FROM: Dong, K., Woo, C., Yamamoto, N., authors of acp-2019-487 "Plant assemblages in atmospheric deposition"
RE: Response to Reviewer #1
DATE: August 2, 2019

The authors thank high-quality comments, especially regarding OTU clustering for plant ITS, by the Reviewer #1. Please find our responses to the reviewers' comments. The page numbers in our responses refer to those of our revised manuscript.

Reviewer #1:

Comment #1: General Comments:
The authors report a wide-ranging study on the two basic types of deposition for aerosol plant particles. They use high-throughput sequencing for identification and additionally qPCR for quantification of the different samples investigated in this study. The adaption of the clustering of OTUs with a similarity of 97% as known for bacteria might for plants be seen critical. References from literature, showing that this is a working method for clustering plant OTUs, are missing. The reached sequencing depth is sufficient as indicated via rarefaction curves and subsampling at 6,142 reads is comprehensible. The methods concerning the qPCR are in good agreement with the standard. Especially the deposition flux calculations give interesting results, but the authors name the weaknesses of the method like variation in the number of ITS gen regions and others (P7 L20). The scientific methods and assumptions are clearly outlined and not redundant. In general, the data of this manuscript are helpful and this paper might fits to the scope of ACP, but my personal point of view is that it is more related to the main topic of BGS. The overall writing of the paper appears somehow tedious, mainly because of redundant parts within introduction and discussion, whereas the story could be very attractive with some more focus on the needs of the reader. I want to name two examples comparing the beginning of the introduction and discussion as both start in the same manure, to strengthen my point:

Response #1: Regarding our selection of 97% as a threshold for OTU clustering of plant ITS, two previous studies have been cited (Page 4 Line 20). We admit that our selection of 97% as a threshold was operational since there is no consensus threshold available for plant ITS. However, Cornman et al. (2015) reported that most plant species were represented by multiple OTUs of ITS at 97% similarity, suggesting that most plant species are representative based on OTU clustering at 97% similarity. This means that the species-level identifications are likely possible with our method, but we restricted our analyses at the genus and family levels due to possible species-level misidentifications caused by the selection of OTU clustering.

References

Cornman, R. S., Otto, C. R. V., Iwanowicz, D., and Pettis, J. S.: Taxonomic characterization of honey bee (*Apis mellifera*) pollen foraging based on non-overlapping paired-end sequencing of nuclear ribosomal loci, PLoS ONE, 10,

e0145365, https://doi.org/10.1371/journal.pone.0145365, 2015.
Núñez, A., Amo de Paz, G., Ferencova, Z., Rastrojo, A., Guantes, R., García, A. M., Alcamí, A., Gutiérrez-Bustillo, A. M., and Moreno, D. A.: Validation of the Hirst-type spore trap for simultaneous monitoring of prokaryotic and eukaryotic biodiversities in urban air samples by next-generation sequencing, Appl. Environ. Microbiol., 83, e00472-00417, https://doi.org/10.1128/aem.00472-17, 2017.

As the Reviewer #1 pointed out, a limitation of DNA-based methods lies in the difficulty in comparing with traditional microscopy-based methods due to variation in the number of ITS copies per pollen grain. Nonetheless, we confirmed that our measurements were reproducible (Fig. S2 in the Supplement), suggesting that between-sample comparisons are accurate at least within our present DNA-based study.

Regarding redundant parts of our manuscript, please see our responses to the Reviewer #1's second and third comments below.

Comment #2: Specific comments:
The authors give the global atmospheric estimates of emitted particles in Tg per year. Noticeable here is that on P1L25 the amount of released plant particles is given with 47-84. In the Discussion P7L2 no range is given but the already mentioned amount of pollen from Hoose et al. 2010 is given with 47 Tg.
P1L25 An estimated 47–84 Tg of plant particles are released into the environment each year (Després et al., 2012;Hoose et al., 2010;Jacobson and Streets, 2009),…
P7L2 Large quantities of biological particles are emitted into the global atmosphere, with estimates of 0.75 Tg yr-1 for bacteria, 31 Tg yr-1 for fungi, and 47 Tg yr-1 for pollen (Hoose et al., 2010)

Response #2: The suggested, redundant sentence has been deleted from the discussion section.

