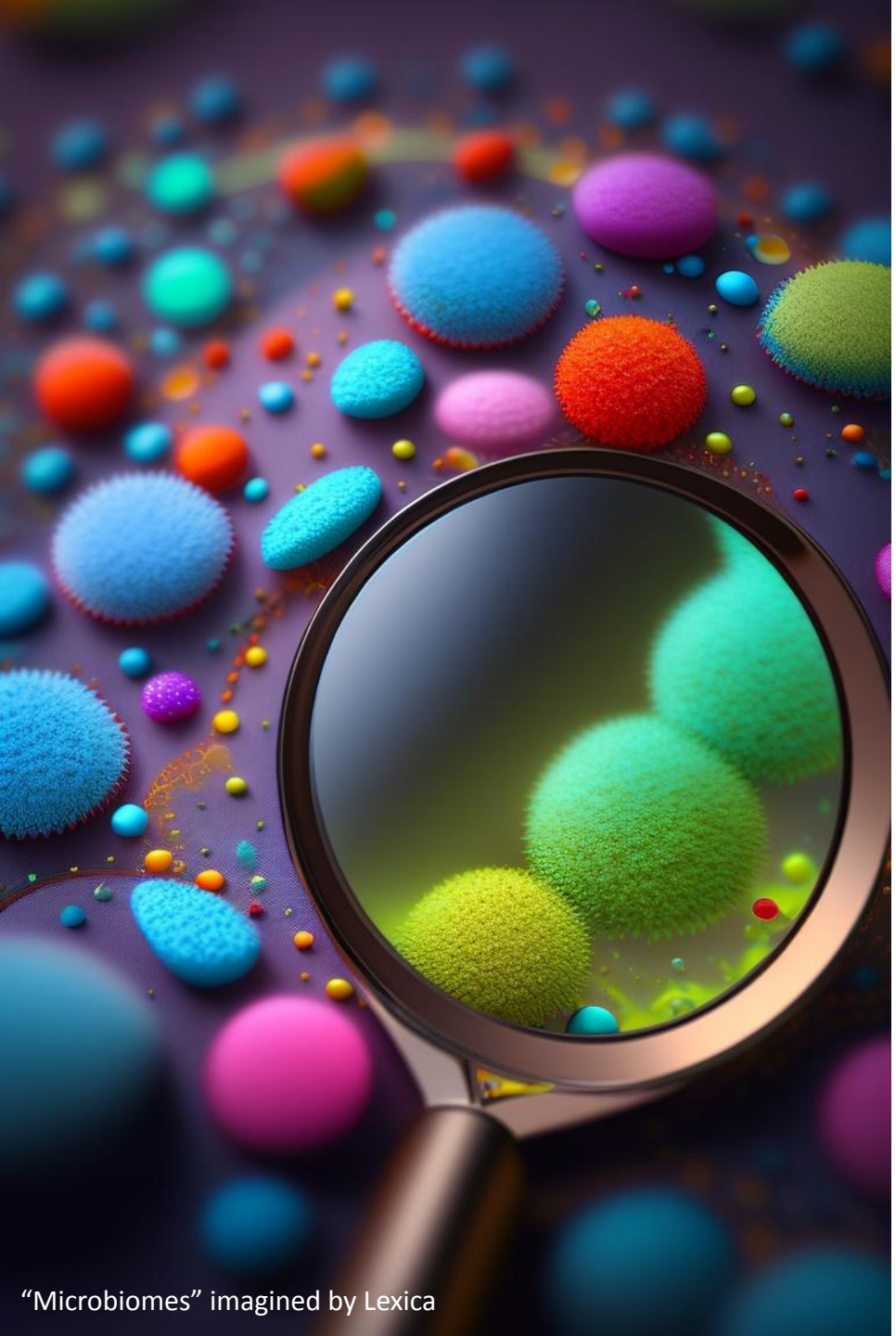


“Microbiomes” imagined by Lexica

Microbiome data analysis

Stats and Plots in R

Evomics - Krumlov

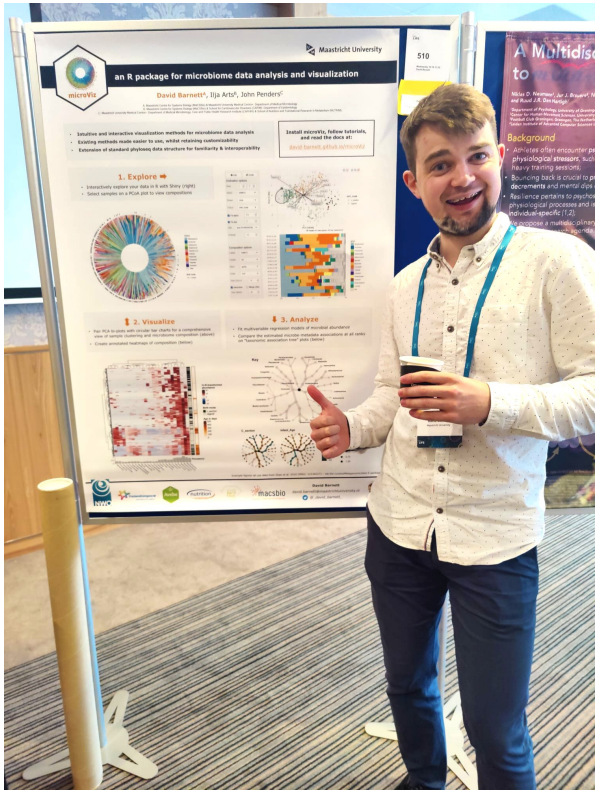
16th Jan 2024

David Barnett

github.com/david-barnett

david.barnett@maastrichtuniversity.nl

David



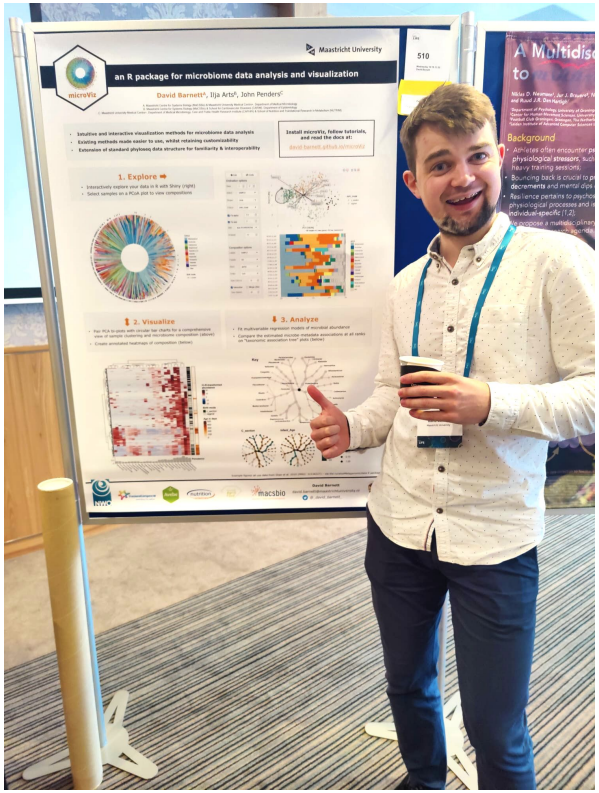
David Barnett

Bioinformatician

Maastricht University, NL

- Epidemiology MSc
- Infant gut microbiome PhD
- Bioinformatics “Postdoc”
  - Medical Microbiology

David



David Barnett

Bioinformatician

Maastricht University, NL

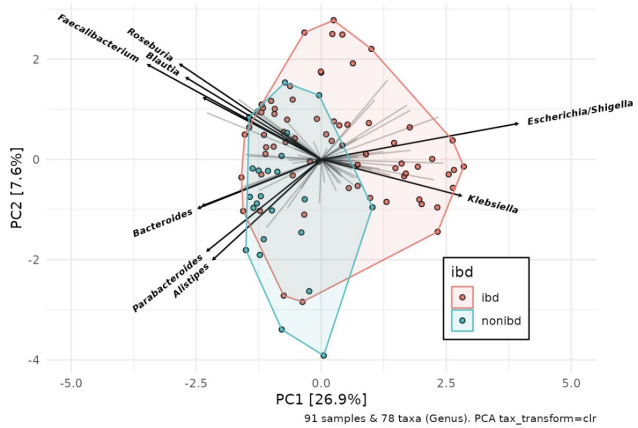
R package

microViz

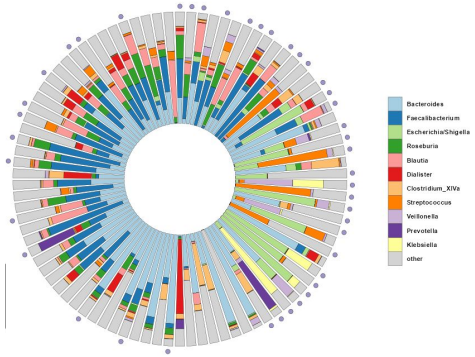
Tools for microbiome data visualization & statistics

microViz

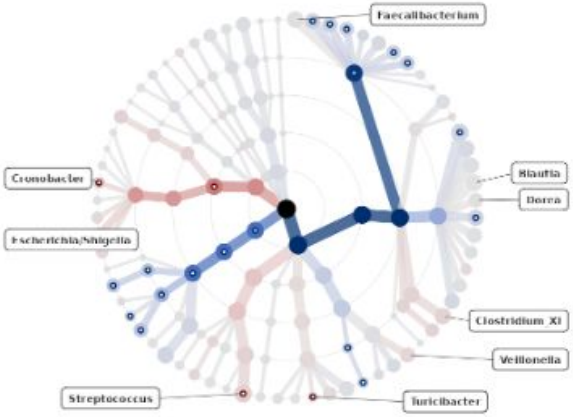
Ordination plots



Bar charts

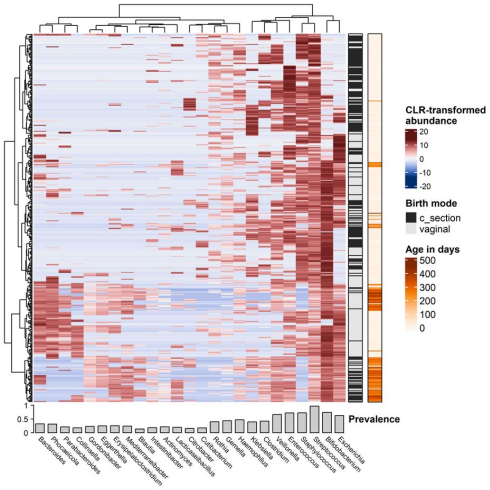


Interactive data exploration



Taxonomic association trees

Annotated heatmaps



The plan: 2 parts

A

- **Key concepts in microbiome taxonomic data analysis**

  19:00 - 19:30 - Lecture

- **Barcharts and Diversity - getting started in R**

  R Exercises - about 45 mins

- - - - - - - - - - - Take a break <u>before</u> 20:30 - - - - - - - - - - -

B

- **Dissimilarity, Ordination, & Differential Abundance**

  20:30 - 21:00 - Lecture

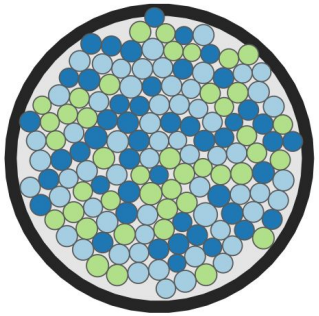
  R Exercises - until 22:00

Key concepts in microbiome taxonomic data analysis

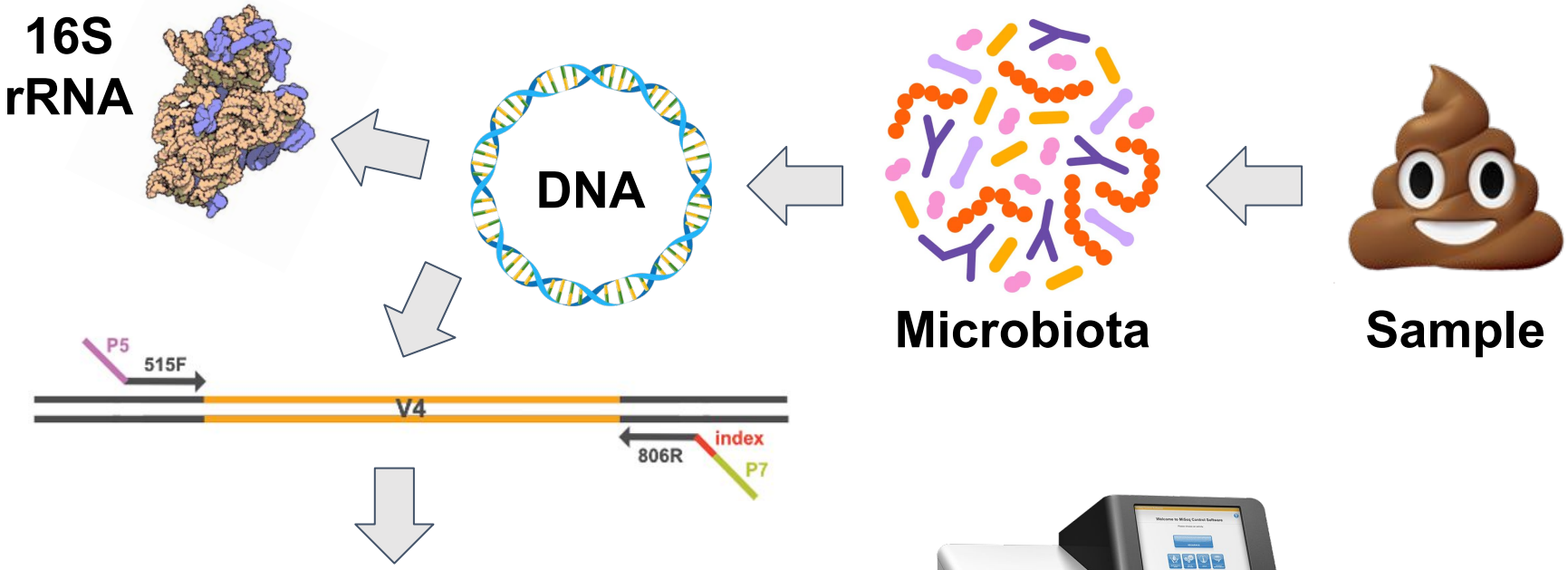
1. **Processing 16S gene amplicon sequencing data**

   - Denoising with: DADA2

2. **Analysing taxonomic info** (16S / ITS / Shotgun / etc...)

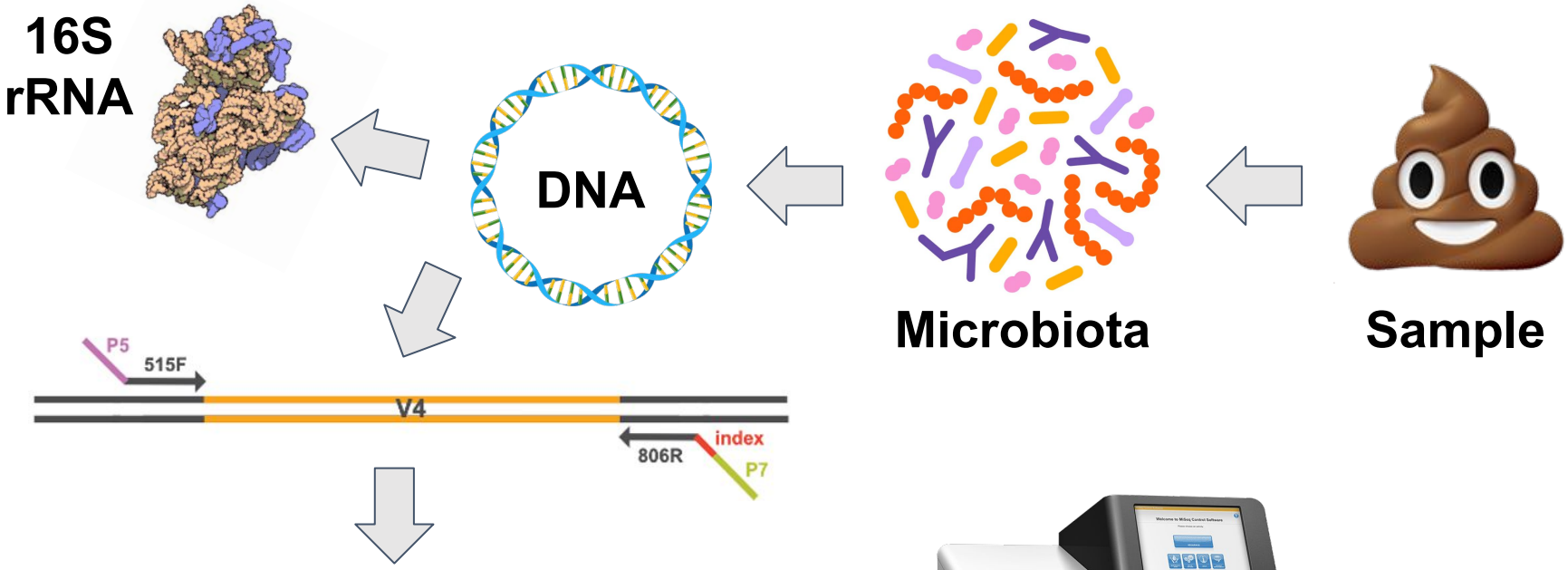


   - Diversity
   - Dissimilarity
   - Differential Abundance



Sequencing: of 16S rRNA gene amplicons

Microbiota profiling - who's there?



Sequencing: of 16S rRNA gene amplicons

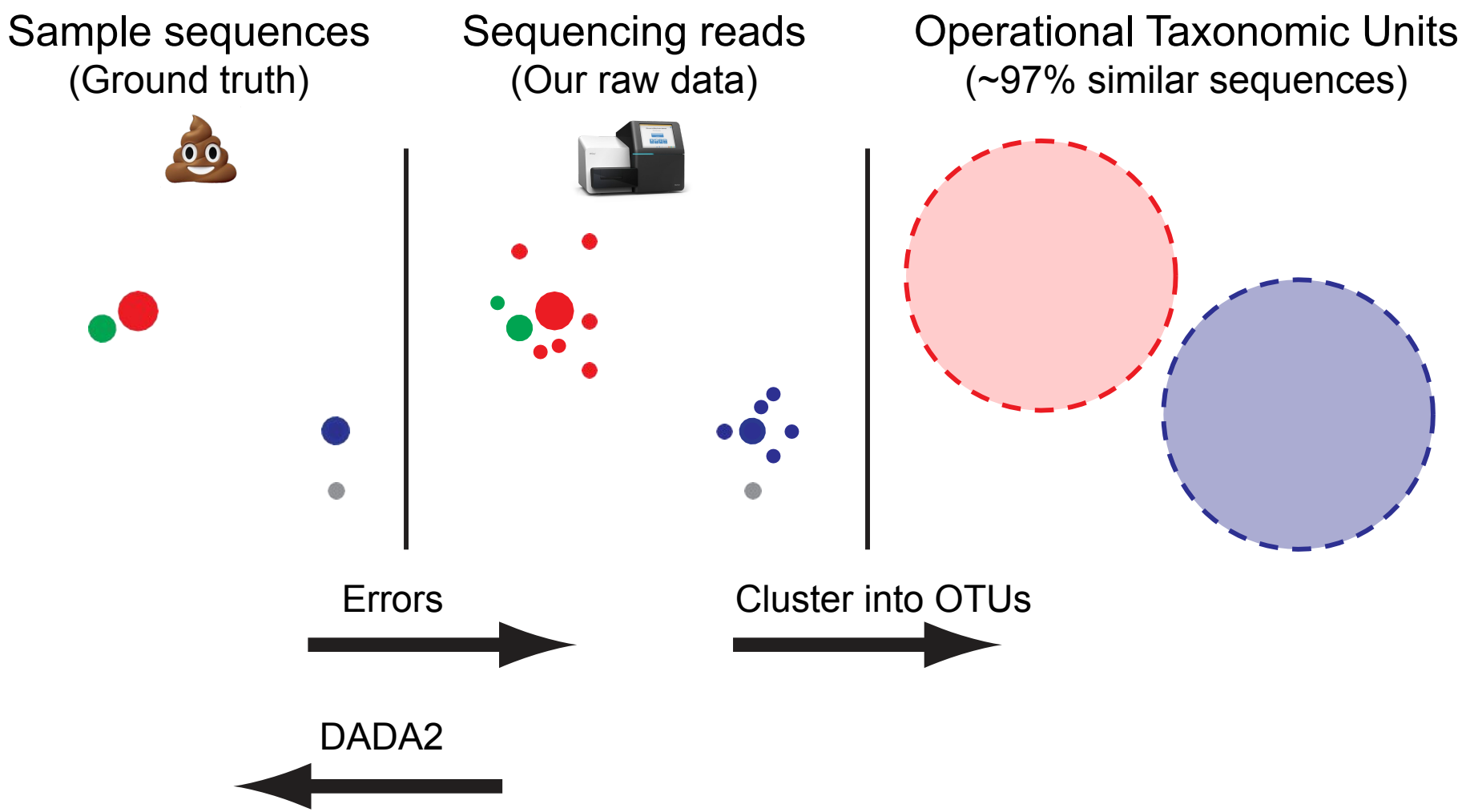
DADA2
Amplicon Sequencing. **Exactly.**

Denoising: Infer Amplicon Sequence Variants

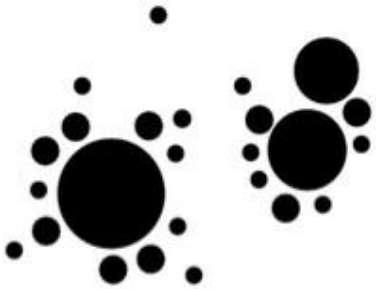
Taxonomic classification: map ASVs to database

silva
high quality ribosomal RNA databases

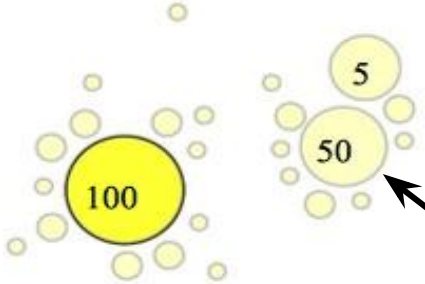
DADA2 vs. OTUs



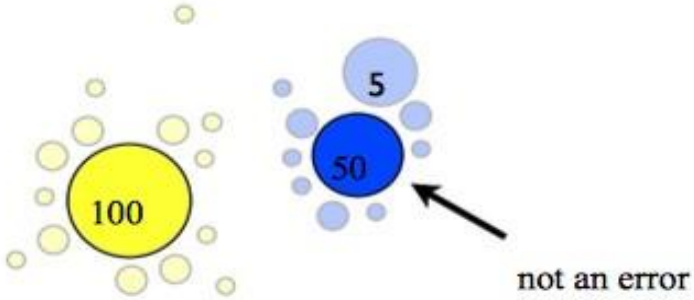
Divisive Amplicon Denoising Algorithm



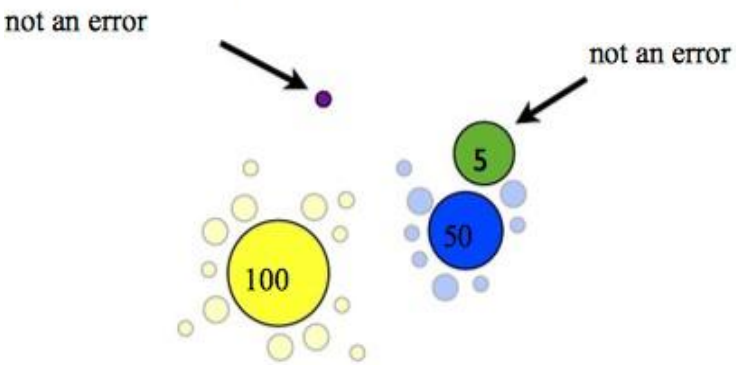
Count unique sequences



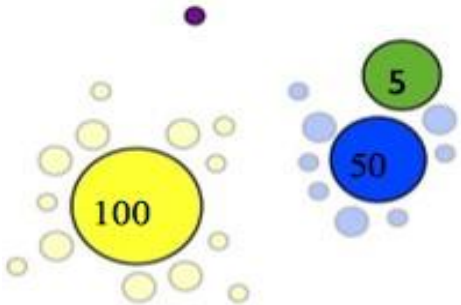
Initial error model
(one partition)



