

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 The paper centres on a very short campaign to detect bioaerosol (and other) found at high-altitude in France. As such it does provide further interesting data, particularly regards the use of the WIBS real-time approach in a field campaign, but I am not sure if any wide-ranging or definitive conclusions about airborne bacteria, pollen, spores etc can be made from the limited study period employed. (v.i. at one point, 3049.25, an important conclusion is given based on just two nights of comparison.) Essentially there must be some more clarification about the way the experiments were performed, why they were performed how the different detection approaches

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complement each other in particle quantification, sizing/shaping and finally the contribution that the work makes to our understanding of airborne bioaerosol distributions in Europe. Detailed comments/questions are found below.

Specific Comments 3033. 16. As discussed in the manuscript at a later point the two excitation wavelengths utilized in WIBS do not uniquely excite tryptophan/NAD(P)H. (NB. some consistency here please on NADH/NAD(P)H use in the paper). Hence I would leave these assignments out here in the abstract...the issue requires the nomenclature discussion given in the main body of text.

Response: We note the inconsistency in nomenclature and this will be addressed in the revised manuscript. We thank the reviewer for their comment. To clarify this we will replace:

“(280 nm; Tryptophan)” With “(280 nm; associated with Tryptophan)”

And “(370 nm; NADH)” With “(370 nm; associated NADH)”

And “The WIBS-3 features two excitation and fluorescence detection wavelengths corresponding to different biological molecules” With “The WIBS-3 features two excitation and fluorescence detection wavelengths designed to detect different biological molecules, although non-biological interferents also contribute.”

3033. 20. Gives two fluorescent populations of interest found: 3um and 2-3 um.... but no real message as to what species they correspond to as an important conclusion from the results.

Response: There are no species assigned directly to the two fluorescent populations discussed here as it is not possible to determine the species without more detailed analysis. Cluster analysis provides further information on the particle populations which allows for species assignment and is discussed in the next sentence of the abstract. This information is worth including so that the particles may be retrospectively identified as more laboratory studies are performed. Additionally fluorescent material

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at the site is unlikely to be strongly influenced by false positive associated with vehicle emissions.

3034. 4 The statement here represents the first reference to the important contribution of non-PBA to the WIBS3 data. Hence it does require the listing of exactly what fluorescent, non-PBA are likely here and what discriminatory WIBS signals they give if the suggested species are introduced to the instrument (say back in the laboratory). This would help pin down some of the important interference issues raised in this paper

Response: Possible conflatory non-PBA materials are discussed in the introduction and references therein (Pg. 3035 In 18). Laboratory characterisation of interferents is ongoing.

3038.4. What were the typical (or high-low limits) of the wind speeds measured at pdD over the campaign period? Is the particle entry point tubing vertical to the WIBS or bent to a right angle? In other words can the incoming PBA be blown away by the wind if it is a vertical arrangement because the WIBS does not pump more effectively than the wind speed? What is the length of tubing used between entry point and WIBS? What is the tubing made from? How high above the ground is the particle inlet point? These matters can lead to major discrepancies between reported "measurements" and actual ambient concentrations unless they have been accounted for. Could/dida right angle capture, for example, provide differing counts to a vertical arrangement on a windy day? That is why knowing the wind speeds present at the site over the campaign period is particularly important to the ultimate counting conclusions of this manuscript. If the wind speeds were high(ish) and only a vertical arrangement was used then the data reported is likely suspect.

Response: Figure 1 shows the wind speed at the site over the measurement period (Mean = 2.2 m/s, STD = 3.9 m/s, Median = 1.9 m/s.)

The WIBS and the impactor were sampling from the WAI and not directly from the ambient air so there will be no losses due to the wind overwhelming the internal pump

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of the WIBS or enhancement from wind ramming. The WAI has an upper cut size of 30 μm for wind speeds of less than 6 m/s which is significantly greater than that of the WIBS (20 μm), thus the WIBS measurement will be representative of the ambient air. WIBS and impactors were placed vertically below the sampling line splitter, which bends are less than 30 ° from the main inlet line (vertical). The inlet entry point is three meters above the roof of the station, the inner tube is made of stainless steel. There is no relationship between the wind speed and the concentrations of NNADH.

