

## ***Interactive comment on “Influence of Rain on the Abundance and Size Distribution of Bioaerosols” by Chathurika M. Rathnayake et al.***

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Reviewer #1 (C. Morris) comments Reviewer 1 general comment: “The overall objective of this work is to assess if rainfall influences the size distribution of biological aerosols and to identify the components of the aerosols – fungal, bacterial or pollen in particular – that contribute to the different size fractions. This question is important because fine aerosol particles move deeper into the respiratory tract thereby more readily setting off allergic reactions and allergies. For this work they have used chemical proxies for fungi, bacteria and pollen based on previously published reports and on additional work on chemical proxies of pollen as reported here.”

The paper is well-written and the results are clear overall. Nevertheless, I have some questions and criticisms about the interpretation of their data and about the novelty of

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their findings that need to be addressed. The specific questions are indicated below. More generally, as a biologist it is difficult to accept that only data about chemical proxies are sufficient for making specific conclusions about the presence, abundance and behavior of bacteria, fungi and pollen. I understand that chemical proxies are used because the nature of filters used for PM measurements are not compatible with microscopy. Furthermore, chemical analyses are more rapid and likely are more sensitive in terms of detection thresholds. But they are not as specific as needed for the many of the conclusions that the authors have made. In many of the studies where these chemical proxies were developed, other types of samplers were used in parallel to validate the results via microscopy. Pollen grains are rather large and have distinguishing features that can be recognized to aide in their identification and to differentiate them from certain fungal spores. The authors also report that pollen grains burst – because of the chemical signals they observed – without ever showing any direct evidence of this phenomenon, something that is also readily visible. Bauer et al 2003 (cited in the manuscript), noted that the relationship (regression coefficient) between the number of fungi in atmospheric samples and the quantities of the chemical proxies varied among different sampling sites and dates. This is likely because of the physiological changes that can occur throughout the life of fungi and especially in the production of different types of spores (ascospores and conidia for ascomycetes; basidiospore, pycniospores, urediospores, aeciospores and teliospores for basidiomycetes, for example). Among their various conclusions, the authors stated that sources other than fungi were responsible for the glucan detected in the samples for cases where glucans and mannitol were not correlated. These are the types of conclusions that should be verified with other data – either direct observations, plating on growth media or through DNA analyses.

My second general question concerns the originality of the conclusions about how rainfall enhances the relative abundance of small aerosol particles as compared to larger particles. There is a growing body of literature describing how rainfall scavenges aerosol particles depending on their size – that have not been cited in this manuscript.

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I have indicated some of those papers below in the specific comments section. The authors state that the medical community is well aware of the increase in cases of asthma after thunderstorms. If the authors have presented new information in understanding this phenomenon, then they should better acknowledge that in the paper. As a last comment, I am not sure why the authors mention CCN, IN and cloud processes in the manuscript. This manuscript concerns bioaerosols that impact human health. Mentioning CCN and IN does not add anything to the manuscript and it distracts a bit from the main message.

Response to reviewer #1 general comment: We thank the reviewer for their input and detailed comments that bring a valuable biological perspective on this data set. We have revised the manuscript in response to each specific comment point-by-point below. We summarize our responses to the main concerns of the reviewer here:

With respect to our use of chemical proxies to study bioaerosols, we note that this approach has been taken previously. We consider a strength of this work to be the combination of chemical tracers and biological assays, which have been combined in only a few prior studies (Rathnayake et al., 2016; Chow et al., 2015). We agree with the reviewer that these methods have limitations, particularly in the ability to identify bioaerosols at the species level, and have clarified this in the manuscript by adding the following text at page 7 line 5-14: "Measurements of chemical tracers and biological markers are used to determine the relative concentrations and distribution of pollens, fungal spores, and bacteria in fine and coarse PM. Only few prior studies have combined chemical tracers and biological markers (Rathnayake et al., 2016; Chow et al., 2015), while many others have relied on either chemical tracers (Fu et al., 2012; Medeiros et al., 2006; Burshtein et al., 2011; Yttri et al., 2007; Zhang et al., 2010) or biological assays (Nilsson et al., 2011; Mueller-Anneling et al., 2004; Pavilonis et al., 2013; Madsen et al., 2011; Singh et al., 2011). Glucose, fructose, and sucrose are major components of pollens, mannitol and fungal glucans are in fungal spores, and endotoxins are in bacteria. In the ambient particulate matter, these species are

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used as bioaerosol tracers, since their concentrations reflect mass concentrations of the corresponding bioaerosol. These species provide general insight to classes of bioaerosols present, but cannot be used for species-level identification, which requires either microscopy imaging or DNA sequencing."

