DTU

DTU Health Technology
Bioinformatics

Quantitative metagenomics

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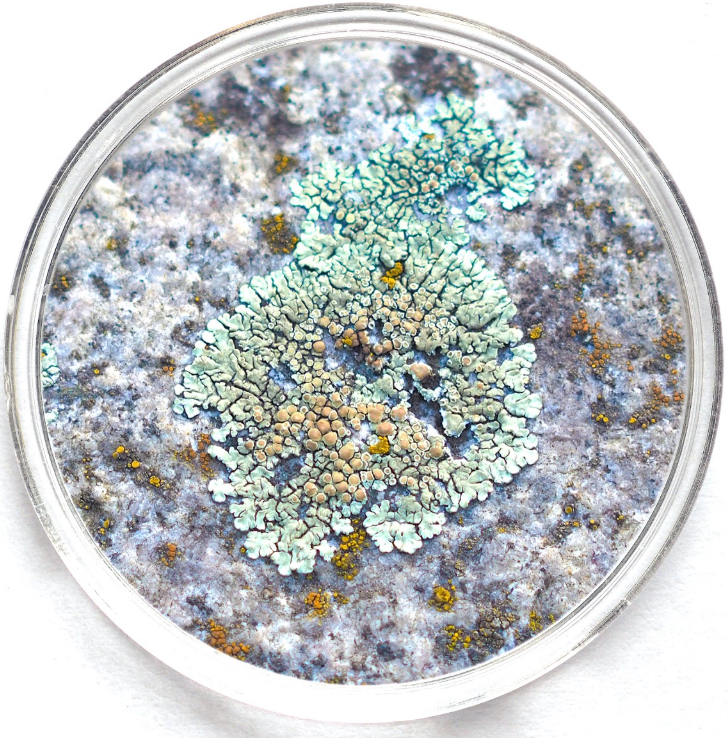
DTU Bioinformatics section

