KICK-OFF MEETING

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Acronym:	BIOLAWEB Boosting Institute of Chemistry, Technology and Metallurgy in Water Biomonitoring
Grant No:	101079234
Type of action:	HORIZON Coordination and Support Actions (HORIZON - CSA)
Starting Date:	01/10/2022
Duration:	36 months

Workshop, Belgrade, October 2023

BIOLAWEB
presentation

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Ecological analysis of metabarcoding data

Beta diversity

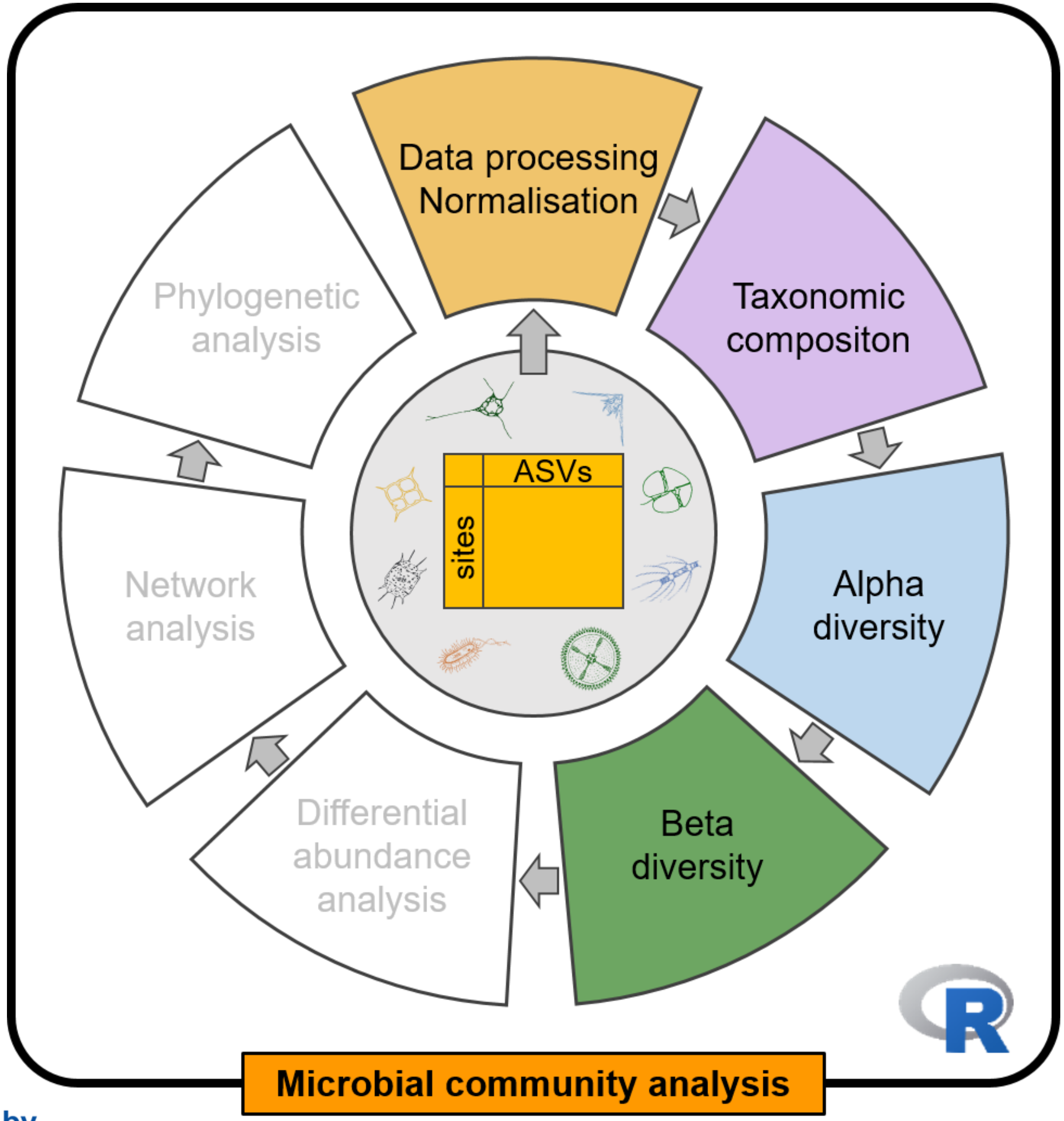
Benjamin Alric

INRAE

ECLA PÔLE ÉCOSYSTÈMES LACUSTRES

OFB OFFICE FRANÇAIS DE LA BIODIVERSITÉ

Statistical analysis workflow from metabarcoding data



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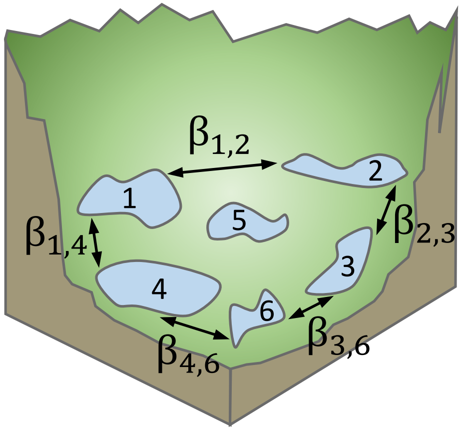
Beta diversity: Analytic workflow



Beta diversity:
Variation in species composition among sites within a geographical area of interest (Whittaker 1960, 1972)

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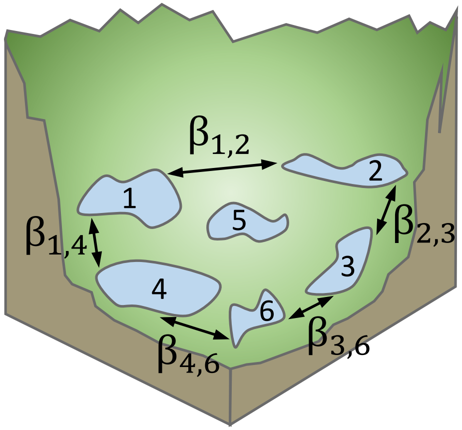

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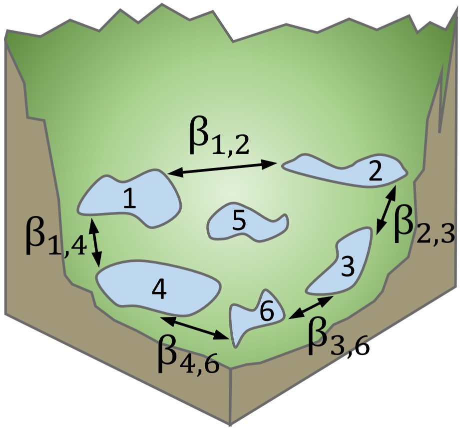

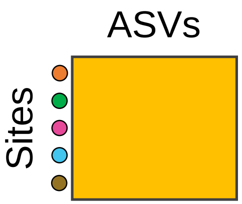

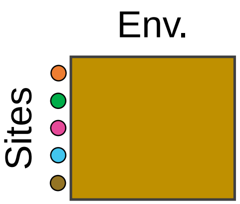

Measuring the variation among commuties

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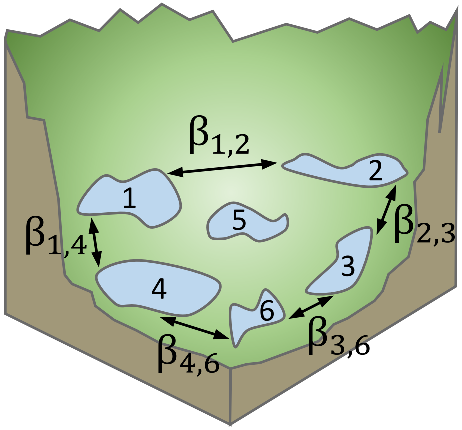

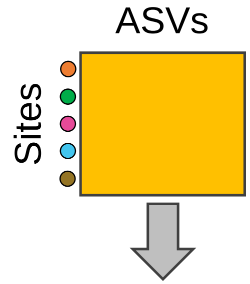

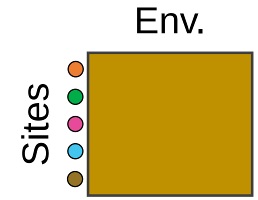

Measuring the variation among commuties

Bringing out a structure

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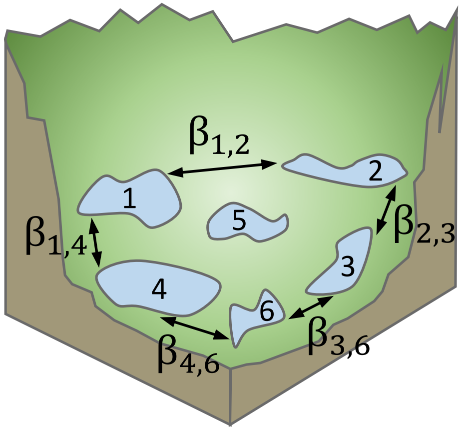

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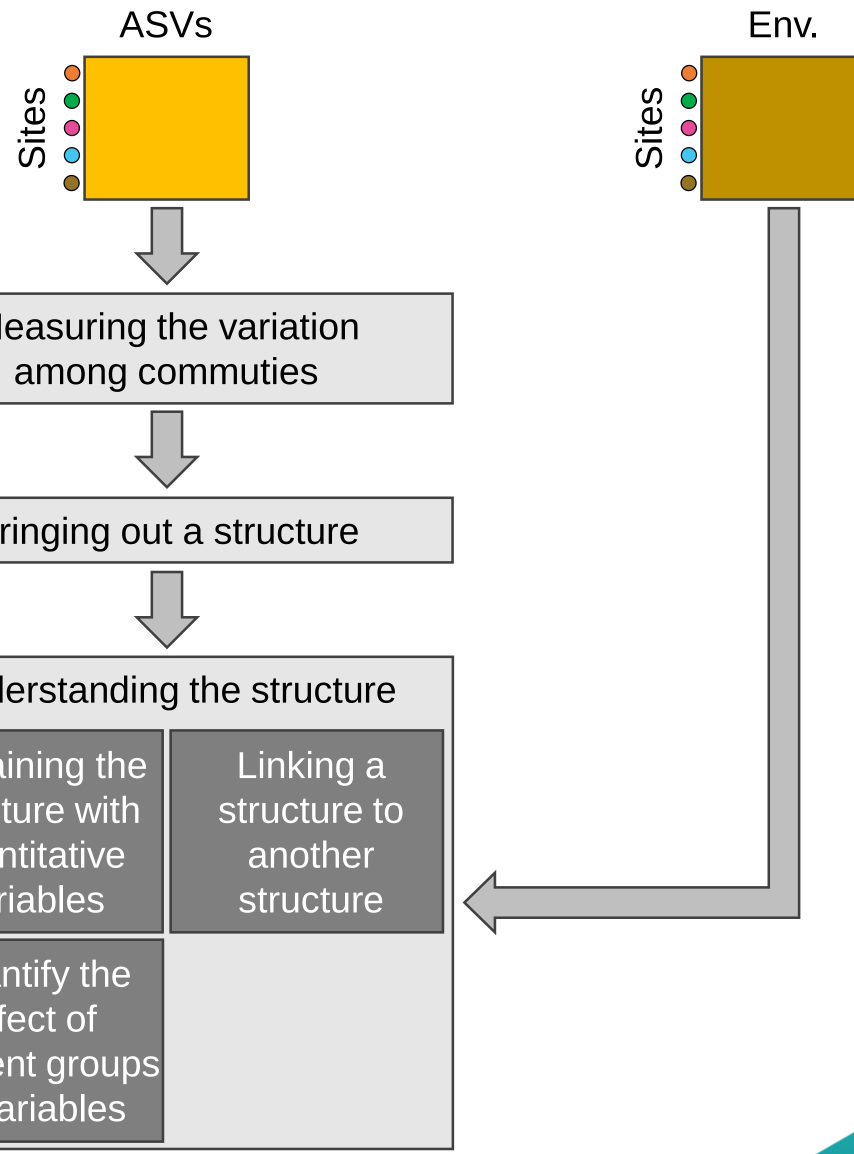

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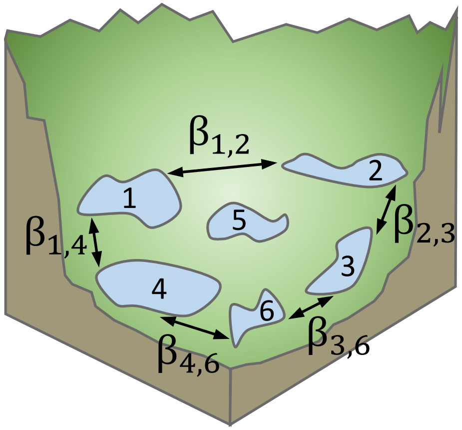
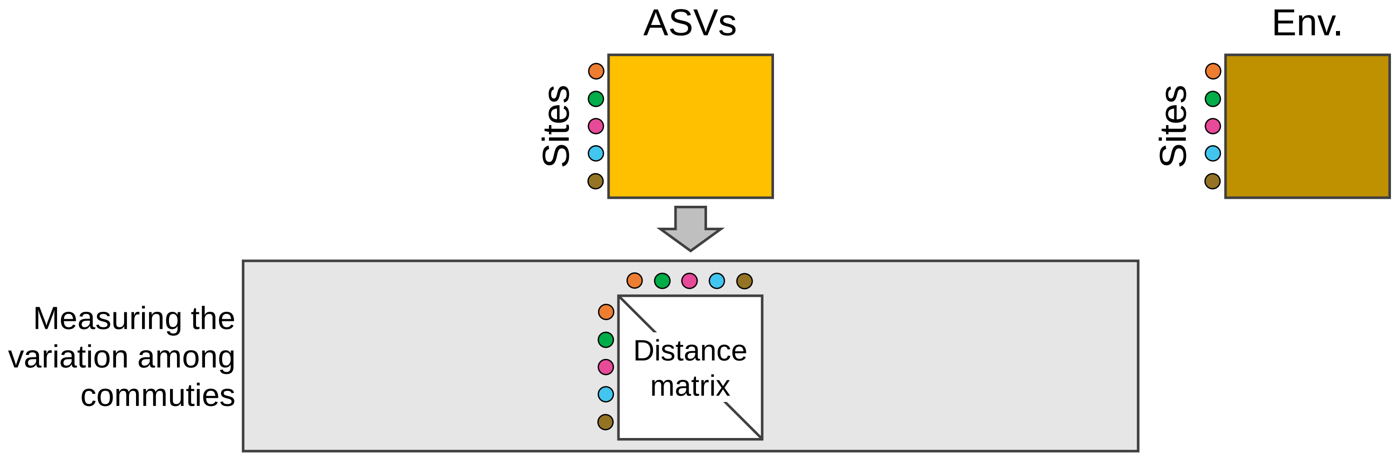
Measuring variation among communities





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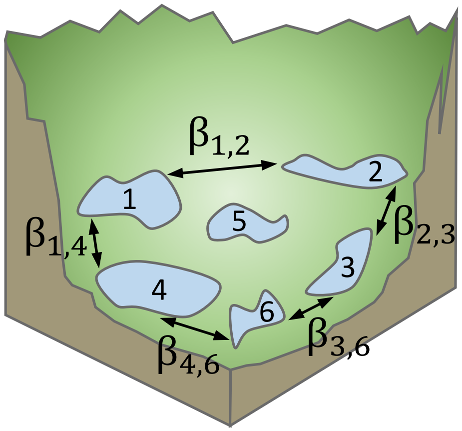

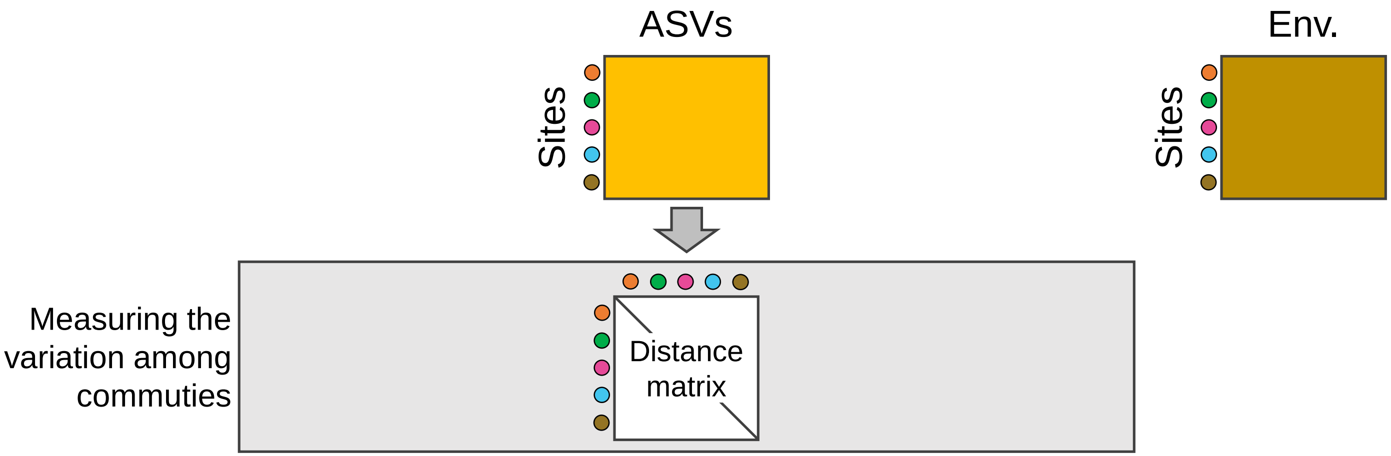

> 20 distance metrics available to estimate dissimilarity between communities (Legendre and Legendre 2012)

Bray-Curtis dissimilarity

$$D_{BC}(x_1, x_2) = \frac{\sum_{j=1}^{p} |y_{1j} - y_{2j}|}{\sum_{j=1}^{p} (y_{1j} + y_{2j})}$$

```
library(vegan)
D-BC <— vegdist(tab, method = "bray")
```

Hellinger distance

$$D_H(x_1, x_2) = \sqrt{\sum_{j=1}^{p} \left[\sqrt{\frac{y_{1j}}{y_{1+}}} - \sqrt{\frac{y_{2j}}{y_{2+}}} \right]^2}$$

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Aitchison distance

$$D_A(x_1, x_2) = \sqrt{\sum_{j=1}^{D} \left[ln \frac{x_{j1}}{g(x_1)} - ln \frac{x_{j2}}{g(x_2)} \right]^2}$$

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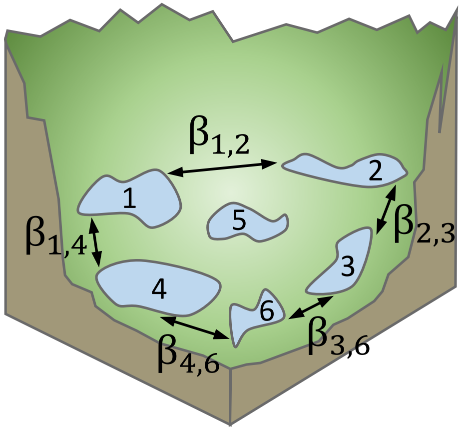
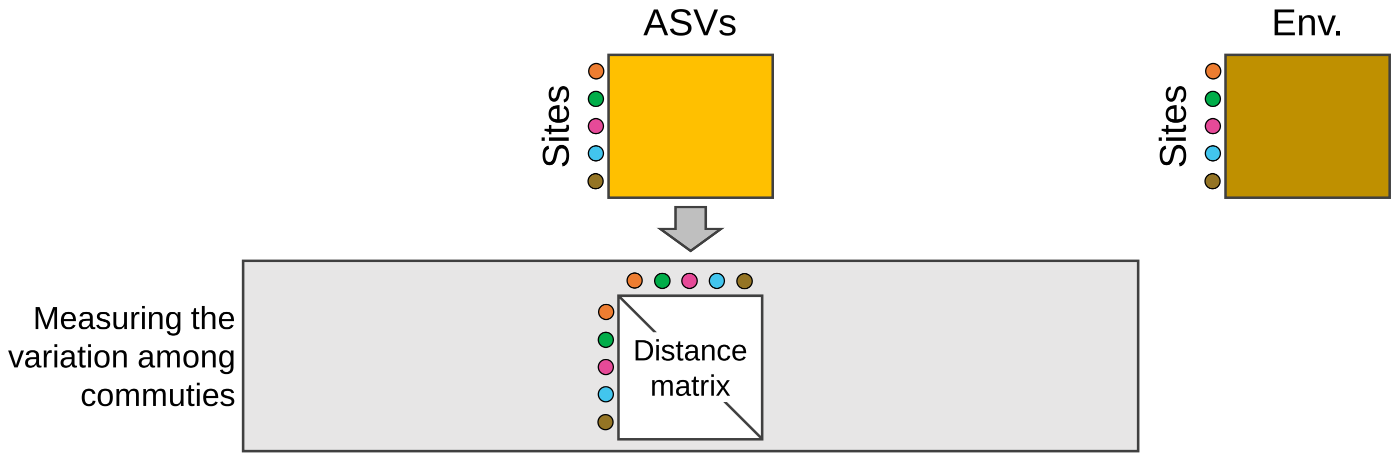
Measuring variation among communities





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Absolute abundances

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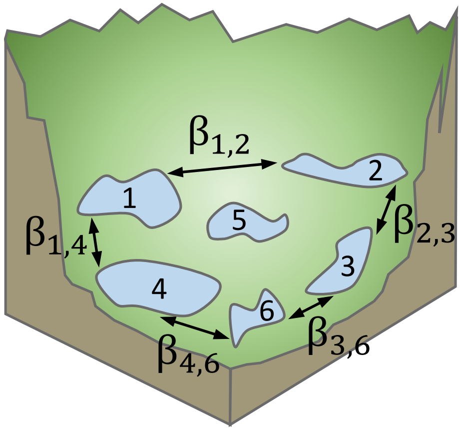

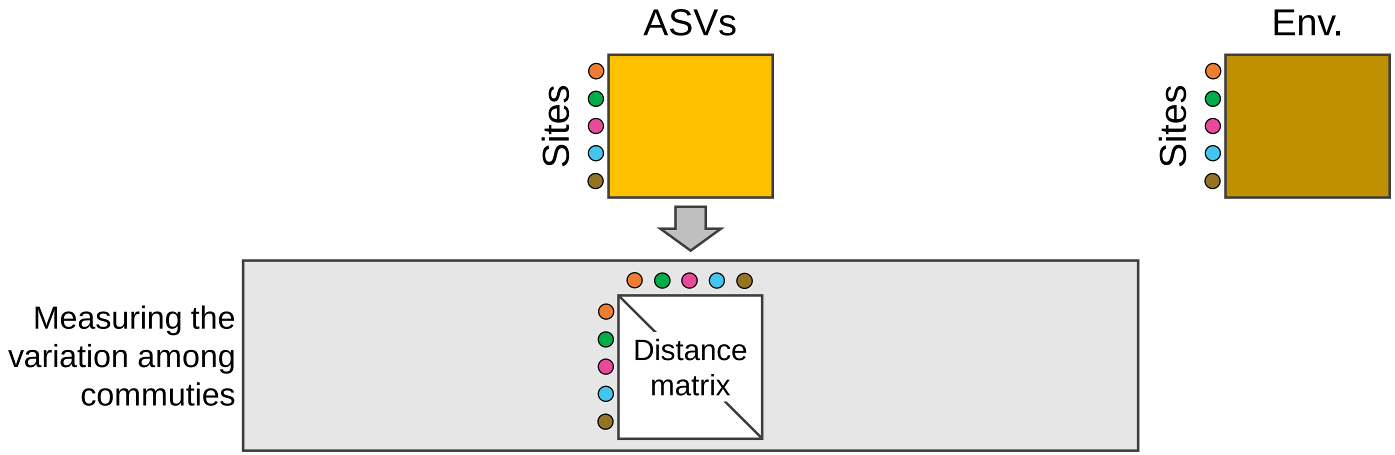

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Log-ratio transformation

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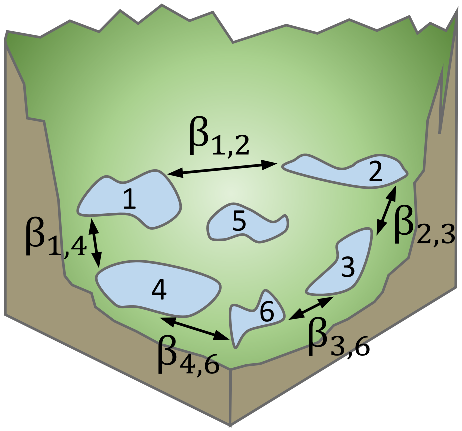
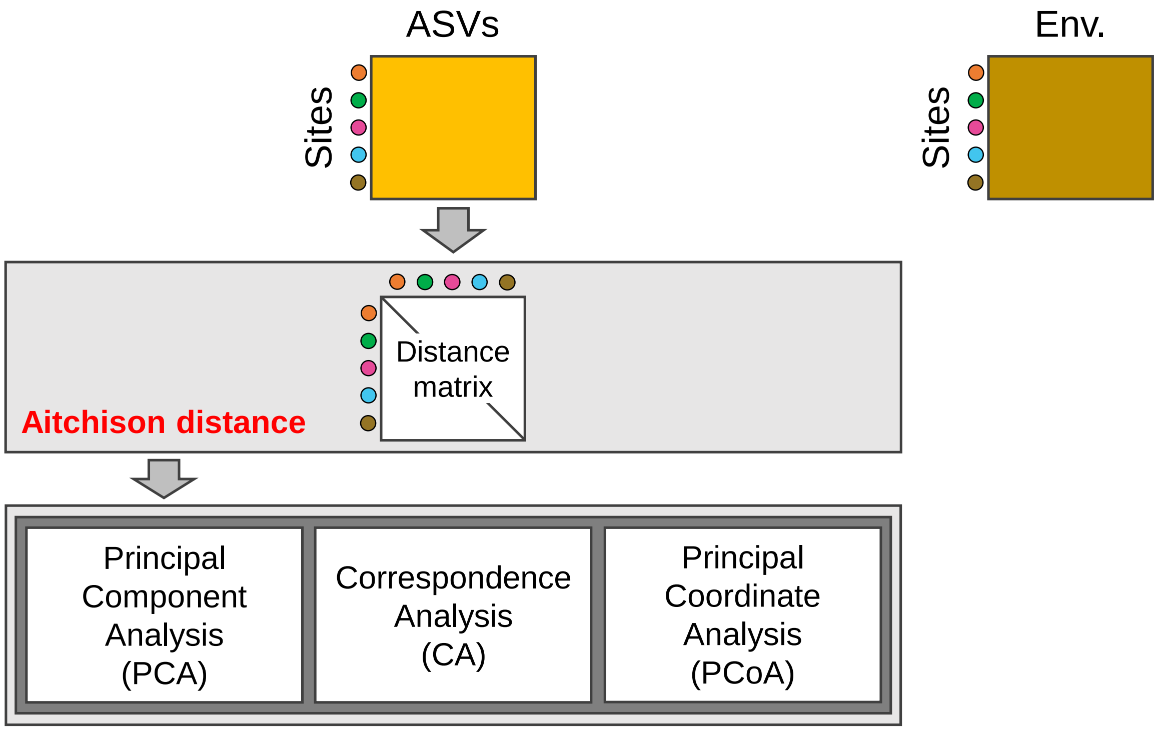
Bringing out a structure



