

## Anonymous Referee #2

For efficient exchange we have copied the review text below in black font and have responded to each section directly, offset and in red font. Italicized text shows changes to manuscript text. Page and line numbers refer to online ACPD version.

This paper is a summary of long-term recordings of fluorescent particles (termed FBAP) from a boreal forest in Finland and a high elevation forest in Colorado. The paper describes the seasonal cycles of total fluorescent particle number concentration contributions and sizes mainly in the coarse aerosol size mode with maxima observed in summer as opposed to winter where snow cover at the Finnish location may have inhibited emissions of some sources of FBAP. Different mode behaviour at the two sites were reported with the broader mode occurring at the US location which the authors attribute to different (or perhaps additional?) FBAP source there. As with many previous studies the FBAP concentrations correlated with RH in each season except at the Finnish site where a plateau was observed followed by a decrease above a threshold at 82% which is attributed, though not verified, by inhibition by dew formation. Finally they show that the both FBAP and total aerosol concentrations scale with rainfall intensity during rain events, a phenomenon which has been reported previously but here an attempt is made to quantify that enhancement. They also attribute an enhancement in fungal spore (assumed) concentration increase following rainfall events due to subsequent RH enhancement as periods advantageous to spore fruiting and germination. The conclusion is that any parameterisations of spore emissions should also account for both diurnal and rainfall/RH enhancements to full capture any “bioaerosol” emission fluxes for model studies.

The paper is a very good summary and analysis of a long-term data set and should be published as an important contribution to the field of study.

We thank the reviewer for his/her constructive comments and for the recommendation that the manuscript be published.

There are a number of presentational issues associated with the figures, in particular the inconsistent use of units, which was confusing to this reader in terms of allowing comparison with other studies and I also had difficulty in reading some of the figures. These are hopefully minor issues that can be addressed in due course. I note these have also been thoroughly highlighted by another reviewer and some corrections have already been addressed by the authors themselves post submission, so I will not repeat these here.

The figures have been cleaned up and units are now consistently in  $\text{cm}^{-3}$ .

Focusing more on the scientific results of the study there are a number of questions and minor clarifications that would help the specialized reader as well as the wider community with regard comparison with related studies and assimilation by emission model studies. These are minor as I believe the authors have covered most of the relevant analyses within the limitation of the instrument used and ancillary data available. There is, as is usually the case with such real-time studies, a general lack of taxonomic fungal spore and bacterial micro biome identification in this paper so it makes it difficult to properly relate the seasonal-modal differences noted to specific and or different bioaerosol sources that might be active at each site. Additional references to previous such studies at the sites would be useful (if available, I note the Huffman study and references therein for the related Colorado study). I do appreciate the limitations of such studies in terms of the non-specificity of real-time UV-LIF for discrimination in this context. This should not however negate the interesting results and discussion points presented.

We have tried to include all references of direct relevance for the presented measurements and interpretation/discussion that are known to us. Beyond that we refer to a number of review and perspective articles with further references of general relevance for atmospheric bioaerosol research. We are not aware of further related studies, but if the reviewer could point us to such studies and provide additional references of direct or general relevance we would be grateful and happy to include them.

Page 17128: Section 2.1 Fluorescence emission is detected in the wavelength region 420-575 nm in the instrument used. It would be advantageous, in order to compare with other studies, to highlight the limitations of using this single UV waveband to infer biological particle type and hence infer contributions and possible emission scenarios due to meteorological response from the observed FBAP. For example this waveband will not provide information on biofluorescent materials dominated by Tryptophan (whose emission spectrum typically lies between 300-400 nm), and so the UV-APS will likely underestimate total FBAP concentrations and hence some emissions that could potentially contribute to the total particle concentration recorded. The response of the two categories, non FBAP and FBAP, to RH and rainfall as shown may therefore be conflated. This caveat should be included as although it is also accepted the situation can be improved by the removal of particles < 1 µm in size from the data set, this assumption is based on similar measurements, that are cited, but from a very different environment and based on removal of possible non-biological fluorescent material due to anthropogenic emissions which, as highlighted in the text correctly, would be minimal at the sites selected here.