Reject sequence least likely to arise from errors
(too abundant and/or different)



Repeat: Reject more sequences and divide into further partitions

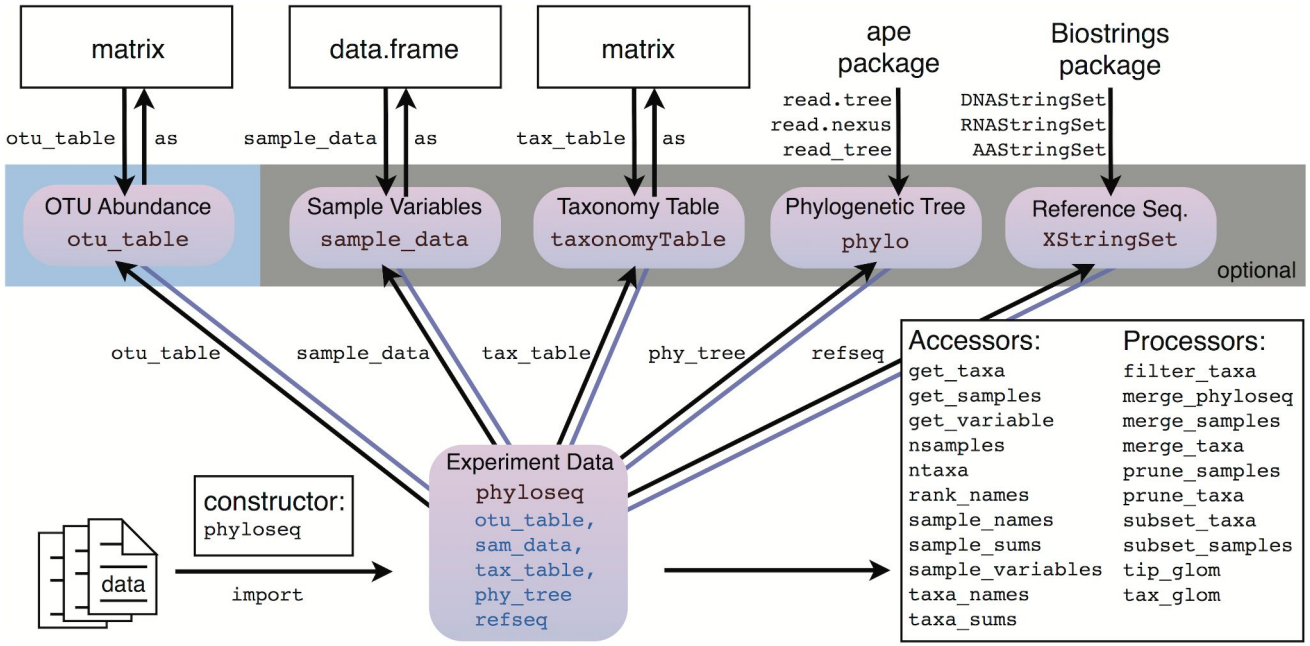


Convergence: All errors are plausible

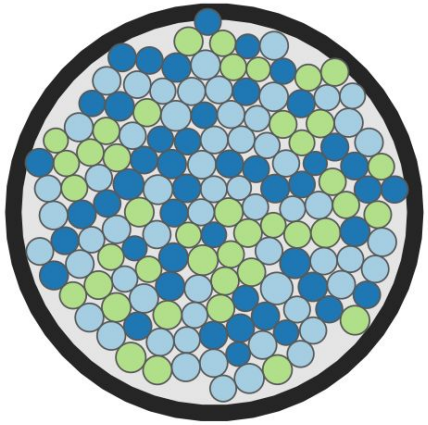
Callahan et al. 2016. Nature Methods

Amplicon Sequencing. **Exactly.**

https://benjjneb.github.io/dada2/tutorial.html



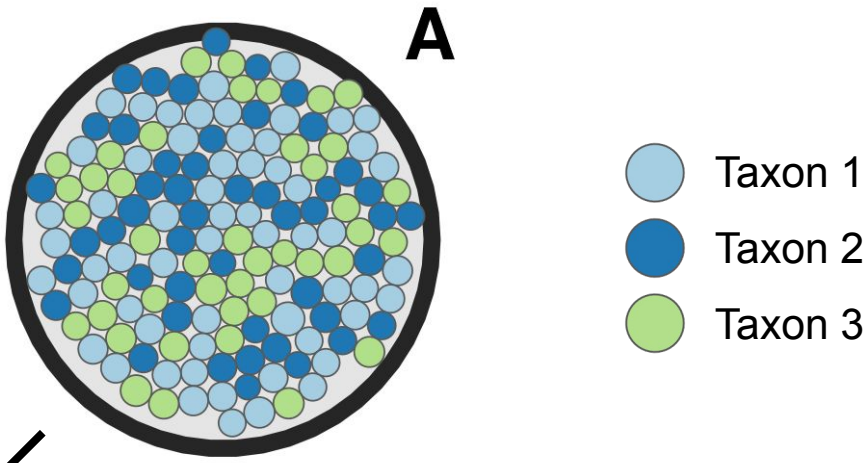
Analysing taxon abundance data



?

1. Diversity
2. Dissimilarity
3. Differential Abundance

Ecosystem **Diversity**

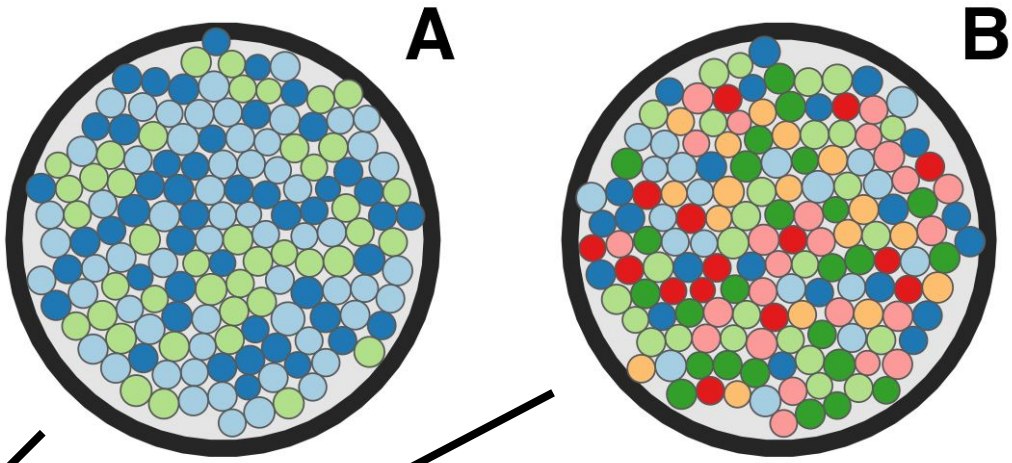


"Observed"

"Pielou's"

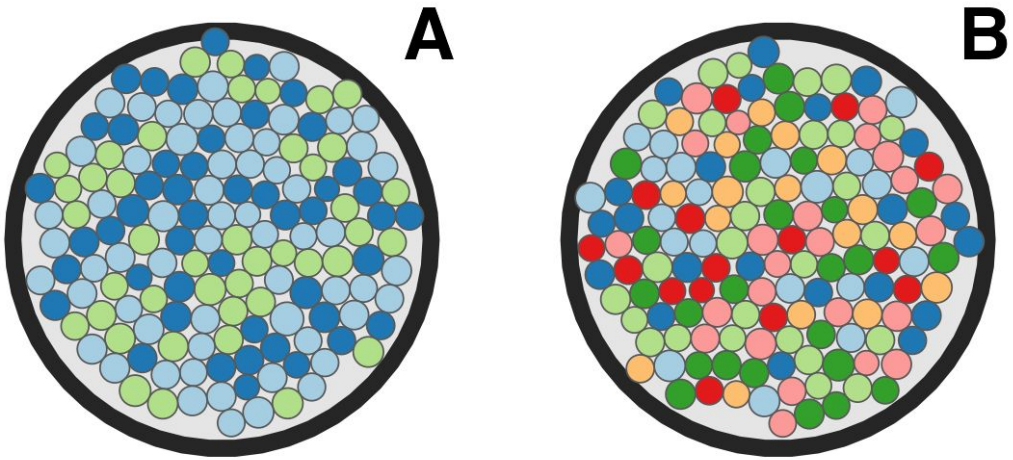
"Effective Shannon"

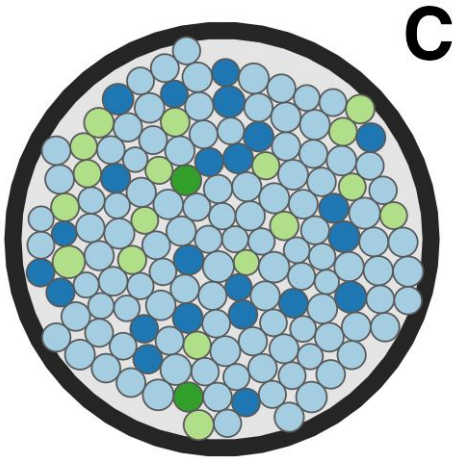
Ecosystem
Diversity



	A
Richness	3.0
Evenness	1.0
Diversity	3.0

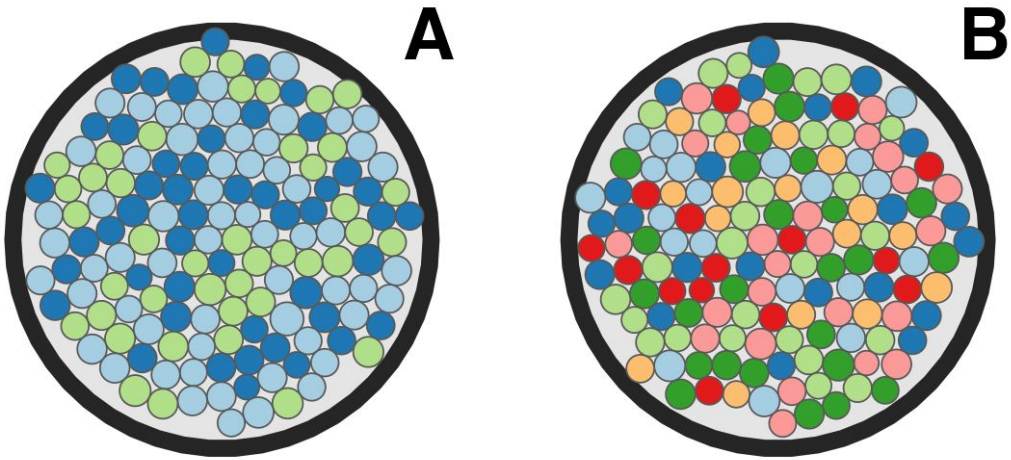
Ecosystem
Diversity

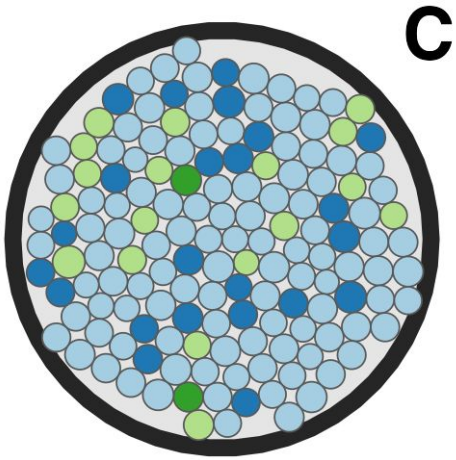




	A	B	C
Richness	3.0	7.0	4.0
Evenness	1.0	1.0	0.6
Diversity	3.0	7.0	2.3

Ecosystem
Diversity





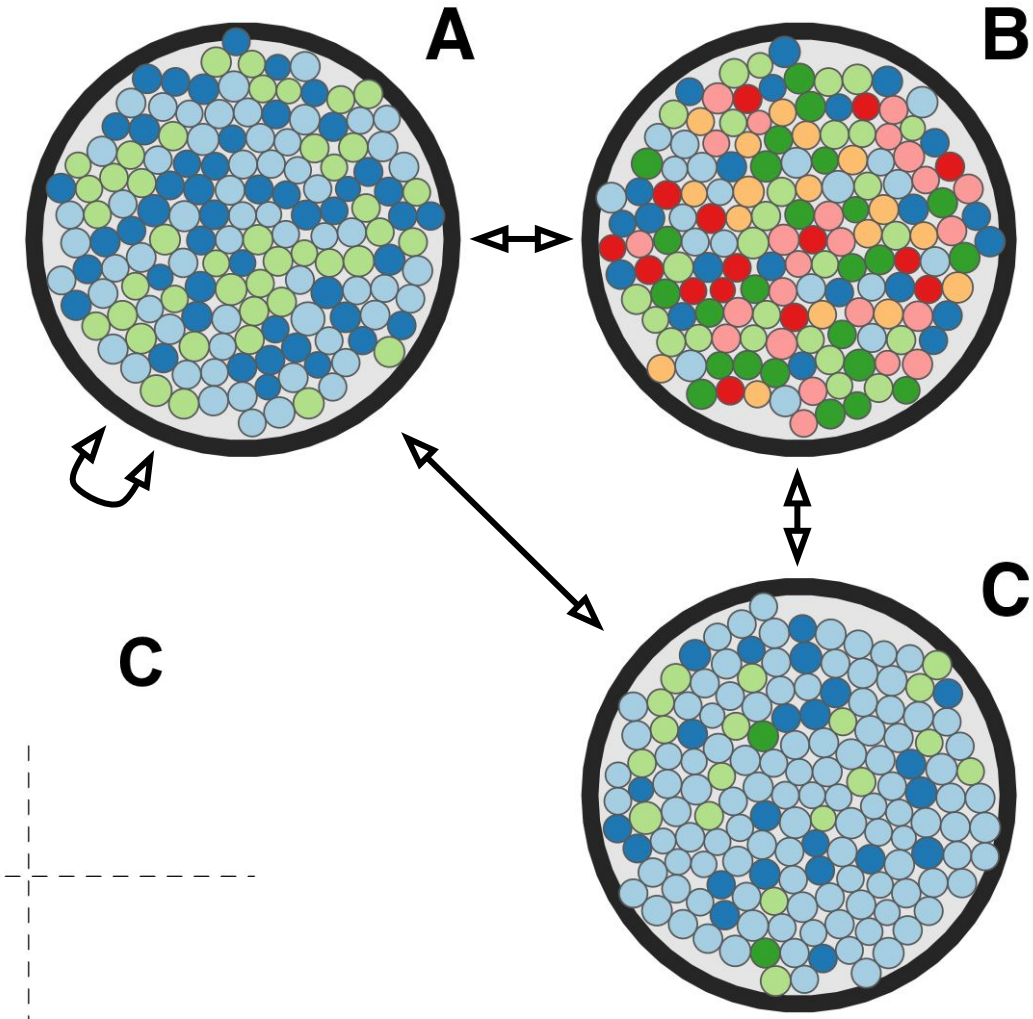
- **Richness** - how many different taxa?
- **Evenness** - how balanced are the taxa?
- **Diversity** - how rich and balanced is the ecosystem?

There are many different ways to calculate these things!

More about diversity indices -> https://www.davidzeleny.net/anadat-r/doku.php/en:div-ind

