

Martin et al. have carefully considered most of the comments and have either addressed them or provided detailed explanations. The manuscript is largely improved compared to the previous version, and the FungalTraits inference is a welcome addition. I have very few textual comments left and consider the manuscript ready for publication after addressing those.

Although the authors argue that rarefaction is an appropriate method for normalizing heterogeneous read numbers among taxa, we believe this approach carries the risk of removing rare taxa that may be ecologically significant. Rarefaction, while widely used, is far from uncontroversial (McMurdie & Holmes 2014 comes to mind..), and the authors did not clarify whether the rarefaction they used were a single random draw or averaged across multiple random rarefactions at the chosen depths. The concern here is particularly because the decrease in alpha diversity over depth could be linked to the decrease in sequencing depth over depth shown in the tables presented in the authors' response.

The authors ruled out that DADA2 ASV clustering is inappropriate for fungal ITS by saying it would likely not affect the results. One would happily trust their word on this, but the phrasing suggests they have not looked into it. Demanding to redo the analyses would be excessive, but acknowledging the limitations of their approach is reasonable.

Fungi exhibit high variability in copy number, ranging from a few to several hundred (<https://doi.org/10.1111/mec.14995>), and bacterial 16S rRNA gene copy numbers are also well known to vary substantially across taxa. Although relative abundance was normalized using ddPCR measurements, the reported read counts may reflect copy numbers originating either from the same taxon or from different taxa. This issue was addressed in Vandeputte et al. (2017), to which the authors refer, but it is not discussed explicitly here. The database referenced by Vandeputte et al. (2017) contains information on bacteria and archaea. It remains unclear which database the authors used to account for fungal ribosomal copy number variation. Therefore, abundance estimates based on copy number, in any form, present inherent challenges. Differences in the quality and completeness of reference data for ribosomal copy numbers, particularly between well-characterized systems such as the human gut microbiome and comparatively understudied environments such as Arctic permafrost, further complicate interpretation. In this context, the authors refer to their estimates as "absolute abundance," although the underlying assumptions do not fully support that designation. Describing the measurements as semi-quantitative would have been a more precise and methodologically consistent way to frame the results.

