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Interactive Comment

Interactive comment on "Observations of fluorescent and biological aerosol at a high-altitude site in Central France" *by* A. M. Gabey et al.

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General Comments This paper was quite easy to read, despite its complex subject matter. The topic was interesting, and the site of the study was unique, which made the results quite thought provoking. My main issue was with the actual motivation for the work. The title employed the term "observations" which raises the question as to the intended purpose of the study. To my mind, use of the term "observations" implies that the results would simply be published, with little/no interpretation. Further analysis would result in a paper entitled "a study of", rather than observations", in my opinion. If this was intended to be a baseline study, then more time should have been allowed for

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a proper longitudinal study, which would reasonably be expected to cover at least a full 12 month period, or samples representative of each of the four seasons. With only 10 days of sampling, it is somewhat inappropriate to be making generalizations from the results, in particular regarding the “diurnal” pattern observed. Air sampling results are generally quite ephemeral – the results depend on so many variables, and thus long-term sampling is considered much more meaningful than a single event sampling. In particular, with the changing weather pattern observed within the campaign period, the actual sampling time was further sub-divided into several even shorter periods. If, however, the purpose of this campaign was a pilot study or similar, then the shorter period would be appropriate. In any case, I would have expected more information about the surrounding topography, and the types of land-use nearby (farms, roads, industry), including distances from the sampling site, to be provided. This data is essential to interpretation of the results.

Response: As referenced in the manuscript the influences on the Puy de dome site have already been characterised and we do not seek to replicate this work (Venzac et. al. 2008). The current data represents a surprising observation: that the fluroscent aerosol fraction is significant downwind of major conurbations and that UV-LIF and bioaerosol counts do not necessarily match better in a clean biogenic emission source region-environment than they do in more polluted locations. This has consequences for comparison of UV-LIF and filter techniques in future studies and we feel this information is useful to the measurement community irrespective of emission season. In this sense it can be considered a pilot study. We state clearly in the conclusions that the data is limioted due to lack of seasonal data and also indicate that the highlighted large fractions may indeed be seasonal which requires further long term measurements to confirm so we do not overstate the representation of other times of year.

Specific Comments The first two paragraphs of the introduction refer to several studies involving PBA counting methods using viable (culture-based) counting methods. Such methods have been variously reported as capable of detecting from <1% to <10% of

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the actual numbers of living bacteria and fungi. This phenomenon is mainly due to the harsh conditions of the atmosphere, which cause sub-lethal damage to most bacteria (more so to Gram negative bacteria than Gram positive) preventing them from growing in culture. For this reason, it is inappropriate to use any numbers obtained by culture techniques to compare with fluorescent-based methods. Referring to your reference (Harrison et al, 2005) it should also be noted that qPCR methods are also inaccurate, as they measure ALL gene copies in a sample, which may include free DNA from dead/lysed bacteria.

Response: This is not correct. The studies referenced are: 1. Harrison et al (2005), which explicitly states total cell counts were performed rather than culturing; 2. Matthias-maser et al (2000), which used "single-particle analysis" – a mixture of electron and optical microscopy; 3. Bauer et al (2002), which used epifluorescence microscopy to enumerate spores. None of these represent culture techniques and, although they are subject to uncertainties, the referenced works actually represent the most sensible candidates for comparison with a particle counter available.

Page 3034, Line 23: the authors conclude that the measurements at sites in Austria and Switzerland are able to be taken as "representative of the background PBA in Europe". I would challenge this assumption. A background study at the actual site is required – surrounding activity and other variables are essential knowledge before such an assumption could be accepted. I would be very surprised if a single background PBA could possibly apply for all of Europe.

Response: There is a paucity of published PBA measurements in the literature and the manuscripts referenced in this paragraph provide consistent results from different field sites.

Page 3037, lines 4 – 9: Mention is made of the vehicular traffic present at the site. Was this traffic logged/recorded? What surface material was on the roads? What type of fuel did the vehicles use? What about the movements of the people present

– numbers, activities, proximity to the recording equipment? What precautions were taken to ensure that any people present in the area did not interact with the sampling? How far away is the military installation and what activity occurs there? Is it manned? If so, how many personnel are present?

Response: Only a few persons have access to the research station and to the military installation. Moreover, local contaminations due to traffic, people activities etc., if they exist, could be routinely tracked and filtered from the analysis using the black carbon and total number concentrations (for traffic) and supermicron aerosol size distributions for eventual dust resuspension. In case of sharp peak concentrations in the time series, which was extremely rare at this site, these local contaminations can be excluded from the data set. Sampling heads were protected in a closed area located on the roof of the station, not accessible to persons other than the scientist in charge of the sampling.

