# PERMANOVA

Eduard Szöcs

Institute for Environmental Sciences - University Koblenz-Landau

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# Introduction

- Assumptions of MANOVA:
  - Independence of the sample units
  - Multivariate normality
  - Homogeneity of variance–covariance matrices
- Euclidean distance useful?
- Generally **not** met for ecological data sets!
- Need a robust method to handle complex data sets.

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# Permutational Multivariate Analysis of Variance Using Distance Matrices



*Austral Ecology* (2001) **26**, 32–46

## A new method for non-parametric multivariate analysis of variance

MARTI J. ANDERSON
*Centre for Research on Ecological Impacts of Coastal Cities, Marine Ecology L*
*University of Sydney, New South Wales 2006, Australia*

**Abstract** Hypothesis-testing methods for multivariate data are needed to make rigo
about the effects of factors and their interactions in experiments. Analysis of variance

- Very influential paper in community ecology
  - Google Scholar: >4500 citations
- Non-parametric approach combined with ecological distance measures!

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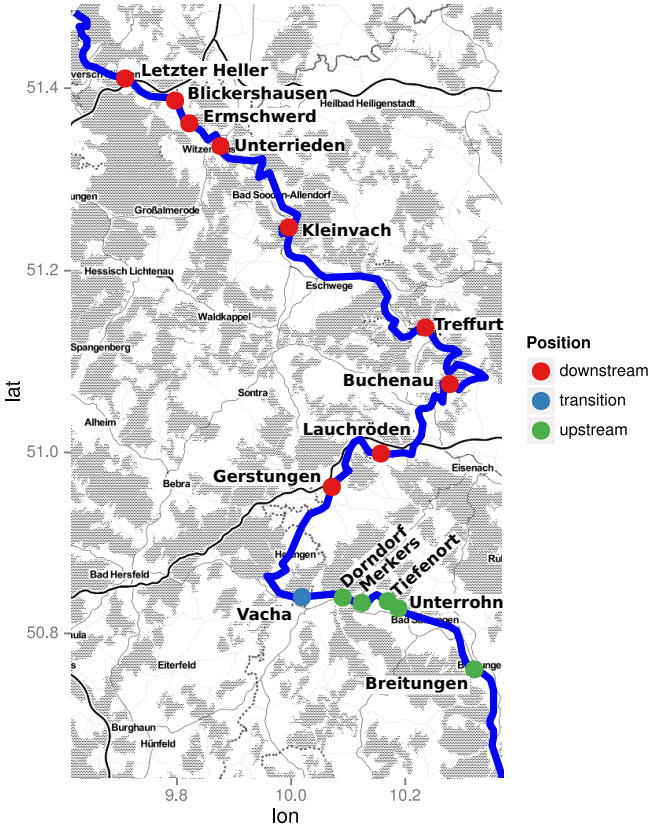
# Data set used in this lecture

Macroinvertebrate data from the River Werra[1]
Aim : Effect of anthropogenic salinisation on macroinvertebrate communities

