

Community ecology and multivariate analyses

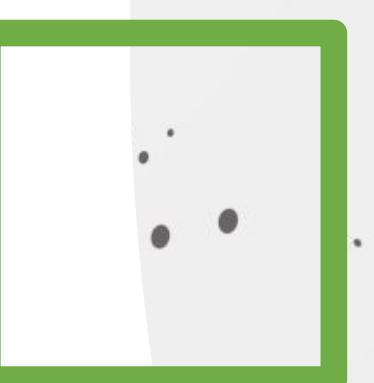
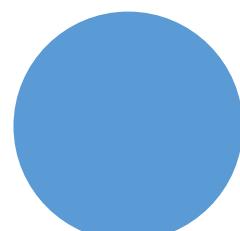
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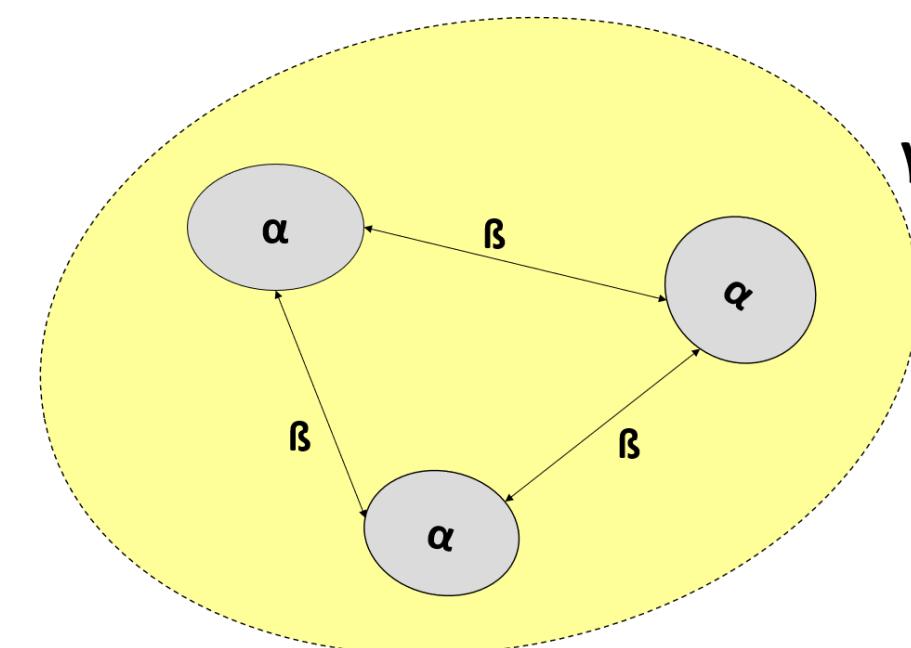
Purpose

- Learn methods for doing **community ecology** analysis of **high throughput metabarcoding** data in R
- Look at some common diversity indexes
- Learn ordination methods
- All analyses are done in R
 - Scripts and lectures can be found on github
 - https://github.com/krabberod/UNIS_AB332_2023

Computer Lab part I : Diversity

Keyterms: Species richness, abundance, evenness, and diversity

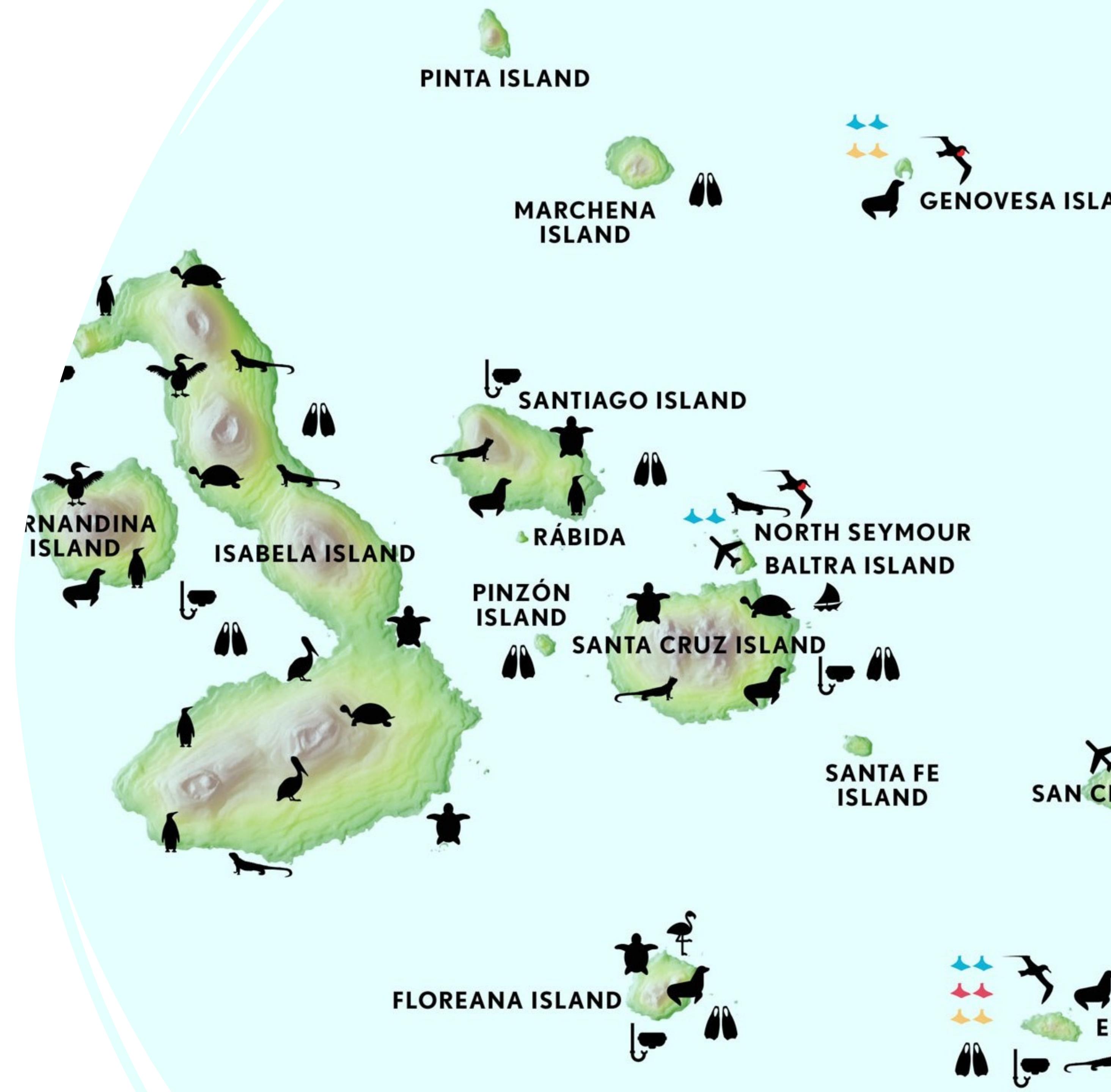
Each forest has the same four tree species (same species richness), but they differ in species evenness (relative abundance of each species).



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Diversity

- **Alpha diversity:** number of species on each island
- **Beta diversity:** species change between islands
- **Gamma diversity:** species on all islands



Some important terms

- **Species richness**
 - the *number of species* in an area
- **Species abundance**
 - the *number of individuals* of each species in an area
- **Species diversity**
 - is a term used for *the different number of species* in an area, their *abundance*, and *the distribution of these species* in that particular ecosystem



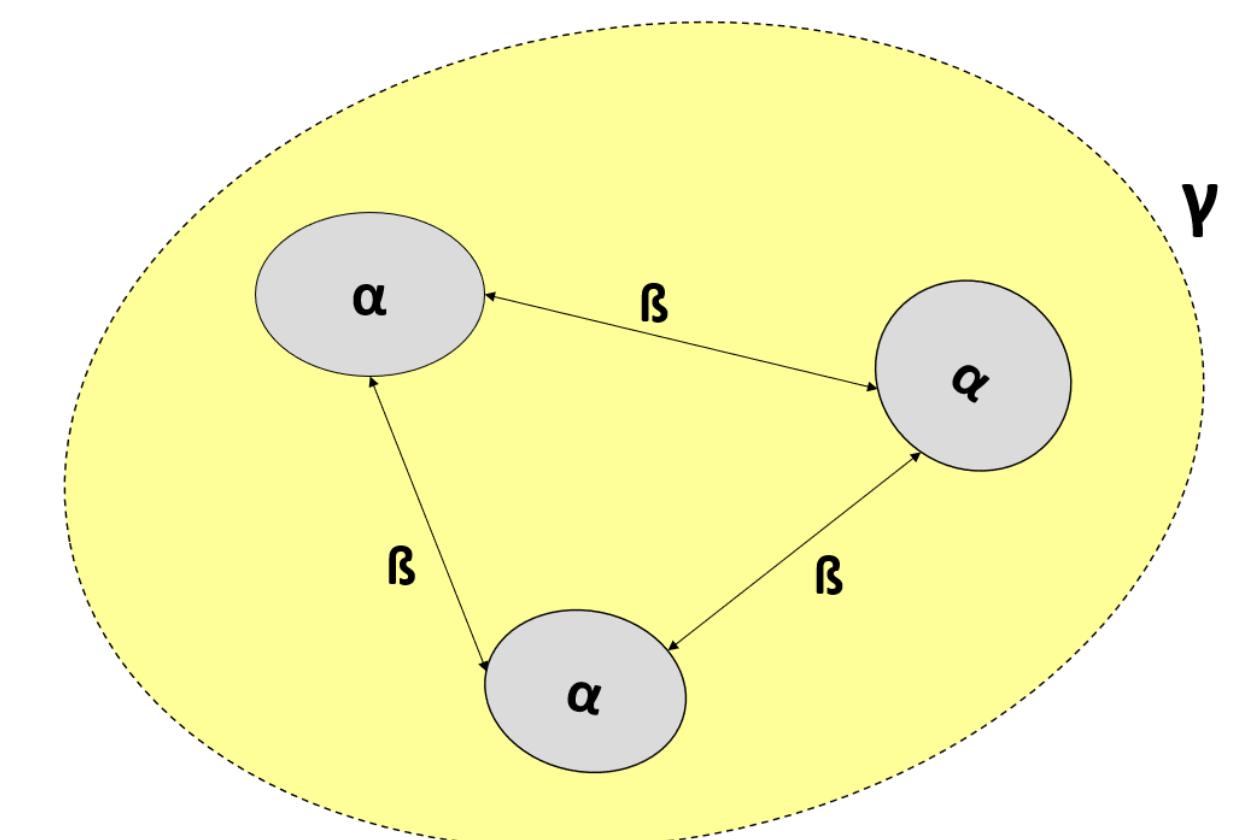
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Diversity

- **Alpha**
 - Richness: number of species in a location/sample
 - Evenness: relative species abundance in a location/sample
- **Beta**
 - Species turnover across locations/time points/samples
- **Gamma**
 - Species in all analysed locations/samples

