

Response to Anonymous Reviewer #1 Comments

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Influence of Rain on the Abundance of Bioaerosols in Fine and Coarse Particles

Chathurika M. Rathnayake¹, Nervana Metwali², Thilina Jayarathne¹, Josh Kettler¹, Yuefan Huang¹, Peter S. Thorne^{2,3}, Patrick T. O'Shaughnessy^{2,3}, and Elizabeth A. Stone¹

¹ Department of Chemistry, University of Iowa, Iowa City, 52242, United States.

² Occupational and Environmental Health, University of Iowa City, 52242, United States.

³ Civil and Environmental Engineering, University of Iowa City, 52242, United States.

*Corresponding author phone: +1-319-384-1863, fax: +1-319-335-1270; email: betsy-stone@uiowa.edu

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 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, picniospores, urediospores, aceiospores 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. 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. 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 used as bioaerosol tracers, since their concentrations are related to 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 condensation 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. Robertson 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 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_{2.5}) and coarse (PM_{10-2.5}) 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 surrounding 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_{2.5} and PM_{10-2.5} were collected using an Andersen dichotomous sampler (Series 241) that included a PM₁₀ cutoff impactor (Anderson Instruments, Model 246b) and virtual impactor. The total air flow rate was 16.67 L min⁻¹ and the coarse flow rate was 1.667 L min⁻¹. PM samples were collected on 37-mm Teflon filters (Pall Corp.) and PM₁₀ was determined as the sum of PM_{2.5} and PM_{10-2.5}. 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_{2.5} 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_{2.5} at a flow rate of 90 L min⁻¹. Rain was excluded from the PM_{2.5} 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 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 Pöhlker 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 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 µm 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 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 glucan 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 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.

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 observations 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 plant surfaces and rapidly increase their populations during raining (Hirano and Upper, 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 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_{2.5} 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_{2.5} samples on filters for chemical analysis. In this way, regions and atmospheric conditions that lead to high levels of PM_{2.5} pollen particles 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 μm) to fine particles (2.5 μm) 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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Response to Anonymous Reviewer #2 Comments

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Influence of Rain on the Abundance of Bioaerosols in Fine and Coarse Particles

Chathurika M. Rathnayake¹, Nervana Metwali², Thilina Jayarathne¹, Josh Kettler¹, Yuefan Huang¹, Peter S. Thorne^{2,3}, Patrick T. O'Shaughnessy^{2,3}, and Elizabeth A. Stone¹

¹ Department of Chemistry, University of Iowa, Iowa City, 52242, United States.

² Occupational and Environmental Health, University of Iowa City, 52242, United States.

³ Civil and Environmental Engineering, University of Iowa City, 52242, United States.

*Corresponding author phone: +1-319-384-1863, fax: +1-319-335-1270; email: betsy-stone@uiowa.edu

Reviewer #2 comments

Reviewer 2 general comments: “In this study, the abundance of different bioaerosols (pollens, fungal spores and bacteria) present in both fine and coarse fraction of atmospheric aerosols is measured using chemical tracer method. The changes in the ambient concentration of bioaerosols and their relative abundance in different size fraction in response to variation in environmental conditions, especially rainfall were also assessed. Additionally the authors have also characterized the chemical profiles of different regionally abundant pollens and have estimated the pollen and fungal spore contribution to PM mass by using CMB modeling.”

On general reading, the findings reported in the paper are quite interesting, however they are inconsistent in certain places. In this study since the authors quantify the atmospheric abundance of different bioaerosols in only two broad size ranges (PM_{2.5} and PM_{2.5-10}), I feel the use of term “size distribution” is inappropriate and misleading. In addition to chemical tracer analysis the authors have not given any other supporting results to further strengthen their finding of presence of smaller fraction of bioaerosols during the rain events.

Response to reviewer #2 general comments: In response we changed the manuscript title from “Influence of Rain on the Abundance and Size Distribution of Bioaerosols” to “Influence of Rain on the Abundance of Bioaerosols in Fine and Coarse Particles”.

The tracer analysis performed during this work was destructive, so additional filter-based measurements, such as microscopy studies were not possible after our analysis revealed high concentrations of pollen tracers in fine PM. We analyzed the diameter and carbohydrate profile of local pollens, which demonstrates that the chemical signature of oak pollens matches the fine PM carbohydrate profile, suggesting that oak pollens may have been the origin of fine pollen PM. Further confirmation by microscopy techniques would be useful, but are beyond the scope of this study, as samples were not collected on substrates conducive to microscopy analysis. We plan to incorporate microscopy into future studies of bioaerosols. In the absence of microscopy, we draw upon scientific literature that demonstrates the bursting of pollens under wet conditions.

Reviewer #2 specific remark 1: “Fig.1, shows the microscopic images of pollen which are >20 µm. How is it relevant to show these images here as the authors are not measuring PM > 10 µm. Also these are not the images of pollens being measured from ambient atmosphere during any of the mentioned measurement periods. Instead of these images it make more sense to show images of ruptured pollens either collected from ambient atmosphere or from laboratory studies, which could further support their argument of presence of pollen fragments < 2.5 µm in size.”

Response to reviewer #2 specific remark 1: We carried-out microscopic imaging to determine diameters of pollens that we chemically profiled in this study. These images were not taken to support any of the ambient PM measurements. To clearly convey the purpose of taking microscopic images we changed our wording as below.

“In the introduction section page 3, lines 31- 36 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, and *iv*) estimate pollen and fungal spore contributions to PM mass by way of chemical mass balance (CMB) modelling.

Moreover we incorporated the purpose of doing microscopy measurements in the method, section 2.5. Now page 6, line 16 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 Pöhlker et al. (2012), and IX-81 inverted microscope (Olympus Corporation, Tokyo, Japan).

We 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. The laboratory studies as suggested by the reviewer that demonstrated pollens releasing fragments of $< 2.5 \mu\text{m}$ described in the introduction, page 2, lines 22-24 as “In rainy conditions, pollen grains absorb water, osmotically rupture, and release cytoplasmic starch granules (D'Amato et al., 2007). Microscopy studies have shown that intact birch pollens of $22 \mu\text{m}$ in size can rupture and release around 400 starch granules (Staff et al., 1999) ranging from $0.03 - 4 \mu\text{m}$ (D'Amato et al., 2007).”

Reviewer #2 specific remark 2: “In Fig. 2, PM_{2.5-10} mass on April 17 and 18 appears to be zero. But there is glucose detected in this size fraction (Fig. 2c). How is this possible?” ?

Response to reviewer #2 specific remark 2: We agree with the reviewer that coarse PM levels on April 17 and 18 are not visible in the Figure 1b (previously, Figure 2b). The coarse PM concentrations during those days were very low, compared to other days. In particular, as indicated in SI Table S4, during April 17 and 18 PM_{10-2.5} was below our detection limit ($< 0.03 \mu\text{g m}^{-3}$). When these filters were subjected to more sensitive chemical analysis we obtained chemical measurements in units of ng m^{-3} . The apparent discrepancy on 17 April and 18 is due to the carbohydrate measurement method being much more sensitive and having lower detection limits than mass measurements done by weighing.

Reviewer #2 specific remark 3: “Page 5, L 27: Correct as Biomarkers.”

Response to reviewer #2 specific remark 3: We agree with the reviewer and in response we revised the text in page 5, line 27. The text in page 5, line 32 now reads as “ Biomarkers were analyzed.....”