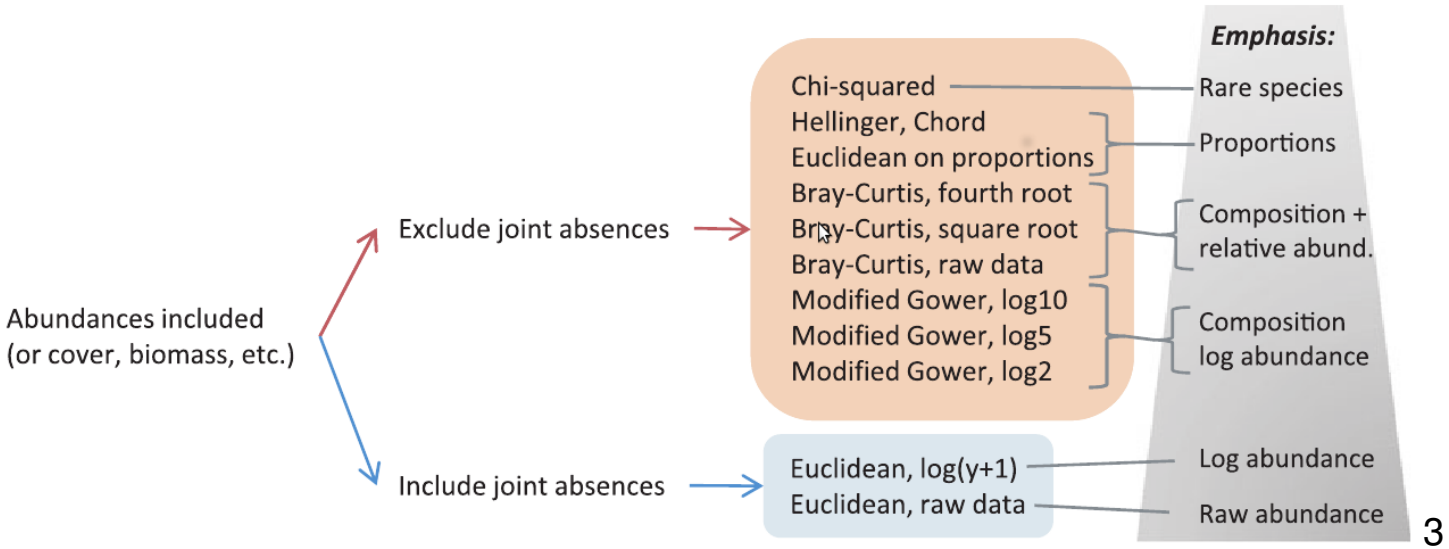
- upstream - downstream design
- salt brine discharge around Vacha
- Do the communities differ between up- and downstream?
- Not the original data (proprietary).




[1] Bäthe, Jürgen, and Eckhard Coring. Biological Effects of Anthropogenic Salt-load on the Aquatic Fauna: A Synthesis of 17 Years of Biological Survey on the Rivers Werra and Weser. Limnologica - Ecology and Management of Inland Waters 41(2): 125-133.

# First impression of the data

- NMDS Bray-Curtis-Distance and $x^{0.25}$ transformation.



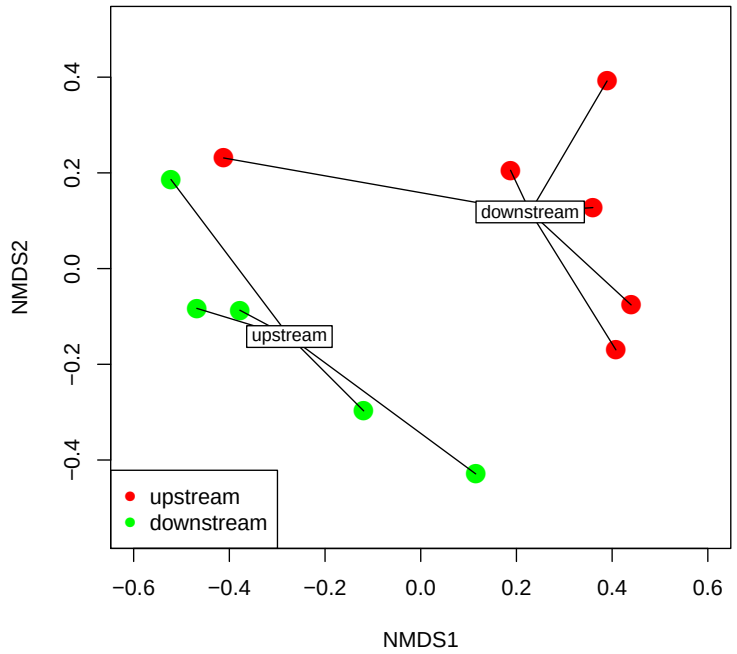

[3]

[3] Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, et al. Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. Ecology Letters. 2011;14(1):19–28.

# First impression of the data - NMDS

- NMDS Bray-Curtis-Distance and $x^{0.25}$ transformation.

- upstream and downstream sites clearly separate in NMDS.
- Spread looks similar.
- Indication of a difference between upstream and downstream.