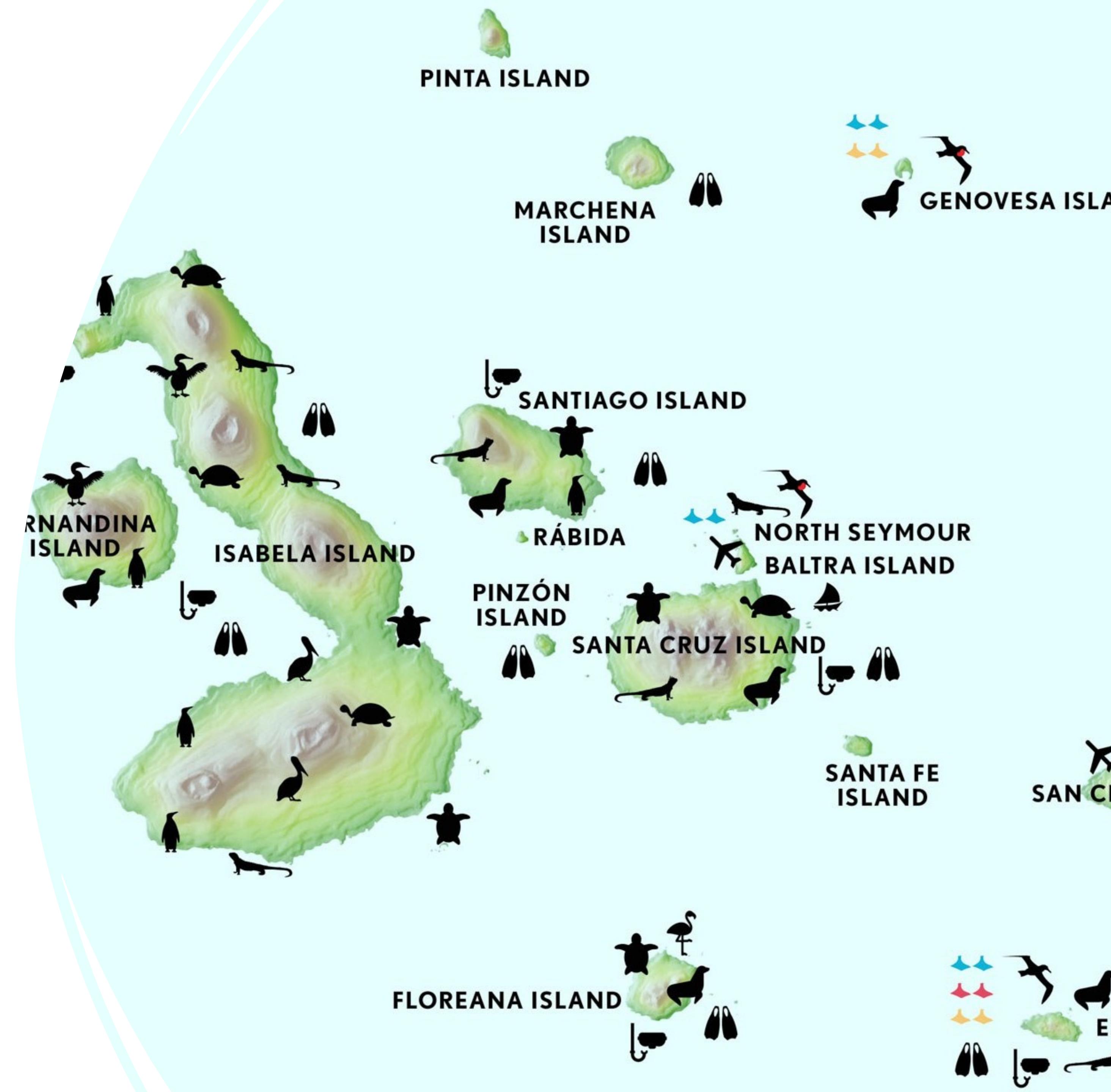


Robert Whittaker

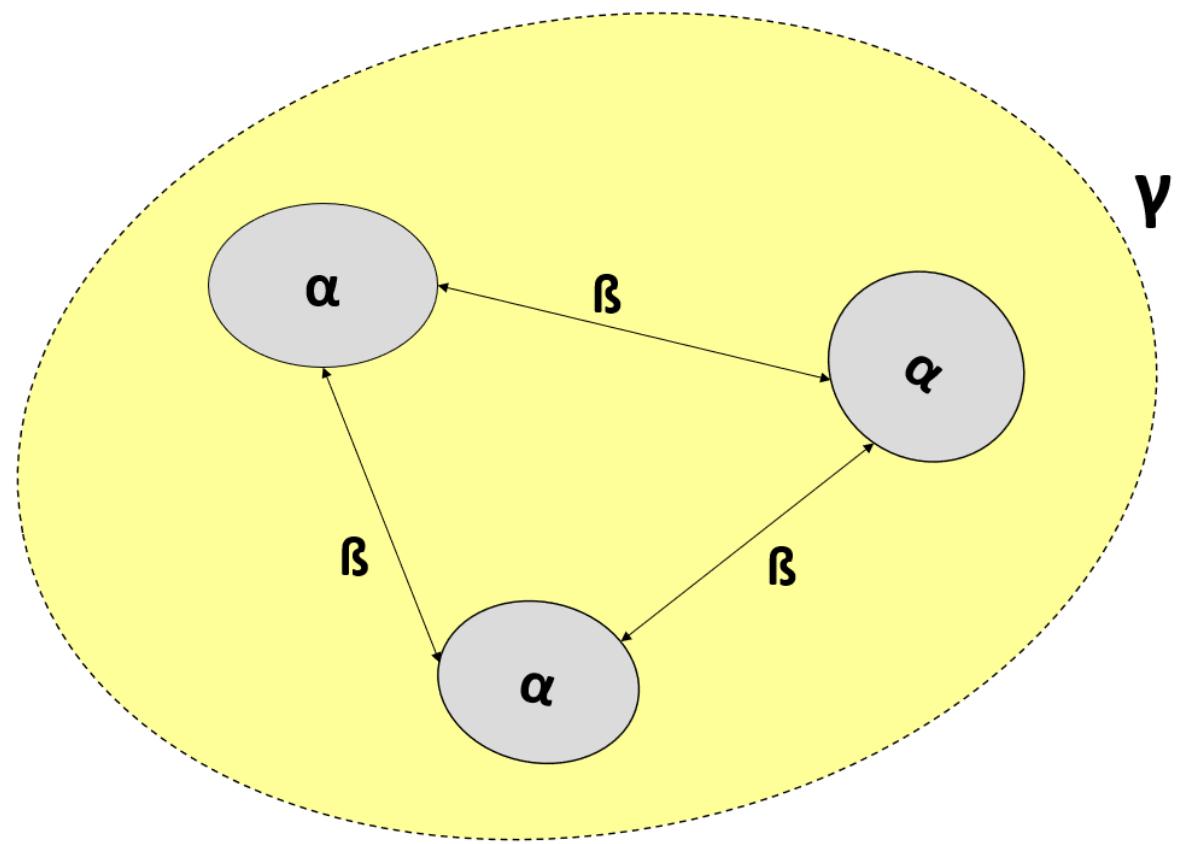


Diversity

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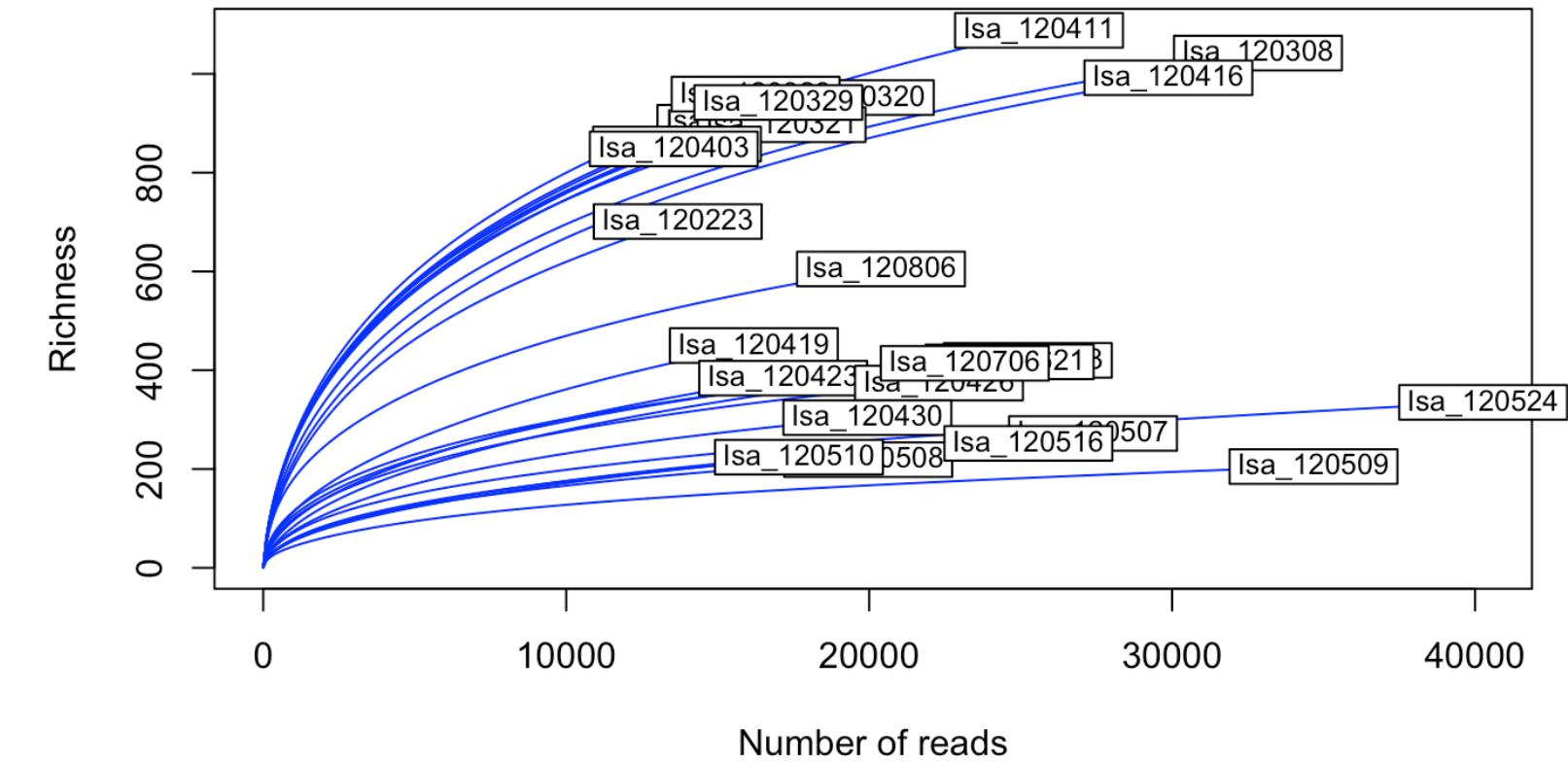
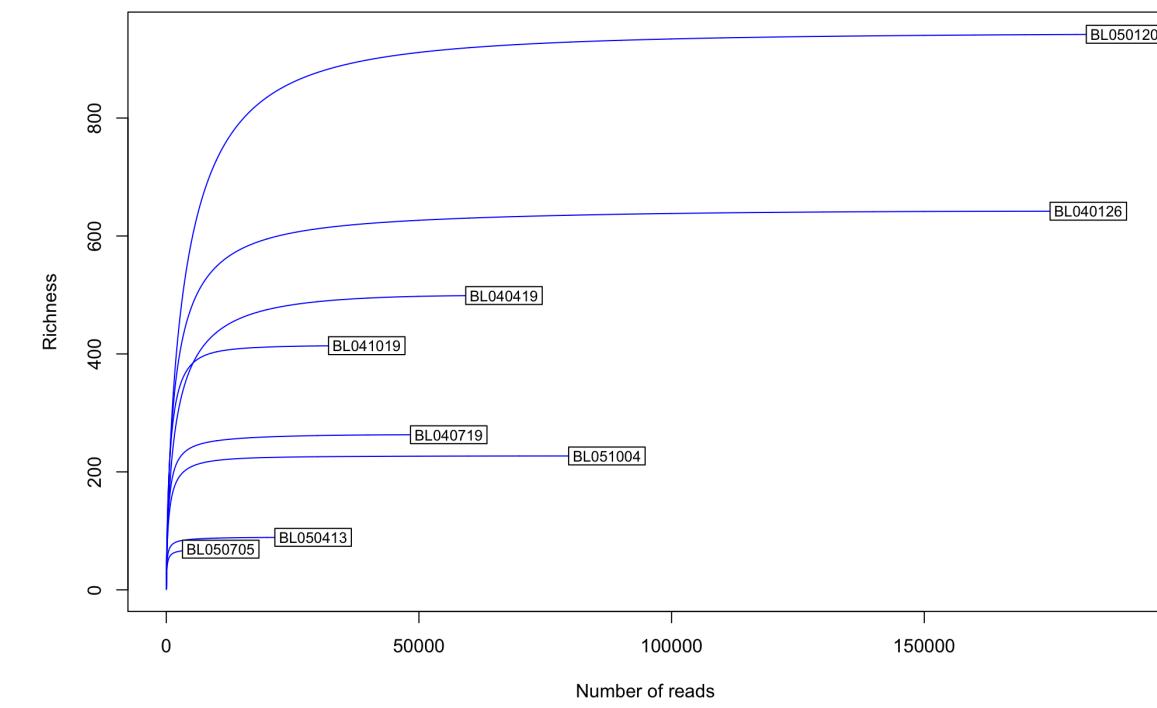
Alpha diversity



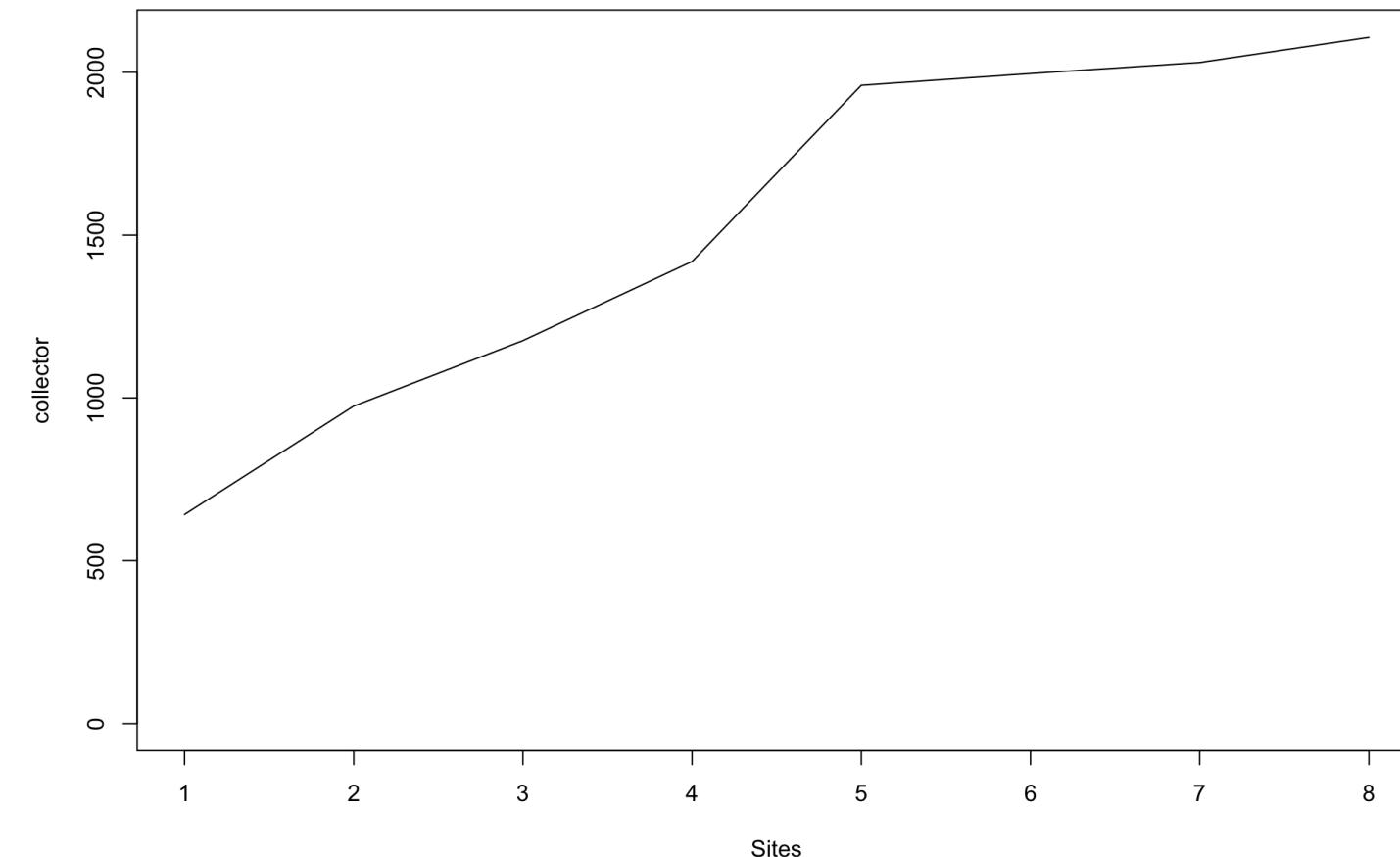
- Number of species in specific samples/location
- Alpha diversity describes the species diversity within a community at a small scale or local scale

Per sample - Alpha

- Rarefaction curves to see if the sequencing effort is sufficient
 - How many new OTUs appear in each sample as we add more reads?

