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 #2

Received: 27 April 2021

[R2.1] We thank the authors for their effort to answer the concerns of the various reviewers and for the corrections of the manuscript. I agree it could be published but I would like the authors to make a few changes to take into account my previous comments.

[A2.1] We appreciate the constructive comments by Referee #2, which have been considered and implemented in the course of this second revision. The referee's comments and our responses are outlined in detail below:

Minor comments:

[R2.2] P3 Line 36 : "Our results demonstrate that FISH has great potential in bioaerosol analysis as it provides number concentrations of specific organism classes (i.e., from domain down to species level) and, therefore, combines bioaerosol identification, enumeration, an visualization" I suggest to delete "great" and keep "FISH has potential in bioaerosol analysis". Indeed we think that this technique is so heavy that its use to analyze a great number of samples will be limited, alternative techniques will be more suitable (qPCR, sequencing, Flow cytometry + targeted probes etc..)

P 11 line 7: "Our study showed that FISH has great analytical potential in bioaerosol analysis." Please delete "great" (for the same reason as before).

P11 line 11: "Here, we propose FISH to be a promising tool". Please change "promising" for "interesting" (same reason).

[A2.2] We have changed the manuscript as the referee suggested:

(P3, L36-L38): Our results demonstrate that FISH has ~~*great*~~ potential in bioaerosol analysis as it provides number concentrations of specific organism classes (i.e., from domain down to

species level) and, therefore, combines bioaerosol *identification, enumeration, and visualization.*

(P11, L7): Our study showed that FISH has ~~*great*~~ analytical potential in bioaerosol analysis.

(P11, L11-12): Here, we propose FISH to be *an interesting* tool, to complement the methods currently established for environmental bioaerosol analysis (Sect. S1.4).

[R2.3] supplement, P 5 line: The authors should add a paragraph about the advantages of flow cytometry to quantify the total number of cells and look at their size distribution. This technique is very fast contrary to DAPI which is time consuming. They should also speak about the combination of flow cytometry with specific staining with targeted probes.

[A2.3] We have added these aspects according to the referee's suggestions in the supplement chapter "Bioaerosol analysis methods" to point out the potential of flow cytometry in relation to FISH/DAPI staining with manual counting. We tried to find the right balance between covering the pros and cons of individual methods, on one hand, and going into too much detail on a single method, on the other, as this is not the focal point of this study. In fact, an appropriate and critical comparison of FISH/DAPI staining, flow cytometry, and qPCR would probably require a dedicated study, which addresses the various aspects to be considered in the choice of the 'right' tool(s) for bioaerosol analysis in a given environment. The referee is right that flow cytometry appears to have clear "advantages" over the manual FISH/DAPI staining and counting approach, which include the fact that it is "very fast" and directly provides particle "size distributions". This portrait would be incomplete, however, without mentioning that flow cytometry (as essentially every technique) has drawbacks at the same time (e.g., uncertainties due to an autofluorescence background, challenging sampling logistics at remote sites, lower precision in quantification).

We agree that flow cytometry works out best in combination with fluorescence staining for environmental bioaerosol analysis. This reduces the influence of autofluorescence and thus the detection of interfering non-biological particles as described in the supplement section S1.4. The occurrence autofluorescence from biological and non-biological materials across wide intensity and wavelength ranges has been well document before and represents a prominent challenge in automated bioaerosol detection (Pöhlker et al., 2012; Savage et al., 2017; Huffman et al., 2020). DNA or RNA staining creates fluorescence in a rather narrow spectral range and, thus, enables a targeted detection via e.g. microscopy counting or flow cytometry. The following text has been added to the supplement.

P6, L3- P6, L 9: *The microscopic analysis of FISH treated samples also bears drawbacks such as the time consuming manual enumeration of fluorescent single particles. Here, automated image generation and software based particle detection or sample analysis with flow cytometry could improve the analysis by speeding up the process. The application of these two techniques is dependent on the careful characterization of sample's properties such as aerosol mixing state and diversity, sample purity or abundance of interfering materials.*

[R2.4] supplement, P5 line 23. We do not agree with this paragraph :impingement is recognized as an efficient tool and no growth is observed with a short time collection, typically less than an hour is need to have enough sample , especially using high volume impingers (Šantl-Temkiv, T., Sikoparija, B., Maki, T., Carotenuto, F., Amato, P., Yao, M., Morris, C. E., Schnell, R., Jaenicke, R., Pöhlker, C., DeMott, P. J., Hill, T. C. J., and Huffman, J. A.: Bioaerosol field measurements: Challenges and perspectives in outdoor studies, Aerosol Science and Technology, 1-27, 10.1080/02786826.2019.1676395, 2019.)