# Recap: ANOVA

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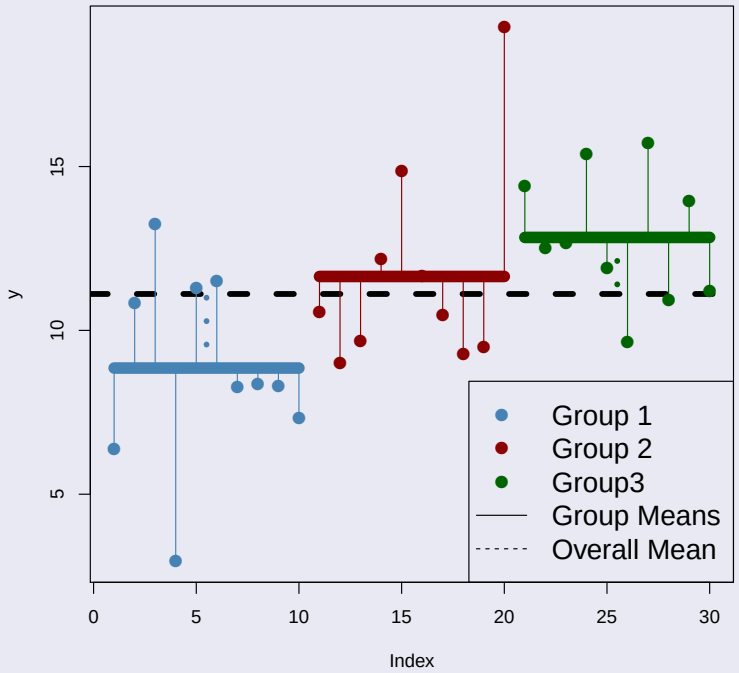
Question : How is univariate ANOVA calculated?

## From univariate...




$$F - ratio = \frac{SS_{group}}{SS_{residual}} \cdot \frac{df_{residual}}{df_{group}}$$

$$SS_{total} = SS_{residual} + SS_{group}$$

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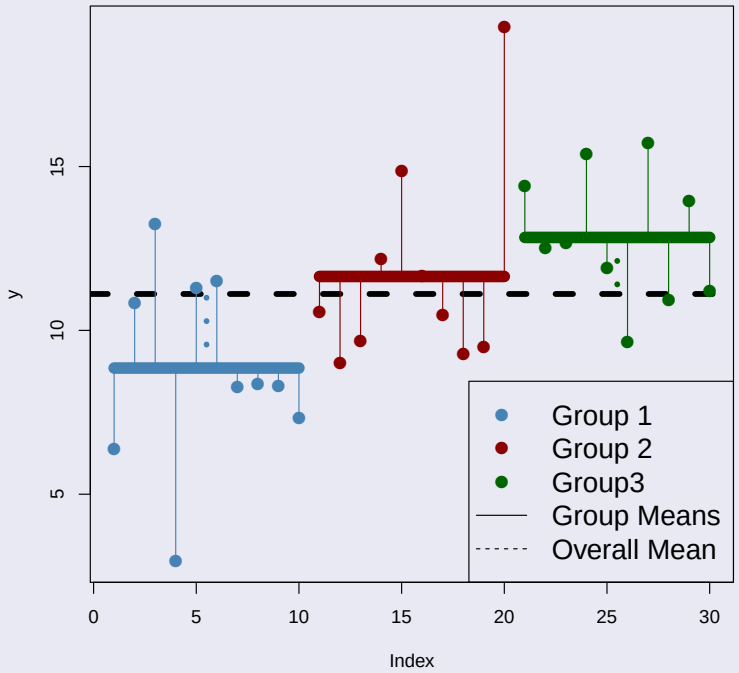


