



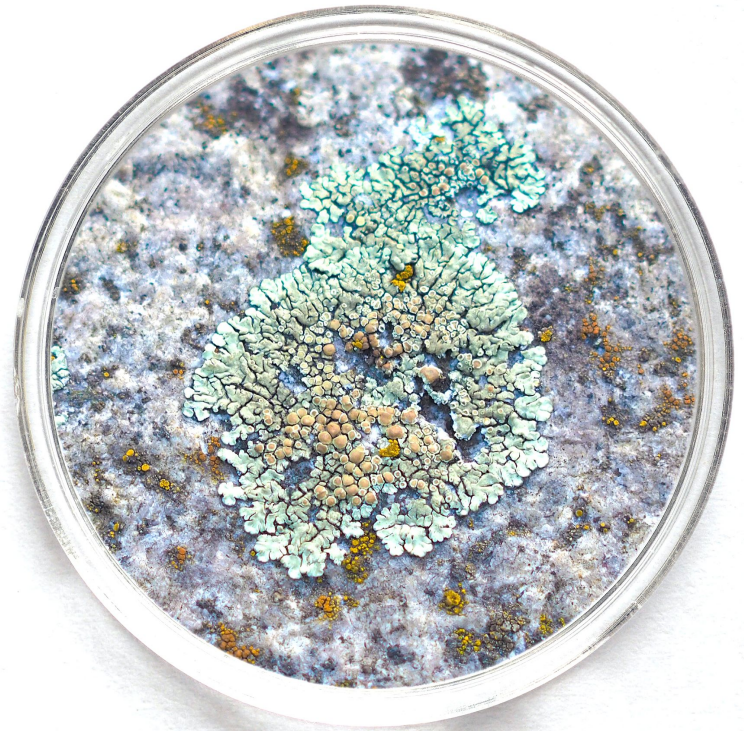
DTU Health Technology
Bioinformatics

Quantitative metagenomics

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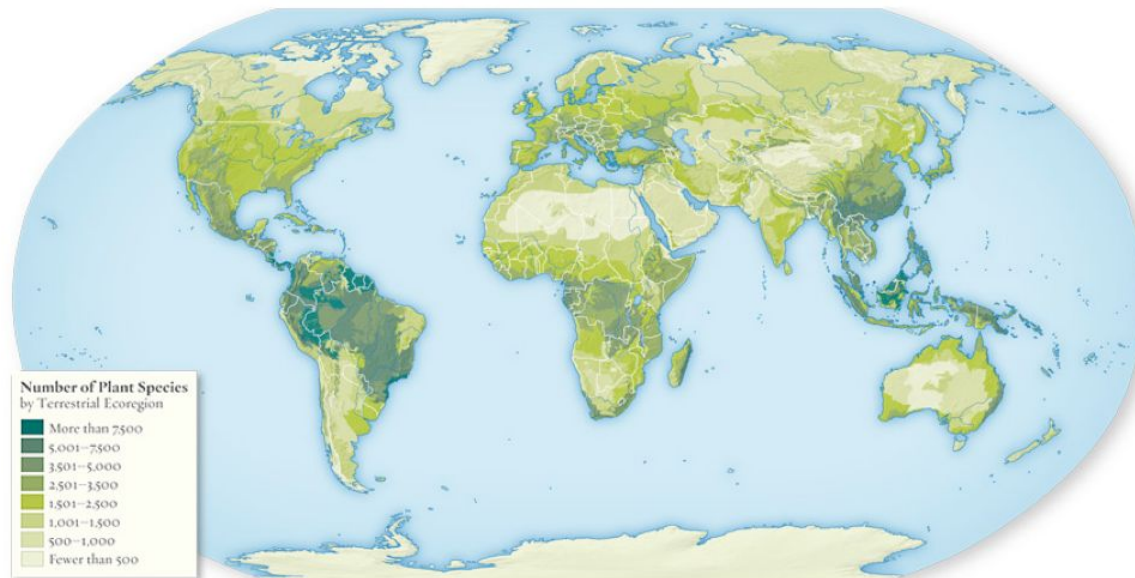
Menu

