acp-2012-724, Huffman et al.
Author response to Reviewer #2
December 7, 2012

Thank you for your thoughtful review and for the recommendation to publish. The "technical tour de force" comment was particularly satisfying. Your comments were helpful to clarify and strengthen certain arguments, and I'm confident the manuscript is significantly improved due to your input. Specifically, the discussion and conclusions have been improved based on the input of the reviewer.

Specific referee comments and point-by-point author responses:

1) *Title:*

The reviewer suggested a stronger title to reflect the work, and so the title was accordingly changed to: "Size distribution and temporal variation of biological aerosol particles in the Amazon rainforest characterized by microscopy and real-time UV-APS fluorescence techniques during AMAZE-08"

2) *P2518, first sentence:*

The point about biological versus biogenic is well taken. This is indeed often a point of confusion within the community. Rigorously, 'biogenic' refers to material emitted from biological origin, but there may be indirect conversions that take place before particles are formed (e.g. biogenic gases react with atmospheric oxidants to eventually form biogenic secondary aerosol). Not all biogenic particles, however, would be considered biological in origin, i.e. originating directly from a biological source. But most instances of the word biological or biogenic in the introduction refer specifically to the word used by the authors of the manuscripts we cite, and we cannot change these from the way they were originally written without re-interpreting the authors' analysis. Beyond this we have been clear to change instances of 'biogenic' to 'biological' where the term applies to our own measurements and conclusions. For example (purple text indicates text additions, strike-through indicates removal from text:

Abstract: "Biogenic gases and particles ~~material~~ emitted into the atmosphere …"
Abstract: "Primary biological aerosol particles (PBAP), a subset of biogenic particles, often referred …"
P25190, L27: biogenic changed to biological

3) *P25185, L14:*

The grammar has been changed exactly as suggested.

4) *P25189, L8-11:*

The reviewer is correct that the 'fine' filter will collect particles essentially between 0.2 – 5 µm in size. However, the filter cut-point was not the determining factor for size categorization in our size distribution analysis. Visual inspection of each filter (fine and coarse) was performed by the analyst such that each particle was individually assigned a size; thus filter size-cut points do not reflect significant artifacts. The two-staged filter approach was used to broadly separate the particles so that the concentration was not so great as to make enumeration difficult. To clarify this issue we modified the text at the point the reviewer suggested: "Aerosol samples for microscopic analysis were collected via the same laminar flow inlet through a two-stage stacked filter unit using 12 mm Nuclepore® polycarbonate filters pre-coated with sputtered gold and with a pore size of 5 µm for coarse particles and 0.2 µm for fine particles, respectively. However, every particle was counted and sized individually and the definition of coarse > 1 µm and fine < 1µm volume equivalent diameter is adhered to for particles counted using the SEM."

5) *P25190, L14-15:*

To clarify the issue of mixing state, the following statement was added at the point the reviewer's question addresses: "Mixing state is a term used to distinguish externally mixed particles found only as separate entities (e.g. PBAP or particles containing pure organic material) from internally mixed particles where mixtures of two or more components are found in the same particle (e.g. PBAP with organic/inorganic coating)."

6) *P25191, L23:*

The word 'a' was removed, as suggested.

7) *P25191, L23:*

The reviewer correctly pointed out that the lactophenol blue stain will not highlight cellulose in the cell walls of species of spores of non-fungal origin (i.e. Chromista, recently re-named as Chromalveolata). This was a particularly important point. In fact, I was not aware of this, and so the reviewer's comment significantly strengthened the discussion and conclusions related to Figure 6. Enlightened by this comment and suggestion to discuss the important distinctions between the classes that may or may not have been enumerated by this staining technique, the following text was added or modified:

Abstract: "This mode may have consisted of single bacterial cells, brochosomes, various fragments of biological material, and small Chromalveolata spores."

P25191, L25: "This technique targets Eumycota (true fungi), but will not identify spores with cellulose-based cell walls, such as those of Chromalveolata (Chromista), a kingdom which evolved separately from the common ancestors of plants, animals and fungi and including *Peronosporomycetes* such as *Phytophthora* spp. that can also form air-borne, pathogenic spores (Bartnicki-Garcia, 1968; Judelson and Blanco, 2005)."

P25198, L15: "Quantitatively, however, the particles positively identified during this 24-hour period using the ~~fungal~~chitin stain comprise only ~18% of the total PBAP observed in the supermicron mode. This discrepancy could indicate the presence of spores of fungus-like species with cell walls comprised of skeletons not made of chitin, such as many yeasts, *Cryptomycota*, and *Peronosporomycota (*formerly *Oomycota*) (Bartnicki-Garcia, 1968; Helbert et al., 1997; Petersen and Rosendahl, 2000; Jones et al., 2011)."

