#### **Reviewer Report**

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

Version: Revision 1 Date: 10/21/2021

**Reviewer name: Mick Watson** 

#### **Reviewer Comments to Author:**

I am dissatisfied with the reviewer response.

I do not think it is at all clear that a DIAMOND search against the NR database (which is full of errors) will accurately reconstruct rumen taxonomy, nor do I believe that a DIAMOND search against KEGG or COG will reconstruct rumen function.

You cannot simply run a bioinformatics pipeline and believe the results, there must be some QC.

The data presented directly contradict previous results (e.g.

https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-015-2032-0) and so the authors need to esnure they are sound.

Both of these papers published rumen microbiome gene catalogues with KEGG information, and these could and should be used to identify functional categories:

https://www.nature.com/articles/s41467-018-03317-6

https://www.nature.com/articles/s41587-019-0202-3

As written, I recommend reject, for methodological problems

## Methods

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

### **Conclusions**

Are the conclusions adequately supported by the data shown? Choose an item.

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Choose an item.

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