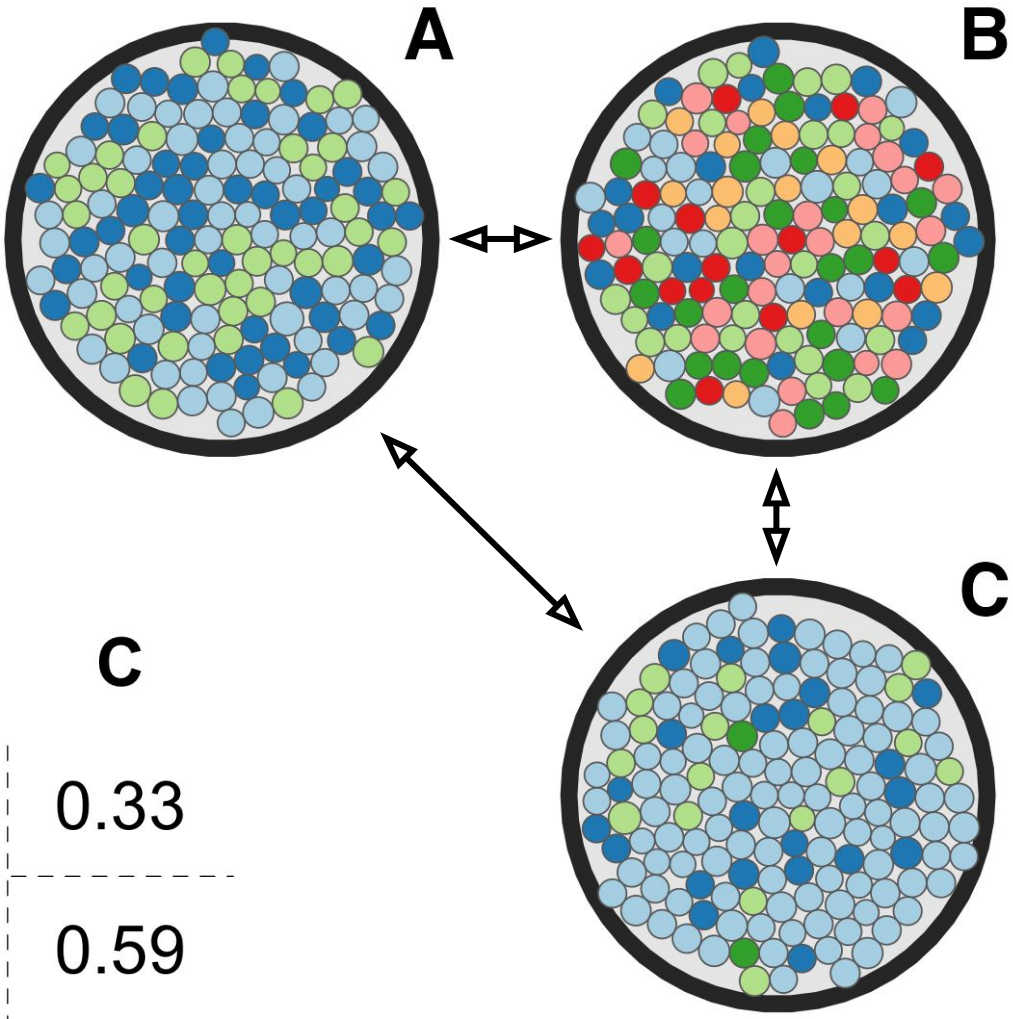
Dissimilarity

between ecosystems



	A	B	C
A			
B			
C			

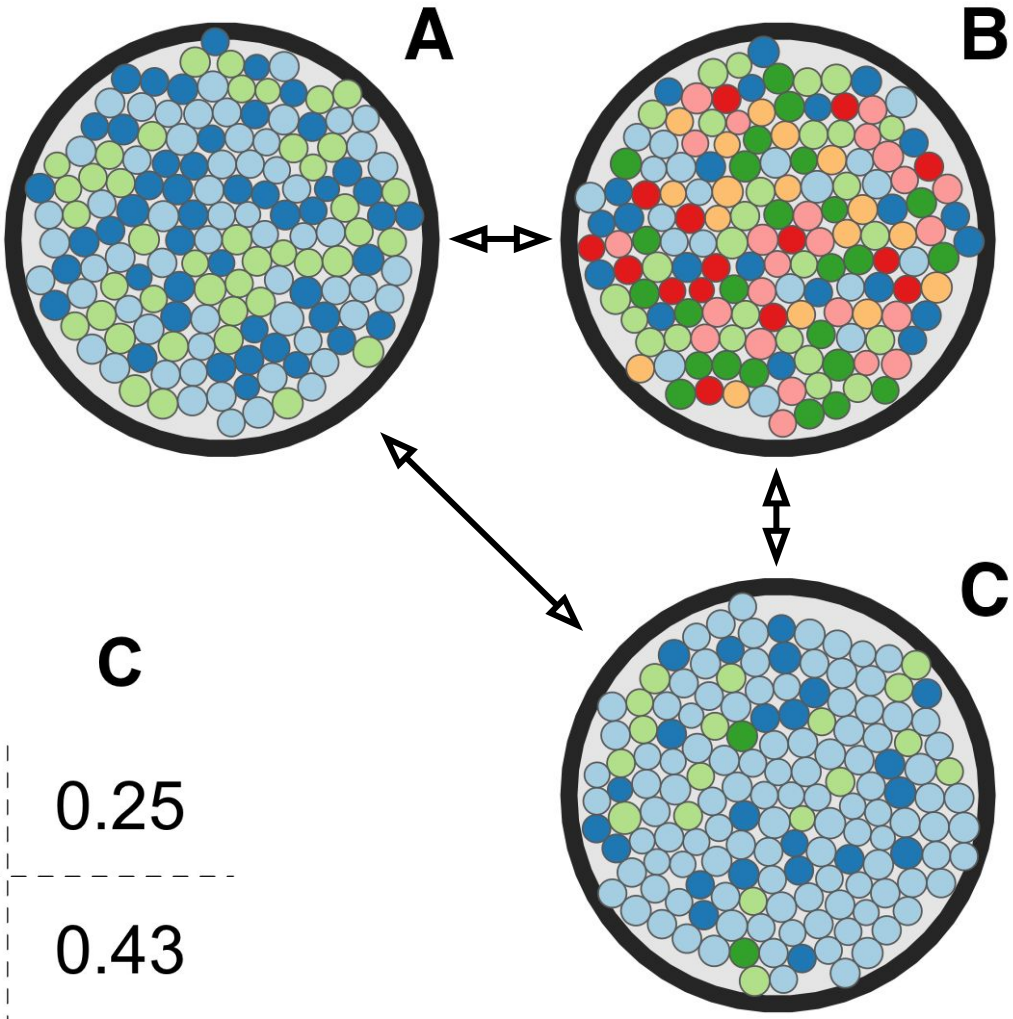
Dissimilarity between ecosystems



	A	B	C
A	0.00	0.54	0.33
B		0.00	0.59
C			0.00

Bray-Curtis Dissimilarity

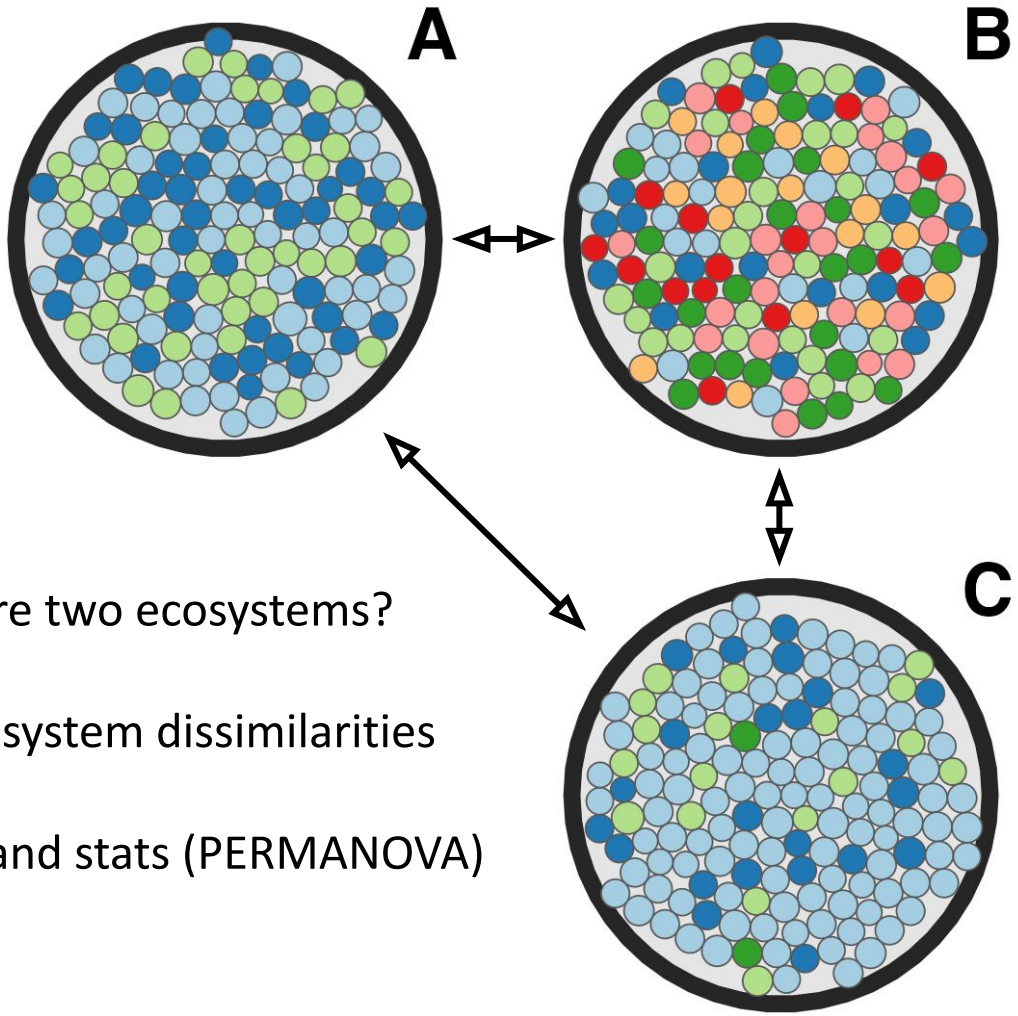
Dissimilarity between ecosystems



	A	B	C
A	0.00	0.57	0.25
B		0.00	0.43
C			0.00

Binary Jaccard Distance

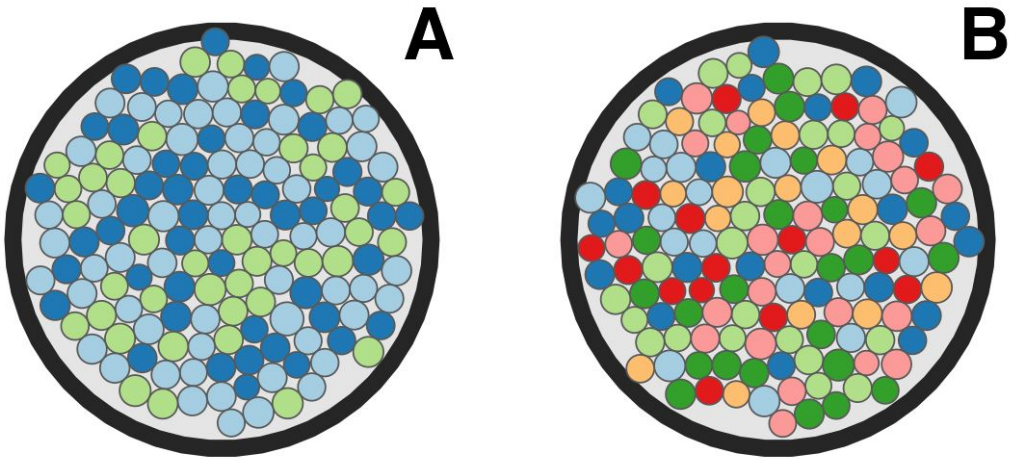
<u>**Dissimilarity**</u> between ecosystems



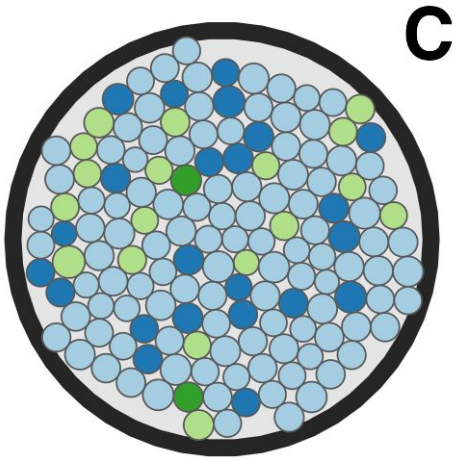
- **Dissimilarity** - how different are two ecosystems?
- **Distance matrix** - pairwise ecosystem dissimilarities
- **Very useful** - for plots (PCoA) and stats (PERMANOVA)

<u>**There are many different dissimilarity measures!**</u>

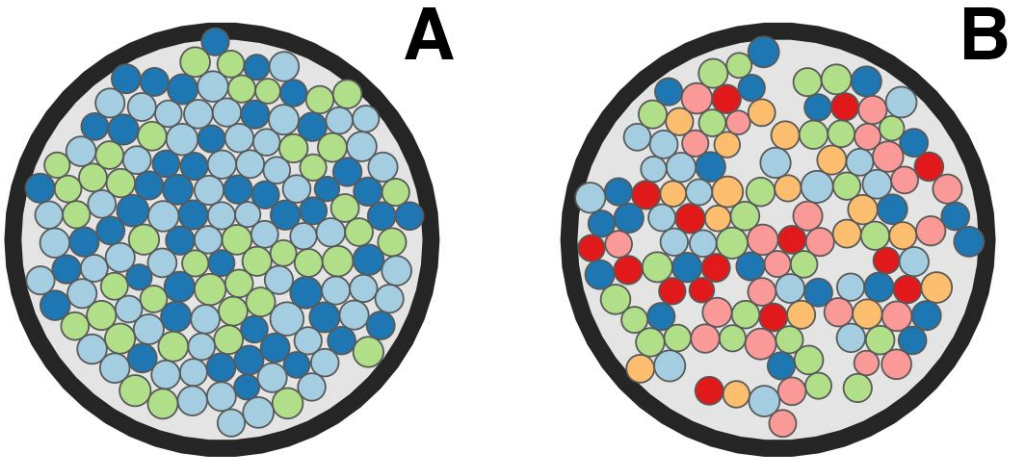
Differential Abundance of each taxon



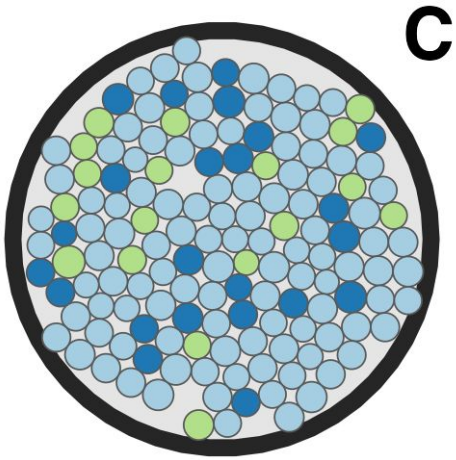
- Compare abundance of each taxon, across ecosystems

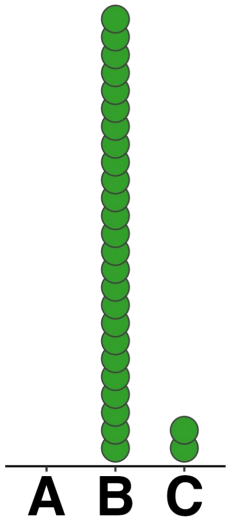


Differential Abundance of each taxon



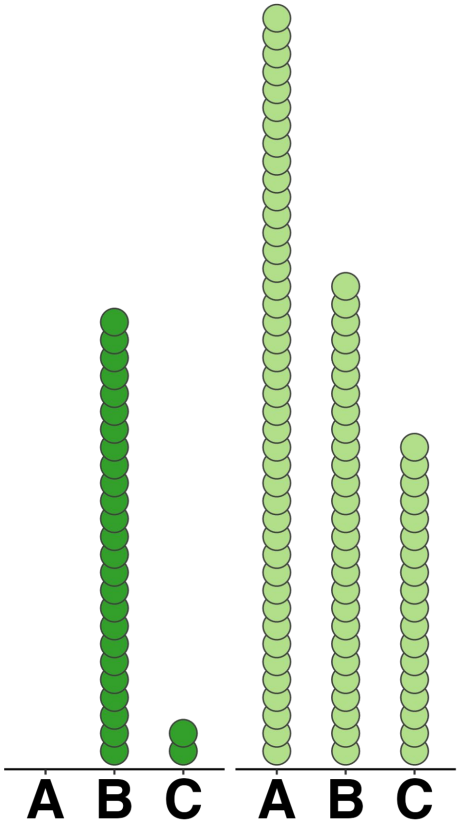
- Compare abundance of each taxon, across ecosystems
- One taxon at a time

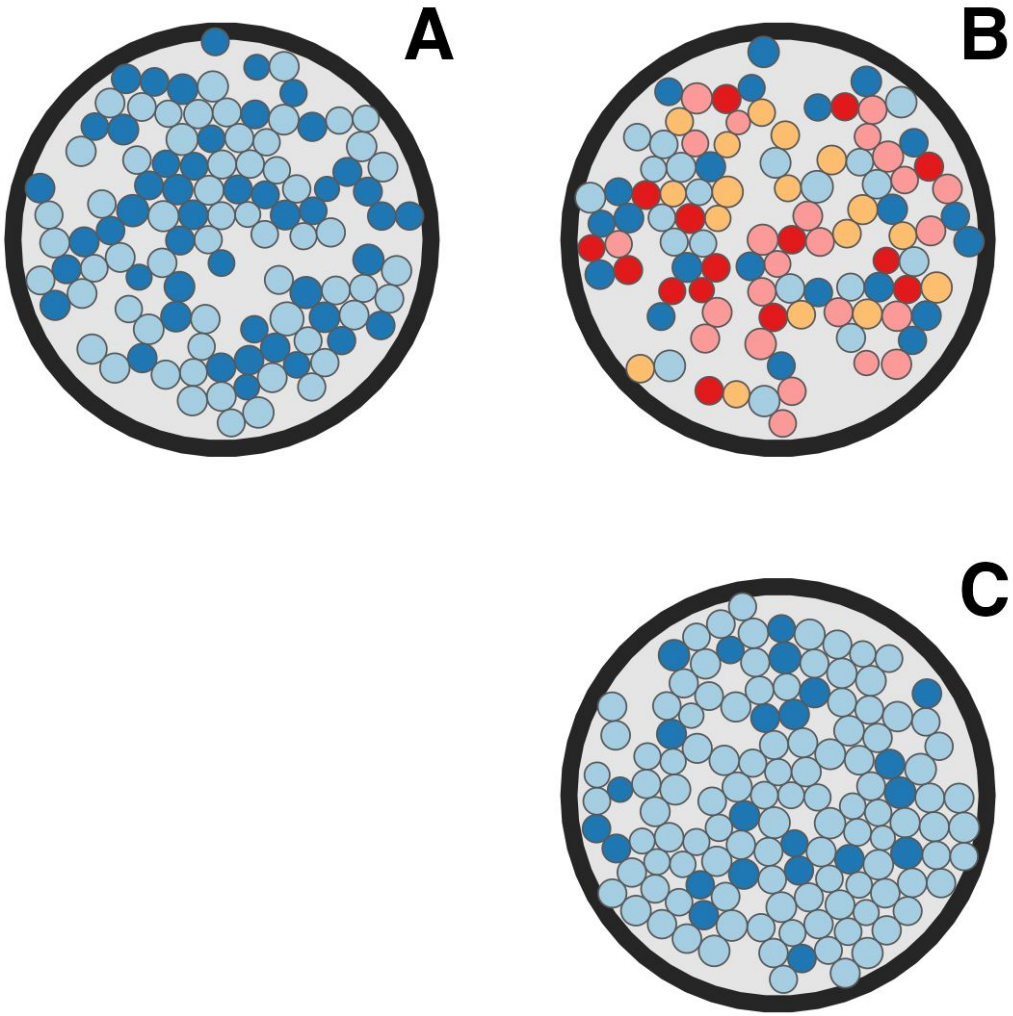




Differential Abundance

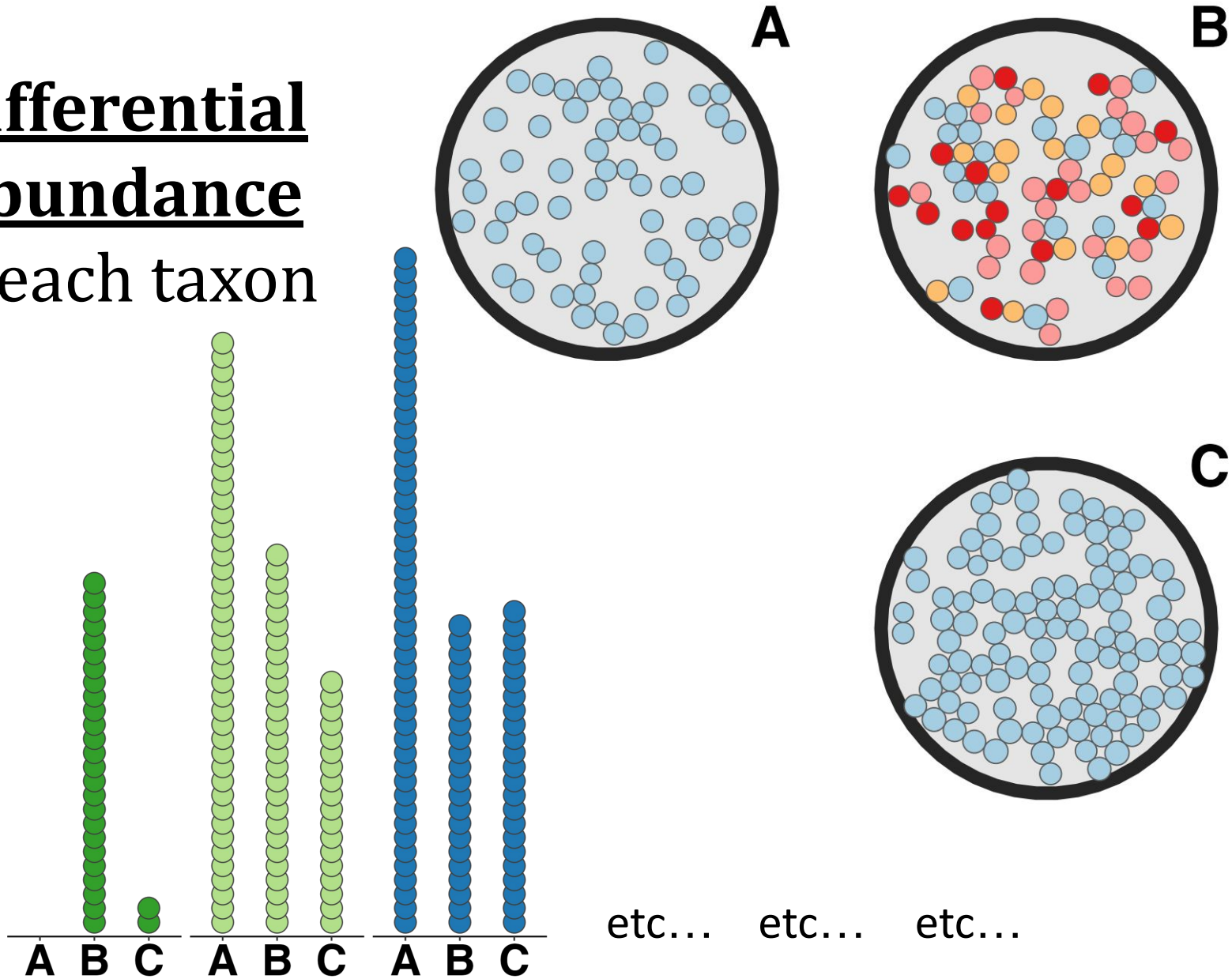
of each taxon





Differential Abundance

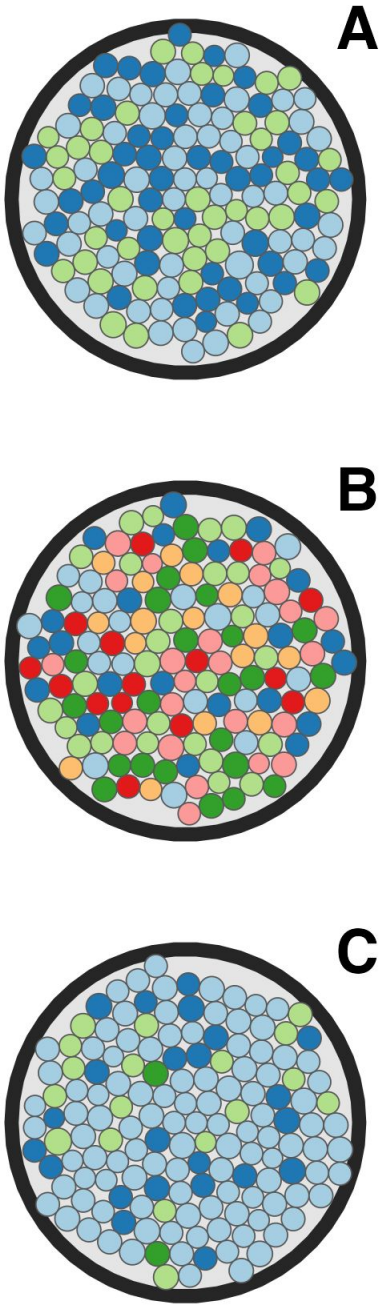
of each taxon



Differential Abundance of each taxon

- **Compare across groups of samples**

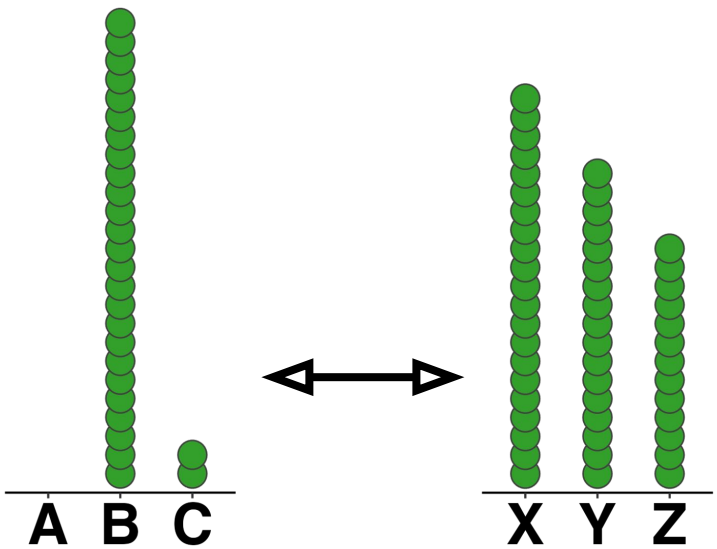
  e.g. - group ABC vs. group XYZ

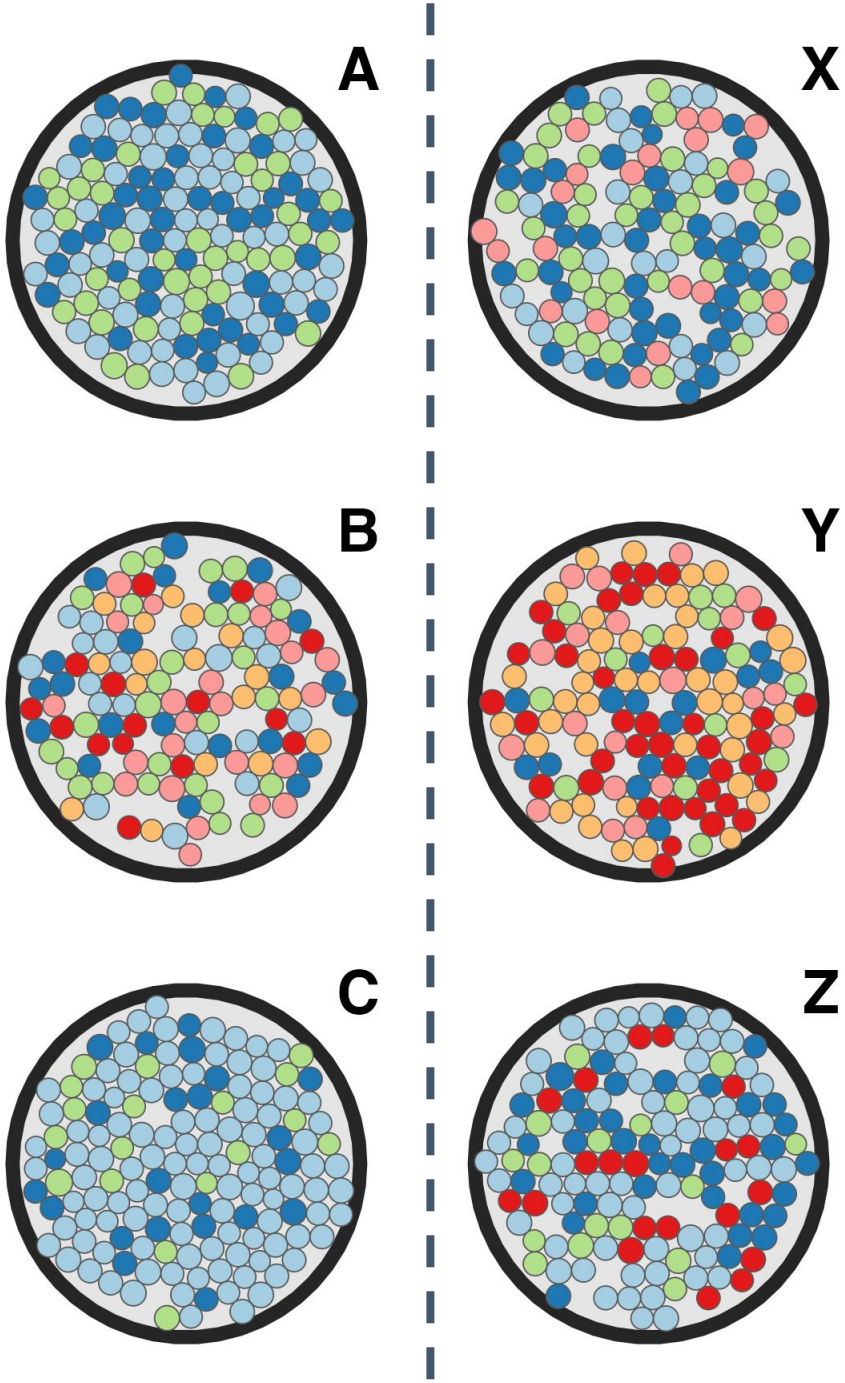


<u>Differential Abundance</u> of each taxon

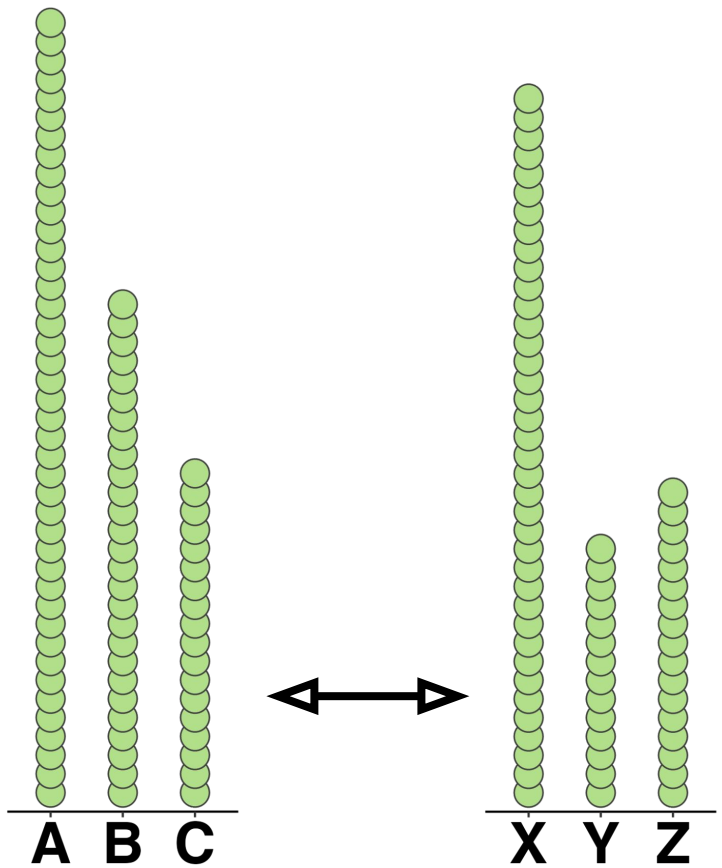
- **Compare across <u>groups</u> of samples**