P25210, L8: "These may have been either non-fungal in nature, or spores from fungal-like species lacking chitin-based cell walls (e.g. organisms of the Chromalveolata kingdom such as plant-pathogenic *Phytophthora* spp.) ~~were non-fungal in nature~~ and may also regularly escape detection by many techniques."

8) *P25193, L11:*

Text changed to remove 'are' as suggested.

9) *P25199, L13-14:*

The insight into the particles shown in Figure 7 was also helpful. The descriptive text was amended as follows: "Figure 7B, in particular, has the appearance of particles suggested by Wittmaack et al. (2005) to be fungal spores like *Cladosporium*, and 7E appears to be a spinose basidiospore such as one of the Agaricales-order mushroom like *Inocybe calospora* (Avis et al., 2006). Figure 7L shows an intriguing image of a particle type not seen often during the study, which may represent an agglomeration of basidiospores or possibly large bacterial cells. This highlights how ~~bacteria, though~~ some biological particles can be individually small, but may ~~can~~ be detected at much larger sizes."

10) *Figure 7 legend:*

The word 'supermicron' has been removed to remove ambiguity.

11) *P25205, L24-27:*

The sentence has been modified exactly as suggested by removing 'other.'

12) *P25206, L3-4:*

The sentence has been modified exactly as suggested to "…were intended to allow us to formulate a rough hypothesis…"

13) *P25207, L6-7:*

The sentence has been modified exactly as suggested to "electrolytes from spores of several species of fungi"

14) *P25207:*

The reviewer's comment about the possible importance of the observed coatings to the in the atmosphere is an important point. S/he suggested adding speculation about the role these coatings could play to microbial survival. We added the following text and citations at the end of Section 3.5 on P25207:

"The presence of coatings on biological particles could provide natural fitness benefits for the species involved by helping to resist against major stresses of the atmosphere: UV radiation and atmospheric desiccation. Particle coatings could both help absorb UV radiation or change their reflective properties, thus protecting the viability of the cell inside (Attard et al., 2012). And whether of primary biological origin or condensed secondary material, coatings could provide the same benefit of encapsulating the PBAP as do natural extrapolysaccharides (EPS): helping to protect cells from death due to desiccation (Potts, 2001). If the coating is more hygroscopic or ice active than the native cell wall material it could provide a fitness benefit for the species by preferentially returning the cells to the Earth surface via precipitation that would provide moisture for cell growth (Sands et al., 1982; Morris et al., 2008). Thus, natural biological selection could reinforce the relationship between microbiology and atmospheric processes via utilization of particle coatings."

15) *P25207, L25-26:*

The sentence was changed, as suggested, to: "Here we summarize our ~~observations with~~ five key ~~areas of~~ conclusions:"

16) *P25208, Conclusion 1:*

The wording was clarified to the following: "Biological and non-biological particles have distinct sources and trends of abundance allowing for separated detection. ~~observed are separable due to unique trends and sources.~~"

17) *P25208, Conclusion 2:*

The wording was clarified to the following: "Biological particles in the Amazon are ~~key~~ important fractions of supermicron aerosol and should be considered when investigating biosphere-atmosphere interactions." The fact that they should be considered when investigating such interactions was added upon suggestion of the reviewer.

18) *P25210, Conclusion 3:*

The wording of this conclusion was changed to reflect the present tense, and also to correct an incorrect formulation of the idea to become: "Biological particles ~~were~~ are ~~often~~ frequently coated with a mixture of organic and inorganic ~~liquids~~ compounds."

19) *P25210, Conclusion4:*

The final two conclusions were merged into one, as suggested to be: "The UV-APS instrument is successfully able to detect biological particles, with some limitations."

Related to this, we also added the following abstract text: "We also show some limitations of using the instrument for ambient monitoring of weakly fluorescent particles < 2 µm."

Additional changes:

Section 2.4 (Clarification here was generally motivated by point 4 above); P25190, L28

"The average footprint to height ratio of each particle type was determined for a small subset of particles using atomic force microscopy for fine mode and the fine focus of the SEM for coarse mode particles. This information was used to convert the 2-D equivalent diameter to a volume equivalent diameter. The size determination was performed on separately acquired high resolution images (pixel size 0.6 nm to 15 nm). SEM size distributions are also based on volume equivalent diameter, which should be similar to aerodynamic equivalent diameter for PBAP (density ~1 g cm^{-3}) but differs by a factor of 1.6 from the aerodynamic equivalent diameter for mineral dust with a density of ~2.1-2.7 g cm^{-3}. Shape factors also need to be considered while converting volume equivalent diameter into aerodynamic diameter. However, due to the complexity of some of the observed shapes we refrained from attempting such a conversion."