3039.2 Could there be an expansion of what is actually meant by EFM uncertainties here...particularly recognition errors? Does this statement indicate issues between bacterial types or between a bacterium and a fungal spore for example? What were the actual proportions of the different types of biological particle observed here over the campaign? There must have been some assignments and size/shape information.

Response: The recognition errors are between a bacterium and the small fungal spores. But the EFM uncertainties of 25% for bacteria and 20% for spores and yeasts are mainly linked to the manual counting. The experimenter counts the biological particles directly through the microscope lens; there was no photographic system capture in the EFM used in this work. For this reason, we are limited to give some assignments and size/shape information.

There was unfortunately a mistake in the manuscript regarding the latter part of this comment:

Page 3042, line 22 "Whilst quantitative size information is not available from the filter data, the bacteria collected were observed during analysis to resemble "bacilli with fluorescent dimensions 0.5 to 2 μm . A smaller number of the bacteria collected were 2 to 5 μm in size." Elliptical fungi and yeasts were typically 10 to 30 μm in size and mainly found on the first (D50%=9.9 μm) stage."

The inner quoted section of the sentence is wrong; it comes from an inversion between the word "cocci" and "bacilli" which was not spotted prior to the final version being

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submitted. This sentence will be replaced with:

“Whilst quantitative size information is not available from the filter data, most of the bacterial cells collected seems to be cocci bacteria (spherical shape) with diameter of 0.5 to 2 μm . And a smaller number of the bacteria collected have a rod-like shape (bacilli) of 2 to 5 μm in length. Elliptical fungi and yeasts were typically 10 to 30 μm in size and mainly found on the first ($D50\%=9.9\mu\text{m}$) stage.”

3040.17. I think it is a mistake for this paper to only briefly discuss the AF data...shown in Figure 3. It could give a lot of information here about bacteria, other PBA or non-PBA. Bacteria are generally spherical when at about 1 μm diameter....that is close in size and shape to certain fluorescent chemical interferants (although these are often $<0.5\ \mu\text{m}$ and spherical). However many bacteria are rod-like at the larger dimensions....maybe 3 μm long and 0.5-1.5 μm wide. (What species are 5 μm in diameter as given on 3042.24?) What clues as to identity are actually given by the EPF results here? (There must be some if only % spherical to % rod-like data). Does the WBS measure some of these PBA on the axis or off the axis thereby giving two different apparent “sizes” for the same species? Also see next point.

Response: The question regarding the EPF analysis was answered in the previous question as a correction to the text.

It is true that rod-like non-spherical particles even particles down to micrometre sizes will tend to align with their long axis parallel to the airflow, especially where a sheath-flow regime is used as in WBS (Kaye et al. 1991, 1996). The degree to which elongated airborne particles align with the flow depends on several variables including particle size, mass, initial orientation, airflow velocity (particle Reynolds number), etc., but for a given instrument the reorientation of elongated particles is predominantly a function of particle aspect ratio – the greater the aspect ratio (directly proportional to AF) the greater the alignment with the flow. For a given species of particle, such as rod-shaped bacteria, the particle orientations will be distributed around the axis of flow, and this

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results in a single distribution of AF values. Generally, an AF of 20 would not be regarded as spherical but rather ellipsoidal, unless the particle size was so small that signal to noise in the AF detectors artificially raised the computed AF value.

3045.24. Reference is made here to Figure 3 and the small feature at AF11 and the larger one at AF20. First of all surely the AF11 peaks are not small in comparison to AF 20! What sizes of particle do these two AF features refer to? In any case AF11/AF20 values also surely indicate spherical particles. (The range of AF is 0-100 from Figure 3). Thus AF20 is still a low value and indicates spherical....but if you have 3 μm sized bacteria then these are more likely rod-like! In summary, I think there should be a more coherent and connected discussion in this paper of AF/size/identity using WBS plus EPF...it would help the reader. Section 4.1 discussion does not include any quantification of the PBA general types likely here or even make comparison to any quantitative EPF results....that should themselves be made more explicit (section 2.3). I began to think that bacteria were not important at all here as currently written....and I should at least be able to understand that by this point in the text. Is it possible that no bacteria were detected by WBS3? This might explain the discrepancy referred to in Section 4.2 regards “clustering”. What evidence is there that airborne bacteria cluster to large sizes in the WBS3...from actual lab experiments? (See next point).