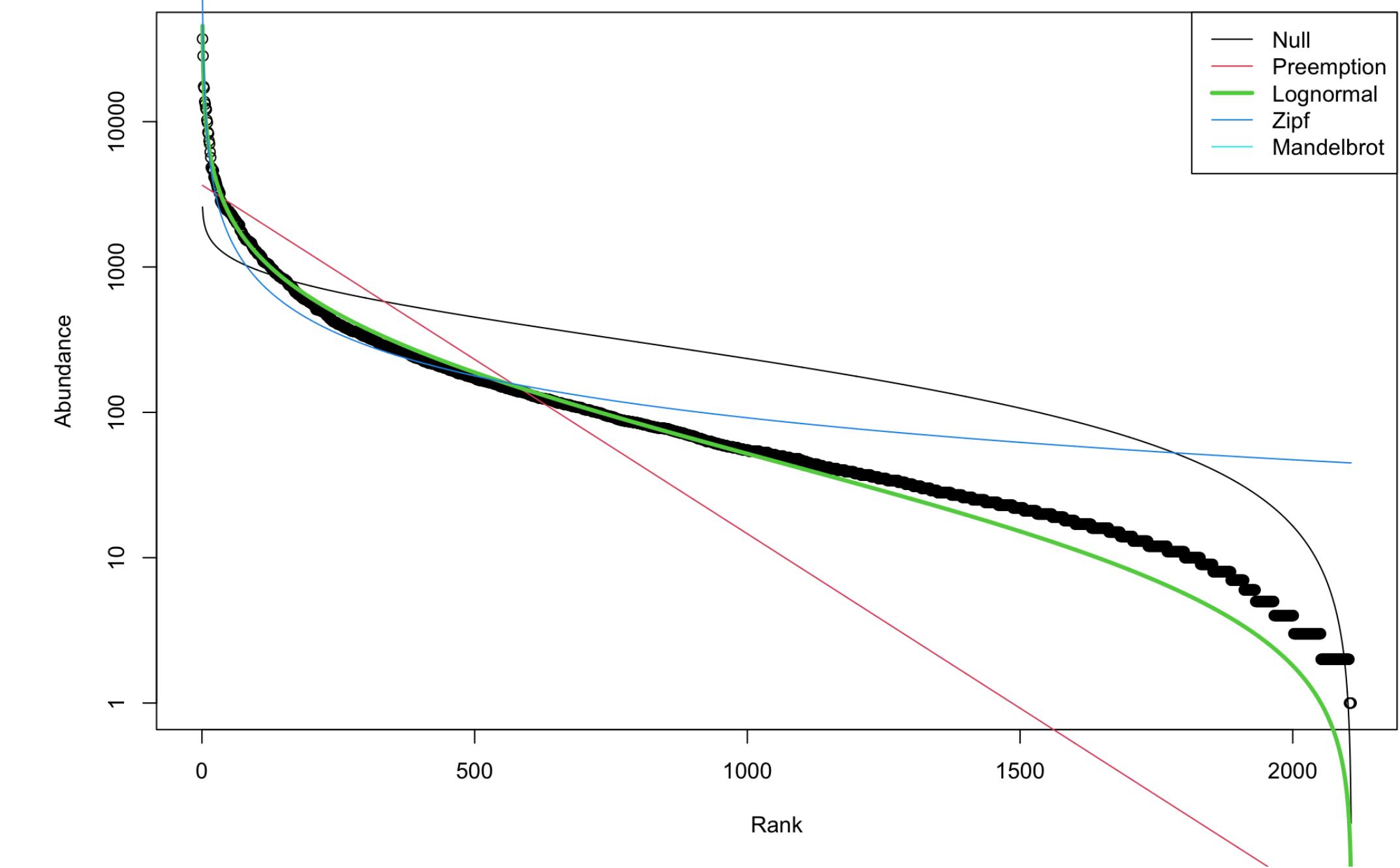
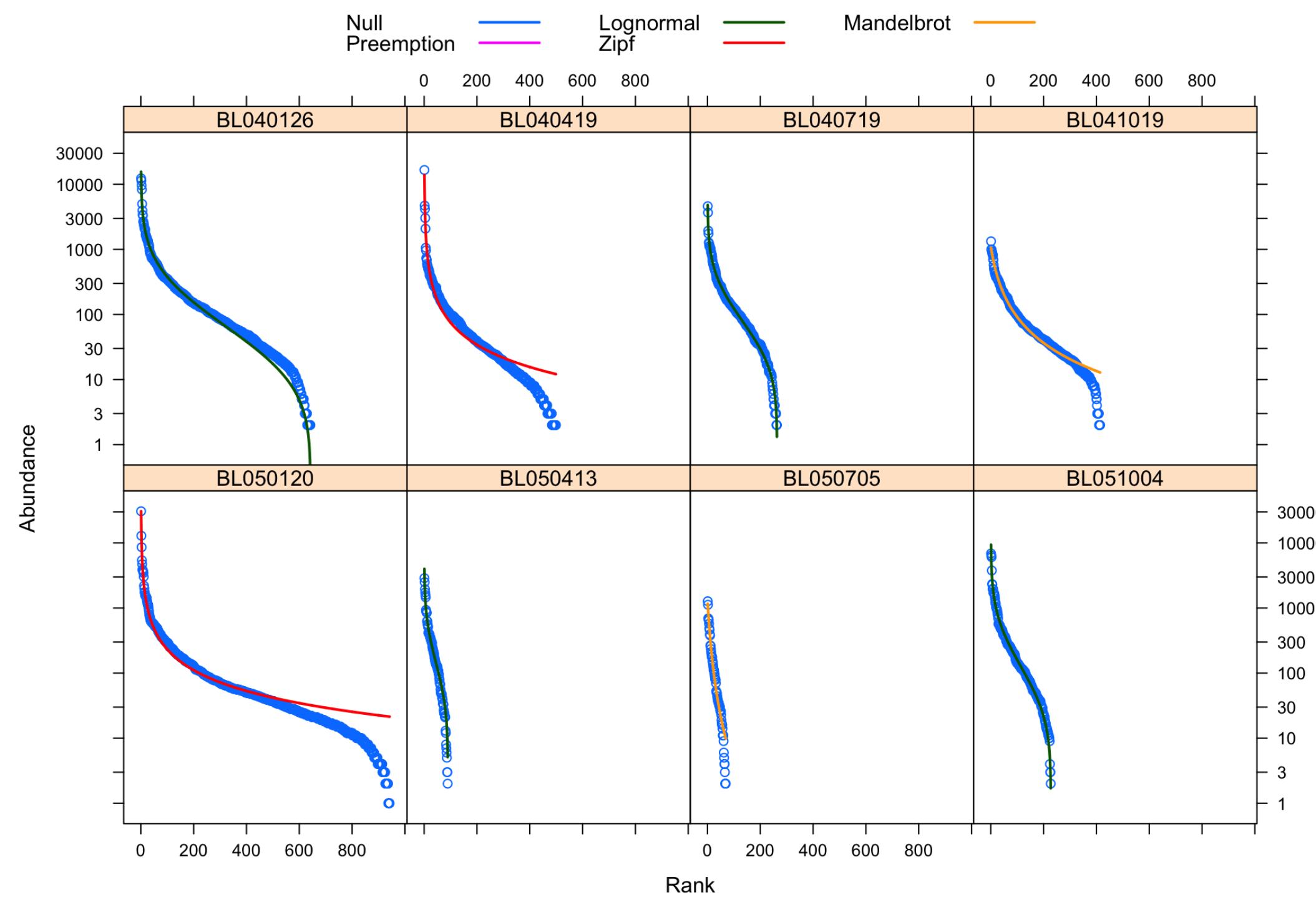


- Richness accumulation curves
 - How much is the richness increasing as we add more samples? Increase in OTUs.

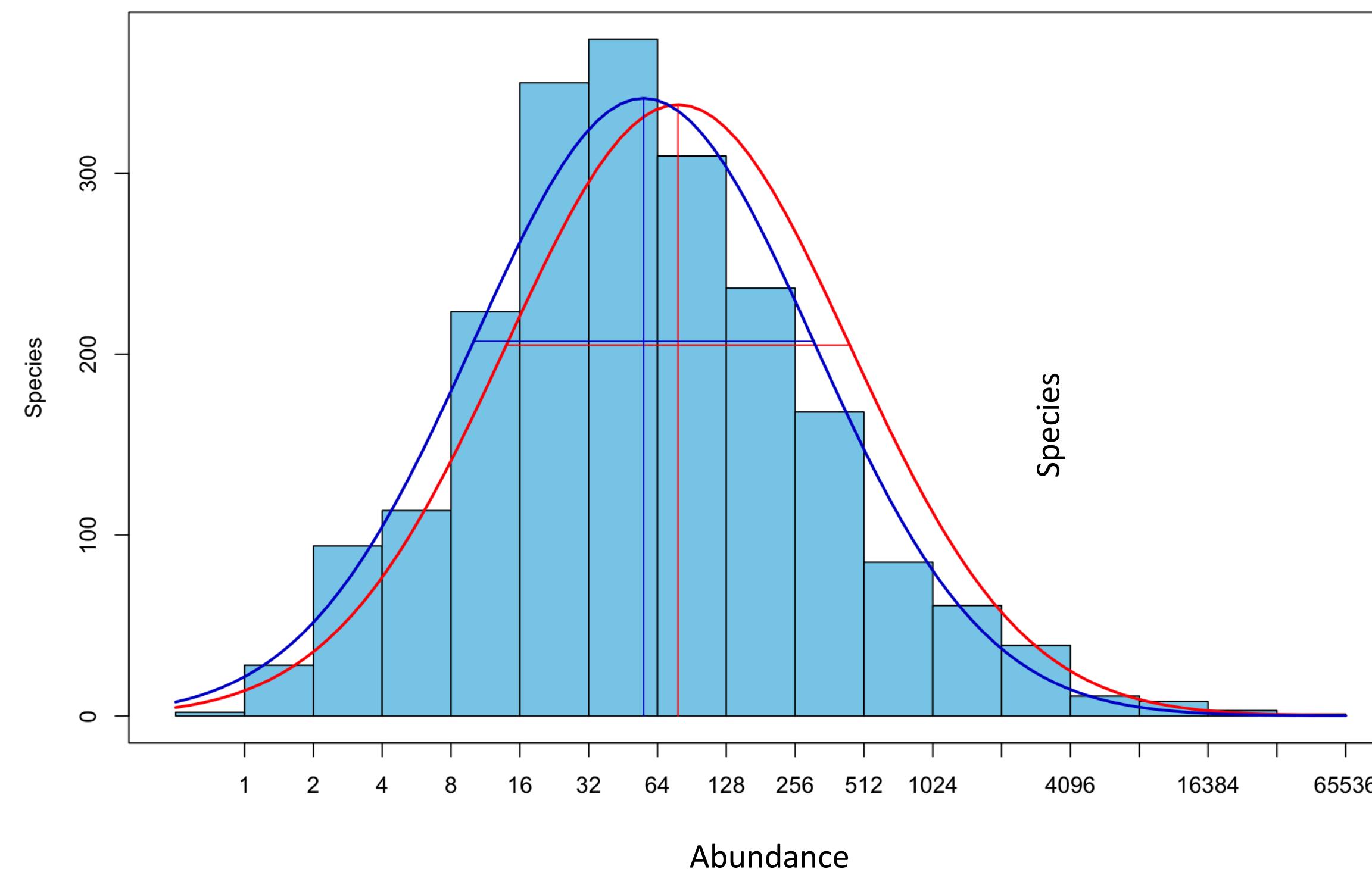


Fitting rank-abundance distributions

- How are the species (OTUs) distributed in each sample/timepoint (alpha) or across all samples (gamma).