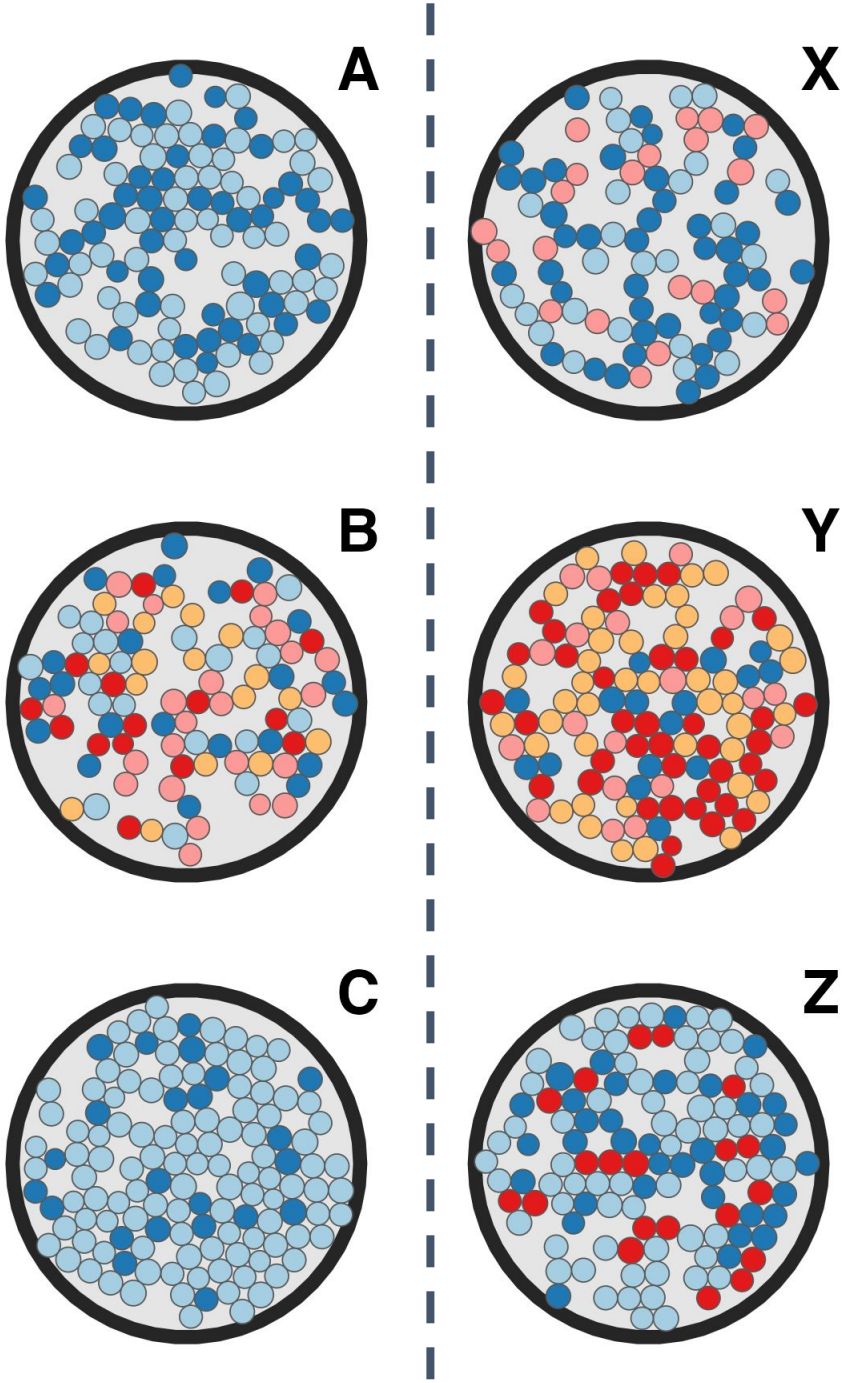
  e.g. - group ABC vs. group XYZ





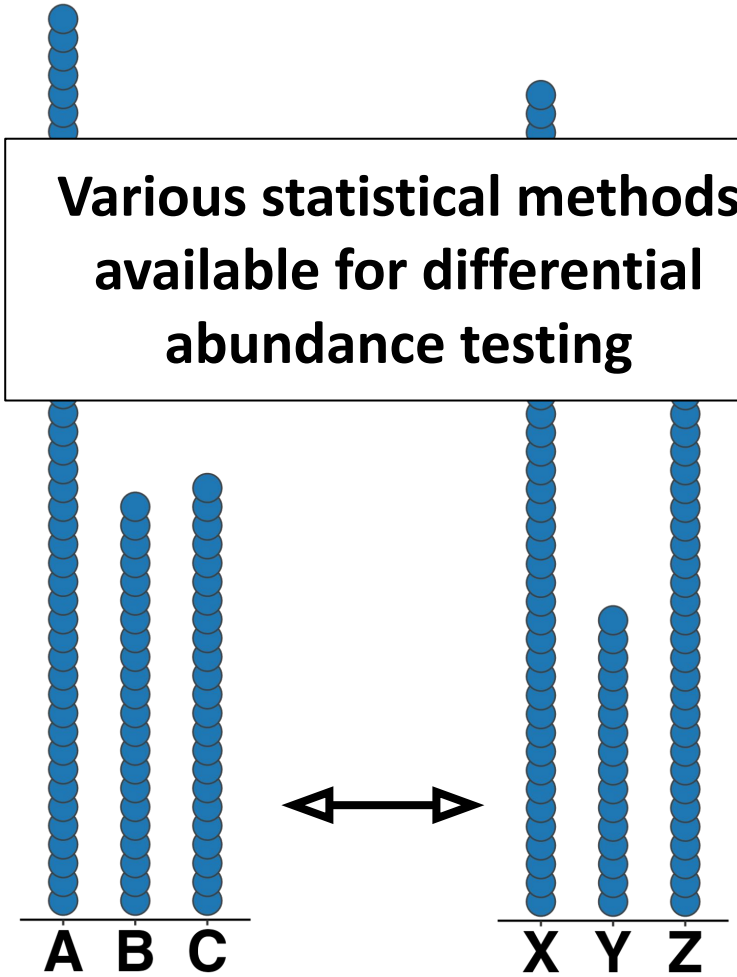
Differential Abundance of each taxon

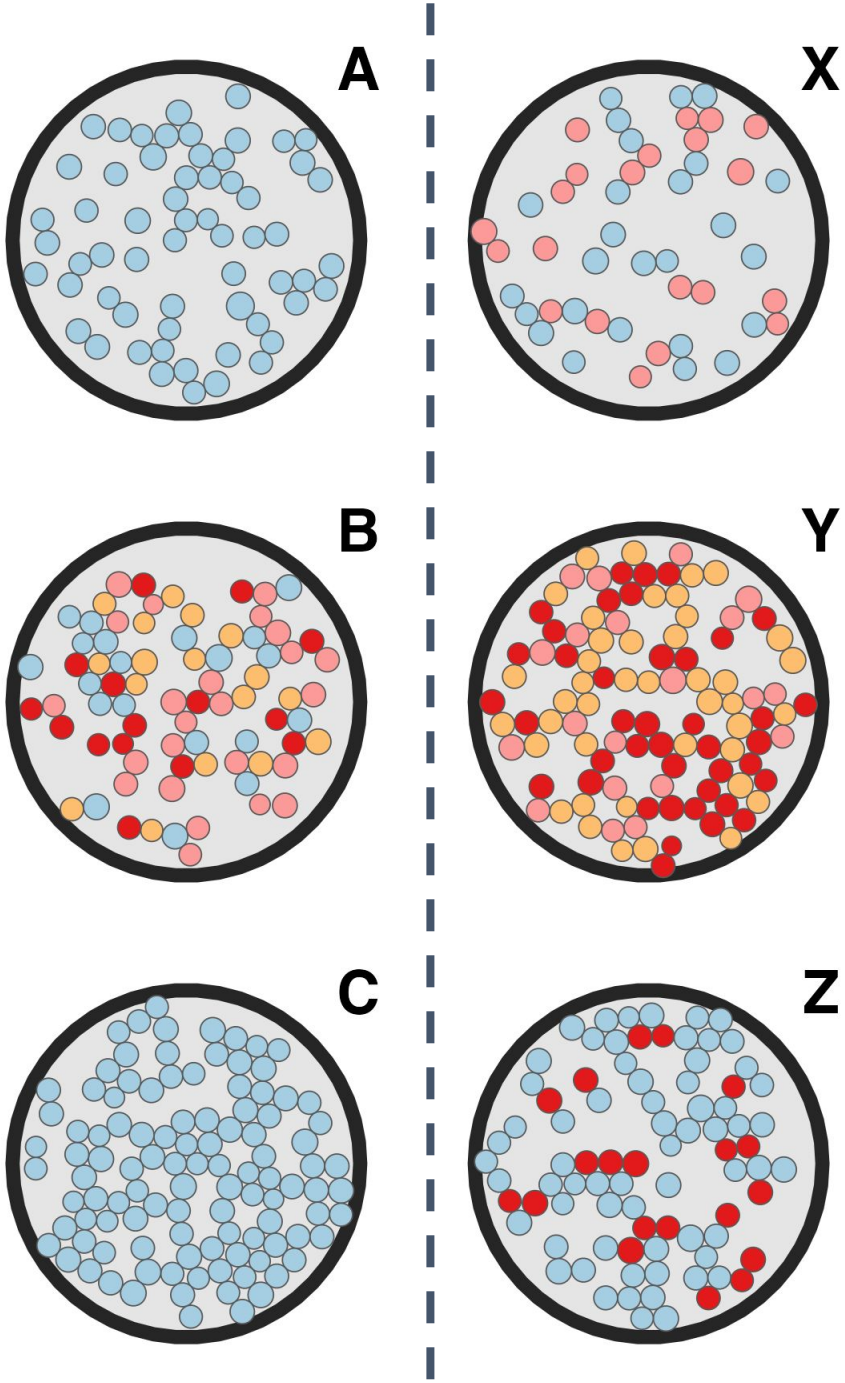




Differential Abundance of each taxon

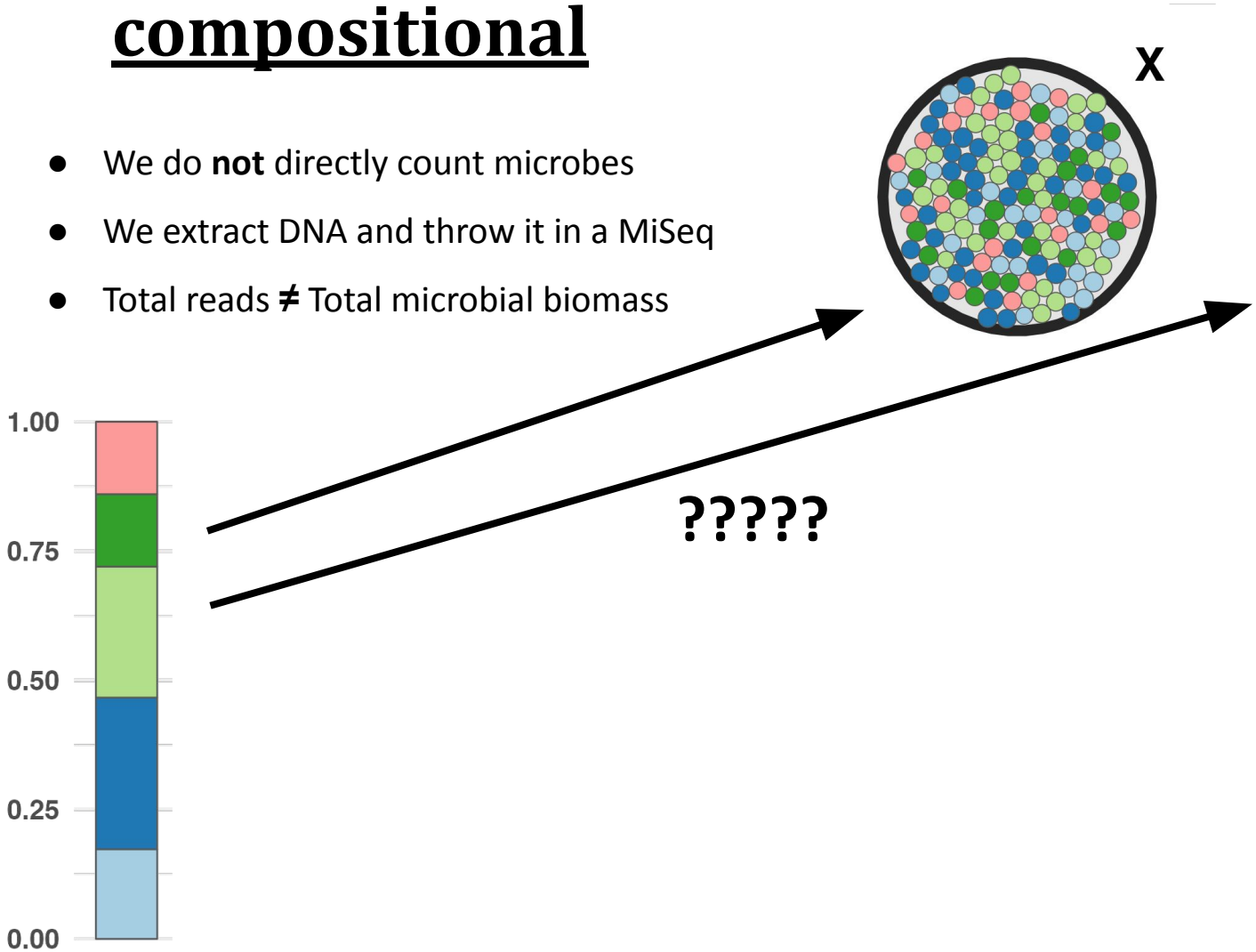
Various statistical methods available for differential abundance testing





Microbiome data are <u>compositional</u>

- We do **not** directly count microbes
- We extract DNA and throw it in a MiSeq
- Total reads ≠ Total microbial biomass



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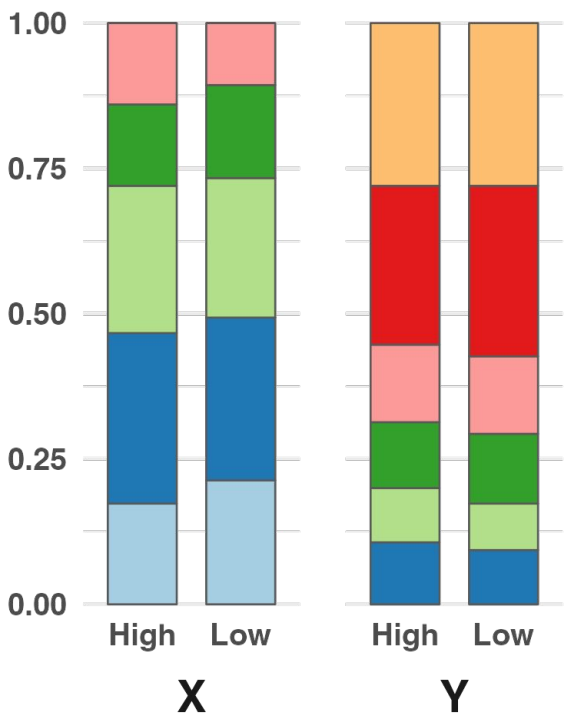

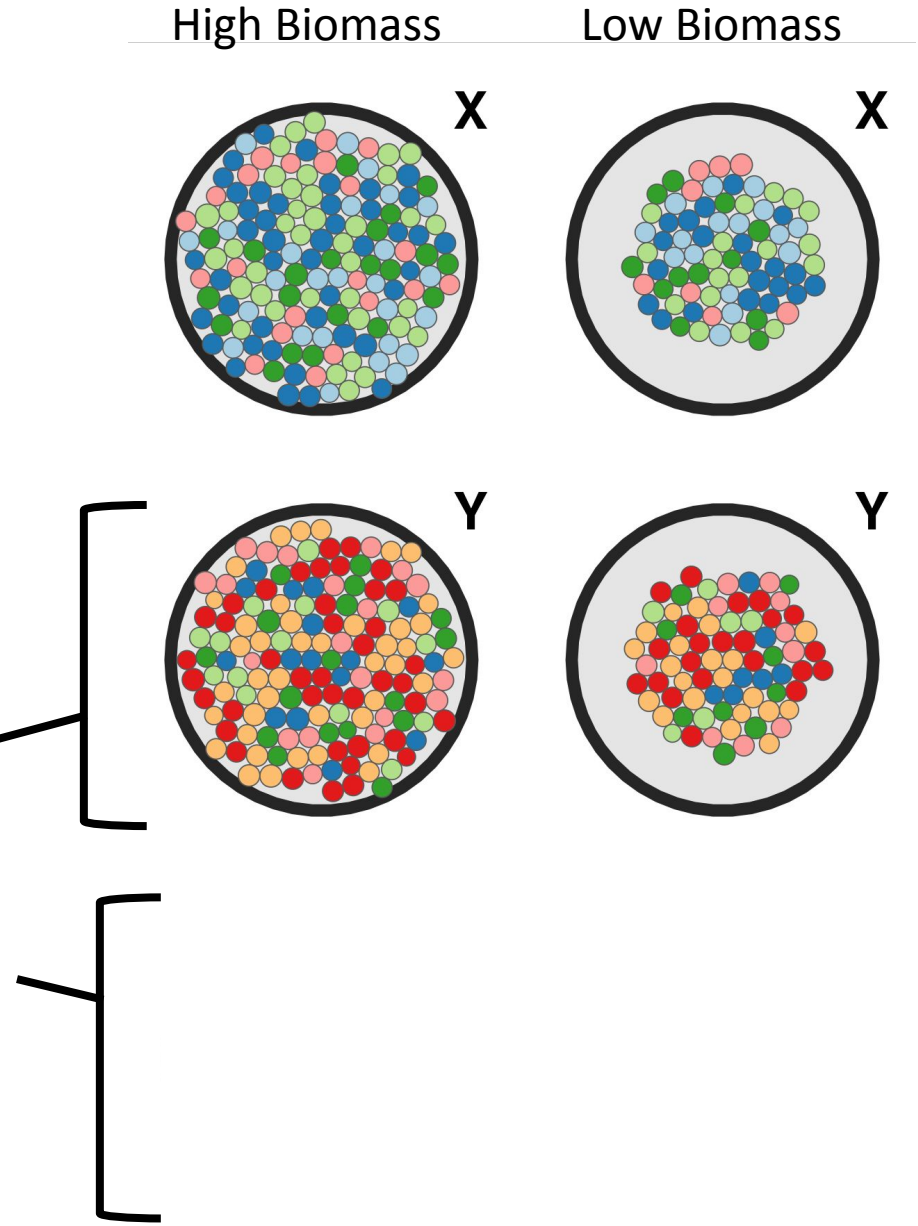

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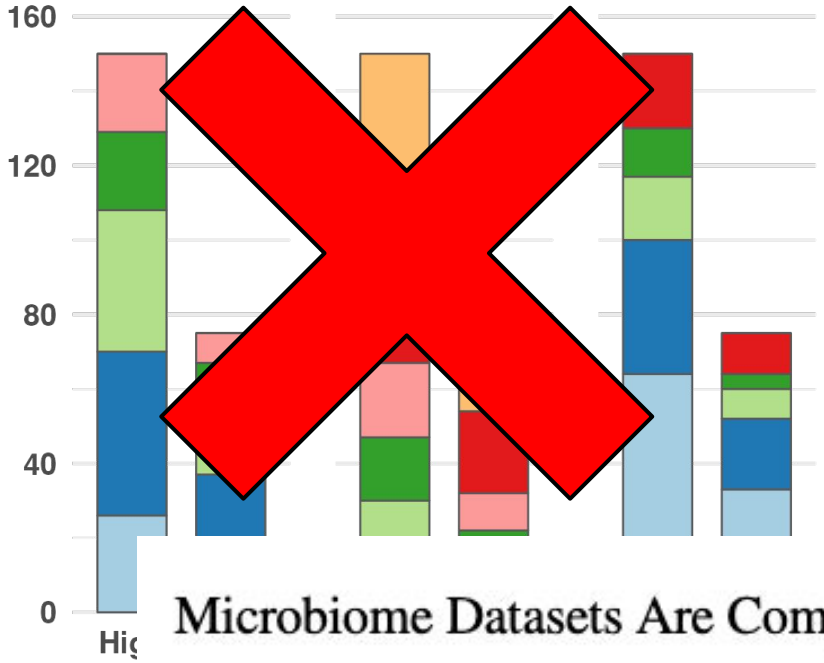

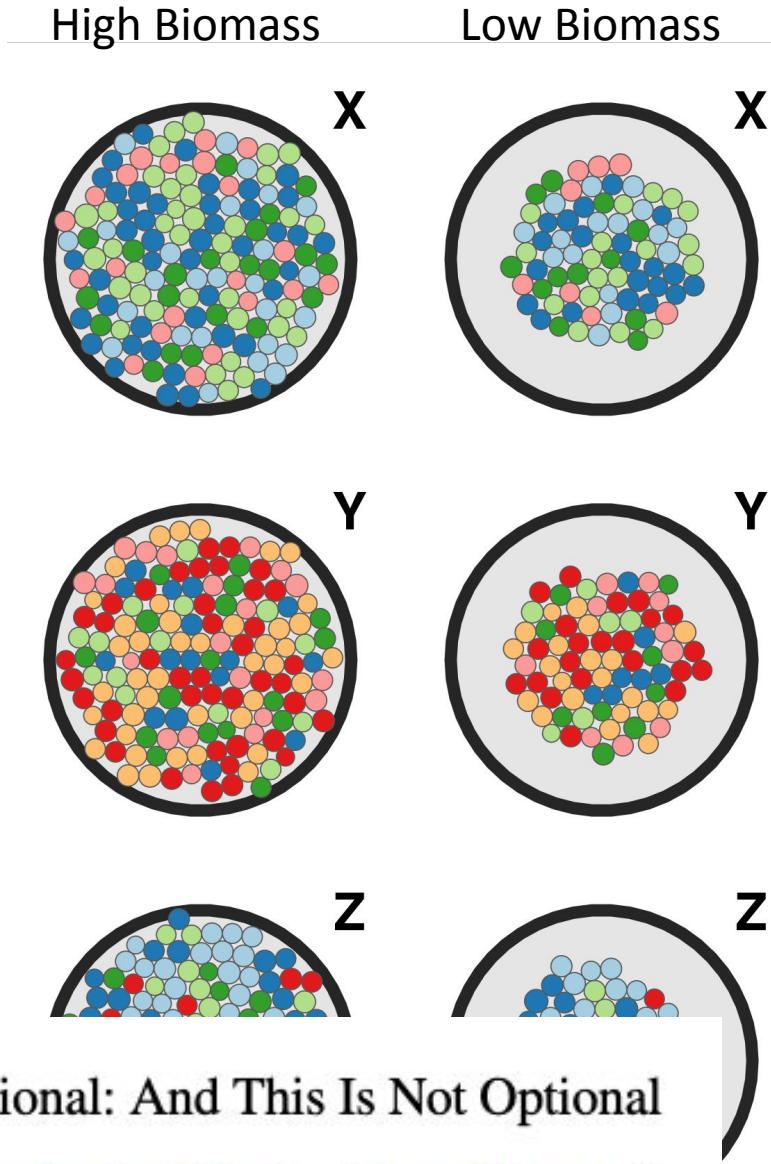

Microbiome Datasets Are Compositional: And This Is Not Optional

Gregory B. Gloor,[1,*] Jean M. Macklaim,[1] Vera Pawlowsky-Glahn,[2] and Juan J. Egozcue[3]

NOW: Barcharts and Diversity - getting started in R

david-barnett.github.io/evomics-material/exercises/exercises_1.html

Next lecture at 20:30

Remember to take a break before then!