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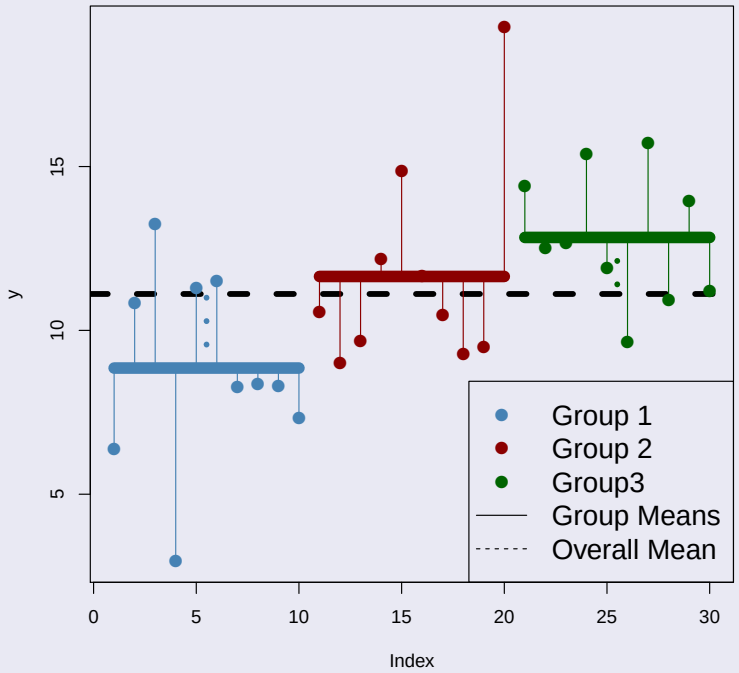


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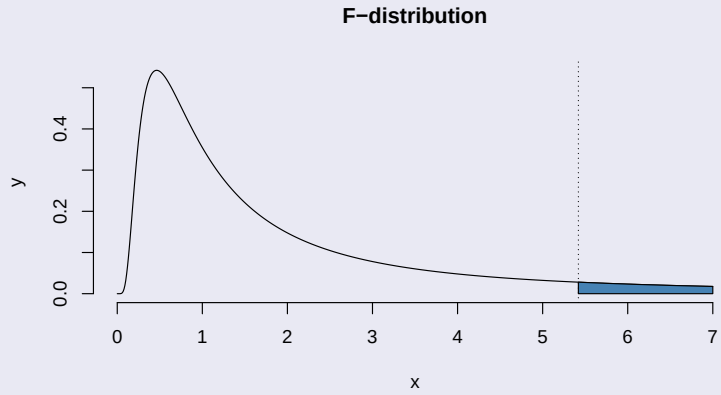

# Distance-based MANOVA

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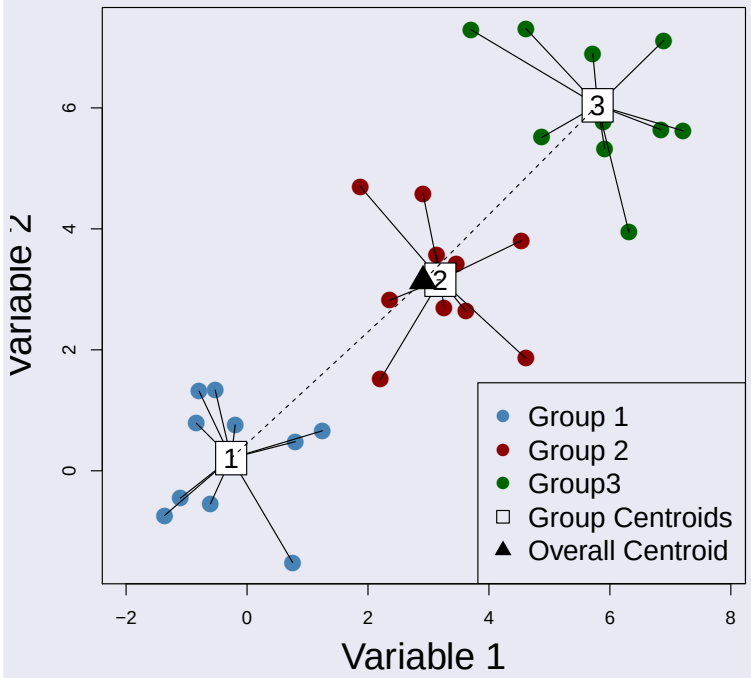
Distance-based MANOVA is analogous!