Reviewer #2 specific remark 4: “Page 7, L 25: “Rain also affected the distribution of particles between the fine and coarse modes, with 48 ± 11 % of PM₁₀ was less than $2.5 \mu\text{m}$ on rainy days compared to 80 ± 13 % on dry days”. This sentence is confusing. Is the author mentioning about %contribution of PM_{2.5} in PM₁₀ during wet and dry days?”

Response to reviewer #2 specific remark 4: We agree with the reviewer and to avoid the confusion we revised the text on page 7, line 25. Now the revised text in page 8, line 5 reads as “Rain also affected the distribution of particles between the fine and coarse modes. PM_{2.5} contributed 48 ± 11 % of PM₁₀ on rainy days compared to 80 ± 13 % on dry days.”

Reviewer #2 specific remark 5: “Page 8, L 23: “passive release of larger pollen particles ranging $2.5-10 \mu\text{m}$ during others”. What could be these larger pollen particles released passively during dry days? The microscopic images shows only pollens $> 20 \mu\text{m}$.”

Response to reviewer #2 specific remark 5: We thank the reviewer for pointing this and in response we revised the text in page 9 line 6 to read as “Together, these data suggest release of pollen fragments less than $2.5 \mu\text{m}$ during some rain events (2–4 May) and the passive release of some pollen particles in the coarse particle size range during others (9 May).”

Reviewer #2 specific remark 6: “Page 9, L 1: From the ratio of glucose and sucrose the authors have related the pollens present on May 9 to that of red oak. Is there any other evidence to support this? The authors have mentioned in section 3.1 that the carbohydrate distribution in pollens are likely to differ with change in environmental factors. Hence it is difficult to relate the pollens to any particular type only based

on carbohydrate ratio. Also the authors have not reported any such match in carbohydrate ratios on any other days or in coarse mode PM fraction.”

Response to reviewer #2 specific remark 6: We have related the ambient carbohydrate measurements of May 2 to oak pollen profiles. May 2 has exceptionally high pollen tracer concentrations and these sugar ratios matched well with red oak profile. Also, oak trees are abundant in the Eastern Iowa and they are known to release pollen during springtime. For clarity we have revised the Page 9, lines 19-21 to read as below. With the information on ambient carbohydrate measurements and pollen profiles, which were done parallelly in spring 2013, our best approximation is that pollens are coming from red oak. We agree that further microscopy measurements would be useful to confirm this, but are beyond the scope of this study, as discussed in response to reviewer 2 general comment.

“On 2 May, the relative ratios of glucose and sucrose (normalized to fructose) in fine PM were 1.4 and 2.5, respectively, close to the ratios of red oak (1.2 and 2.1, respectively). Oak trees are abundant in Eastern Iowa and a prominent pollen type in the springtime, making oak a likely (but unconfirmed) source of pollens in fine PM.”

Reviewer #2 specific remark 7: “Page 9, L 16: “shift in glucose size distribution to 34% in the fine mode”. It is not actually the size distribution, instead the relative contribution of glucose in fine fraction increases.”

Response to reviewer #2 specific remark 7: We thank the reviewer for pointing this out. Page 10, lines 2-4 reads as follows “The single late-summer rain event on 22 August coincided with an increase in fine mode glucose concentration and an increase of the fine PM fraction of glucose to 34%, compared to 16% on dry days.”

Reviewer #2 specific remark 8: “Page 9, L 23-24: “Daily concentrations of coarse mode concentrations of two fungal spore tracers—fungal sugar mannitol and the fungal cell wall component glucan—were significantly correlated with daily average temperature ($r_s > 0.4$, $p < 0.05$).” This statement appears to be significant only for mannitol and not glucan. In L 27, The authors have reported an increase in fungal spore tracer level with increase in temperature. But no significant increase in glucan level can be seen in the graph (Fig. 4b).”

Response to reviewer #2 specific remark 8: To be clear about the statistical results we revised our sentence in page X, lines 10-12 to read: “Daily coarse mode fungal spore tracer concentrations significantly correlated with daily average temperature: fungal sugar mannitol and temperature ($r_s = 0.7$, $p < 0.001$) and the fungal cell wall component glucan and temperature— ($r_s = 0.4$, $p = 0.04$).” The statistical correlations performed here are Spearman’s rank correlations, a non-parametric correlation test that use ranks of the measurements as stated in section 2.7. Due to the differences in resulted r_s ($r_s = 0.7$ vs $r_s = 0.4$) values and the significance of the correlations of temperature with mannitol and glucans it is understandable that reviewer 2 having hard time visualizing trends of temperature and glucans with the graphs where we have plotted both coarse and fine mode concentrations together. Thus we revised our wording as above to clearly mention the statistical results.

Reviewer #2 specific remark 9: “Page 10 L 1: “Fungal spore tracer levels dropped on days when rain fell (e.g. 23 April, 2 May), due to particle removal by wet deposition”. The drop in tracer levels is visible only in coarse PM. The mannitol concentration in fine fraction actually shows an increase on 23 April as compared to the previous day without rain. Same is for glucan on 23 April. Also the relative contribution of fungal tracer in fine PM is high on these rainy days as compared to other dry days. Hence this statement is true only for tracer levels in coarse PM.”

Response to reviewer #2 specific remark 9: We agree with the reviewer and accordingly we changed the text in page 10, line 1. Now page 10 line 25 reads as “Fungal spore tracer levels in coarse PM dropped on days when rain fell (e.g. 23 April, 2 May), due to particle removal by wet deposition.”

Reviewer #2 specific remark 10: “Page 10 L19: “which suggests an alternative non-fungal source of glucan”. Pollen is a likely source’. If glucan can have other non-fungal source including pollen, then how can it be used as tracer for fungal spore. The high increase in glucan in PM10 on 22 April (Page 9, L 26) might be due to pollens which are generally released due during higher temperature”

Response to reviewer #2 specific remark 10: Assessment of ambient fungal glucan level is very important as they are directly associated with negative health impacts. In response to this reviewer comment we expanded our discussion in page 11, lines 6-21 to read: “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)). The glucan content in fungal cell walls also vary with the fungal species (Foto et al., 2004). Collectively, these differences could give rise to weak or negligible correlations of ambient mannitol and glucan concentrations. Alternatively, non-fungal sources of either mannitol or glucans would confound their correlation. For instance, higher plants and some algae contain mannitol in their structure (Loescher et al., 1992; Shen et al., 1997). Ragweed pollens contain glucans (Foto et al., 2004), is a possible glucan source in late summer when ragweed pollens are prevalent and glucans significantly correlate with sucrose ($r_s=0.5$, $p=0.04$). Alternatively glucans may have derived from bacterial cells (McIntosh et al., 2005; Rylander and Lin, 2000), although their correlation was not significant ($r_s=0.4$, $p=0.1$). Although glucans appear to have been influenced by bacterial and pollen levels in addition to fungi, the assessment of their ambient concentrations remains important, because they are immunostimulants that negatively impact human health (Thorn, 2001; Bonlokke et al., 2006).”

Reviewer #2 specific remark 11: “Section 3.5: It is interesting to note that 92% of bacterial endotoxin is present in coarse fraction of PM. Generally one would expect bacterial endotoxin to be abundant in fine fraction of PM as bacteria are smaller in size. Authors have not given any satisfactory explanation for this low concentration of endotoxin in fine PM.”