We agree with the reviewer that additional, corroborating measurements of bursting pollens in the PM samples collected would be very useful; however the PM samples were collected on filters that were not conducive to microscopy analysis and the chemical tracer analysis and biological assays were destructive, so additional measurements, such as microscopy or DNA sequencing were not possible. The study of intact pollens by chemical methods links the chemical tracers to local pollen types (namely oak) and the scientific literature base provides evidence of pollen rupturing that we draw upon in discussing our results. In future studies of bioaerosols, we plan to incorporate additional analytical tools, as suggested by the reviewer.

In regards to the reviewer's comment on glucan and mannitol correlations, we have re-worded our sentences as described in reviewer 1 specific remarks 18 and 19. To incorporate reviewer 1 comments on fungal spore tracer ratios of different types of fungal spores as a likely reasoning for the lack of correlation of mannitol and glucans we revised our discussion at page 11, lines 8-12 as described in reviewer 1 specific remark 12.

In regards to the reviewer's second general comment, we have made a number of modifications to the text. To address the comment about rain suppressing atmospheric PM, we have added the suggested references and expanded the discussion as suggested by the reviewer in response to reviewer 1 specific remark 9. In order to acknowledge the novelty of this study and new insights to thunderstorm asthma, we incorporated a paragraph to the manuscript as described in response to reviewer 1, specific remark 17. We agree with the reviewer that the main implication of the observations in this study relate to human health and asthma, although the release of bioaerosols to fine PM has also important implications for meteorology as they can be effective cloud con-

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densation nuclei (CCN) and ice nuclei (IN). We believe that this is important to include, albeit briefly, as noted in response to reviewer 1's specific remarks 4 and 15. The changes made to this manuscript in response to these suggestions are detailed below.

Reviewer #1 specific remark 1: "Pg 2, Ln 16 : There is probably better terminology than "growing cycle". "Plant phenology" would be more appropriate."

Response to reviewer #1 specific remark 1: We agree with the reviewer and changed the wording in page 2, line 16. Now the text in page 2, line 16 reads as "Ambient levels of pollens vary seasonally with plant phenology (Galán et al., 1995; Targonski et al., 1995)."

Reviewer #1 specific remark 2: "Pg 3, Ln 1. What do the authors mean by "Bacteria in the atmosphere are typically settled on soil or vegetative surfaces" ?

Response to reviewer #1 specific remark 2: We thank the reviewer for pointing this out. In order to make our statement more clear we changed the wording in page 3, line 1. Now page 3 line 1 reads as "Bacteria in the atmosphere are typically attached to soil or vegetative surfaces as agglomerations of cells (Jones and Harrison, 2004)."

Reviewer #1 specific remark 3: "Pg 3, Ln 3-6 : The authors state: "In vegetation covered areas, atmospheric bacterial concentrations peaked after approximately 1 h of rain relative to areas with bare soil (Robertson and Alexander, 1994)." However, this statement is not supported by this paper. Roberston and Alexander studied one single bacterial species (a nitrogen fixer that nodulates stems) and rainfall was simulated in their study. So it is not appropriate to make such generalizations from this one work.

Response to reviewer #1 specific remark 3: We appreciate the reviewer pointing this out and have added additional information and citations to support a more general statement. The text at page 3, line 4-6 now reads as "In vegetation covered areas, atmospheric bacterial concentrations have been shown to increase during and after simulated rain events (Graham et al., 1977; Robertson and Alexander, 1994) as well

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as natural rain events (Constantinidou et al., 1990; Huffman et al., 2013)."

Reviewer #1 specific remark 4: "Pg 3, Ln 9-10 : In support of the statement "bioaerosols in the atmosphere promote cloud and ice nucleation" the authors cite Pope, 2010; Sun and Ariya, 2006; Franc and Demott, 1998. However, these papers concern CCN and do not support the statement about ice nucleation. Please add a reference about ice nucleation if you are going to maintain information in the introduction and discussion about cloud physical processes. But as noted above in the general remarks, the focus of this work seems to be on aerosols that affect human health. The statements about aerosols that influence cloud processes seem irrelevant to the point of this research.