Measuring the variation among commuties



Unconstrained ordinations

Bringing out a structure

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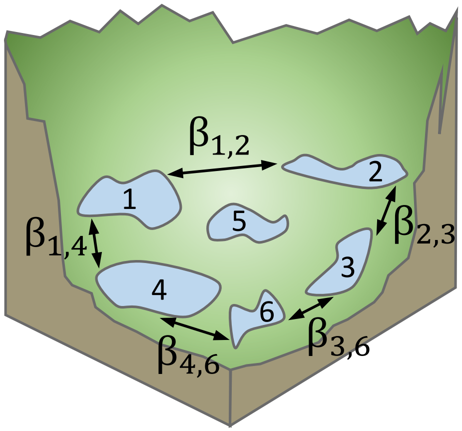
Bringing out a structure



Measuring the variation among commuties

Distance matrix

Aitchison distance

Bringing out a structure

Principal Component Analysis (PCA)	Correspondence Analysis (CA)	Principal Coordinate Analysis (PCoA)

Unconstrained ordinations

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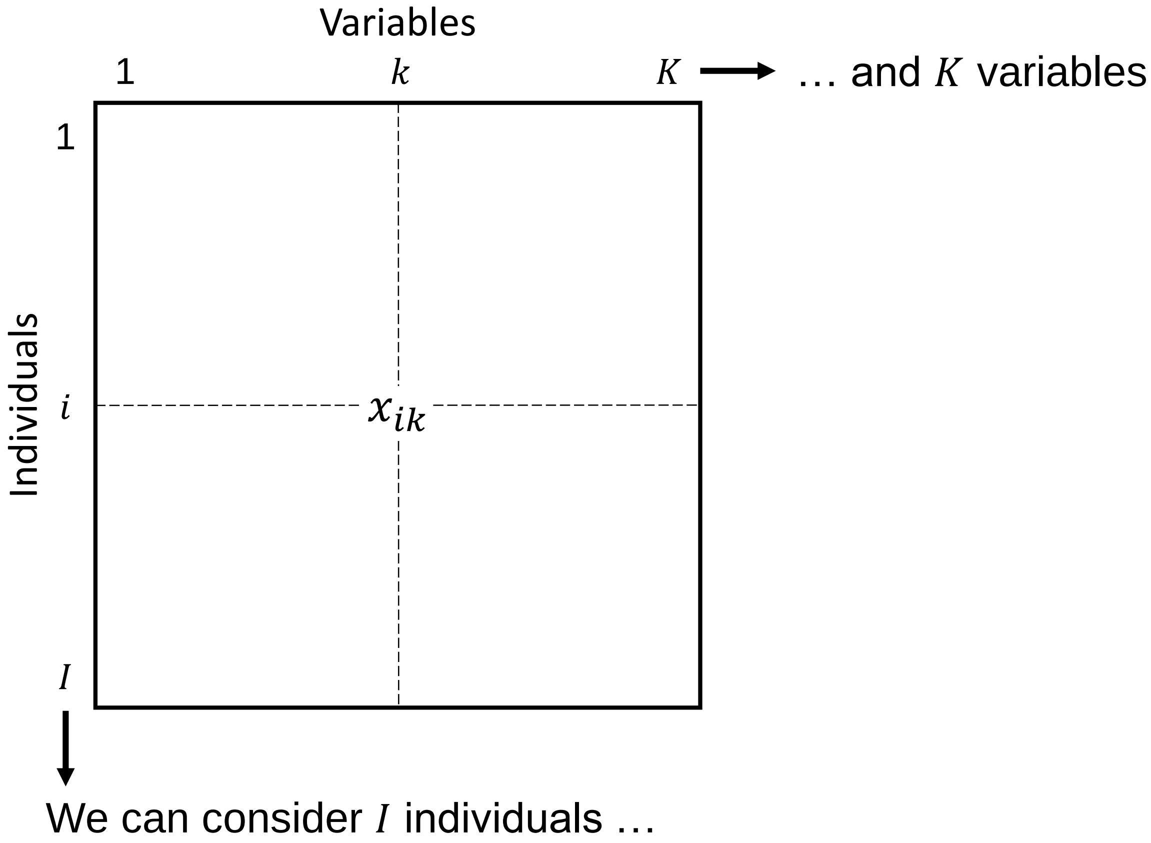
Bringing out a structure – PCA – type of data



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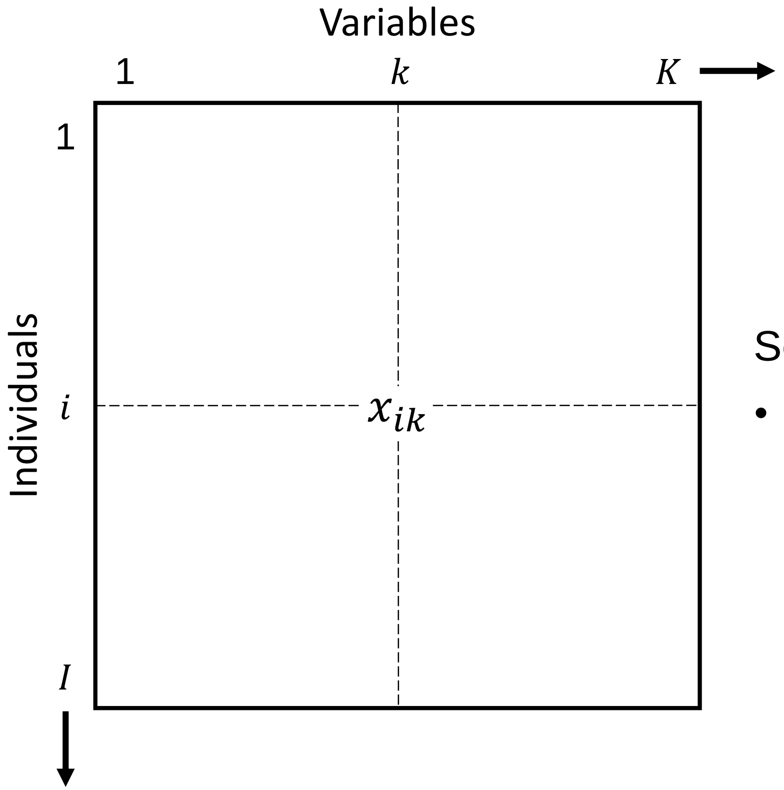
Bringing out a structure – PCA – type of data



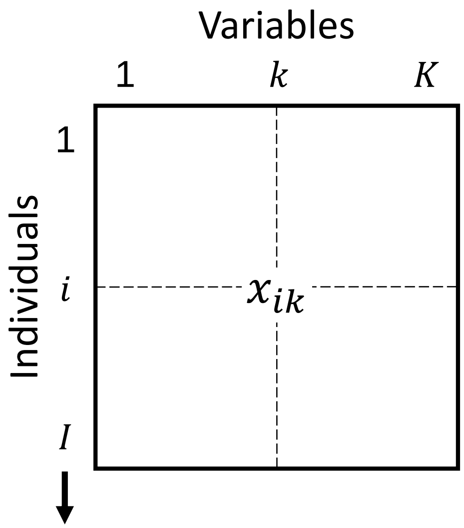
… and K variables

We can consider I individuals …

Several fields:

- Ecology: abundance of species k in lake i

Bringing out a structure – PCA – problems and objectives



I individuals $\in \mathbb{R}^K$

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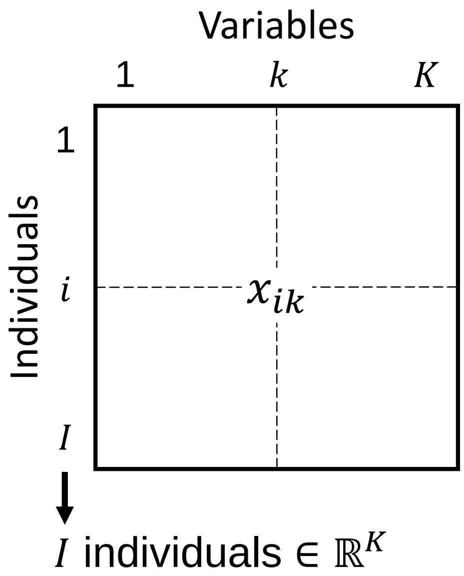

	Sp. 1	Sp. 2	Sp. 3	Sp. 4
Lake 1	12	8	14	7
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Lake 3	10	7	12	8
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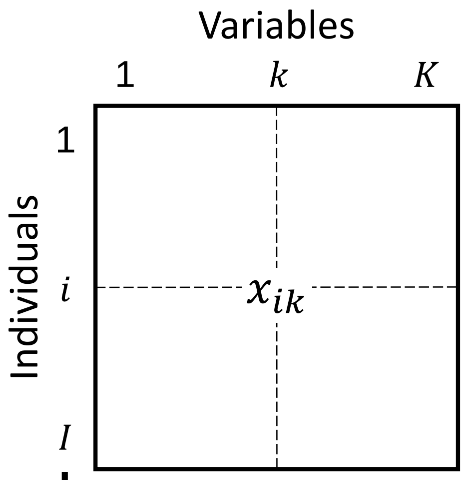

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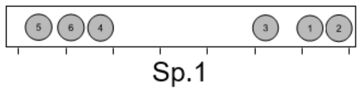
$K = 1$

If K = 1:
axial representation



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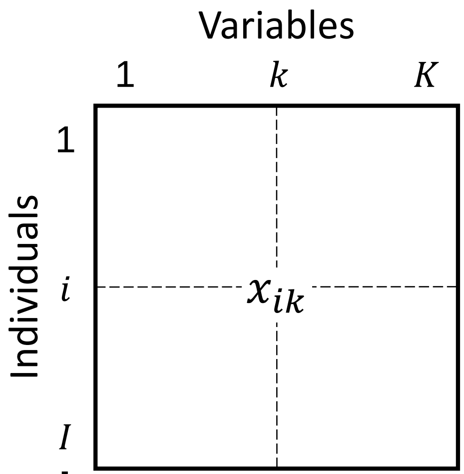

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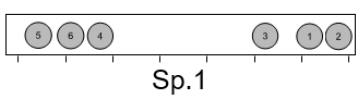
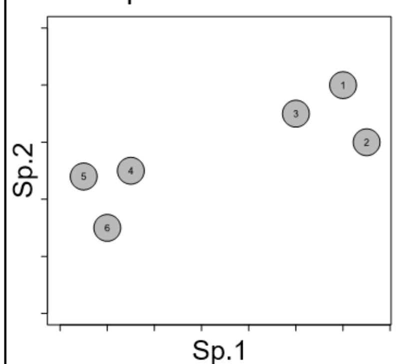
K = 2

If K = 1:
axial representation



If K = 2:
scatter plot



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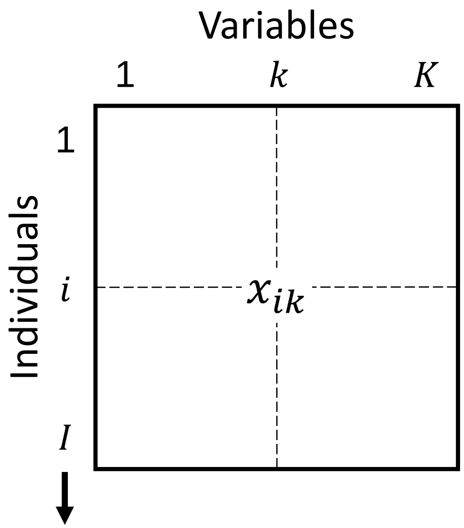

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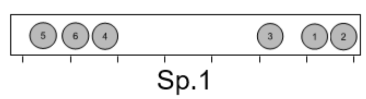
$K = 3$

If K = 1:
axial representation



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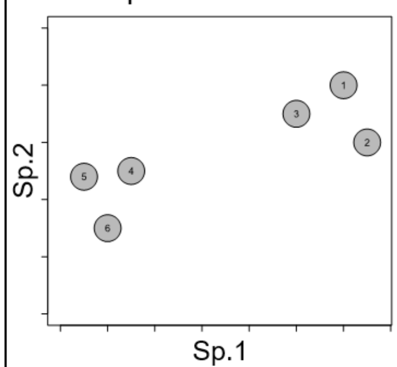
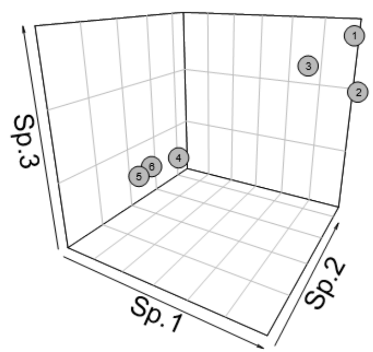
scatter plot



If K = 3:
3-dimensional graph



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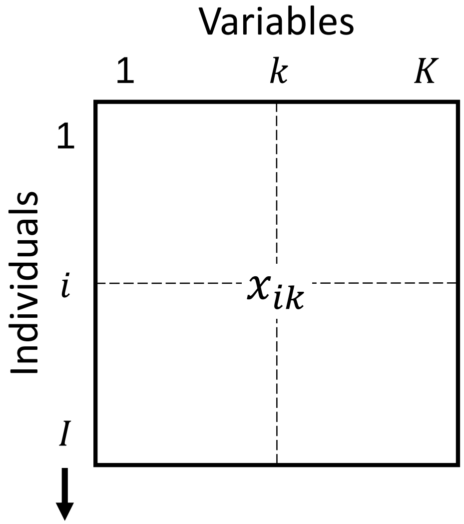
Bringing out a structure – PCA – problems and objectives



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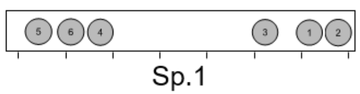
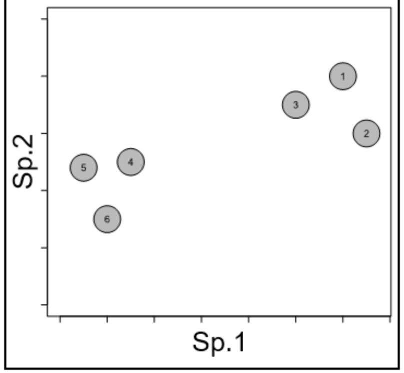
$K = 4$

If $K = 1$:
axial representation



If $K = 2$:
scatter plot



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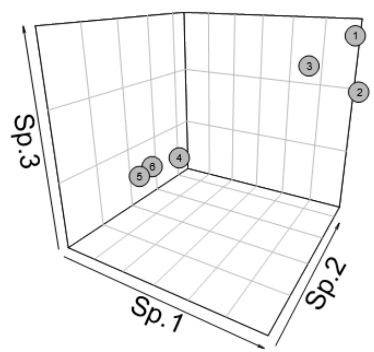

If $K \geq 4$:

Impossible to represent or even imagine, BUT simple mathematical concept: K coordinates on K dimensions

How can we visualize a scatterplot, if it evolves in a very large space ($K \geq 4$)

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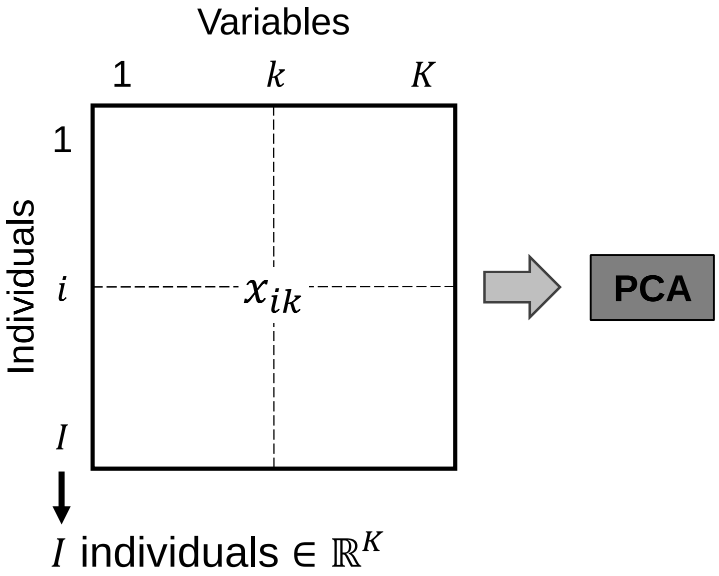
Bringing out a structure – PCA – problems and objectives

Variables: species

Individuals: lakes



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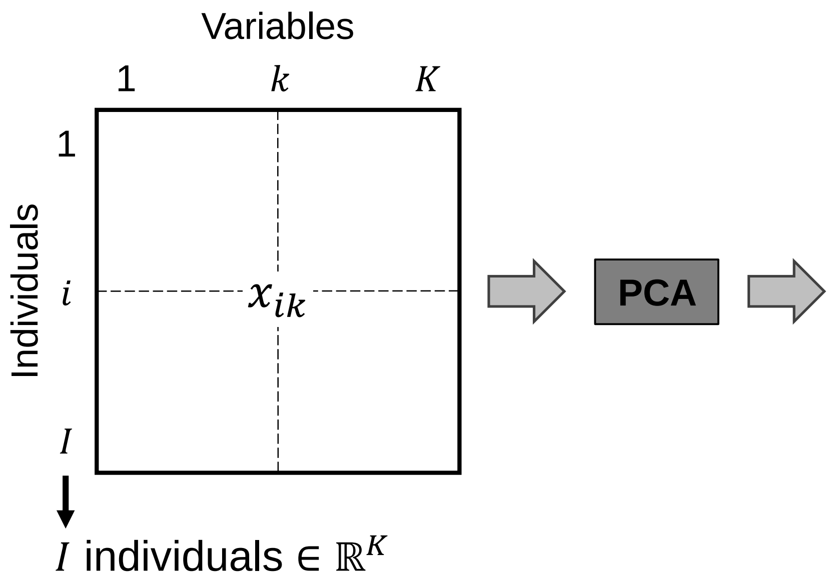

- Exploratory statistics, multidimensional descriptive statistics
- Synthetize, summarize and rank information contained in a table (high dimension, $K \geq 4$)
- Visualize data tables using simple grapics (2D, 3D)

PCA ≡ Analyze a table with K dimensions (or variables, ≥ 4) measured on I individuals

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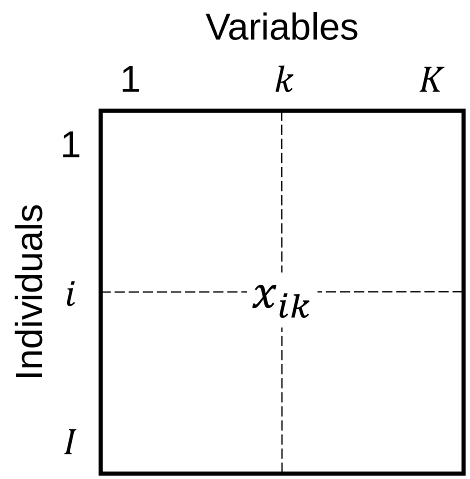
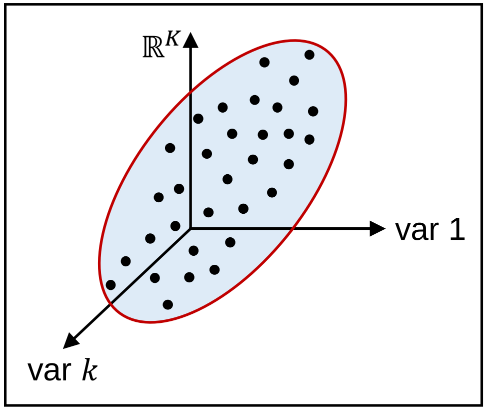
Bringing out a structure – PCA – cloud of individuals

Variables: species

Individuals: lakes



A set of lines …



i point-lines $\in \mathbb{R}^K$

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Bringing out a structure – PCA – cloud of individuals

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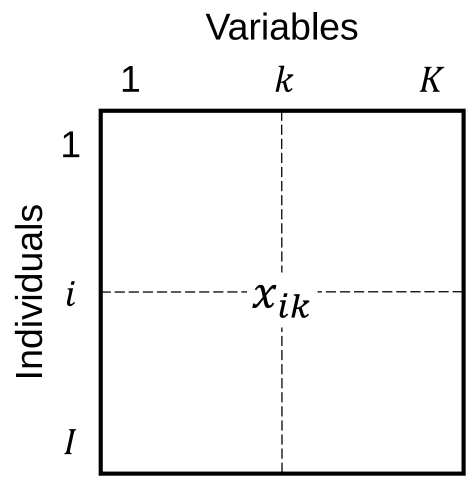

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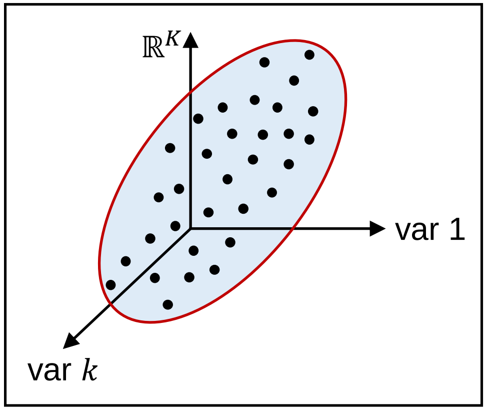

i point-lines $\in \mathbb{R}^K$

- Similarity between individuals as a function of variables
- Groups of individuals

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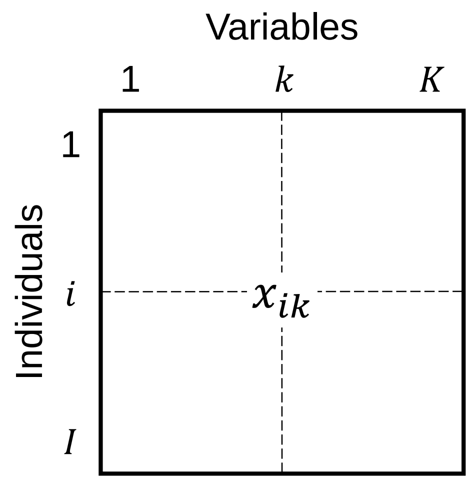
Bringing out a structure – PCA – study of individuals (N^I)

Variables: species

Individuals: lakes



i point-lines $\in \mathbb{R}^K$

Notion of **similarity** between individuals i and i'.