Response to reviewer #2 specific remark 11: We thank the reviewer for pointing this out. In response, we added the requested explanation to our discussion of the size distribution of bacterial endotoxins. Now page 11, line 32 reads as “On average, 92 ± 5 % of PM_{10} endotoxins were in the coarse mode (Figure 3c). The distribution of bacterial endotoxins as well as bacterial cells towards larger particles has been demonstrated previously (Nilsson et al., 2011; Monn et al., 1995; Shaffer and Lighthart, 1997). Such observations reflect the association of bacteria with particles prominent in coarse mode such as plant parts, animal parts, soil, spores or pollen surfaces (Jones and Harrison, 2004; Shaffer and Lighthart, 1997). In addition, it has been suggested that bacteria settled on particles are more likely to survive in the atmosphere compared to a single bacterium (Lighthart et al., 1993).”

Reviewer #1 specific remark 12: “Page 13, L 21-22: “The release of pollens, fungal spores, and Gram negative bacteria in fine particles during rain events, as observed surrounding spring and late-summer rain events in Iowa, has the potential to influence human health”. This statement cannot be generalized at least for gram-negative bacteria during spring season where no increase in bacterial endotoxin in fine PM was observed during rain event.”

Response to reviewer #2 specific remark 12: We agree with the reviewer on this comment. In response we revised the text. Page 14, line 22 now reads as “In general, the release of pollens, fungal spores, and Gram-negative bacteria in fine particles during rain events in Iowa, have the potential to influence human health”

Reviewer #2 specific remark 13: “Page 14, L 21-22: “Airborne fungal spore tracers, however, were suppressed by spring rain and increased in concentration following rain events”. I feel this line contradicts the statement given in page 13, L 21, ‘The release of pollens, fungal spores, and Gram negative bacteria in fine particles during rain events, as observed surrounding spring and late-summer rain events in Iowa, has the potential to influence human health”.

Response to reviewer #2 specific remark 13: We agree with this reviewer comment and page 14, line 21-23 is revised. Page 16, line 6 now read as “Airborne fungal spore tracers in coarse PM fraction, however, were suppressed by spring rain and increased in concentration following rain events”

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Influence of Rain on the Abundance ~~and Size Distribution~~ of Bioaerosols in Fine and Coarse Particles

Chathurika M. Rathnayake¹, Nervana Metwali², Thilina Jayarathne¹, Josh Kettler¹, Yuefan Huang¹,
Peter S. Thorne^{2,3}, Patrick T. O'Shaughnessy^{2,3}, and Elizabeth A. Stone¹

5 ¹ Department of Chemistry, University of Iowa, Iowa City, 52242, United States.

² Occupational and Environmental Health, University of Iowa City, 52242, United States.

³ Civil and Environmental Engineering, University of Iowa City, 52242, United States.

10 *Correspondence to:* Elizabeth A. Stone (betsy-stone@uiowa.edu)

Abstract. Assessing the environmental, health and climate impacts of bioaerosols requires knowledge of their size and abundance. These two properties were assessed through daily measurements of chemical tracers for pollens (sucrose, fructose, and glucose), fungal spores (mannitol and glucans) and Gram-negative bacterial endotoxins in fine particulate matter (PM_{2.5}), coarse PM (PM_{10-2.5}) and PM₁₀ (as the combination of PM_{2.5} and PM_{10-2.5}) during the spring tree pollen
15 season (mid-April to early-May) and late summer ragweed season (late-August to early-September) in the Midwestern US in 2013. Under dry conditions, pollen and fungal spore tracers were primarily in coarse PM (>75%), as expected for particles greater than 2.5 μm. Rainfall on May 2 corresponded to maximum atmospheric pollen tracer levels and a redistribution of pollen tracers to the fine PM fraction (>80%). Both changes were attributed to the osmotic rupture of pollen grains that led to the suspension of fine-sized pollen fragments. Fungal spore tracers peaked in concentration following spring rain events and
20 decreased in particle size, but to a lesser extent than pollens. A short, heavy thunderstorm in late summer corresponded to an increase in endotoxin and glucose levels, with a simultaneous shift to smaller particle sizes. Simultaneous increases in bioaerosol levels and decrease in their size has significant implications for population exposures to bioaerosols, particularly during rain events. Chemical mass balance (CMB) source apportionment modelling and regionally-specific pollen profiles were used to apportion PM mass to pollens and fungal spores. Springtime pollen contributions to PM₁₀ mass ranged from
25 0.04–0.8 μg m⁻³ (0.2–38%, averaging 4%), with maxima occurring on rainy days. Fungal spore contributions to PM₁₀ mass ranged from 0.1–1.5 μg m⁻³ (0.8–17%, averaging 5%), with maxima occurring after rain. Overall, this study defines changes to size distributions and concentrations of pollens, fungal spores, and endotoxins in response to rain in the Midwestern United States and advances the ability to apportion PM mass to pollens.

1 Introduction

Inhalable bioaerosols (<100µm) act as aeroallergens, triggering mild to severe allergic respiratory diseases (D'Amato et al., 2007a; Dales et al., 2003). Types of bioaerosols include viruses (<0.3 µm), bacteria (0.25-8 µm), fungal spores (1-30 µm), and plant pollens (~5-100 µm) (Jones and Harrison, 2004; Matthias-Maser and Jaenicke, 1995). Once inhaled, bioaerosols reach different regions of the respiratory system based on their size (Oberdörster et al., 2005; Brown et al., 2013), which is dependent on the route of breathing, age, gender, and activity level (Brown et al., 2013). In general, particles of 3 µm and 5 µm for adults and children, respectively, travel beyond the larynx (Brown et al., 2013). Human immune system produces antibodies against inhaled aeroallergens that initiate airway symptoms (e.g., cough and runny nose), and exacerbate diseases like asthma and allergic rhinitis. Allergic respiratory diseases are estimated to affect 334 million people worldwide, particularly children (GAN, 2014). These respiratory illnesses are predicted to increase in response to global trends of increasing carbon dioxide concentrations (Singer et al., 2005; Ziska and Caulfield, 2000) and temperatures (Beggs, 2004) that enhance the allergenicity (Singer et al., 2005) and quantity (Ziska and Caulfield, 2000) of pollens, and duration of pollen seasons (Beggs, 2004; Beggs and Bambrick, 2006). The protection of sensitive populations from bioaerosols requires understanding environmental exposures to bioaerosols as a function of their type, size, and temporal variation.

Ambient levels of pollens vary seasonally with growing-eyesplant phenology (Galán et al., 1995; Targonski et al., 1995). Springtime in the Midwestern United States is generally characterized by high levels of tree pollens (Targonski et al., 1995), such as oak (Wallner et al., 2009), birch (Emberlin et al., 2002), alder, and hazel (Niederberger et al., 1998). Summertime has elevated concentrations of grass pollens (e.g., Timothy and Rye grass) and weed pollens, especially ragweed (Targonski et al., 1995). Daily pollen levels are affected by temperature, with warmer conditions favouring pollen development, maturation, and active release (van Vliet et al., 2002). Rainfall promotes the passive release of intact pollens by agitation (Taylor and Jonsson, 2004). In rainy conditions, pollen grains absorb water, osmotically rupture, and release cytoplasmic starch granules (D'Amato et al., 2007b). Microscopy studies have shown that intact birch pollens of 22 µm in size can rupture and release around 400 starch granules (Staff et al., 1999) ranging from 0.03 - 4 µm (D'Amato et al., 2007b). Consequently, human exposures to pollens in the atmosphere are highly dependent on pollen type, season, and local meteorology.

