

Comments to the Referee 2#

We acknowledge the reviewer for his/her valuable and constructive comments stating “this study is an important contribution to the growing field of online bioaerosol measurements”. We addressed all questions carefully and revised our manuscript under the light of all suggestions. Referee comments (given in italic and blue) and our specific responses can be found below.

My feeling is that the title would be clearer and stronger without the abbreviation “FBAPs”. FBAP can be introduced and explained in the main text. Also, the use of the abbreviations FBAP and PBAP versus FBAPs and PBAPs is not consistent throughout the text. Please decide if you want to use the plural ‘s’ or not and align it in the entire text.

The reason for using the abbreviation “FBAP” is because of the fact that previous studies from Gabey et al. (2010) and Huffman et al. (2010) used the same acronym to present primary biological aerosol particles, which are detected by using the ultraviolet light induced fluorescence (UV-LIF) method. Therefore, “FBAP” is a well-known acronym, which represents an underestimated fraction of bioaerosols. However, we will follow the referee’s suggestion and remove it from the title. Suggestions concerning the acronyms “FBAP” and “PBAP” will be taken into account in the revised manuscript.

p. 17608 / l. 5-8: The sentence “This study aims to investigate the sensitivity of WIBS-4 to biological and non-biological aerosols, performance of WIBS-4 for discrimination of several types of aerosols, and the detection and identification of biological particles in the ambient aerosol.” is misleading. In particular, the words “detection and identification” suggest that beyond the “detection” of FBAP an “identification” of their nature (i.e. pollen, spore, bacteria) is performed. Also “discrimination of several types of aerosols” is not what the WIBS does - it provides a more or less reliable discrimination between biological and non-biological particles, but not really more.

Concerning the “identification of biological particles”, we agree with the referee and will rearrange the sentence as follows: “This study aims to investigate the sensitivity of WIBS-4 to biological and non-biological aerosols and detection of biological particles in the ambient aerosol.”

p. 17608 / l. 9: Please replace “spores” by “fungal spores” to make sure that you are not talking about bacterial or plant spores

Corrected

p. 17608 / l. 9: Here, “pollen” (also “fluorescent polystyrene spheres”) are mentioned as biological standard particles for the chamber experiments but the results of these experiments are not shown in this paper. If aspects are omitted in the study, please do not advertise them in the abstract. Please also decide if you want to use the plural ‘s’ or not: in p17608 / l. 9 it is “pollen”, in p. 17609 / l. 8 it is “pollens”. By the way, is there a specific reason to not

implement the pollen results in the study? Pollen are one of the major PBAP classes and it would be interesting to see how the WIBS responds to them.

We used the fluorescent PSL spheres only for calibration purpose and one asymmetry factor (AF) plot for these spheres are presented on Figure 1. Because of their large sizes, it was not possible to disperse pollen successfully into the chamber. Therefore, we did not implement the pollen results in this study and will exclude from the abstract as well.

p. 17609 / l. 10: Please add an appropriate reference for PBAP as CCN.

A reference will be added here. (Petters and Kreidenweis, 2007)

p. 17609 / l. 12: What is meant with “atmospheric processes”? The cloud microphysics (CCN and IN) is already mentioned earlier.

Avoiding going into details, we wanted to admit that aerosol particles could affect the abundance and distribution of atmospheric trace gases by heterogeneous chemical reactions and other multiphase processes. “Atmospheric processes” include also these different processes.

p. 17609 / l. 19-20: Replace “Mannitol” by “mannitol”. What does “organic carbon” mean in this context?

Elbert et al. (2007) analyzed aqueous droplets containing carbohydrates for the investigation of wetly discharged fungal spores. Here, organic carbon represents this organic aerosol.

p. 17609 / l. 25: Mention the “specific size range”.

Please see the answer to Review1#, P17609, L25

p. 17610 / l. 3: Jaenicke et al. 2007

Corrected

p. 17610 / l. 28-29: Ascomycota have also developed active wet discharge mechanisms (e.g. Trail et al., 2005) and do occur in substantial concentrations in the atmosphere [e.g. 30% (Fröhlich-Nowoisky et al., 2009)]. Your argumentation is very focused on Basidiomycota – is there a reason?

We focused mostly on Basidiomycota (BMC) rather than Ascomycata (AMC). The reason for that, Nowoisky et al. (2012) found that in Germany (a continental area) diversity of BMC is greater (65%) than of AMC (35%). Nevertheless, we agree to include Ascomycata to the manuscript and state that AMC has also the similar properties like BMC and can develop active wet discharge mechanisms. This will be added to the revised manuscript.

p. 17612 / l. 8: I would not call four months a "short period".

Please see the answer to Review1#, P17612, L8

p. 17612 / l. 15: Make "FBAP" instead of "FPAB"

Corrected

p. 17613 / l. 19-20: The sentence "These individual channels provide essential information about the nature of the detected particles." is imprecise and too strong. The words "essential information" and "nature" sound as if the WIBS provides information about the type of particle (e. g. species, composition, etc.) which is not the case.

We agree with the referee and will tone down the language here. First of all, we aim to discriminate biological aerosol from non-biological aerosol. On the other hand, WIBS-4 provides multi-dimensional single particle data, which can be analyzed with cluster analysis methods. A first study has shown that more information about the nature of biological aerosol particles can be extracted from WIBS data in this way (Robinson et al., 2012).

p. 17613 / l. 20-22: It would be helpful to add a statement why the combination F1/F3 is promising and why channel F2 is not used for the analysis although it provides additional information. Isn't it possible that the combination F1/F2/F3 provides an even higher sensitivity for biological particles?

