

Basic Reporting

The article is well structured and well written. The authors use clear and unambiguous, professional English throughout the article. However, in some occasions sentences are unnecessary long and could be simplified, such as in lines 207-210, 315-318, or typing errors occurred, line 240.

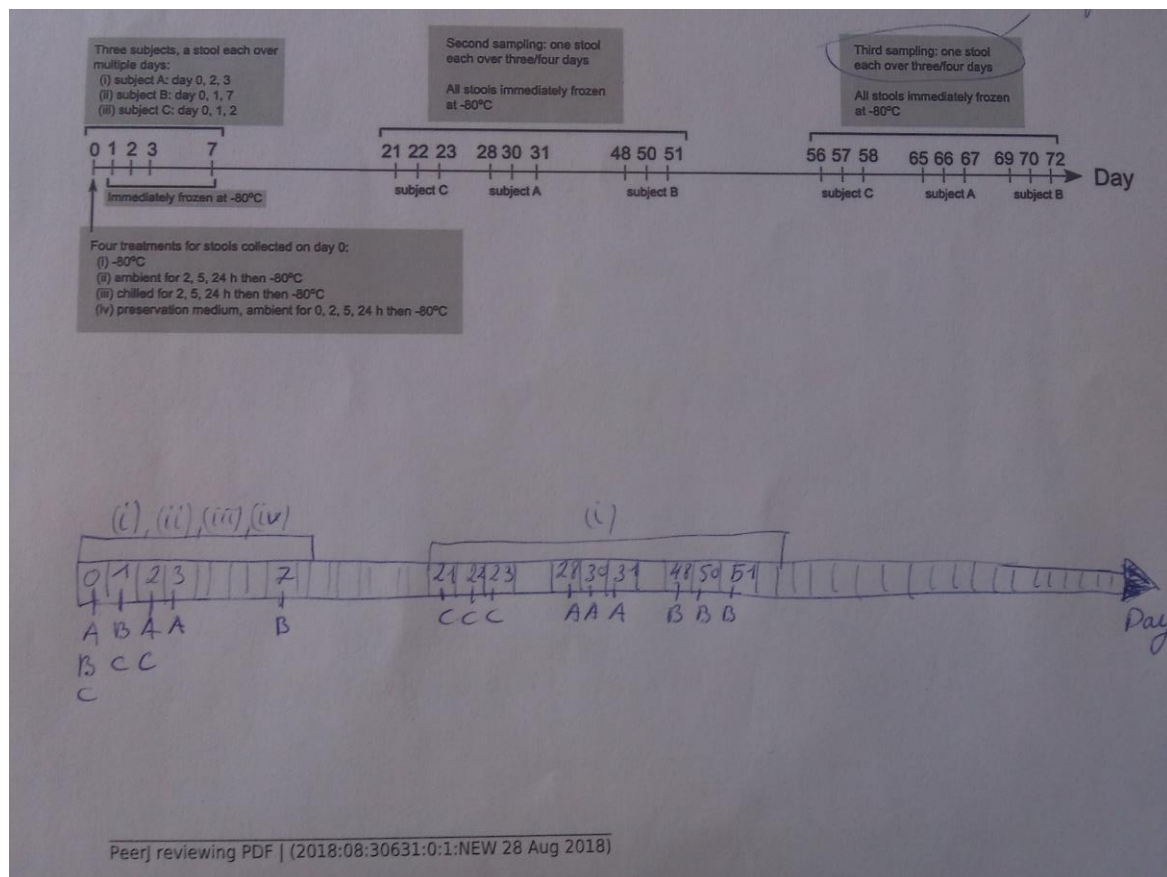
In general, literature references are up to date and sufficient field background/context is provided. The section on reduction of community diversity in the case of highly abundant taxa in the discussion (lines 301-303) would benefit from a brief explanation of the mechanisms at play. In addition, the finding on dominant *Prevotella* abundances being severely reduced with storage conditions, is a major one in the light of the importance of this taxon in the determination of gut community types. Referencing to papers describing these gut community types (or enterotypes) would demonstrate how the work fits into the broader field of knowledge. Next to temperature, the possible role of (an)aerobic conditions should also be discussed as diverging factor in the discussion on specific taxa abundance changes.