Fungal growth and spore release is also promoted by elevated temperatures (Corden and Millington, 2001) and wet conditions (Pasanen et al., 2000). Fungi discharge spores via splash-induced emission, as is the case for *Cladosporium*, a prominent fungal genus (Troutt and Levetin, 2001; Oliveira et al., 2009) that releases spores by mechanical shock and fast air currents produced by rain drops (Elbert et al., 2007; Allitt, 2000). Fungi that belong to the division Ascomycetes disperse spores in moist conditions (Jones and Harrison, 2004) leading to elevated spore levels several hours after rain (Allitt, 2000; Packe and Ayres, 1985). The release of bioaerosols during and after rain events can trigger significant changes to ambient bioaerosol numbers (Knox, 1993; Huffman et al., 2013) and mass concentrations (Marks et al., 2001).

Bacteria in the atmosphere are typically ~~settled on~~ attached to soil or vegetative surfaces ~~and are found in~~ agglomerations of cells ~~agglomerates~~ (Jones and Harrison, 2004). Taxonomic analysis has revealed that soil and plant surfaces serve as sources of bacteria in the Midwestern US (Bowers et al., 2011). Ambient bacterial levels increase with temperature (Carty et al., 2003) due to conditions that favour vegetation and bacterial habitat (DeLuca and Palmgren, 1986; 5 Romantschuk, 1992). In vegetation covered areas, atmospheric bacterial concentrations have been shown to ~~increased~~ peaked after approximately 1 h of rain relative to areas with bare soil (Robertson and Alexander, 1994) during and after ~~stimulated rain events~~ (Graham et al., 1977; Robertson and Alexander, 1994) as well as natural rain events (Constantinidou et al., 1990; Huffman et al., 2013). This response to precipitation has been attributed to rain moving plants and aerosolizing bacteria (Jones and Harrison, 2004). With strong dependences on local meteorology, bacteria, are likely to exhibit high 10 temporal variability.

Once released, bioaerosols in the atmosphere promote cloud and ice nucleation (Pope, 2010; Sun and Ariya, 2006; Murray et al., 2012). Intact birch, walnut and willow pollens have been demonstrated to be cloud condensations nuclei (CCN) (Pope, 2010), with cytoplasmic pollens granules ranging 0.05-0.3 μm being the most CCN active, due to their hygroscopicity and longer residence time (Steiner et al., 2015). Bacteria also are CCN, at relatively low supersaturations 15 (Sun and Ariya, 2006; Franc and Demott, 1998). Because of their ordered structures, bioaerosols are effective ice nuclei (IN) forming ice crystals at sub-cooled temperatures, including intact pollens (Diehl et al., 2001; Diehl et al., 2002), pollen extracts (Augustin et al., 2013), fungal spores and bacteria (Murray et al., 2012). ~~and bacteria (Sun and Ariya, 2006; Pouleur et al., 1992)~~. Their ability to act as CCN and IN affects the earth's climate through changes to cloud albedo and precipitation cycles (Diehl et al., 2001; Sun and Ariya, 2006).

20 Atmospheric levels of bioaerosols can be assessed through measurements of specific chemical and biological tracers. Glucose, fructose and sucrose are main energy storage material in plants, major contributors to pollen mass (Speranza et al., 1997; Fu et al., 2012) and have been used as pollen tracers in China and the United States (Fu et al., 2012; Jia et al., 2010a; Jia et al., 2010b) Although not unique to pollens, these three sugars also comprise a minor fraction of suspended soil (Rogge et al., 2007), road dust (Simoneit et al., 2004) and biomass burning (Medeiros and Simoneit, 2008). 25 Mannitol and arabitol are sugar alcohols that serve as energy storage materials in fungi and are used to identify the presence of airborne fungal spores and to quantify their contributions to PM mass (Bauer et al., 2008; Zhang et al., 2010). 1,3- β -D-glucans are immune-active polysaccharides in fungal cell walls (Thorn et al., 2001; Bonlokke et al., 2006) that are also tracers of fungal spores that have been used to assess exposure levels in indoor and outdoor environments (Madsen, 2006; Crawford et al., 2009). Endotoxins are lipopolysaccharides in Gram-negative bacterial membranes that induce respiratory 30 inflammations (Douwes et al., 2003; Thorne et al., 2015). Ambient levels of endotoxins have been measured in outdoor (Pavilonis et al., 2013) and occupational settings (Thorne et al., 2009). Measurement of these bioaerosol tracers allows for the evaluation of the atmospheric concentrations and size distributions of pollens, fungal spores, and Gram-negative bacteria.

Given the important role of bioaerosols in the health of sensitive populations and in atmospheric processes, a robust understanding of bioaerosol types and their response to changing meteorological conditions is needed. Our central objectives

were *i*) to assess temporal variations in pollens, fungal spores and endotoxin concentrations and size distributions, *ii*) evaluate environmental conditions including rain and temperature that lead to high levels and decreases in bioaerosol sizes across fine (PM_{2.5}) and coarse (PM_{10-2.5}) modes, *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, and *iv*) estimate pollen and fungal spore contributions to PM mass by way of chemical mass balance (CMB) modelling. The outcomes of this study include an improved understanding of changes in ambient concentrations and PM size distributions of bioaerosols in response to rain events and their contributions to PM mass.

2 Methods

10 2.1 Sample collection

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_{2.5} and PM_{10-2.5} were collected using an Andersen dichotomous sampler (Series 241) that included a PM₁₀ cut-off impactor (Anderson Instruments, Model 246b) and virtual impactor. The total air flow rate was 16.67 L min⁻¹ and the coarse flow rate was 1.667 L min⁻¹. PM samples were collected on 37-mm Teflon filters (Pall Corp.) and PM₁₀ was determined as the sum of PM_{2.5} and PM_{10-2.5}. 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_{2.5} 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_{2.5} at a flow rate of 90 L min⁻¹. Rain was excluded from the PM_{2.5} 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.

To assess the representativeness of 2013 PM levels to typical conditions in Iowa, PM_{2.5} and PM₁₀ mass measurements were compared to measurements from 2010-2015 downloaded from the Technology Transfer Network (TTN) Air Quality System (AQS) Data Mart (USEPA, 2013). The federal reference method (FRM) site for Johnson County, Iowa is located at Hoover Elementary School, (+41.6572, – 91.5035), 6.3 km east of the University of Iowa air monitoring site. PM_{2.5} concentrations were compared to average levels over the sampling period calculated from hourly measurements while PM₁₀ data were compared to filter measurements collected from midnight to midnight every three days.

2.2 PM mass measurement

PM mass was determined by the difference of pre- and post-sampling Teflon filter weights. Filter measurements made in a temperature (21.9 °C) and humidity controlled (25±5%) room using an analytical microbalance (Mettler Toledo XP26) after conditioning 48 hours. Standard deviations of triplicate measurements were used as the error associated with the mass measurement.