Slides adapted from Trine Zachariasen

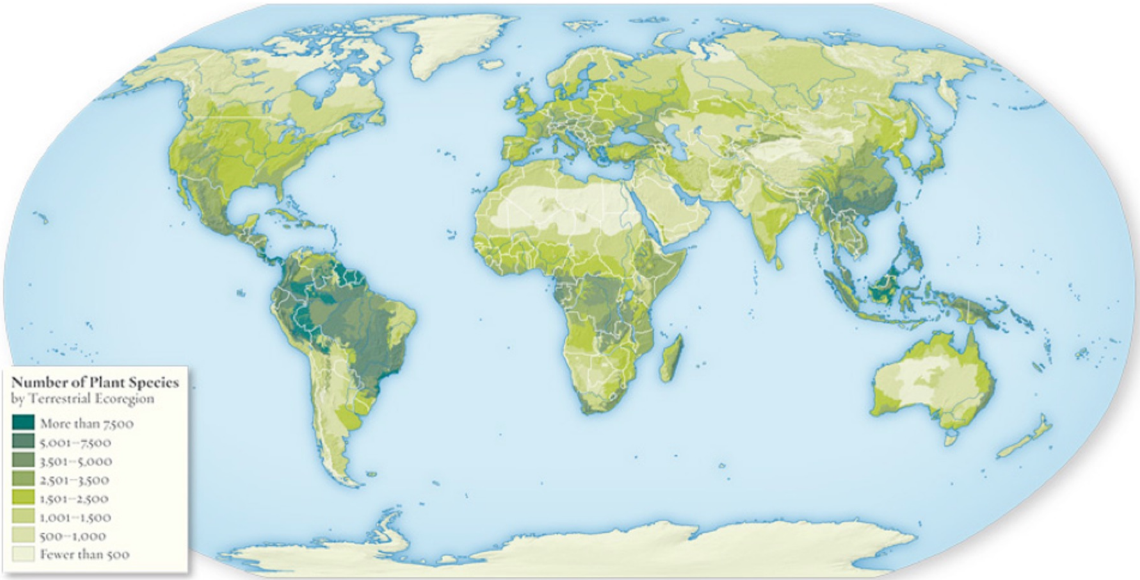
Menu

- Diversity measurements
  - Abundance
  - Alpha & beta diversity



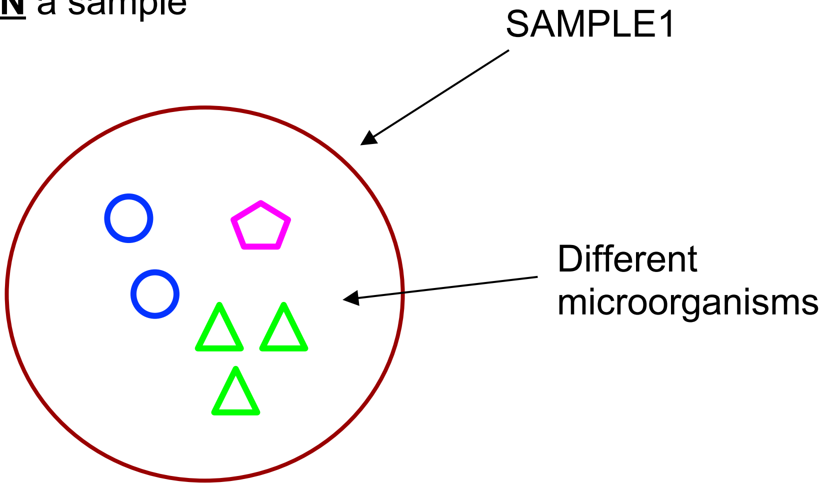
Classical measures of diversity

- Richness
- Rarefaction
- Diversity
  - Alpha
  - Beta



Describing biodiversity: Alpha-diversity

Describes the diversity **WITHIN** a sample



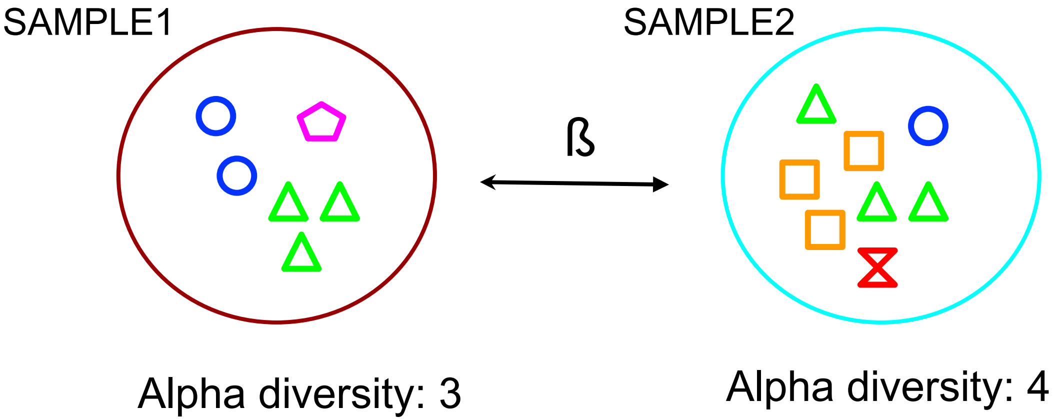
Alpha diversity: 3

Describing biodiversity: Beta-diversity

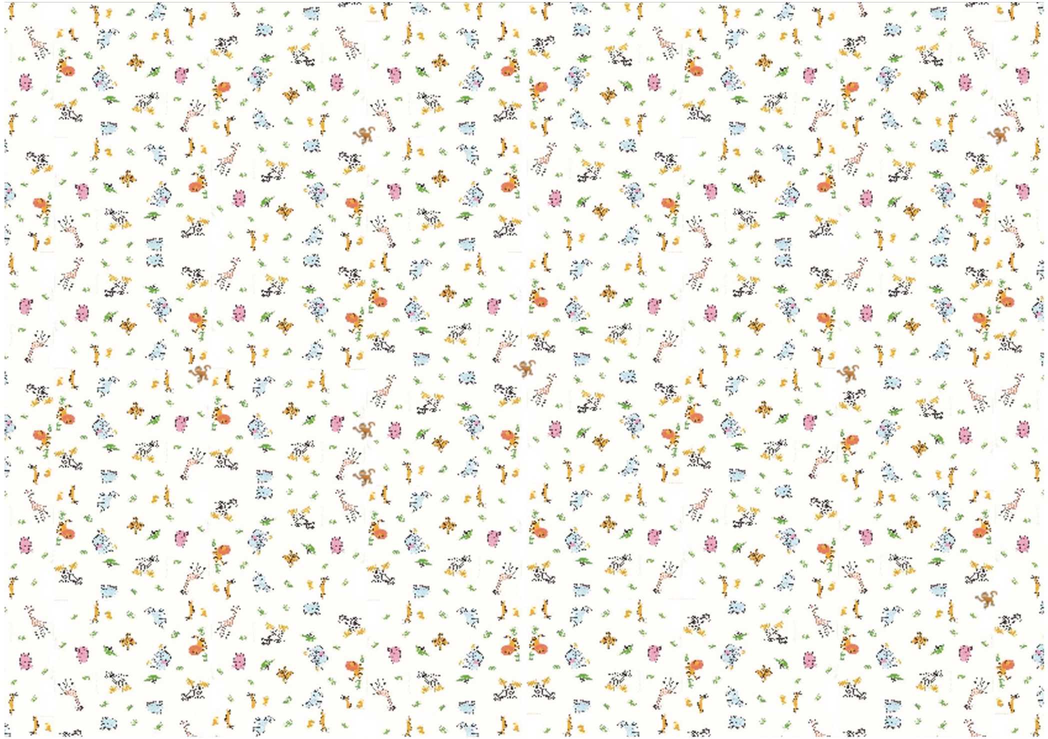
Describes the diversity **BETWEEN** samples,

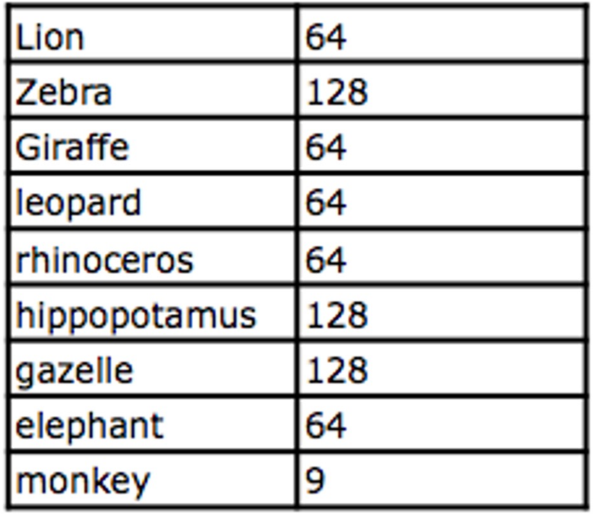
$$(\alpha_{Sample1} - c) + (\alpha_{Sample2} - c) = \beta$$

c = species in common



Abundance (counts)

