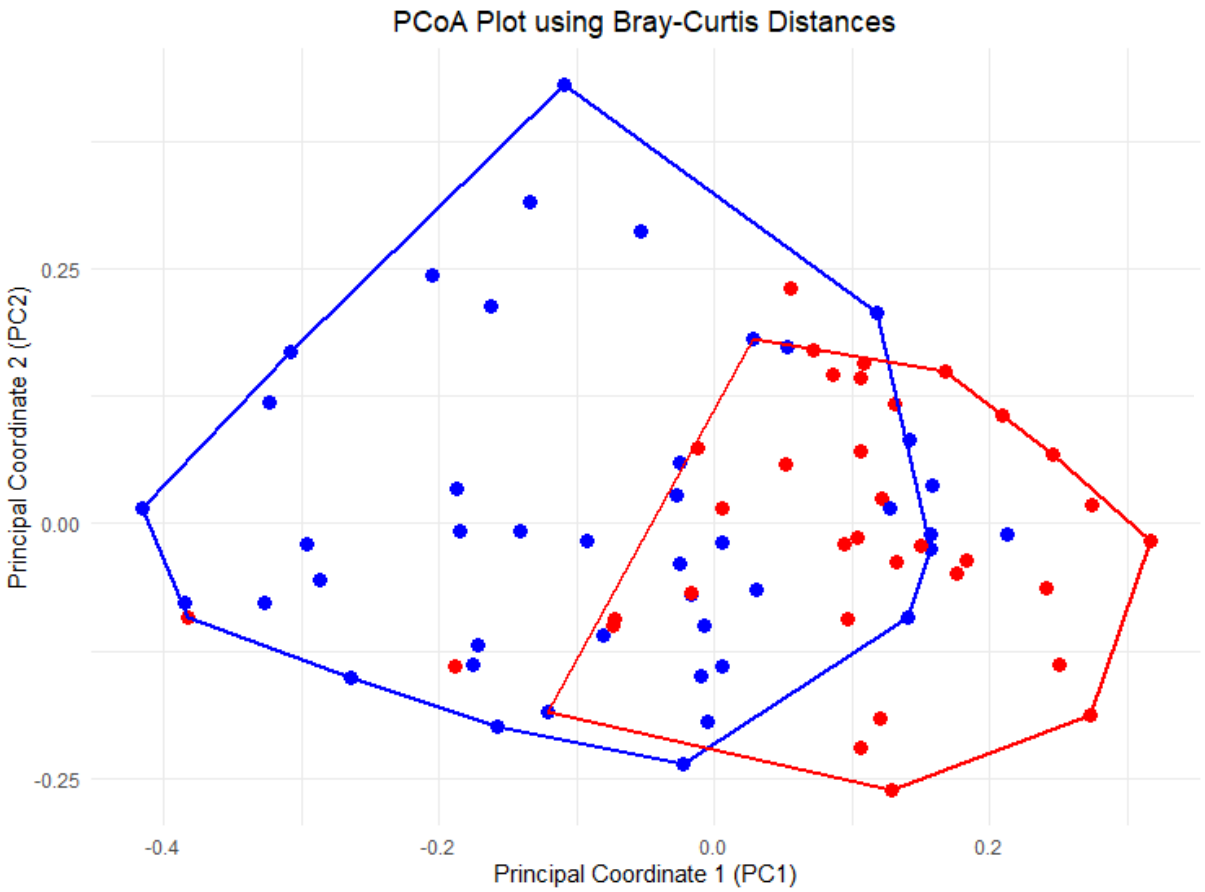
Results

Cluster analysis: groups based on the dissimilarity coefficients calculated.