5

2.3 Analysis of carbohydrates and inorganic ions

All glassware was prebaked at 500°C for 5 hours, while plastic vials used were pre-rinsed with ultrapure (UP) water (resistivity >18.2 MΩ cm⁻¹) (Barnstead EasyPure II, 7401). Teflon filters (containing PM_{10-2.5} samples) were cut in half using ceramic scissors on a clean, guided glass surface. Prior to extraction, Teflon filters were pre-wet with 100 µL of acetone (Sigma Aldrich). Subsamples of Teflon and quartz ~~fiber~~ fiber filters (containing PM_{2.5}) were extracted into 4.00 mL of UP ~~water~~ by water by rotary shaking for 10 min at 125 rpm, ultra-sonication for 30 min at 60 Hz (Branson 5510, Danbury, CT, US), and then rotary shaking for 10 additional min. The extract was then filtered through a 0.45 µm polypropylene syringe filter (GE Healthcare, UK).

Carbohydrate concentrations were determined by high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD, Dionex ICS 5000, Thermo Fisher, Sunnyvale, CA, USA). The HPAEC-PAD instrument consisted of an eluent organizer, dual pump, degasser, column compartment, electrochemical detector (ED50), AS-DV autosampler, CarboPac PA20 analytical column (3 X 150 mm, Dionex), guard column (3 X 30 mm), and a 10 µL injection loop. An isocratic separation of carbohydrates (erythritol, arabitol, fucose, trehalose [Alfa Aesar], glucose, fructose, arabinose, xylitol, xylose [Sigma Aldrich], rhamnase, mannose, ribose [Acros], sucrose and mannitol [Fisher Scientific]) was achieved with 10 mM sodium hydroxide (NaOH, Fisher Scientific) that was stored under N₂ (Praxair). The detector cell contained a gold disposable working electrode, to which quadruple waveform A was applied relative to a pH-Ag/AgCl reference electrode (Rocklin et al., 1998; Jensen and Johnson, 1997). Chromeleon 7 software was used for instrumental control, data acquisition and analysis. Carbohydrates were quantified against seven-point calibration curves ranging from 0.0100–2.50 ppm. Each analysis batch consisted of eight PM samples, two field blanks, one lab blank and one spike recovery sample. Summarized in Table S1 are carbohydrate extraction efficiencies (94-103%), instrument detection limits, and method detection limits.

Inorganic ion concentrations were determined using ion exchange chromatography with suppressed conductivity detection (ICS-5000, described above) following Jayarathne et al. (2014). Briefly, anions were separated on an Ionpac AS22 analytical column (4 X 250 mm, Dionex) preceded by a guard column and followed by a suppresser (Dionex AERS 500). Cations were separated on an Ionpac CS12A analytical column (3 X 150 mm, Dionex) preceded by a guard column, and followed by suppresser (Dionex CERS 500). Seven-point calibration curves were prepared from Seven Anion Standard and Six Cation-II Standard (Dionex) over the range of 0.010–10.0 ppm. Method performance metrics are summarized elsewhere (Jayarathne et al. 2014).

2.4 Analysis of biomarkers

Biomarkers were analyzed in extracts from the remaining halves of Teflon filters containing coarse PM and entire Teflon filters containing fine PM. Filters were extracted via shaking into 2 mL of sterile pyrogen-free (PF) water for 1 h at 22°C. Extracts were then centrifuged (5 min at 600g at 4 °C).

5 For analysis of fungal glucans, one aliquot of the supernatant was transferred into a PF borosilicate tube, mixed with 10x PF phosphate buffered Saline containing 0.05% Tween-20 (a surfactant), shaken for 1 h, autoclaved for 1 h, shaken for 20 min shaking, and then centrifuged for (600g at 4°C) 20min. Glucans were quantified by enzyme immunoassay as previously described by Blanc et al. (2005). A 12-point calibration curve prepared from (1-3, 1-6)-β-D-glucan (*scleroglucan*) ranged from 3-5000 ng mL⁻¹. The solution absorbance was measured at 450 nm (SpectraMax Plus 384; 10 Molecular Devices, Sunnyvale, CA, USA).

For analysis of endotoxins, a second aliquot of the supernatant was subjected to the kinetic chromogenic *Limulus* amoebocyte lysate assay (LAL) (Lonza, Inc., Walkersville, MD) as described in Thorne (2000). The 12-point calibration curve was generated utilizing endotoxin standard (*Escherichia coli* 055:B5) at concentrations ranging from 0.024-50 Endotoxin Units (EU) mL⁻¹. The solution absorbance was measured at 405 nm (SpectraMax M5, Molecular Devices).

15

2.5 Collection and analysis of pollens

Oak pollens were harvested from pin and red oak trees in park areas surrounding Iowa City during the spring of 2013 into pre-cleaned aluminium foil lined bags. Cotton and giant ragweed pollens were collected in late-summer of 2015 from bushes near roadways in residential areas of Iowa City. Cotton ragweed and corn pollens were purchased (Polysciences 20 Inc., Warrington, PA). Pollen images were taken to determine pollen grain diameters using a Zeiss LSM 710 fluorescence microscope (Carl Zeiss Microscopy GmbH, 07745 Jena, Germany) following Pöhlker et al. (2012), and IX-81 inverted microscope (Olympus Corporation, Tokyo, Japan). Prior to extraction and chemical analysis, pollens were desiccated overnight and weighed (Mettler Toledo XS204 and XP26 balances). Pollens (~0.005–0.015 g) were extracted and analysed following the methods described in section 2.3.

25 2.6 Chemical Mass Balance (CMB) modeling

PM mass was apportioned to fungal spores and pollens using EPA-CMB model (version 8.2). PM_{2.5} and PM₁₀ mass (from the sum of PM_{2.5} and PM_{10-2.5}) was apportioned to bioaerosols using sucrose, glucose, fructose, and mannitol as fitting species. Input source profiles included one pollen profile selected from red oak, pin oak (this study), white birch, Chinese willow, or Peking willow (Fu et al., 2012) and one fungal spore profile (Bauer et al., 2008). Sensitivity tests were conducted 30 to assess the fit of different pollen profiles to ambient measurements, focusing on sampling days from 26 April–9 May when pollen tracer levels were highest.

2.7 Statistical analysis

Prior to statistical analysis, data points below detection limits were substituted with the limit of detection (LOD)/ $\sqrt{2}$ (Hewett and Ganser, 2007). Concentration measurements were tested for normality and log-normality using the Anderson-Darling test in Minitab (version 16). Species concentration measurements were not normally distributed, thus Spearman's rank order correlation was employed for non-parametric comparisons (r_s) in ~~SPSS (Statistical Package for the Social Sciences 21)~~Minitab (version 16). PM measurements were normally distributed thus t-tests comparing PM means from dry and rainy periods was conducted in Minitab (version 16). Significance was assessed at the 95% confidence interval ($p \leq 0.05$).

3 Results and discussion

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 rare in bacteria. In the ambient particulate matter, these species are 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.

3.1 Characterization of pollens common to the Midwestern US

Red oak, pin oak, corn, cotton ragweed and giant ragweed pollen ranged in average diameter from 20 - 90 μm (Figure S1, Table 1). Together, glucose, fructose and sucrose accounted for an average of 5–14 % of pollen mass, while erythritol, arabinose, mannitol and rhamnose were detected in trace amounts (Table 1). Due to the relatively high mass fraction of glucose, fructose, and sucrose in pollens in the present and in prior studies (Fu et al., 2012; Speranza et al., 1997) these carbohydrates are the best candidates for assessing pollen contributions to ambient PM. Notably, the carbohydrate distributions in corn pollens differ from those previously reported (Speranza et al., 1997), with differences likely resulting from genetics (Speranza et al., 1997) and environmental factors (e.g. temperature, availability of water, and CO_2 levels) that are known to affect the synthesis and storage of carbohydrates (Aloni et al., 2001; Yoshida et al., 1998; Vesprini et al., 2002). Across different pollen types, the relative abundances of glucose, fructose and sucrose varied. For instance, the most abundant carbohydrate was sucrose for red oak, pin oak, and Polysciences cotton ragweed, fructose for corn pollen, and glucose for local cotton and giant ragweed. Sucrose to fructose ratios across different pollen types may serve to identify pollen types in ambient PM, in cases when a single pollen type is dominant (as discussed in section 3.6.1).