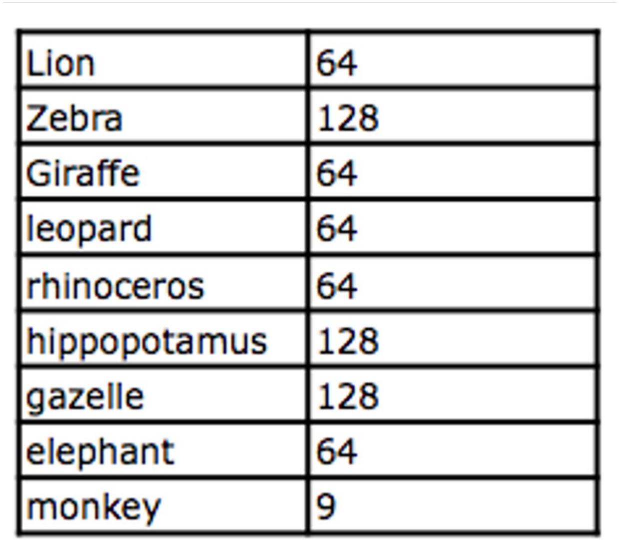




Lion	64
Zebra	128
Giraffe	64
leopard	64
rhinoceros	64
hippopotamus	128
gazelle	128
elephant	64
monkey	9

Species richness

- The number of different species in a system



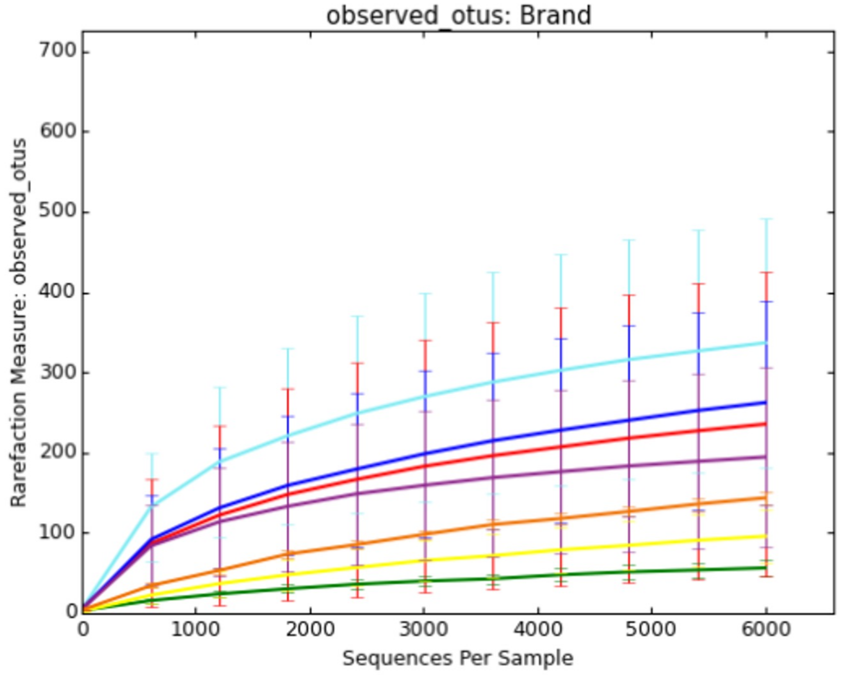
Lion	64
Zebra	128
Giraffe	64
leopard	64
rhinoceros	64
hippopotamus	128
gazelle	128
elephant	64
monkey	9

9 observed species

Rarefaction

- Species richness is a function of our no. observations
- When have we sampled enough?
- Mostly used for 16s rRNA amplicons…why?

Operational Taxonomic Unit



Shannon index

- Incorporates species richness & eveness
- Quantify the entropy (information content)
- Quantifies the uncertainty (degree of surprise)
- The Shannon index increases as both the richness and the evenness of the community increase
- Typical values are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4

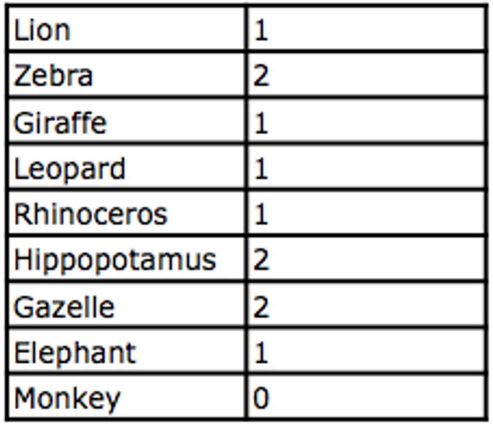
$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

$$H' = -(\ln p_1^{p1} + \ln p_2^{p2} + \ln p_3^{p3} + \cdots + \ln p_R^{pR})$$

P_i = species proportion

R = observed species = richness

Shannon index



Lion	1
Zebra	2
Giraffe	1
Leopard	1
Rhinoceros	1
Hippopotamus	2
Gazelle	2
Elephant	1
Monkey	0

