Microbiome diversity analysis

Analysis of alpha and beta diversity using phyloseq R package

2024.04.24. Phytobiome symposium

Session 2
Dong-A University Master course
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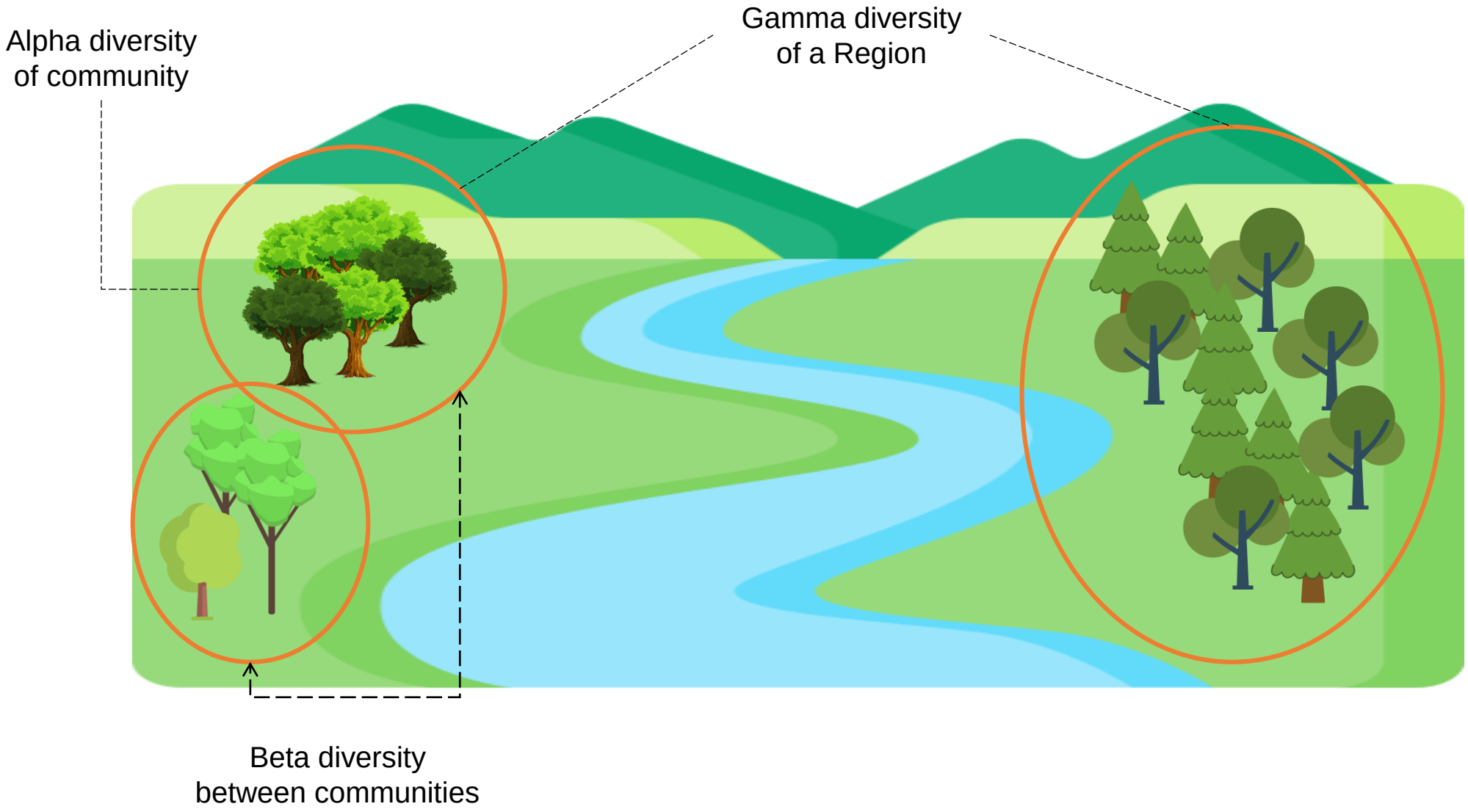
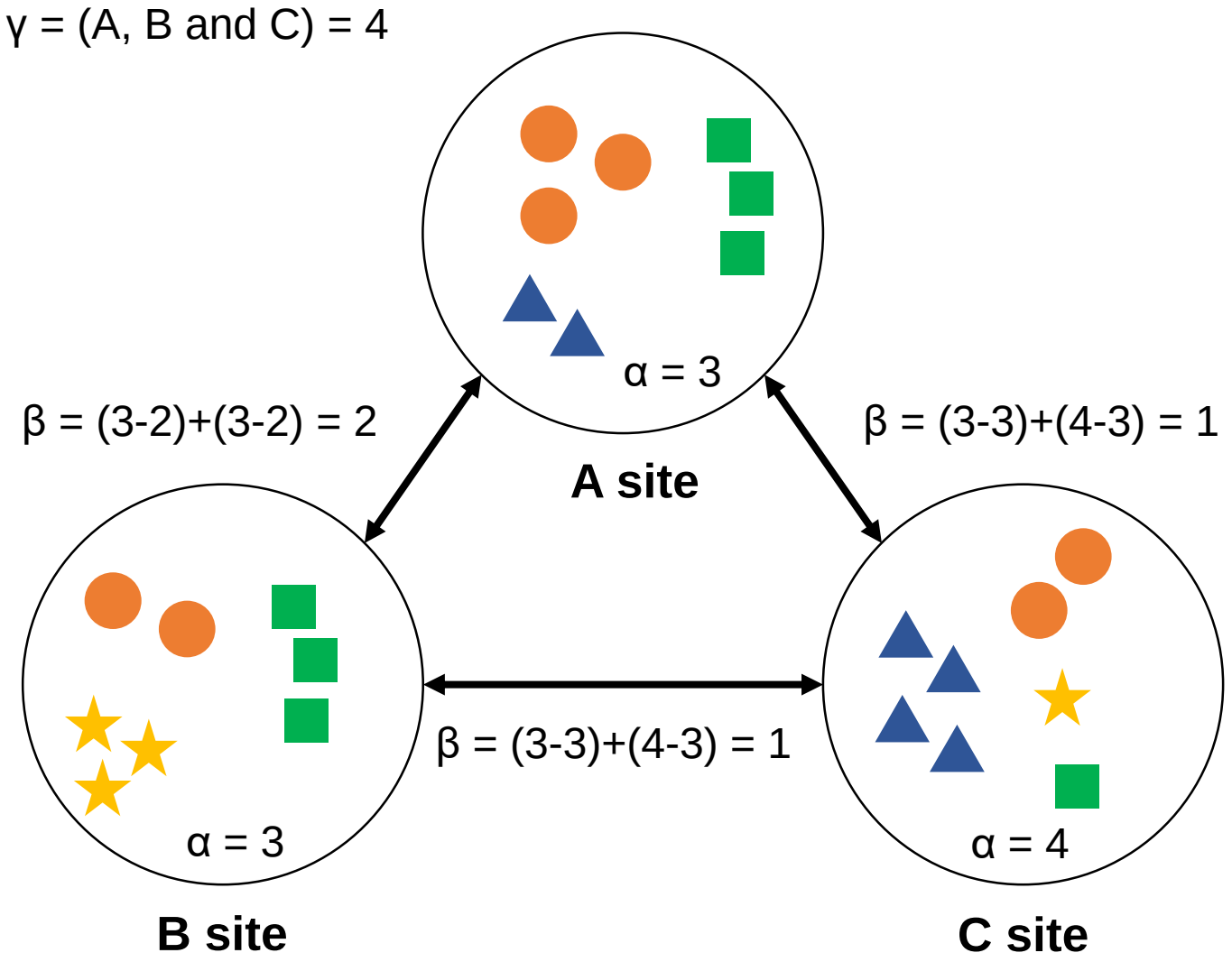
1. Diversity