3.2 Fine and coarse PM concentrations

3.2.1 Spring

From 17 April to 9 May, 2013, daily PM₁₀ levels in Iowa City ranged from 2–32 µg m⁻³ (with an average of 15±8.9 µg m⁻³), and fine PM ranged from 2–13 µg m⁻³ (with an average of 7.1±3.0 µg m⁻³). Comparison to PM levels at a nearby FRM site (located 6.3 km to the east) from 2010–2015 (Table S2), demonstrated that spring 2013 PM levels were typical for the surrounding years.

On 15 of the 23 spring sampling days, conditions were dry and no rain occurred (Figure 2a1a). On the remaining 8 days, daily rainfall totalled 0.3–85 mm. Rainfall ~~corresponding~~ corresponded to low PM concentrations with average fine PM levels decreasing from 8.3±2.6 µg m⁻³ on dry days to 4.7±2.2 µg m⁻³ on rainy days and coarse PM levels decreasing from 10±5.6 µg m⁻³ to 1.9±1.5 µg m⁻³ (Figure 2b1b). The PM reduction on rainy days was statistically significant (p<0.01) and was driven by wet deposition of PM in both size modes. Rain also affected the distribution of particles between the fine and coarse modes. PM_{2.5} contributed, ~~with~~ 48±11 % of PM₁₀ ~~was less than 2.5 µm~~ on rainy days compared to 80±13 % on dry days. 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 consistent with Pprevious ambient studies that have demonstrated coarse PM is more effectively scavenged than fine particles also demonstrated better particulate scavenging efficiencies of coarse particles compared to fine PM (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- µm have a higher tendency to collide with falling rain drops and are are effectively scavenged via inertial impaction (Wang et al., 2010; Andronache, 2003; Mircea et al., 2000).

3.2.2 Late summer

Only one brief rain occurred during the three-week campaign, on August 22 when a thunderstorm brought 1.0 mm between 10–11 am (Figure 3a2a). From 15 August to 4 September, 2013, Iowa City daily PM₁₀ levels as shown in Figure 3b2b, ranged from 21–50 µg m⁻³ (averaging 33±8 µg m⁻³) and fine PM levels ranged from 3–17 µg m⁻³ (averaging 12±4 µg m⁻³). On average, fine PM accounted for 39±12 % of PM₁₀. Compared to adjacent years (2010–2015), the late-summer of 2013 exhibited higher PM levels (Table S3). This is attributed to unusually dry conditions that reduce soil moisture leading to increase soil resuspension, and lack of wet deposition.

3.3 Pollen tracers

3.3.1 Spring

The temporal variations of pollens were assessed utilizing the combination of glucose, fructose and sucrose as chemical tracers. Ambient concentrations of these pollen tracers were relatively low from 17–25 April when lower temperatures (averaging 7 °C) and rainy conditions prevailed. Pollen tracers levels were relatively higher from 26 April–9 May, coinciding with warmer temperatures (averaging 15 °C) that marked the transition from winter to spring (Figure 2e1c-

e, Table S4). Temperature and coarse mode glucose and sucrose were significantly correlated ($r_s \geq 0.8$, $p < 0.001$), reflecting that warmer temperatures promote the development, maturation, and release of pollens.

After the onset of spring, rain events increased pollen levels. For instance, maximum fructose and sucrose levels occurred on 2 May and maximum glucose on 9 May; rain occurred on both of these days, following a dry period with relatively high temperatures. Remarkably, rain events substantially altered the size distribution of pollen tracers. On a typical dry day, more than 80% of pollen tracers were present in coarse PM, which is expected for pollen particles that have geometric diameters in the range of 5-100 μm (Huffman et al., 2010). However, when pollen markers peaked on 2 May, mass fractions of glucose, fructose and sucrose in the fine mode reached 83%, 91% and 93%, respectively (Figure 2e1c-e, right axis). With continued rainfall on 3-4 May, pollen markers remained elevated in the fine mode relative to coarse PM. After the rain stopped, coarse mode pollens increased in concentration and resumed the typical size distribution by 5 May. Light rainfall on 9 May coincided with increases in glucose in both size modes, with only 14% of these tracers in the fine mode. Together, these data indicate the possible suggest release of pollen fragments less than 2.5 μm during some rain events (2-4 May) and the passive release of some larger pollen particles in the coarse particle size range ranging 2.5-10 μm during others (9 May). Notably, this is the first observation of the release of fine particle pollen fragments to the atmosphere using chemical tracers. Most field measurements include analysis of either $\text{PM}_{2.5}$ or PM_{10} , while measurements in both size modes are required to capture this phenomenon.

The likely explanation for the increase in airborne pollens and simultaneous decrease in their size on May 2 is the rupturing of pollen walls as a result of the osmotic pressure that builds up inside the pollen due to absorbed moisture during rain (Taylor et al., 2004; Taylor et al., 2002). Osmotic shock has been previously demonstrated to cause rupturing of grass and birch pollens that releases cytoplasm (Taylor et al., 2004; Taylor et al., 2002; Suphioglu et al., 1992). Gusty winds can loft pollen fragments (Wallis et al., 1996) and strong winds on 2 May are likely to have contributed to the elevated fine pollen levels.

Differences in the size distributions of pollen tracers during the rain events on 2 May (mostly fine PM) and 9 May (mostly coarse PM) are expected to result from different pollen types predominating as evidenced by differing ratios of carbohydrates. On 2 May, the relative ratios of glucose and sucrose (normalized to fructose) in fine PM were 1.4 and 2.5, respectively, close to the ratios of red oak (1.2 and 2.1, respectively). Moreover oak trees are abundant in Eastern Iowa and a prominent pollen type in the springtime, making oak a likely (but unconfirmed) source of pollens in fine PM. Meanwhile, the respective carbohydrate ratios on 9 May (18 and 0.7, respectively) did not match any of the local or literature available pollen profiles. These data suggest that certain pollen types undergo osmotic rupturing and release fine particles, while others do not. Further studies are needed to identify the types of pollens that rupture and conditions under which osmotic rupturing occurs.

3.3.2 Late summer

From mid-August to early-September, average temperature was moderately correlated with coarse mode glucose, fructose and sucrose ($r_s > 0.5$, $p < 0.02$). In the fine mode, glucose was frequently detected, while fructose and sucrose not (Figure 3e2c-e); this is likely due to the predominant pollen type having higher glucose concentrations relative to fructose and sucrose, as is the case for ragweed pollens (Table 1). The potential of glucose deriving from soil (Rogge et al., 2007; Simoneit et al., 2004) suspended in the air by splashing (Joung and Buie, 2015) was eliminated because there was no corresponding change in calcium, a well-established soil-tracer. Consequently, glucose is considered to be a tracer for pollens even in the absence of the other two pollen tracers, and the discussion of pollen size distribution relies solely on glucose for this time period. On average, 83% of glucose mass concentration was found in coarse mode (Figure 3e2c), consistent with typical size range of intact pollens (Huffman et al., 2010). The single late-summer rain event on 22 August coincided with an increase in fine mode glucose concentration and an increase of the fine PM fraction of glucose to contribution of glucose in fine fraction increased shift in glucose size distribution to 34% in the fine mode, compared to 16% on dry days. The late summer single rain event indicated passive release of pollen fragments in response to rain that was similar to spring (section 3.3.1). However, with only one rain event occurring in the late summer study in 2013, additional studies are needed to validate these trends and identify the responsible pollen types.