- Frank W. Preston (1948) proposed that species abundances (when binned logarithmically) follow a normal distribution.
- This leads to a lognormal abundance distribution.
- Before using ASVs, these plots were rarely observed for microbial data



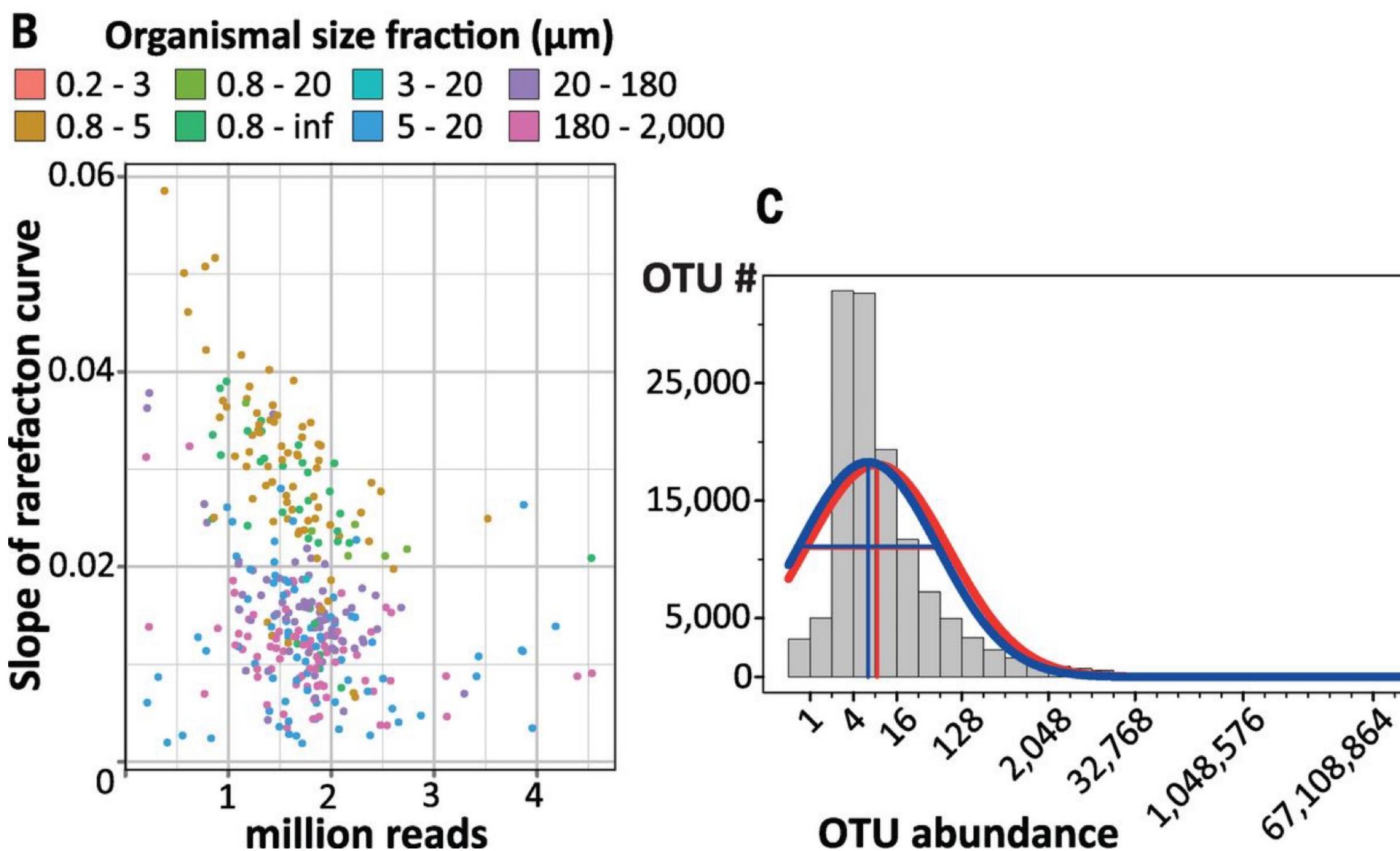
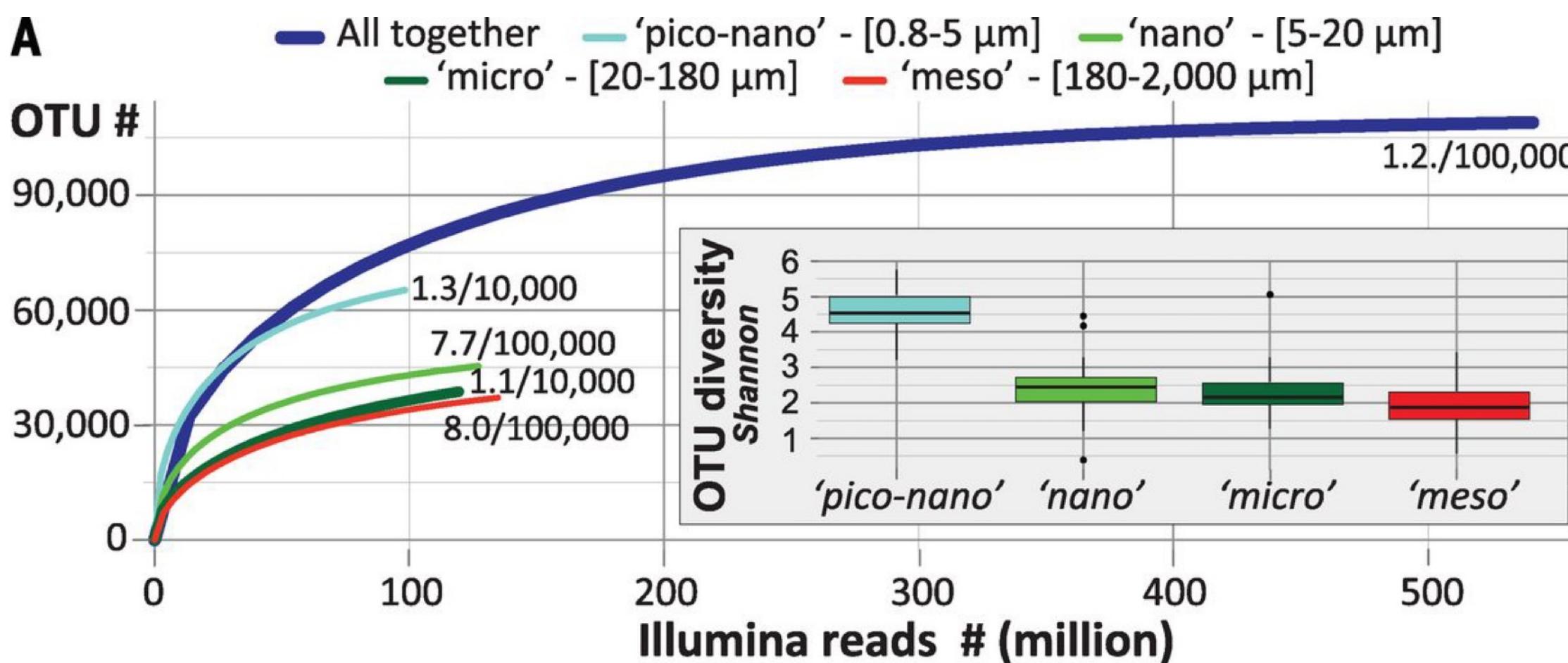
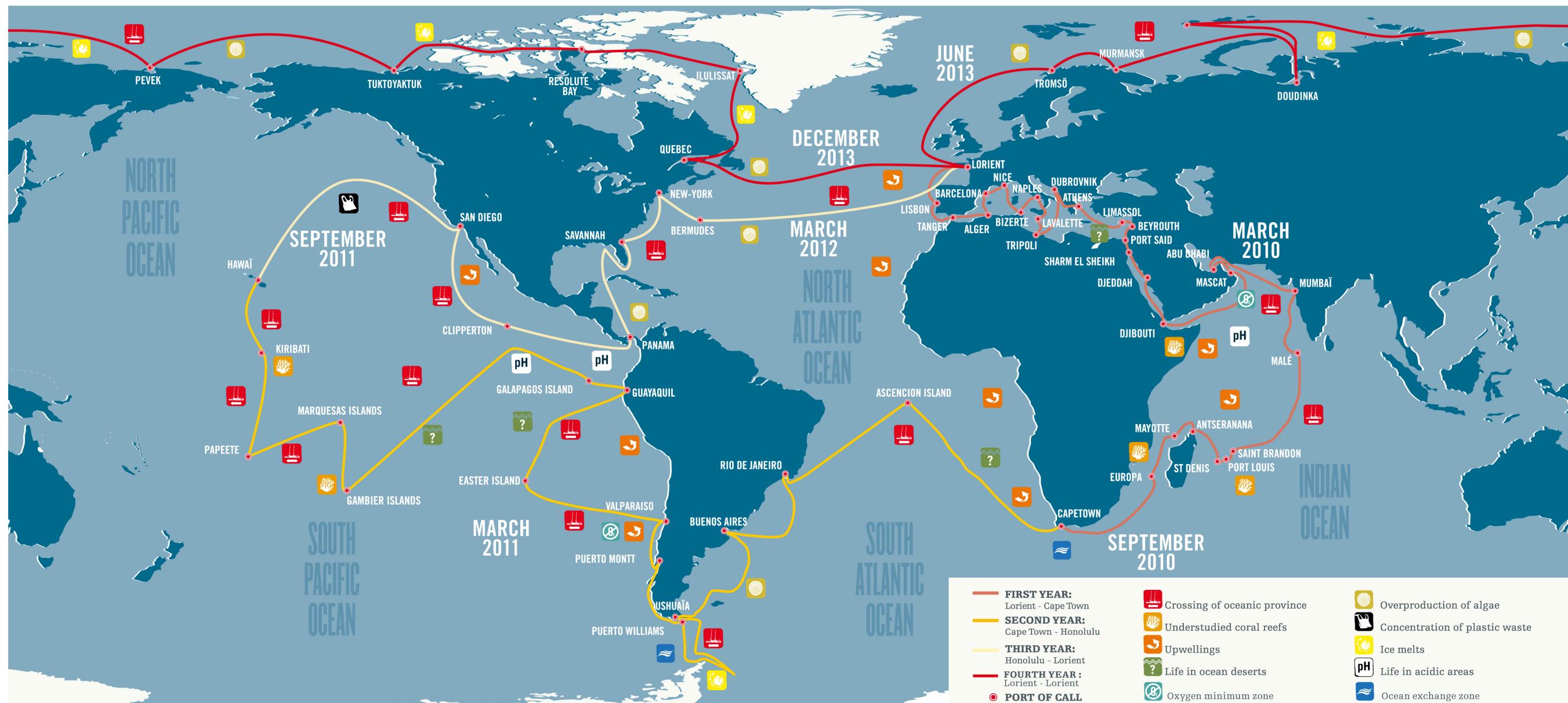
RESEARCH ARTICLE

Eukaryotic plankton diversity in the sunlit ocean

Colomban de Vargas^{1,2,*†}, Stéphane Audic^{1,2,†}, Nicolas Henry^{1,2,†}, Johan Decelle^{1,2,†}, Frédéric Mahé^{3,1,2,†}, Ramiro Logares...

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Science 22 May 2015:
Vol. 348, Issue 6237, 1261605
DOI: 10.1126/science.1261605



Diversity Indices

Shannon index H (entropy).

A diversity index considering the number of individuals as well as the number of taxa. Varies from 0 for communities with only a single taxon to high values for communities with many taxa, each with few individuals

-Originally proposed by Claude Shannon in 1948 to quantify the entropy in strings of text

Pielou's index of evenness

A calculated value of Pielou's evenness ranges from 0 (no evenness) to 1 (complete evenness).

Inverse Simpson's D index

The value of Simpson's D ranges from 0 to 1, with 0 representing infinite diversity and 1 representing no diversity, so the larger the value of D , the lower the diversity. For this reason, Simpson's index is usually expressed as its inverse

$$H' = - \sum_{i=1}^R p_i \ln p_i$$



Claude Shannon

$$J' = \frac{H'}{H'_{\max}}$$



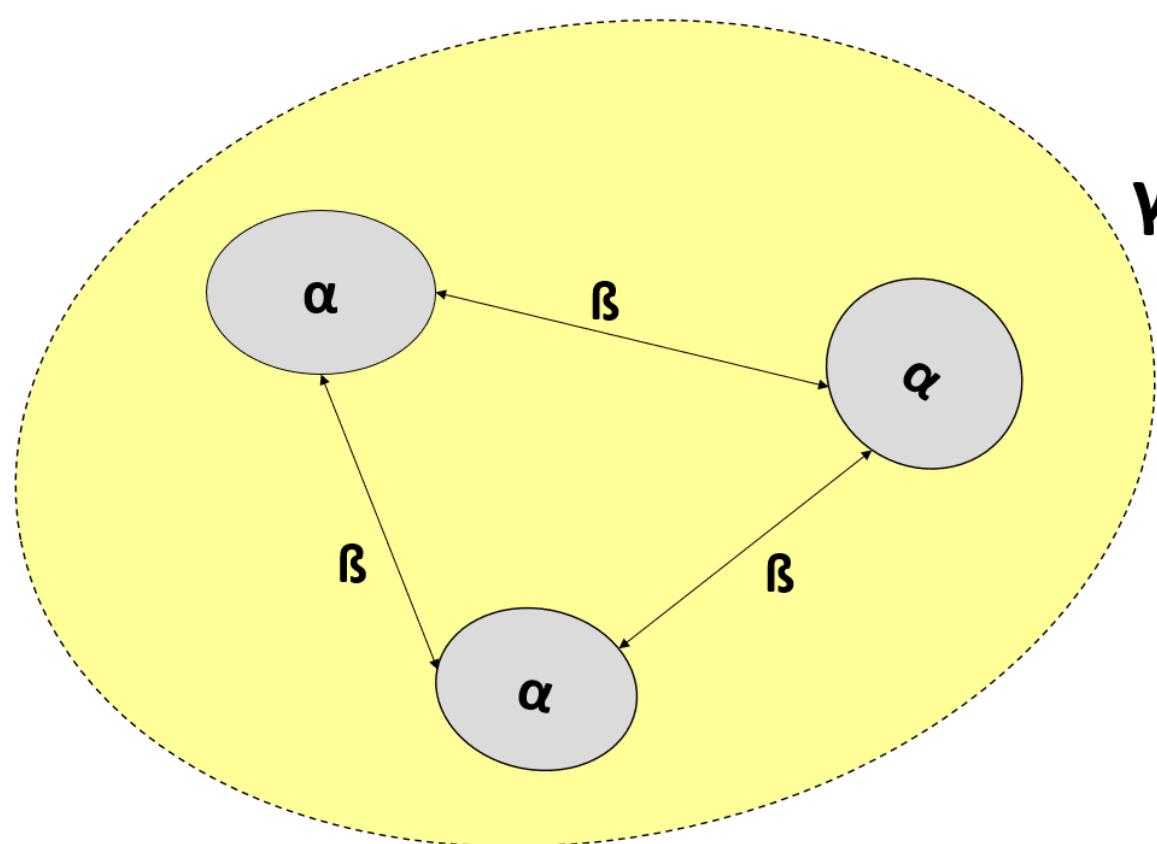
E.C. Pielou

$$\frac{1}{\lambda} = \frac{1}{\sum_{i=1}^R p_i^2} = {}^2 D$$



Edward Simpson

Beta diversity



- Species turnover between samples/location
- Beta diversity analyses measure how communities change between different samples (time points, locations, etc.)
- Analyses can be biased if different samples have different sequencing efforts (or sequencing depth)
- High throughput sequencing (HTS/NGS) data are compositional

