

Comparison of multivariate power and sample-size estimation approaches using different distance measures on 16S rRNA gene amplicon sequencing based on chicken gut data

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The aim of this study was to show how, and to what extent, different sample-sizes influence the alpha and beta diversity metrics for gut microbiota studies.

Methods

- Broiler chickens were raised in three different experimental units
- Within each unit 35 broilers were sacrificed of feed treatment A or B
- 16S ribosomal RNA gene amplicon sequencing was performed on cecal content
- NG-Tax was assessed to identify operational taxonomic units (OTU) [1]
- In total three data sets, set 1: 1011 OTU, set 2: 743 OTU, set 3: 753 OTU
- Power was calculated as the fraction of time the null hypothesis was rejected over 100 or 1000 repetition of the test (threshold $p < 0.01$)

Results

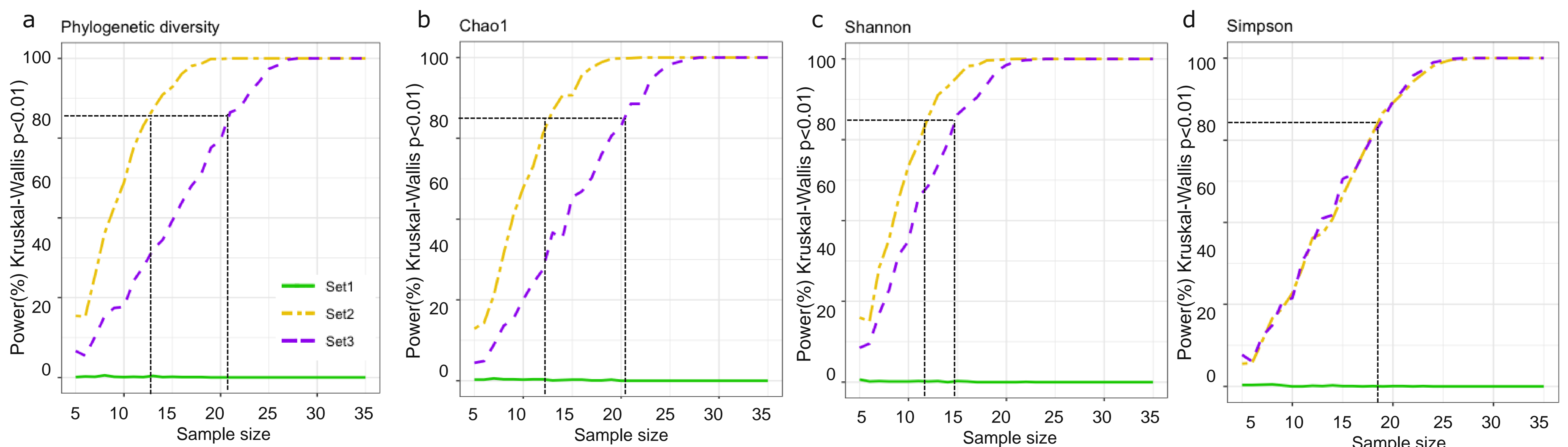


Figure 1: Across different alpha diversity metrics' data set 2 is most sensitive to observe the difference between the groups based on their microbial communities ($n=1000$ repetitions, power 0.80). **a, b.** Phylogenetic diversity and Chao1 showed comparable sensitivity, and a sample size of 13 or 20 individuals per group. **c.** Shannon index showed the smallest sample size needed to observed difference between the microbial communities. **d.** Simpson index showed no difference in samples size between data sets.

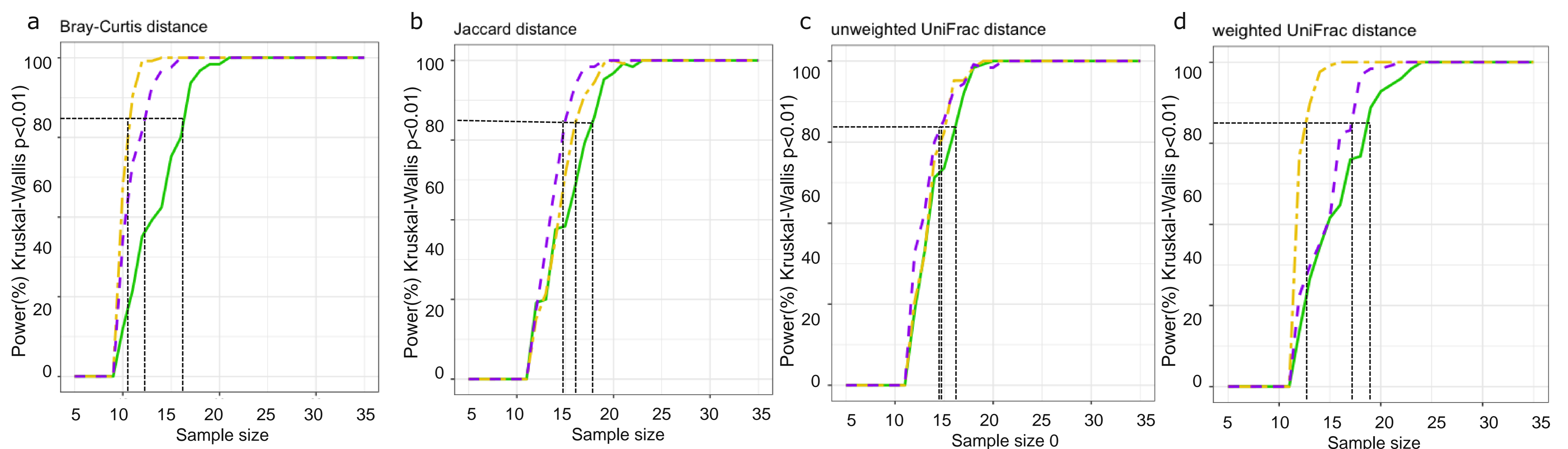


Figure 2: Different beta diversity metrics to estimate different sample sizes ($n=100$ repetitions). **a.** Bray-Curtis distance metrics is most sensitive to observe difference between groups (16 samples needed). **b.** Jaccard distance is the only metrics that showed that data set 3 needed the smallest sample size, indicating that between the groups in data set 3 specific OTU are absent between the groups. **c, d.** Weighted UniFrac is more sensitive compared to unweighted UniFrac to observe a difference between the groups based on their microbial communities.

Conclusions and future research

- Based on this data set we observed that the phylogenetic diversity, chao1 and bray-curtis metrics are most sensitive to observe difference between groups, resulting in a lower sample size.
- Future research will have to investigate different data sets in further detail, and on different taxonomic levels, to observe if the trends observed can be generalized.