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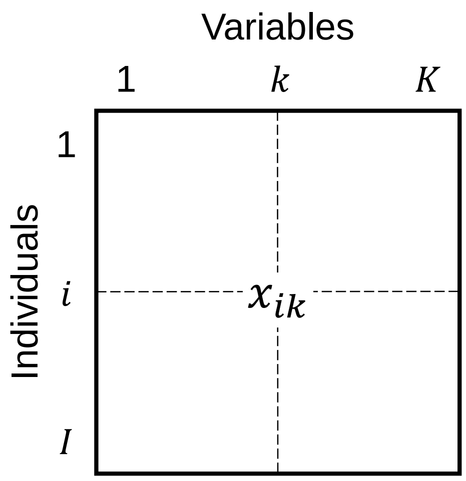

i point-lines $\in \mathbb{R}^K$

Notion of **similarity** between individuals i and i'.

$$d^2(i,i') = \sum_{k=1}^{K} (x_{ik} - x_{i'k})^2$$ [Pythagoras]

i: individual 1

i': individual 2

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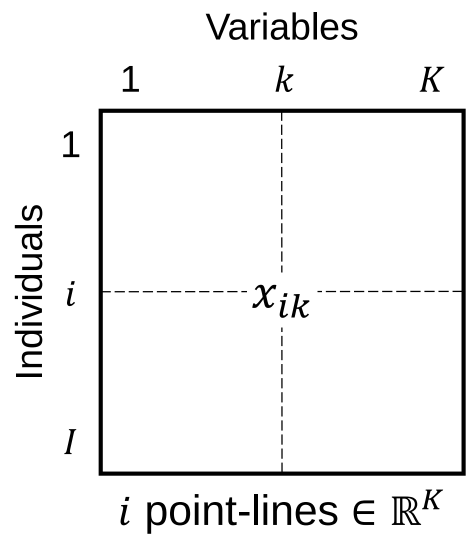
Bringing out a structure – PCA – study of individuals (N^I)

Variables: species

Individuals: lakes



i: individual 1

i': individual 2

Notion of **similarity** between individuals i and i'.

$$d^2(i,i') = \sum_{k=1}^{K} (x_{ik} - x_{i'k})^2 \quad \text{[Pythagoras]}$$

Study of individuals $\equiv$ Study of distance between individuals $\equiv$ Study of the shape of cloud N^I

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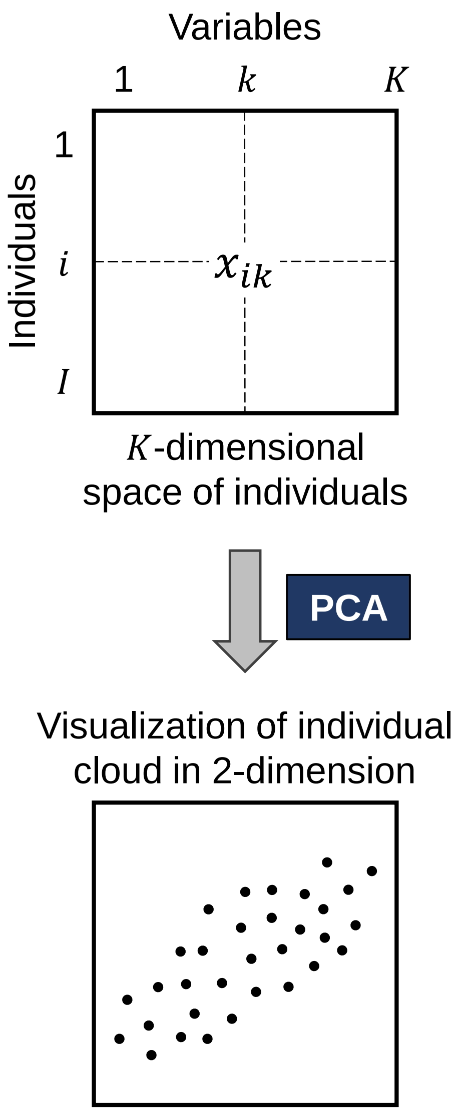
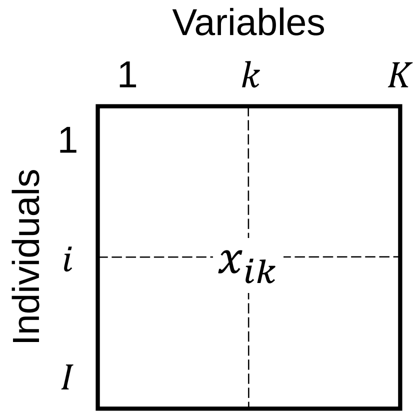
the European Union

Bringing out a structure – PCA – study of individuals (N^I)



Bringing out a structure – PCA – study of individuals (N^I)



K-dimensional space of individuals

PCA

Visualization of individual cloud in 2-dimension

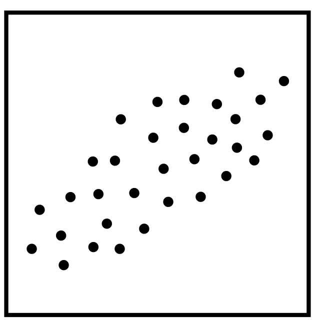
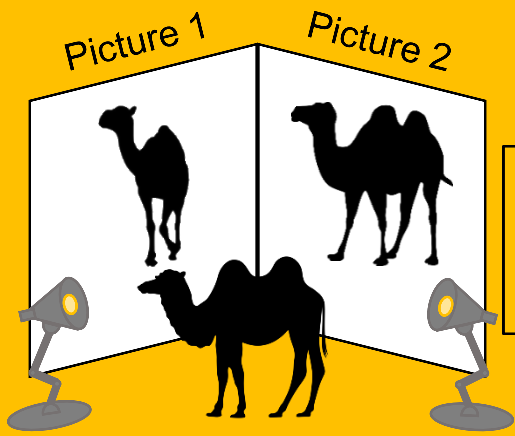


Image quality



PCA

- Accurately reproduces cloud shape
- Idea of distance between idividuals
- Distances as close as possible

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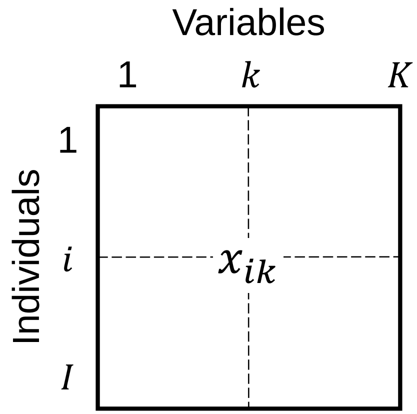
Bringing out a structure – PCA – study of individuals (N^I)



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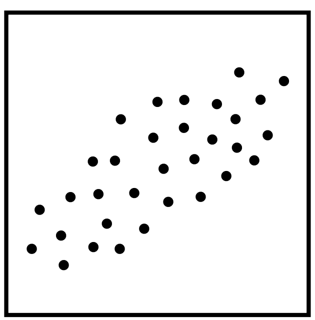

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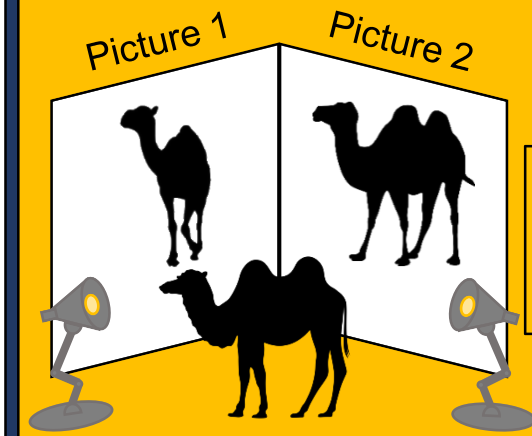

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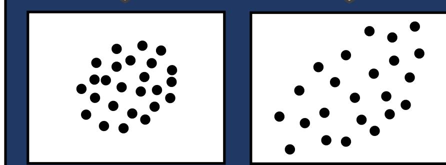

Quantify quality

Low dispersion → Poor-fitted picture

High dispersion → Well-fitted picture

Notion of dipersion ≡ Variability ≡ Inertia (several dimensions)

High dispersion ≡ High variability ≡ Well-fitted picture

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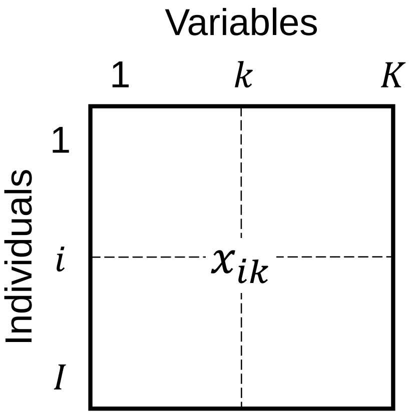
Bringing out a structure – PCA – study of individuals (N^I)



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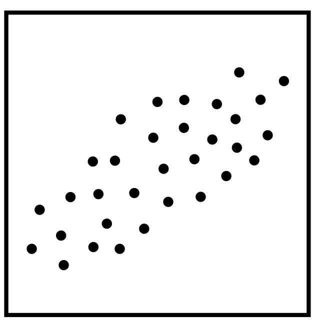

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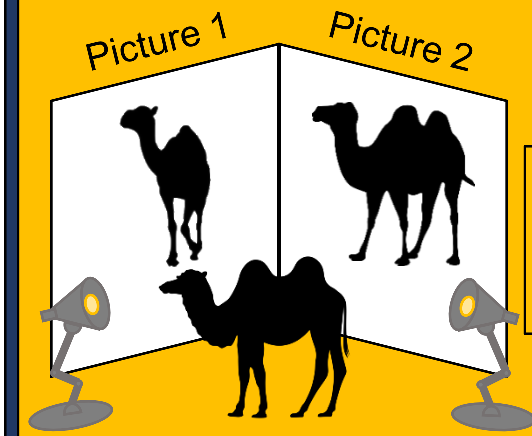

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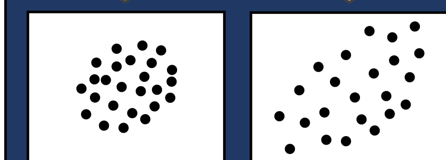

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Objective

Search for a subspace that best summarizes the data (i.e., principal components)

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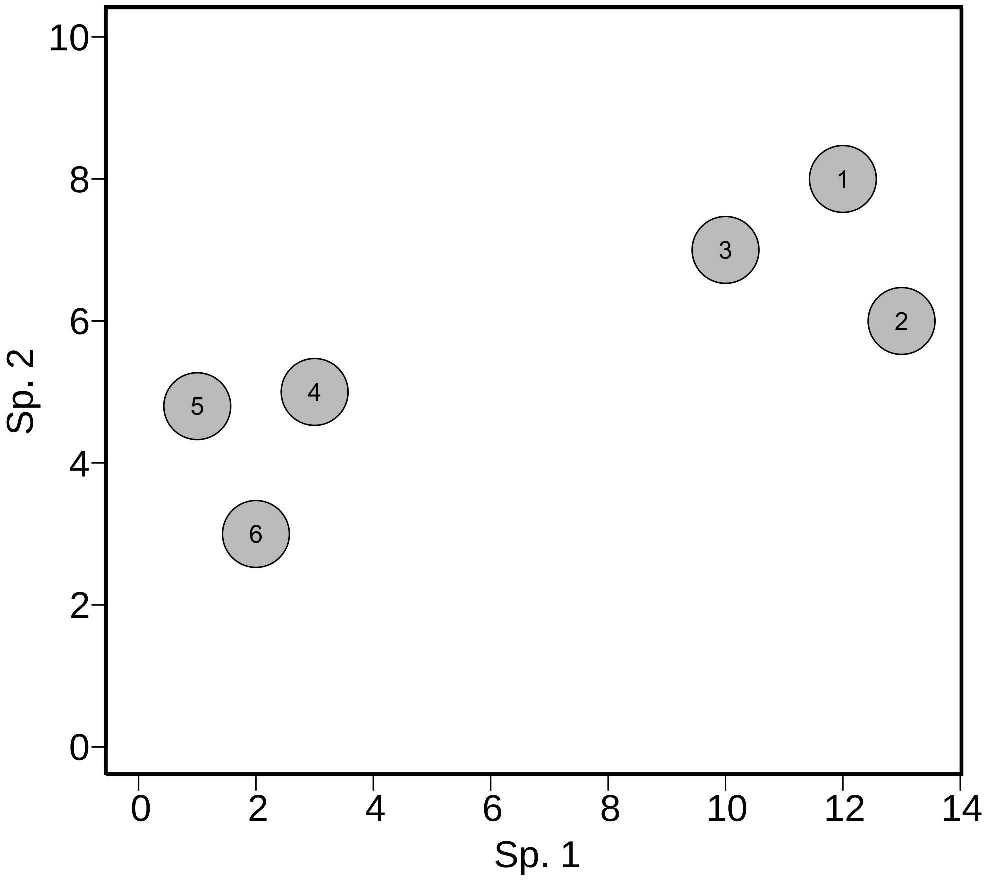
Bringing out a structure – PCA – study of individuals (N^I)

We start by plotting the data …

	Sp. 1	Sp. 2
Lake 1	12	8
Lake 2	13	6
Lake 3	10	7
Lake 4	3	5
Lake 5	1	4.8
Lake 6	2	3



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Bringing out a structure – PCA – study of individuals (N^I)

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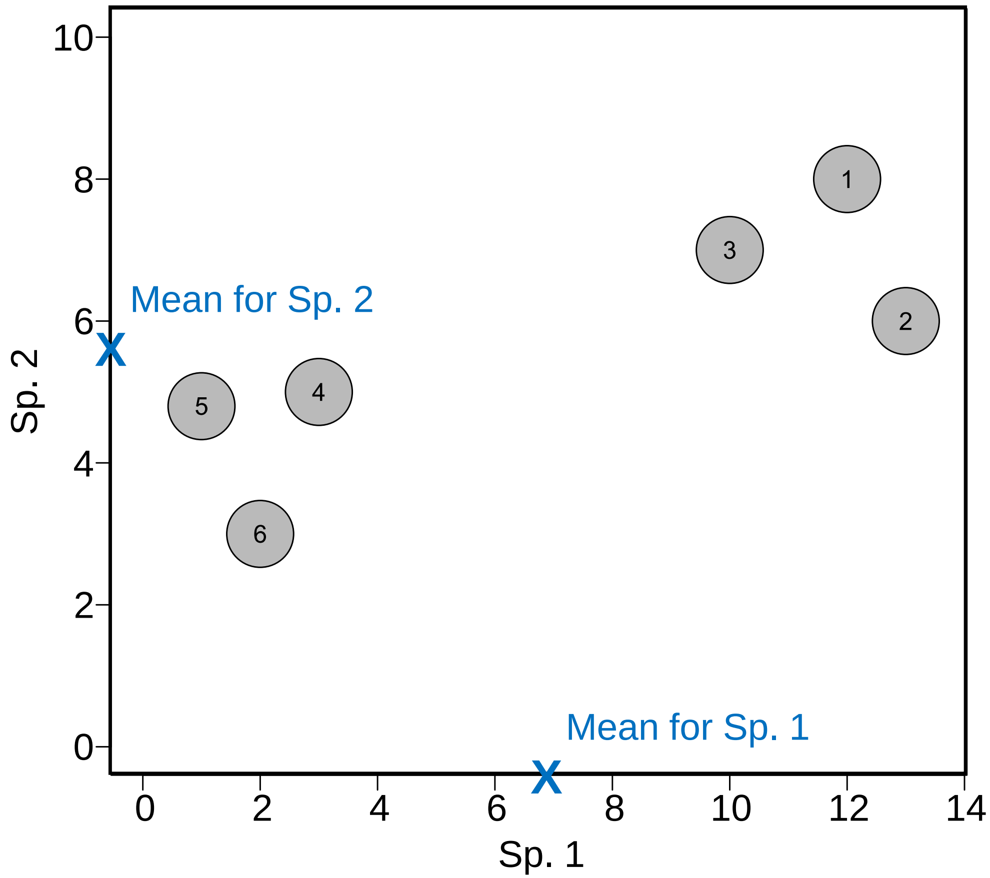

Then we calculate the average values for Sp. 1 and Sp. 2 …

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Bringing out a structure – PCA – study of individuals (N^I)

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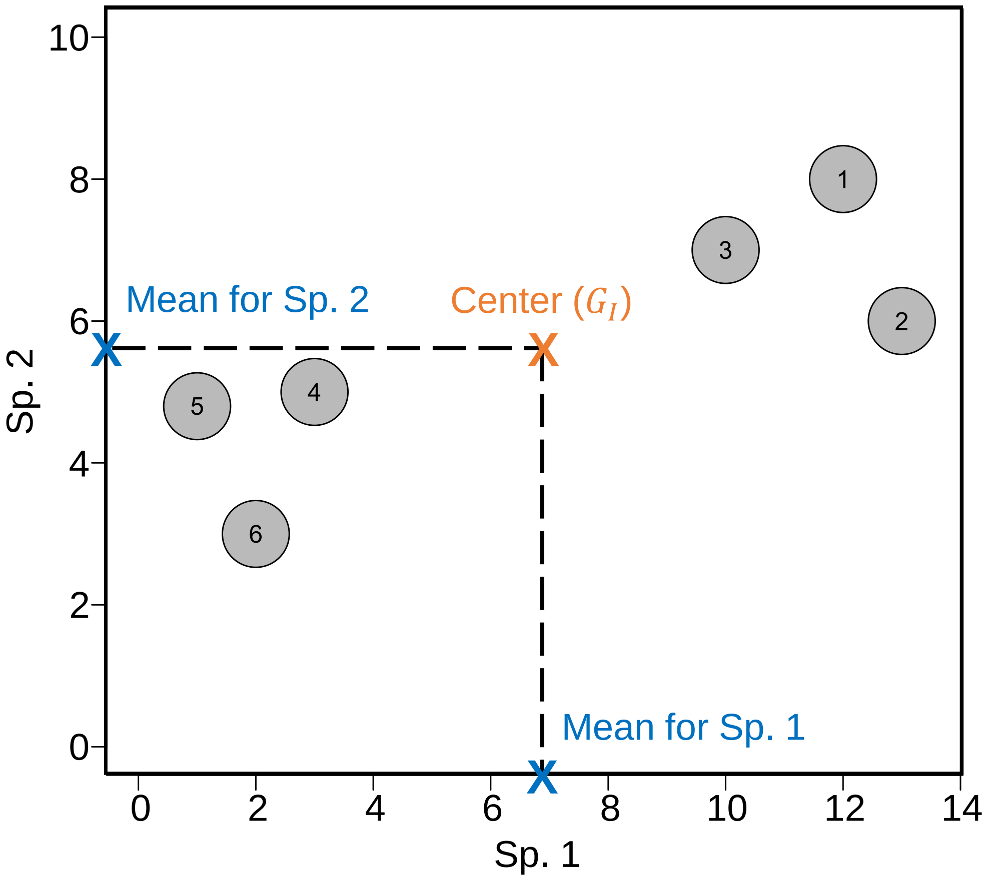

Then we calculate the average values for Sp. 1 and Sp. 2 …

With the average values, we can calculate the center of the data.

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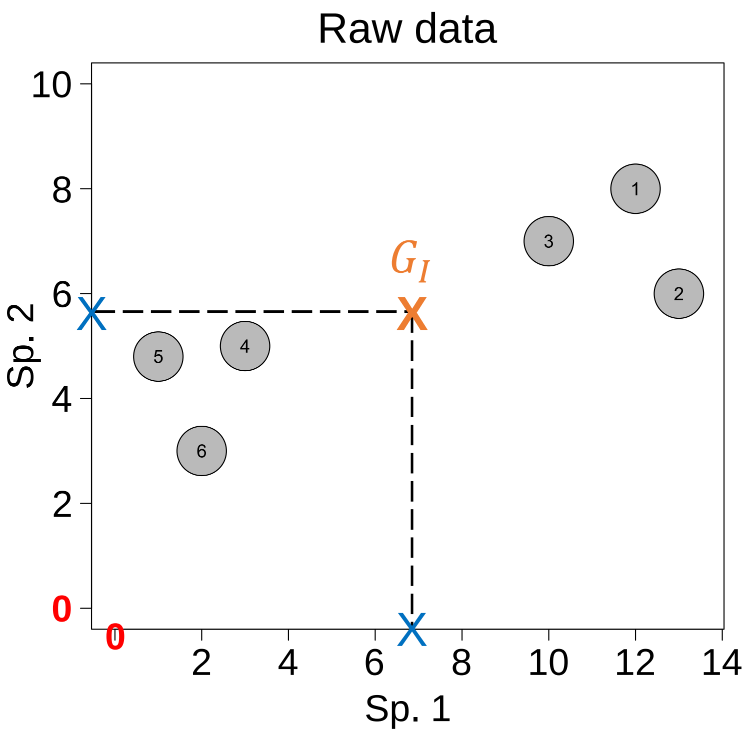
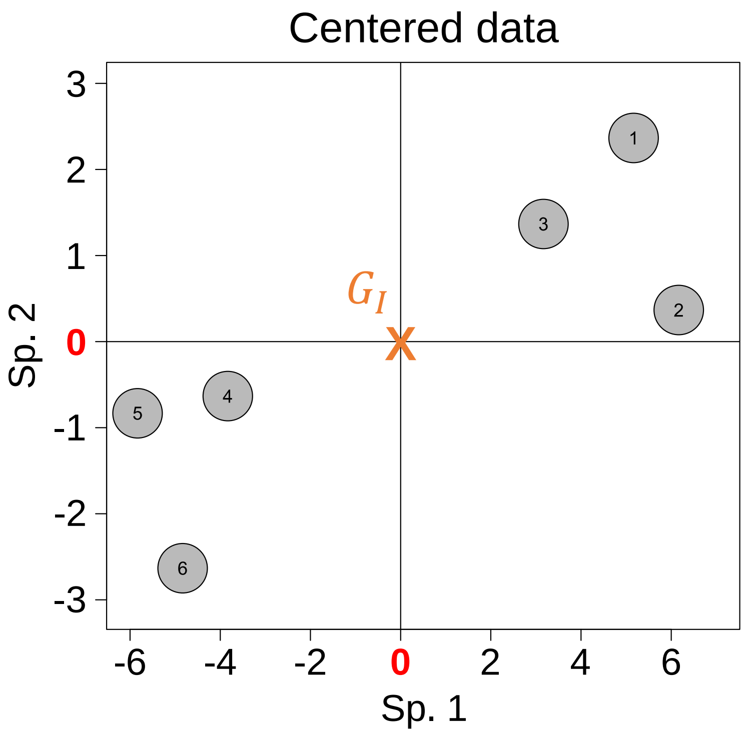
Bringing out a structure – PCA – study of individuals (N^I)





Then we centered the data … ⟶ $y_{ik} = x_{ik} - \overline{x}_{ik}$

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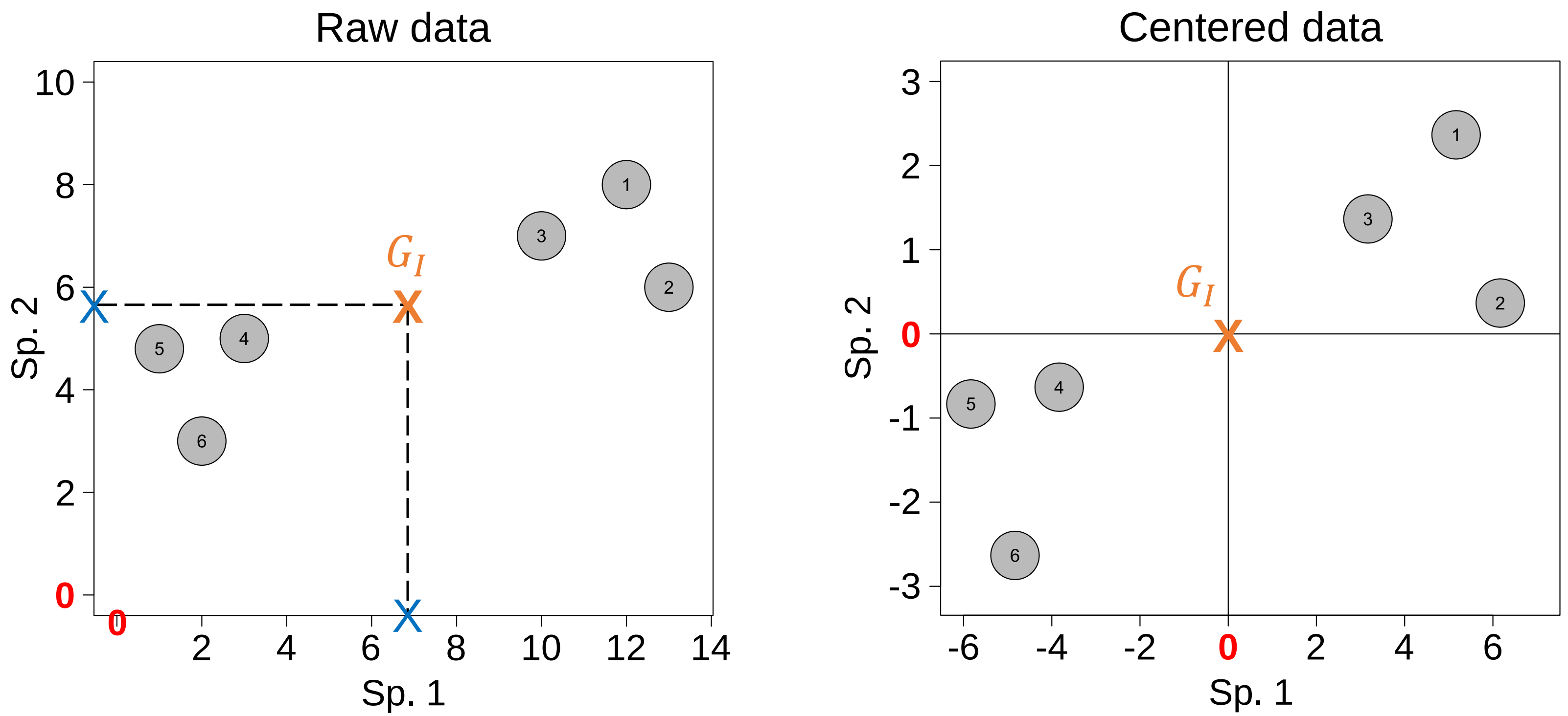
Bringing out a structure – PCA – study of individuals (N^I)