HTS data are compositional

- HTS datasets are compositional, due to the total limits imposed by sequencers. Then, the increase of one OTU means the decrease of another OTU in the HTS dataset.
- The total read count in an HTS run is a fixed-size, random sample of the relative abundance of the molecules in the underlying ecosystem
- The count cannot be related to the absolute number of molecules in the input sample

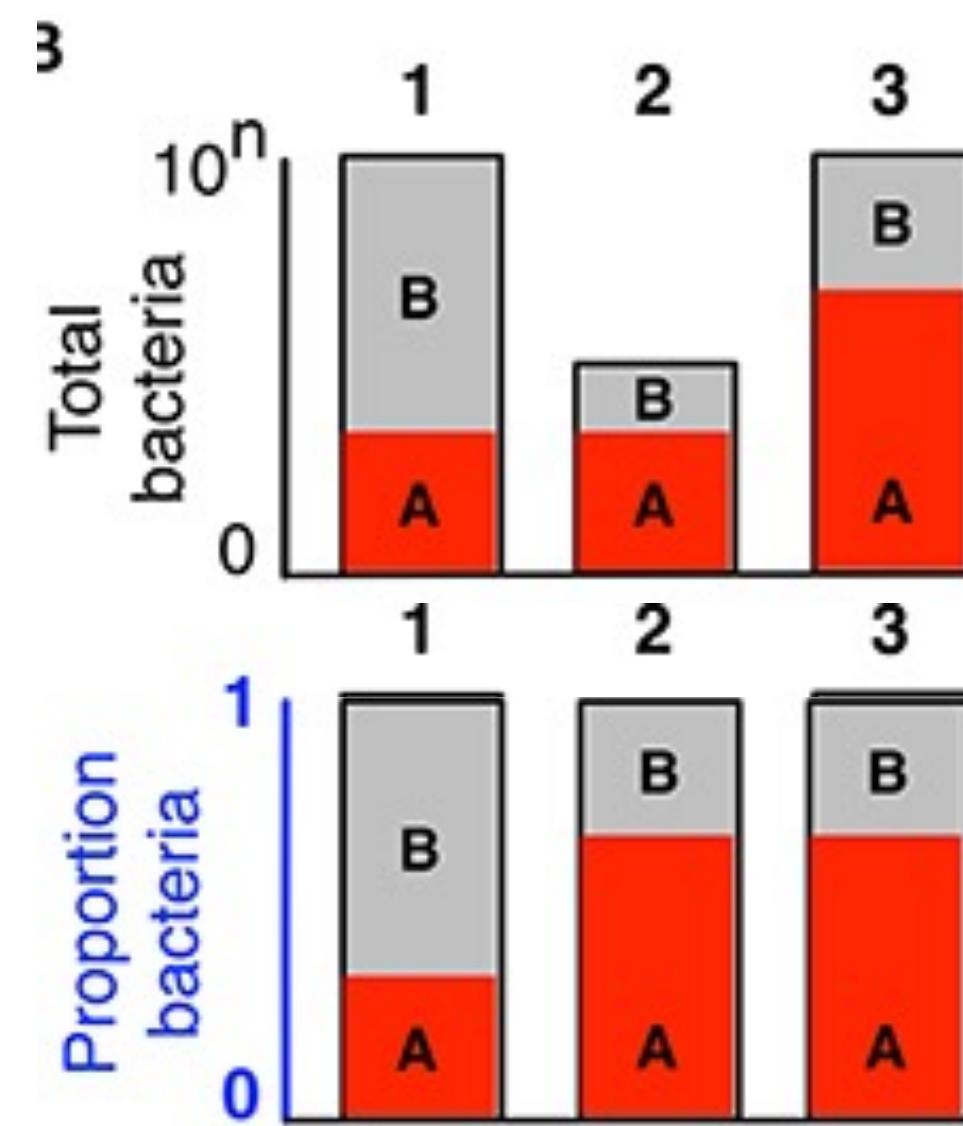
HTS data are compositional



- This is implicitly acknowledged when microbiome datasets are converted to **relative abundance** values, or **normalised counts**, or are **rarefied**
- Data described as proportions or probabilities, or with a constant or irrelevant sum, are referred to as **compositional data**
- Compositional data is all about **the relationships between the parts** (can't inform on absolute abundances of molecules)
- The abundance of one OTU is only interpretable relative to another

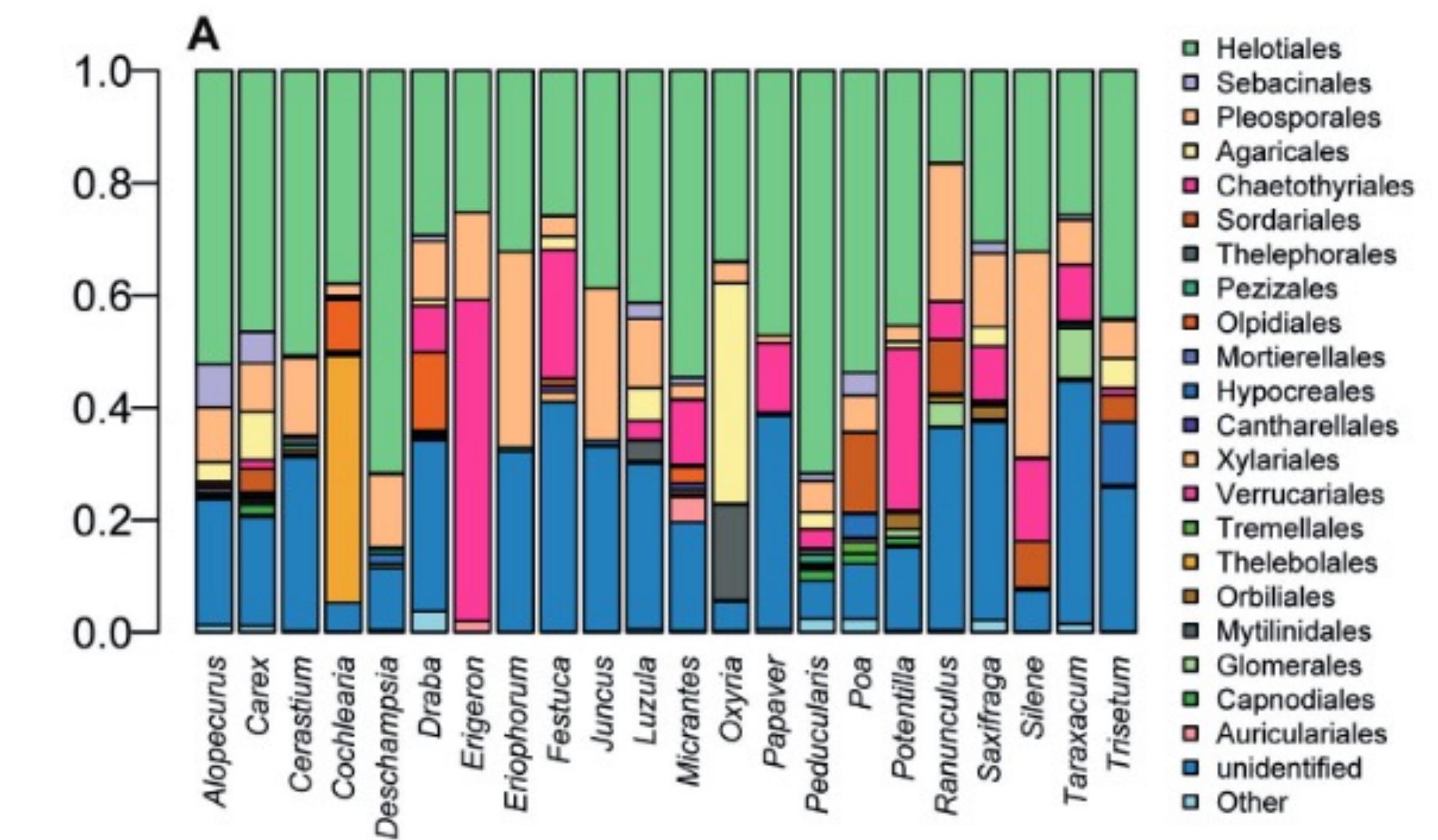
HTS data are compositional

- After samples are sequenced we lose the absolute count information and only have relative abundances, proportions, or “normalised counts”



Absolute abundance
before sequencing

Relative abundance
after sequencing



HTS data have different sequencing depth

- Different sequencing depths may bias the calculation of distances for multivariate analyses
- One way to mitigate this is to subsample or “rarefy” samples to the same sequencing depth
 - But, it has been criticised due to loss of information and precision
- Function `rrarefy()` in `vegan` generates a randomly rarefied (without replacement) community data frame or vector of given sample size.

Other ways of transforming

- The clr transformation is becoming more popular
- centered log-ratio (clr) transformation
- Ratio transformation of the data: captures relationships between features (e.g. OTUs)
- Taking the logarithm of the ratios (log-ratios) makes data symmetric and linearly related
- In a composition, all components (OTUs) are mutually dependent, and can not be understood in isolation
- Analyses of individual components (OTUs) is done with respect to a reference
- The clr transformation uses the geometric mean as a reference

Computer Lab II:

