Review 1: Shiftwork, Functional Bowel Symptoms and the Microbiome

Ann E. Rogers[1], Yi-Juan Hu[2], Ye Yu[3], Emily F. Wissel[4], Robert A. Petit III[5], Simone Jarrett[6], Jennifer Christie[7], Timothy D. Read[8]

The manuscript by Rogers et al. aims to determine the differences in the microbiota's composition and diversity between night and day shifts and among workers with functional bowel symptoms. A relation that has not been previously established by previous literature.

It is a well-written manuscript with a good experimental design. However, a few minor issues with the data analysis should be addressed before accepting the manuscript for publication.

1. Please give more details on the bioinformatics pipeline used in the study. The authors mention they used OTUs for taxonomy assignation (line 212); however, in the differential analysis, the taxa mentioned have ASV in the name, as in Amplicon Sequence Variant). Also, the reference mentioned in the methodology, Callahan 2016 (line 217), corresponds to the publication of the software DADA2, which is used for ASV analysis. Please clarify if the use of OTUs or an ASVs taxonomy assignation, as these, are two different analysis pipelines.

2. The authors mention gut samples and fecal samples throughout the manuscript. Some readers might find this confusing, as some authors use "gut samples" to refer to gastrointestinal tissue. Please, clarify this point and use only one term (preferably fecal samples) in the manuscript to avoid confusions,

3. An interesting aspect of the study is the analysis of workers with IBS diagnoses. Please enrich the discussion section in lines 342-345, where authors compare their findings with previous reports of this syndrome.

4. Clarify the sequencing depth used to calculate the Chao and Shannon índices (line 264-265).

5. Consider adding a Figure showing the taxonomical composition. Maybe a bar chart with the most abundant genera and their relative abundances.

6. Do Figures 1 and 3 differ only in the scale? If so, please consider removing Figure 1, as they both show no significant differences.

7. In line 138, the authors mention, "Those with recent antibiotic exposure were asked to delay their enrollment in the study for two weeks." Please clarify if this delay was after the patients suspended the antibiotics treatment.

8. Please include the p values in all the boxplots to facilitate the interpretation of the figures.

9. Please consider rewriting lines 249-256. It was complicated to understand as it is.

10. Make sure to include the definitions for all the abbreviations. For example, GI is not defined in the abstract or the main text.