

## Referee: 1

Comments to the Author

Thank you for the improvements: I have no further comment.

I provided a suggested version for figure 5 (as well as the original excel file so you can easily play with it) - it was more easy for me to show you what I meant with an actual example. I think the actual number of studies per year is still an interesting thing to show. But it is really just a suggestion.

Regards,  
J-B Leducq.

We really appreciate the reviewer taking the time to supply an example figure idea! We also agree that the number of studies is interesting to show, and have updated Figure 5 as you suggested. In order to keep it to a single plot, we've opted not to show information regarding the proportion of studies with sequenced controls, etc. Thank you again for your constructive and valuable comments during the peer review process!

## Referee: 2

Comments to the Author

Dear Authors,

Thank you very much for providing your feedbacks and clarifying your thoughts concerning my comments.

In general, I am sincerely pleased to have read the new version of the manuscript and happy to see that the authors brought our comments to improve the manuscript.

In general, there are yet rooms for the improvement such as sources of publications that seem to be quite selective from particular countries. I am also not fully believed by their search thread to extract previous publications from a specific set, and to extents as if their search would have been strong to be checked:

(1) An accumulation of previous publications potentially from the same lab could be a searching bias that came from an original set of selected publications ( $n = 450$ ). Although it is apparent from the data that some countries, and individual labs, produce a majority of the data, this was not actively selected and is due to some countries (and research groups) producing more research outputs than others, and thus being more likely to be retrieved from search strings. As the main objective of our review looks at the systemic underuse of contamination controls in phyllosphere

microbiome research, and less so the prevalence per country, we believe standardising the included papers across country of origin would cause the data to no longer be representative of the field as a whole.

(2) Their search approach to test if there can be a confounding (such as a usage of PNA clamp, as a control) factor that somehow introduces a ratio metric difference in V3V4 16S rDNA relative abundance, because PNA clamps (for both mitochondria and chloroplast) have a competitive inhibition to block 341F – 799R priming site. These two perspectives, in my view, can be tested with their approach to identify not only sole publications that lack of controls (as the author did), but also to further possibly demonstrate that although V3V4 is used across different publications, there is still heterogeneity of data that can be contributed by a neglect of PNA usage. Nevertheless, this expectation will be based on my assumption that the search thread may bring something confounding from their selected publications.

The focus of the review is on exogenous contamination sources, as described from LN: 43-46, and less so towards host contamination such as mtDNA and cpDNA. We really appreciate the reviewer highlighting the issue of mtDNA/cpDNA contamination in the previous revision, and did incorporate their suggestions by stressing this important problem in the 'RECOMMENDATIONS FOR FUTURE PHYLOSOPHERE RESEARCH' section. We believe that focusing more on this issue would dilute the clarity and impact of this review. However, we agree with the reviewer that the issue is important for the field, and as such, deserves its own focused review/paper.

As a methodological review and case study, this work gives raise concerns regarding lack of controls for phyllosphere microbiome data generations and brings critical points of views for improving quality of data.

As I also understand the authors' goal and clarity of the work, I would be quite OK to support their publication.

Best wishes,

We truly appreciate the feedback the reviewer has provided us with. It has challenged our work and given us much to consider that we previously did not think of – which we believe has strengthened the review.

**Referee: 3**

Comments to the Author

The authors have addressed my comments and I have no further suggestions

Thank you again for your constructive suggestions.