Response to reviewer #1 specific remark 4: We agree with the reviewer that the current set of references only supports the CCN activity of bioaerosols. CCN and IN are a very active research field, although of secondary importance to health, the results of this study suggest that the pollen bursting phenomenon would impact CCN and IN levels. Therefore in response to this comment, we expanded the reference list to include references that showed IN activity of bioaerosols. Now page 3, line 9 reads as "Once released, bioaerosols in the atmosphere promote cloud and ice nucleation (Pope, 2010; Sun and Ariya, 2006; Murray et al., 2012)"

Reviewer #1 specific remark 5: "Pg 3, Ln 31: The authors do not state objectives that specifically mention the role of rain or the response of bioaerosols to rain. Why not?

Response to reviewer #1 specific remark 5: We thank the reviewer for pointing this and in response we have revised our objectives to be more specific. Page 3, lines 31-33 now reads as "Our central objectives were. . . .ii) evaluate environmental conditions including rain and temperature that lead to high levels and decreases in bioaerosol sizes across fine (PM<sub>2.5</sub>) and coarse (PM<sub>10-2.5</sub>) modes. . . ."

Reviewer #1 specific remark 6: "Pg 4, Ln 9. In the methods section the authors do not indicate where the Andersen sampler is positioned relative to the ground and surround-

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ing objects. How high above the ground was the Andersen sampler placed? What was the surrounding area like? Where there hedges, etc. Can the authors describe the footprint? the fetch? How was the sampler protected from rain? Did air circulate freely around the sampler? The authors need to provide information so that the reader can assess the representativeness of the air sampler relative to the surroundings.

Response to reviewer #1 specific remark 6: As suggested by the reviewer, we have added details to the site and sampler descriptions at page 4, lines 8-22: "Daily (24 h) PM samples were collected from 17 April–9 May (springtime) and 15 August–04 September (late-summer) in 2013, at the University of Iowa air monitoring site in Iowa City, Iowa, US (+41.6647, – 91.5845). The site was located at the University of Iowa Practice Fields in a suburban landscape in an open area surrounded by woods, agricultural fields, meadows and a parking lot. PM<sub>2.5</sub> and PM<sub>10-2.5</sub> were collected using an Andersen dichotomous sampler (Series 241) that included a PM<sub>10</sub> cutoff impactor (Anderson Instruments, Model 246b) and virtual impactor. The total air flow rate was 16.67 L min<sup>-1</sup> and the coarse flow rate was 1.667 L min<sup>-1</sup>. PM samples were collected on 37-mm Teflon filters (Pall Corp.) and PM<sub>10</sub> was determined as the sum of PM<sub>2.5</sub> and PM<sub>10-2.5</sub>. The dichotomous sampler had a UMLBL (the University of Minnesota-Lawrence Berkeley Laboratory) type inlet which is equipped with a rain guard and a mesh-screen to exclude rain drops and insects. An additional set of PM<sub>2.5</sub> samples were collected on to 90-mm quartz fibre filters (Pall Life Sciences) using a medium-volume sampler (URG Corp.) equipped with a sharp-cut cyclone to select PM<sub>2.5</sub> at a flow rate of 90 L min<sup>-1</sup>. Rain was excluded from the PM<sub>2.5</sub> sampler primarily by positioning the inlet downward and secondarily by the cyclone. Both samplers were affixed to a platform 3 m above ground level and were unobstructed. Flowrates were measured using a rotameter at the beginning and the end of each sampling period; average flowrates were used to calculate air volumes. Filters were changed at 08:00 local time (CST) and one field blank was collected for every 5 samples. After sample collection, filters were stored at -20 °C in the dark." Reviewer #1 specific remark 7: "Pg 6, the section starting on Ln 9: What was the purpose of the microscopy? How

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was this used in the study? Furthermore, why do the authors show a few images of pollen grains as one of the figures?

Response to reviewer #1 specific remark 7: We agree with the reviewer the need to clarify the use of microscopy in the manuscript, which was specifically to determine the diameter of pollen grains. We expanded our objectives to include why we took microscopy measurements of pollens. In the introduction section page 3, line 31-34 reads as "Our central objectives were. . . . iii) determine intact pollen diameters and chemically profile regionally-important pollen types (red oak, pin oak, cotton ragweed, giant ragweed and corn) for use in source apportionment. . ."