Then we centered the data … ⟶ $y_{ik} = x_{ik} - \overline{x}_{ik}$

NOTE 1: Centered ≡ Translation of cloud ⟶ No change in shape of cloud

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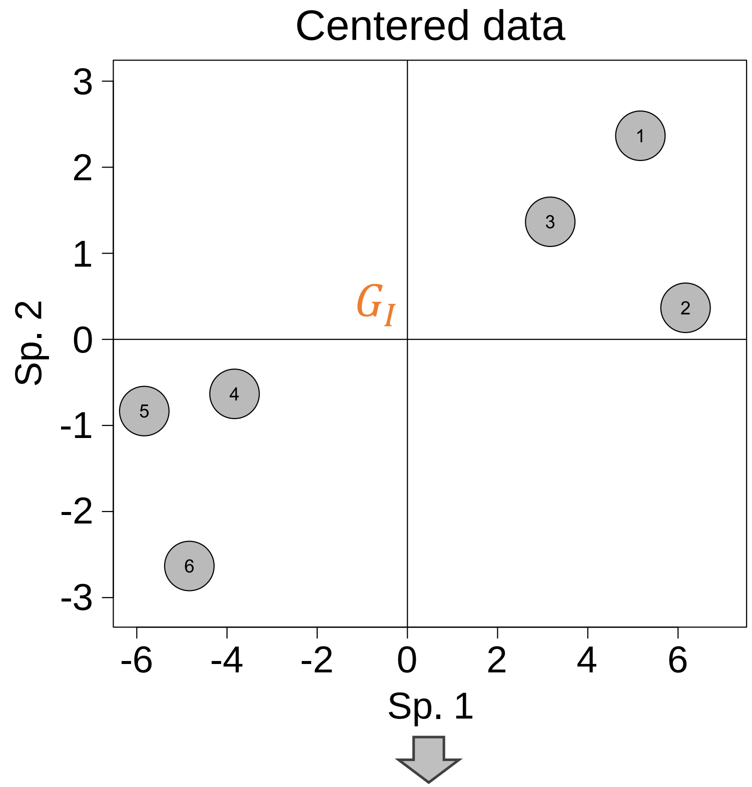
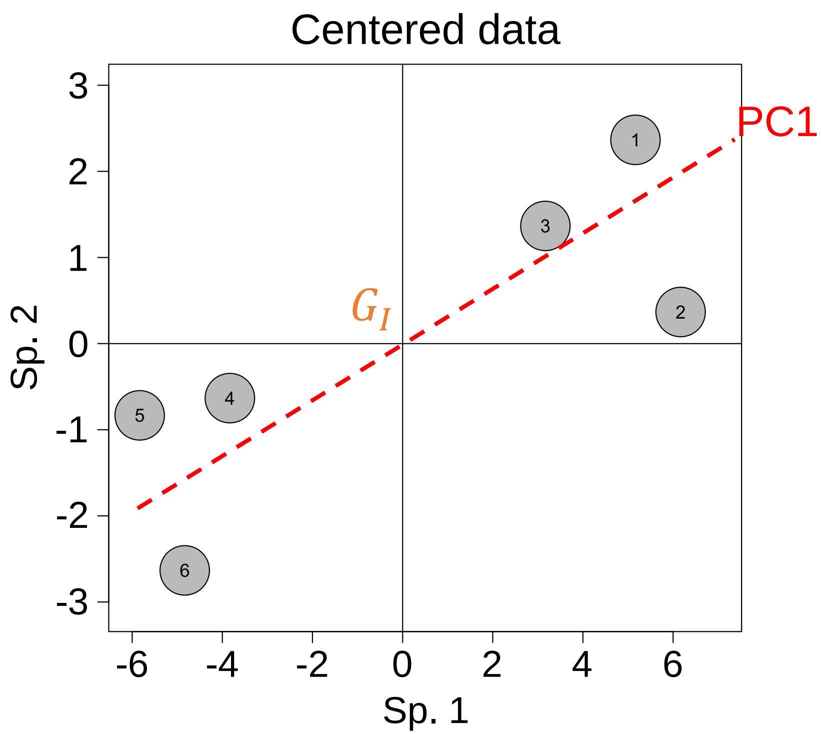
Bringing out a structure – PCA – study of individuals (N^I)



Subspace providing the best approximate picture of the cloud of individuals

Bringing out a structure – PCA – study of individuals (N^I)



Subspace providing the best approximate picture of the cloud of individuals

- Find the first axis (PC1) goes through the origin (0, 0) in the K-dimensional variable space

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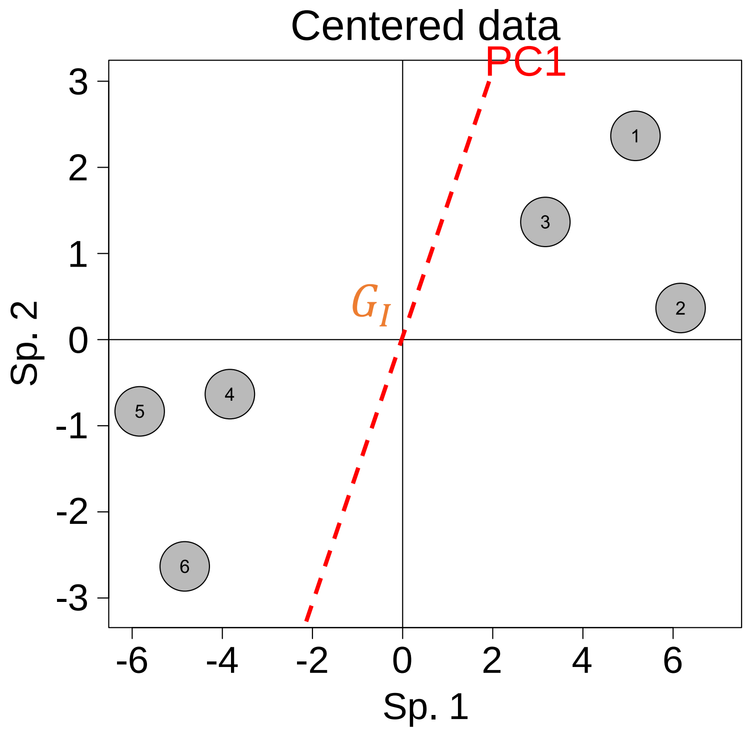
Bringing out a structure – PCA – study of individuals (N^I)



Subspace providing the best approximate picture of the cloud of individuals

- Find the first axis (PC1) goes through the origin (0, 0) in the K-dimensional variable space
- Best possible fit to the data, in the least-squares sense

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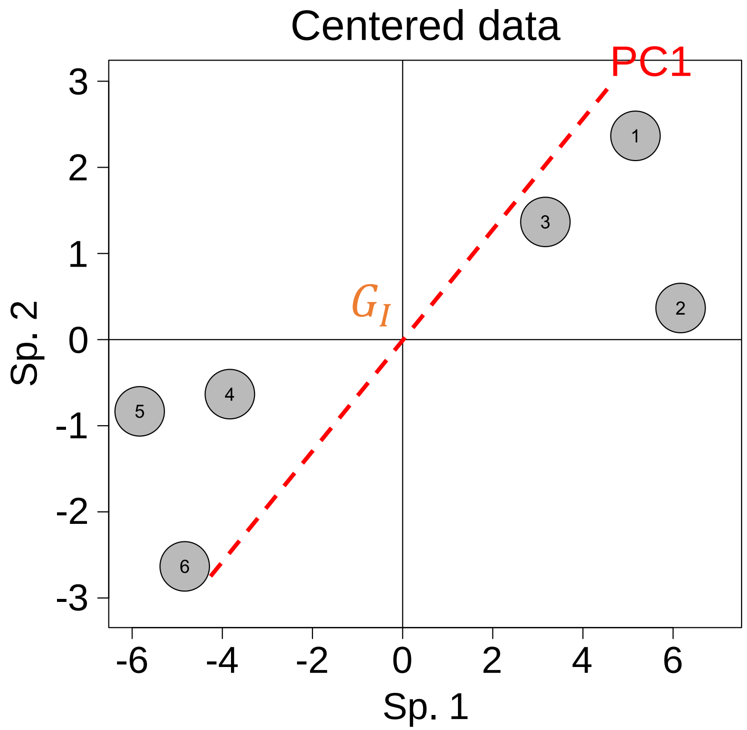
the European Union

Bringing out a structure – PCA – study of individuals (N^I)



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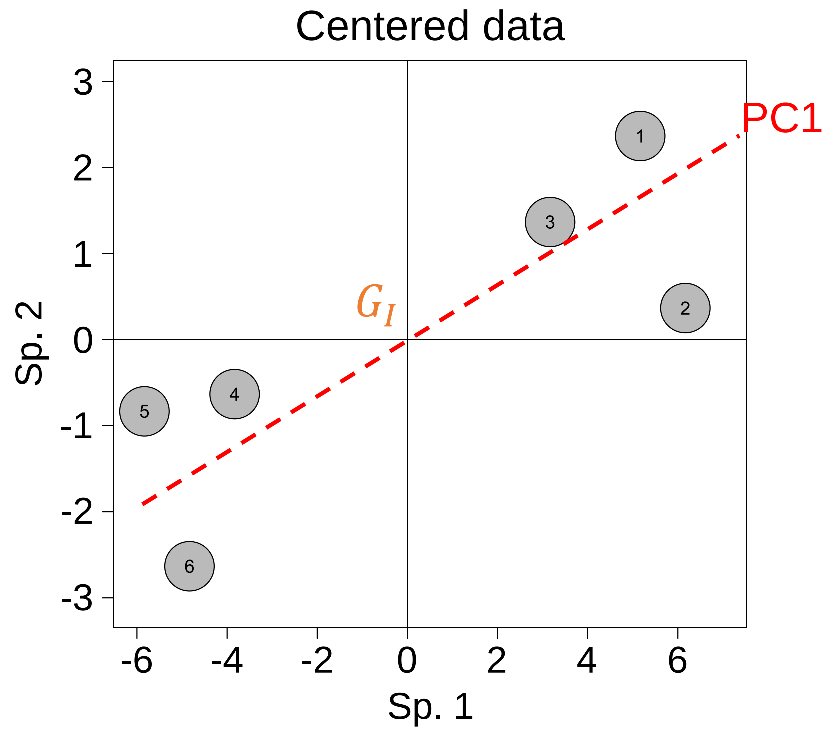

Subspace providing the best approximate picture of the cloud of individuals

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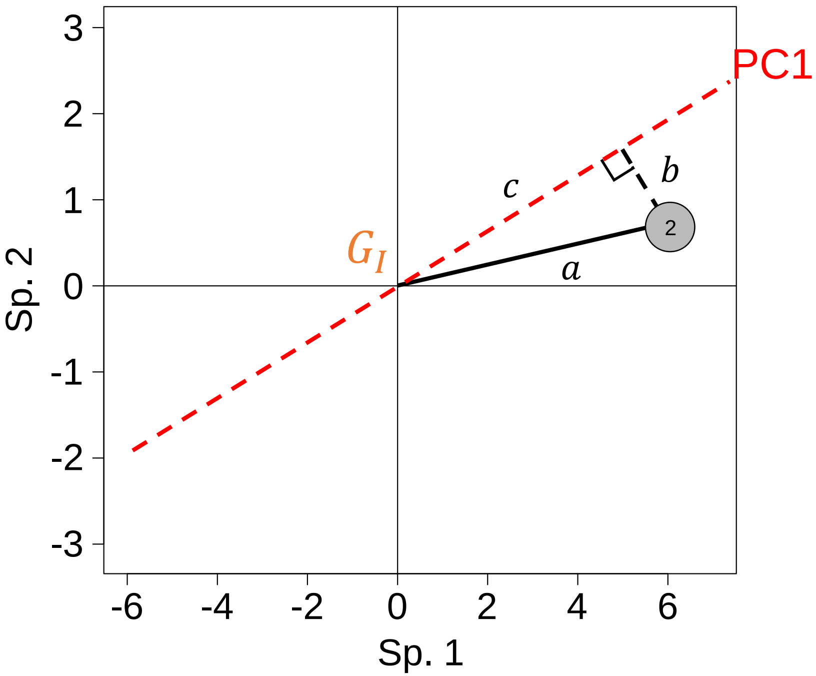

- $a \longrightarrow$ distance from the point to the origin
- $b \longrightarrow$ distance from the point to its projection on the axis
- $c \longrightarrow$ distance from the projection to the origin

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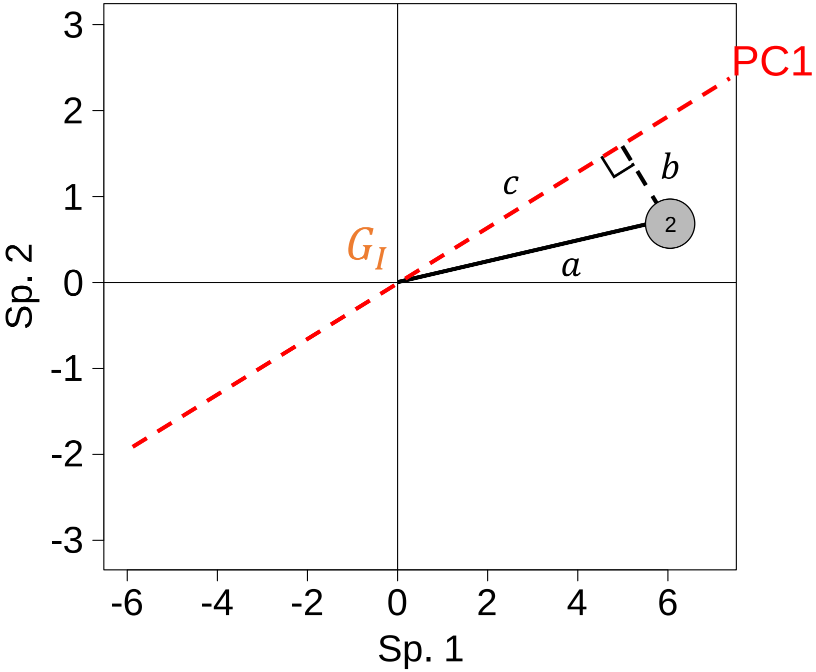
Bringing out a structure – PCA – study of individuals (N^I)



- $a \longrightarrow$ constant distance
- b is small <=> c is large

  $a^2 = b^2 + c^2$ [Pythagoras]
- Minimizing b (or b^2) ≡ Maximizing c (or c^2)
- $I_{total} = I_{PC1} + I_{residual}$

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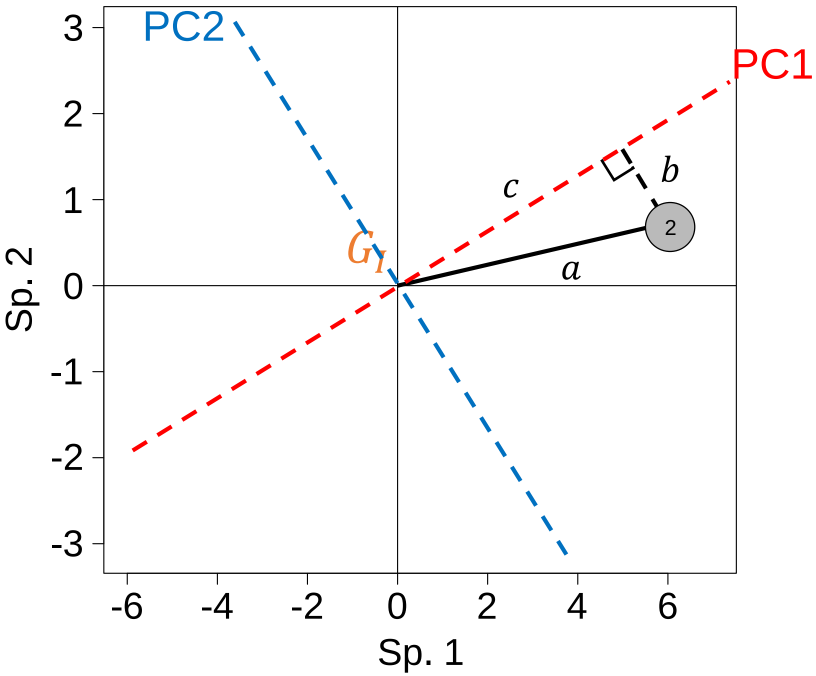
Bringing out a structure – PCA – study of individuals (N^I)



- We look for PC2 ⊥ PC1

  ⊥ (orthogonal) ≡ no correlation ≡ independent
- PC1 + PC2 ≡ best projection plane for the cloud of individuals
- Look for a 3th axis and sequentially the others (⊥)
- $I_{total} = I_{PC1} + \underbrace{I_{PC2} + I_{PC3} + \cdots + I_{PCK}}_{I_{residual}}$

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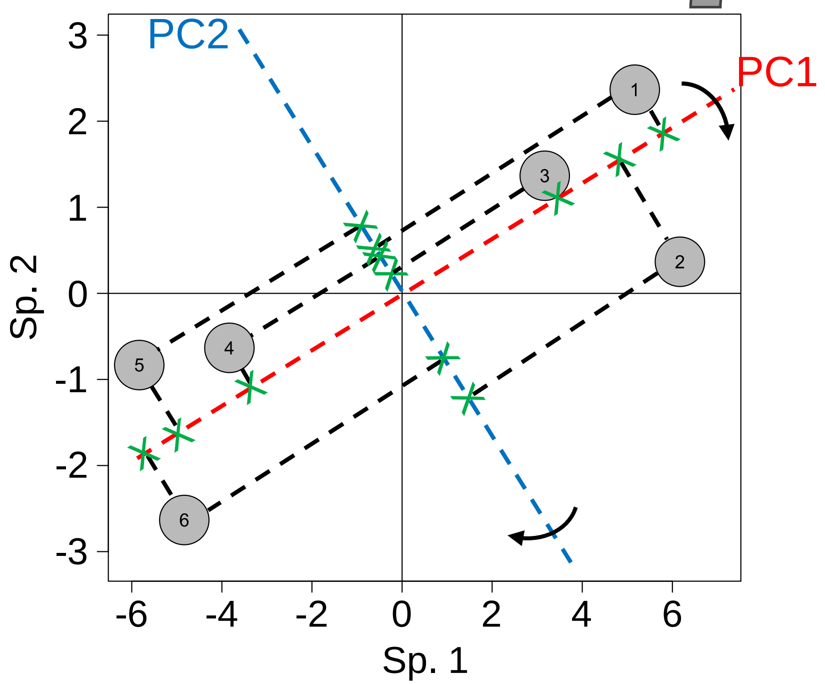
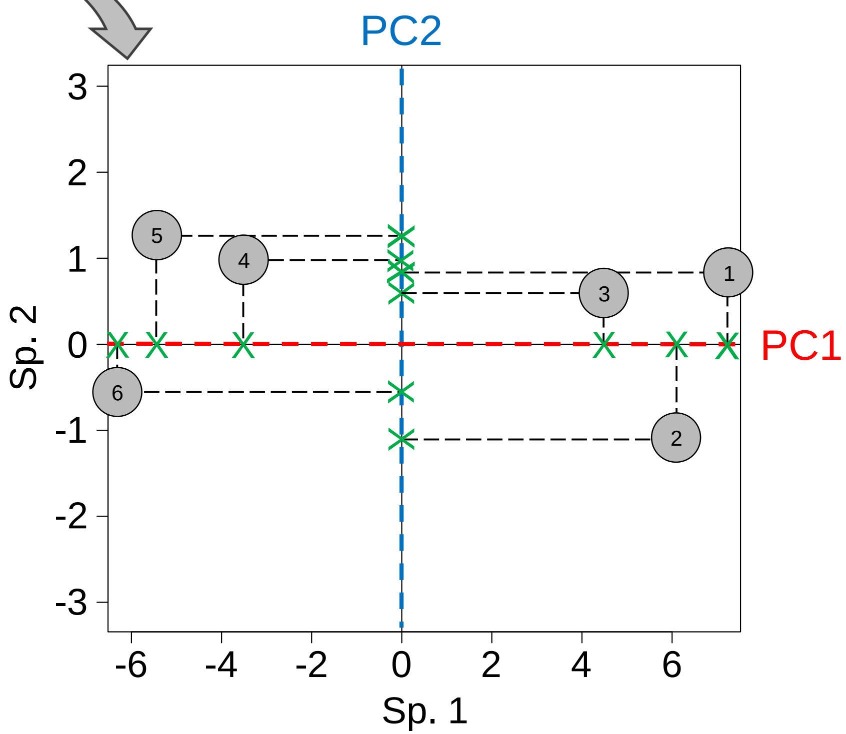
Bringing out a structure – PCA – study of individuals (N^I)

Rotate axes so that
PC1 is horizontal





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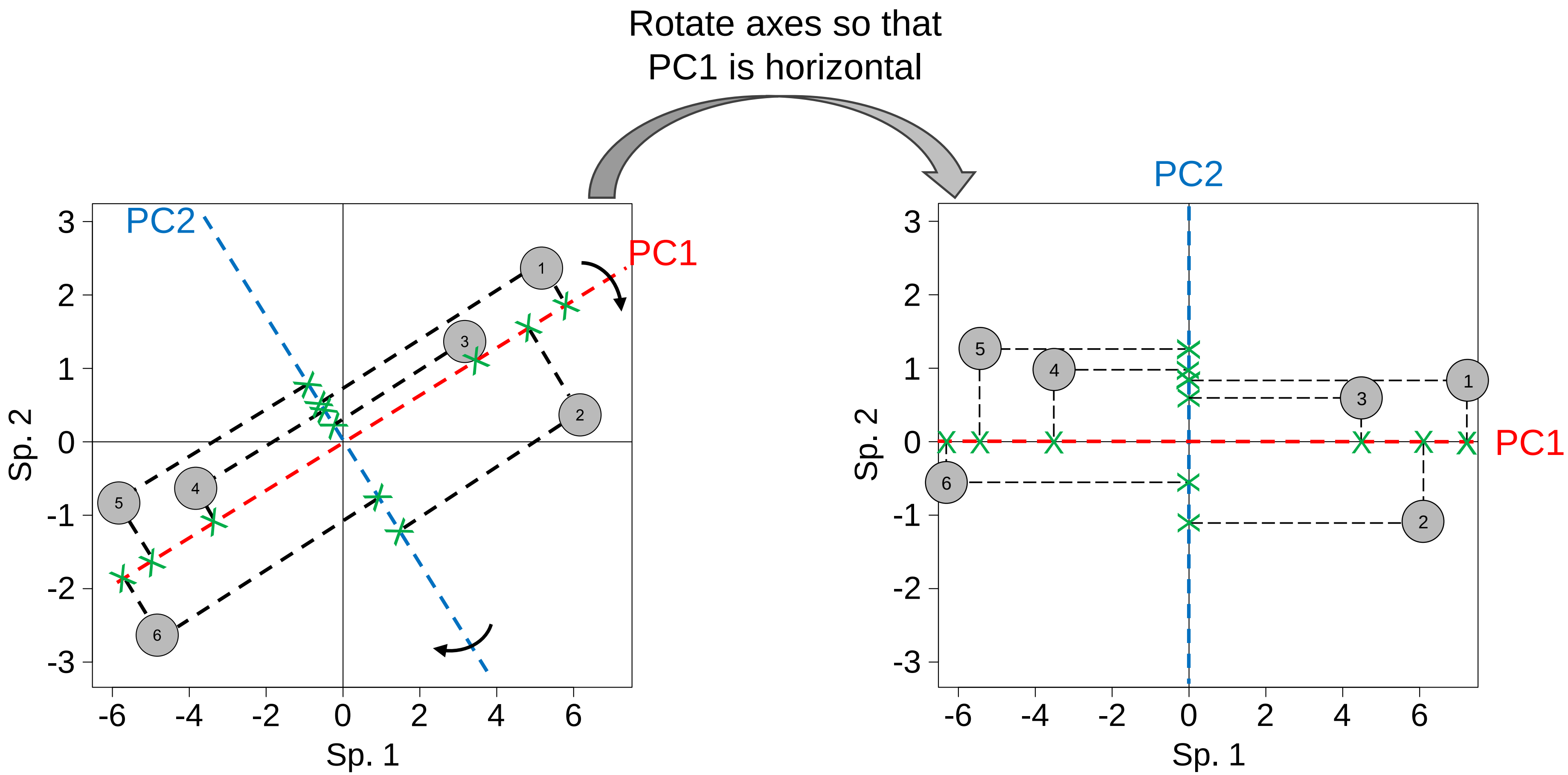
Bringing out a structure – PCA – study of individuals (N^I)



Great, we made a PCA

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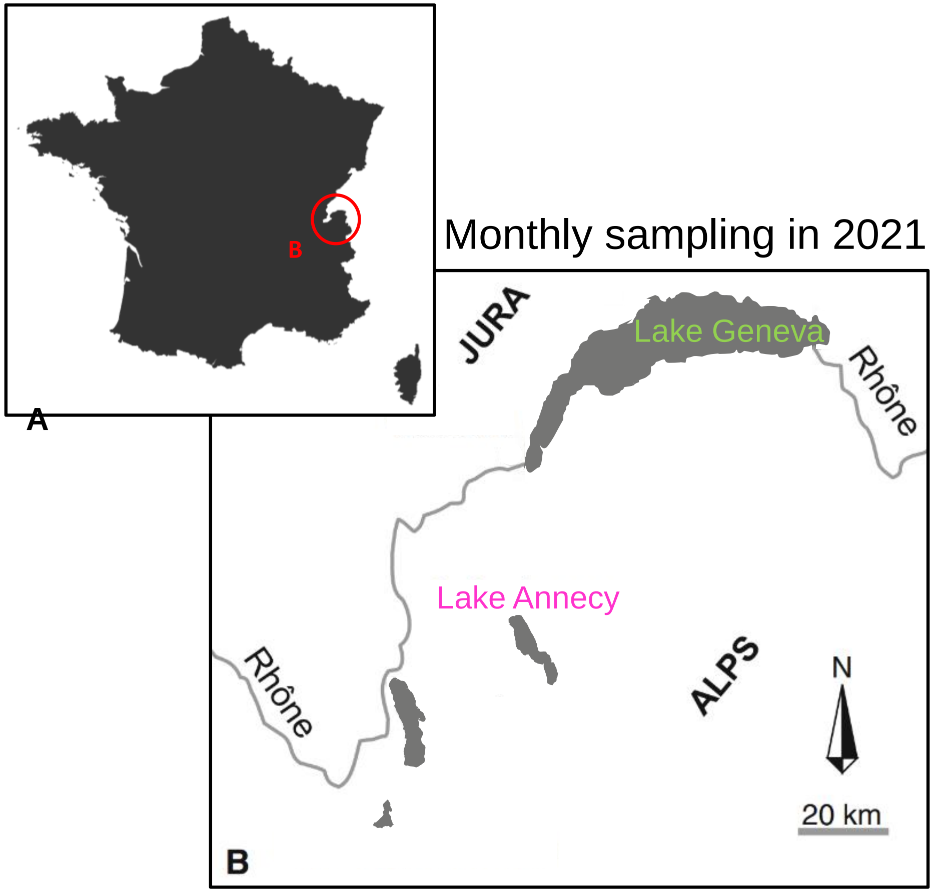
Bringing out a structure – PCA

Case of study: Spatio-temporal monitoring of microalgae in lakes Geneva and Annecy

Practical course on R

Monthly sampling in 2021



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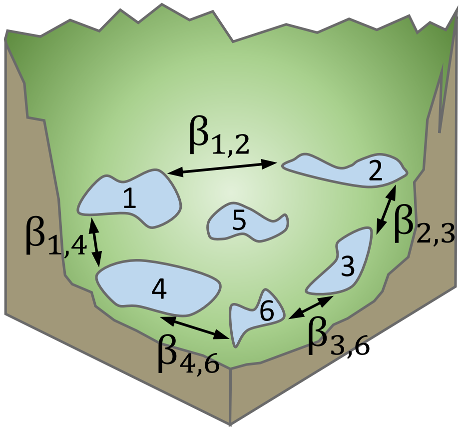
Understanding the structure



ASVs

Env.