The reason why we choose to use the combination of F1 and F3 to measure the biological aerosol is that we wanted to be comparable with previous WIBS studies (Gabey et al., 2010). Moreover, due to the high cross-sensitivity of F2 to non-biological we did not include channel F2 to the manuscript.

p. 17616 / l. 17: "... bacteria and spores, never saturate the fluorescence detectors" – I guess this refers to the samples the authors have investigated in the chamber experiments. Or does this statement mean that all bacteria and spores in ambient air do "never" saturate the detectors? This is a strong statement – what is the evidence the authors can provide?

The statement "...bacteria and spores, never saturate the fluorescence detectors" refers to the samples we investigated in the chamber experiments. In the case of non-biological aerosols, we observed some false positives which are most likely due to the high number concentration of aerosol in the chamber. On the other hand, these false positives (saturated fluorescence channels) were not observed in the ambient data. So, we assumed that for our data set, it would make sense to exclude the events which saturate the fluorescence detectors. However, this may be different for other type of bacteria or fungal spores in ambient air and the dependence of the fluorescence intensity on particle size should be further investigated.

p. 17618 / l. 19: Please provide some information in the experimental section where the biological standard particles (e.g. Penicillium notatum) are derived

from. Are the fungi cultivated to harvest the spores? Are all fungal hyphae separated from the spores?

Please see the answer to Review1#, P17618, L19

p.17618/l.25 to p.17619/l.13 and Fig. 3: How do you explain the obvious difference in the FBAP size distribution between Fig. 3a and Fig. 3b? For the F1-mode: Fluorescent particles occur in wide size range 1-2.5 μm whereas in the F1/F3-mode there is only a narrow peak of fluorescent particles $\sim 2 \mu\text{m}$. The fungal spores should have a defined and similar size distribution in both modes. The contribution of 'fluorescent' ammonium sulfate particles is too weak (1%) to explain it. Moreover, I am not really convinced by the viability explanation. Fungal spores as a reproductive unit do not really have an active metabolism. Do they provide a measureable NADH-signal? Moreover, it has been reported that there is a negative correlation between viability and autofluorescence intensity of fungal spores (Wu and Warren, 1984).

Fig. 3a and Fig. 3b represent the fluorescence data for the same aerosol mixture. However, the main difference between two plots is, Fig. 3a uses only the F1 detection, while Fig. 3b uses F1&F3 detection. A likely explanation for the discrepancy between Figs. 3a and 3b is that channel F3 has a lower sensitivity than channel F1. However, it's also conceivable that a fraction of fungal spores (the smaller particles) does not exhibit NADH-like fluorescence (channel F3). Either of these explanations would result in different N_{F1}/N_T and $N_{F1,F3}/N_T$ ratios which are $\sim 35\%$ and $\sim 20\%$ respectively. Note that in our mixture of spores with ammonium sulphate particles a value of 40% corresponds to 100% detection of the spores.

In the case of viability, we agree with the referee. It might be really speculative at this point to say that $N_{F1,F3}$ represents only viable bioaerosol. Although we measured NADH-like signal from fungal spores, we do not have any other evidence to prove our hypothesis about viability.

p.17622: A heading is missing here: "3.1.4 Bacteria"

That section will be separated by a heading as suggested.

p. 17622: Again, it would be helpful to have some information how the bacteria were cultivated and prepared for aerosolization. What bacterial species have been used? What is their physical size? How long did they stay in the chamber?

Pseudomonas syringae type of bacteria isolated from cloud water was used for AIDA cloud experiments. Bacterial solution was nebulized into the chamber within several minutes and was characterized for ~ 30 minutes after. Meanwhile, WIBS-4 sampled directly from the AIDA chamber for 25 minutes. After the aerosol characterization, a cloud activation of the aerosol was performed before the chamber was evacuated and cleaned. Typical AIDA cloud expansions take about 10 minutes (details of the AIDA experiments can be found e.g. in Möhler et al., 2008). Average residence time of the bacterial

species in the chamber was approximately 1 hour. The physical size of the bacterial strains was around 1.0 μm . This information will be added to the caption of Figure 3.

p. 17622/l.14: Maybe you mean "cells" instead of "species".

We used bacterial strains. This will be also added to the revised manuscript.

p. 17622/l.1-21: In Fig. 6a and b there is again a pronounced difference in the particle size distribution. Fig. 6a shows a large amount of material in the size range of 0.8-1.5 μm . Is this an artifact of the aerosolization process? Do you have any information about the identity of this material? Moreover, in Fig. 6b a constant signal around 1.5 μm is shown. The narrow peak seems to indicate the presence of the bacterial cells. What is the average physical size of the bacterial cells that have been sampled? Is this consistent with the observed FBAP signal?

Pronounced difference in the particle size distribution in Fig. 6a and b is most probably because of the lower sensitivity of the channel F3 compared to the channel F1. Although rather speculative at this point, the difference between the two figures could also be an indication for the viability of the bacterial cells. While Fig. 6a shows all bacteria, Fig. 6b represents only the viable fraction.

References:

Robinson, N. H., Allan, J. D., Huffman, J. A., Kaye, P. H., Foot, V. E., and Gallagher, M.: Cluster analysis of WIBS single particle bioaerosol data, Atmos. Meas. Tech. Discuss., 5, 6387-6422, 2012.