$$H' = -(\ln p_1^{p1} + \ln p_2^{p2} + \ln p_3^{p3} + \cdots + \ln p_R^{pR})$$

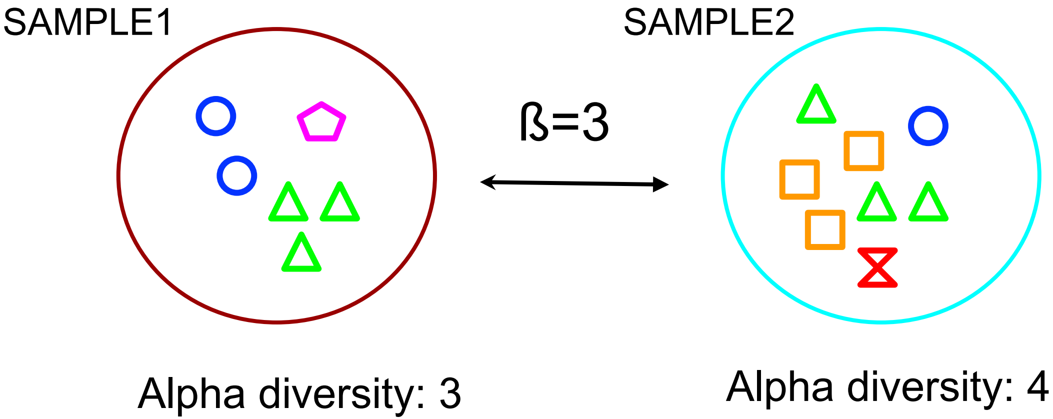
11 animals (NOT species) meaning each animal is 0.09 of the total abundance

$H' = -(\ln(0.09^{0.09}) + \ln(0.18^{0.18}) + \ldots = 2.0$

Describing biodiversity: Beta-diversity

$$(\alpha_{Sample1} - c) + (\alpha_{Sample2} - c) = \beta$$

(3-2) + (4-2) = 3



Bray-curtis dissimilarity

$0 \leq B \leq 1$

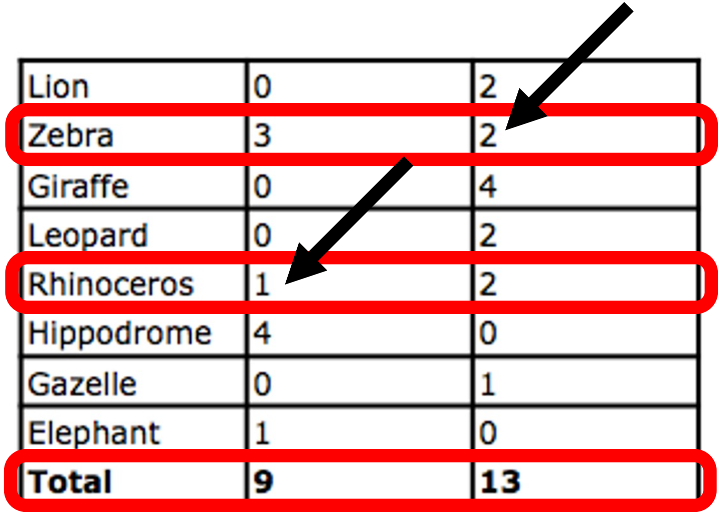
$B_{ij} = 1 - 2C_{ij} / (S_i + S_j)$

C = sum of the lowest count of all common species

S = total count of the sample

1 means that they do not share anything

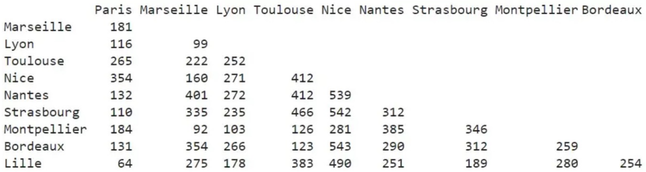
$B_{s1s2} = 1 - 2*(2+1) / (9 + 13) = 0.73$



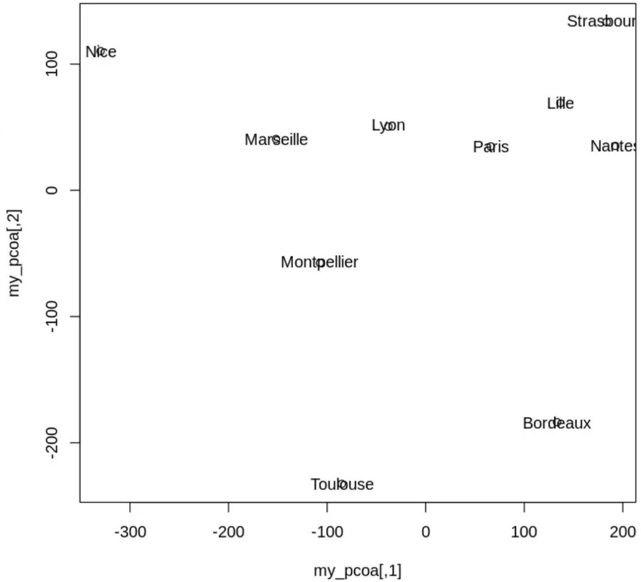
Lion	0	2
Zebra	3	2
Giraffe	0	4
Leopard	0	2
Rhinoceros	1	2
Hippodrome	4	0
Gazelle	0	1
Elephant	1	0
Total	**9**	**13**