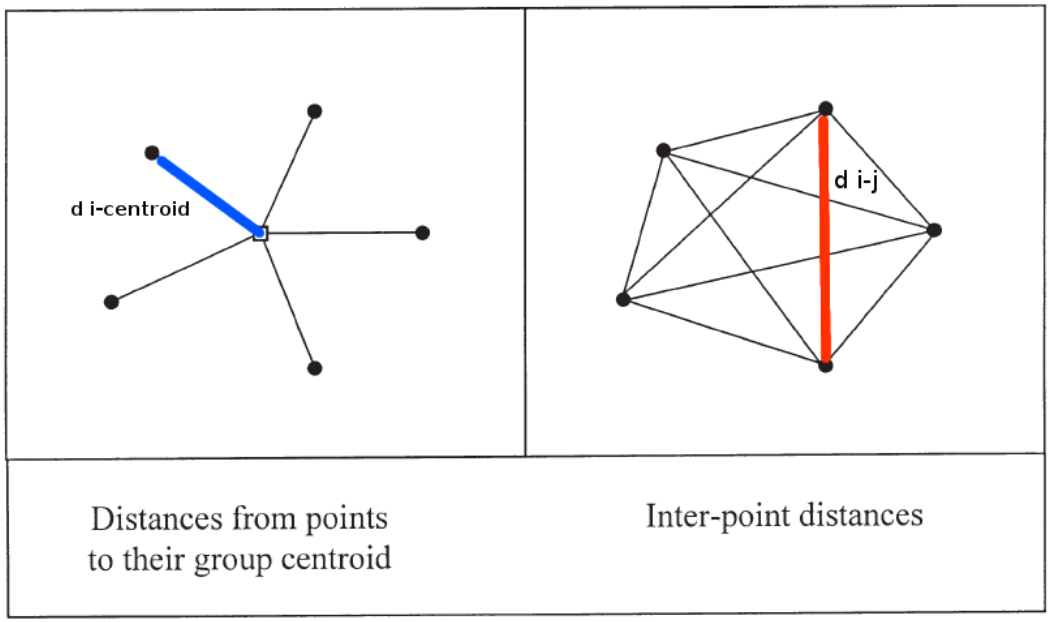
## ... to multivariate ANOVA




- Partitioning into variance components:
  $SS_{total} = SS_{group} + SS_{residual}$
- **centroids**
- p-value by **permutations**

# Variance Partitioning

- We can use any **Distance Matrix** to partition the variance.
- Sum of squared distances from individual points to their centroid is equal to the sum of squared interpoint distances divided by the number of points.
- $\sum d^2_{i-centroid} = \frac{1}{N} \sum d^2_{i-j}$



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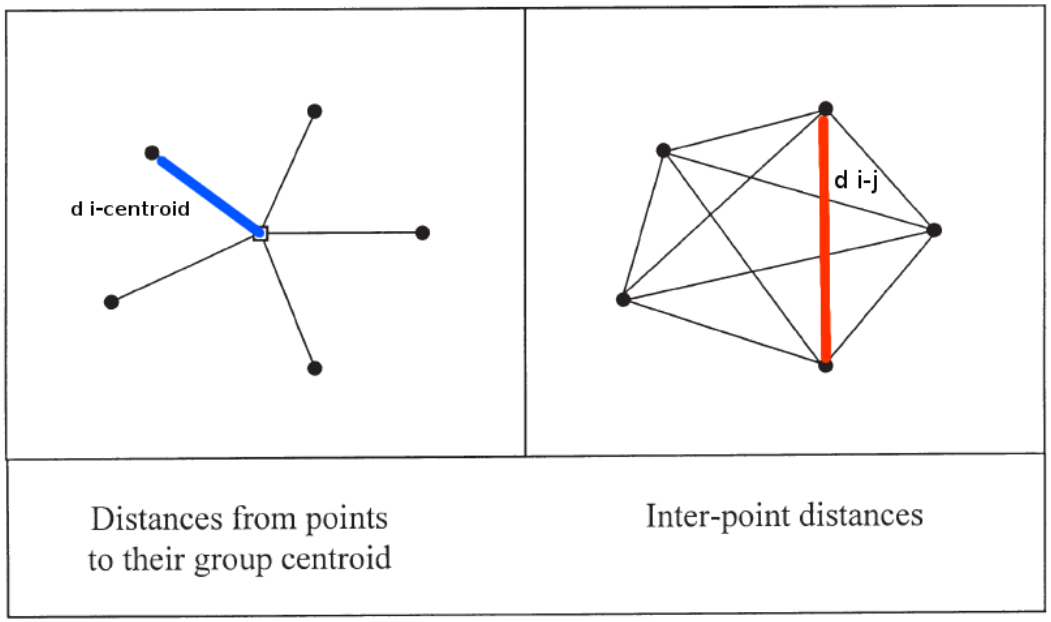