Sites

Sites

Measuring the variation among commuties

Distance matrix

Aitchison distance

Bringing out a structure

Principal Component Analysis (PCA)	Correspondence Analysis (CA)	Principal Coordinate Analysis (PCoA)

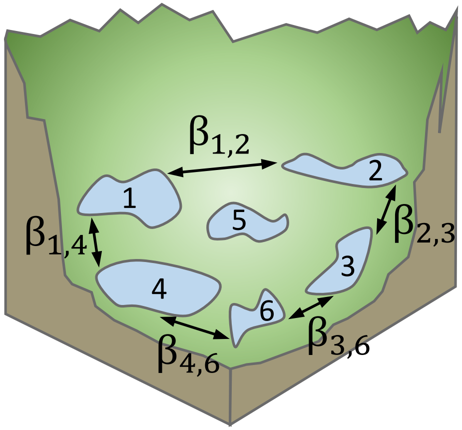
Explaining the structure with external variables

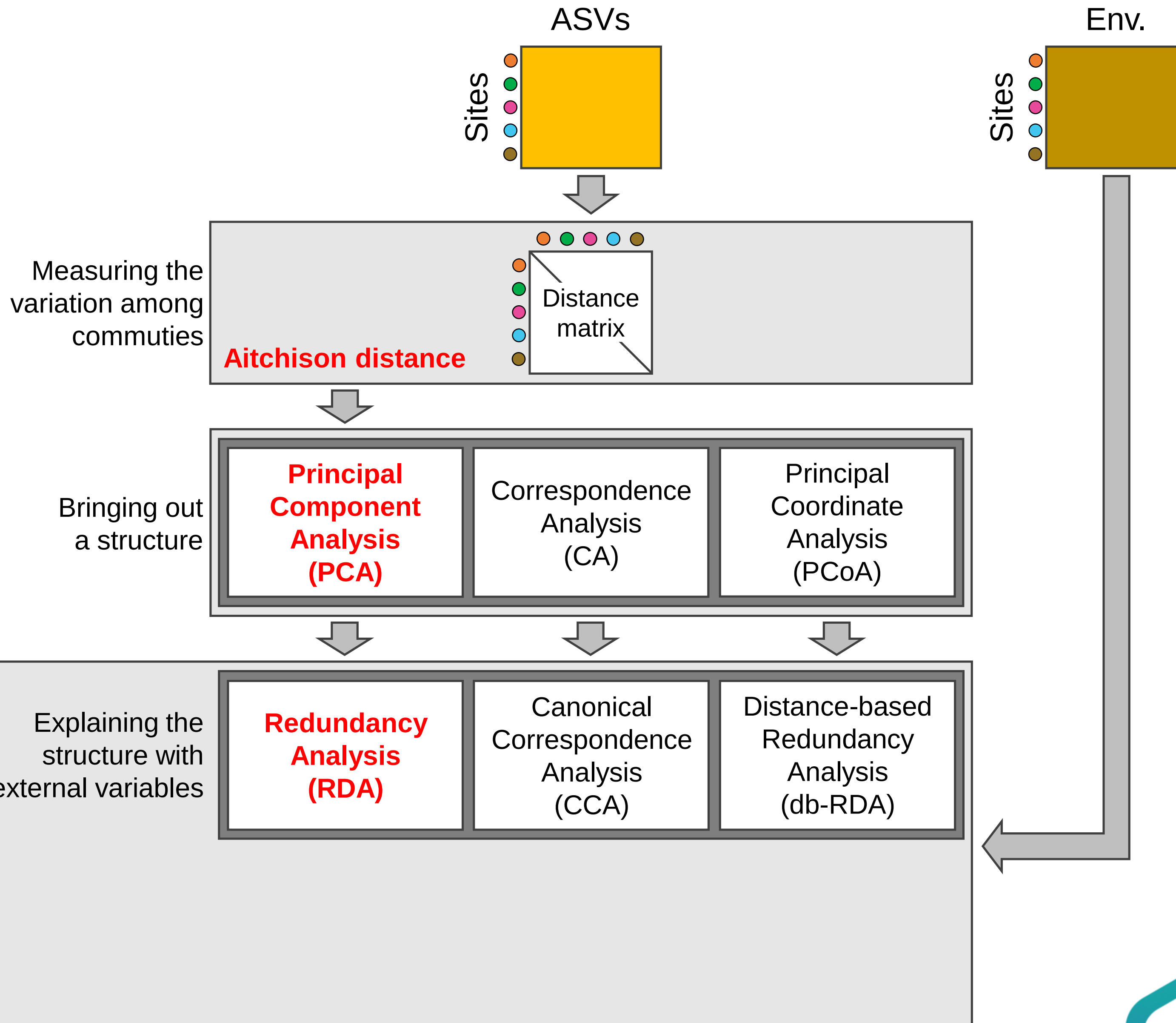
Redundancy Analysis (RDA)	Canonical Correspondence Analysis (CCA)	Distance-based Redundancy Analysis (db-RDA)

Understanding the structure

Funded by the European Union

Understanding the structure





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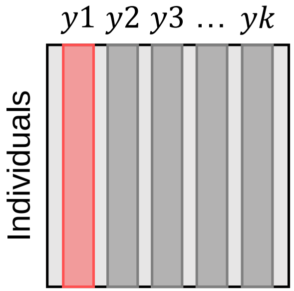
Understanding the structure – RDA

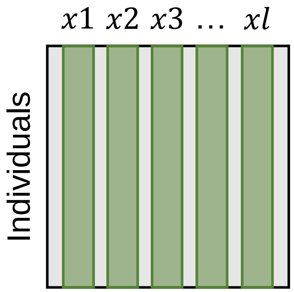
Data table $\boldsymbol{Y}$
(response variables)



Individuals: lakes

Response variables: species

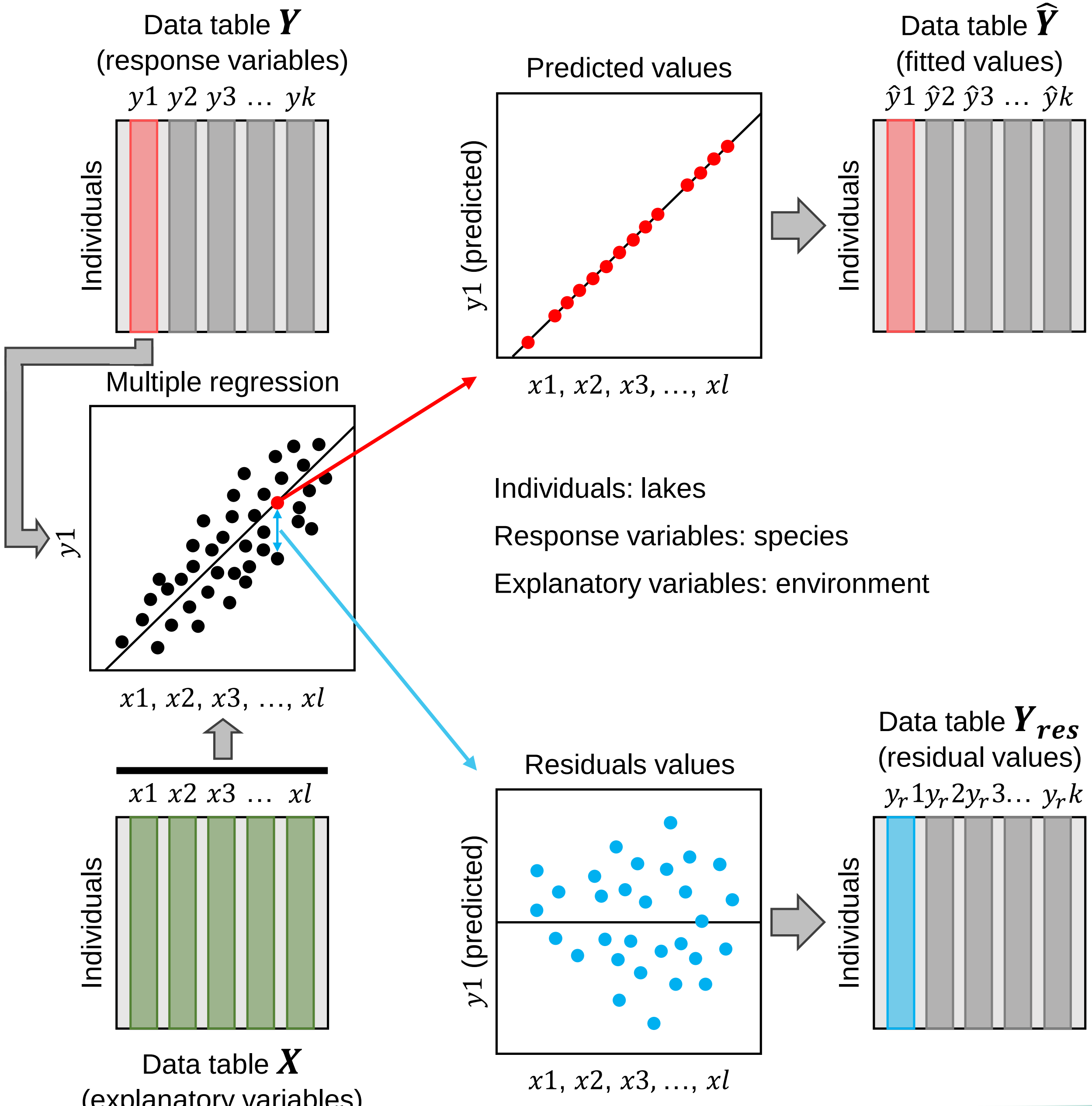
Explanatory variables: environment



Data table $\boldsymbol{X}$
(explanatory variables)

Understanding the structure – RDA

Step – 1



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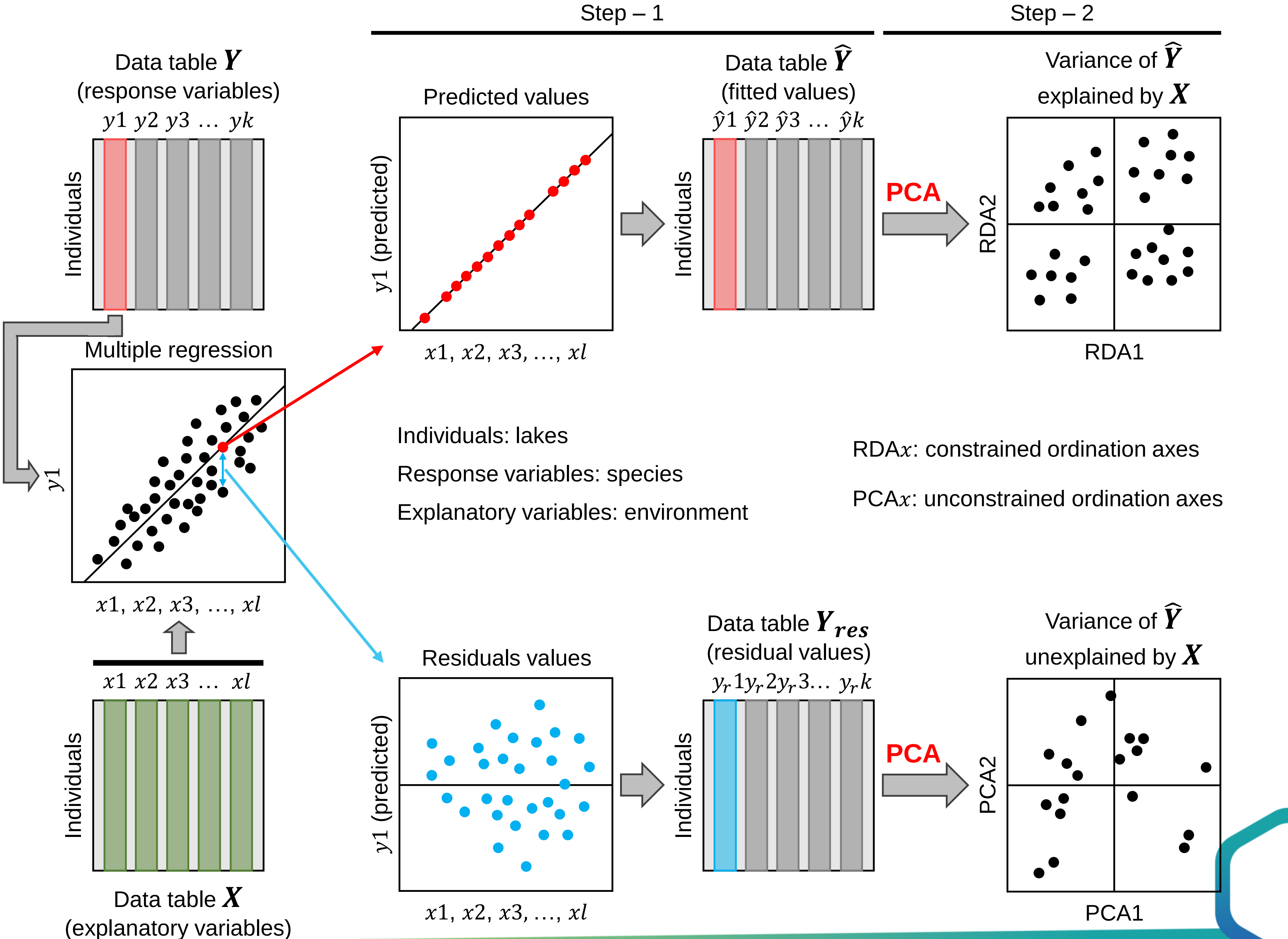
Understanding the structure – RDA



Individuals: lakes

Response variables: species

Explanatory variables: environment

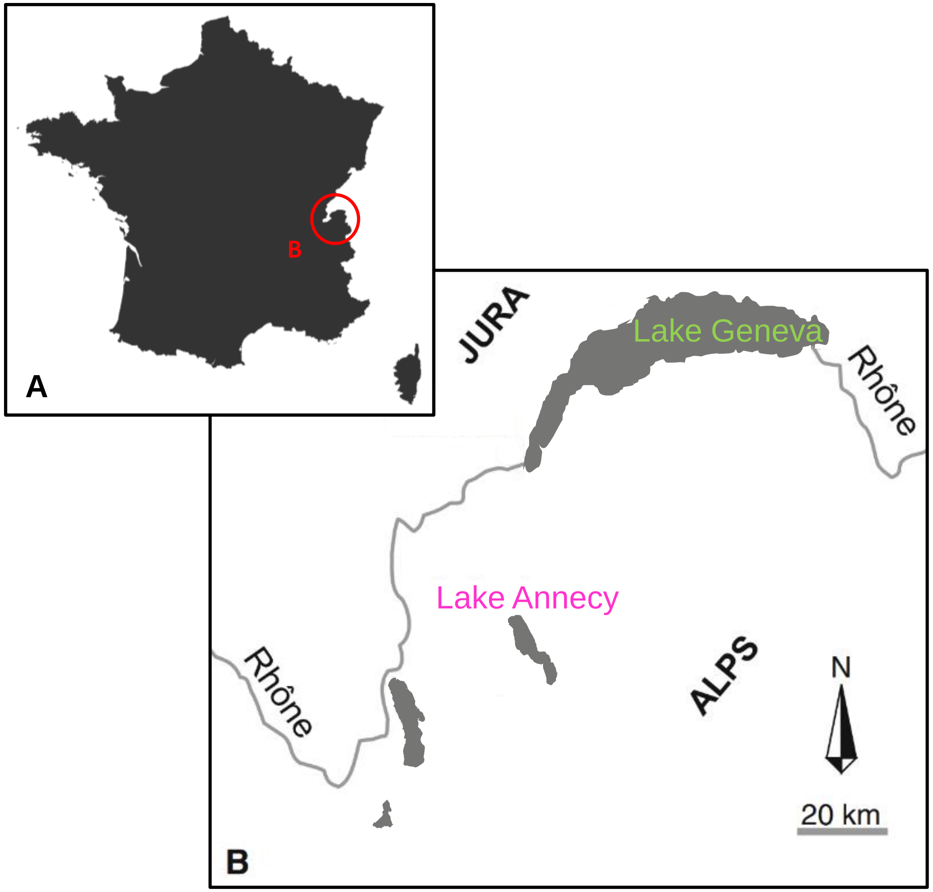
RDAx: constrained ordination axes

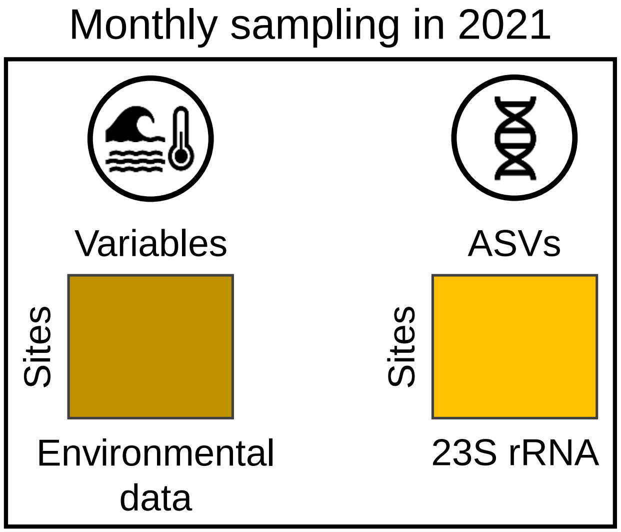
PCAx: unconstrained ordination axes

Understanding the structure – RDA

Case of study: Spatio-temporal monitoring of lakes Geneva and Annecy

Practical course on R





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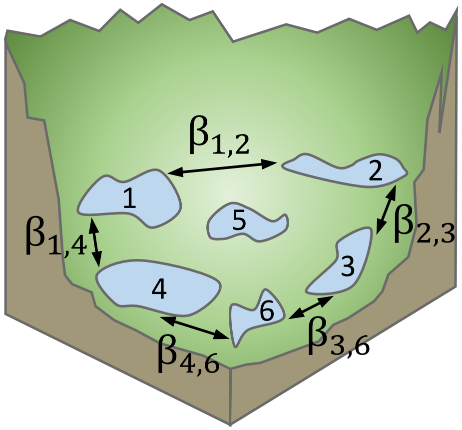
Understanding the structure



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Quantify the effect of different groups of variables

Variation partitioning

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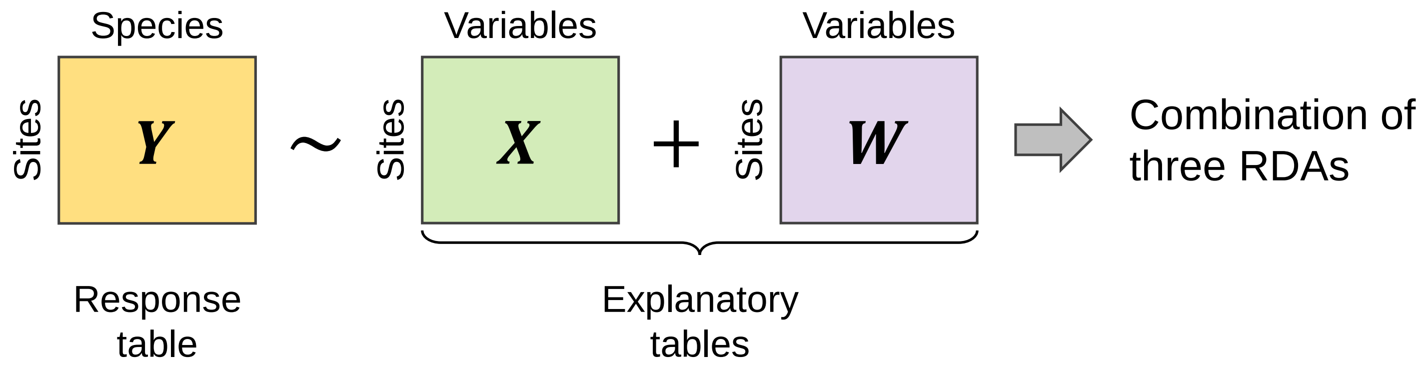
Understanding the structure – Variation partitioning

Variation partitioning:
Quantify the relative effects of different groups
of explanatory variables on the response variables



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Understanding the structure – Variation partitioning

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Quantify the relative effects of different groups
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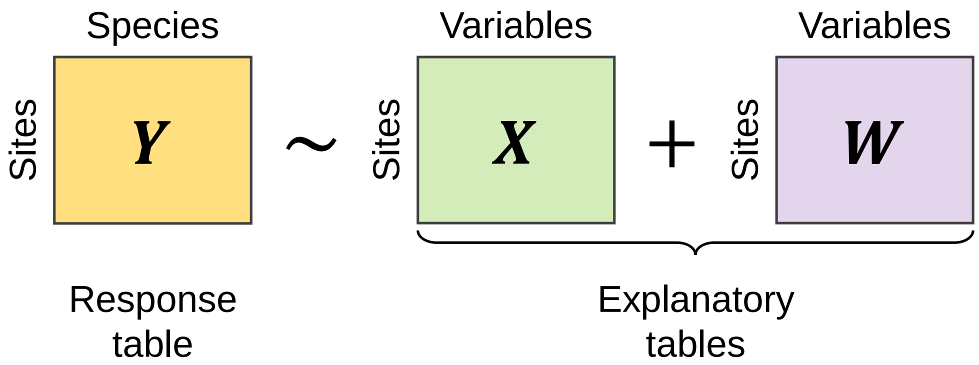
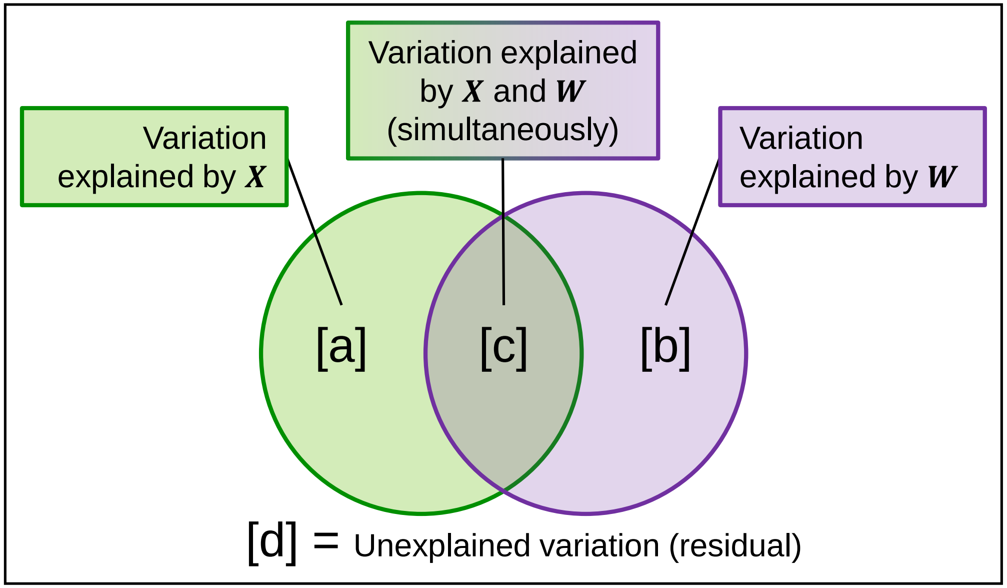