PCoA analysis

Lille
Paris
Strasbourg
Nantes
Lyon
Bordeaux
Nice
Montpellier
Toulouse
Marseille



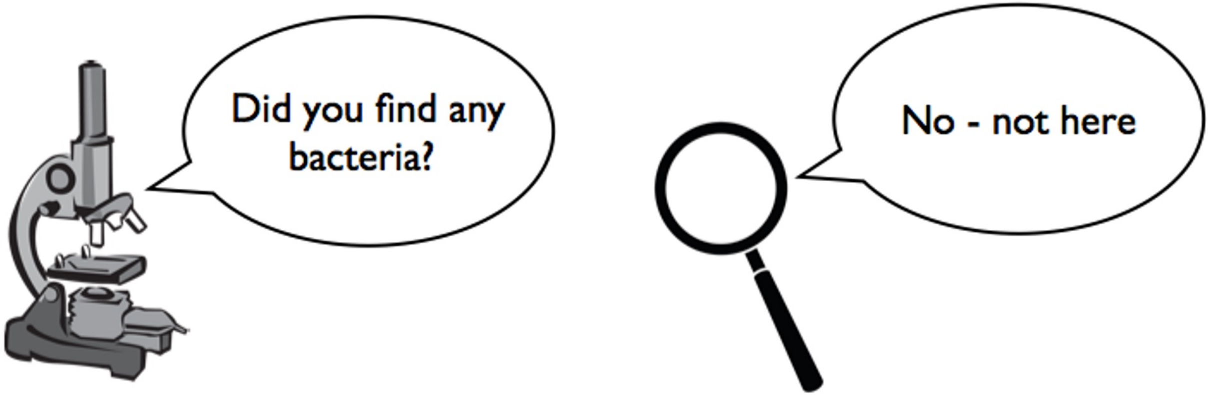
```
            Paris Marseille Lyon Toulouse Nice Nantes Strasbourg Montpellier Bordeaux
Marseille     181
Lyon          116        99
Toulouse      265       222  252
Nice          354       160  271      412
Nantes        132       401  272      412  539
Strasbourg    110       335  235      466  542    312
Montpellier   184        92  103      126  281    385        346
Bordeaux      131       354  266      123  543    290        312         259
Lille          64       275  178      383  490    251        189         280      254
```



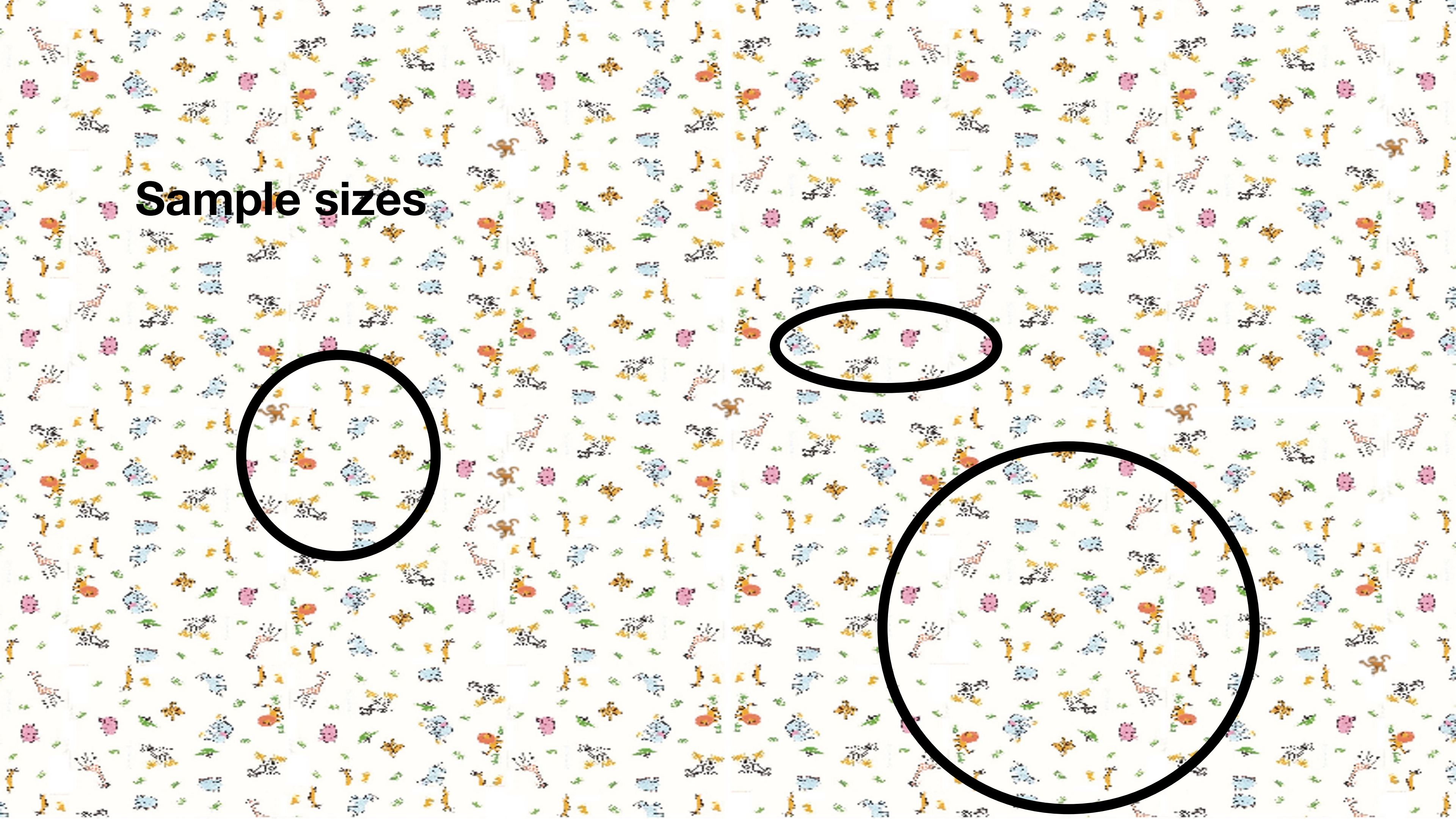
https://towardsdatascience.com/principal-coordinates-analysis-cc9a572ce6c

