Response to referee comments and suggestions on bg-2020-469', by Prass et al.

Manuscript format description:

Black text shows the original referee comment, blue text shows the authors response, and red text shows quoted manuscript text. Changes to the manuscript text are shown as *italicized and underlined*. We used bracketed comment numbers for referee comments (e.g., [R1.1]) and author's responses (e.g., [A1.1]). Line numbers refer to the discussion/review manuscript.

Anonymous Referee #3,

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The manuscript by Prass and colleagues investigates the altitude distribution of bioaerosols in the Amazon rain forest. In summary, there are several strengths to this study: 1. bacteria, archaea, and eukarya were enumerated using fluorescent microscopy and FISH, 2. the site is an undisturbed forest environment, 3. collection was done at several different heights. There are also several weaknesses to this study: 1. only one week of data collection, 2. only one site of collection, 3. only one time/day of bioaerosol collection for FISH analysis, 4. Only one analytical technique (FISH) used to identify particles as bacteria, archaea, or eukarya.

We thank Reviewer #3 for their positive and critical evaluation. The reviewer's comments were very helpful to clarify several statements and arguments.

[R3.1] While FISH is a laborious technique and it has been used before on some aerosols to distinguish bacterial species from each other, it has not been used for atmospheric bioaerosols and not to distinguish bacteria, archaea, or eukarya from each other. In particular, the ability to observe and enumerate assemblages of different composition (for example, assemblages that consist of only bacteria or assemblages that consist of eukarya and bacteria) is a clear strength of this technique. Figure 4H is a beautiful example.

[A3.1] We thank Reviewer #3 for this positive overall evaluation.

[R3.2] The determination of absolute and relative numbers of bacteria, archaea, or eukarya at different heights in the Amazon rain forest is by itself another important result. However, it is clearly limited by the fact that collection was restricted to a one-time seven-day

period and that the results were not compared with any other analytical technique. Because FISH was not used before to determine concentrations of bacteria, archaea, or eukarya at different heights at other sites, the results cannot be compared with other studies that used different techniques.

[A3.2] We agree that the six-day sampling period (covering 3 sampling heights) analyzed here is comparatively short. Note, however, that more samples were collected in this sampling campaign and that the six days were analyzed on purpose as this time window had ideal conditions to investigate pristine atmospheric conditions in the Amazon (P4, L15-17, Supplement P2, L9-26, Figures S1, S2). Pristine episodes in the central Amazonian wet season are relatively rare and have an episodic character (for definition and details, see Pöhlker et al., 2018). Further note, that the sampling and analysis were embedded in a broad set of other meteorological, trace gas, and aerosol measurements running continuously at ATTO (e.g., (Andreae et al., 2015), which provided us a comprehensive context of data and, thus, allowed us to choose particularly interesting periods. In this sense, the present work can be regarded as a targeted case study on the bioaerosol population in the pristine Amazonian wet season as well as a proof of concept of the feasibility of our sampling and FISH protocols under challenging field conditions. Clearly, the results presented here spark a variety of follow-up questions (e.g., higher temporal resolution, day vs night differences, seasonal variations), which will be addressed in ongoing or planned follow-up campaigns. To emphasize these aspects more clearly in the manuscript, we rearranged and added the following statements in the main text:

(P4; L15-18) Revised: The samples analyzed in this study were collected during prevailing clean wet season conditions in the Amazon. *The six-day sampling period was chosen for detailed analysis as the aerosol mixture approximated a pre-industrial state with the bioaerosol population originating from the primary rain forest region within the ATTO site's footprint. A detailed characterization of the conditions can be found in the supplement.*

(P14, L10-14) The Amazonian bioaerosols were investigated on domain level by quantifying eukaryotic, bacterial, and archaeal cells as well as the overall concentrations of airborne cells as a function of time and height within and above the forest canopy. *These bioaerosol abundances are characteristic for naturally and clean background aerosol conditions as during the analyzed sampling period local emissions from the primary rain forest dominated.*

Regarding the reviewer's statements that "results were not compared with any other analytical technique" and that "results cannot be compared with other studies that used different techniques":

We agree here, though would like to point out a few aspects. Studies with quantitative bioaerosol analyses – especially in the Amazon – are very sparse. In this sense options for comparison with previous studies are inherently limited. Nevertheless, we conducted a literature synthesis and comparison with previous studies as far as possible by collecting all published number concentrations for biological aerosols (e.g., from microscopy and autofluorescence detection) in tropical (and boreal) forests in Table S3. We found that these results are largely consistent with our findings. Note in this context that the authors regard the uncertainties involved in autofluorescence-based (bio)aerosol detection as much larger as the uncertainties of the FISH approach. The agreement between Table S3 and the DAPI and FISH results made us confident that our data is a robust first data set e.g. on bacterial number concentrations in the Amazonian atmosphere.