Results

PCoA (Principal coordinates analysis)



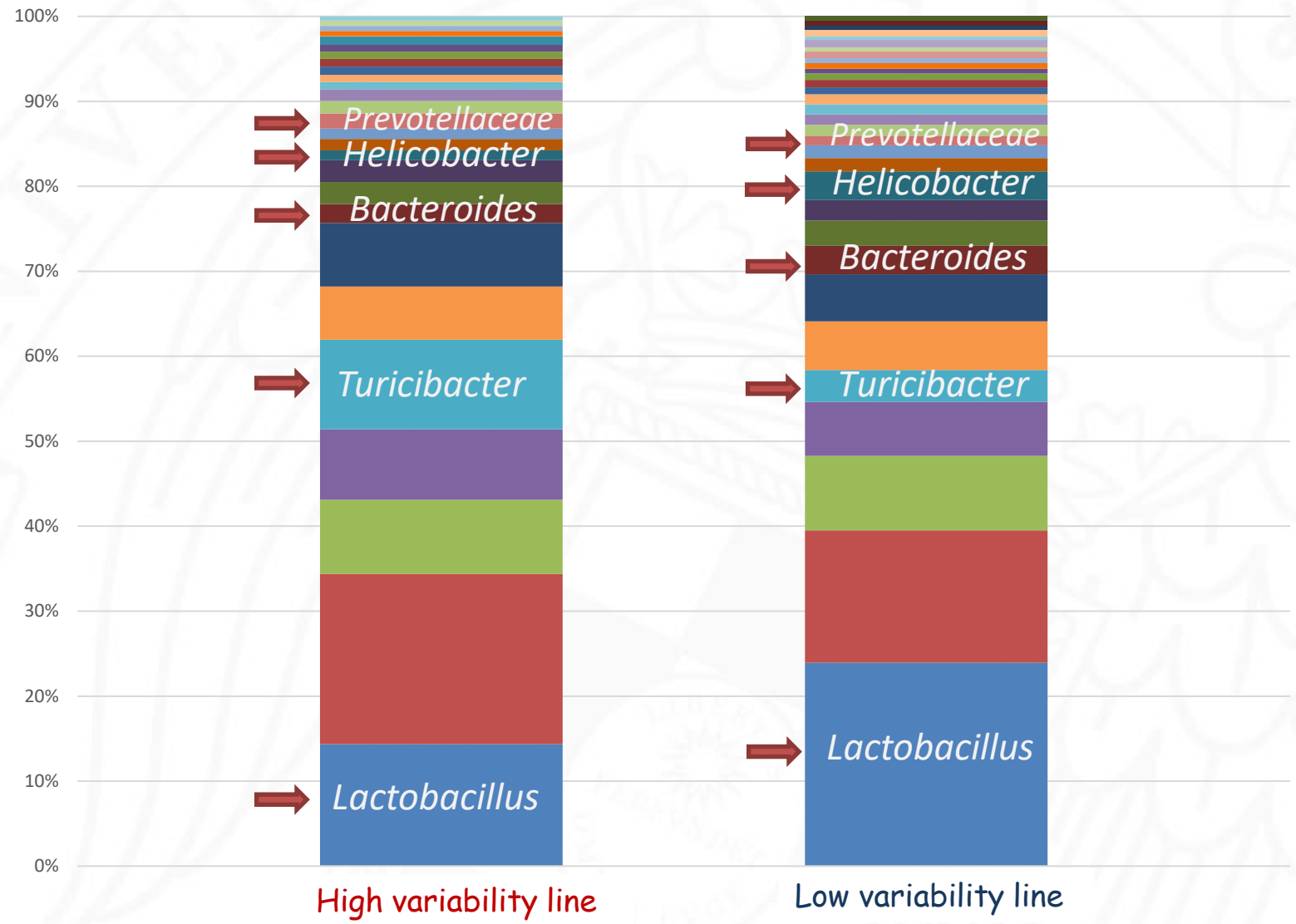
High line
Low line

PERMANOVA

	Df	SumOfSqs	R2	F	Pr(>F)	
line	1	0.8905	0.05549	4.4065	1e-04	***
Residual	75	15.1565	0.94451			

Results

Genus abundances



Conclusions

- ✓ Possible differences between lines due to the selection, more analyses needed.
- ✓ Differences in the abundances of genus between lines: more abundance of *Lactobacillus*, *Bacteroides*, *Helicobacter* in Low line than in High line and less abundance of *Turicibacter* and *Prevotellaceae UCG-1*.

Thank you!

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