Moreover we incorporated the purpose of doing microscopy measurements in the method, section 2.5. Now page 6, lines 16-18 reads as "Pollen images were taken to determine pollen grain diameters using a Zeiss LSM 710 fluorescence microscope (Carl Zeiss Microscopy GmbH, 07745 Jena, Germany) following Póghlker et al. (2012), and IX-81 inverted microscope (Olympus Corporation, Tokyo, Japan).

We also agree with the reviewer that these microscopy measurements were not used in this study other than to visualize the pollen size and shape thus we moved the images of pollens (Figure 1) to the supplementary information Figure S1."

Reviewer #1 specific remark 8: "Pg 7, Ln 22: change "Rainfall corresponding to low PM" to "Rainfall corresponded to : : :"

Response to reviewer #1 specific remark 8: We agree with the reviewer and text in page 7, line 22 is revised. Now page 8 line 2 reads as "Rainfall corresponded to low PM concentrations with average. . ."

Reviewer #1 specific remark 9: "Pg 7, Ln 26-28 : The authors state that "The shift in the PM size distribution of PM reflects that rain was more effective at scavenging and/or suppressing the release of coarse particles compared to fine particles." This is what should be expected. They should cite the relevant references here and in their

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discussion. The differential effect of scavenging according to particle size has been reported as early as the 1960's in the work of Gregory [Gregory, P. H. 1961. *The Microbiology of the Atmosphere*. New York: Interscience Publishers, Inc.]. For a more recent example, the authors should refer to [Li et al. 2016. Observed changes in aerosol physical and optical properties before and after precipitation events. *Advances in Atmospheric Sciences* 33: 931–944].

Response to reviewer #1 specific remark 9: We agree with the reviewer's suggestion to provide citations in support of this statement. The revised text in page 8, lines 6-12 reads: "The shift in the PM size distribution reflects that rain was more effective at scavenging and/or suppressing the release of coarse particles compared to fine particles. This is consistent with previous ambient studies that have demonstrated coarse PM is more effectively scavenged than fine particles (Guo et al., 2016; Li et al., 2016). Particle removal via rainfall depends on many factors including a strong dependence on the particle size (Gregory, 1962; Baklanov and Sørensen, 2001); airborne particles with diameters greater than 3  $\mu\text{m}$  have a higher tendency to collide with falling rain drops and are effectively scavenged via inertial impaction (Wang et al., 2010; Andronache, 2003; Mircea et al., 2000)."

Reviewer #1 specific remark 10: "Pg 7, Ln 32: Change "levels are shown in Figure 3b" to "levels as shown: : :."

Response to reviewer #1 specific remark 10: We agree with the reviewer and we revised the text in page 7, line 32 accordingly. Now page 8, line 16 read as "...levels as shown in Figure 2b..."

Reviewer #1 specific remark 11: "Pg 9, Ln 31-32 : The authors state that "Rain influenced ambient concentrations and the size distributions of fungal spore tracers, by triggering passive and active release mechanisms." This is a very strong statement about mechanisms that is not supported by any biological observations in this work. This is a possible mechanism and it should be stated as a conjecture. Are there any

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other possible explanations such as growth, breaking of fungal hyphae, etc ?

Response to reviewer #1 specific remark 11: We appreciate this reviewer for highlighting this. We agree with the comment and we revised the text in page 9, line 31-32 accordingly. Now the revised text in page 10, lines 19-23 reads as "Rain influenced ambient concentrations and the size distributions of fungal spore tracers, likely by triggering passive and/or active release mechanisms and/or promoting fungal growth. Maximum mannitol and glucon levels occurred on 5 May, which followed three days with rain (Figure 3a-b). Rainfall facilitates fungal growth promoting fungal germination and hyphal growth (Schulthess and Faeth, 1998; Morris et al., 2016) and wet conditions that follow rain are favourable for active release of fungal spores (Rodriguez Rajo et al., 2005; Van Osdol et al., 2004)."

