BioMed Central

Microbiome

Normalization and microbial differential abundance strategies depend upon data characteristics

Sophie Weiss, Zhenjiang Zech Xu, Shyamal Peddada, Amnon Amir, Kyle Bittinger, Antonio Gonzalez, Catherine Lozupone, Jesse R. Zaneveld, Yoshiki Vázquez-Baeza, Amanda Birmingham, Embriette R. Hyde and Rob Knight

Microbiome 2017 5:27 | https://doi.org/10.1186/s40168-017-0237-y

Received: 9 October 2015 | Accepted: 27 January 2017 | Published: 3 March 2017

1. Effect of normalization method and uneven library size on beta diversity

2. Effect of normalization, uneven library size, and differential abundance test on differential abundance results

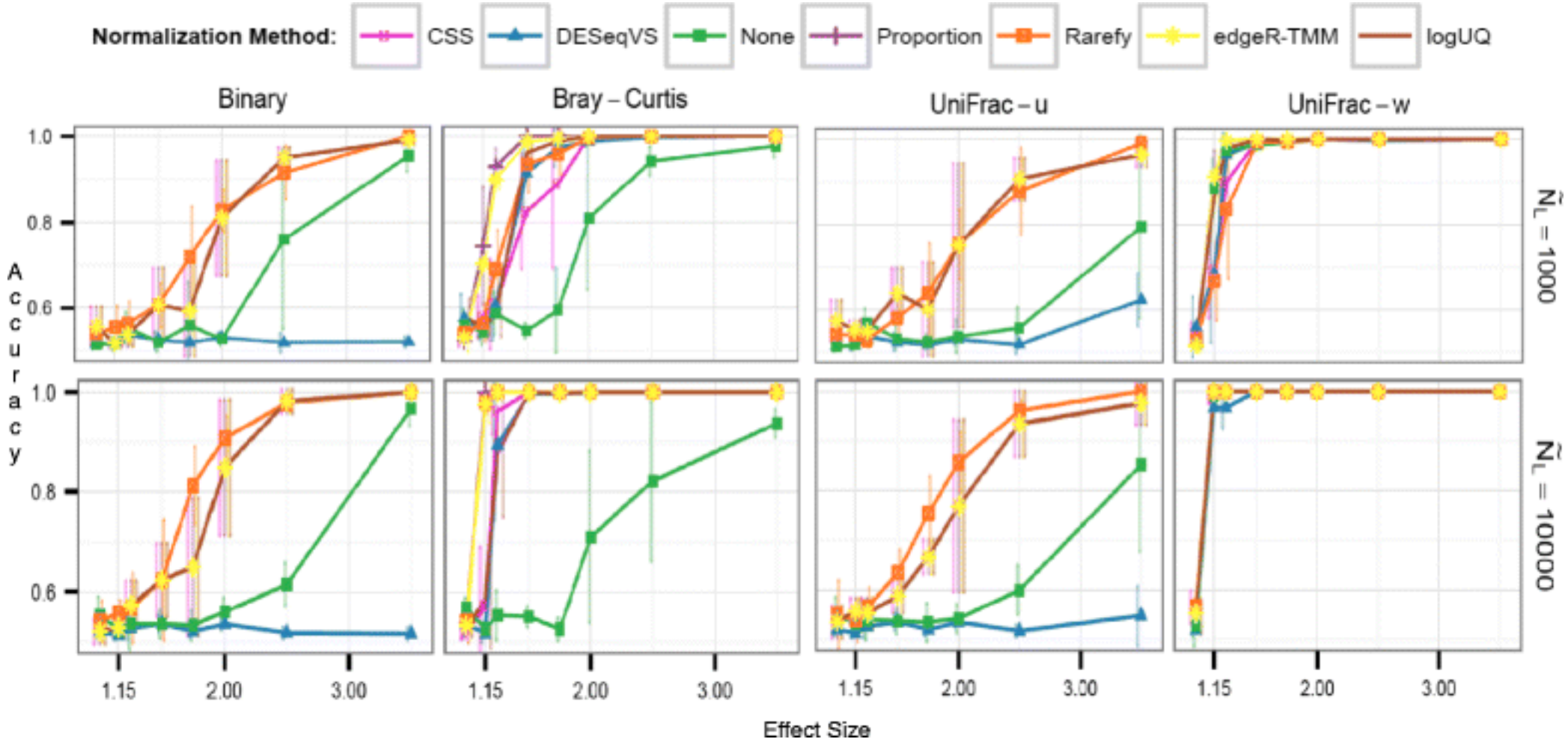
---

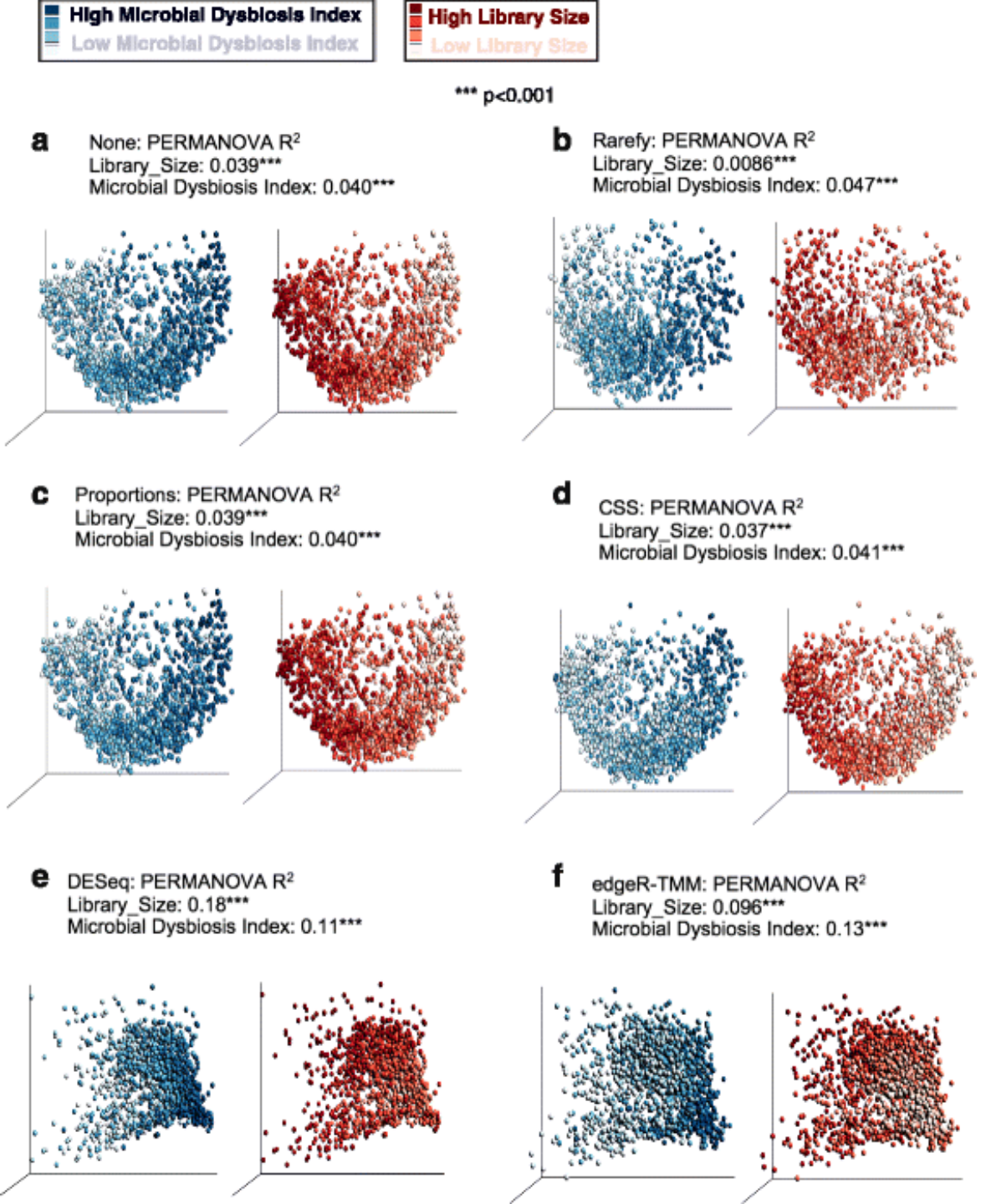
tl;dr: if your library sizes are super uneven, you should probably do something about it.