Further, we conducted a careful comparison with online aerosol data and especially the data from an optical particle sizer, which provides a reference for the overall aerosol abundance in the DAPI and FISH relevant size range. We found a good agreement here as well. In fact, we consider consistent results with the overall aerosol variability (size distributions and total coarse mode concentrations) as equally important than a consistency for other molecular biological techniques as this is key for a comprehensive understanding of atmospheric processes at the interfaces of atmospheric physics, chemistry, and biology. The reviewer's comment made us aware that aforementioned aspects are probably not as clear as necessary in the manuscript in its current form. Thus, we revised the text accordingly and changes are specified under [A3.3].

[R3.3] Of course, it is impossible to change the study itself at this point. It is the opinion of this reviewer that some improvements to the manuscript itself can make this an important and interesting contribution. The main recommendation is to more clearly acknowledge the limitations I listed above. In particular, the authors should not state at the same time that their data provide "unprecedented insights" and are "highly consistent with … previous studies". The authors should instead acknowledge that the absence of an independent verification using

other techniques, such as sequence-based techniques or qPCR, and the absence of similar studies performed at other sites during other season limits the ability to compare their results and verify the accuracy of their results.

[A3.3] We appreciate this constructive recommendation and changed several statements in the manuscript accordingly. As described under A3.2, we agree that the comparability of this data set is partly hampered by the absence of studies from either the same location or studies using the same method. Nevertheless, we found bioaerosol number concentrations in tropical rainforest reported in a comparable range. We pointed out these limitations as follows:

Supplement, P6, L6-8): Accordingly, number and mass concentrations derived from different measurement techniques and sampling locations are comparable only within certain limits and similarities as well as deviations have to be evaluated carefully (see Table S3).

(P 2, L14-16) Before: The observed concentrations and profiles provide unprecedented insights into the sources and dispersion of different types of Amazonian bioaerosols as a solid basis for model studies on biosphere-atmosphere interactions such as bioprecipitation cycling.
(P 2, L14-16) Revised: The observed concentrations and profiles provide new insights into the sources and dispersion of different types of Amazonian bioaerosols as a solid basis for model studies on biosphere-atmosphere interactions such as bioprecipitation cycling.

(P 4, L22-30) Revised: A focal point of this study has been the careful cross-validation and comparison of the obtained FISH results with online aerosol data as well as a synthesis with existing literature knowledge. This validation is important since FISH is experimentally demanding and prone to various artifacts (i.e. false positive or false negative counts) and thus may yield biased results (Thiele et al, 2011). *A comparison with data from different locations or obtained by different methodologies is meaningful only within certain limits (S1.4). Though, we overall* found a high consistency with complementary online data from the ATTO site as well as from previous studies, which underlines that the obtained organism concentrations are a solid representation of the Amazonian wet season bioaerosol population.

(P 12, L25-28) Before: Overall, the results of this study greatly extend the knowledge on the life cycle of the Amazonian aerosols and provide a solid experimental basis for model investigations of bioaerosol-related processes, such as the role of biological ice nuclei or giant cloud condensation nuclei in cloud microphysics and potential bio-precipitation cycling.

(P 14, L24-27) Revised: Overall, the results of this study extend the knowledge on the life cycle of the Amazonian aerosols and provide a solid experimental basis for model investigations of bioaerosol-related processes, such as the role of biological ice nuclei or giant cloud condensation nuclei in cloud microphysics and potential bio-precipitation cycling.

(P 14, L33-34): For this purpose, a broader statistical basis of FISH results and comparisons *with bioaerosol analysis techniques (such as sequencing or qPCR)* along with meteorological observations is needed.

(P15, L4-6): Future studies should use the analytical potential of FISH by targeting organism classes on lower taxonomic levels (e.g., theoretically down to species level) *in combination with and on the basis of sequencing-based techniques.*

References:

Andreae, M., Acevedo, O., Araùjo, A., Artaxo, P., Barbosa, C., Barbosa, H., Brito, J., Carbone, S., Chi, X., and Cintra, B.: The Amazon Tall Tower Observatory (ATTO): overview of pilot measurements on ecosystem ecology, meteorology, trace gases, and aerosols, Atmospheric Chemistry and Physics, 15, 10723-10776, 2015.

Pöhlker, M. L., Ditas, F., Saturno, J., Klimach, T., Hrabě de Angelis, I., Araùjo, A. C., Brito, J., Carbone, S., Cheng, Y., and Chi, X.: Long-term observations of cloud condensation nuclei over the Amazon rain forest–Part 2: Variability and characteristics of biomass burning, long-range transport, and pristine rain forest aerosols, Atmospheric Chemistry and Physics, 18, 10289-10331, 2018.