- Diversity measurements
 - Abundance
 - Alpha & beta diversity



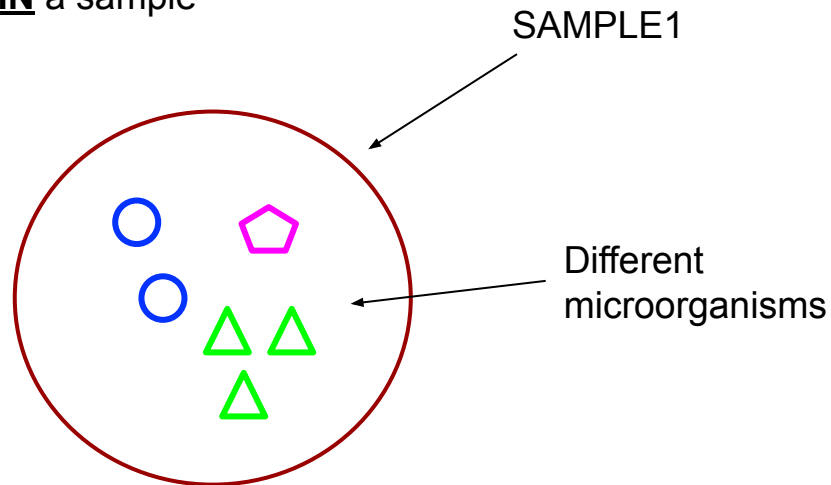
Classical measures of diversity

- Richness
- Rarefaction
- Diversity
 - Alpha
 - Beta



Describing biodiversity: Alpha-diversity

Describes the diversity WITHIN a sample



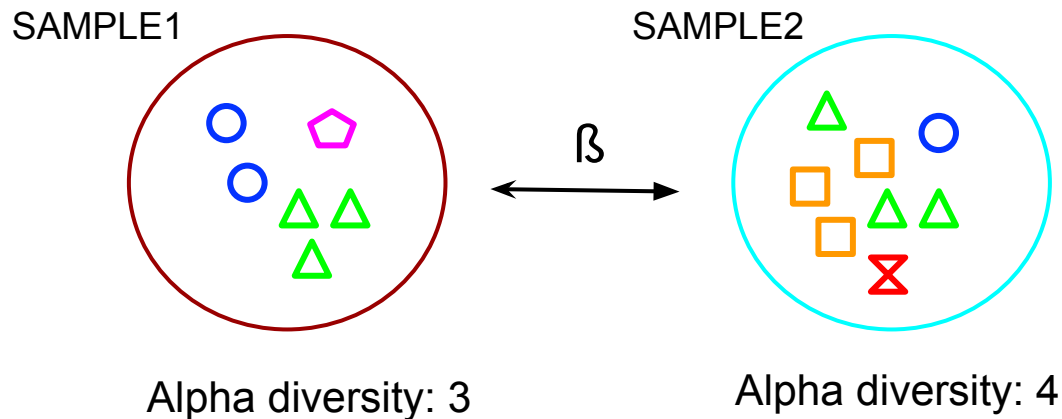
Alpha diversity: 3

Describing biodiversity: Beta-diversity

Describes the diversity **BETWEEN** samples,

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

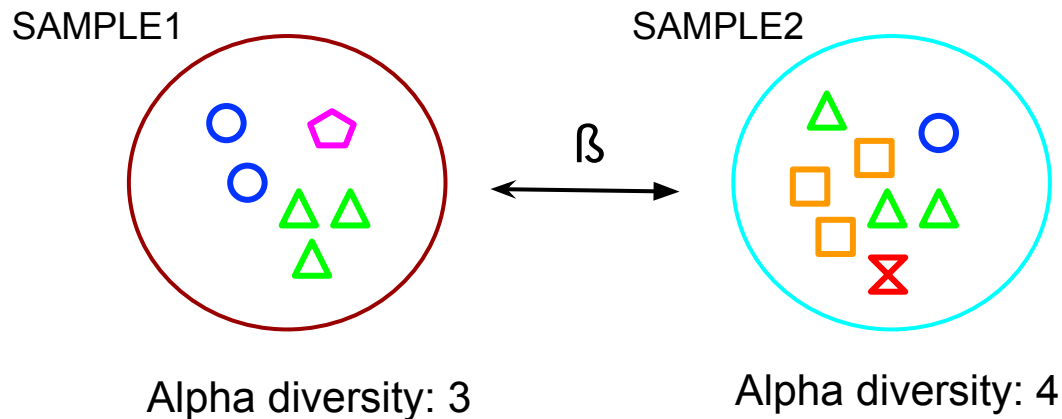
c = species in common