We ask the authors to change this paragraph.

[A2.5] We thank reviewer 2 for this comment. As written in the text, the experimental issues play a role when liquid evaporation over time takes place because of long sampling periods. Since we purposefully collected diel average samples in this study, liquid evaporation became a relevant issue here. We modified the following sentence to point out this aspects more clearly. (Supplement, P5, L24- P6, L 3): However, *for long-term sample collections as conducted for this study* varying collection efficiency due to liquid evaporation over time and therefore changes in chemical composition (e.g., pH or fixative concentration) as well as microbial growth within the liquid *can play a role* and have to be considered (Lin et al., 1997) ~~taken into account~~.

Further note that we do not question that liquid impinges are an efficient bioaerosol sampling device in a range of applications. There are multiple studies analyzing their performances and proofing their advantages (e.g.: Lin et al, 1997; Dybwad et al., 2014; Šantl-Temkiv et al., 2017). However, we found that dry aerosol filtration was more appropriate than impingement for this study in the Amazon for the reasons given in the supplement. In fact, we conducted the first sampling for FISH in the Amazon with the so called BioSampler, which is a broadly used impinger (Willeke et al., 1998; Lin et al., 2010), and compared its performance with filter sampling. We found that for the relevant sampling period varying liquid levels and therefore a varying collection efficiency and varying concentration of the fixative occurred. Furthermore, microbial growth has been an omnipresent challenge in the Amazon, as the high humidity and

abundant airborne fungal and bacterial spores foster rapid microbial growth. Everything, even the laboratory equipment, was affected by microbial, especially fungal, growth on its' surface, if it was not disinfected and cleaned very often. As a result, if there is no or not enough fixative in the sampling medium, there indeed is a high potential for microbial growth in the sampler.

References

Dybwad, M., Skogan, G., and Blatny, J. M.: Comparative Testing and Evaluation of Nine Different Air Samplers: End-to-End Sampling Efficiencies as Specific Performance Measurements for Bioaerosol Applications, Aerosol Science and Technology, 48, 282-295, 10.1080/02786826.2013.871501, 2014.

Huffman, J. A., Perring, A. E., Savage, N. J., Clot, B., Crouzy, B., Tummon, F., Shoshanim, O., Damit, B., Schneider, J., Sivaprakasam, V., Zawadowicz, M. A., Crawford, I., Gallagher, M., Topping, D., Doughty, D. C., Hill, S. C., and Pan, Y.: Real-time sensing of bioaerosols: Review and current perspectives, Aerosol Science and Technology, 54, 465-495, 10.1080/02786826.2019.1664724, 2020.

Lin, X., Willeke, K., Ulevicius, V., and Grinshpun, S. A.: Effect of sampling time on the collection efficiency of all-glass impingers, American Industrial Hygiene Association Journal, 58, 480-488, 1997.

Lin, X., Reponen, T., Willeke, K., Wang, Z., Grinshpun, S. A., and Trunov, M.: Survival of Airborne Microorganisms During Swirling Aerosol Collection, Aerosol Science and Technology, 32, 184-196, 10.1080/027868200303722, 2000.

Pöhlker, C., Huffman, J. A., and Pöschl, U.: Autofluorescence of atmospheric bioaerosols - fluorescent biomolecules and potential interferences, Atmos. Meas. Tech., 5, 37-71, 10.5194/amt-5-37-2012, 2012.

Šantl-Temkiv, T., Amato, P., Gosewinkel, U., Thyrhaug, R., Charton, A., Chicot, B., Finster, K., Bratbak, G., and Löndahl, J.: High-Flow-Rate Impinger for the Study of Concentration, Viability, Metabolic Activity, and Ice-Nucleation Activity of Airborne Bacteria, Environmental Science & Technology, 51, 11224-11234, 10.1021/acs.est.7b01480, 2017.

Savage, N. J., Krentz, C. E., Könemann, T., Han, T. T., Mainelis, G., Pöhlker, C., and Huffman, J. A.: Systematic characterization and fluorescence threshold strategies for the wideband

integrated bioaerosol sensor (WIBS) using size-resolved biological and interfering particles, Atmos. Meas. Tech., 10, 4279-4302, 10.5194/amt-10-4279-2017, 2017.

Willeke, K., Lin, X., and Grinshpun, S. A.: Improved aerosol collection by combined impaction and centrifugal motion, Aerosol Science and Technology, 28, 439-456, 1998.