Distance measures

Ordination, and

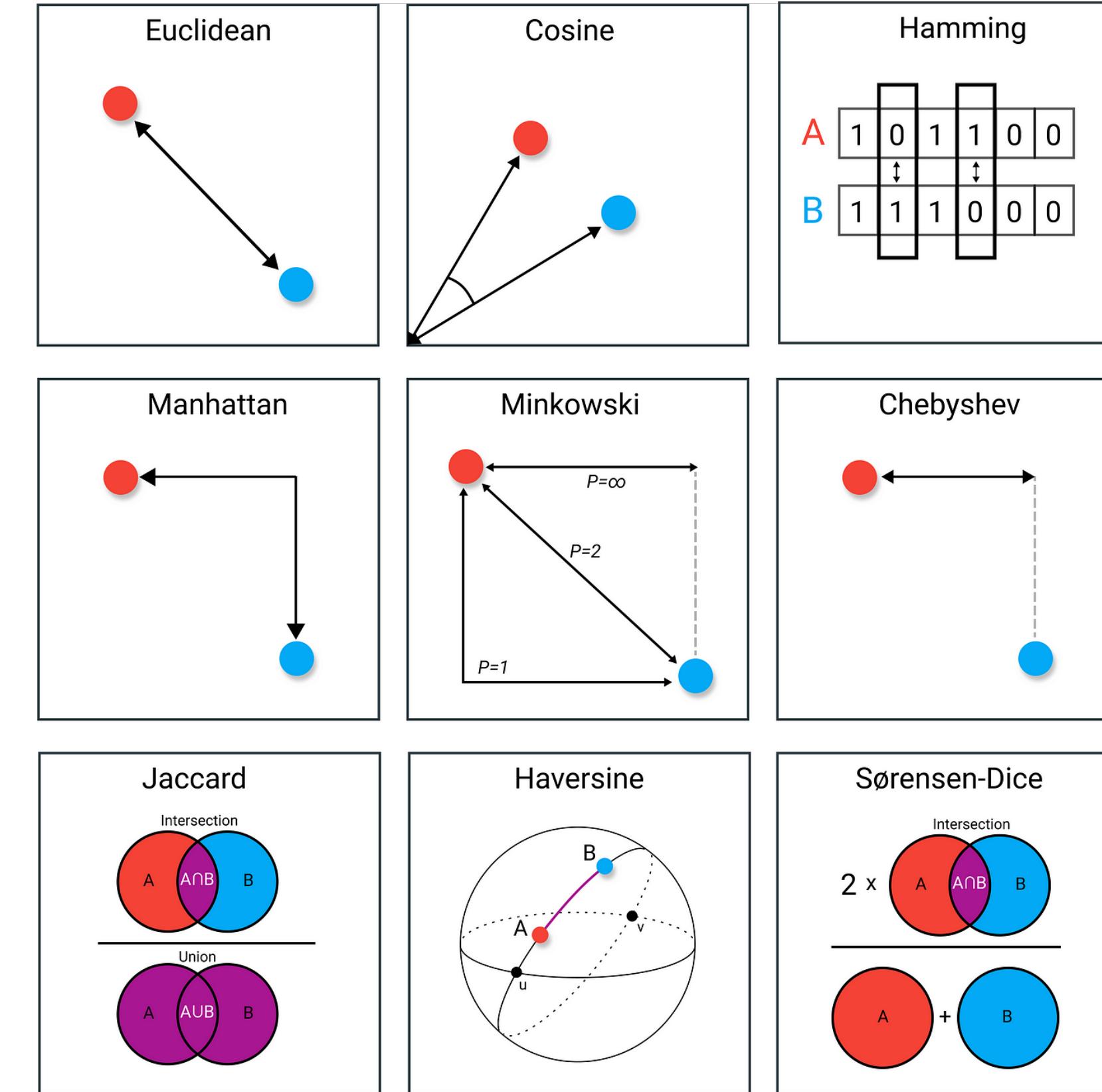
clustering

- **Keyterms:** Bray-curtis,
PCA, NMDS, UPGMA

Distance metrics

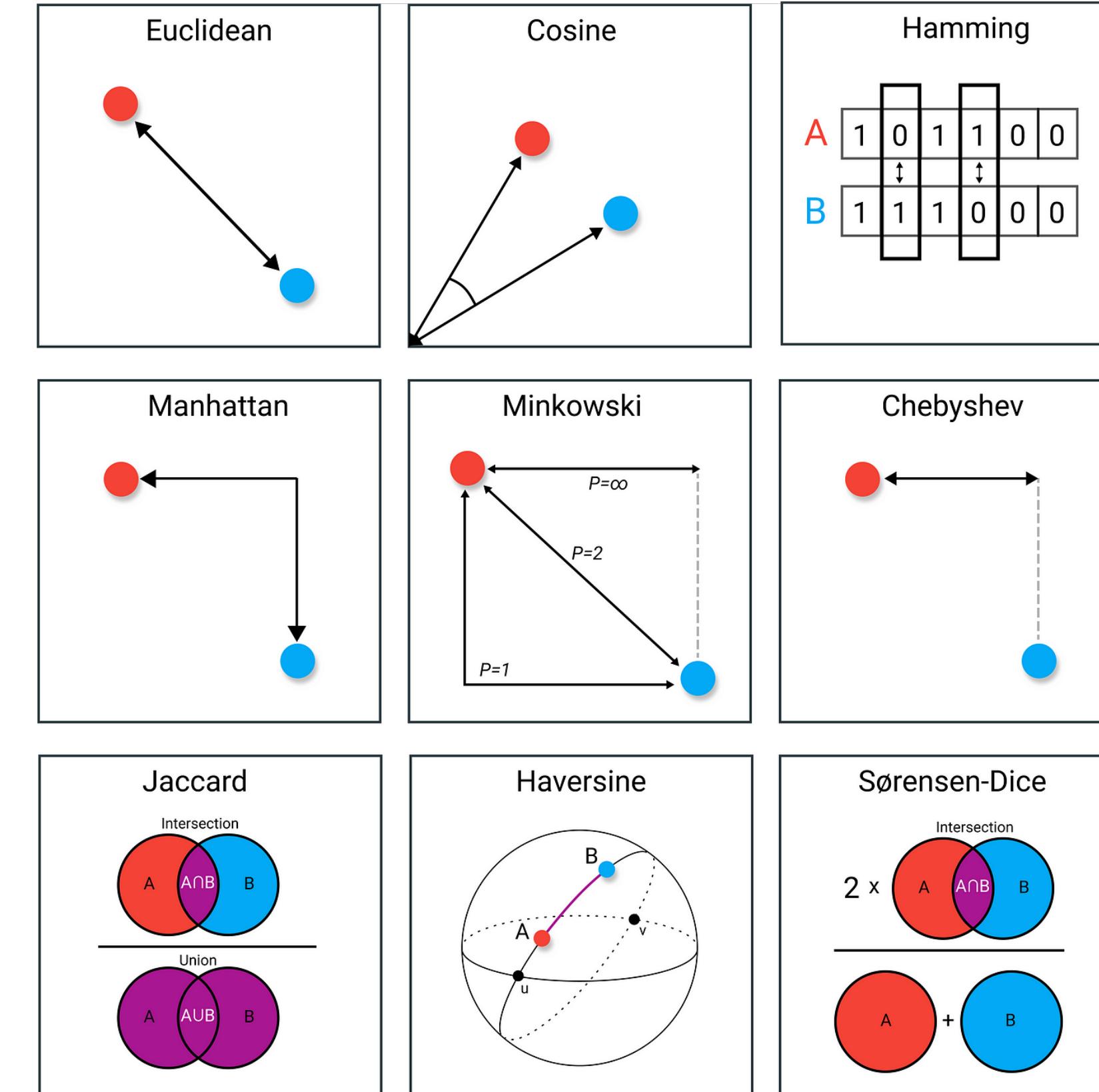
- Statistical distance: distance between variables
- Distance metrics in ecology: allow measuring the dissimilarity between communities composed by several species (OTUs)
- Several distance metrics available in R
- Often used: Euclidean, Jaccard, Sørensen, Simpson, **Bray Curtis**

$$BC_d = \frac{\sum |x_i - x_j|}{\sum (x_i + x_j)}$$



Distance metrics

- It is important to think what is the most appropriate distance metric for the data
- Different distance metrics can have different ranges
- Bray-Curtis: influenced by abundant taxa (but normally used)
 - Ranges between 0-1 (1 most dissimilar)
- Euclidean and Sørensen: influenced by large differences in species abundances, data sparsity (lots of zeros) and many observations
- Euclidean: no upper limit of values



Ordination

- Is a collective term for multivariate techniques which summarise a multidimensional dataset in such a way that when it is projected onto a two dimensional space any intrinsic pattern the data may possess becomes apparent upon visual inspection (Pielou, 1984)
- In ecological terms: ordination serves to summarise community data (such as species abundance data) by producing a *low-dimensional ordination space in which similar species and samples are plotted close together, and dissimilar species and samples are placed far apart.*

Ordination

- Ordination is used in ecology to investigate relationships between species composition patterns and environmental variability.
- Many times, these techniques are used to address the question: what environmental variables shape communities?
- The relative importance of environmental gradients in shaping communities can be estimated

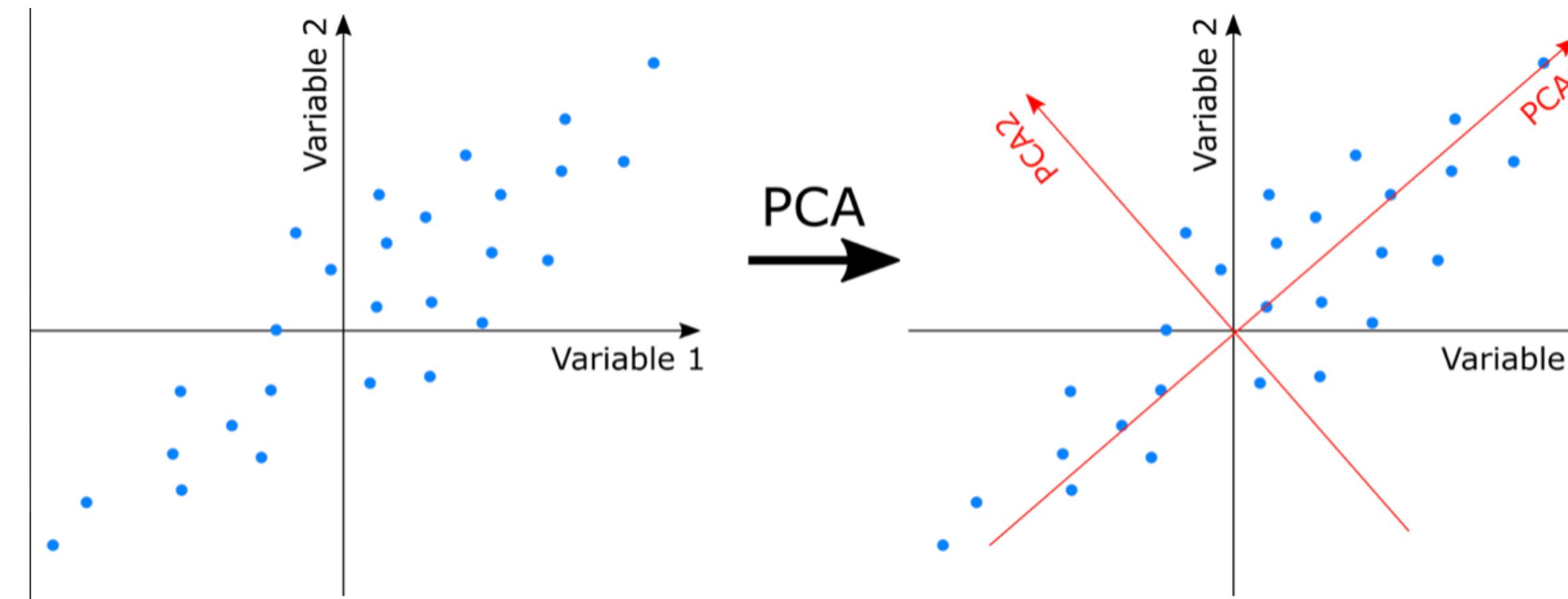
Ordination techniques

Two commonly used unconstrained techniques

- Principal Component Analysis (**PCA**)
 - Assumes linear relationship between features, variances, and correlations (i.e. euclidean space)
 - Deterministic, always the same outcome for the same dataset
 - Orthogonal linear transformation
- Non-metric Multidimensional Scaling (**NMDS**)
 - Does not assume linear relationship between features, which allows the use of any (suitable) distance measure (for instance bray-curtis)
 - NMDS relies on rank orders (distances) for ordination
 - Iterative and non-parametric process
 - Outcome will vary for each run

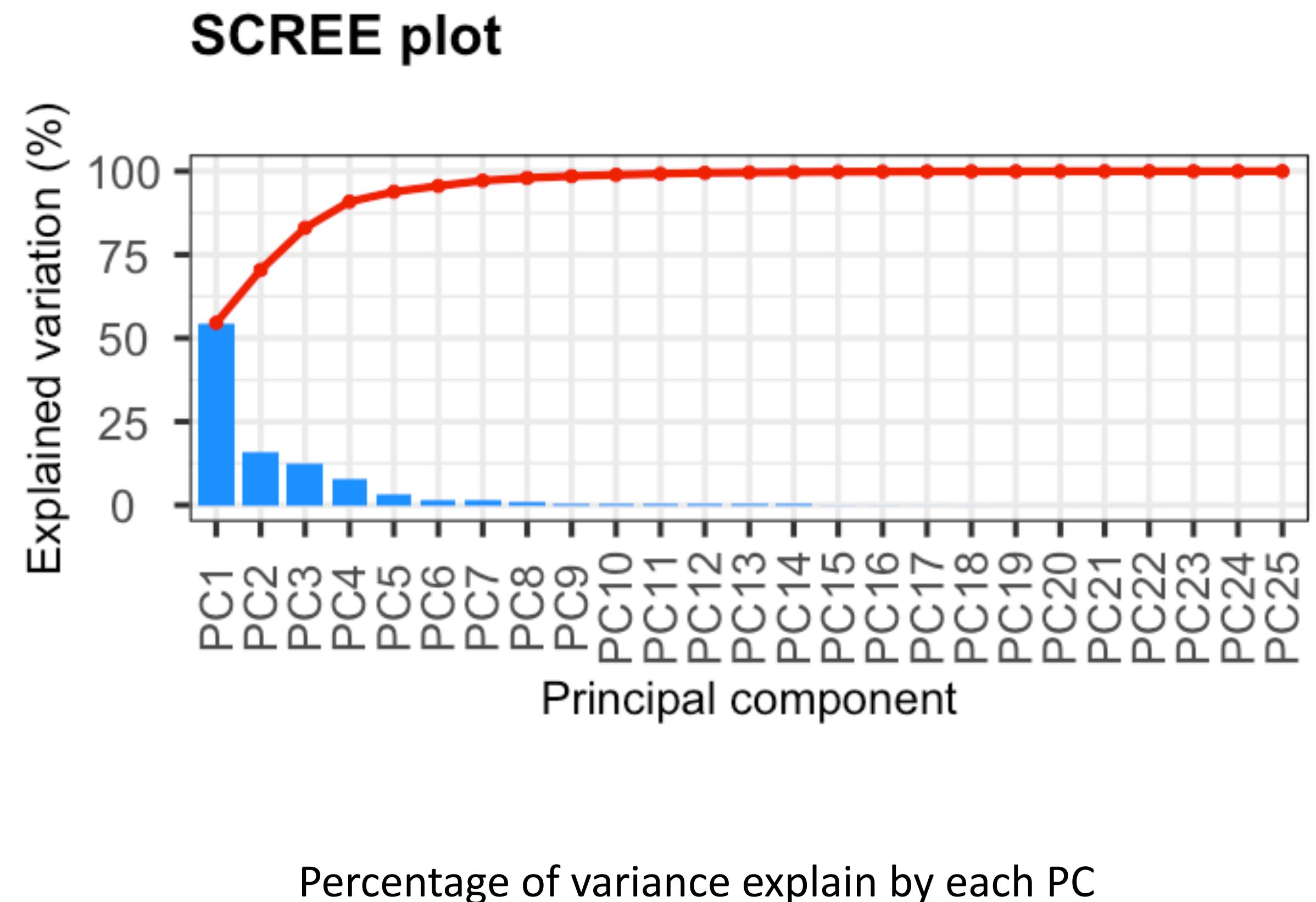
PCA

- A rotation of the coordinate axes, chosen such that each successful axis captures as much variance as possible
- The first principal component (PC) will be placed in the direction of the maximum variability and subsequent axes (PCs) will be generated in the same manner



PCA

- Typically the first 1-4 axes represents the majority of the variation in the data set. The rest is often considered noisy