Same page, line 10: as a typical "rural" background, what crops or other plants were present, at what distance?

Response: The station is located in a protected area where crops are not permitted. The area is forested over several tens of km west, and up the city of Clermont Ferrand on the eastern part (16 km).

Same page, line 24-26: air masses are variously reported as being "anthropologically influenced". How was this factored into the measurements?

Response: This information is provided to help the reader interpret the only significant change in the size distribution that occurs during the measurement period.

Page 3039, line 1-2: the counting uncertainty of the DAPI stained filters is noted as being 25% for bacteria and 20% for spores and yeasts. How were these figures determined? Both sound about right, but are high for basing analytical work upon. Any comparison with other methods (WIBS-3) would be highly doubtful with such margins for error. This is particularly of concern when we read on page 3040 (line 9) that

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the study intends to challenge the “common assumption . . . of the link between NNADH and the bacterial count”. Given the issues involved in obtaining an accurate bacterial/fungi count, not addressed in this paper, this study would not be capable of supporting such a challenge.

Response: From a filter of a microbial culture, 30 optical fields were counted under EFM by 3 different experimenters, and the counting uncertainties for bacteria and for spores and yeasts were obtained from the standard deviation and the average of cells counts per fields. Obtained bacteria counts vary from one sample to the other by a factor of 10, which is higher than the variability within a sample. Hence, we believe that the variability in bacteria/fungi counts between samples can be compared to the variability of NNADH between samples.

Page 3046, lines 19-25: I am concerned that the assumption of a measurement declining progressively with each successive impactor sample is accepted without question. This should have been followed up, perhaps with some laboratory simulation testing of the equipment. Such a trend may also be due to decreasing sensitivity of the equipment over time, or any one of a number of other factors. When testing indoor air, the phenomenon of “stripping” is generally observed, whereby the number of particles is reduced over time due to their removal by the testing equipment. This is less common in outdoor air, where the air is replenished quickly (especially if the site is windy), but it is at least one reason why numbers of particles fall over time. The authors might try a bit harder to prove an actual reduction, rather than an instrumental anomaly.

Response: A slow decreasing trend in concentration is observed over the period of impactor sampling for all three instruments (WIBS, APS, and impactor samples). This consistent behaviour between different techniques cannot be a phenomenon of stripping in the APS instrument. Stripping cannot occur on impactor samples, as impaction substrates are changed for every sample, and the flow of large aerosols is not in contact with the impactor walls. Simialr comparisons between WIBS and other instruments over long periods (e.g Gabey et al. 2010, Huffman et al. 2013, ACPD and Robinson et

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al. 2013, AMT) can show increasing trends. Furthermore the WIBS, a single counter, was calibrated regularly with fluorescent particles to test any change in detection limits. There was no change over the period of sampling.

Page 3048, line 15: What is “casual” observation? This size range is recorded in basic microbiology textbooks, so it is not a surprise. The presence of clusters, chains and other groups, as well as adherence to dust particles for example, so that larger particles are detected, is also well documented.

Response: It was noted at the time of measurement that most of the cells were this size, but a more detailed analysis of size was not performed. We will rephrase this for clarity.

Page 3050, line 10: Statement “pollen in unlikely to be found in such concentrations” requires explanation and support. Why is this so? In any case, pollen is easily recognized microscopically, so should have been detected in the EFM slides, better evidence for its absence than being “unlikely”.

Response: The referee is correct that pollen is easily recognised microscopically. Additionally the DAPI staining technique employed is suitable for pollen. Analysis found no pollen on the filters. We will include a short comment in the revised manuscript to clarify this point.

Same page, line 11: as mentioned previously, the lack of systematic agreement between NNADH and bacterial counts is likely due to the lack of accuracy in the latter.

Already answered on the comment on page 3039

Page 3051, line 1: Once again, the comparison with culturable bacteria is not appropriate. In the case of air testing, culturable does not equal viable!

Response: This reference is in place for the purpose of comparing the size, rather than the concentration, of bacteria measured at continental sites to that of cluster 2 to show that they are consistent.

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Page 3053, line 23: Here the authors mention the “seasonal agricultural contribution” to which I was referring earlier. This should have been included in the site description, and the extent to which it may have affected the outcomes should have been measured and included in the results analysis.

Response: We feel that the limitation of the study sample period is suitably caveated as described above and in the conclusions and that the results are representative of the site during summertime for the air-masses sampled.

Technical comments The text of the document is largely written in the present tense. This is not appropriate, according to scientific publication conventions, and also to the implication that the results are happening currently, rather than in 2010.

Response: We will address this where appropriate during the preparation of a revised manuscript.

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