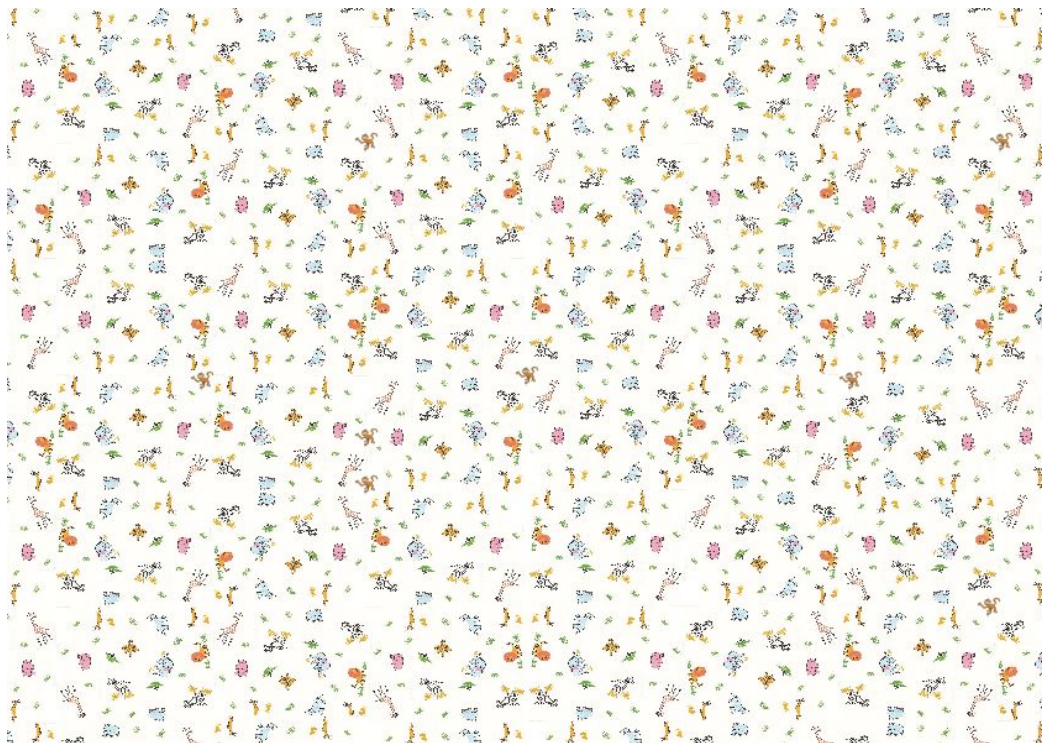
Describing biodiversity: Beta-diversity

$$(\alpha_{\text{sample1}} - c) + (\alpha_{\text{sample2}} - c) = \beta$$

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



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

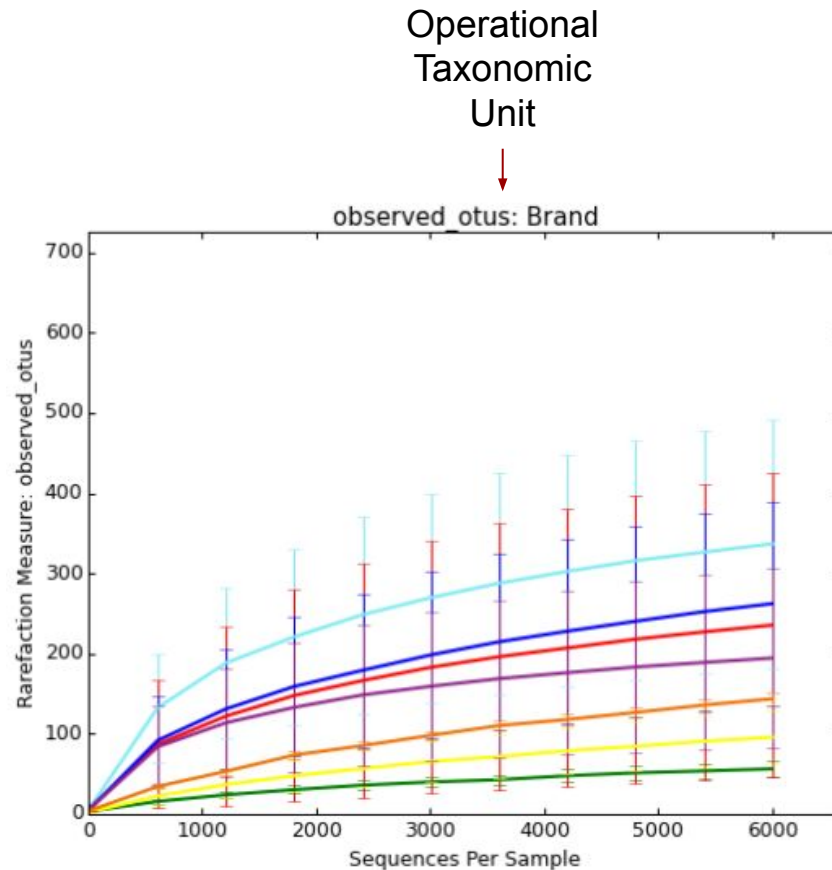
Lion	64
Zebra	128
Giraffe	64
leopard	64
rhinoceros	64
hippopotamus	128
gazelle	128
elephant	64
monkey	9



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?



Shannon index

- Incorporates species richness & evenness
- 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