Combination of three RDAs

Variation in table Y =



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Understanding the structure – Variation partitioning

Variation partitioning:
Quantify the relative effects of different groups
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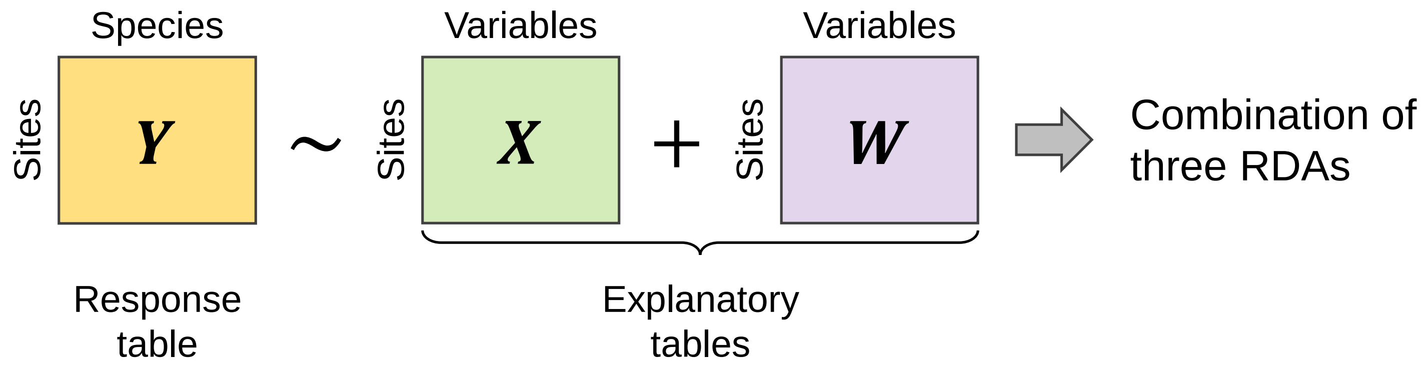
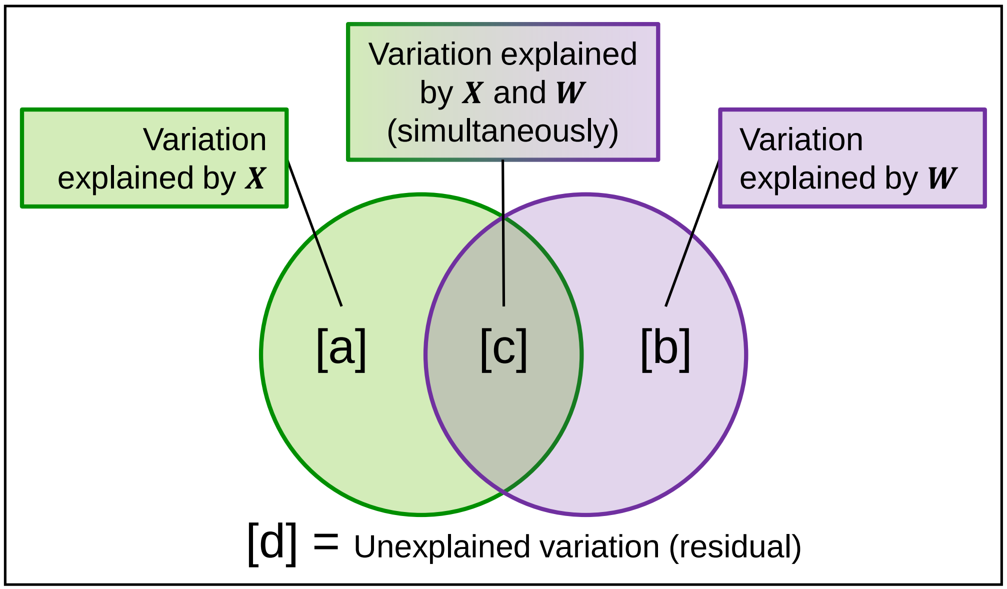


Variation in table $\boldsymbol{Y}$ =



1. RDA of $\boldsymbol{Y}$ by $\boldsymbol{X}$: [a + c]
2. RDA of $\boldsymbol{Y}$ by $\boldsymbol{W}$: [b + c]
3. RDA of $\boldsymbol{Y}$ by $\boldsymbol{X}$ and $\boldsymbol{W}$: [a + b + c]

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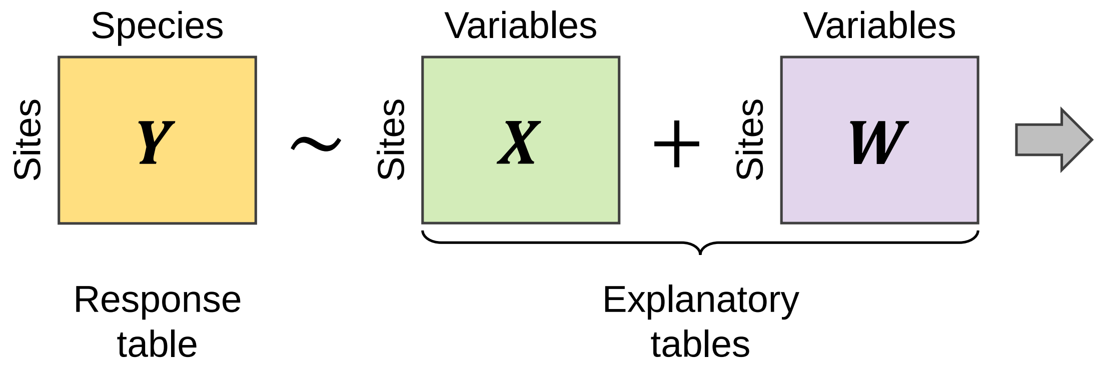
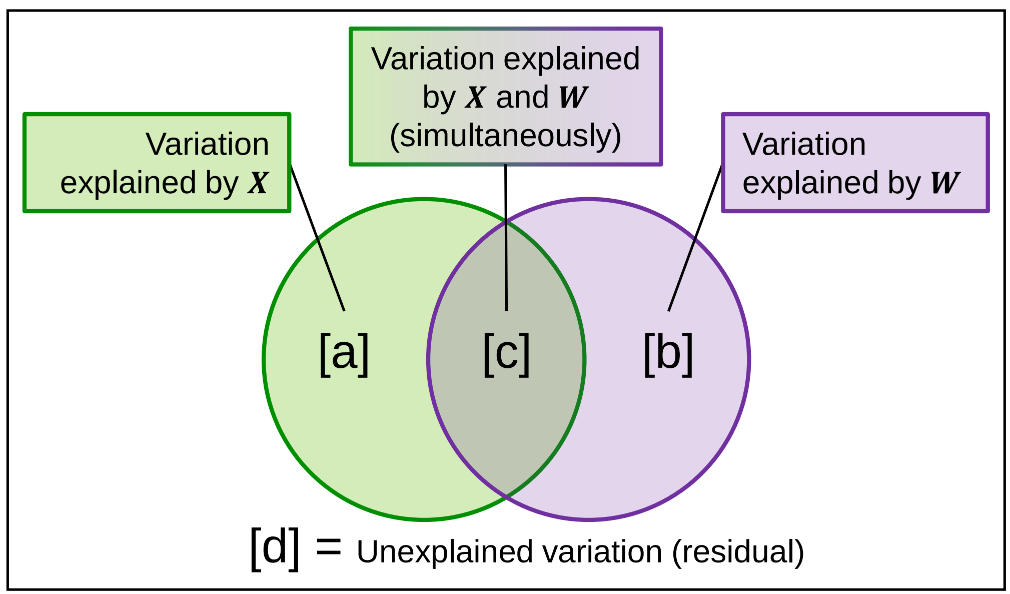
Understanding the structure – Variation partitioning

Variation partitioning:
Quantify the relative effects of different groups
of explanatory variables on the response variables



Combination of three RDAs

Variation in table Y =



1. RDA of Y by X: [a + c]
2. RDA of Y by W: [b + c]
3. RDA of Y by X and W: [a + b + c]

4. [a] = [a + b + c] – [b + c]
5. [b] = [a + b + c] – [a + c]
6. [d] = 1 – [a + b + c]
7. [c] = [a + c] – [a] = [b + c] – [b]

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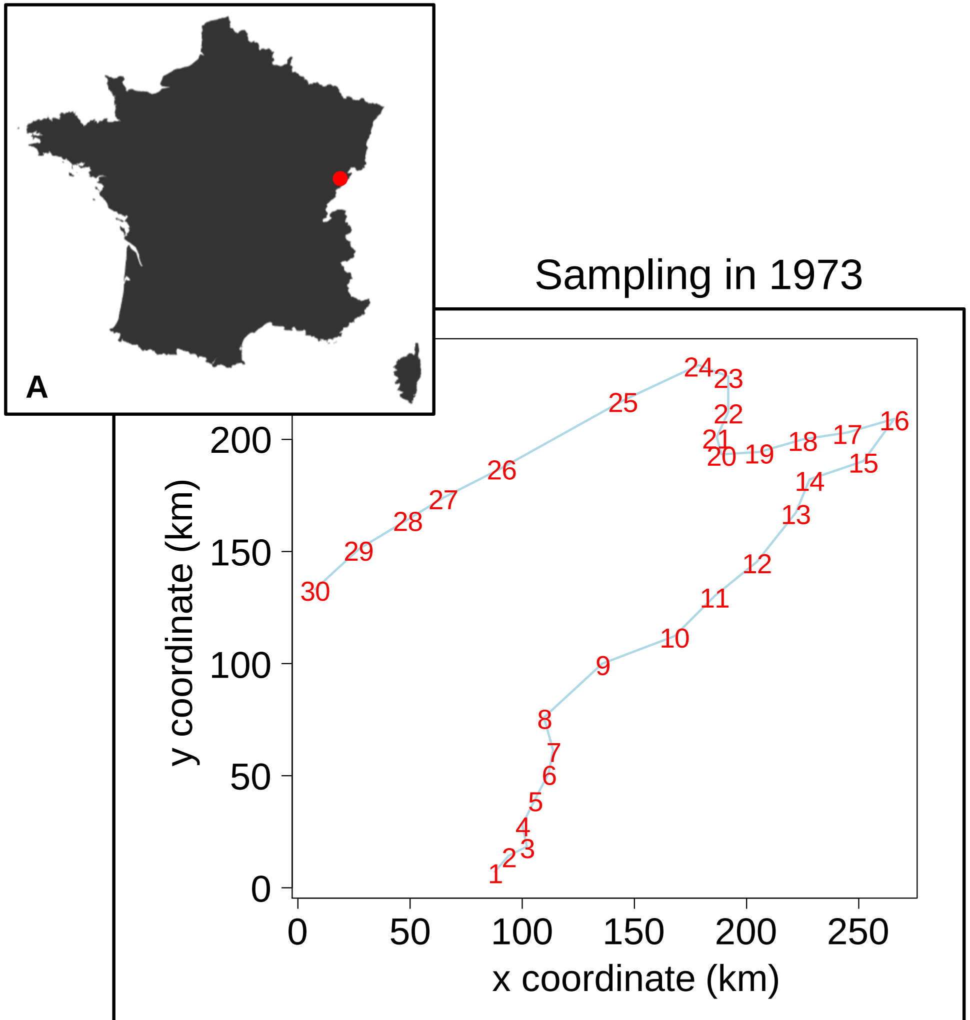
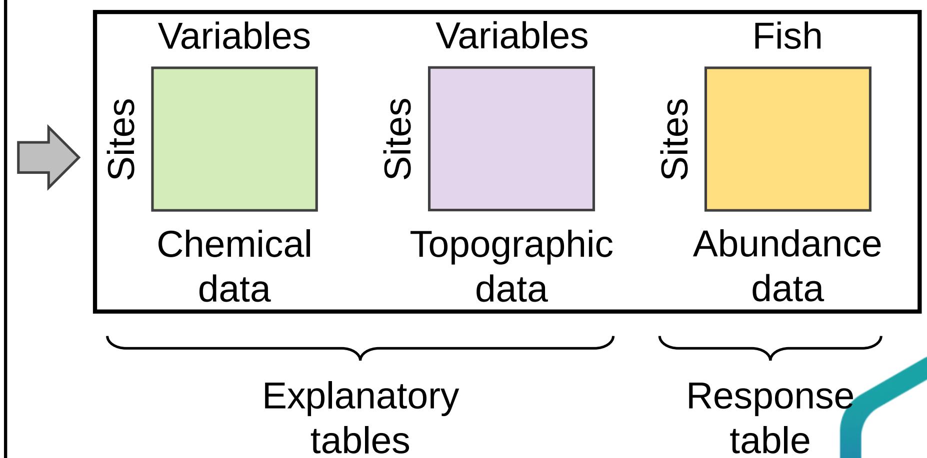
Understanding the structure – Variation partitioning

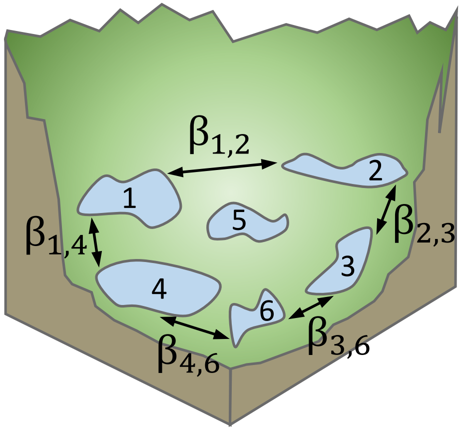
Case of study: Data from Verneaux (1973) → Doubs river (France), fish communities

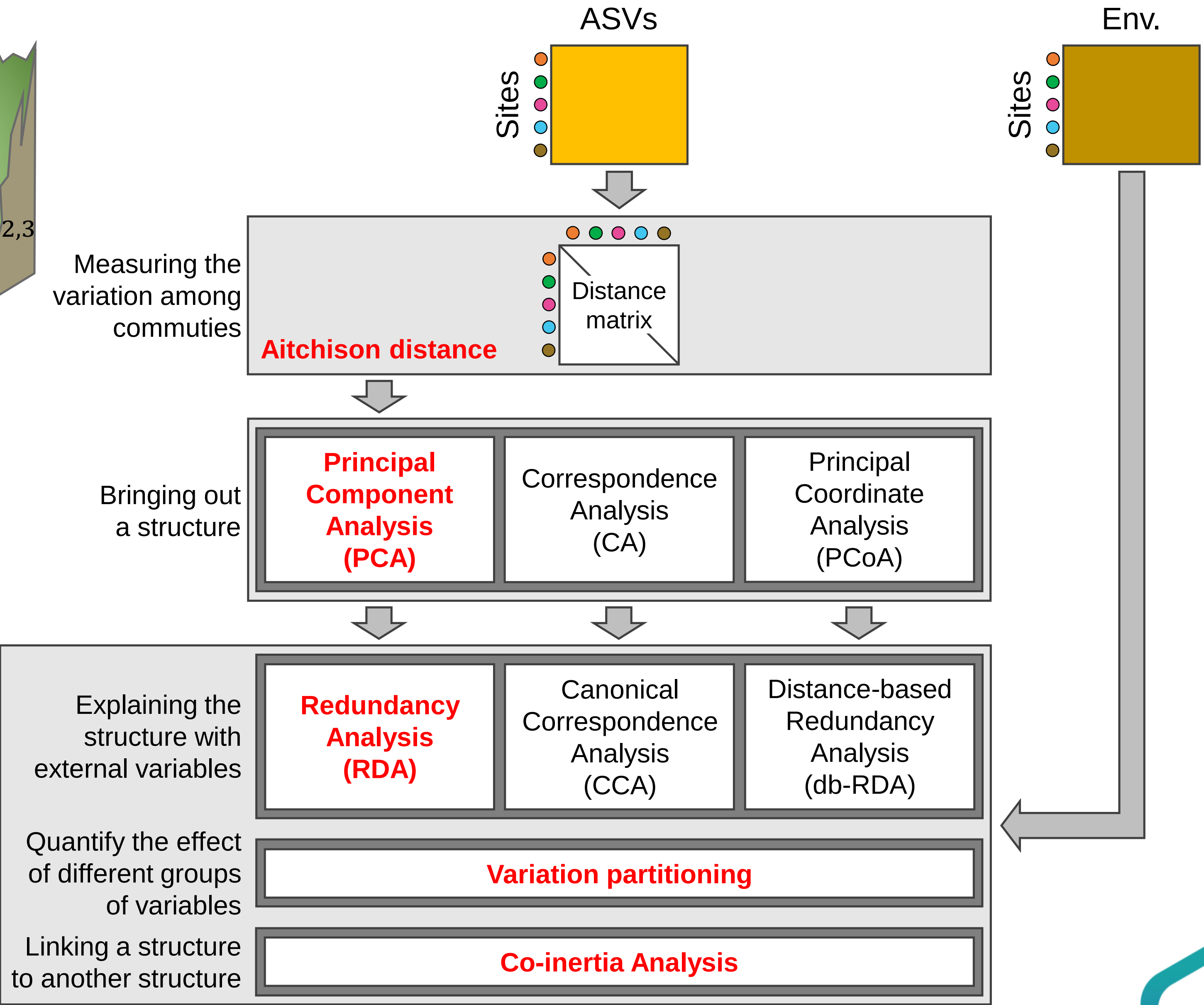
Practical course on R





Understanding the structure





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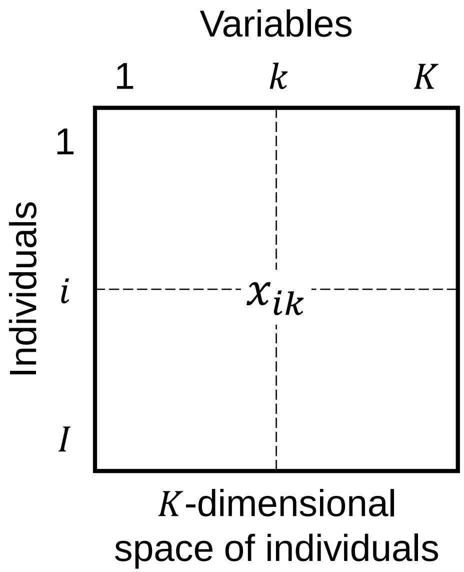
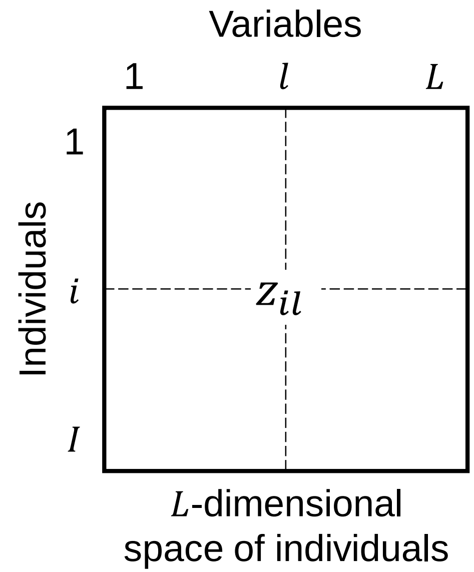
Understanding the structure – Co-inertia analysis



Co-inertia analysis:
Enables finding common structures
between two set of variables



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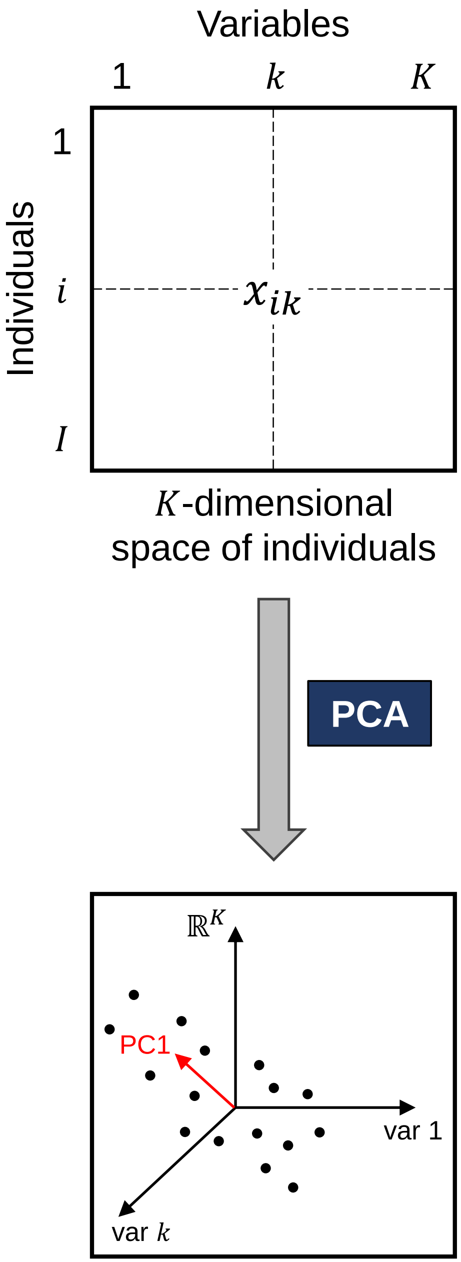
Understanding the structure – Co-inertia analysis



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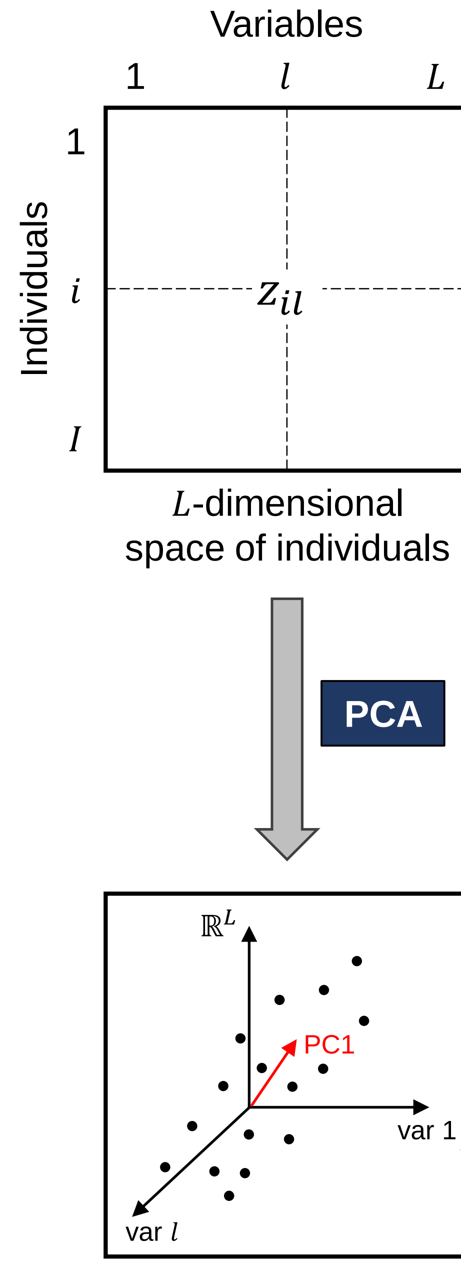
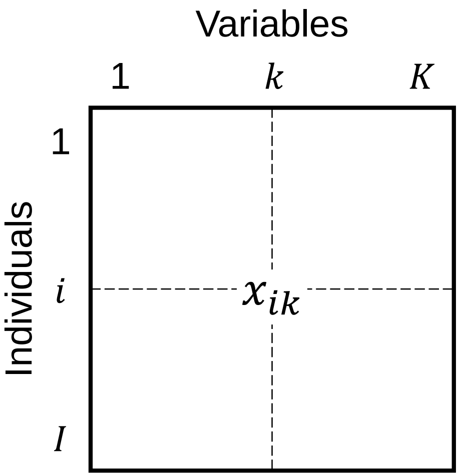
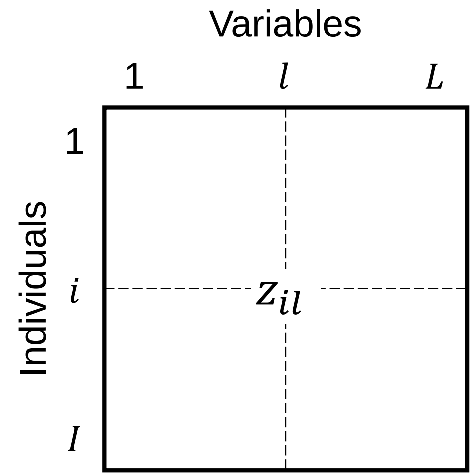