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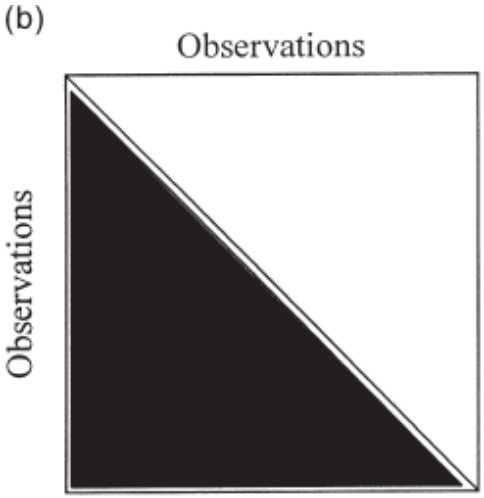
Like in univariate ANOVA variance can be partitioned:




$$SS_{total} = \frac{1}{N} \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} d_{ij}^2$$

N = total number of observations

$$SS_{residual} = \frac{1}{n} \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} d_{ij}^2 \epsilon_{ij}$$

n = number of observations per group

$$\epsilon_{ij} = \begin{cases} 1, & \text{if if observations i and j are in the same group} \\ 0, & \text{otherwise} \end{cases}$$

$$SS_{group} = SS_{total} - SS_{residual}$$

$$(Pseudo-)F = \frac{SS_{group}}{SS_{residual}} \frac{N-a}{a-1}$$

a = no. groups

p-value is assessed via permutations.

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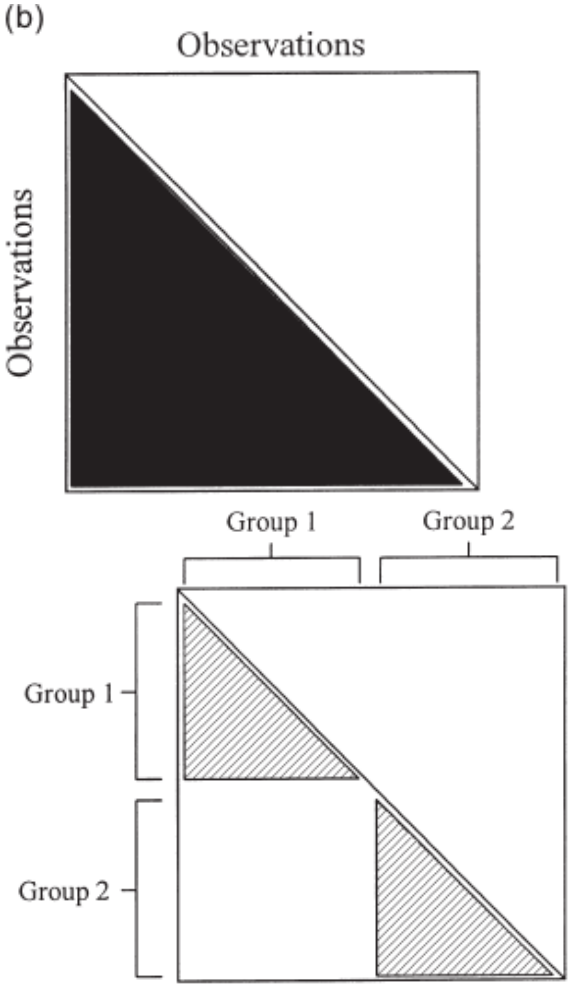