$$H' = - \sum_{i=1}^R p_i \ln p_i \qquad H' = -(\ln p_1^{p_1} + \ln p_2^{p_2} + \ln p_3^{p_3} + \dots + \ln p_R^{p_R})$$

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

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

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

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

Bray-curtis dissimilarity

$$0 \leq B \leq 1$$

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

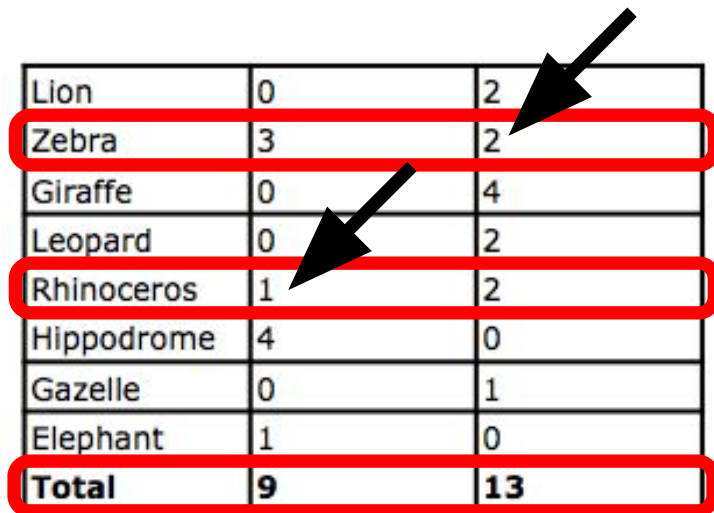
C = sum of the lowest count of all common species