Response: The significance here is that the AF from the two phases of the diurnal cycle are different, suggesting that the particles are not made of the same material.

From laboratory experiments it was found that an AF of 20 is aspherical/ellipsoidal (Kaye et al. 1991, 1996). Additionally as rod-like bacteria are aligned with the flow it would be expected that the measured AF would be lower than if they weren't aligned due to the cross-section they would present to the detector. For these reason it is important to not overstate the AF parameter as it proves only an estimate of asymmetry and it is not an absolute measurement.

At no point do we suggest that bacteria forms clusters in the WBS.

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3047.9. Reference is made to WIBS3 limited sensitivity at small sizes. What does this mean...what do the calibration curves look like for sizes <2 and >2 um? In other words if you cannot detect <2 um particles effectively then you will not detect many bacteria...even some spores....likely important here....and what does that all mean to the overall analysis given in this paper?

Response: Figure 2 shows the information required on the WIBS3 sizing function that relates the light scatter signal magnitude to particle size. The red plot is a combination of the light flux on the forward and side scatter detectors. This curve is produced using Mie calculations based on the WIBS3 geometry taking into account any electronic and software adjustments made when combining the forward and side scatter signals. The WIBS3 software is coded with a polynomial fit to the red plot and a scaling factor to match the WIBS3 response to 3um PSL with the equivalent size on the plot. The polynomial fit to the red plot is effectively the WIBS3 size calibration curve. The limit of detection (in terms of particle size) is dependent on the magnitude of elastic scattering from the particle when it passes through the CW laser beam. In the case of WIBS3, the determination of particle size from this scattering is based on a combination of forward scatter received by the QUAD-PMT and side scatter received by the FL2 channel detector. The smallest particles WIBS3 can detect from the light scattering signals are around 0.7 to 0.8 um, depending on particle refractive index. The magnitude of the fluorescence from such a particle could be very small or even undetectable, depending on the particle material. The 'limit of detection' as far as fluorescence is concerned is determined by the variability of the 'background' fluorescence signal when the xenons are fired in the absence of a particle (ie: in Forced Trigger mode). In our experience, the fluorescence from individual airborne bacteria, vegetative cells or spores, was sufficient to be detected above this background variability, so single bacterial cell fluorescence detection was achievable. The instrument can distinguish between fluorescent and non-fluorescent particles at small sizes. Section 5 of Robinson et al. (2013) demonstrates this using fluorescent and non-fluorescent 1 um polystyrene latex spheres. Laboratory experiments performed in the AIDA chamber have shown that

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the WIBS3 could reliably detect fluorescence from bacteria as small as 1 um (Gabey, 2011).

3048.23. The diurnal cycle referred to generally has a relationship to relative humidities. What were the RH as a function of day/time measured here?. Such data is important...as well as temperatures.

Response: We will include a discussion of the diurnal cycles of temperature and humidity and their relationship to the observations. The observed diurnal trends are shown in figure 3.

3050.3 Is enough known about the relative intensities of fluorescence for any airborne PBA to say that all bacteria can be discriminated from fungal spores etc by signals originating from the two excitations? Again....the two excitation wavelengths used in WIBS do not correspond to tryptophan and NAD(P)H contents entirely....many other absorbers can be present in different ratios for different species.

Response: No. This caveat is addressed in the introduction and further laboratory and field validations are required. See Pg 3035 In 28. Preliminary characterisations can be found in Gabey (2011.)

3050.23 How can the cluster technique come up with a distribution set at 1.5 um if WIBS3 is not sensitive at <2 um? This point, in a sense, goes to the heart of the issue that must be clarified in this paper for readers. Is this set being assigned as non-PBAP because they are non-fluorescent even though the WIBS3 is not sensitive to detecting such particles? What were the previous laboratory characterizations, using WIBS3 that leads to the conclusion made about Cluster1?