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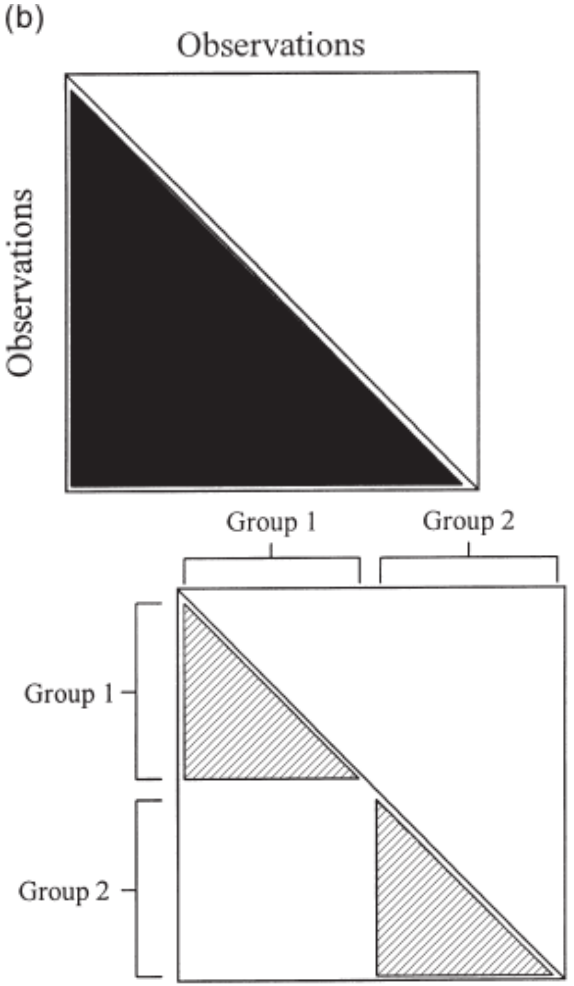


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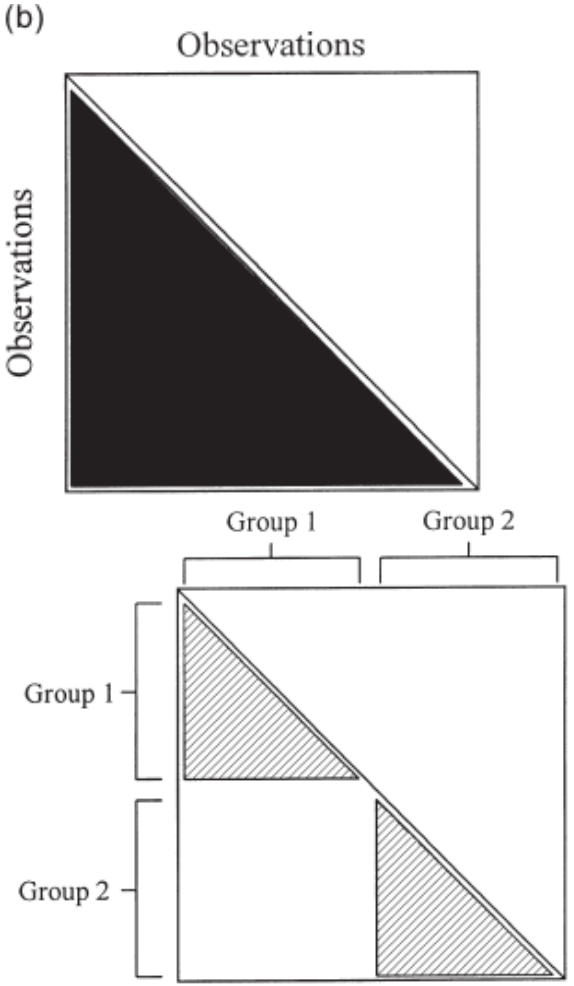


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# p-values using permutations

- Cannot use Fisher's F-ratio
  - normal distribution?
  - euclidean distance?
- Instead use **permutations**
  - shuffle data randomly
  - compute F-Ratio ($F_{perm}$)
  - repeat many times
- compare F of randomized data with original F.
- $p = \frac{\text{No. of } F_{perm} \geq F}{\text{No. of permutations} + 1}$