Weighted Unifrac is good no matter what you do.

Differential abundance methods are all not that great?

Weighted Unifrac is the best beta diversity metric.
For unweighted metrics, DESeqVS and “No normalization” are not great.





Rarefying reduces the effect of library size best.

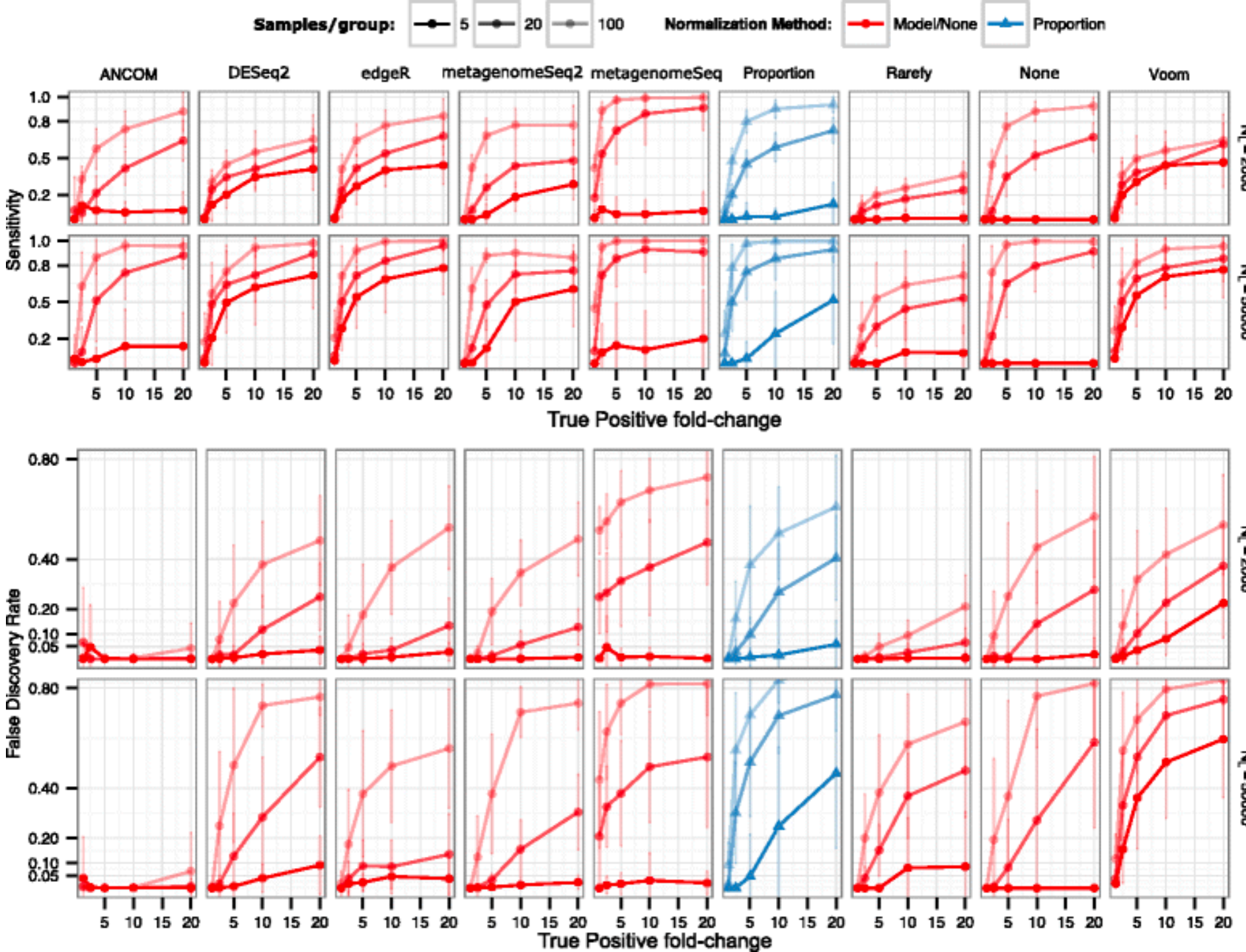
a-d are unweighted metrics
e-f are weighted unifrac

Word before "PERMANOVA R^2" is the normalization

There are trade-offs in sensitivity (top) and FDR (bottom).