Alpha diversity
of community
Gamma diversity
of a Region
Beta diversity
between communities

1. Diversity



2. Alpha diversity

- **Within-sample diversity**
- **Two components of alpha diversity**
  - Richness (number of taxonomic groups)
  - Evenness (distribution of abundances of the groups)

2-1. Observed OTU or Taxa (Richness)

- Count of different species/OTUs

2-2. Shannon's diversity Index

- p_i: ratio of i species, S: number of speices in a sample
- Consider both species richness and evenness
- Associated with entropy concept (Shannon diversity)
- Quantify the uncertainty in predicting the taxa identity of an individual selected at random from the sample

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

2. Alpha diversity

2-3. Simpson's index

$$D = \sum_{i=1}^{s} {p_i}^2$$

$$\boldsymbol{Gini\text{–}Simpson} = 1 - D$$

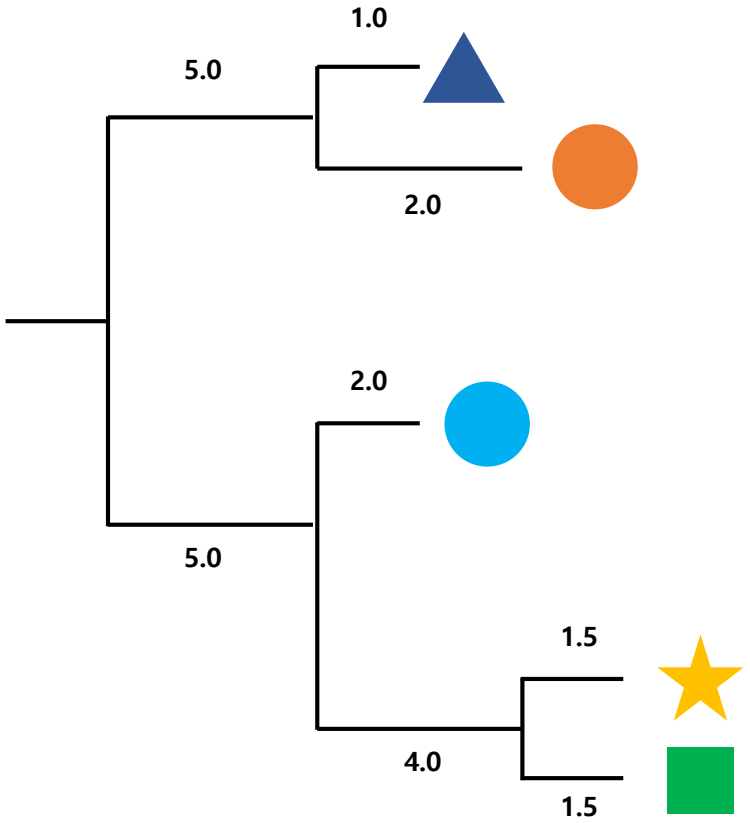
$$\boldsymbol{Inverse\ Simpson} = \frac{1}{D}$$

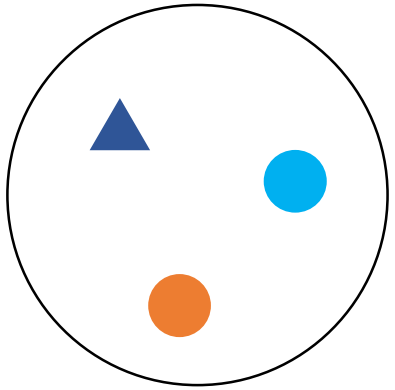
- p_i: ratio of i species
- Probability that two individuals belong to the same taxa randomly chosen
- Higher D value suggest that community has low diversity
- More sensitive to evenness than richness
- So called, Simpson's evenness index.