*In response to the reviewer's comment we added the following text:*

*It has also been shown that ambient UV-APS measurements can inadvertently conflate biological material into the non-fluorescent category based on the fact that many microorganisms fluoresce only weakly and thus escape real-time LIF detection (Huffman et al., 2012). [Inserted at P17128, L14]*

Page 17126: There is some confusion between the description of UV-APS and WIBS. The latter is not used in this study. If this is a general comparison and summary of available technology (there are other instruments cited in the literature) then the difference should really be highlighted. The WIBS is a multi-waveband fluorescence and particle shape spectrometer as opposed to the UV-APS, which is a single waveband. The references cited for WIBS did not use the commercial DMT version of the instrument as implied here but rather the prototype WIBS-3 provided by Kaye et al. 2005. The technical and discrimination limitations of the newer WIBS-4 (which is more closely aligned with the cited commercial DMT version) and which were used in the cited references are described by Robinson et al. 2013 (AMTD). I would suggest the later reference be included.

*The WIBS is only mentioned twice, both in the second paragraph of the introduction as means of suggesting that both the UV-APS and WIBS generally detect fluorescent biological aerosol particles. We don't go into details comparing the instruments and do not intend to do a thorough review here of LIF bioaerosol instrumentation. Thus, we had not considered it appropriate to refer to the paper of Robinson et al., 2013 (co-authored by J. A. Huffman) discussing an advanced cluster method for WIBS data analysis. As the reviewer seems to consider the reference relevant, we are happy to include it in the introduction together with the reference to Kaye et al. 2005.*

Section 2.2. I understand the authors wish to be brief and have used references to provide details of the sample locations elsewhere, however even basic information such as the height of the forest canopy with respect to the measurement heights used in each location together with basic leaf area index for each site would be of benefit to those modellers wishing to make use of their observations for flux estimates.

*The following text was added to the manuscript following the reviewer's suggestion:*

*The average tree canopy height at the sampling site was 16.8 m, with leaf area index averaging between 2.5 and 3. For collection of meteorological data, the inlet was placed at 16.8 meters, however, the tops of the trees immediately surrounding the tower were below that of the inlet. [Inserted at P17129, L3]*

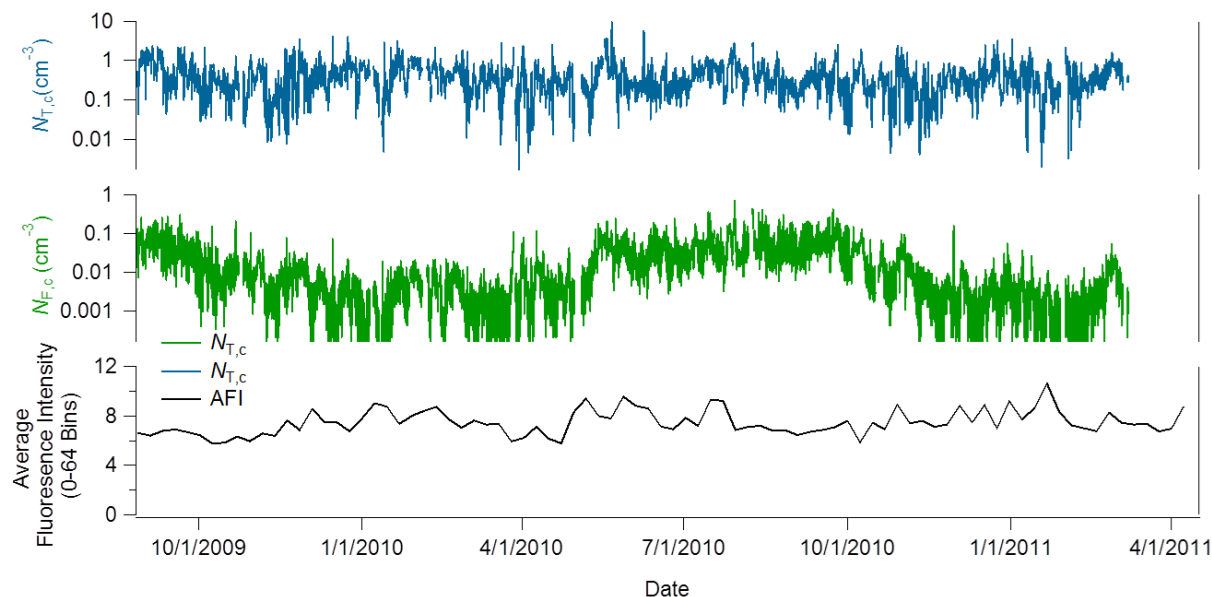
*Ponderosa pines sparsely covered the study area, averaging 10-20m in height, with leaf area index variable, but averaging ~1.9 (DiGangi et al 2011). [Inserted at P17129, L23]*

Page 17135 Line 6: Can the authors simply quote the relevant Dp-50% for their instrument here please? It is not clear from the statement here what this is. Can the authors provide some brief information on how and how often the instrument was calibrated and in particular how the fluorescence detector efficiency was monitored over this extensive period. Was there any decline in detection efficiency over this period (also see comment below).? For example did the mean fluorescence intensity in the dominant size modes from which they are inferring particle type etc change over the year?