Comment #3: Another example:
P2 L1-5… and/or by serving as ice nuclei (IN) and cloud condensation nuclei (CCN) (Pöschl et al., 2010;Pope, 2010). Finally, atmospheric pollen is involved in global cycling of substances (Després et al., 2012) by long-range transport and subsequent settlement to the planetary surface (pedosphere) by dry or wet deposition, i.e., sedimentation or precipitation, respectively.
P7 L2-6 The emitted particles are involved in global cycling of substances, including the bioprecipitation cycle in which organisms emit airborne particles (or are emitted as airborne particles) that serve as cloud nuclei and promote precipitation (Morris et al., 2014;Sands et al., 1982).

Response #3: The suggested, redundant sentence has been deleted from the discussion section.

Comment #4: Further question:
I wonder why the results of the Anderson sampler especially for the Pinidae (found within all chambers for all aerodynamic diameter) is not discussed by the authors

as it might indicate pollen rapture or the co-emittance of non-pollen particles? Or is this an effect due to the mentioned air-filled sacci of for example Pinus?

Response #4: It is not completely clear to us about what is asked in this question, but we assume that the Reviewer #1 asked about the reasons of why we did not discuss about particle size distribution for each plant taxon, including Pinidae, based on our particle size-resolved measurements by the Andersen sampler. In our previous fungal studies (e.g., Yamamoto et al., 2014; Woo et al., 2018), we computed a representative geometric mean of aerodynamic diameters for each fungal taxon based on their particle size distributions characterized by the Andersen sampler. It was possible for fungi since their particle size distributions were normally (i.e., log-normally) distributed with their peaks situated between d_a = 2.1–11 μm for most taxa. Meanwhile, size distributions of plant particles were highly skewed and right-truncated with peaks typically situated at d_a > 11 μm (Figure 1a), and it is difficult to accurately compute representative aerodynamic diameters with these highly skewed, right-truncated particle size distributions. This is the reason why we discussed our results based on the literature-derived diameter values of pollen grains rather than based on the experimental particle size distribution data that can be but not accurately characterized by particle size-resolved measurements by the Andersen sampler for large-sized plant particles.

References

Woo, C., An, C., Xu, S., Yi, S.-M., and Yamamoto, N.: Taxonomic diversity of fungi deposited from the atmosphere, ISME J., 12, 2051–2060, https://doi.org/10.1038/s41396-018-0160-7, 2018.

Yamamoto, N., Nazaroff, W. W., and Peccia, J.: Assessing the aerodynamic diameters of taxon-specific fungal bioaerosols by quantitative PCR and next-generation DNA sequencing, J. Aerosol Sci., 78, 1–10, https://doi.org/10.1016/j.jaerosci.2014.08.007, 2014.

Comment #5: Technical corrections:
In Table 1 : asterids and rosids are not capitalized in table 2 they are. Please unify.

Response #5: Corrected (Page 18).

Comment #6: Table 2: Please optimize the dimension of the table in a way that no single characters appear as for "Chenopodium".

Response #6: Corrected (Page 18). Thanks for careful reading.

Comment #7: Figure 1: A method is missing, giving some information how data for the plots were generated. I guess 1A) qPCR and 1B) NGS? This should be added to the caption. Overall seems this figure caption somehow unfinished when compared to all others and scientific standards. One opening sentence would improve this.

Response #7: The sentences were added to explain how the data were calculated for each panel. The revised caption reads as follows:

“Figure 1: (a) Particle size-resolved concentrations based on plant classes or clades in terms of copy number (CN) of ITS2 from atmospheric samples from Seoul in South Korea. Monthly results from May to November 2015 are shown, except for August when air sampling failed. The data shown are obtained by multiplication of DNA sequencing-derived relative abundance of each family by a total plant concentration measured by the universal plant-specific qPCR assay. (b) Principal coordinate analysis plot for plant assemblage structures based on Bray-Curtis distance. The data shown are based on DNA sequencing.” Page 19