Activate RStudio Project in microbiome_analysis directory

File Edit Code View Plots Session Build Debug Profile Tools Help

genomics

Go to file/funct Addins

Project: (None)

Console Terminal Background Jobs

R 4.3.2 · ~/

```
R version 4.3.2 (2023-10-31) -- "Eye Holes"
Copyright (C) 2023 The R Foundation for Statistical Computing
Platform: x86_64-pc-linux-gnu (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

Session restored from your saved work on 2024-Jan-07 15:59:32 UTC (6 days ago)
>
```

Environment History Connections

Import Dataset 178 MiB List

R Global Environment

Environment is empty

Files Plots Packages Help Viewer Presentation

New Folder New Blank File Upload Delete Rename More

Home > workshop_materials > microbiome_analysis

Name	Size	Modified
..		
.gitignore	40 B	Dec 28, 2023, 5:53
.Rprofile	101 B	Dec 28, 2023, 5:53
data		
evomics-material.Rproj	229 B	Dec 28, 2023, 5:53
exercises		
README.md	1.2 KB	Dec 28, 2023, 5:53
setup		
slides		

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Home > workshop_materials > microbiome_analysis

Name	Size	Modified
..		
.gitignore	40 B	Dec 28, 2023, 5:53
.Rprofile	101 B	Dec 28, 2023, 5:53
data		
evomics-material.Rproj	229 B	Dec 28, 2023, 5:53
exercises		
README.md	1.2 KB	Dec 28, 2023, 5:53
setup		
slides		

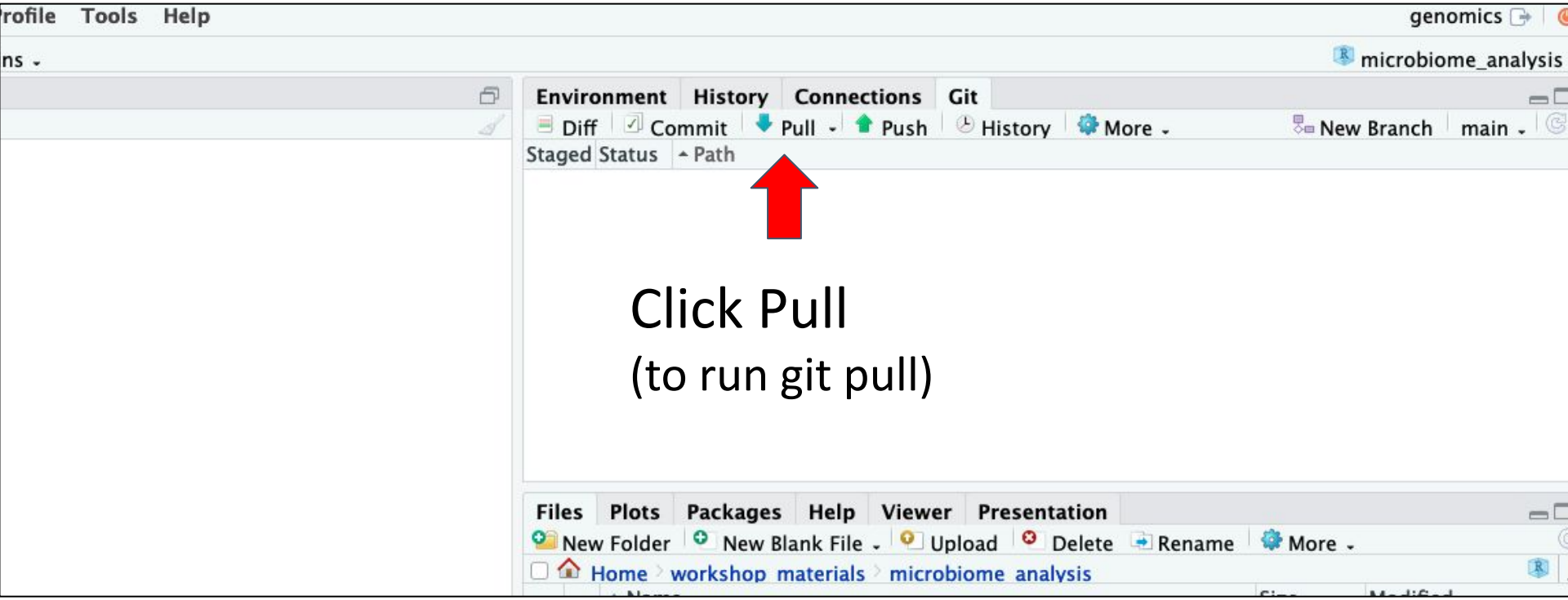
Confirm Open Project

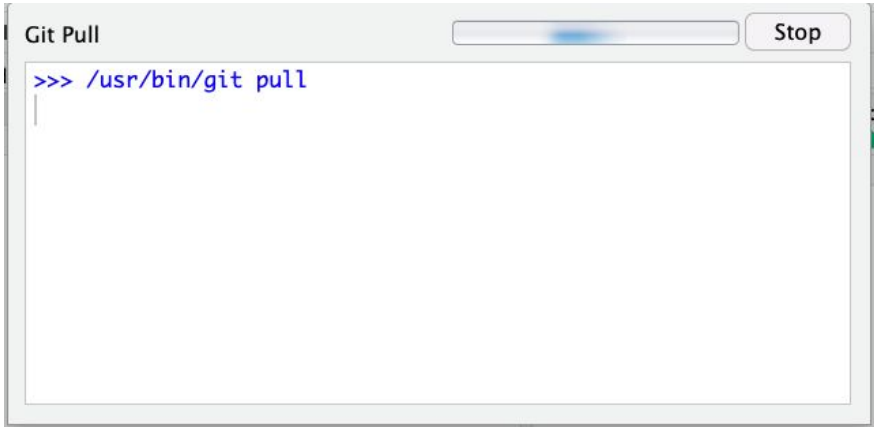
Do you want to open the project ~/workshop_materials /microbiome_analysis?

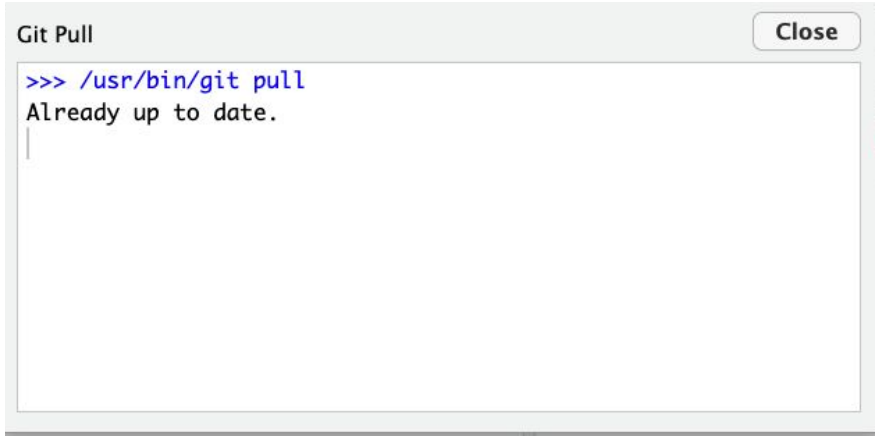
Yes No

Learn more about RStudio projects?
https://rstats.wtf/projects

Ensure you have the latest version of the project git repo







More about using git with R & RStudio?
https://happygitwithr.com/

NOW: Barcharts and Diversity - getting started in R

david-barnett.github.io/evomics-material/exercises/exercises_1.html

Next lecture at 20:30

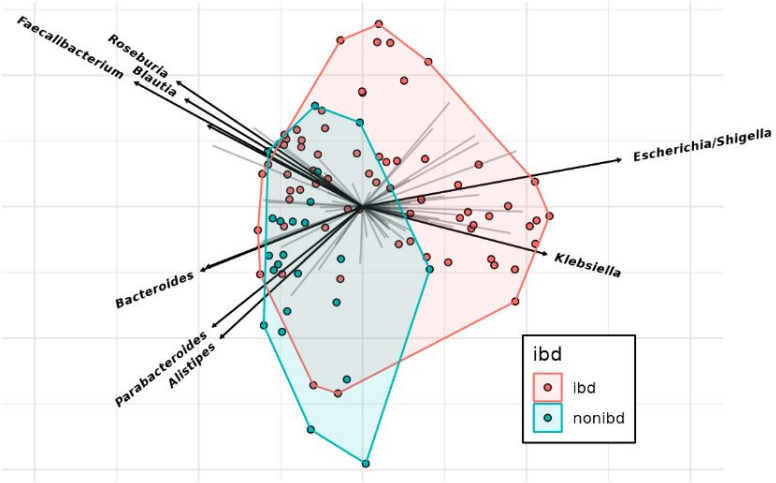
Remember to take a

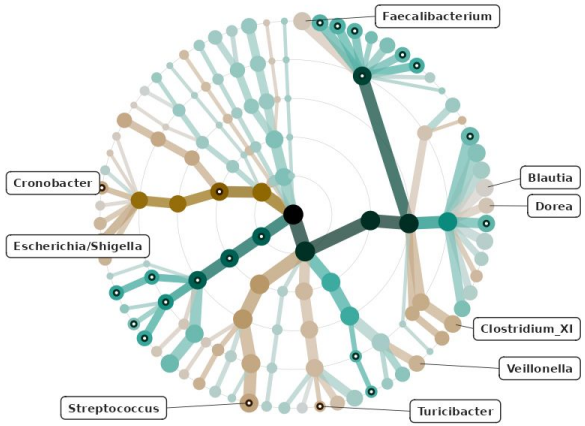
break before then!

Dissimilarity, Ordination, and Differential Abundance

3. From Dissimilarity to Ordination

- Common dissimilarity measures
- PCoA, PERMANOVA, and PCA

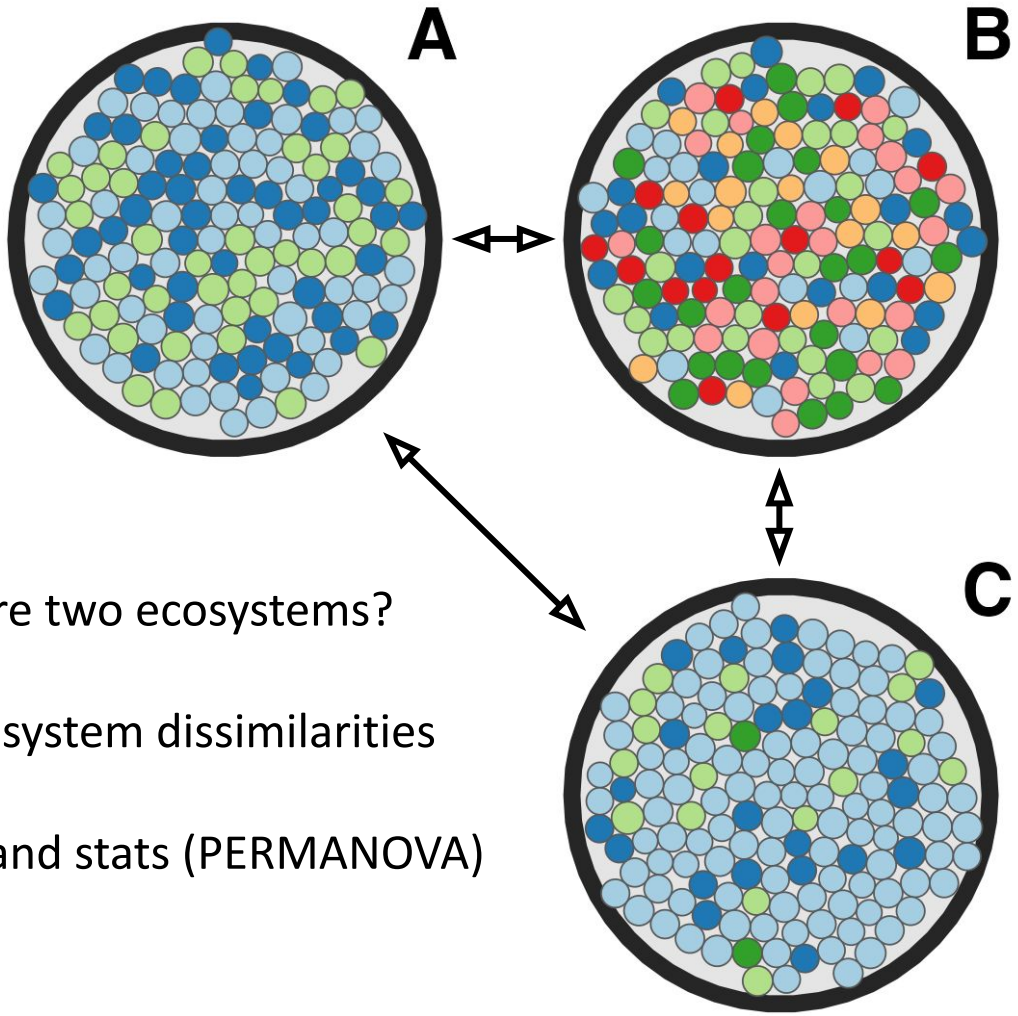




4. Differential Abundance testing

- A gentle intro to modelling individual taxa

<u>**Dissimilarity**</u> between ecosystems



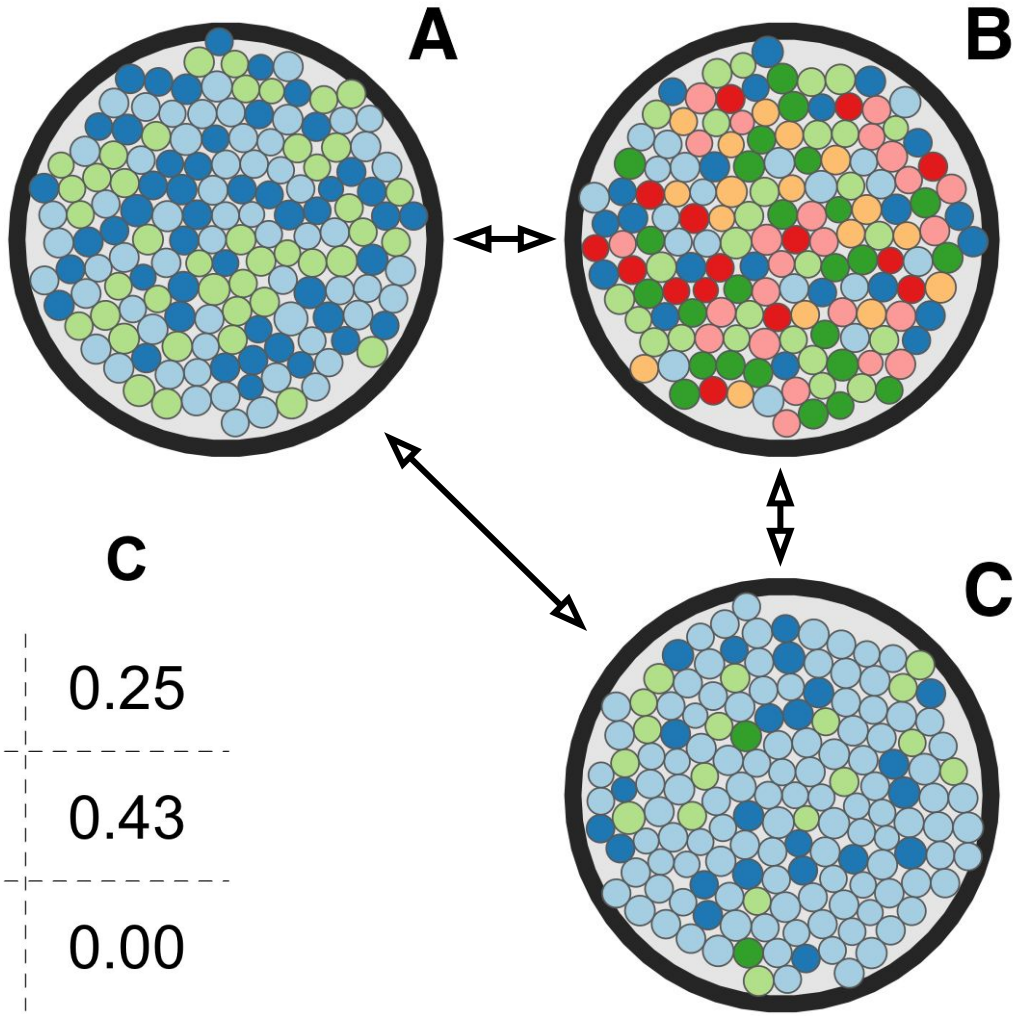
- **Dissimilarity** - how different are two ecosystems?
- **Distance matrix** - pairwise ecosystem dissimilarities
- **Very useful** - for plots (PCoA) and stats (PERMANOVA)

<u>**There are many different dissimilarity measures!**</u>

Dissimilarity Measures

1. **Binary Jaccard Distance**
   - an “unweighted” measure

	A	B	C
A	0.00	0.57	0.25
B		0.00	0.43
C			0.00



Dissimilarity Measures

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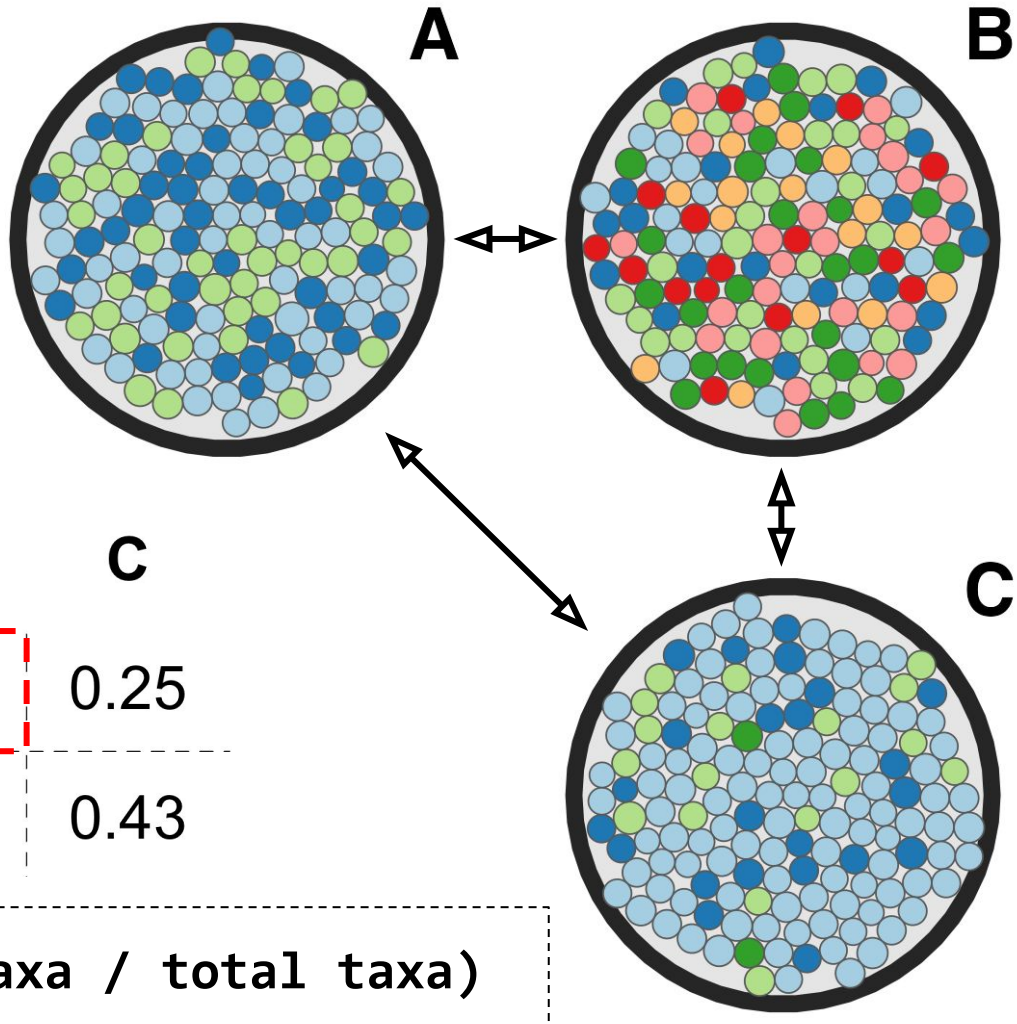
   - an "unweighted" measure



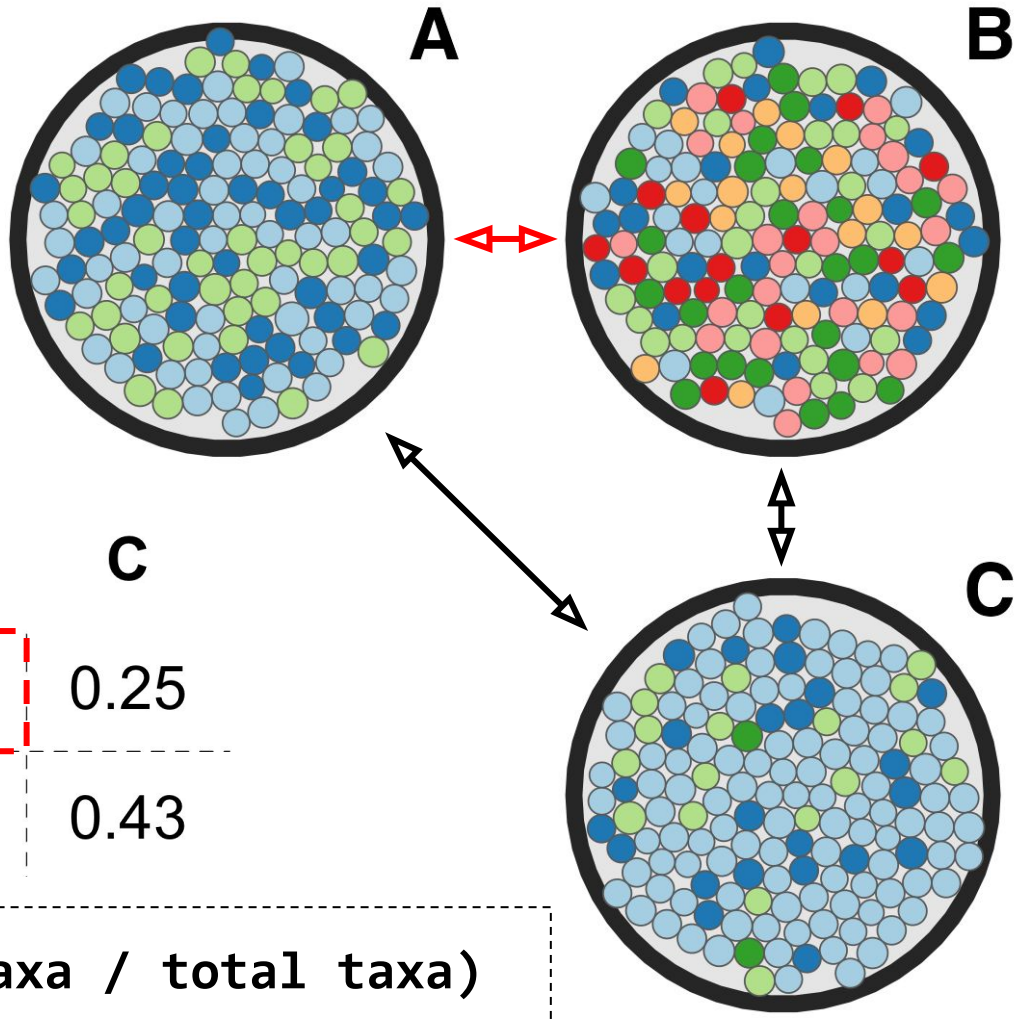
	B	C
A	0.57	0.25
B		0.43

d_{ij} = 1 - (shared taxa / total taxa)

➡ AB = ???