The text was modified with the following information at P17128, L8 as suggested (italicized text added):

*“Though particles as small as  $\sim 0.3 \mu\text{m}$  can be detected, the particle collection efficiency drops below unity at approximately  $0.8 \mu\text{m}$  ( $D_{50} \sim 0.5 \mu\text{m}$ , Huffman et al., 2010; 2012).”*

The instrument response to particle size and fluorescence was monitored periodically using fluorescent and non-fluorescent polystyrene latex spheres (PSLs). The instrument was operated at a relatively low level of sensitivity, and so there was no drop-off in fluorescence detection as a function of time. The plot below shows the average fluorescence intensity of ambient particles observed at the Finland site (black curve) alongside the total (blue) and fluorescent (green) particle concentrations. The figure shows that there was no systematic change in average fluorescence intensity during the campaign, suggesting that there is no obvious downward trend that one would expect if the fluorescence detector was slowly losing sensitivity. The black curve was integrated over particles  $1.5\text{-}4.0 \mu\text{m}$  in diameter. The results are qualitatively similar for the Colorado site, with average fluorescence intensity averaged over  $1.0\text{-}3.0 \mu\text{m}$  and  $3.0\text{-}8.0 \mu\text{m}$ , but were not shown here for simplicity.



Page 17125. Line 27. The recent study of the upper troposphere microbiome by DeLeon-Rodriguez et al. (2013) [PNAS] over the US showed that the bioaerosols lofted to altitude by convective systems were in fact dominated by ice active methanobacteria which were taxonomically identified as being derived from plant surfaces (and better adapted to high UV environments). Fungal spores did not represent a key component so their contribution as IN for cloud processes and precipitation patterns is thus speculative at best. The potential contribution to health effects is well stated though.

The following text was modified [P17126, Line 4]:

*PBAP, as ice nucleators, could thus influence precipitation patterns through human- or nature-induced landscape changes (Morris et al., 2013), though it is uncertain what fraction of ice-active bioparticles observed at ground level may be lofted to form clouds. Biological particles can also be inhaled into the lungs or deposited in the nasal passages, causing respiratory disease, allergies, and other health effects (e.g., Franze et al., 2005; Pöschl 2005; Polymenakou et al., 2008; Despres et al., 2012).*

#### General Comments

With regard to the interpretation of fungal spore emissions. It has previously been demonstrated that “fresh” fungal spores exhibit significantly higher fluorescence intensity than the same species when aged by three months reflecting the decline in NADH metabolism as nutrient availability changes with season. Given the duration of the experiment how would such behaviour influence the authors quantification and interpretation of the “decline” observed? (see comment above, did the average fluorescence intensity within the dominant size modes at each site change?)

*The reviewer stated that ‘fresh fungal spores exhibit significantly higher fluorescence intensity than the same species when aged’. First, the relationship between fluorescence intensity is not always as clear as this. It has been reported in some cases that fluorescence intensity increases when fungal spores become inactivated (e.g. Wu and Warren 1984a,b). More importantly, however, the suggestion implies that the spores released in the forest will remain aloft for 3+ months before collection and detection. Particles of this size will have a short atmospheric lifetime and so we assume that spores observed in the air are likely to have been released recently (at least within days). There is uncertainty in this assumption, but the likelihood of a large fraction of spores remaining aloft, or even being re-suspended, after such a long aging process seems very unlikely. Following on that, see the figure and comment above about fluorescence intensity measured as a function of time through the studies.*

The different modal contributions at the two sites do indeed suggest different relative contributions from different emission sources. However, based on the very different leaf area indexes as well as the likely very different understory vegetation amount (negligible at the Colorado site I suspect) and hence fungal spore emission sources at each, can the authors suggest whether there are possibly different contributions from spore and bacteria sources above as opposed to below from the surface the measurement heights within the two canopies? Previous work has shown that fungal spore sources within canopies can be significant (Gilbert, G., *Biotropica*, 27, 2005) and hence the contribution from rain induced splash mechanisms can be very different in different forest types.