Reviewer #1 specific remark 12: "Pg 9, Ln 34: The authors wrote: "Known for releasing spores after rain are some Ascospores: : :". "Ascospores" is not the correct terminology here. Ascospores are a type of spore. Here you mean Ascomycetes, i.e. a name for the group of fungi that produces ascospores during their sexual stage of reproduction. But although Ascomycetes are abundant, many of them produce mostly conidia that are formed on fungal "stems" called conidiophores and do not involve the formation of asci (sacs) containing ascospores and the accompanying fluids that are released into the atmosphere upon ascospore ejection. The relative prevalence of different types of spores (ascospores vs. conidia for the Ascomycetes and basidiospores vs. picnia, aeciospores and urediospores for Basidiomycetes) could be part of the reason that Bauer et al 2003 observed different relationships between the amount of chemical proxy and amount of atmospheric fungi depending on site and season.

Response to reviewer #1 specific remark 12: We agree with the reviewer and appreciate their explanation of fungal spore types in prominent fungal species. We revised the text in page 10, line 22-25 to read "...wet conditions that follow rain are favourable for active release of fungal spores (Rodriguez Rajo et al., 2005; Van Osdol et al., 2004). For instance, actively discharged ascospores peak after rain in wet conditions (Troutt

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and Levetin, 2001; Elbert et al., 2007; MacHardy and Gadoury, 1986).”

To address the reviewer comment about the relative prevalence of different types of spores and chemical proxies (both here and in their general comments), we have incorporated the likelihood of different spore types into our discussion of fungal spore tracers. Now page 11, lines 8-12 reads as “Coarse mode glucan concentrations in late summer were neither correlated with temperature ( $r_s=0.01$ ,  $p=1$ ), nor mannitol ( $r_s=0.2$ ,  $p=0.3$ ). Mannitol concentrations and fungal spore counts have spatial and seasonal differences from one another (Bauer et al., 2008), likely due to differences in mannitol emission per spore across fungal types (Elbert et al., 2007; Bauer et al., 2008) and/or mannitol concentrations in spores from within a species (e.g. ascomycetes releases ascospores during sexual reproduction and conidia during asexual reproduction (Nauta and Hoekstra, 1992)).”

Reviewer #1 specific remark 13: “Pg 10, Ln 23-24: The authors state: “and prior observations that pathogenic bacteria that grow on crops (i.e. *Agrobacterium* spp., and *Rhizobium* spp.) contain glucans in their structure”. In this section the authors are trying to provide information about sources other than fungi for glucans in the atmosphere. Glucans are widely distributed in the microbial world and in biology in general. Here they give an example of 2 bacterial species. Although the information is accurate that these species contain glucans, they are soil-borne microorganisms. Furthermore, *Rhizobium* is not a pathogen, but rather it is a symbiotic nitrogen-fixing bacterium that is considered to be very beneficial to plants (NB: being beneficial or not has nothing to do with the likelihood of being airborne. I mention this only to clarify that it is not a pathogen). It is not logically obvious that these soil-borne bacterial species would be readily in the air. There have been reports of aerial dissemination of *Rhizobium* between African and the Canary Islands, but this is also associated with loss of soils. It would be more appropriate to find a reference for the presence of glucans in bacteria in general, or to find references about bacteria that are common on aerial plant surfaces and more likely to be regularly in the atmosphere in agricultural contexts.

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Response to reviewer #1 specific remark 13: We agree with this reviewer comment and page 10, line 23-24 is revised to reflect the presence of glucans in bacteria in general. Page 11, line 18 now read as “Alternatively glucans may have derived from bacterial cells (McIntosh et al., 2005; Rylander and Lin, 2000), although their correlation was not significant ( $r_s=0.4$ ,  $p=0.1$ ).”

Reviewer #1 specific remark 14: “Pg 10, Ln 24-25: The authors state: “Agricultural crops are abundant in Iowa during the growing season and the mechanical agitation of plant surfaces by wind can aerosolize surface bacteria”. Perhaps this is just awkward phrasing, but it should be changed because it suggests that the authors do not know that this is common knowledge. The “growing season” generally means the season during which crops grow. If Iowa were covered by forests, one would talk about the seasons (spring, summer, etc.). So, saying that agricultural crops are abundant during the growing season is redundant. Furthermore, I think that it is common knowledge that the Midwestern states of the US such as Iowa, Nebraska, Kansas, etc. are mostly covered by agriculture (corn, wheat, alfalfa). In this context, this sentence is surprising. It is sort of like reminding us, for example, that China or India have large populations of people.