Dissimilarity Measures

1. **Binary Jaccard Distance**
   - an “unweighted” measure



	B	C
A	0.57	0.25
B		0.43

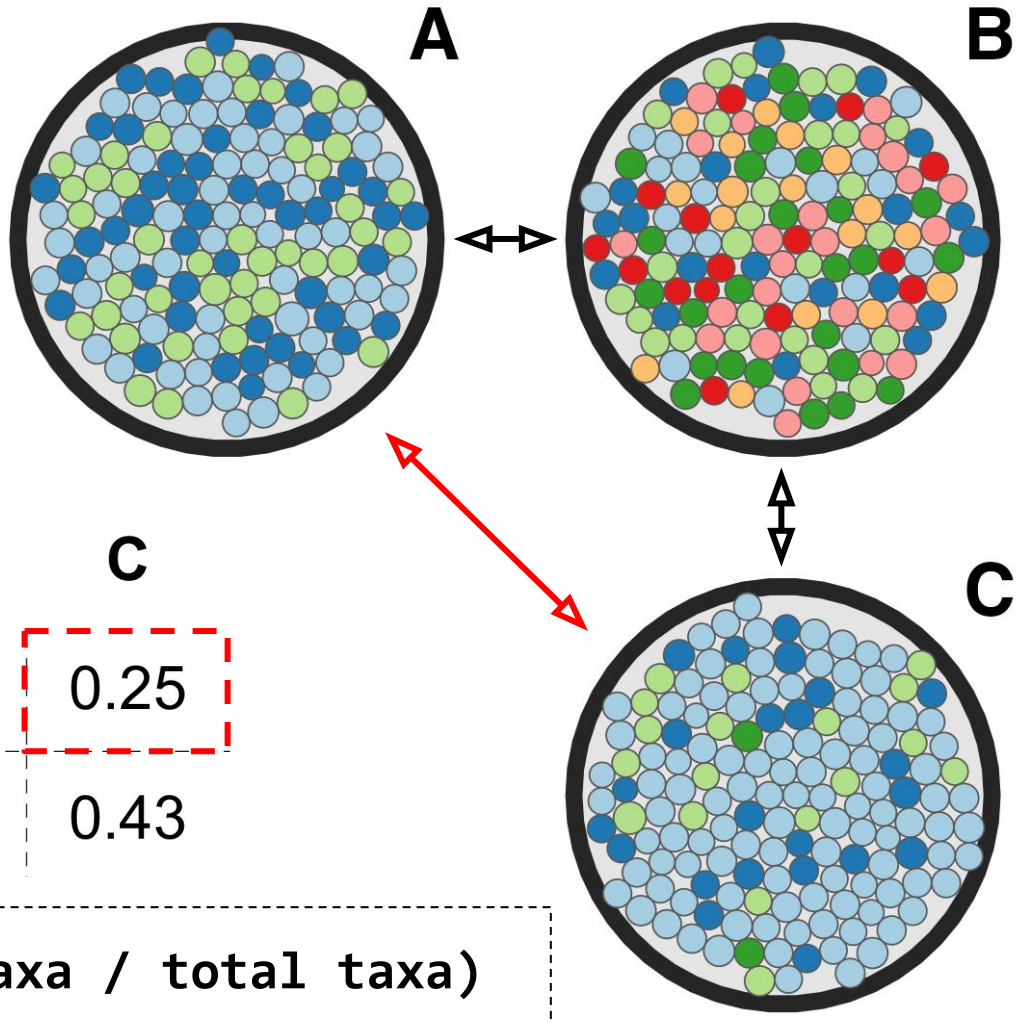
d_{ij} = 1 - (shared taxa / total taxa)

➡ AB = 1 - (●●● / ●●●●●●●)

➡ AB = 1 - (3 / 7) = 4 / 7 = **0.57**

Dissimilarity Measures

1. **Binary Jaccard Distance**
   - an "unweighted" measure



	B	C
A	0.57	0.25
B		0.43

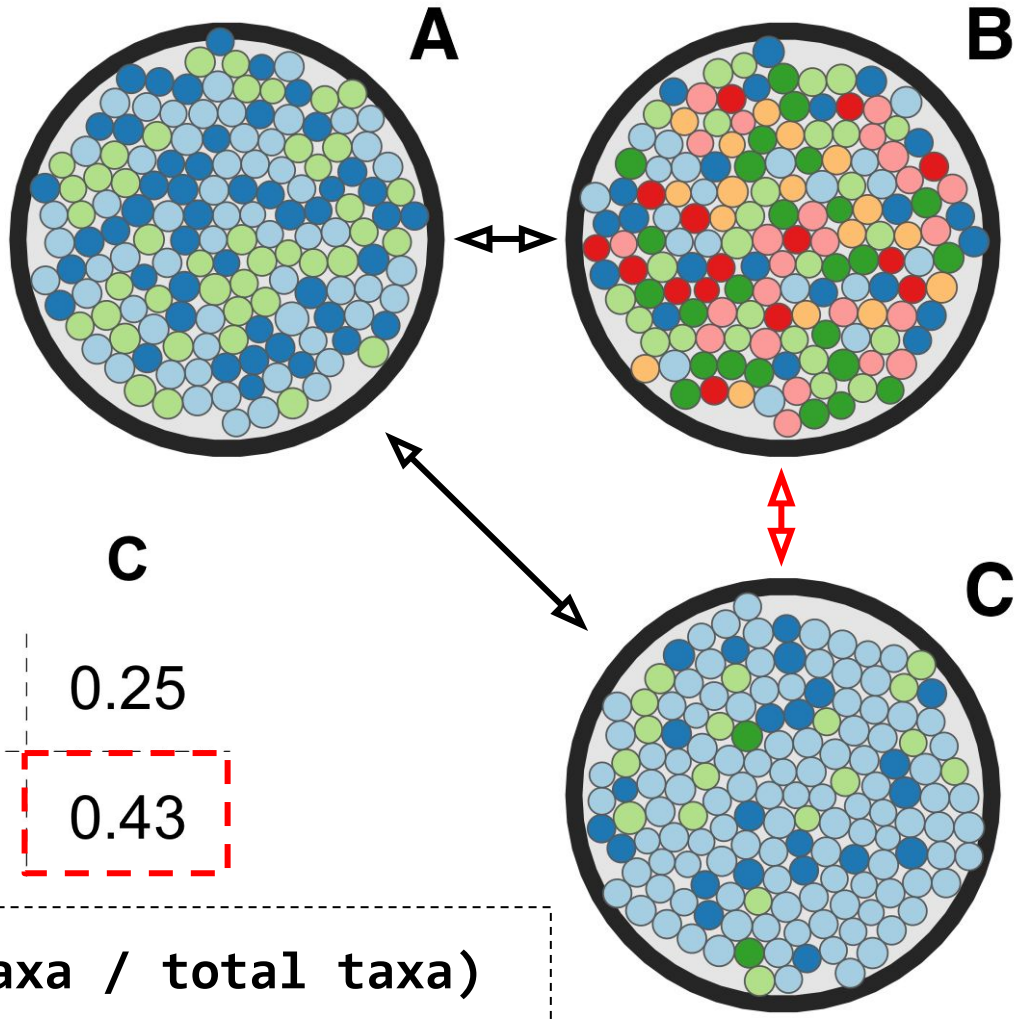
d_{ij} = 1 - (shared taxa / total taxa)

➡ AC = 1 - ()

➡ AC = 1 - (3 / 4) = 1 / 4 = **0.25**

Dissimilarity Measures

1. **Binary Jaccard Distance**
   - an “unweighted” measure



	B	C
A	0.57	0.25
B		0.43

d_{ij} = 1 - (shared taxa / total taxa)

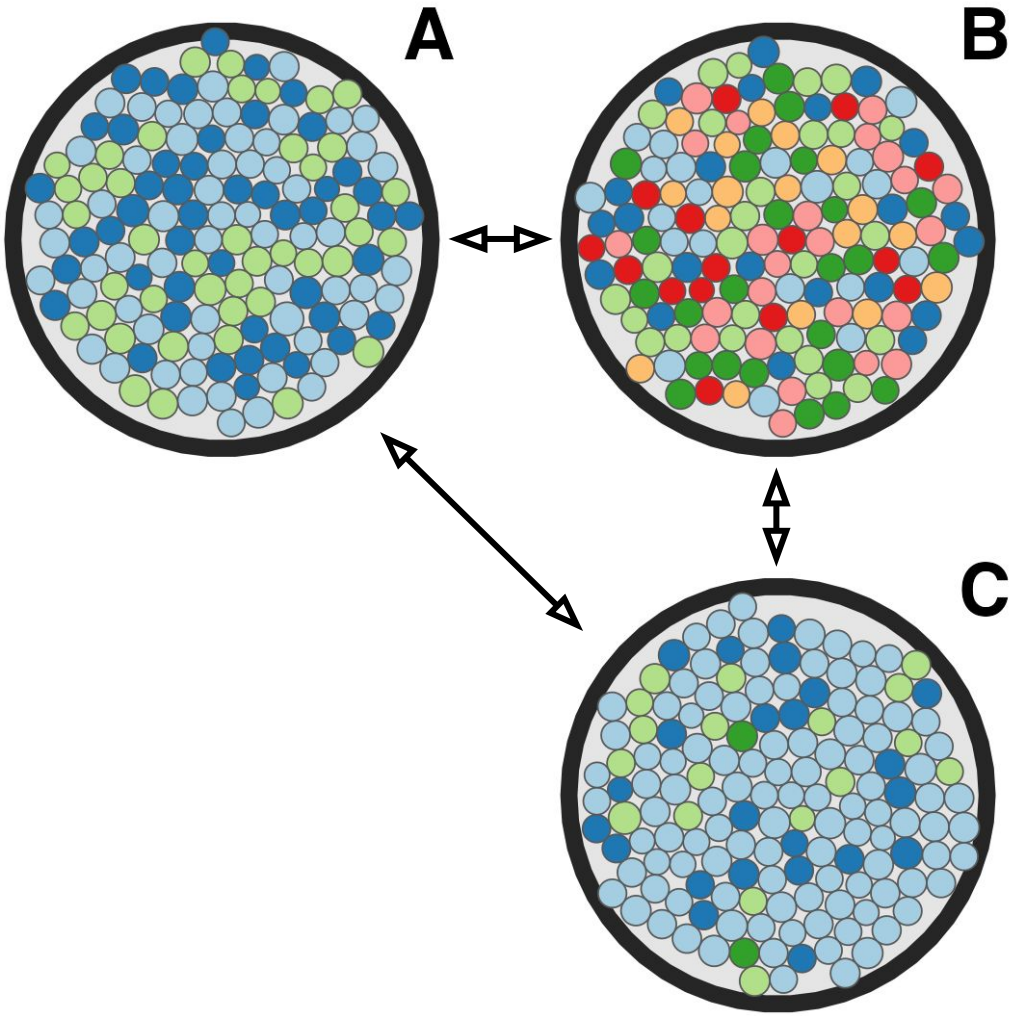
➡ BC = 1 - ()

➡ BC = 1 - (4 / 7) = 3 / 7 = **0.43**

Dissimilarity Measures

2. Bray-Curtis Dissimilarity

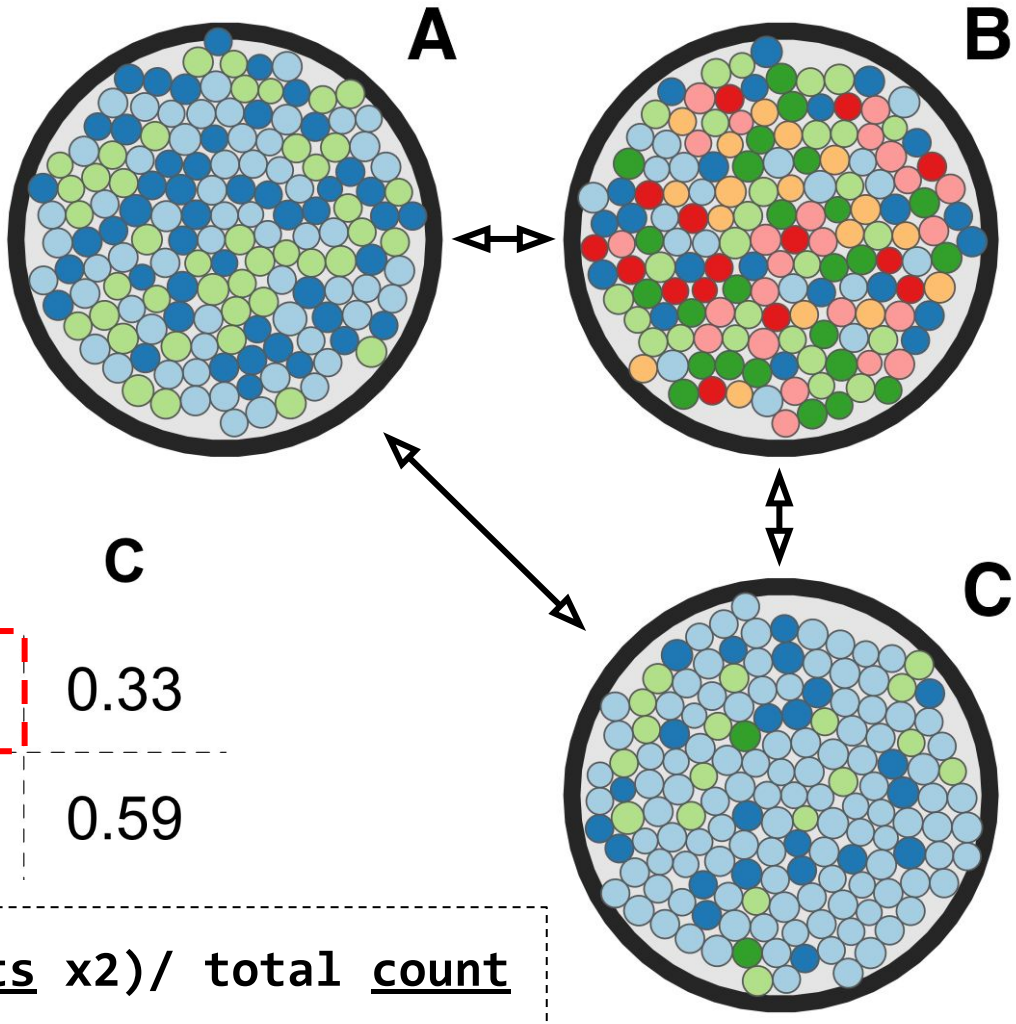
- "abundance-weighted"



Dissimilarity Measures

2. Bray-Curtis Dissimilarity

- "abundance-weighted"



	B	C
A	0.54	0.33
B		0.59

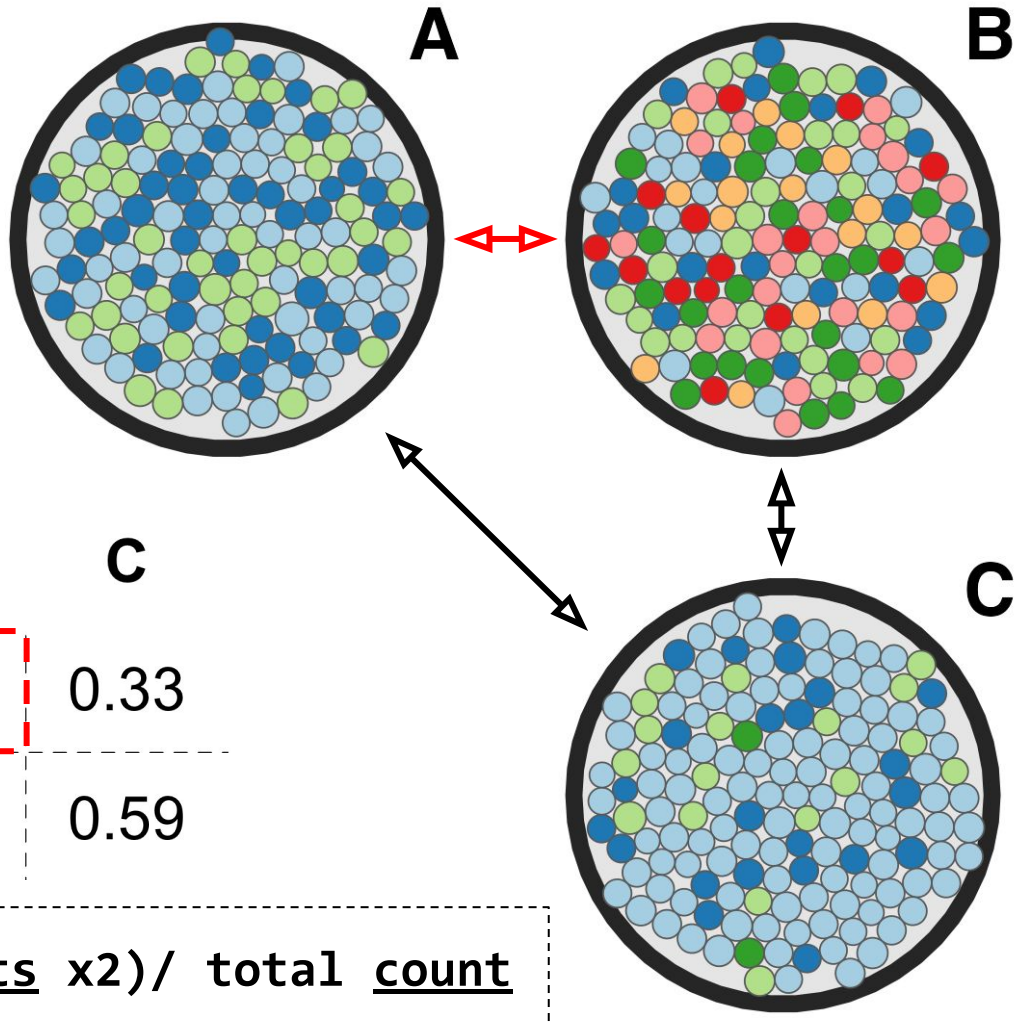
d_{ij}= 1 - (shared <u>counts</u> x2)/ total <u>count</u>

➡ AB= ???

