

## Reviewer Report

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

**Version: Original Submission**    **Date: 9/8/2021**

**Reviewer name: Mick Watson**

### Reviewer Comments to Author:

My main point of concern is the suitability of the SqueezeMeta pipeline, and the choice of databases (nr and KEGG) for rumen data.

For example, many groups have now published data showing rumen metagenome genes are very dissimilar to those found in public databases e.g. RefSeq.

The most up-to-date rumen microbiome datasets are:

- the Hungate collection <https://www.nature.com/articles/nbt.4110>

- Anaerobic fungi from JGI

<https://mycocosm.jgi.doe.gov/neocallimastigomycetes/neocallimastigomycetes.info.html>

- MAG collections (many summarised here:

<https://www.biorxiv.org/content/10.1101/2021.04.02.438222v1.full.pdf>)

- more MAGs and a rumen gene catalogue here:

<https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-021-01078-x>

As this analysis is the fundamental basis of most of the rest of the manuscript, I need to be reassured it is producing accurate results, and without including the latest rumen genomes and gene catalogues, I am unsure that it is

## 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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