## Response to Reviewer 2

We thank reviewer for meticulously reading our manuscript and for providing a thorough and thoughtful review. Our responses to the issues raised follow.

**Reviewer comment:** "Line 510: LNA size distributions are dominated by 2-4 µm particle. Authors suggested that bacteria can contribute to this group. Are you sure about that? I believe bacteria are smaller in size."

***Answer:*** *Although many individual bacteria are likely in the order of ~ 1µm, the median aerodynamic diameter of culturable bacteria in continental sites has been ~ 4µm (Despres et al., 2012). Bacteria in the atmosphere can be co-emitted together with bigger particles (e.g. soil, plant fragments) and sometimes they are observed as clumps of bacteria cells (Burrows et al., 2009). For these reasons 2-4µm biological particles observed in the LNA population suggest large bacteria cells contribute to it. In addition, several bacterial species observed in the atmosphere (Microbiology of Aerosols, p.9; Monier and Lindow, 2003; Baillie and Read, 2001) are within this sizes range, like: Sphingomonas spp.(1.0-2.7µm), Methylobacterium spp. (1.0–8.0µm), Pseudomonas spp. (e.g. Pseudomona syringae, ~2.5µm) and Bacillus spp. (e.g. Bacillus anthracis, 3 - 10µm).*

*Therefore, we can not ensure the LNA population is solely composed by bacterial cells; the size range of the LNA population and epifluorescence microscopy results, however, support bacteria cells between 2 - 4µm contribute to the LNA population.*

*The above discussion will be reflected in the revised text.*

**Reviewer comment:** "Line 497: Authors discussed about pollen cluster of the FCM results in Figure 2. It is not clear to me the pollen cluster. I don't see a clear cluster."

***Answer:*** *A pollen cluster is present in Figure 2, but it is not well defined because of small counting statistics (~200 counts) compared to the total counts (~50,000 counts). Pollen particles constitute less than 1% of the total particle number; given this, flow Jo cannot cluster it using the 2% contour plots (look Figure S3a). However, the pollen population showed very high autofluorescence when no SYTO-13 was added (look Figure S11 in the supplemental information), consistent with the literature (Pöhlker et al., 2012); given their autoflurescence, size and the low counts strongly points to pollen.*

**Reviewer comment: "**Line 527: The authors suggested that pollen fragmentation will have negligible effect on LNA concentrations. However, previous studies suggested that pollen grain can rupture into many fragments. I am not sure about Ragweed pollen but different species of pollen rupture at high humid condition. If FCM protocol is used as a tool for detection and quantification of bioparticle in other location where different species of pollen are present. Then how should we interpret the FCM data?"

***Answer:*** *Although 0.2µm – 5µm pollen fragments can be generated upon rupture, pollen (e.g. Birch, Ryegrass, Oak, Olive) mainly breaks apart into submicron fragments by hydrolysis and favors fragmentation into small submicron (<1µm) particles (Taylor et al., 2002; Taylor et al., 2007; Bacsi et al., 2006; Grote et al., 2003) that are not considered in our FCM analysis. An additional factor to consider in pollen fragmentation is the number of fragments generated per pollen grain. Given pollen concentrations are 100-1000 times lower than bacteria concentrations in the atmosphere (Hoose et al.,2010), at least 100 supermicron (>1µm) pollen fragments will have to be released per pollen grain to considerably influence the LNA population, which has not been observed. This discussion, although mentioned in the supplementary material, will be further emphasized in the revised text.*

**Reviewer comment: "**Line 532: How did you compare the pollen concentrations and LNA concentrations?"

***Answer:*** *Each pollen and LNA cluster defines their respective number concentration, and that was used for comparison; calculations were performed for each of the analytical triplicates. Comparisons was conducted without taking in consideration the threshold approach because it takes into account the whole LNA population, not just the "bioLNA"(LNA above threshold). On average pollen number concentration is 0.54 ± 0.48% of total LNA number concentration (min: 0.16%; max: 1.70%). After threshold application, pollen number concentration constitutes on average 1.70±1.36% (min: 0.36% max:4.06%) of the bioLNA number concentration. Overall, bioLNA number concentration (~$10^4$ $m^{-3}$) is two order of magnitude higher than pollen number concentration (~$10^2$ $m^{-3}$) throughout the 15 sampling events, and will be further discussed in the revised manuscript.*

**Reviewer comment:** "Line 539: How do you get the size information in Figure 2? Discussion of figure 2 and 3 needs improvement.

***Answer:*** *This is a good point. In Figure 2 the FL1-A vs SSC-A plot shows the SYTO-13 fluorescence intensity vs. 90° scattering intensity (SSC-A; related to "internal complexity") for each single particle in a density plot; green and red zones denote the most populated regions. FSC-A value is related to particle size, and is determined based on a calibration (supplemental information, Figure S9, Equation S3) using standardized (e.g. 1µm, 2µm, 4µm,6µm,10µm &15µm) beads. Figure 2 does not show FSC-A-derived sizes, but we nevertheless report them.*

**Reviewer comment:** "Line 560: is it possible that "unclassified" bioparticles contribute from secondary bioparticles such as fragments from fungal spores and pollen? Fragmented particles might have broad size distributions and may change their chemistry?"

***Answer:*** *The "unclassified" bioparticles are those not constrained by Flow Jo 2% contour gating, and most of these particles are far from the centroids of the gated populations. They can indeed be formed by fragmentation or accretion, or also be related to plant debris (i.e., irregular*

*bioparticles) that are characterized by a very broad size, internal complexity and nucleic acid content distributions. We will include these points in the revised manuscript.*

**Additional References:**


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**Revised Figure 2**