The Effects of Normalization, Transformation, and Rarefaction on Clustering of OTU Abundance

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Introduction

Metagenomic clustering presents a unique opportunity to associate and understand communities. Working with Operational Taxonomic Units (OTUs), however, often requires a strategy for handling OTUs that may be over or under represented in a given sample, which is thought of as "erroneous". PCR amplification, for example, is known to sometimes non-linearly over-represent more common species *Gonzalez et al. 2012*. Strategies dealing with this bias include Normalization, Rarefaction, and Log Transformation. Here, we examine how methods to handle potential outlier observations affect *de novo* estimation of groups using both clustering and matrix factorization methods.

While log transformations may affect parametric tests *O'Hara and Kotze 2010* our primary interest is in clustering, where relative abundances among samples could affect the derived clusters *Weiss et al. 2017*. We examine similarity between the adjusted OTU tables and the original ones to determine if relationships are preserved after post-processing.

We consider four methods: Bray-Curtis Similarity, *de novo* determined clusters by K-means and PCA, determining groups using Non-Negative Matrix Factorization (NMF). Our goal is to adjust for potential over or under representation while keeping relative sample abundances as similar as possible, a problem that finds analogy in Single Cell sequencing *Vallejos et al. 2017*. We find that Rarefaction and then Transformation have higher mantel statistics, as a matrix correlation *Legendre and Legendre 1998*, in relation to an unmodified OTU table than a Normalized OTU table, and this same observation applies also to K-means. Only for NMF does Log Transformation results appear similar to an unedited table.

Methods

We estimate a Bray-Curtis distance matrix from each of four OTU tables *Fig. 1* and compare these matrices with a mantel statistic *Fig. 2* to see if Normalization, Rarefaction, or Log Transformation

disrupts groups. Data was chosen that had three distinct sampling locations and three distinct OTU profiles *Crits-Christoph et al. 2013*. We used k=3 for K-means and NMF, which is described below.

K-means, PCA of Abundance

Using OTU tables we generate *de novo* clusters with K-means and then compare these clusters using a Jaccard-driven similarity *Fig. 3*. We test these clusters using a silhouette plot, which shows the distance

of each sample to the closest other cluster. The clusters are plotted with a PCA on abundance, using the DC algorithm *Fig. 4*. Rand Similarities show the same relationships between OTU tables, and when a Wilcoxon signed-rank test is conducted between all tables, log transform is seen to have the highest statistic to the un-edited OTU table *Molik 2017*.

Non-negative Matrix Factorization

Using the OTU tables we generate *de novo* clusters in NMF and compare these clusters with Jaccard Similarity Fig. 3. NMF groups had higher silhouette coefficients than their K-means counterparts Fig. 5. All groups had an above .8 silhouette coefficient.

Discussion

Distance matrices are used in hierarchical clustering. In Figure 2 we can observe that "normalized" is a fairly distinct distance matrix, and has been shown to cause a substantial difference in OTU composition *Weiss et al. 2017*. These differences appear using mantel statistic assessment, as well as in K-means analysis and in NMF clustering. Similarly, the expectation in Rarefaction is that relative abundances will change according to the amount of rarefaction; a presence absence OTU table will have different sample to sample relationships than a slightly scaled down OTU table. We look at the later and, as expected, our rarefied table is shown to be more similar based on a mantel test on distance matrices, as well as in PCA/K-means assessments.

The optimal method for handling outliers and erroneous abundances depends on data and application *Weiss et al. 2017*. In clustering and dissimilarity both rarification and log transformation seems to produce results that approximate the original OTU table; however, formatrix factorization only log transformation maintains original OTU table structure.

Data and Code Availability

Source sequence data came from National Center for Biotechnology Information Sequence Read Archive SRA accession number SRA091062 *Crits-Christoph et al. 2013*. Analysis was conducted in R and is available at <https://github.com/status-five/The-Effects-of-Normalization-Transformation-and-Rarefaction-on-Clustering-on-OTU-Abundance>, the DOI:10.5281/zenodo.896927 version was used *Molik 2017*. We used the R vegan package *Dixon 2003* for log transformation, normalization, and rarefaction of the OTU table. The NMF package was used for applying NMF *Gaujoux and Seoighe 2010*. The Corrplot R package was used for matrices visualization *V. Simko 2016*. The fpc R package was used for PCA analysis and visualization *Hennig 2015*. The clusteval package was used for determining Jaccard similarities between clusters *Ramey 2012*.

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