2. Alpha diversity

2-4. Faith's phylogenetic diversity

- Sum of branch length between observed species in the phylogenetic tree

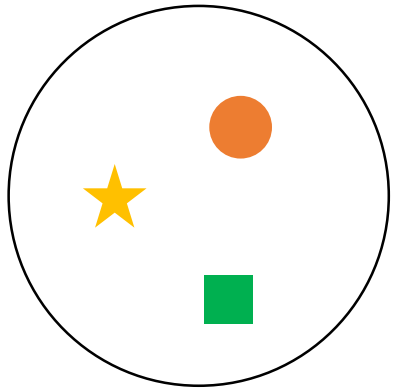




Faith's PD

= 5 + 1 + 2 +7

= 15



Faith's PD

= 5 + 2 + 5 + 4 + 1.5 + 1.5

= 19

3. Rarefaction

- Low sampling depth vs High sampling depth
- Briefly, it is unfair
- Amplicon sequencing data is not total DNA sequence data
- Randomly choose observations without replacement from our samples up to a specific depth

	Sample 1	Sample 2
Species 1	148	36
Species 2	53	10
Species 3	2	4
Species 4	48	24
Species 5	6	3
Total	257	77

Rarefaction

	Sample 1	Sample 2
Species 1	34	36
Species 2	23	10
Species 3	1	4
Species 4	18	24
Species 5	1	3
Total	77	77

Is it fair to compare samples with different sequencing depth?

Can say that the Species 5 exists more in Sample 1 than in Sample 2?

4. Beta diversity

- **Between-sample diversity**
- **How different is it between different communities**

4-1. Bray-Curtis Dissimilarity

$$\boldsymbol{BC} = 1 - \frac{2C_{ij}}{S_i + S_j}$$

- Measure the compositional dissimilarity between the communities of two samples
- C_{ij} is sum of the lesser values for given taxa in common between sample i and j
- S_i and S_j are the total number of taxa counted in i and j, respectively
- Ranges between 0 (two samples share all taxa) and 1 (two samples do not share any taxa)
- Computed pairwise between all samples

4. Beta diversity

4-2. Jaccard distance

- Based on presence or absence of species
- Ratio between the number of members that are common between the two samples and the number of members that are distinct
- Ranges between 0 (the communities are identical) and 1 (the two communities are different)

$$\boldsymbol{Jaccard\ coefficient} \quad J(i,j) = \frac{|i \cap j|}{|i \cup j|}$$

$$\boldsymbol{Jaccard\ distance} \quad Jd(i,j) = 1 - J(i,j)$$

4. Beta diversity

4-3. UniFrac distance

- Based on phylogenetic tree, UniFrac distance is calculated as sum of the branch length
- Measure community dissimilarity based on the presence or absence of branch

  -> **Unweighted UniFrac**

$$UU(A,B) = \frac{sum\ of\ unique\ branch\ length}{sum\ of\ observed\ branch\ length} = fraction\ of\ total\ unshared\ branch\ lengths$$

- Consider the abundance of sequences

  -> **Weighted UniFrac**

$$WU(A,B) = \sum_{i}^{n} b_i * \left|\frac{A_i}{A_T} - \frac{B_i}{B_T}\right|$$

- n is the total number of branches in the tree
- b_i is the length of branch i
- A_i and B_i are the numbers of sequences that descend from branch i in A and B
- A_T and B_T are the total numbers of sequences in A and B

4. Beta diversity

4-4. Summary

	Consider abundance	**Not consider abundance (Presence/Absence)**
Not consider phylogenetic relationship	Bray-Curtis	Jaccard
Consider phylogenetic relationship	Weighted UniFrac	Unweighted UniFrac

5. Ordination

- Statistical method for transforming multidimensional data into a concise and interpretable form for visualization and analysis purposes
- Represent sample and species relationships in a low-dimensional space

5. Ordination

Distance-based approach

- Rely on a square, symmetric distance matrix or similarity matrix

• PCoA (Principal Coordinates Analysis)

- To describe the data by reducing the dimensions of a distant matrix among objects
- Maximize linear correlation distance measures and distance in the ordination
- Cannot indicate combinations of variables

• NMDS (Nonmetric Multidimensional Scaling)

- To describe data by reducing the number of dimensions
- To discover nonlinear relationships
- Maximizing the rank-order correlation between distance measures and distance in ordination space
- Points are moved to minimize: stress (a measure of the mismatch between the two kinds of distance)
- To increase the likelihood of finding the correct solution

Thank you !

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