Dissimilarity Measures

2. Bray-Curtis Dissimilarity

- "abundance-weighted"



	B	C
A	0.54	0.33
B		0.59

d_{ij}= 1 - (shared <u>counts</u> x2)/ total <u>count</u>

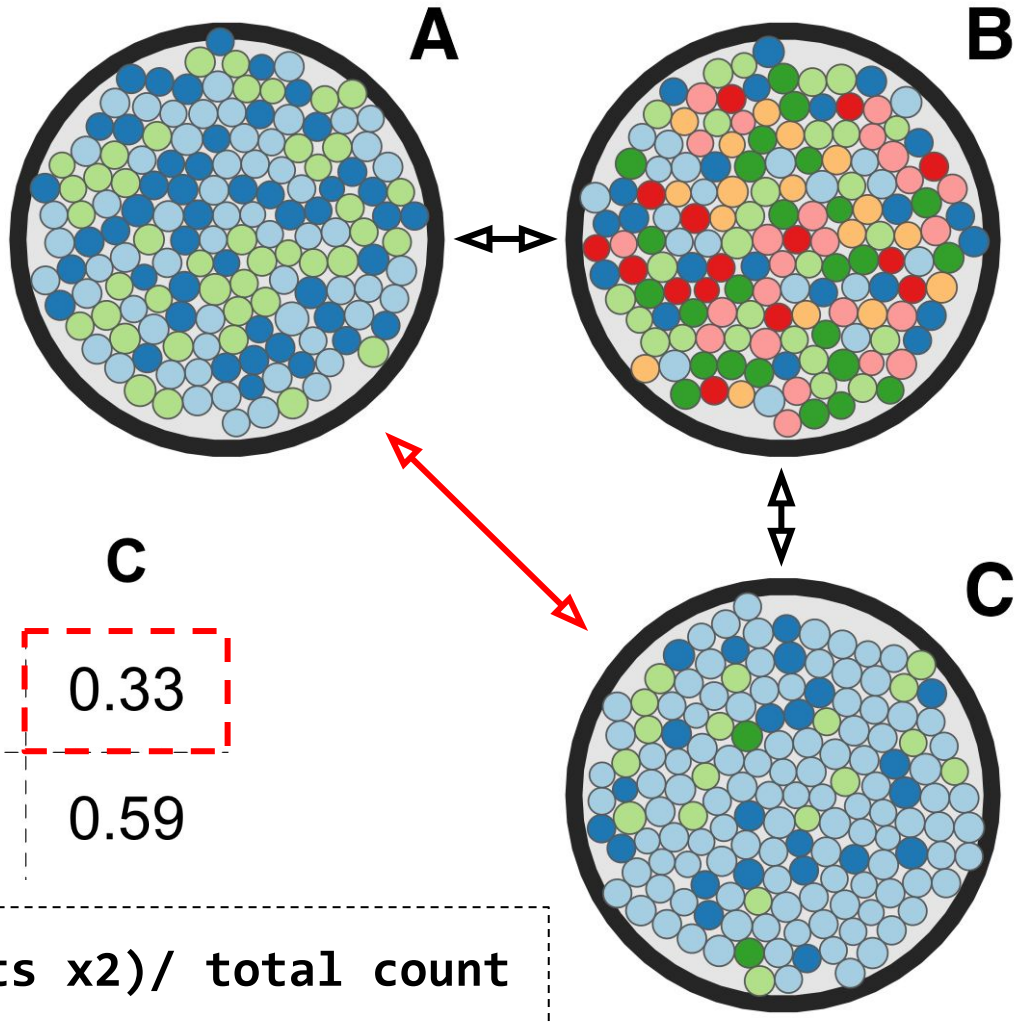
➡ AB= 1 - (20 +22 +27)x2 / (+)

➡ AB= 1 - 138/300 = 1 - 0.46 = **0.54**

Dissimilarity Measures

2. Bray-Curtis Dissimilarity

- "abundance-weighted"



	B	C
A	0.54	0.33
B		0.59

d_{ij}= 1 - (shared counts x2)/ total count

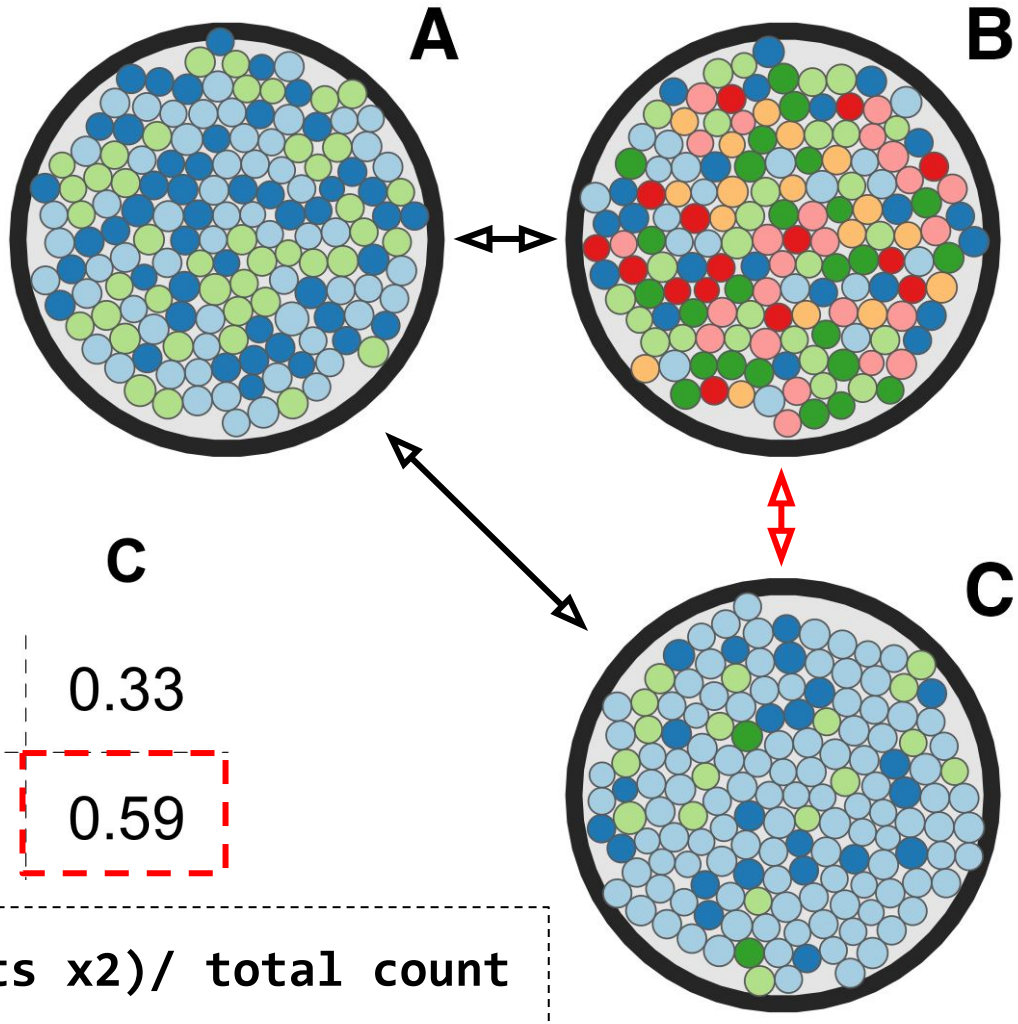
➡ AC= 1 - (60 +23 +18)x2 / (+)

➡ AC= 1 - 202/300 = 1 - 0.67 = **0.33**

Dissimilarity Measures

2. Bray-Curtis Dissimilarity

- "abundance-weighted"



	B	C
A	0.54	0.33
B		0.59

d_{ij}= 1 - (shared counts x2)/ total count

➡ BC= 1 -(20 +22 +18 +2)x2/(+)

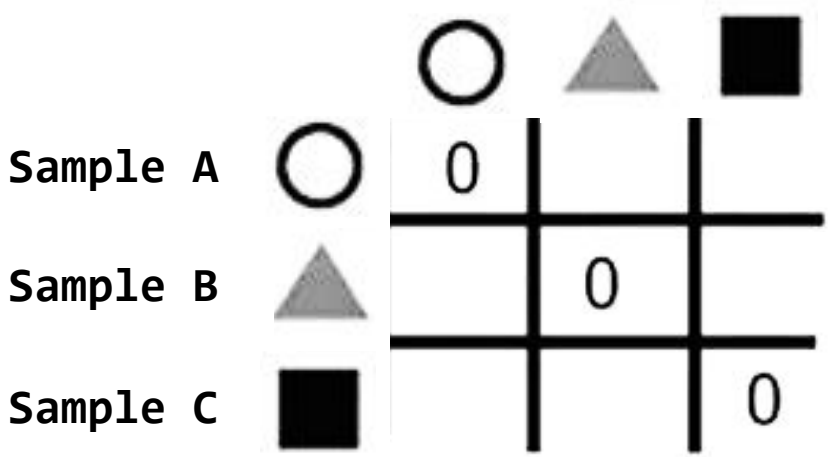
➡ BC= 1 - 124/300 = 1 - 0.41 = **0.59**

Dissimilarity Measures

3. UniFrac distance family

- "phylogenetic" distances

- Samples share the same taxa ➡ UniFrac is very low (or zero)
- Samples contain distinct but **related** taxa ➡ UniFrac is low
- Samples contain **unrelated** taxa ➡ UniFrac is higher



Example adapted from Lozupone and Knight 2005

Dissimilarity Measures

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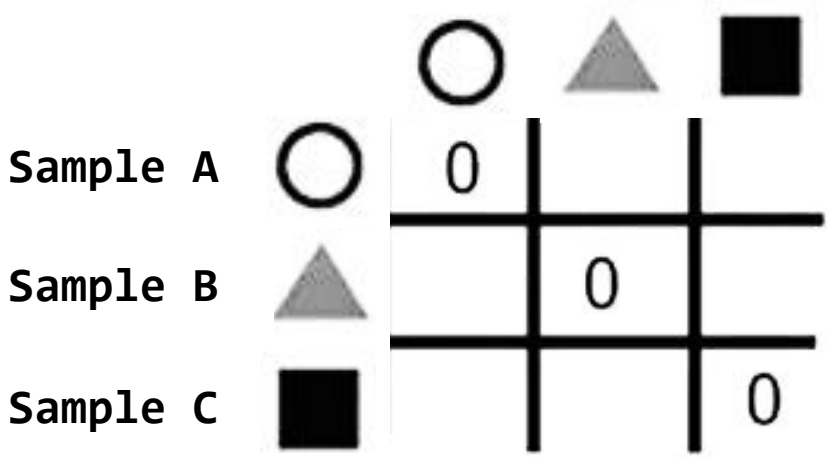

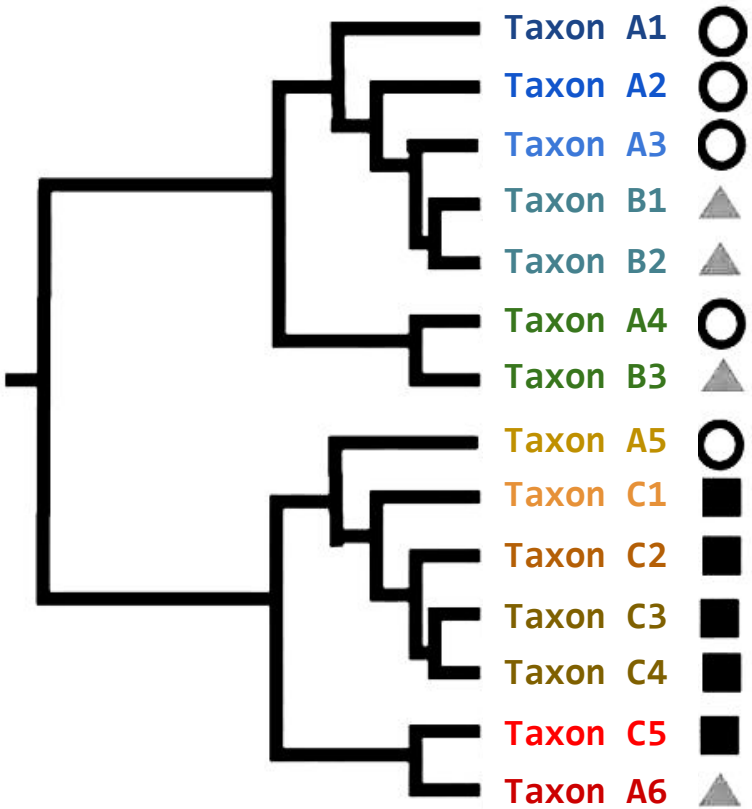

Tree tips are **taxa**!
Shape shows their **source**

<u>**UniFrac** = "**Uni**que **Frac**tion"</u>
of branch length leading to
taxa from only one sample

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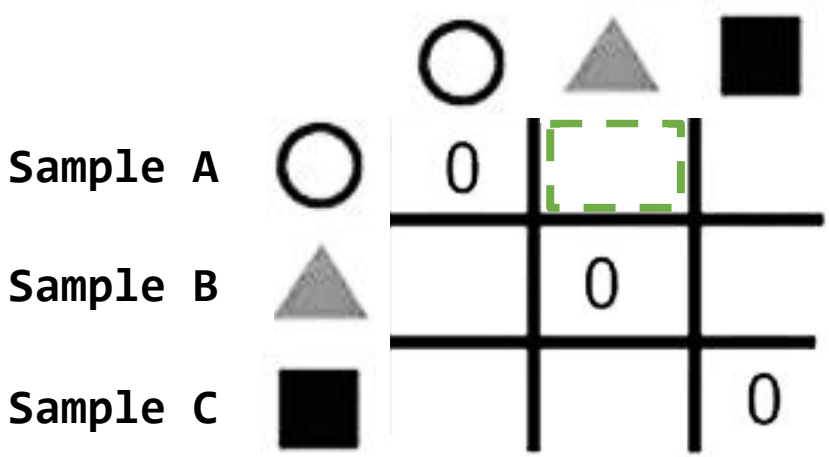

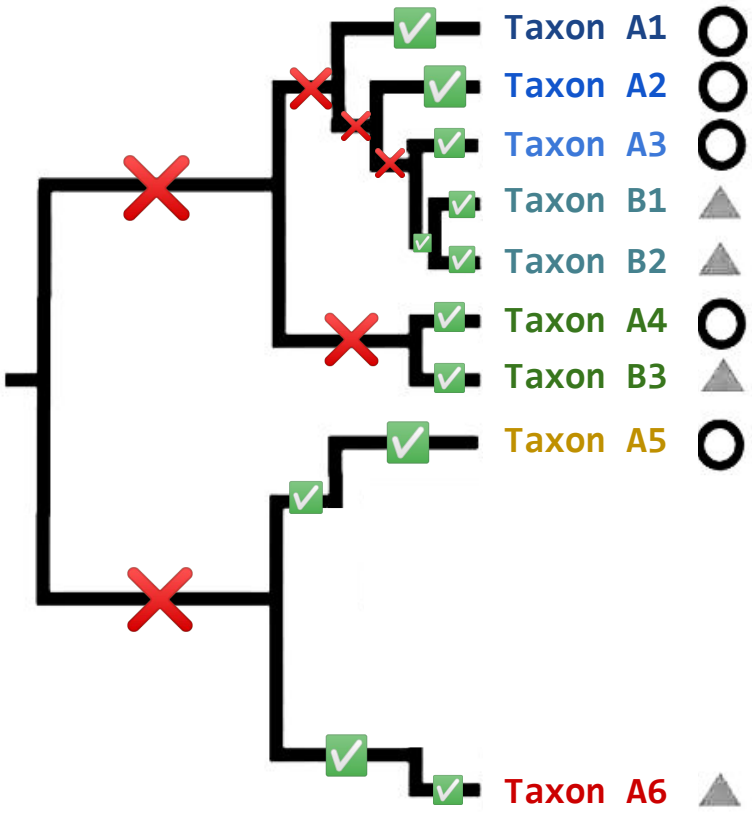

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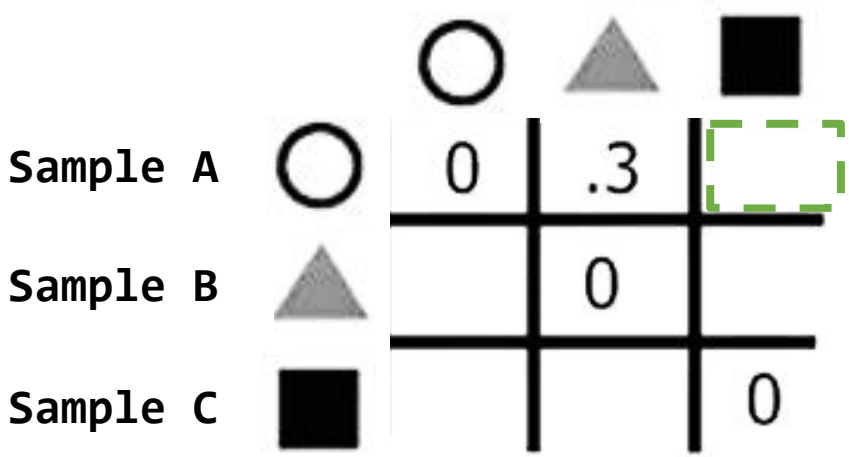

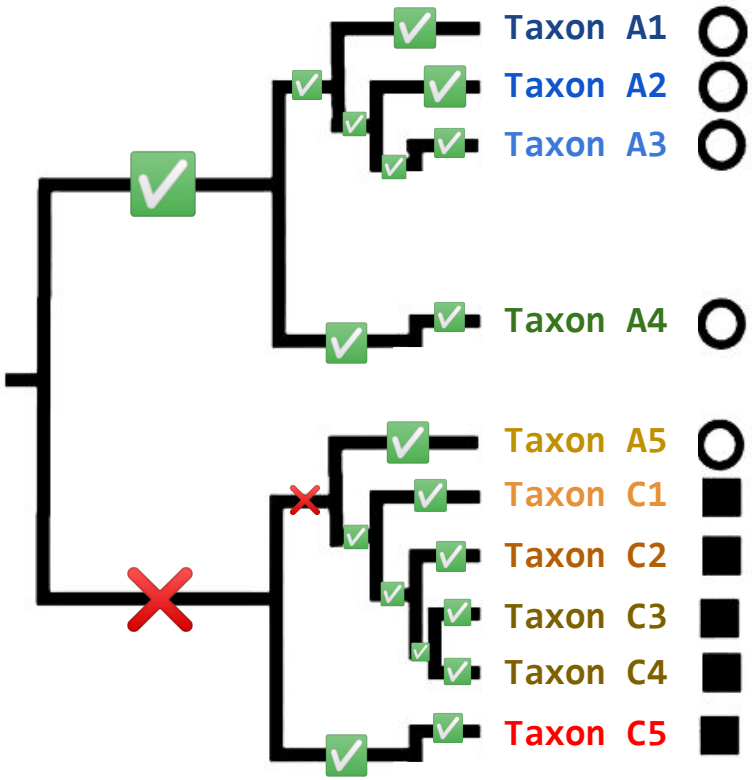

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Dissimilarity Measures

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- "phylogenetic" distances

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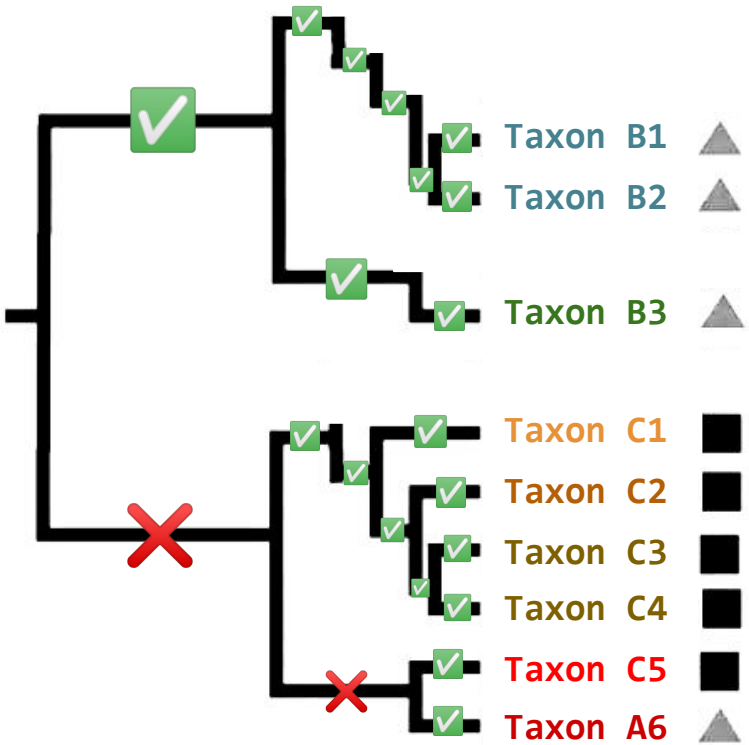
- … distinct but **related** taxa ➡ UniFrac is low
- … **unrelated** taxa ➡ UniFrac is higher

	○	▲	■
Sample A ○	0	.3	.7
Sample B ▲		0	
Sample C ■			0



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Dissimilarity Measures

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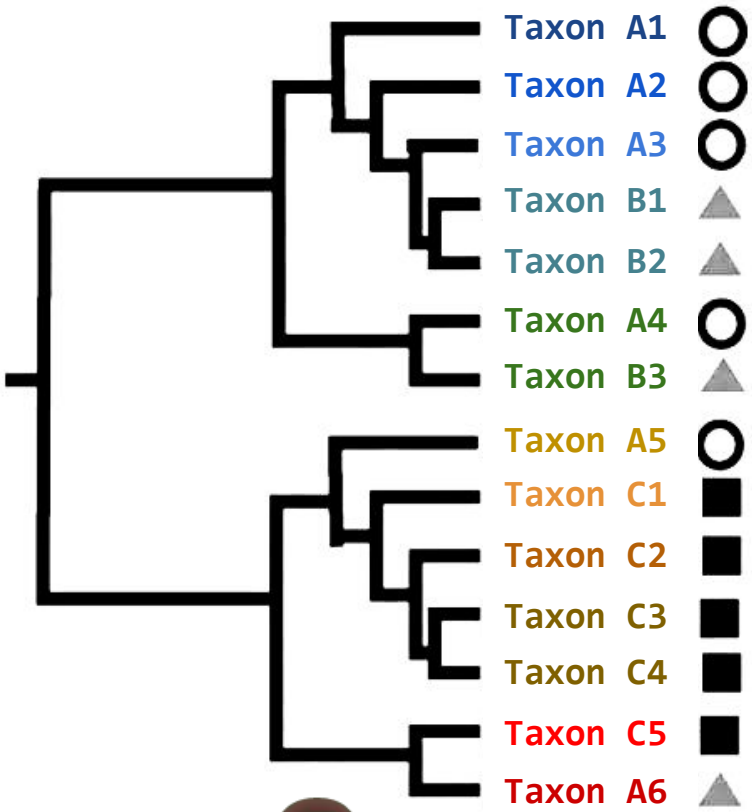
- "phylogenetic" distances

- share the same taxa ➡ UniFrac is very low (or zero)
- … distinct but **related** taxa ➡ UniFrac is low
- … **unrelated** taxa ➡ UniFrac is higher

The UniFrac family:

1. UniFrac (unweighted)
2. Abundance-weighted UniFrac
3. Generalised UniFrac (balanced)



Tree  s are **taxa**!
Shape ss their **source**

<u>UniFrac</u> = <u>"**Uni**que **Frac**tion"</u>
of branch length leading to
taxa from only one sample

Principal Coordinates Analysis

Dissimilarities ➡ Ordination

<u>Distances</u> (Kilometres)

Now, place the cities on map …?