*The reviewer comments are intriguing ideas, however speculating on the contributions above and below the canopy would be beyond the scope of these measurements and this paper. However, an upcoming paper by Crawford et al. (to be submitted to ACPD in 2013) will, in part, address this issue.*

Section 3.1.3 Diurnal patterns. Although the diurnal patterns are shown in the SOM, it would be useful to show a graph of the average diurnal concentrations of total and FBAP for each site for general reference to the data tables shown in this paper. This would help place the discussion in better context rather than the Table used.

*We discussed this reviewer comment at length and made additional plots to show the diurnal aerosol patterns for each season and site. The message is not immediately obvious and clear, however, and we feel that no matter how we presented the plot the message was difficult to summarize quickly. Adding the plots would seem to confuse the already many messages of the manuscript. As a result, we have chosen to keep the diurnal plots in the supplement, with a brief summary of the key peaks in the table. Also since the observations seen in the diurnal patterns presented in the SOM are not different than what we discuss in the text, we found it unnecessary to repeat these observations in the text.*

Pages 1737-1738: The dew inhibition hypothesis coupled with the T/RH gradient discrepancy between surface and measurement height seems reasonable. However, one point of clarification from reading one of the cited references I believe is needed:

The review by Jones et al. (Sci. Total Environ 2004;326:151-80) (which the authors cite in another context) concluded that previous chamber and field observations of spore emissions are “consistent with moisture on the leaf surface preventing removal of spores due to surface tension effects.” It would be useful to reference the (Jones et al.) hypothesis as originally stated in this regard and then state that the data presented here also support this hypothesis rather than state “We hypothesize”. Or is your hypothesis different? Please clarify or expand.

The following text has been added:

*Jones and Harrison (2004) also summarized reported observations of reduced spore concentration after dew formation or rainfall, suggesting that rates of spores release were tied closely to changes in surface wetness. [Inserted at P17138, L3]*

The different RH responses are always interesting to see for spores, however again, whilst reference is made to the SOM it would be helpful to quote the spline fit results in a table in the main paper. Can the authors add uncertainty envelopes based on standard deviations on the curves in Figure 6, please. The data have been averaged into 100 RH bins. What is the accuracy of the RH measurements please, i.e. is this number of bins justified?

The spline curves added to Figure 6 are not discussed in the SOM, and are added to the figure simply to guide the eye along the trend. The spline curve is an empirical fit, without mathematical function to report.

The following text was added to the manuscript at P17130, L18:

*Reported accuracy for meteorological measurements made at the Colorado site are as follows: temperature +/- 0.3°C; RH +/- 3% for all RH values <90% and +/- 5% for all RH values >90%; precipitation +/- 0.25 mm. Reported accuracy for RH at the Finland site is within +/- 3%.*

In response to the reviewer comment we re-plotted Figure 6 in 25, 50, 100, and 200 bins with no qualitative change in the trend.

Some additional information: With respect to the response of spores to humidity changes. Some studies (Adams et al. 1986, see the review by Jones cited) suggest that in the field diurnal growth and changes in metabolism may in fact mask the effects of temperature and RH on emission. For example it is well known that leaf surface temperature in high insolation cases (e.g. Colorado) may be 10°C higher than measured in ambient air hence supporting the contention by the authors that the ambient T/RH readings may have been decoupled from the actual leaf surface conditions and hence would not be capable of fully describing the release mechanisms and so will not be adequately captured by the spline analysis as shown. I think this caveat needs to be emphasised, highlighting the need for further work in this field.

We agree that the temperature dynamics at and near the surfaces of vegetation will impact the likelihood of dew formation and the conclusions that one might draw from meteorological measurements. We modified the existing text (below) with the italicized text [P17137, L24]:

Measurements of temperature and RH allow estimation of dew point for each sample, though surface temperature and RH *on or* immediately above vegetative surfaces may be somewhat different from

measurements at 8 m above the forest floor. RH of ~82% corresponds, on average, to a temperature ~2.7°C above the dew point of water (Fig. S6).

Good study overall.

References:

Wu, C. H. and Warren, H. L.: Induced autofluorescence in fungi and its correlation with viability- Potential applications of fluorescence microscopy, *Phytopathology*, 1353-1358, 1984a.

Wu, C. H. and Warren, H. L.: Natural autofluorescence in fungi, and its correlation with viability, *Mycologia*, 1049-1058, 1984b.