Sampling effect

- To be fair we should sample equally in the systems we investigate



Sample sizes

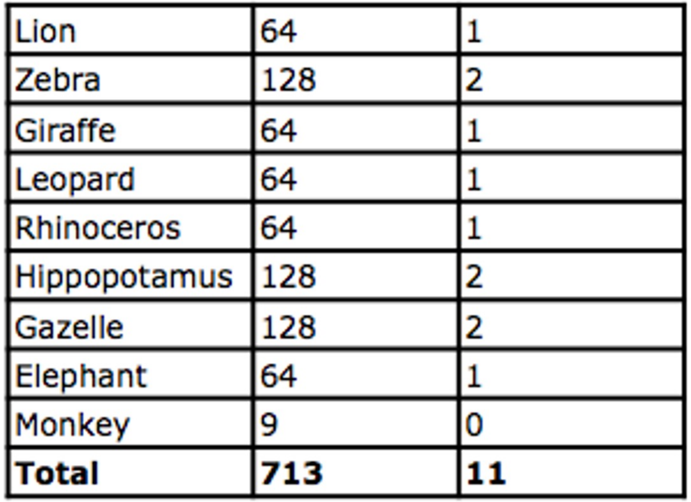


Sample sizes

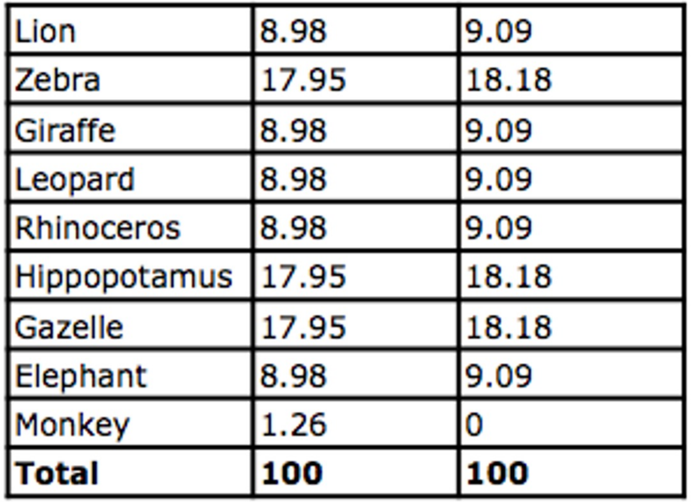
- Accounting for different sample sizes:
  - –Normalize to sample size
  - –Rarefy (downsize) samples
  - –Statistically model the variance

Normalizing

$$N = n_i/n_{tot}$$



Lion	64	1
Zebra	128	2
Giraffe	64	1
Leopard	64	1
Rhinoceros	64	1
Hippopotamus	128	2
Gazelle	128	2
Elephant	64	1
Monkey	9	0
Total	**713**	**11**

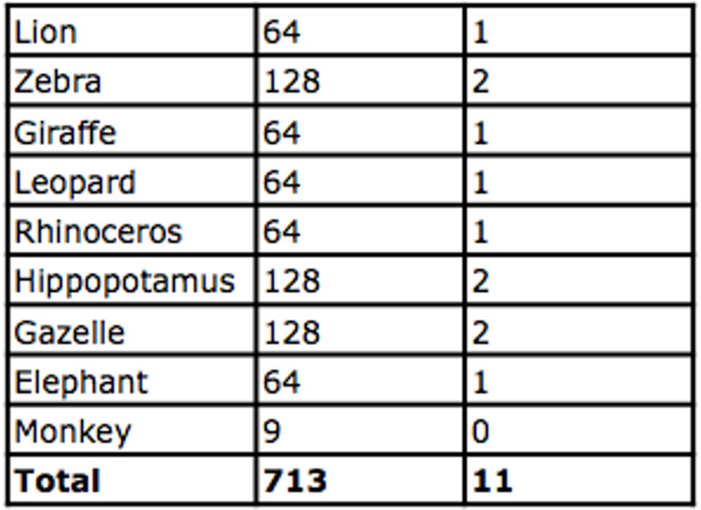


Lion	8.98	9.09
Zebra	17.95	18.18
Giraffe	8.98	9.09
Leopard	8.98	9.09
Rhinoceros	8.98	9.09
Hippopotamus	17.95	18.18
Gazelle	17.95	18.18
Elephant	8.98	9.09
Monkey	1.26	0
Total	**100**	**100**

Issue with different sampling power (higher chance of observing rare species) and does not take compositional nature into account

Downsize / rarefy

Resample x amount of observations



Lion	64	1
Zebra	128	2
Giraffe	64	1
Leopard	64	1
Rhinoceros	64	1
Hippopotamus	128	2
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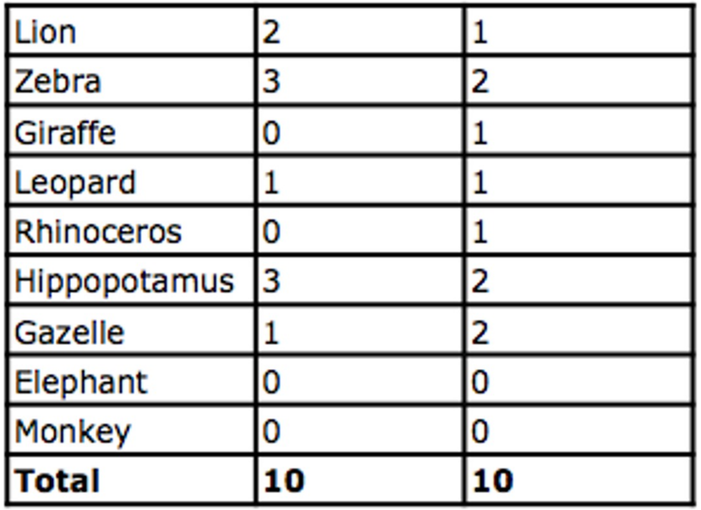
Elephant	64	1
Monkey	9	0
Total	**713**	**11**