Response to reviewer #1 specific remark 14: In response to reviewer 1, specific comment 13, we generalized our discussion, and the sentence in question has been deleted.

Reviewer #1 specific remark 15: “Pg 13, Ln 13: The information on CCN and IN seems out of place in this paper because the authors are focusing on impacts on human health. For more detailed information about the possible sources of bioaerosols during and after rainfalls, I suggest that the authors refer to: Morris et al 2016 (<http://journals.ametsoc.org/doi/abs/10.1175/BAMSD-15-00293.1>).

Response to reviewer #1 specific remark 15: We think the discussion of CCN and IN activity of bioaerosols to be relevant to this work, particularly with respect to observa-

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tions of pollen tracers in fine PM that are more CCN active than coarse PM. Consequently, we have retained this component of the manuscript. As suggested, we refer to Morris et al., 2016 about possible sources of bioaerosols during and after rain. The revised text follows.

In the revised manuscript, the text at page 14, lines 9-21 reads: "The release of fine sized bioaerosols can influence cloud formation, by acting as CCN and IN. Pollen fragments are effective CCN and IN (Pope, 2010; Diehl et al., 2001). During rain intact pollen particles can swell and rupture, producing hundreds of fine-sized pollen particles (D'Amato et al., 2007), significantly increasing the number of CCN and IN active particles in the atmosphere. Bacteria and fungal spores also active IN and CCN (Murray et al., 2012; Sun and Ariya, 2006; Hassett et al., 2015). Bacterial strains with higher IN activity (mostly Gram-negative bacteria that inhabit plant surfaces (Murray et al., 2012), such as *Pseudomonas syringae*) increase in population during rain (Hirano et al., 1996), which can substantially increase airborne IN (Morris et al., 2016) that can persist in the atmosphere for weeks following rain (Bigg et al., 2015). Rainfall in general favours fungal growth (Schulthess and Faeth, 1998; Morris et al., 2016) as well as passive and active release of spores (Rodriguez Rajo et al., 2005; Van Osdol et al., 2004; Allitt, 2000; Elbert et al., 2007; Huffman et al., 2013) thereby increasing CCN and IN active particles in the atmosphere. When decreased in size ( $< 2.5 \mu\text{m}$ ), these bioaerosols are more effective IN (Murray et al., 2015; Huffman et al., 2013). Because smaller particles have longer atmospheric lifetimes, fine bioaerosols will be transported longer distances before deposition, and thus may have effects in areas downwind of their release."

We also revised text in page 12, lines 9-23 to incorporate information from suggested references: "On 22 August, the only late summer day with rain, fine mode endotoxin concentrations reached a maximum (Figure 5c). Meanwhile, the endotoxin fraction in the fine mode increased to 36% relative to an average of 5% on dry days. Rainfall promotes bacterial growth, such as *Pseudomonas syringae* that are common on

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plant surfaces and rapidly increase their populations during raining (Hirano and Uper, 1990; Hirano et al., 1996). The release of endotoxin to fine PM is expected to be caused by the aerosolization of Gram-negative bacteria living on plant surfaces (e.g., *Pseudomonas syringae*, *Pseudomonas fluorescens*, and *Pseudomonas viridiflava* etc. (Murray et al., 2012)) by agitation of plants or fungi by falling rain (Jones and Harrison, 2004; Constantinidou et al., 1990). Soil resuspension was suggested as an important source of bacterial endotoxins in spring (section 3.5.1), however coarse mode endotoxins were not significantly correlated with calcium in late summer ( $r_s=0.2$ ,  $p=0.33$ ), suggesting that this is not the case. Consequently, non-soil bacterial sources were likely responsible, such as plant surfaces (Romantschuk, 1992; Jeter and Matthyse, 2005; Murray et al., 2012) that are probably agricultural row crops (Lindemann et al., 1982; Hirano et al., 1996) in the agricultural state of Iowa. This link could be further explored by examining the co-occurrence of bacterial endotoxins with markers of plant waxes (i.e. odd-numbered n-alkanes), but is beyond the scope of the present study. The comparison of spring and late-summer endotoxin behavior in response to rain suggests that soil bacteria are dominate in springtime, while bacteria residing on plant surfaces dominate in late-summer." The revisions done to the discussion of fungal spores are described in reviewer 1 specific remark 11.