Understanding the structure – Co-inertia analysis



K-dimensional space of individuals

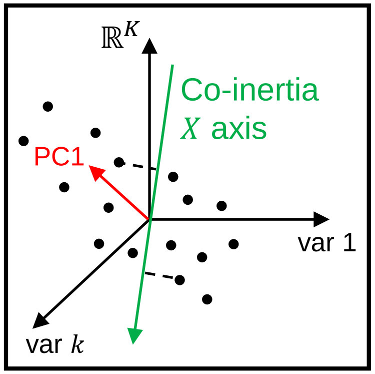
Co-inertia analysis:
Enables finding common structures between two set of variables



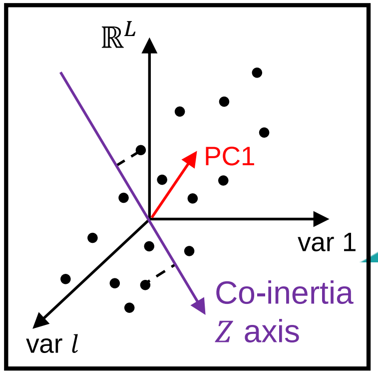
L-dimensional space of individuals

PCA

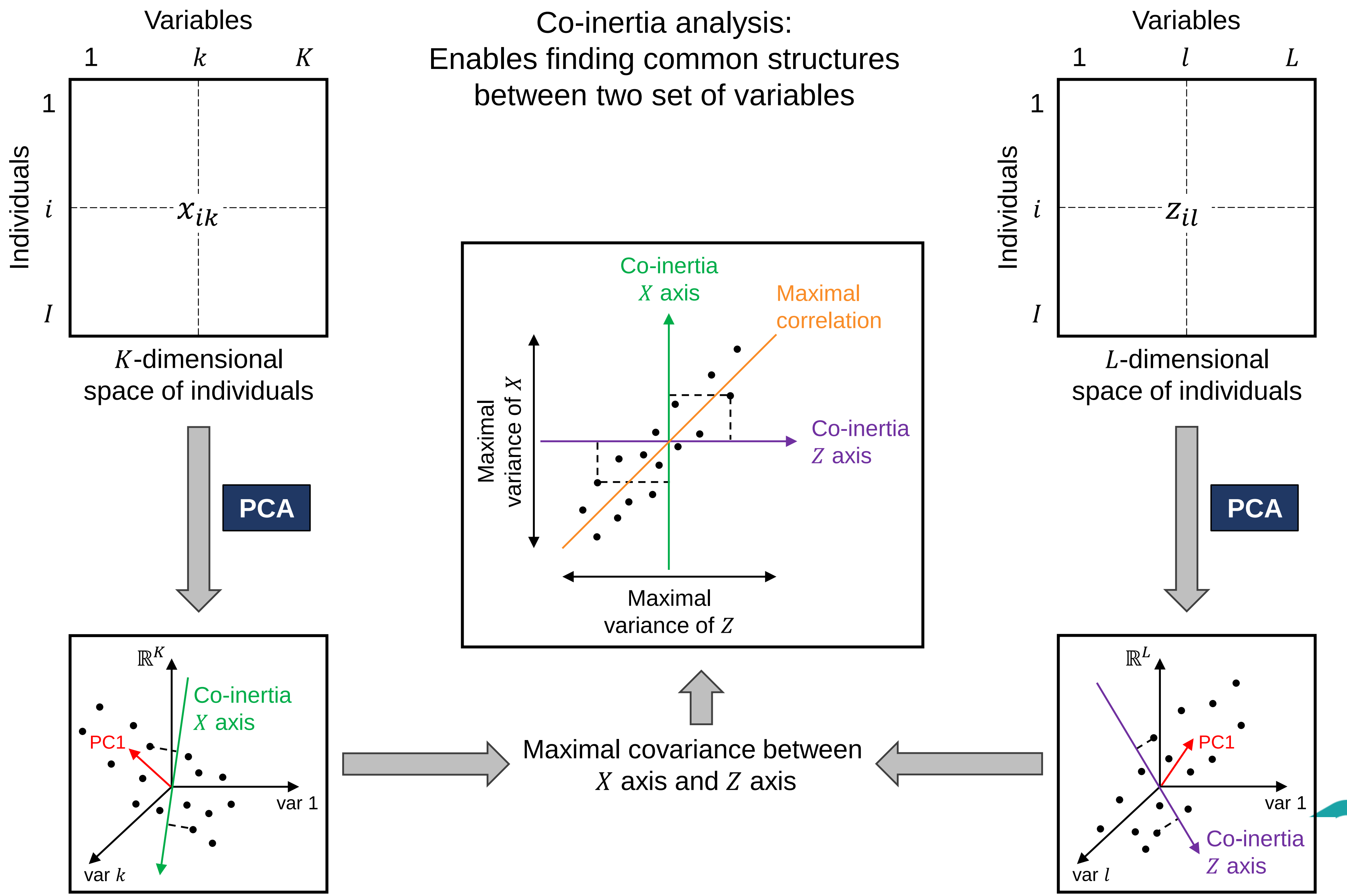
PCA



Maximal covariance between X axis and Z axis

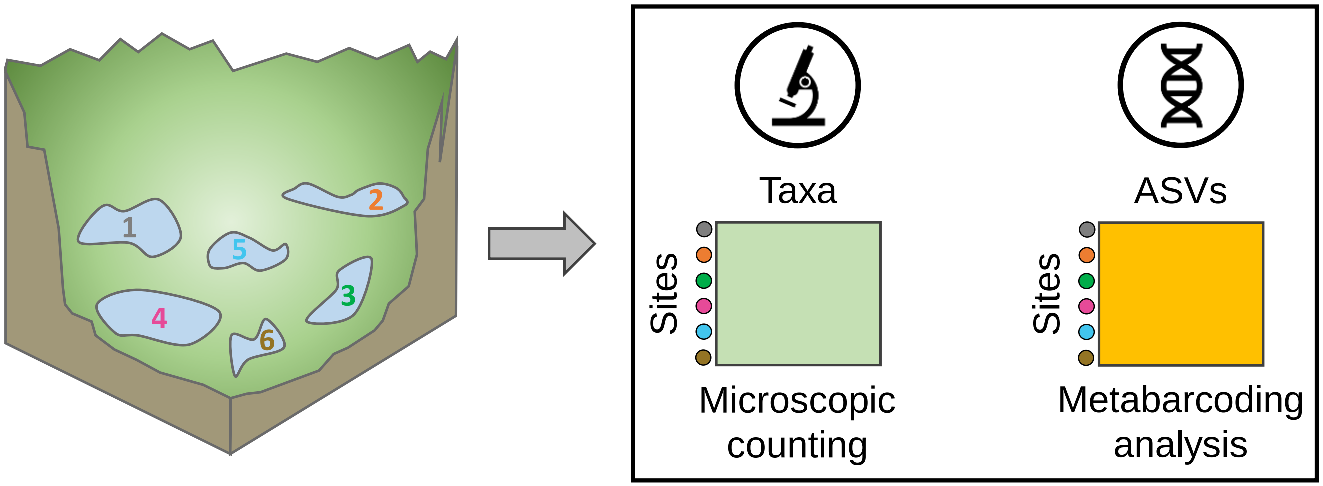


Understanding the structure – Co-inertia analysis



Understanding the structure – Co-inertia analysis

Co-inertia analysis:
Enables finding common structures between two set of variables



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Understanding the structure – Co-inertia analysis

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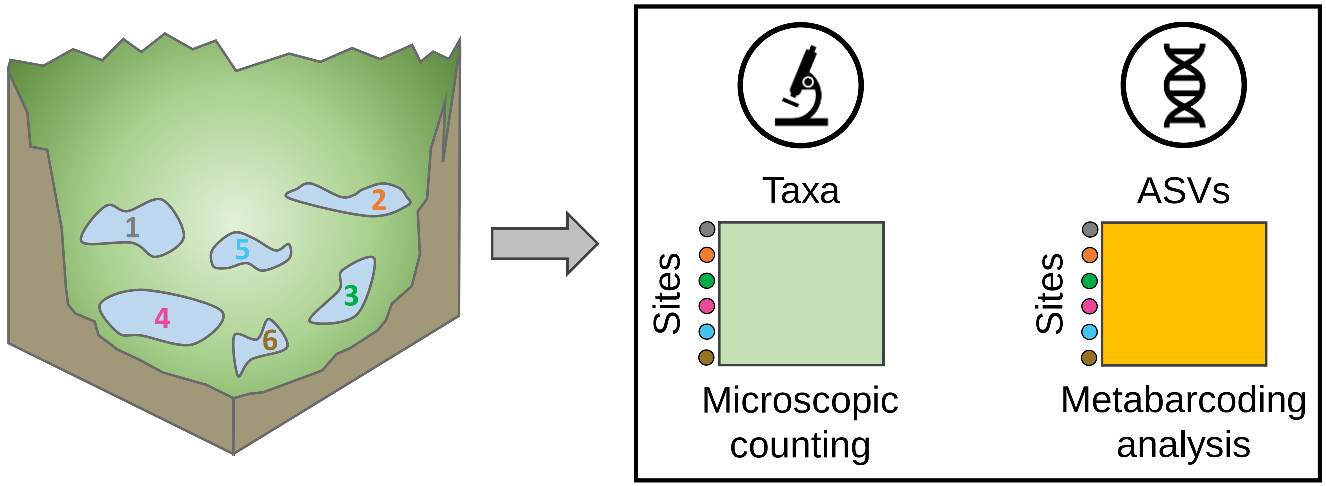
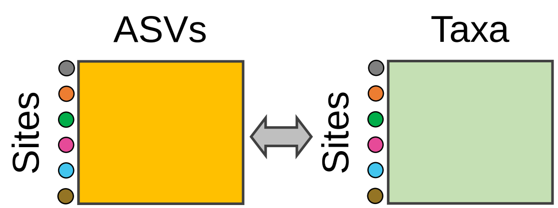
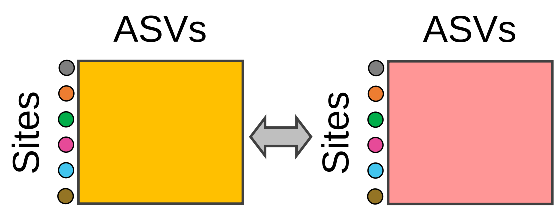


1. Concordance between eDNA and microscopy data



e.g., Nicolosi et al. (2023)
STOTEN, *in review*

2. Congruence between two biological communities



e.g., Alric et al. (2020)
Mol. Ecol. Res.

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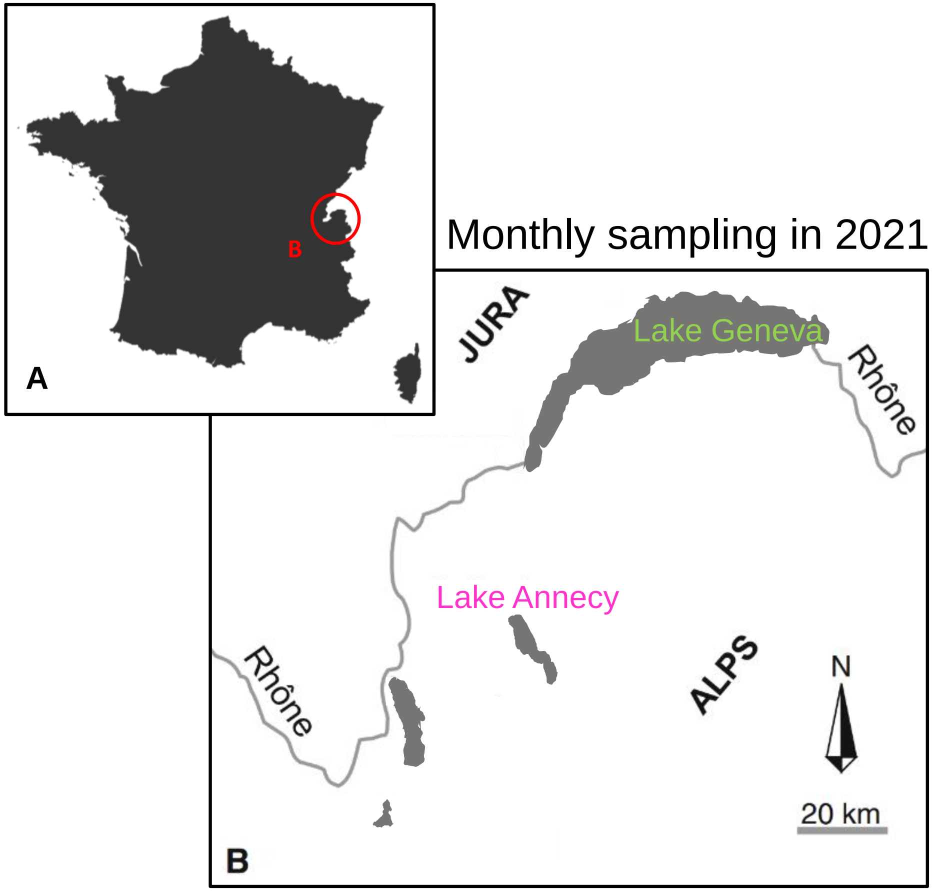
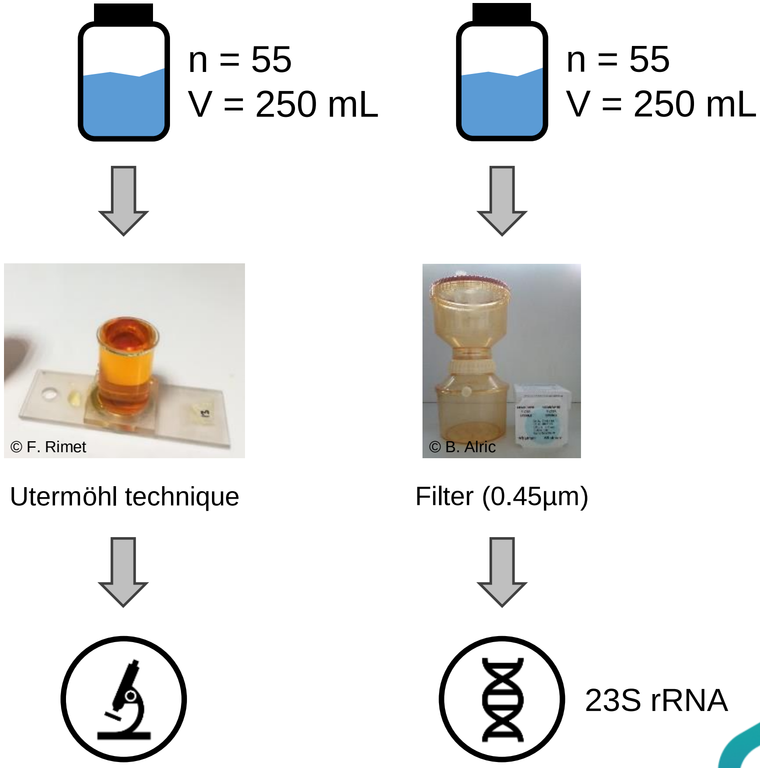
Understanding the structure – Co-inertia analysis

1. Concordance between eDNA and microscopy data

Practical course on R

Monthly sampling in 2021





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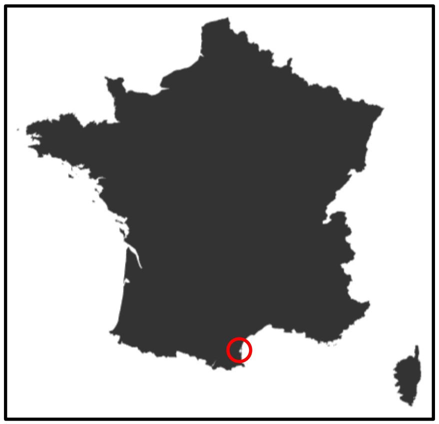
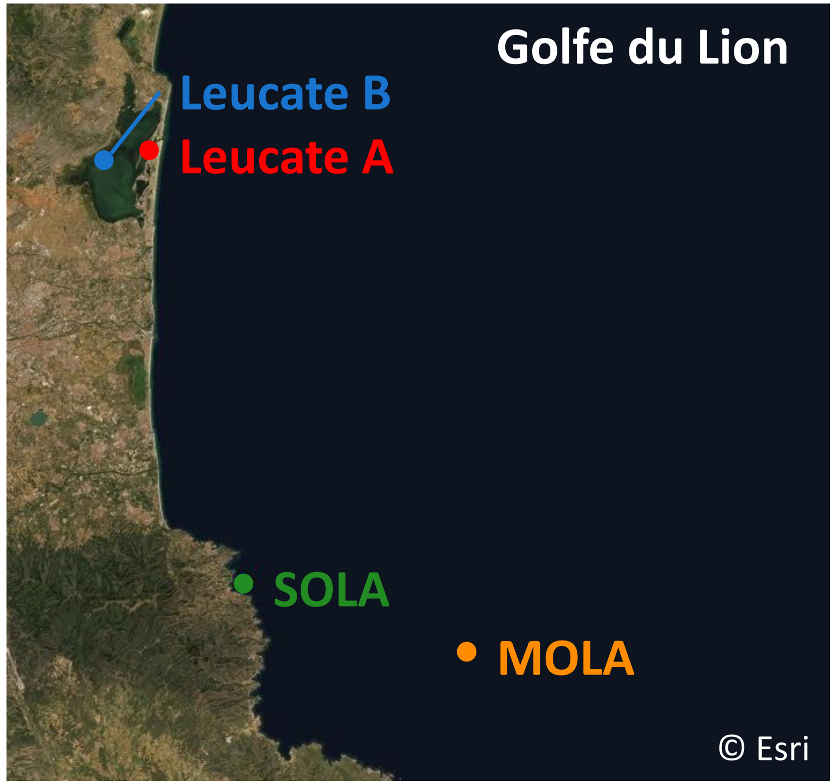
Understanding the structure – Co-inertia analysis

2. Congruence between two biological communities
   e.g., Alric et al. Mol. Ecol. Res., 20 (2020) – Host-virus association in marine environment





Monthly monitoring over 2 years

Microalgae (Mamiellophyceae): 18S rRNA
Virus (*Prasinovirus*): *PolB*

Eutrophication gradient

Understanding the structure – Co-inertia analysis

2. Congruence between two biological communities

e.g., Alric et al. Mol. Ecol. Res., 20 (2020) – Host-virus association in marine environment

Co-correspondence Analysis (CoCA) ≡ co-inertia based on two CA

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Understanding the structure – Co-inertia analysis

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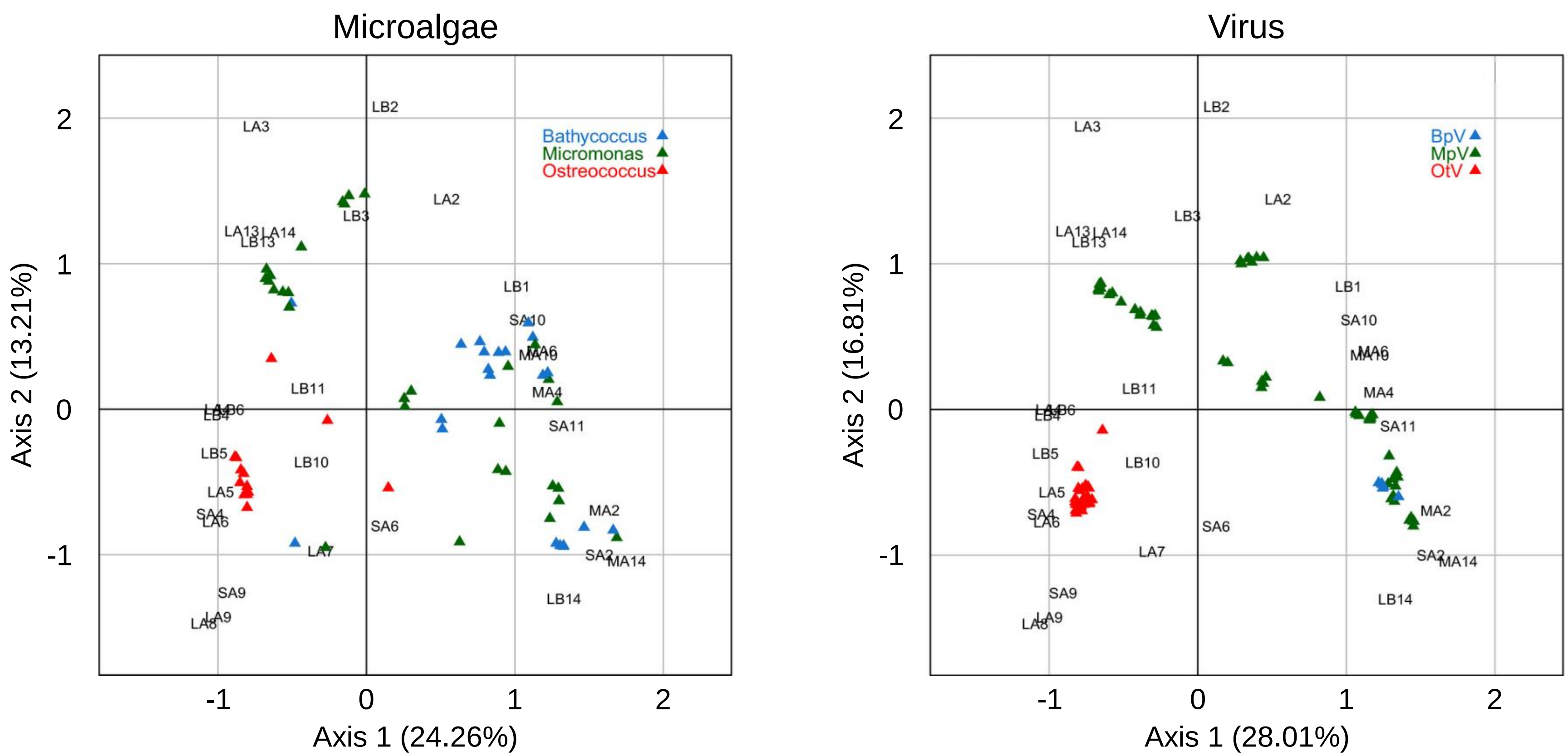
Co-correspondence Analysis (CoCA) ≡ co-inertia based on two CA
Predictive power = 32.02%, p = 0.001 (Axis 1, Axis 2)



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Understanding the structure – Co-inertia analysis