3.4 Fungal spore tracers

3.4.1 Spring

Daily ~~concentrations of~~ coarse mode fungal spore tracer concentrations significantly correlated with daily average temperature: of two fungal spore tracers—fungal sugar mannitol and temperature ($r_s = 0.7$, $p < 0.001$) and the fungal cell wall component glucan and temperature—were significantly correlated with daily average temperature ($r_s \geq 0.4$, $p \leq 0.0504$). From 17–21 and 23–25 April, cooler temperatures prevailed (averaging 6 and 7 °C, respectively) and PM₁₀ mannitol and glucan concentrations were relatively low (Figure 4a-3a and b). An exceptionally high PM₁₀ glucan level occurred (Figure 4b3b) on April 22, when temperature increased to a local maximum of 14 °C. From 26 April, temperatures warmed to an average of 15 °C, concurrent with an increase fungal spore tracer levels. The correlation of temperature with fungal spore tracers is consistent with warmer temperatures favouring fungal growth (Corden and Millington, 2001; Rodriguez Rajo et al., 2005). The two tracers were moderately correlated with one another ($r_s = 0.5$, $p < 0.02$), signifying their origin from the same source.

Rain influenced ambient concentrations and the size distributions of fungal spore tracers, possibly likely by promoting their growth, triggering passive and/or active release mechanisms and/or promoting fungal growth. Maximum mannitol and glucan levels occurred on 5 May, which followed three days with rain (Figure 4a3a-b). The wet Rainfall conditions 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). For instance, actively wet discharged ascospores shown to peak after rain occurred in wet conditions. Known for releasing spores after rain are some Ascospores (Troutt and Levetin, 2001; Elbert et al., 2007;

MacHardy and Gadoury, 1986)²~~which are abundant in the US (Shelton et al., 2002)~~. Fungal spore tracer levels in coarse PM dropped on days when rain fell (e.g. 23 April, 2 May), due to particle removal by wet deposition. The size distributions of fungal spores, which typically have intact diameters in the range of 1–30 μm (Jones and Harrison, 2004), also were influenced by rain. During dry days, 13% of fungal spore tracers were in the fine PM fraction. On rainy days, the fraction of
5 fungal spore tracers in the fine mode reached local maxima at 41% (23 April), 36% (24 April), and 54% (2 May) for mannitol and 38% for glucans (23 April; Figure 4a3a-b, right axis). The relative decrease in the size of fungal spores is attributed to a combination of the passive release of fungal spores less than 2.5 μm via rain splash and mechanical agitation of vegetative surfaces by rain drops (Allitt, 2000; Elbert et al., 2007; Huffman et al., 2013), and the removal of coarse fungal spore particles by droplet scavenging. Compared to pollens (section 3.3.1), rain events impacted the size distribution of
10 fungal spores to a much lesser extent.

3.4.2 Late summer

From mid-August to early-September atmospheric concentrations of mannitol correlated with temperature ($r_s=0.5$, $p=0.01$), consistent with increased fungal growth with elevated temperatures (section 3.4.1). Fine mode mannitol reached a
15 maximum on 22 August when rain fell during a one hour period (Figure 5a4a), likely due to fungal spore release via rain splash ~~and mechanical~~and mechanical agitation (section 3.4.1). Coarse mode mannitol also increased on 22 August, most likely due to release of fungal spores after rain subsided in response to wet conditions. Mannitol in fine PM accounted for an average of $9\pm 4\%$ of the total PM_{10} concentration and was not substantially different on 22 August (14%).

Coarse mode glucan concentrations in late summer were neither correlated with temperature ($r_s=0.01$, $p=1$), nor
20 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)). The glucan content in fungal cell walls also vary with the fungal species (Foto et al., 2004). Collectively, these differences could give rise to weak or negligible—a poor correlations of ambient mannitol and glucan concentrations. Alternatively, non-fungal sources of either mannitol or glucans would confound their correlation. For instance, higher plants and some algae contain mannitol in their structure (Loescher et al., 1992; Shen et al., 1997). Ragweed pollens contain glucans (Foto et al., 2004), is a possible glucan source in late summer when ragweed pollens are prevalent and likely source of glucans in late summer, due to the correlation of glucans significantly correlate with sucrose ($r_s=0.5$, $p=0.04$) and prior demonstration of glucans in ragweed pollens (Foto et al., 2004), which is a prevalent pollen in Iowa during the late summer. Alternatively glucans may have derived from bacterial cells (McIntosh et al., 2005; Rylander and Lin, 2000), as suggested by the moderate, but not statistically significant correlation of coarse mode glucans with bacterial endotoxins although even though their correlation was not significant ($r_s=0.4$, $p=0.1$), and prior observations work pathogenic show glucans in some bacterial cells that grow on crops (i.e. *Agrobacterium spp.*, and *Rhizobium spp.*) contain glucans in their structure. Agricultural crops are abundant in Iowa during
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~~the growing season and the mechanical agitation of plant surfaces by wind can aerosolize surface bacteria.~~ Although glucans appear to have been influenced by bacterial and pollen levels in addition to fungi, the assessment of their ambient concentrations remains important, because they are immunostimulants that negatively impact human health (Thorn, 2001; Bonlokke et al., 2006).

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3.5 Bacterial endotoxins

3.5.1 Spring

Coarse mode bacterial endotoxins, measured in endotoxin units (EU) against an *Escherichia coli* (055:B5) standard, were significantly correlated with daily average temperature ($r_s=0.7$, $p<0.001$). Lower temperatures averaging 7 °C from 17-25 April, led to low endotoxin levels compared to a warmer period averaging 11-23 °C from 26 April-1 May. The correlation of endotoxins with temperature agrees with prior ambient studies (Carty et al., 2003; Guan et al., 2014; Degobbi et al., 2011; Rathnayake et al., 2016) and is attributed to warmer temperatures increasing vegetative surfaces that serve as substrates for bacterial growth (Romantschuk, 1992; DeLucca and Palmgren, 1986; Carty et al., 2003). Heavy rain on 2 and 3 ~~May~~ ~~led~~ ~~May~~ ~~led~~ to a drop in PM₁₀ endotoxin concentrations, due to wet deposition and suppression of soil dust particles upon which bacteria settle. On average, 92±5 % of PM₁₀ endotoxins were in the coarse mode (Figure 3c). The distribution of bacterial endotoxins as well as bacterial cells towards larger particles has been ~~observed~~ demonstrated previously (Nilsson et al., 2011; Monn et al., 1995; Shaffer and Lighthart, 1997). ~~previously~~, Such observations reflecting reflected the association of bacteria with particles prominent in coarse mode such as plant parts, animal parts, soil, spores or pollen surfaces ~~by settling on their surfaces which themselves become airborne~~ (Jones and Harrison, 2004; Shaffer and Lighthart, 1997). ~~and agglomeration~~ (Jones and Harrison, 2004; Lighthart et al., 1993) In addition, it is has been suggested that bacteria settled on particles are more likely to survive in the atmosphere compared to a single bacterium (Lighthart et al., 1993). Coarse mode endotoxins demonstrated a moderate positive correlation with calcium, the crustal element ($r_s=0.7$, $p<0.001$), which suggests soil resuspension as a source of endotoxins in Iowa City, which has been demonstrated previously in the Midwestern US (Bowers et al., 2011; Rathnayake et al., 2016).