The authors provide most of the necessary information throughout the text and in figures and tables. All appropriate raw data has been made available in a public database.

At some occasions, information on the statistical tests and its results are missing, such as in lines 180-181 and 283.

Figures and tables are of sufficient resolution, but description and labeling can be improved. Suggested improvements:

- Figure 1. Reduction in text and better use of symbols to make the study set up easier to understand at a glance. I have attached an example.
- Figure 2. Place data of Subject A in panel A, B in B, C in C and overall in D. Take legend of PCA overall out of the plot and move it to the side, as it applies to all of the plots. Number all sample points according to the day they were taken, remove text-box. Add info on storage duration (2,5,24h) in individual plots. Add info on the exclusion of samples (failed sequencing, ...) in the figure legend – referring to the methods.
- Table 1: Heading could be turned into 'all samples'. Sampling occasion might be better formulated as sampling time point. It could be useful to include which kind of levels the parameters include within the table of table legend. (e.g. for sampling occasion: is it all days, or period 1/2/3? Especially to compare with subject:sampling occasion). At this moment it seems like the column names of the data tables used during analysis by the researchers - who are fully accustomed with the research design – are used. Parameters should be turned into easy understandable text and convey all necessary information in an easy way for the reader.
- Table 2: Idem to suggestions Table 1.



Experimental Design

This paper tries to assess the impact of sample storage conditions on stool community profiles of Asian individuals. The research question is well defined, relevant & meaningful. The authors state in the abstract and introduction how their research fills an identified knowledge gap. They performed a rigorous investigation, complying with the ethical standard and using the latest available methods (e.g. application of the Dada2 algorithm for sequence variance identification).

The methods are well described and provide enough detail to repeat the analysis. I would suggest to also include information on the number of unassigned taxa at each taxonomical level at the end of the paragraph '16S sequencing data processing'. The authors chose to apply a centered log-ratio transformation for the PCA and state this in the result section, yet I think it is more appropriate to include this in the method section, together with a brief explanation on the reasons to do so.

Validity of the findings

The authors used the appropriate materials and methods and performed appropriate statistical tests, including multiple testing correction where necessary. Some notes:

- Lines 180-182, statements are not completely supported by the results (I think one cannot state that the specific durations of 2,5 and 24 hours are associated with shifts in community composition but only duration in general).
- Lines 230-232: A much higher amount of taxa is enriched versus depleted over time, could the authors elaborate on this? This might be a consequence of the relative data.

Importantly, the data on which the conclusions are based are made available for the community.

General comments for the authors

This work contains some important messages for our field. Thank you for taking the time to look into this subject and report the results of your analysis in a clear way. In order to improve the manuscript further, I would like to make the following suggestions.

- Include a ranking of the variables according to their effect on microbiota composition.
- Make optimal use of the longitudinal set up of the study by determining not only the inter-individual variation but also intra individual variation. For instance, using Bray Curtis dissimilarity or another distance metric, variation between samples could be determined between persons (inter-ind.), between time-points of the same time-period (intra ind.), storage methods (for the same person, at the same time point), intra-sample.
- For the title, 'delivery conditions' does not really convey what it should. With the numerous reports on the microbiome of infants, the wrong association with childbirth is easily made. 'Storage conditions' might be more appropriate. In order to make the title and text more comprehensible a distinction between biological relevant variation (inter-ind, temporal, intra-sample) and induced variation by storage conditions could be made.
- To be completely correct, one should make a distinction between the taxonomic units obtained using the Dada2 workflow and those obtained by previous methods (Operational Taxonomic Units (OTUs), defining taxonomic units based on a cut off value for sequence similarity). With dada2 the taxonomic units are of a higher resolution and can be named amplicon sequence variants (ASVs).