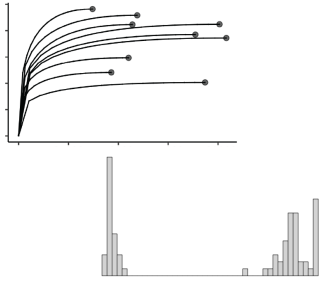
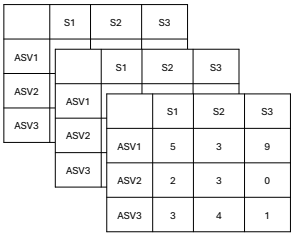
1. Assess sequencing depth



- Examine distributions of sequencing depth
- Use rarefaction curves to estimate saturation
- Remove samples with too few reads (this will be dependent on richness)

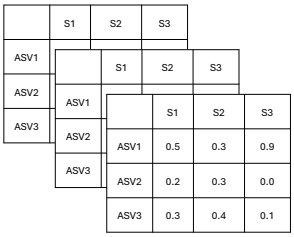
2. Rarefy to lowest sample **reads** (many iterations)



x100

- Repeatedly subsample ASV abundance matrix to an even sequencing depth
- Use 100-1000 iterations

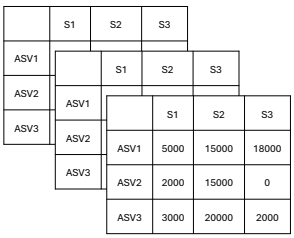
3. Convert to relative abundance



x100

- Divide the abundance of each ASV by the normalized sequencing depth

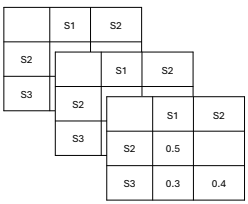
4. Convert to absolute abundance



x100

- Multiple the relative abundance of each ASV by the total cell count or 16S copy number of each sample

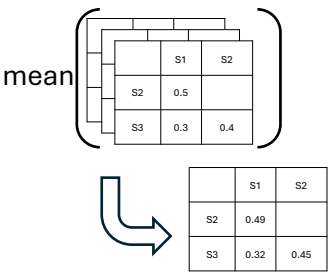
5. Calculate distance or dissimilarity



x100

- Repeatedly calculate distance or dissimilarity metric across all iterations of rarefied ASV abundance matrices

6. Average distances or dissimilarities



- Average distance/dissimilarity matrices across all iterations of rarefaction