Reviewer #1 specific remark 16: "Pg 13, Ln 22-23: The authors state: "Elevating ambient fungal spore levels, particularly from species like Ascospores and Cladosporium, trigger allergenic respiratory diseases : : " Here again, note that "Ascospores" is not a species. You cannot replace it with "Ascomycetes" because this is the name given to the members of the phylum Ascomycota. Perhaps you meant *Aspergillus*?"

Response to reviewer #1 specific remark 16: We agree with this reviewer comment and now page 14, lines 23-26 read as "Elevating ambient fungal spore levels, particularly from species like *Penicillium*, *Aspergillus* and *Cladosporium*, trigger allergenic respiratory diseases like allergic rhinitis and asthma (Garrett et al., 1998; Tillie-Leblond et al., 2011; Knutsen et al., 2012) and high environmental exposures may lead to asthma

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exacerbations (Dales et al., 2003).”

Reviewer #1 specific remark 17: “Pg 13, Ln 32, the authors describe the well-known phenomenon of thunderstorm asthma where allergies increase because of the abundance, after a storm, of small particles that penetrate deep into the respiratory system. In light of the previous research on this phenomenon, the originality of this present work is not clear. They authors should point out more strongly how the work presented in this manuscript goes beyond what was currently known.

Response to reviewer #1 specific remark 17: To clarify the novelty of this work, we have added the following paragraph to the section 3.7 on page 15, lines 15-29: “The results of this study provide new insight and tools to better understand the potential scope of thunderstorm asthma. While thunderstorm asthma has been documented in a number of locations, the data presented herein provide the first evidence of this phenomenon occurring in the Midwestern US. Thunderstorms and heavy rain are common in this region during spring, and thus it is anticipated that conditions characteristic of thunderstorm asthma likely occur several times annually. Pollen prediction indices do not currently account for the release of fine pollen fragments during rain, and consequently sensitive populations are not forewarned. To understand the potential for conditions that trigger thunderstorm asthma more broadly, chemical tracer approaches, as used here, are a useful tool. Chemical tracers provide a sensitive method of detecting fine pollens particles that may be useful in monitoring conditions that precede PM<sub>2.5</sub> pollen release. Because carbohydrates are not expected to undergo chemical alternation by the pollen bursting, they also provide a means of tracking pollens across PM size fractions and associating pollens with their species of origin. Microscopy-based methods are challenged by changes to particle size and morphology upon bursting, which may require use of multiple microscopy techniques suitable for different particle sizes. Chemical tracer methods have potential to be broadly applied, as national monitoring programs routinely collect PM<sub>2.5</sub> samples on filters for chemical analysis. In this way, regions and atmospheric conditions that lead to high levels of PM<sub>2.5</sub> pollen particles

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may be better defined.” Reviewer #1 specific remark 18: “Pg 14, Ln 18-19: The authors state: “Warmer temperatures promoted pollen, fungal and bacterial growth leading to higher ambient levels of these bioaerosols during both spring and late summer periods.” They state this in the Conclusion section as if they had observed this in this work. But isn't this what they infer from their observations of chemistry ? It would be more appropriate to say that the warm temperatures promoted increases in the proxies that are assumed to represent these organisms.

Response to reviewer #1 specific remark 18: We agree that this statement should be restated to align with the data we present. In response we edited page 13, line 18-19. Now page 16, lines 3-5 reads as “Elevated bioaerosol tracer levels were observed when temperatures are warmer suggesting increased pollen, fungal and bacterial concentrations during both spring and late summer periods.”

Reviewer #1 specific remark 19: “Pg 14, Ln 35-36: The authors state “The fragmentation of pollens due to osmotic rupture, shown previously only through microscopy methods, is demonstrated in this study for the first time by way of chemical tracers.” However, in this current work they have not made any microscopic observations to verify the phenomenon of fragmentation. Without direct observation they cannot make this conclusion. They can only speculate.

Response to reviewer #1 specific remark 19: We agree with the reviewer, in response, we re-worded the text in page 14, line 35-36. Now page 16, line 10-13 read as “The fragmentation of pollens due to osmotic rupture, shown previously through microscopy methods. For the first time, we demonstrate a shift of coarse particle pollens (2.5-10  $\mu\text{m}$ ) to fine particles (2.5  $\mu\text{m}$ ) by way of chemical tracers during a major rain event and propose that this is due to osmotic rupture of pollens.”

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