

Reviewer Report

Title: Fungal and ciliate protozoa are the main rumen microbes associated with methane emissions in dairy cattle

Version: Original Submission **Date: 9/13/2021**

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Reviewer Comments to Author:

This study has generated the largest ONT ruminal metagenomic dataset currently available, and found that ciliates and fungi are closely associated with methane emission. The findings is novel and have wide interest, but the manuscript need revision to be a good manuscript.

1. In all the Figures, the text are not clear. For me, I can't distinguish most of the characters, words, so it must be resolved.

2. In the Abstract Part:

"Background: This study analyzed whole rumen metagenome using long reads and considering its compositional nature in order to disentangle the role of rumen microbes in methane emissions." Is this really background? I can't see any backgroud and logic.

Methods: Line 33-38, can be removed from Abstract. Abstract needs concise, methods is not necessary here.

3. In the Results Part:

"3.1 Cohort description

Our cohort included 437 Holstein lactating cows sampled at 14 different herds from northern Spain (Cantabria, Euskadi, Navarra and Girona regions)." This paragraph is very short and only have one sentence. I suggest to put it into methods part, and remove it from the results part. It can be merged with Methods 6.1 "Animal housing and feeding".

"3.7 Interaction networks" and "3.8 Taxonomy of genes" do not like sub-title names, they should be revised, to focus on the novel discoveries, which be more meaningful for the readers.

4. The Discussion Part:

The biggest problem is that it is too long. I suggest either make them concise, or move some into the results part. Those discussion contents that are closely with the results can be merged into the results part, and further disscussions can be kept in the Discussion part.

5. In the Methods Part:

"6.6 Bioinformatics" this sub-title is not meaningful, better change it to "Reads filtering, taxonomic and functional assignment" or others.

6. The last suggestion: try to assemble the ONT long reads, generate gene set, and chromosome results by binning or other technologies. Maybe this will get a lot more useful sequence data.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

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