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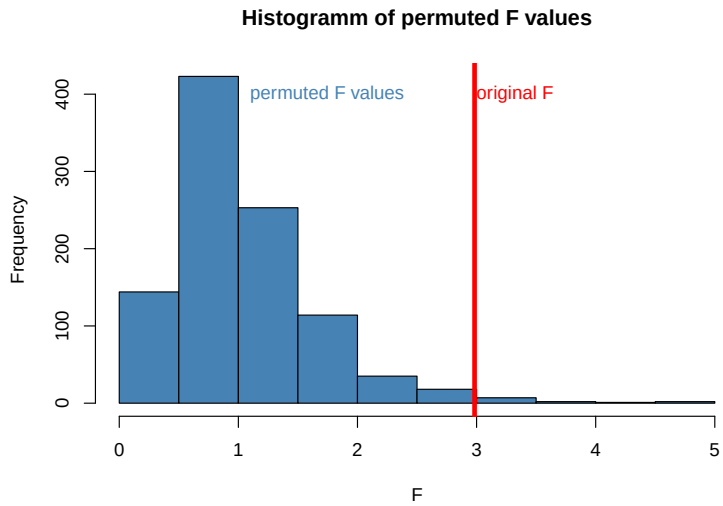
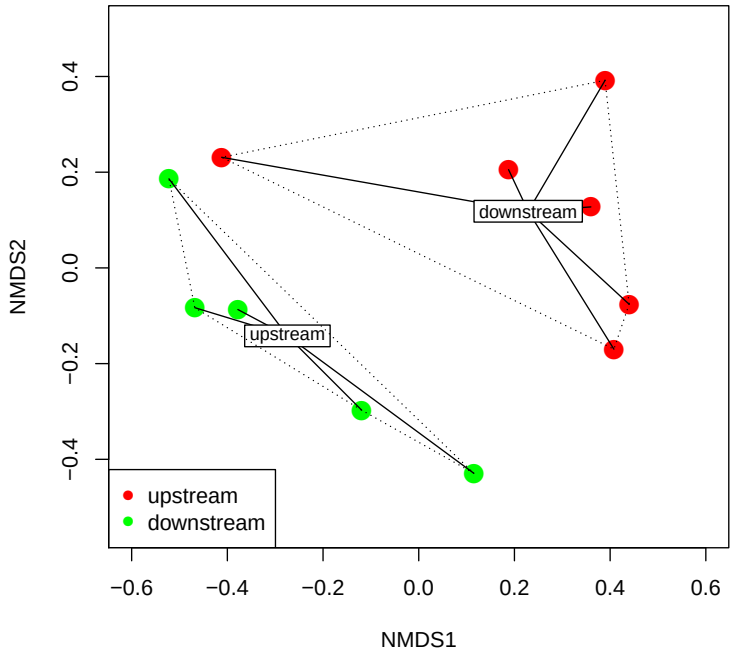


# Assumptions of PERMANOVA

- equal **dispersions**
- Visual inspection
- Multivariate analogue to Levene's test available.[4]
- Multivariate Dispersion
  - $\beta$-diversity
  - functional diversity
  - see literature folder

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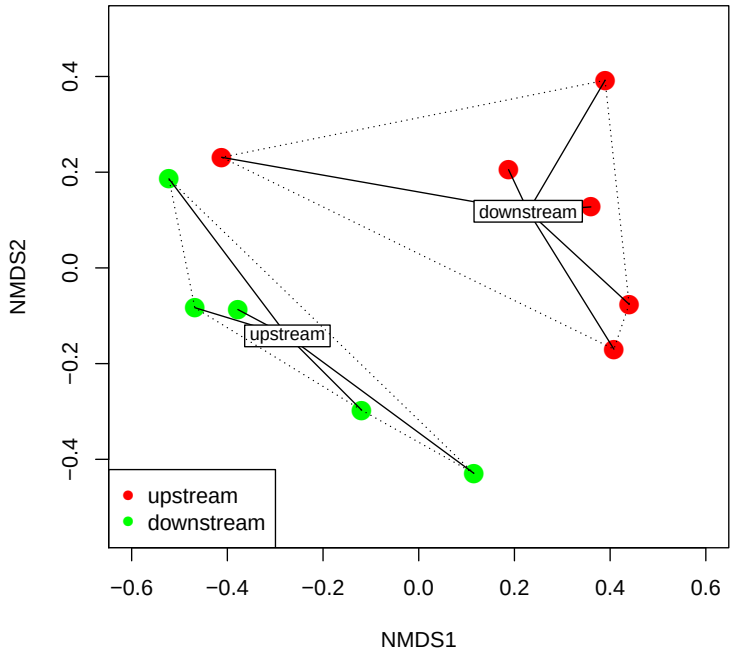


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PERMANOVA is a

- flexible (any distance measure) and
- easy (analogue to univariate Anova)

tool for ecologists.

However,

- non-parametric does no mean assumption free.

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# Lets do it in R!

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