Lion	2	1
Zebra	3	2
Giraffe	0	1
Leopard	1	1
Rhinoceros	0	1
Hippopotamus	3	2
Gazelle	1	2
Elephant	0	0
Monkey	0	0
Total	**10**	**10**

Downsize / rarefy

- Select the target depth carefully
- The more reads we keep the more sensitive
- We may have to remove samples with few counts
- We might throw away a lot of data
- Still does not take compositional nature of data into account

Compositional data

- Arbitrary total
  - Sequencing depth never 100%
- Species can co-exist without abundance inter-influences
  - Independence between abundance is affected by the capacity of the sequencing instrument
  - Sequencing instrument has fixed number of slots

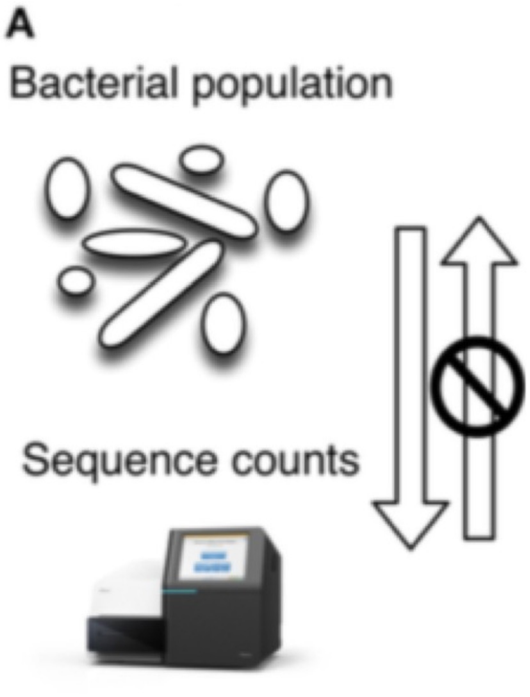
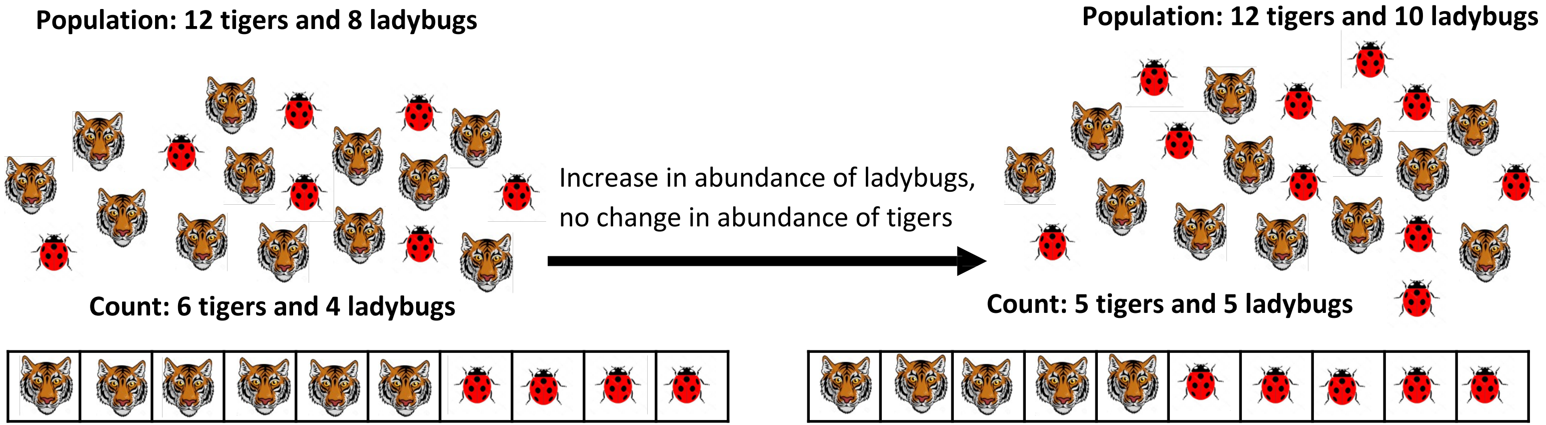


Figure from: Gloor, Gregory B. *et al.*, Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in Microbiology* **8** (2017)

Compositional data problems

- **Example**: an environment containing both tigers and ladybugs
  - The abundances of the two are not affected by each other
  - If the abundance of the ladybugs increases some of the slots with tigers must instead be filled by ladybugs
  - i.e. the two environmentally independent species are affecting the read count of each other