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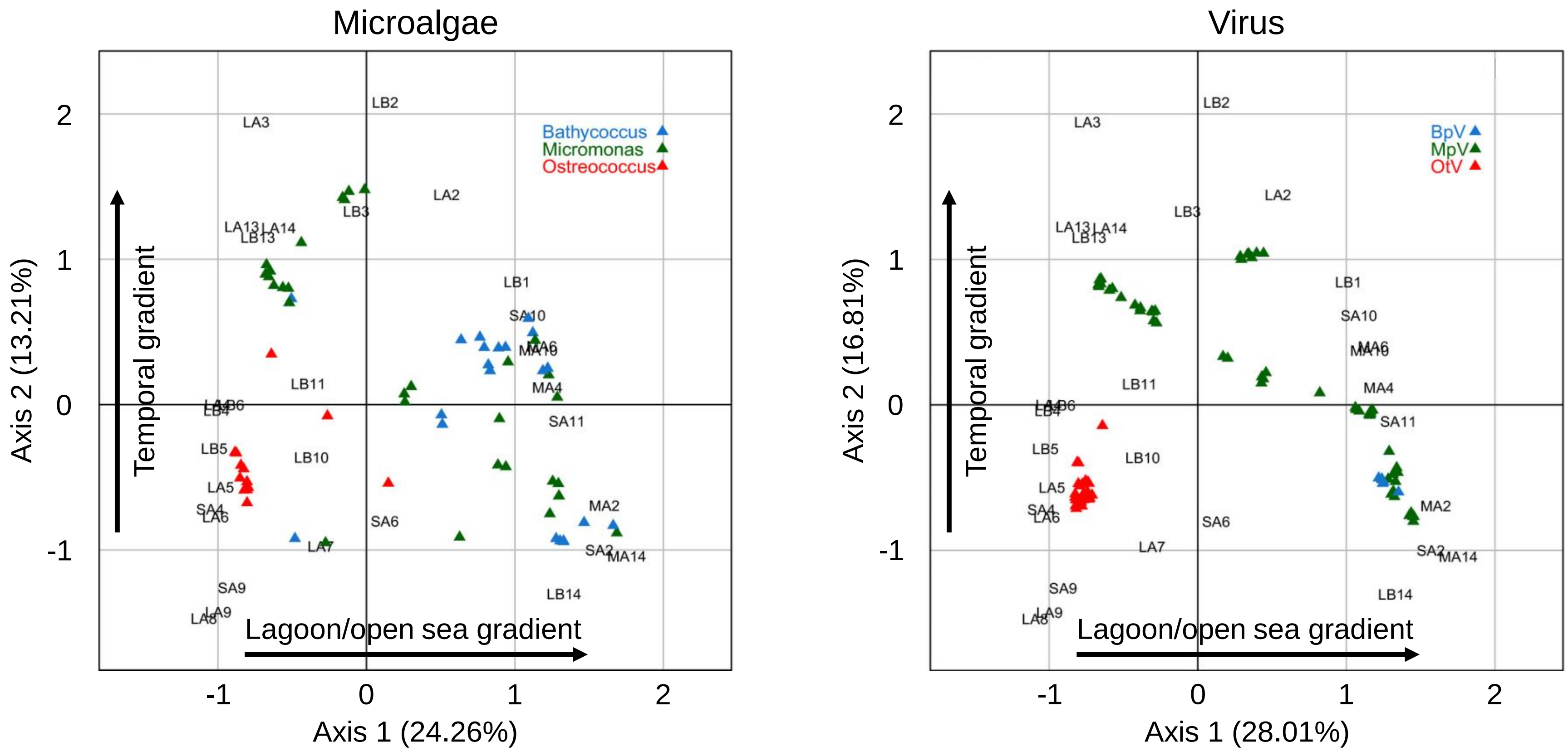
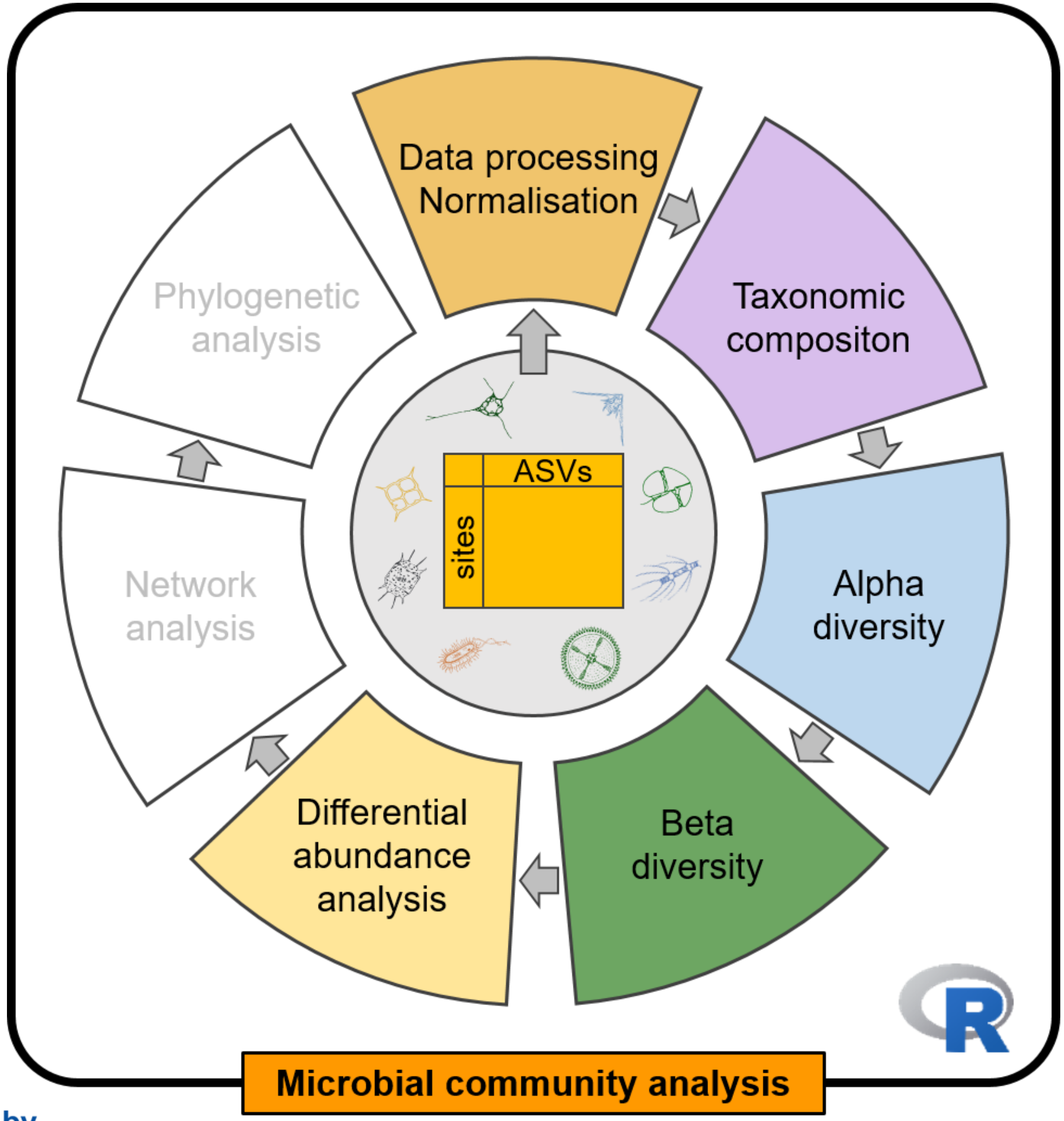


Covariation of two communities along a lagoon/open-sea gradient (Axis 1) and a temporal gradient (Axis 2)

Statistical analysis workflow from metabarcoding data



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Differential abundance analysis

Differential abundance analysis:
Identify biomarker taxa (i.e., taxa whose relative abundance is significantly higher under given environmental conditions)

Method	Model assumption	Normalization	References	Availability	
edgeR*	Negative binomial	TMM	[42]	Bioconductor	2012
metagenomeSeq	Zero-inflated normal or log-normal	CSS	[24]	Bioconductor	2013
DESeq2*	Negative binomial	RLE	[39]	Bioconductor	2014
ANCOM	ANOVA	ALR	[40]	GitHub	2015
ZIBseq	Zero-inflated beta	TSS	[5]	CRAN	2016
ZIGDM	Zero-inflated generalized Dirichlet-multinomial	None†	[6]	CRAN	2019
corncob	Beta-binomial	None†	[41]	GitHub	2020
mixMC	PCA/sPLS-DA‡	CSS/TSS+CLR	[43]	Bioconductor	2016
maSigPro*	Generalized linear models	User specified††	[44]	Bioconductor	2014
NBME*	Negative binomial mixed effects	User specified††	[45]	CRAN	2016
MetaSplines	Gaussian + SS-ANOVA	CSS	[46]	Bioconductor	2017
MetaDprof	Gaussian + SS-ANOVA	TMM	[47]	Online	2017
MetaLonDA	Negative binomial + SS-ANOVA	TMM/CSS‡‡	[48]	CRAN	2018
NBZIMM	Negative binomial or Gaussian mixed effects	See below**	[49]	GitHub	2020

Lutz et al. Front. Appl. Math. Stats. 8 (2022)

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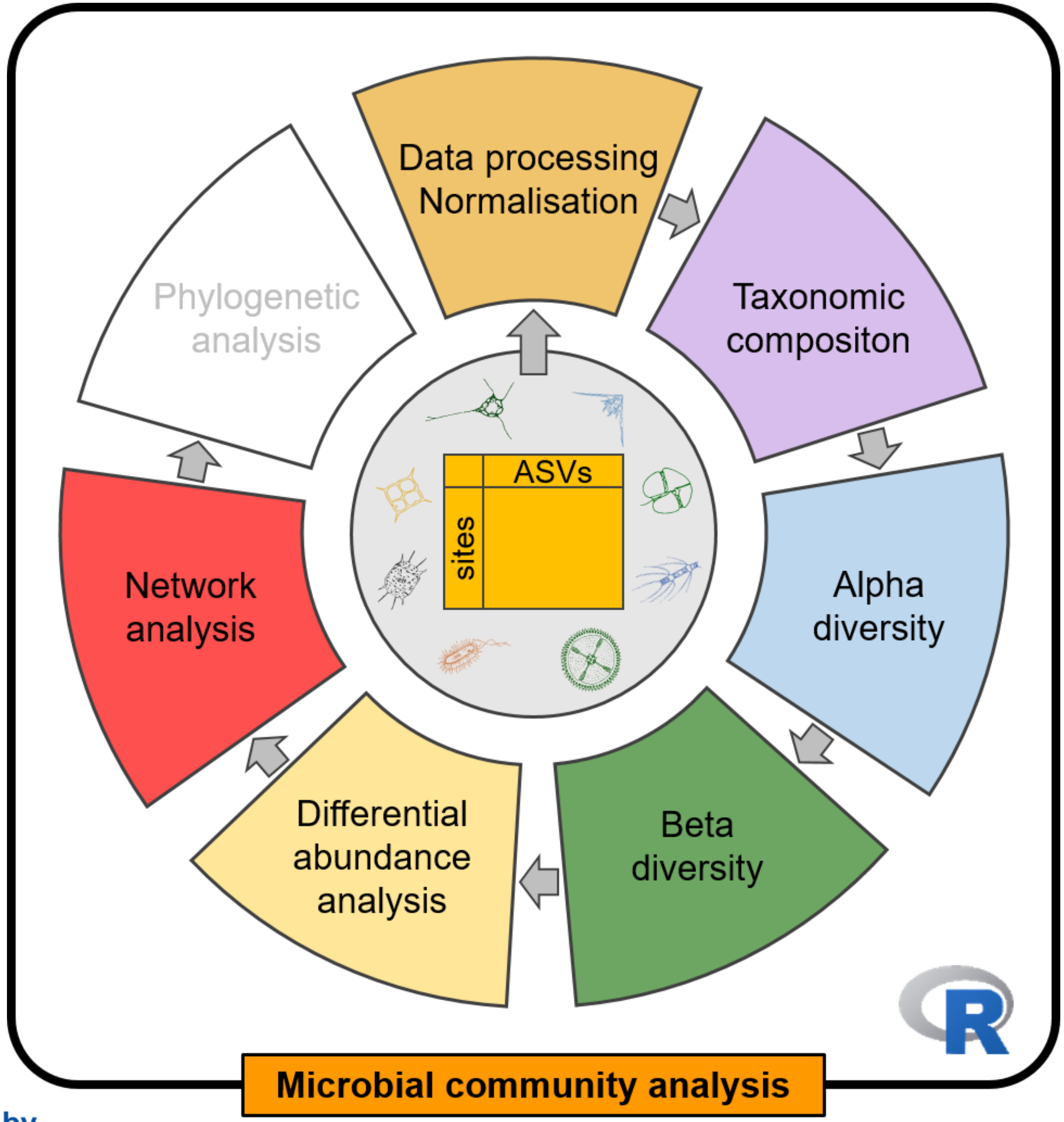
Statistical analysis workflow from metabarcoding data



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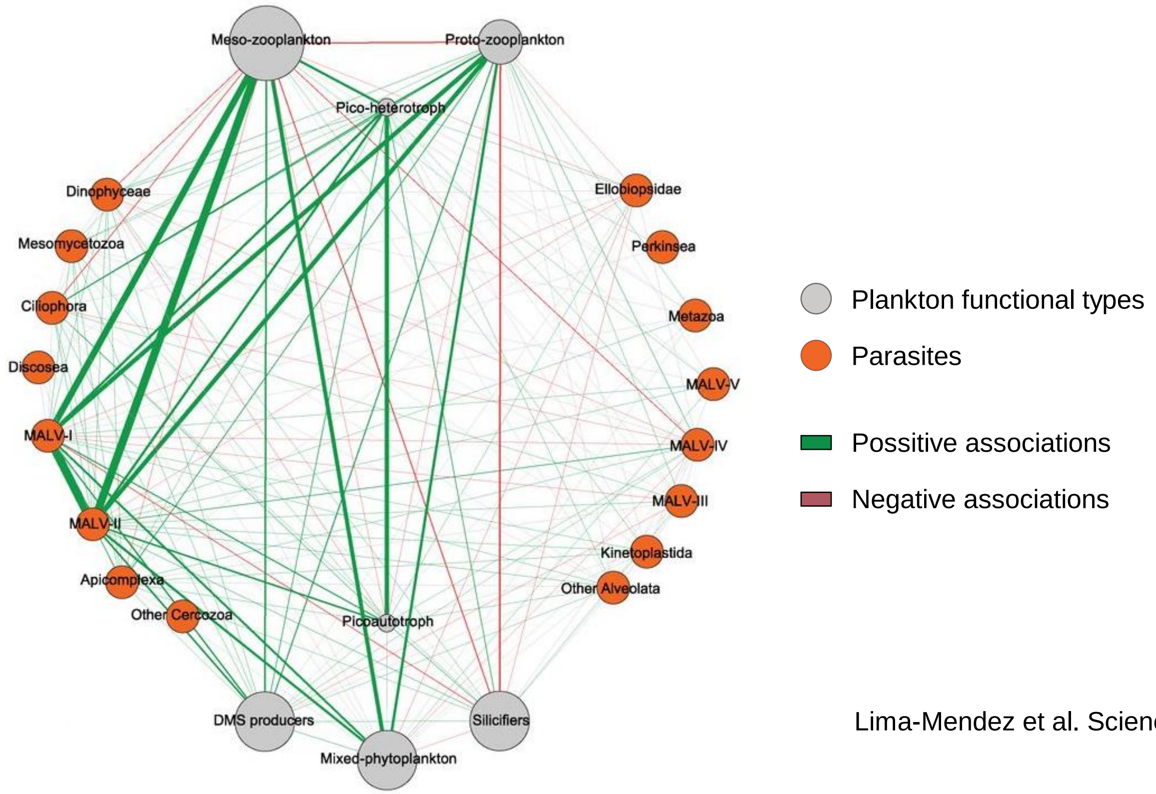
Network analysis

Co-occurrence network:



Lima-Mendez et al. Science 348 (2015)

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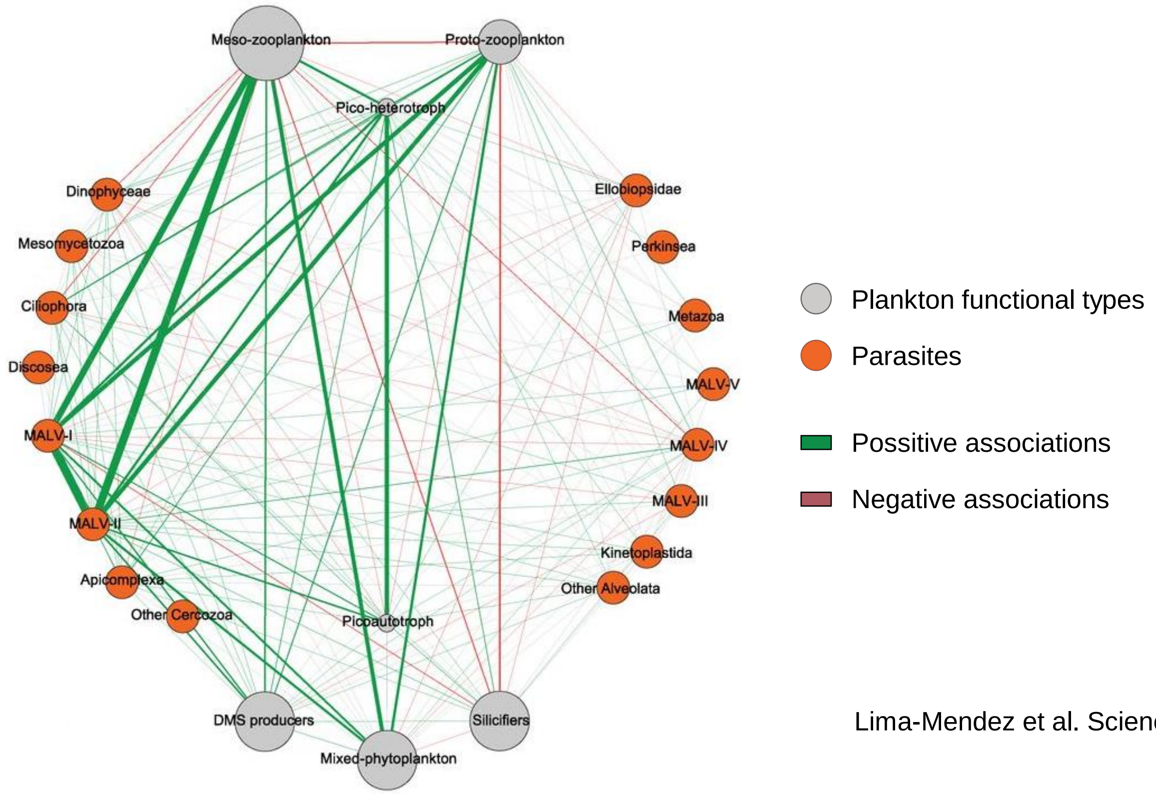
Network analysis

Co-occurrence network:



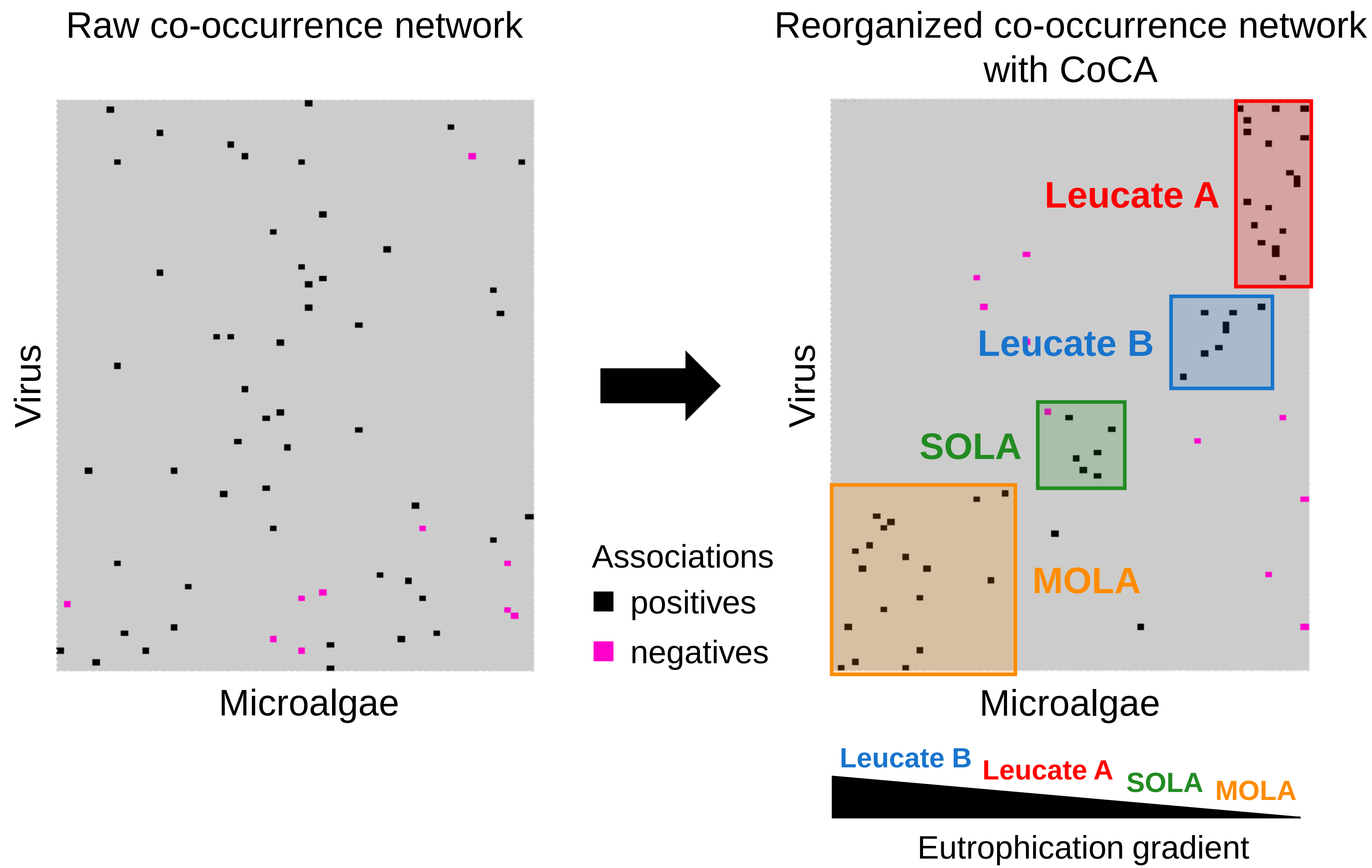
Lima-Mendez et al. Science 348 (2015)

Method	Network type	Method	Normalization	References	Application	
SparCC	Correlation	Iterative estimation of correlation	ALR	[69]	GitHub	2012
CCLasso	Correlation	Least squares with l_1 penalty	ALR	[70]	GitHub	2015
REBACCA	Correlation	Fast l_1-norm shrinkage	ALR	[71]	Online	2015
SpiecEasi	Partial correlation	Gaussian graphical model	CLR	[72]	GitHub	2015
SPRING	Partial correlation, SPR correlation	Truncated Gaussian copula model	Modified CLR	[7]	CRAN	2019
HARMONIES	Partial correlation	Gaussian graphical model	DPP	[73]	GitHub	2020

Lutz et al. Front. Appl. Math. Stats. 8 (2022)

Network analysis

Similarity between two biological communities: Alric et al. (2020) Mol. Ecol. Res.



Spatial structuring of microalgae-virus association networks in relation to eutrophication gradient

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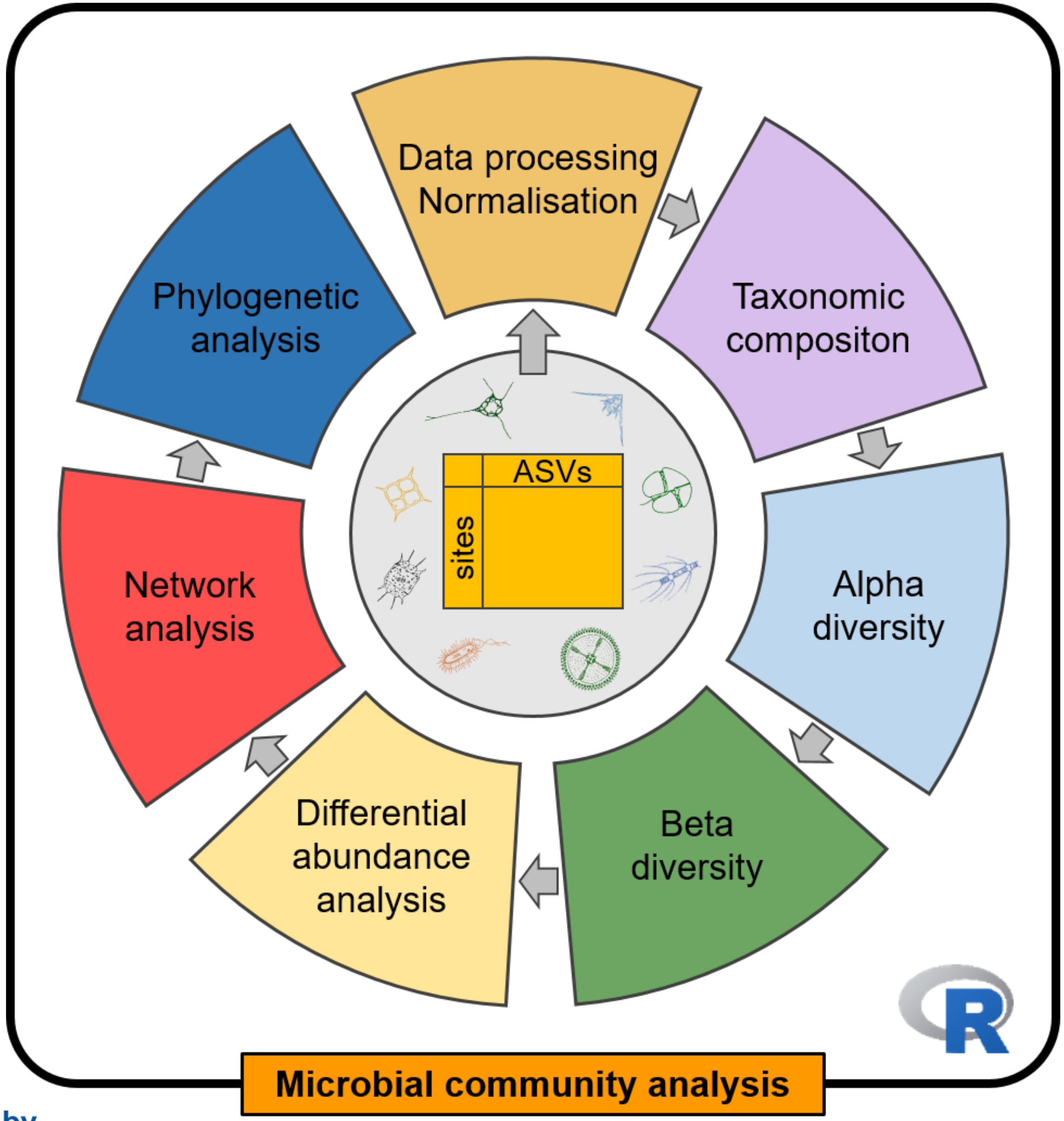
Statistical analysis workflow from metabarcoding data



Acknowledgement

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Thank you for your attention!

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