Some methods are better at small sample sizes.

Other than ANCOM they all kind of look the same



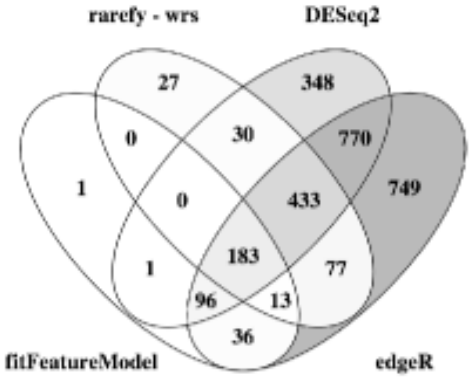
Differential abundance methods don’t find the same things.

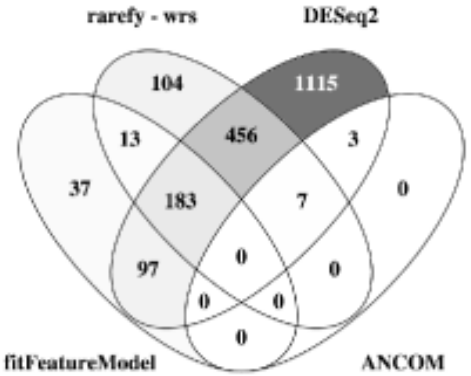
DESeq2 and edgeR give a lot of hits

Venn diagrams with more than 3 circles are really hard to interpret

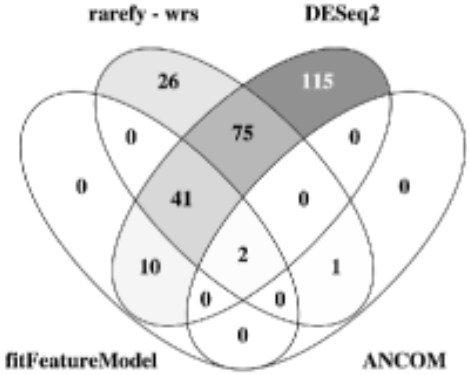
We really need gold-standard datasets to know what is true

a Caporaso *et al.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms *ISME* (2012).
~ 6 skin samples, 8 soil samples
mean sequences per sample: 1.3 million

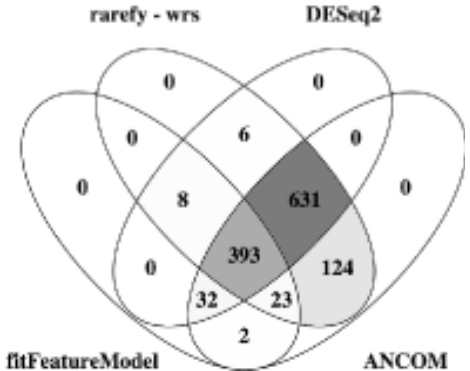




b Piombino *et al.* Saliva from Obese Individuals Suppresses the Release of Aroma Compounds from Wine. *PLoS One* (2014).
~28 samples per category (lean vs. obese)
mean sequences per sample: 75,580



c Caporaso *et al.* Moving Pictures of the Human Microbiome. *Genome Biology* (2011).
~500 samples per category (tongue vs. left palm)
mean sequences per sample: 25,600



skbio.stats.composition.ancom()

Performs a differential abundance test using ANCOM.

This is done by calculating pairwise log ratios between all features and performing a significance test to determine if there is a significant difference in feature ratios with respect to the variable of interest.

In an experiment with only two treatments, this test tests the following hypothesis for feature ii

$$H_{0i} : \mathbb{E}[\ln(u_i^{(1)})] = \mathbb{E}[\ln(u_i^{(2)})]$$

where $u^{(1)}_{\mathrm{i}}$ is the mean abundance for feature i in the first group and $u^{(2)}_{\mathrm{i}}$ is the mean abundance for feature i in the second group.