Population: 12 tigers and 8 ladybugs

Count: 6 tigers and 4 ladybugs

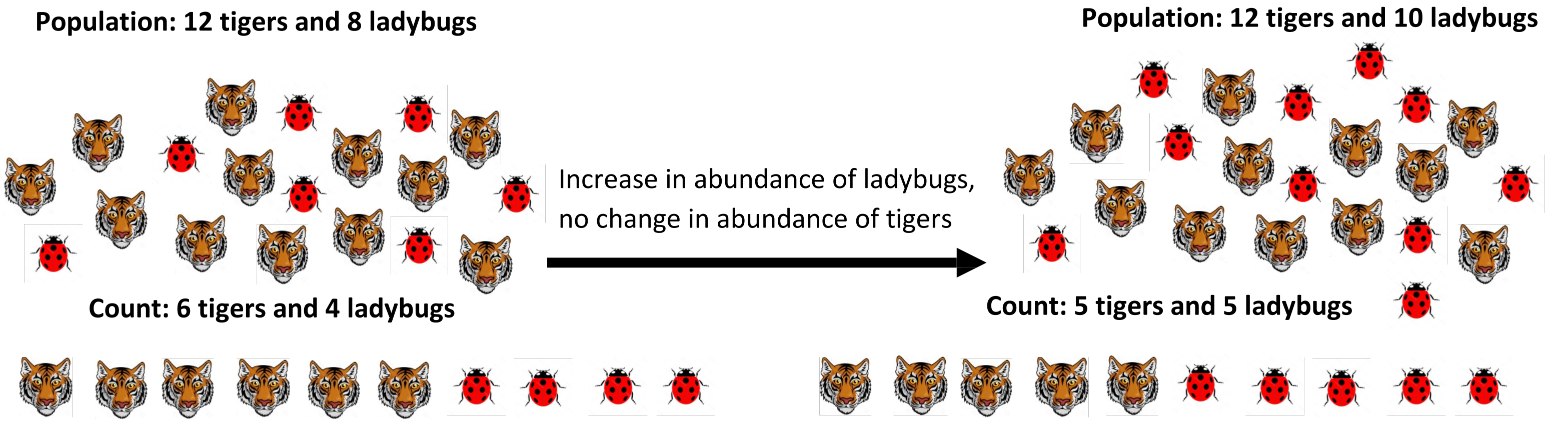
Increase in abundance of ladybugs, no change in abundance of tigers

Population: 12 tigers and 10 ladybugs

Count: 5 tigers and 5 ladybugs

Relative abundance

- The counts we get is not the absolute abundance, but their proportions relative to each other



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Count: 6 tigers and 4 ladybugs

Increase in abundance of ladybugs, no change in abundance of tigers

Population: 12 tigers and 10 ladybugs

Count: 5 tigers and 5 ladybugs

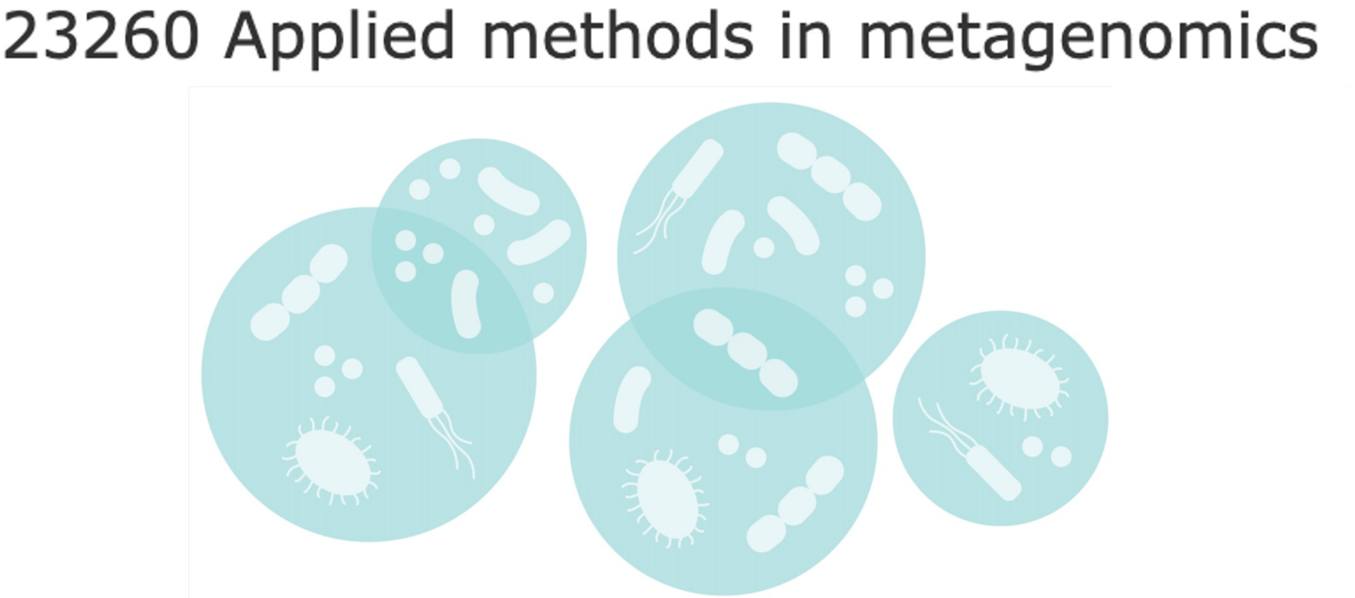
Dealing with compositional data

- Statistically model the variance & heteroscedasticity

- Use packages developed for RNA-seq such as DESeq2 and edgeR

- DESeq2 takes raw counts divided by sample-specific size factors determined by median ratio of gene counts relative to geometric mean per gene
(See this link for a brilliant explanation)

If you found it interesting check out the course at DTU Food



23260 Applied methods in metagenomics