Response: The topic of the WIBS sensitivity at small sizes has been addressed in an earlier response. Robinson et al. (2013) describes the frame work for classifying cluster analysis results. We will include a reference to Robinson et. al. (2013) in this sentence for clarity.

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3051.7. Data in table 1 do not indicate high AF for clusters 1 and 3....they are likely spherical at 22 and less...Cluster 2 at 33 is perhaps on the edge of being slightly asymmetric. At a size of 7.6 um,could cluster 3 be associated with any pollen or spores monitored by the EFM approach?

Response: See previous comment regarding the interpretation of the AF parameter. The AF presented here is consistent with ellipsoidal particles.

We didn't measure any pollen on the filters so it is likely to be associated with spores, however, no quantitative size information was available from the filter measurements for comparison to the WIBS3 optical sizing. But biological cells of 7.6 um could be associated to fungal spores (mainly yeasts).

How did the team calibrate the WIBS3 for AF relationship to shape for the types of PBA proposed here e.g. *P.syringae* ?

Response: The WIBS3 is not calibrated for particle type or AF. Only size and fluorescence are calibrated using polystyrene latex spheres. PBA types are classified by statistical analysis of the single particle data as presented in Robinson et al. (2013).

My summary conclusion for this paper is that it will have to make a more convincing case for showing that in this campaign the WIBS3 instrument detected any bacteria at all, and if so what types they were...including the likelihood of them becoming agglomerated.....possibly by parallel laboratory studies on the WIBS3. Once this is established then the important point that this paper makes about non-fluorescent particle contribution to the overall burden in this campaign in France can be better evaluated. Currently I cannot do this but with a clarified manuscript I hope to be able to attempt to do so because it represents a very important topic for on-line analyses of ambient bioaerosol.

Response: Further laboratory analysis of bacteria will be required if further sub-types of bacteria are to be successfully categorised using the WIBS in conjunction with cluster analysis, however, we feel that we have adequately demonstrated that the WIBS has

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detected particles consistent with bacteria at the measurement site. At no point in the paper do we suggest that bacteria has become agglomerated in the instrument. We only refer to agglomeration as a statistical method to employed by the specific cluster analysis technique we use to classify particles into groups of similar types. For clarity we will ensure that the revised manuscript caveats the WIBS results such that we don't overstate the results of the cluster analysis in terms of particle classification. We will only state that the cluster averages are consistent with previous measurements of particular particle types.

References

Gabey, 2011, 'Laboratory and field characterisation of fluorescent and primary biological aerosol particles' PhD thesis, University of Manchester.

Kaye , P H , Eyles , N A , Ludlow , I K & Clark , J M., 1991 , ' An instrument for the classification of airborne particles on the basis of size, shape and count frequency ' Atmospheric Environment , vol 25A , no. 3/4 , pp. 645-654 .

Kaye , P H , Alexander-Buckley , K , Hirst , E & Saunders , S., 1996 , ' A real-time monitoring system for airborne particle shape and size analysis ' Journal of Geophysical Research , no. Atmospheres , pp. 19215-19221.

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, doi:10.5194/amtd-5-6387-2012, 2012.

Interactive comment on Atmos. Chem. Phys. Discuss., 13, 3031, 2013.

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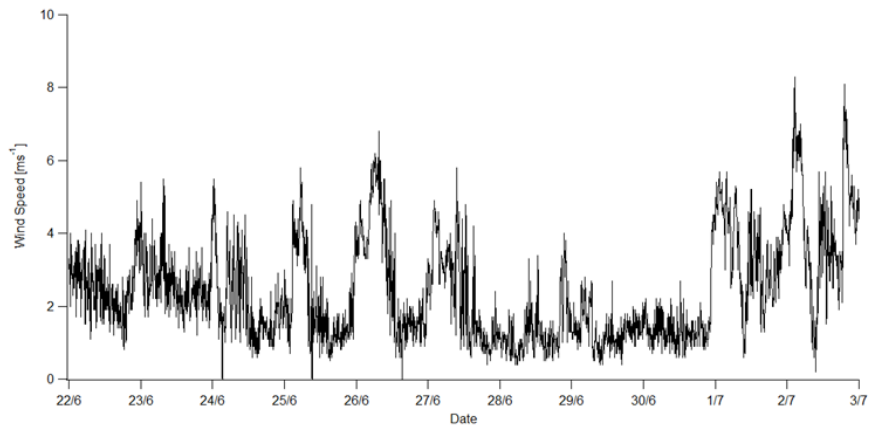


Fig. 1.