Non-metric Multidimensional Scaling (NMDS)

- NMDS is more robust than PCA (e.g. is not affected by the arch effect)
- NMDS attempts to represent the **pairwise dissimilarity between objects in a low-dimensional space**
- Any distance metric can be used to build the distance matrix
- NMDS is a rank approach, meaning that distances are replaced by ranks
- The stress value indicates how well the ordination summarises the observed distances among the samples

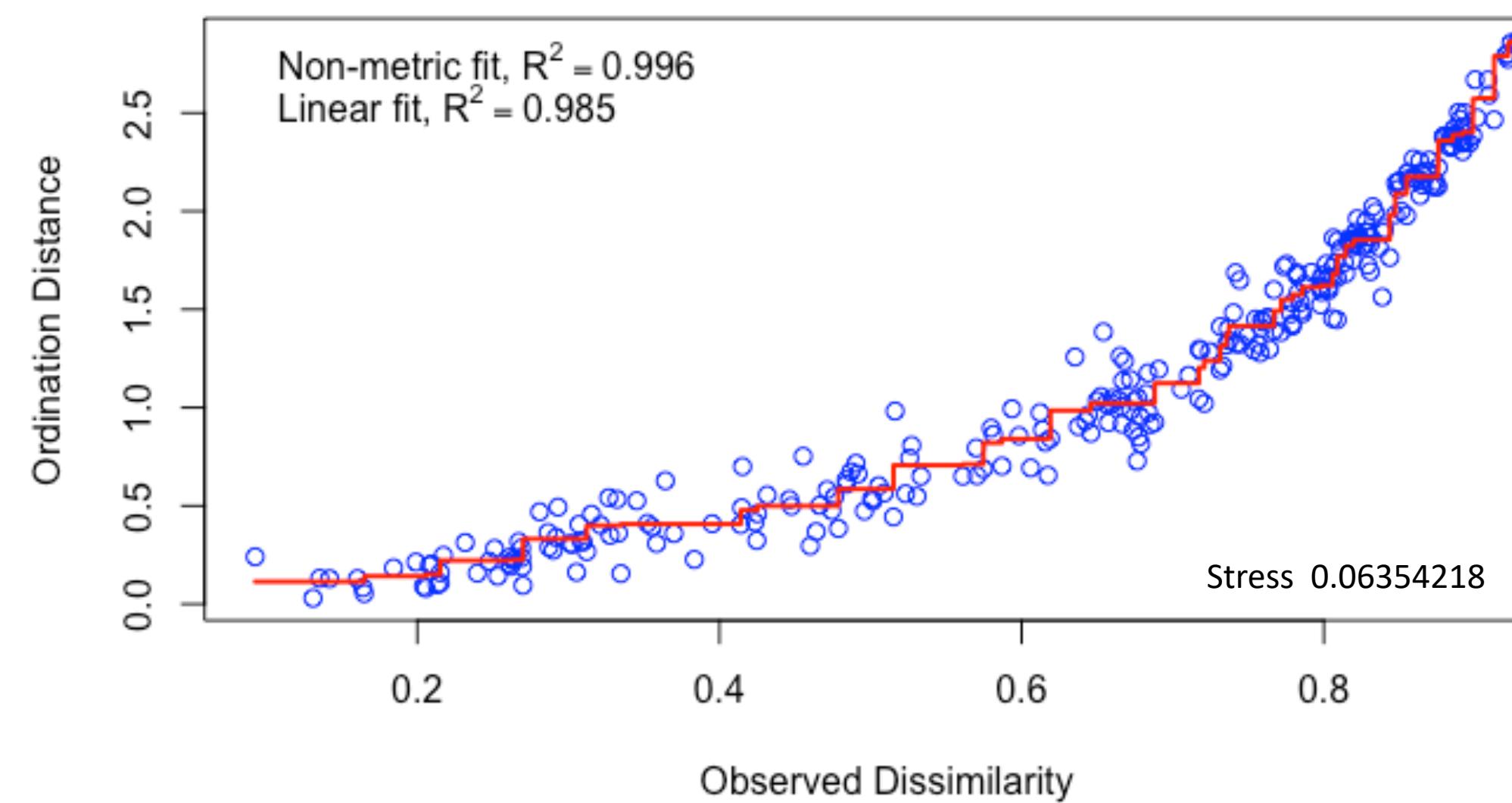
Non-metric Multidimensional Scaling (NMDS)

- NMDS differs from PCA in that:
- There is not a unique ordination result (thus, algorithms run NMDS multiple times)
- The axes of the ordination are not ordered according to the variance they explain (but metaMDS() in Vegan rotates final results to make Axis 1 correspond to the greatest variance among samples)
- The number of dimensions of the low-dimensional space must be specified before running NMDS
- Plotting stress (goodness of fit) vs. dimensionality can be used to assess the choice of dimensions. Stress values should be <0.2. We choose the minimum number of dimensions

Non-metric Multidimensional Scaling (NMDS)

- Running NMDS:

- Step 1: run NMDS with e.g. 2 to 10 dimensions
- Step 2: Check stress vs. dimension plot
- Step 3: Choose optimal number of dimensions (typically k=2)
- Step 4: Check for convergent solution and final stress

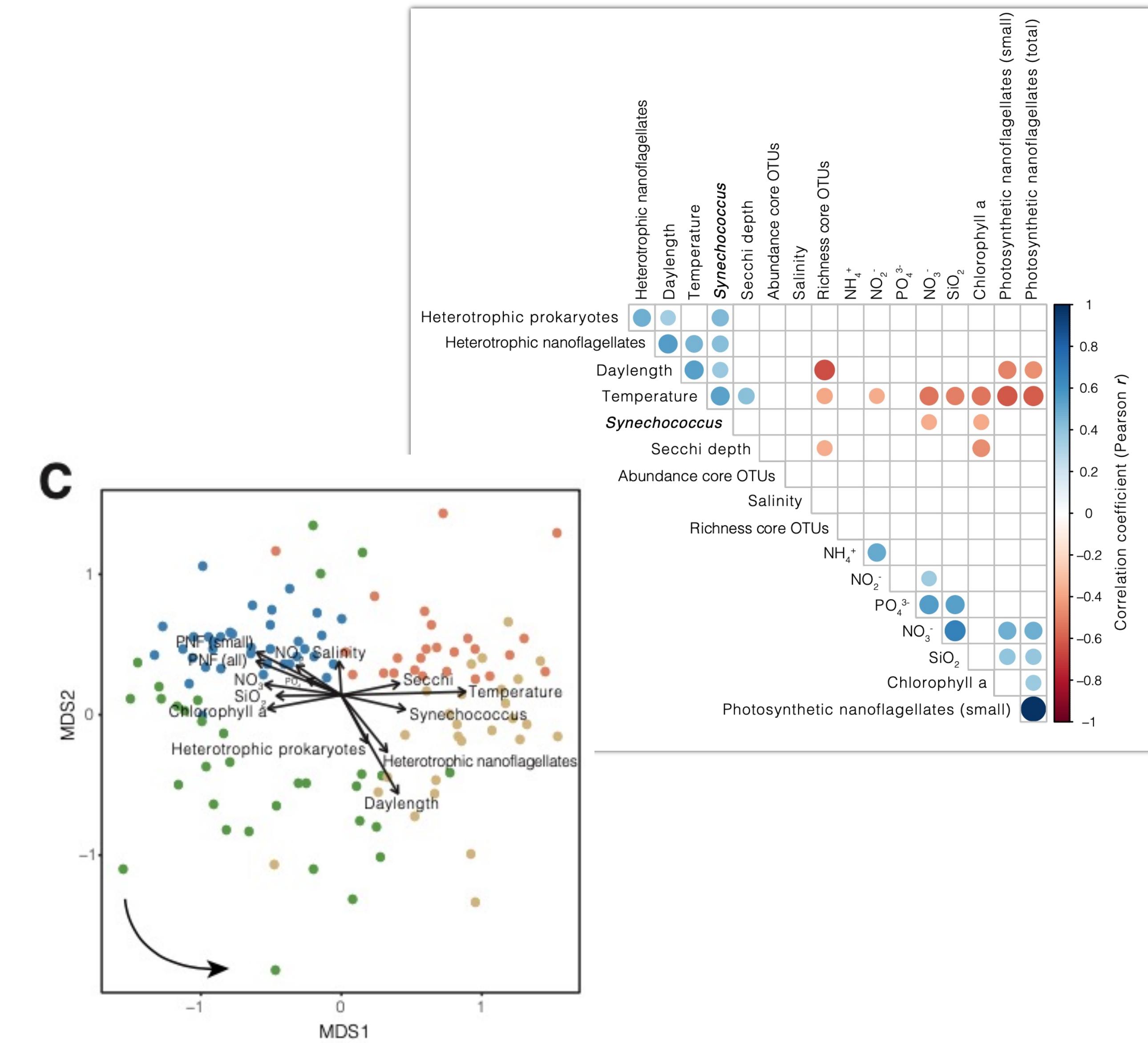


Hierarchical Clustering

- Hierarchical clustering: groups are organised in ranks according to their similarity
- UPGMA (unweighted pair group method with arithmetic mean): agglomerative (bottom-up) hierarchical clustering
- Based on a dissimilarity matrix

Computer Lab III: Incorporating environmental data

- **Keyterms:**
Unconstrained and
Constrained ordination



Environmental data

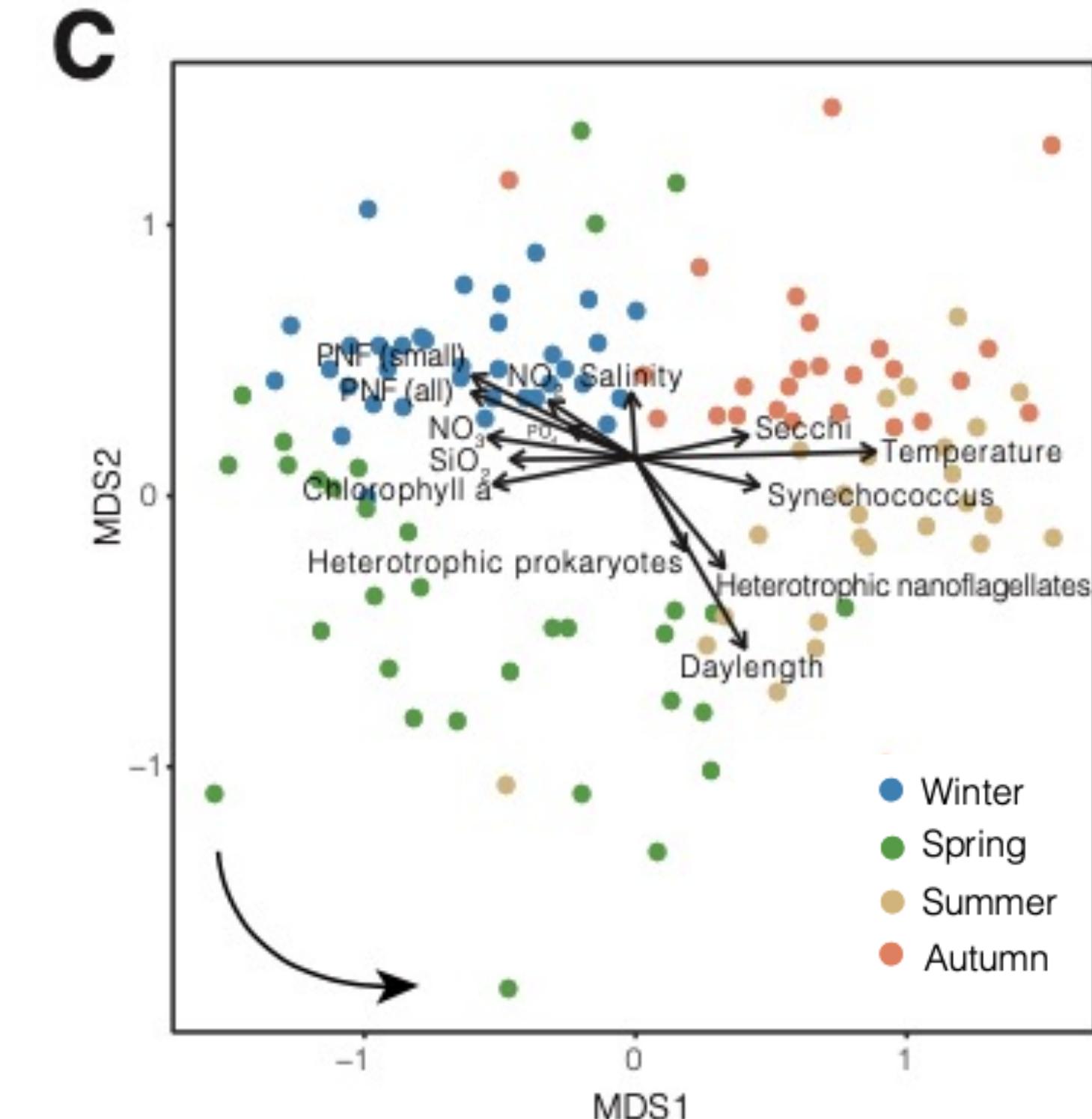
- We aim at investigating whether environmental variability could explain community variance
- Environmental variables are **standardised** to have comparable ranges of variation

Unconstrained vs. Constrained ordination

- In unconstrained ordination we first find the major compositional variation, and then relate this variation to observed environmental variation (envfit e.g.)
- In constrained ordination we do not want to display all or even most of the compositional variation, but only **the variation that can be explained by a set of environmental variables, or constraints**
- The constrained ordination is non-symmetric: we have independent variables or constraints (environmental data) and we have dependent variables or the community