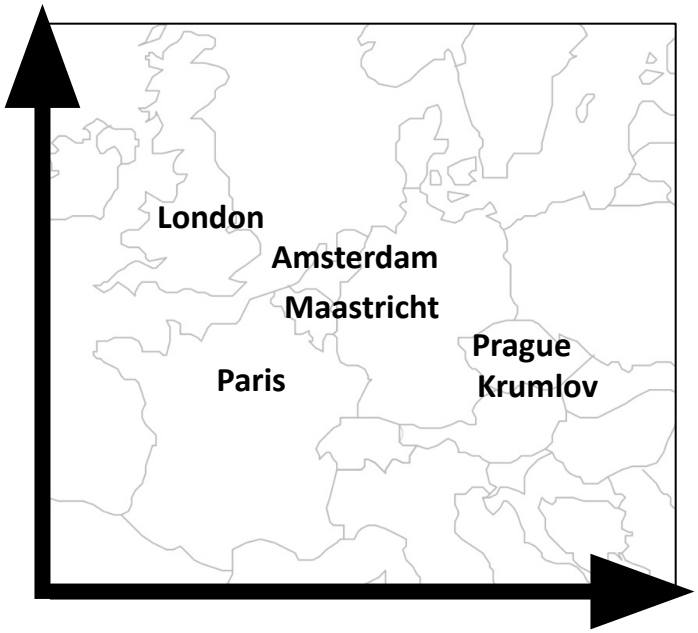
Principal Coordinates Analysis

Dissimilarities ➡ Ordination

<u>Distances</u> (Kilometres)

	Lon.	Par.	Ams.	Maa.	Pra.	Kru.
London	0	…	…	…	…	…
Paris	…	0	…	…	…	…
Amsterdam	…	…	0	…	…	…
Maastricht	…	…	…	0	…	…
Prague	…	…	…	…	0	…
Krumlov	…	…	…	…	…	0

<u>Map</u> (Coordinates)



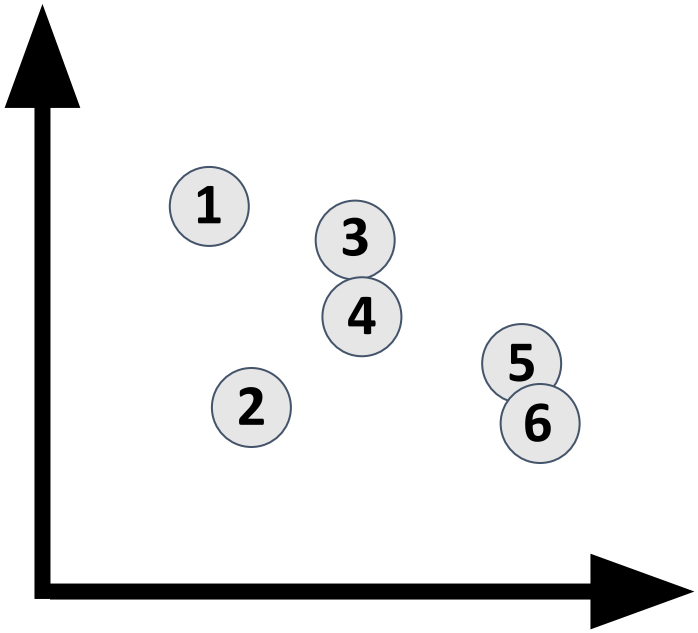
Principal Coordinates Analysis

Dissimilarities ➡ Ordination

<u>Dissimilarities</u> (Bray-Curtis)

	S1	S2	S3	S4	S5	S6
Sample 1	0	…	…	…	…	…
Sample 2	…	0	…	…	…	…
Sample 3	…	…	0	…	…	…
Sample 4	…	…	…	0	…	…
Sample 5	…	…	…	…	0	…
Sample 6	…	…	…	…	…	0

<u>Plot</u> (Principal Coordinates)

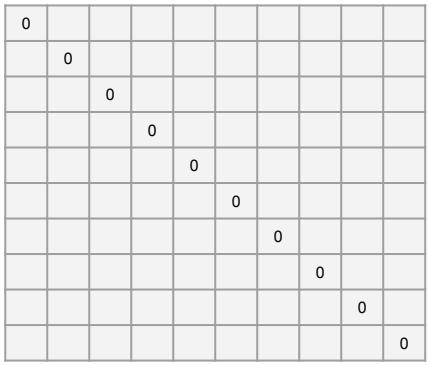


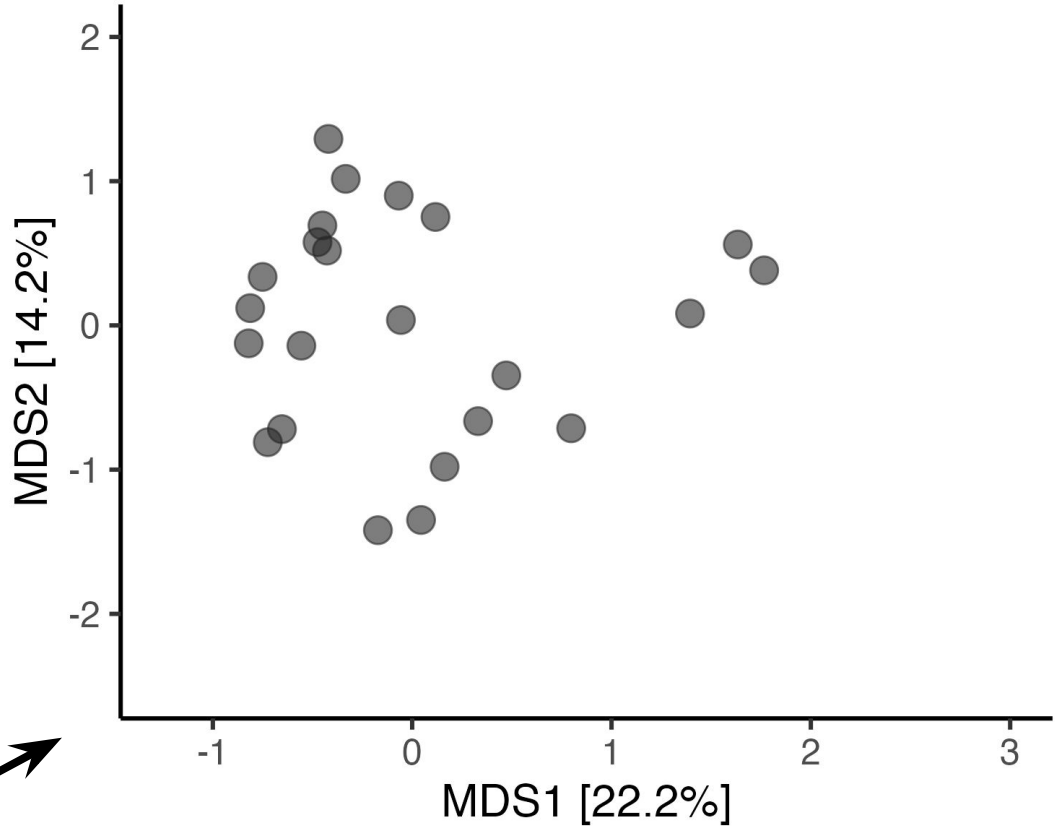
PCoA
on real data

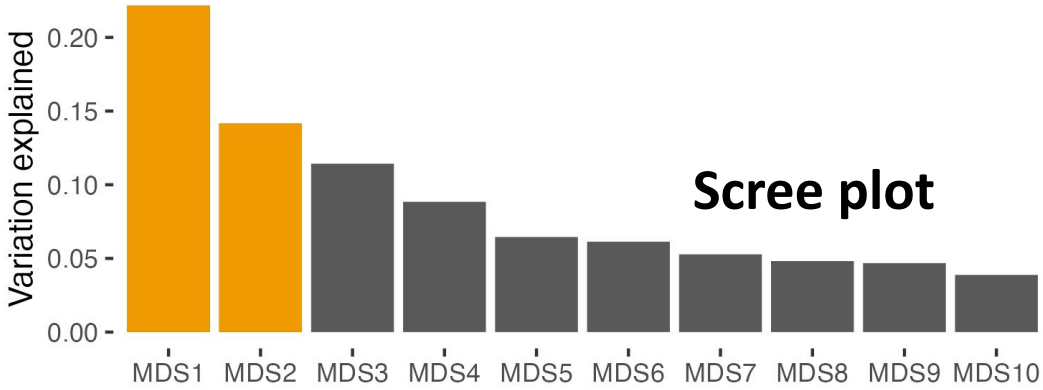
Ulcerative Colitis patients and Healthy Controls

Stool samples --> 16S microbiota abundances

Compute Bray-Curtis dissimilarities with genera







PCoA
on real data

Ulcerative Colitis patients and Healthy Controls

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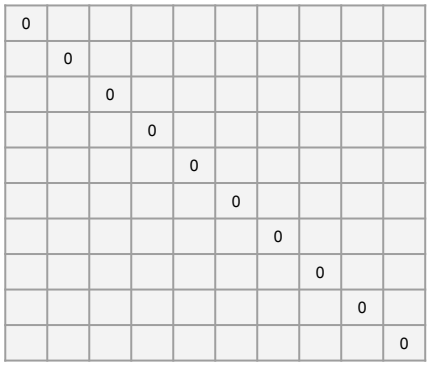

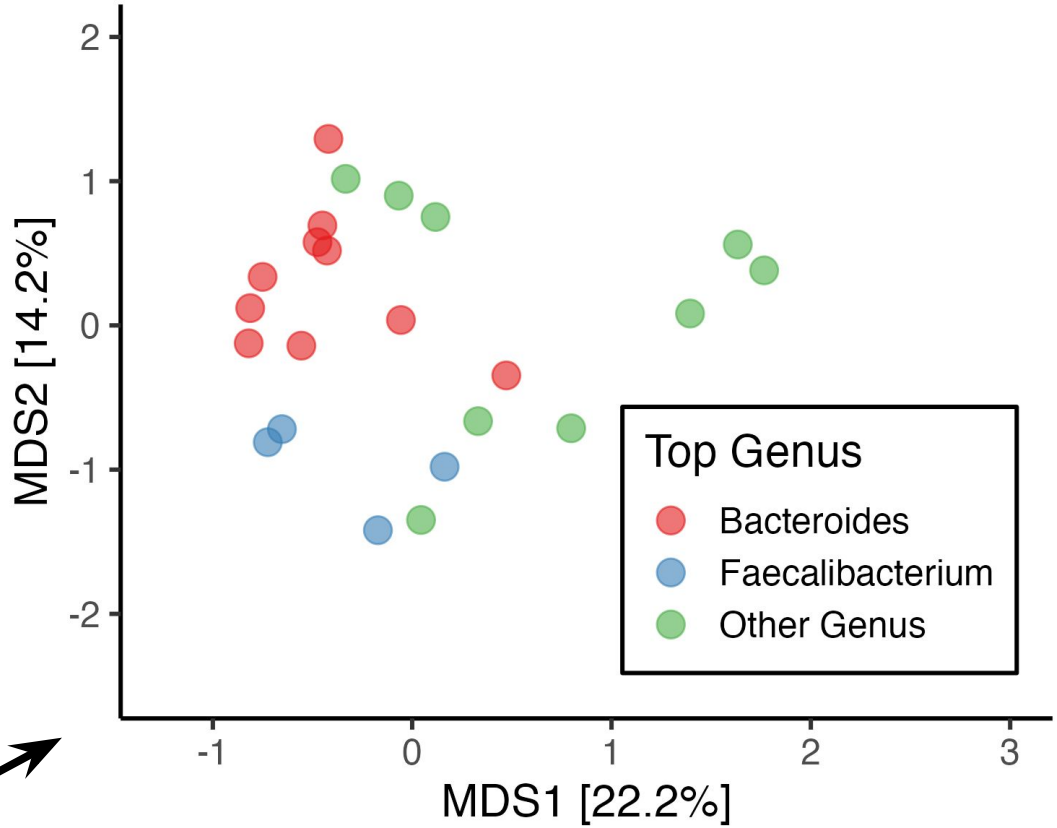

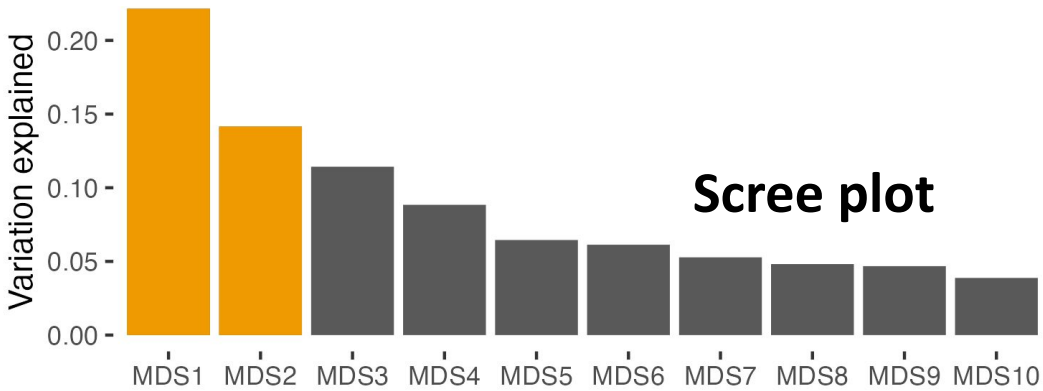

PCoA
on real data

Ulcerative Colitis patients and Healthy Controls

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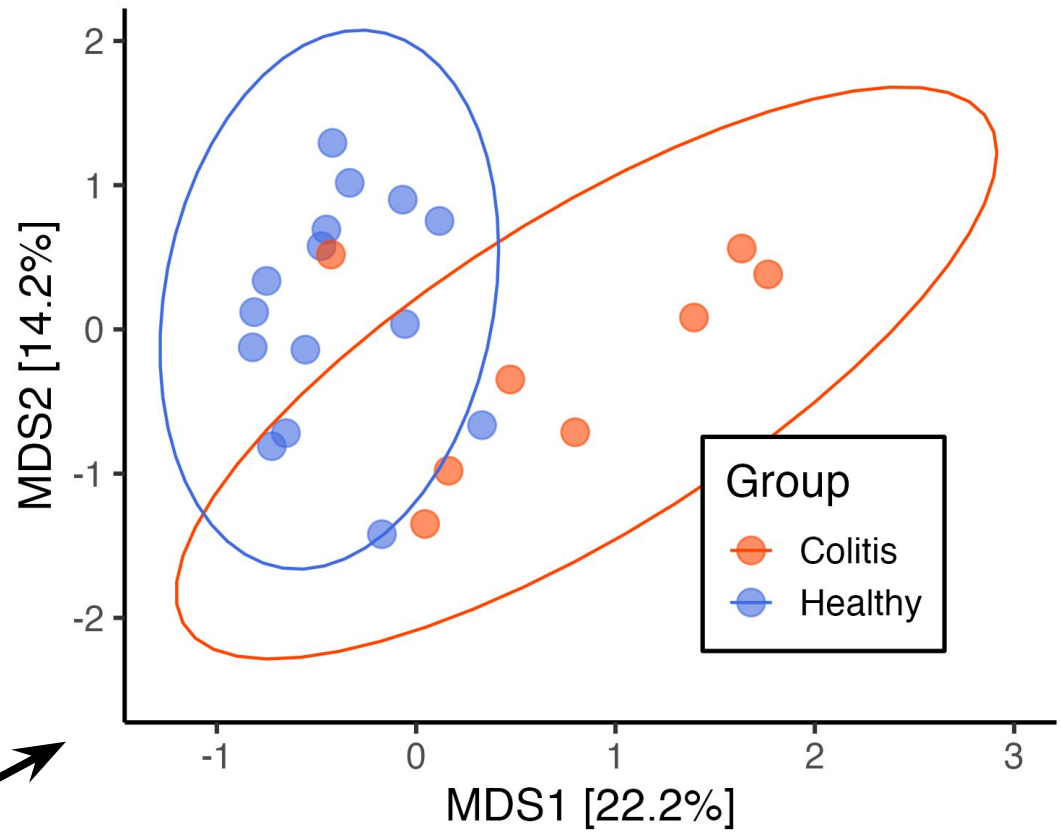
Compute Bray-Curtis dissimilarities with genera

0									
	0								
		0							
			0						
				0					
					0				
						0			
							0		
								0	
									0



PERMANOVA: $p < 0.01$

- Permutational Multivariate ANOVA
- Does average composition differ by Group?

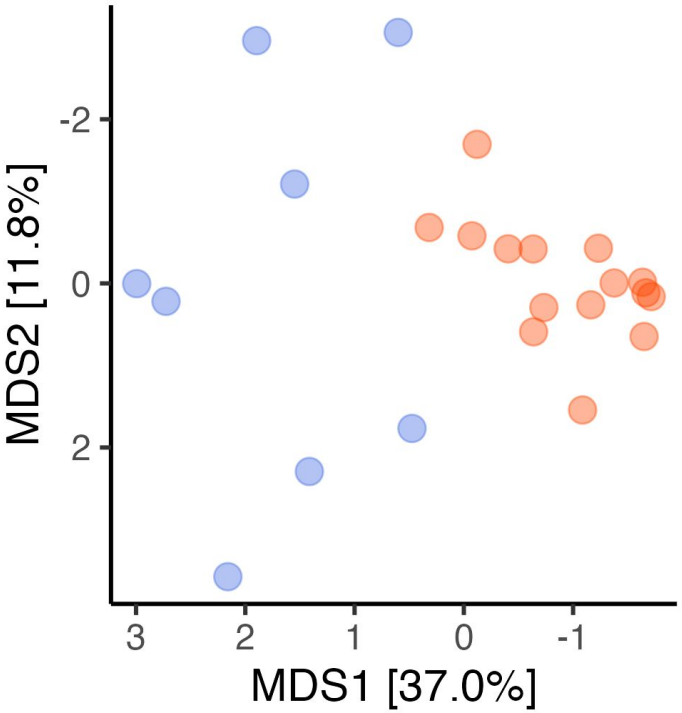
Interactive Ordination!

PCoA Principal <u>Coordinates</u> Analysis	**VS.**	**PCA** Principal <u>Components</u> Analysis
Taxon abundances		Taxon abundances
Calculate Dissimilarities	↓	**Transform abundances**
Distance Matrix	↓	Transformed abundances
PCoA / MDS ordination	↓	**PCA ordination** (rotate input)
New dimensions (Coordinates)	↓	New dimensions (Components)
Plot first 2 or 3 dims	↓	**Plot first 2 or 3 dims**

PCoA

Coordinates

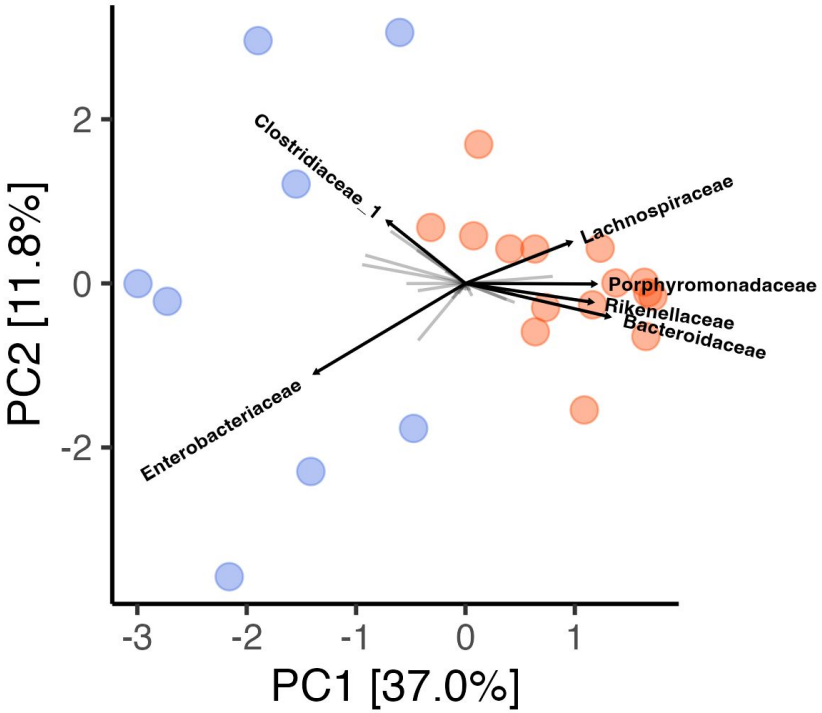
on Aitchison distances



PCA

Components

on CLR-transformed taxa

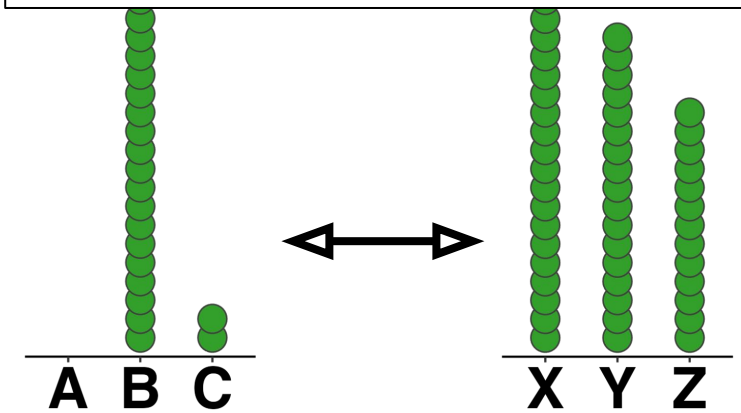


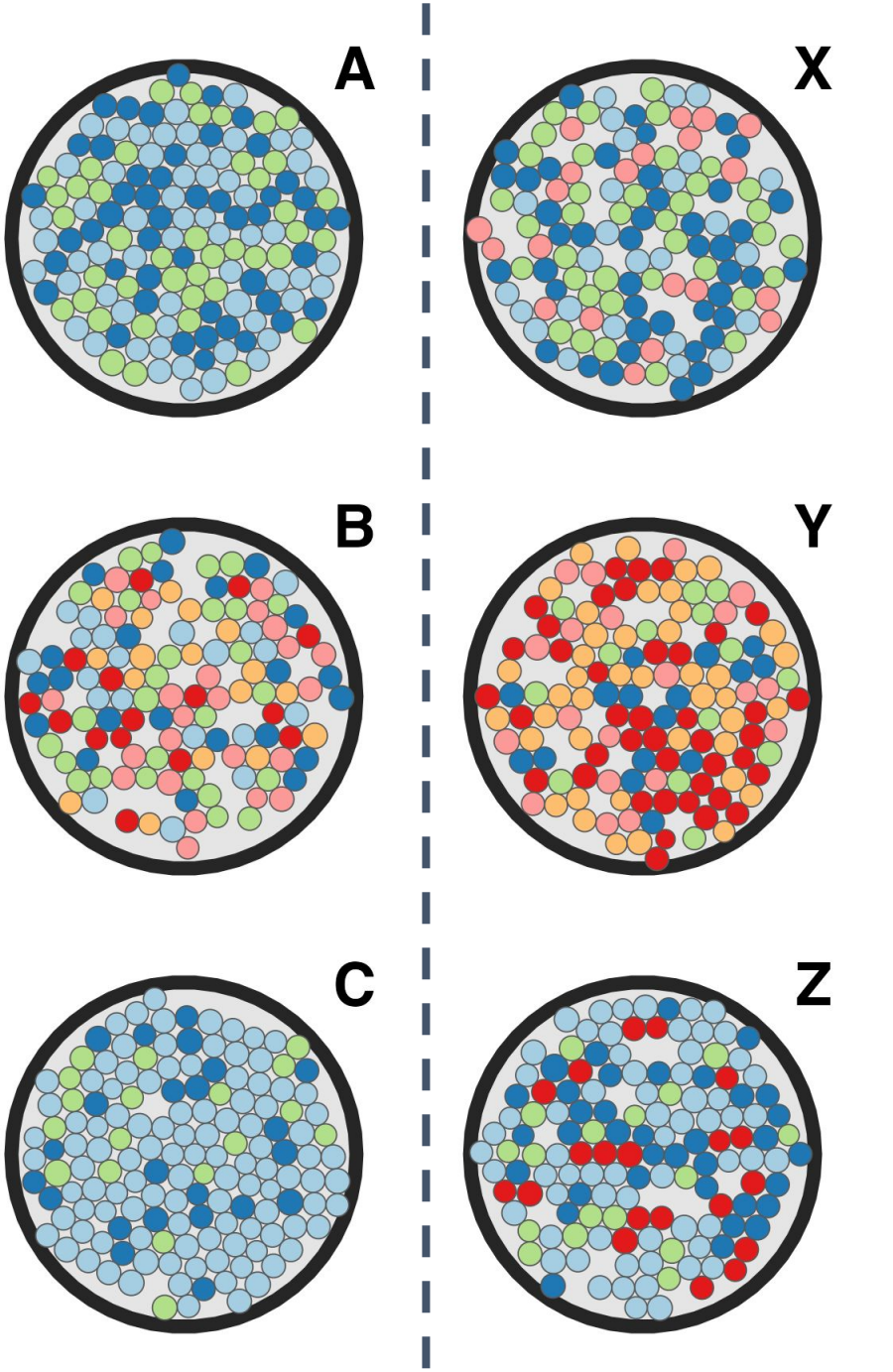
<u>Differential Abundance</u> of each taxon

- **Compare across <u>groups</u> of samples**

  e.g. - group ABC vs. group XYZ

Various statistical methods available for differential abundance testing





Differential Abundance

- Just comparing two groups of numbers, how hard can it be?
- But, we have compositional, noisy, and zero-inflated abundance counts…
- And about 15 different specialist methods to choose from…

Microbiome differential abundance methods produce disturbingly different results across 38 datasets

nature COMMUNICATIONS

Microbiome differential abundance methods produce different results across 38 datasets

Jacob T. Nearing[1,7]✉, Gavin M. Douglas[1,7], Molly G. Hayes[2], Jocelyn MacDonald[3], Dhwani K. Desai[4], Nicole Allward[5], Casey M. A. Jones[6], Robyn J. Wright[6], Akhilesh S. Dhanani[4], André M. Comeau[4] & Morgan G. I. Langille[4,6]

Microbiome differential abundance methods produce different results… 😬 😱 😵

So what can you do?

1. **Filter out the noise**

   - Most methods perform poorly on rare taxa (which are also often less relevant)

2. **Keep it simple, and visualise your data!**

   - Check for visible patterns and start with non-parametric measures (Spearman correlations or Wilcox tests)

3. **Try a couple of common DA methods** (not DESeq2)

   - Pick methods that suit your dataset (covariates? repeated samples?)
   - Check where the methods agree on overlapping results

NOW: PCoA, PCA, and DA exercises in R

david-barnett.github.io/evomics-material/exercises/exercises_2.html

Continue until 10

Or until the need for beer or bed becomes too strong…

Thank you & good luck

Microbiomes, imagined by Lexica