S = total count of the sample

1 means that they do not share anything

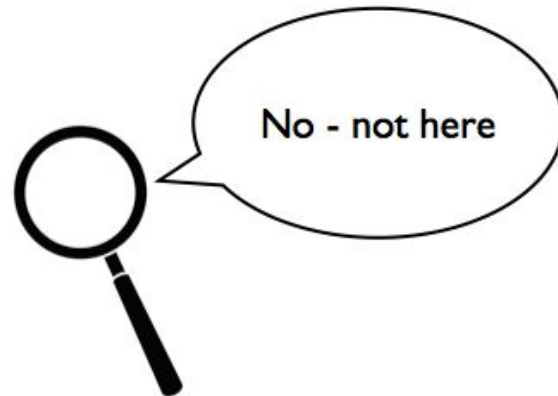
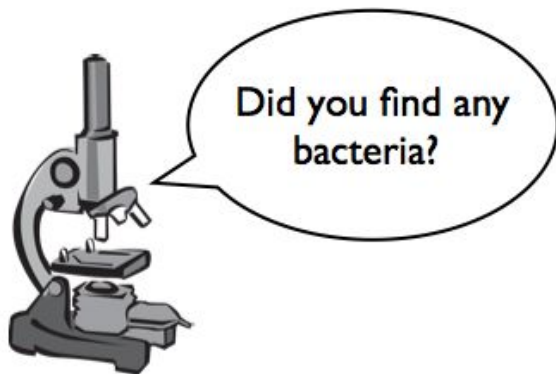
$$B_{s_1s_2} = 1 - 2*(2+1) / (9 + 13) = 0.73$$

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

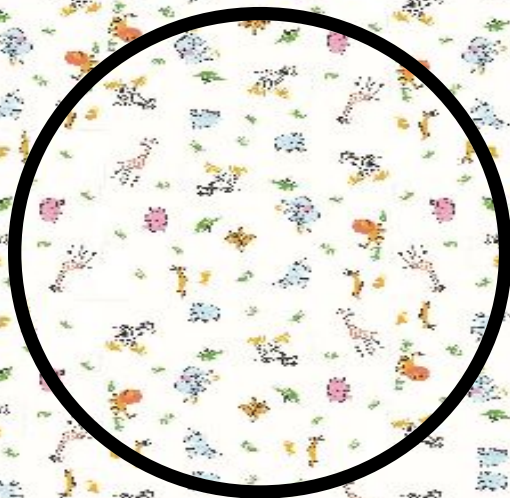


Sampling effect

- 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

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

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
Gazelle	128	2
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%
- Independence between abundance is affected by the capacity of the sequencing instrument
- Sequencing instrument has fixed number of slots

Bacterial population



Sequence counts



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

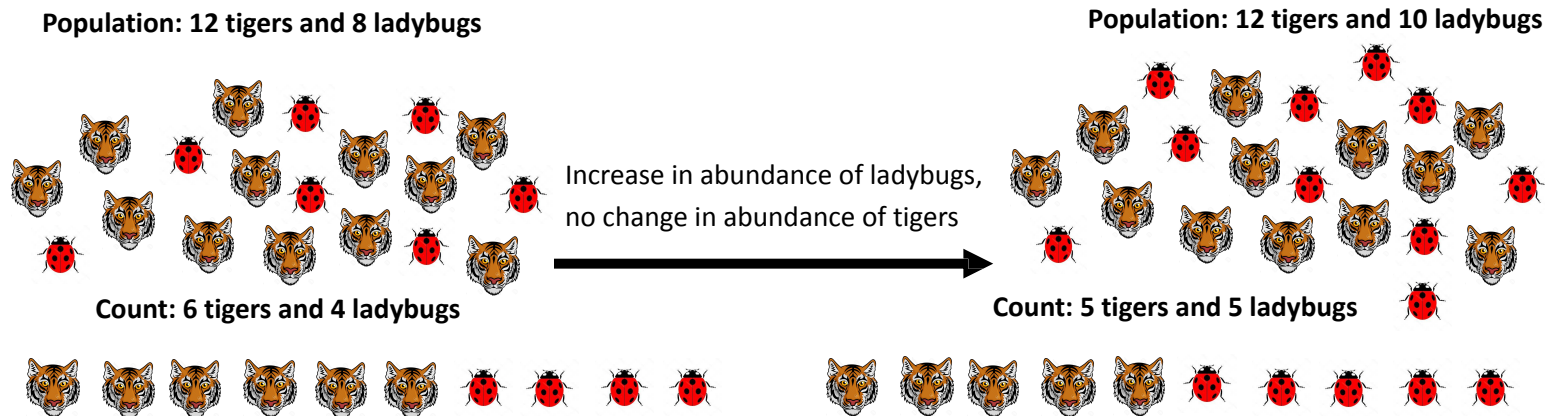


Count: 5 tigers and 5 ladybugs



Relative abundance

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



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