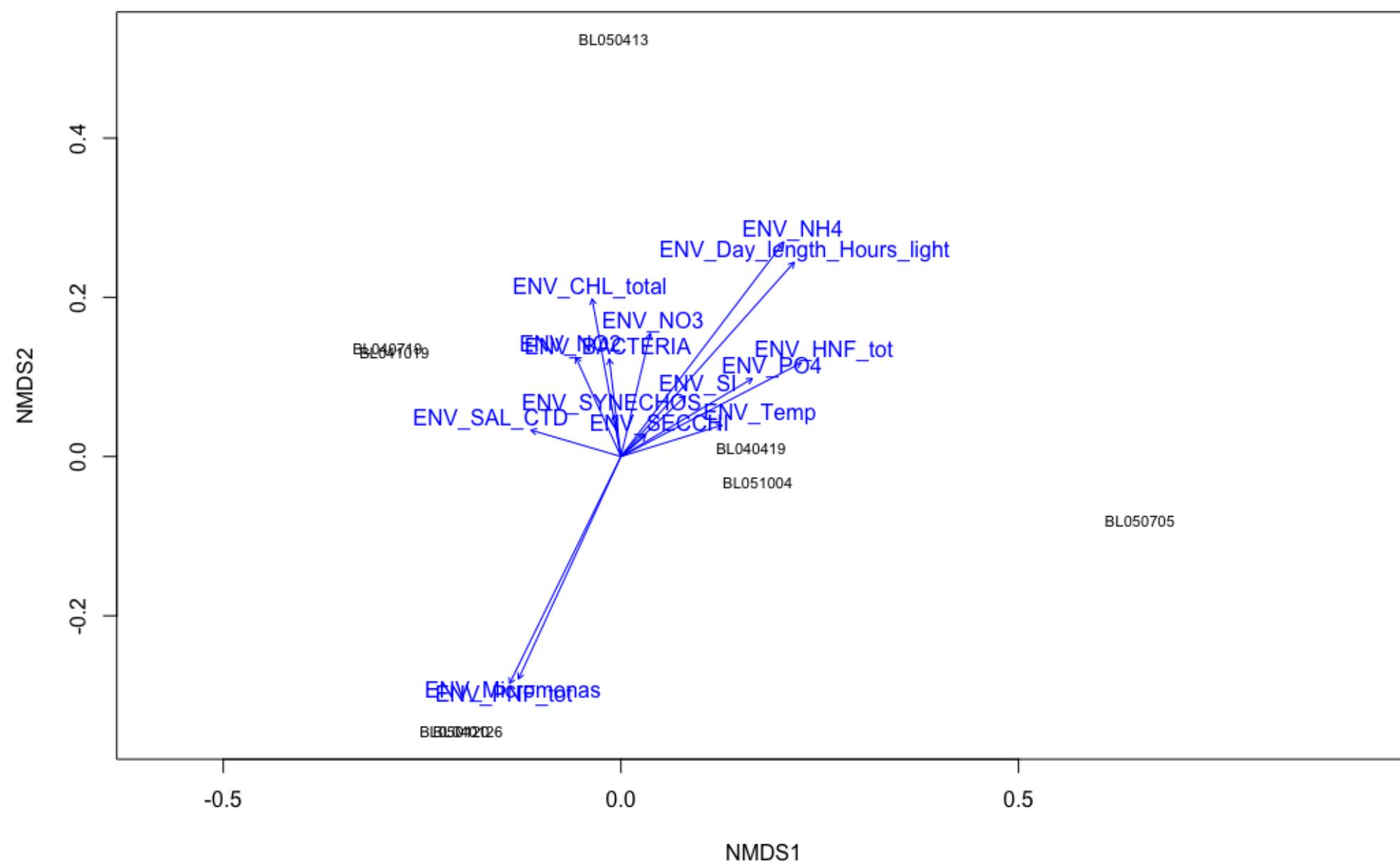
Unconstrained vs. Constrained ordination

- We correlate environmental variables with ordination axes
- The arrow points to the direction of most rapid change in the environmental variable. Often this is called the direction of the gradient
- The length of the arrow is proportional to the correlation between ordination and environmental variable. Often this is called the strength of the gradient

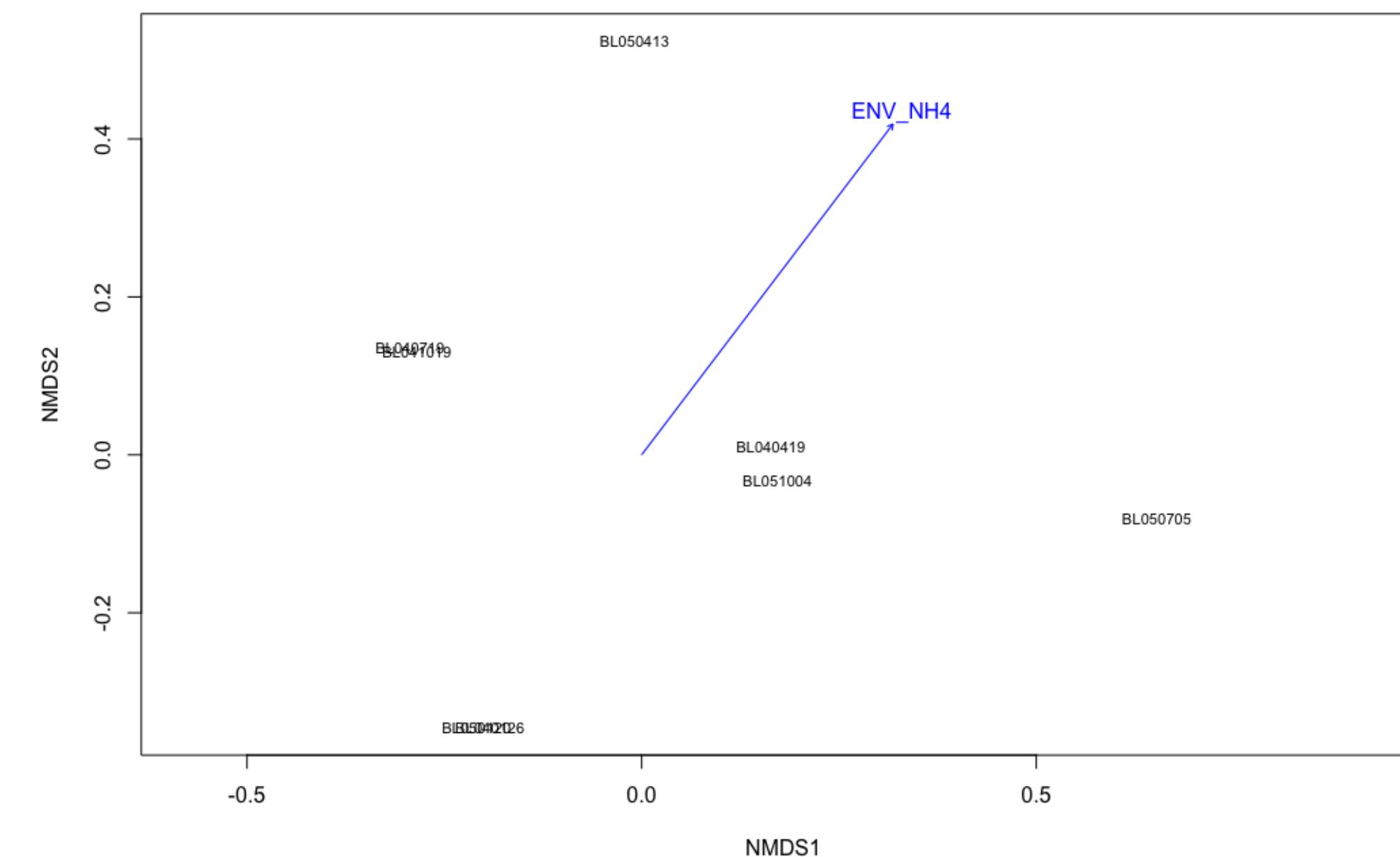


doi: 10.1101/2021.03.18.435965

All



p<0.1



vegan (version 2.4-2)

envfit: Fits an Environmental Vector or Factor onto an Ordination

Description

The function fits environmental vectors or factors onto an ordination. The projections of points onto vectors have maximum correlation with corresponding environmental variables, and the factors show the averages of factor levels.

Usage

```
"envfit"(ord, env, permutations = 999, strata = NULL, choices=c(1,2), display = "sites", w = weights(ord), na.rm = FALSE, ...)
"envfit"(formula, data, ...)
"plot"(x, choices = c(1,2), labels, arrow.mul, at = c(0,0), axis = FALSE, p.max = NULL, col = "blue", bg, add = TRUE, ...)
"scores"(x, display, choices, ...)
"vectorfit"(X, P, permutations = 0, strata = NULL, w, ...)
"factorfit"(X, P, permutations = 0, strata = NULL, w, ...)
```

Constrained ordination

Redundancy Analysis (RDA) can be considered as a constrained version of PCA

- Distance based RDA: allows calculating RDA with a chosen distance matrix

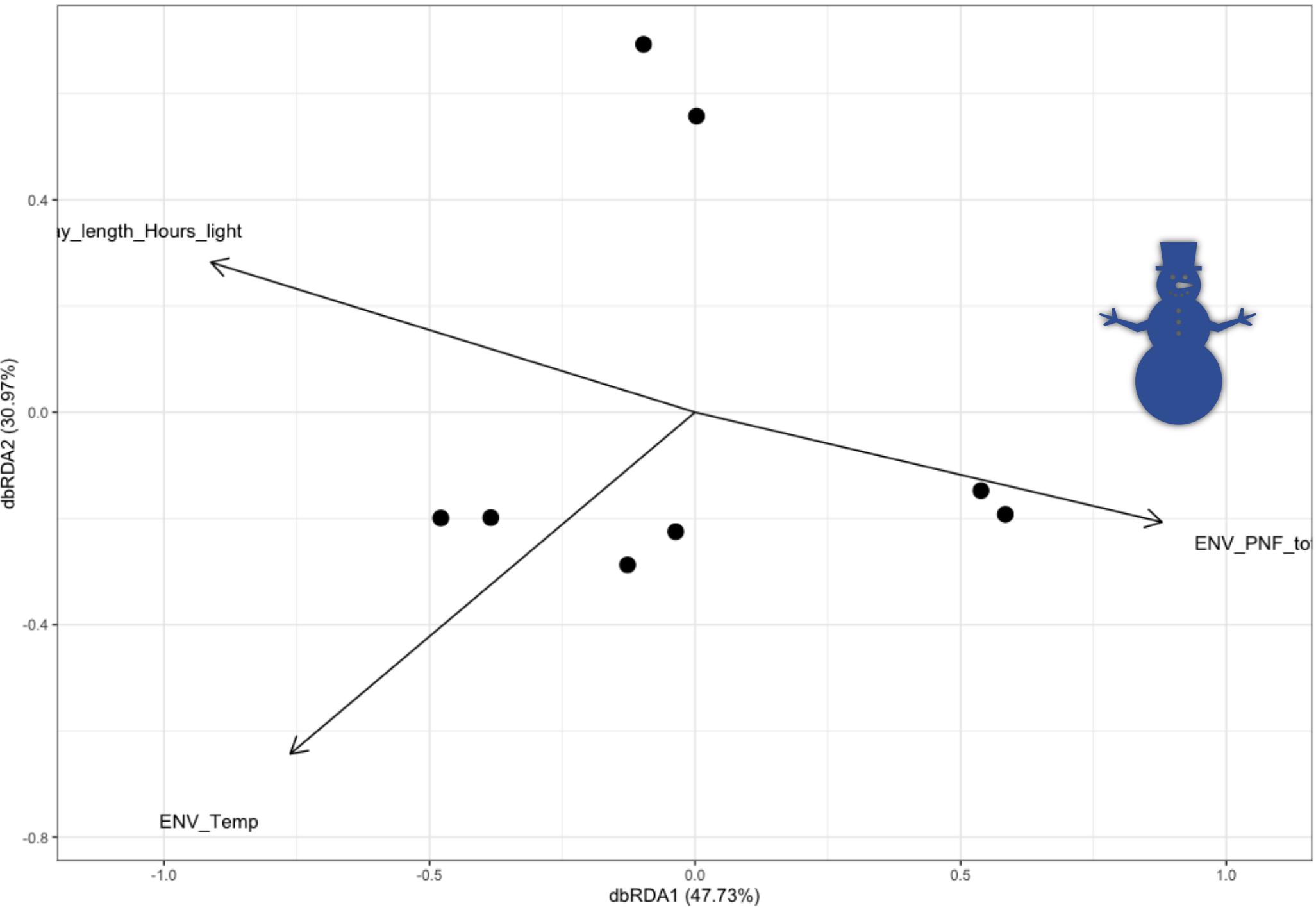
Constrained ordination

Which environmental should be selected?

- Selecting environmental variables that explain most community variance:
 - **Forward selection:** begin with an empty model and add variables one by one. In each step forward, add the one variable that gives the single best improvement to the model.
 - **Backwards elimination:** start with a model that includes all variables and eliminate variables with low explanatory power one by one

Constrained ordination

- The function `Ordistep()` in Vegan performs a step-wise selection of environmental variables based on two criteria:
 - If their inclusion into the model leads to a significant increase of the explained variance
 - If the AIC (Akaike Information Criterion) of the new model is lower than the AIC of the more simple model
 - AIC: estimates the quality of models relative to other models (model selection). It is an estimator of prediction error



```
dbrda(formula = otu.tab.simple.ss.nozero.bray ~ ENV_PNF_tot +
ENV_Day_length_Hours_light + ENV_Temp, data = as.data.frame(bbmo.metadata.course.15vars.zscores))
```