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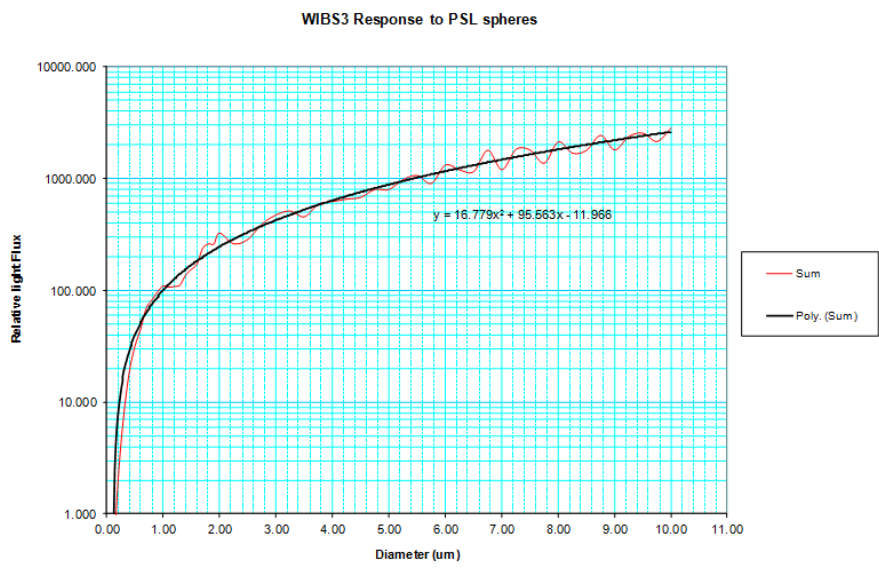


Fig. 2.

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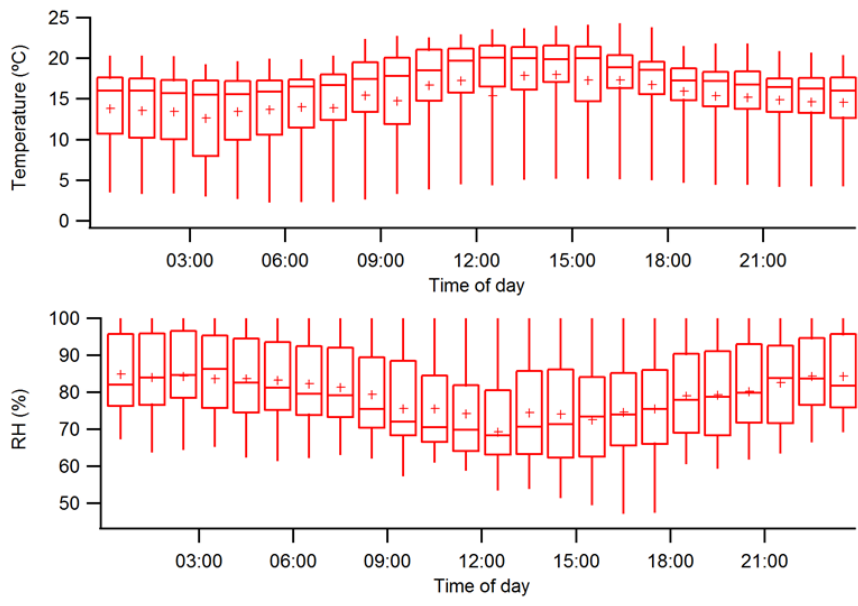


Fig. 3.

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