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3.5.2 Late summer

In late ~~summer~~ summer, ambient endotoxin concentrations had a positive moderate correlation with coarse mode endotoxins ($r_s=0.5$, $p=0.02$) similar to springtime (section 3.5.1). ~~—~~ On 22 August, the only late summer day with rain, fine mode endotoxin concentration s reached a maximum (Figure ~~5e4c~~). Meanwhile, the endotoxin fraction in the fine mode increased to 36% relative to an average of 5-% on dry days. Rainfall promotes bacterial growth. (Hirano et al., 1996) such as *Pseudomonas syringae* that are common on ~~plant surfaces and rapidly~~ increase their populations during raining (Hirano and Upper, 1990; Hirano et al., 1996). The release of endotoxin to fine PM is expected to be caused by the ~~aerosolization dissemination~~ 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,

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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/behaviour in response to rain suggests that soil bacteria are dominate in springtime, while bacteria residing on plant surfaces dominate in fall/late-summer.

3.6 Contributions of pollens and fungal spores to PM mass

CMB source apportionment modelling was applied to estimate mass contributions of pollens and fungal spores to PM_{10} and $PM_{2.5}$. This work extends the application of fungal spores tracer-to-mass ratios to estimate their contributions to PM mass (Di Filippo et al., 2013; Zhang et al., 2010) to pollens for the first time. The CMB model requires representative source profiles for sources, which were drawn from the literature in the case of fungal spores (Bauer et al., 2008), birch, and willow pollens (Fu et al., 2012), and from this study (section 3.1).

3.6.1 Source apportionment in spring

The pollen profiles that explained the greatest fraction of the variance in the springtime measurements (assessed by the CMB R^2 value) were pin oak and red oak (Figure S1S2). The resultant R^2 value further increased when fungal spores were added to the model (Figure S1S2). Birch and willow profiles, which showed an excess of sucrose (Fu et al., 2012) explained a substantially lower fraction of the variance in ambient data, where glucose and fructose concentrations outweighed sucrose. Hence, birch and willow pollen profiles were not considered further. Model results from using pin oak or red oak profiles in concert with the fungal spore profile produced consistent source contributions that were strongly correlated (Figure S2S3). Because red oak and pin oak fit ambient data to a comparable extent and both are sources of atmospheric pollens in Iowa, the best estimate of pollen contributions was calculated as the average contribution from red oak and pin oak.

Pollen and fungal spore contributions to PM_{10} and $PM_{2.5}$ estimated by the CMB model are shown in Figure 6-5 (and Table S6). Overall, contributions to fine PM after onset of spring, from 26 April-09 May ranged from $0.01-0.7 \mu\text{g m}^{-3}$ for pollens and $0.03 - 0.1 \mu\text{g m}^{-3}$ for fungal spores, while contributions to PM_{10} were consistently higher at $0.04-0.8 \mu\text{g m}^{-3}$ for pollens and $0.13-1.5 \mu\text{g m}^{-3}$ for fungal spores. On dry days, pollens contributed an average of 0.7% of $PM_{2.5}$ and 3.3% of PM_{10} . On rainy days, pollen contributions to fine PM averaged 11% and reached a maximum of 42% on May 2. Fungal spore contributions to fine PM averaged 0.5% on dry days and 1.7% on days with rain. Meanwhile, fungal spores had the greater contributions to PM_{10} mass on days following rain, reaching 8.7% on May 5. These source apportionment results

demonstrate that bioaerosol contributions to PM_{10} mass in spring are typically low with averages of 4% and 5% for pollens and fungal spores, respectively), but can be significantly greater on days with rain, when bioaerosols are released and PM is removed by wet deposition. The distribution of bioaerosols in fine and coarse PM during spring is shown in Figure 76. For dry conditions, ~11% of pollens and fungal spores were observed in fine PM. However, during rainy days, 62% of pollen mass and 20% of fungal spore mass were observed in fine PM. These results indicate the importance of rain in shifting the size distribution of bioaerosols by affecting release mechanisms (i.e. passive release by splashing and mechanical agitation, or osmotic rupture of pollens).

Bioaerosol contributions to PM in this study were relatively in good agreement with prior studies. The average fungal spore contribution to PM_{10} in spring (5%) was 1.6 times higher than suburban site of Vienna, Austria, and 1.6 times lower than a tropical rainforest in China (Zhang et al., 2010), which were measured during springtime. Collectively, contributions from pollens (3.3%) and fungal spores (0.9%) to fine PM was ~2 times lower than contributions reported in US which determined in summertime (Coz et al., 2010). The slight variations of contributions could be attributed to the differences in ambient bioaerosol levels and geographical differences.

15 3.6.2 Source apportionment in late-summer

PM mass could not be apportioned to pollens in late summer, because of poor agreement between ambient data and source profiles. Fewer than 10% of the ambient PM samples had relative ratios of sucrose, fructose, and glucose in the range ragweed pollen profiles, which is a dominant pollen type in the Midwest. This lack of agreement could result from mixtures of pollen in the atmosphere that are not represented when utilizing a chemical profile for a single pollen type, and/or other dominant pollen types during late summer (e.g. Timothy grass and rye grass).

Fungal spore contributions to PM were estimated using the average mannitol conversion factor of 1.7 pg mannitol spore⁻¹ (range from 1.2-2.4 pg mannitol spore⁻¹) and a spore mass of 33 pg from Bauer et al. (2008). Resultant fungal spore mass contributions to $PM_{2,5}$ and $PM_{10-2,5}$ ranged from 0.04-0.31 $\mu\text{g m}^{-3}$ and 0.45-3.44 $\mu\text{g m}^{-3}$, respectively, (Table S7). The contribution of fungal spores to $PM_{2,5}$ averaged 1% on dry days, and 3% on 22 August when it rained. Meanwhile, fungal spore contributions to $PM_{10-2,5}$ averaged 6% and reached to 16% on 22 August. The maximum fungal spore contributions to PM on 22 August is likely due to fungal spores released during rain by passive mechanisms and after rain by active mechanisms (section 3.4.1). This leads to an increase in fine sized fungal spores when raining, and coarse sized spores post-rain (Huffman et al., 2013; Hjelmroos, 1993).

30 3.7 Implications of the release of fine bioaerosols surrounding rain events

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., 2007b), 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 µm), 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.

In general, the release of pollens, fungal spores, and Gram-negative bacteria in fine particles during rain events in Iowa, as observed surrounding spring and late-summer rain events in Iowa, have, has the potential to influence human health. ~~Elevating~~ Elevating ambient fungal spore levels, particularly from species like ~~Aseospores~~ *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 exacerbations (Dales et al., 2003). Likewise, endotoxins induce inflammations in the respiratory tract (Dales et al., 2006; Liebers et al., 2008; Thorne et al., 2015). When pollen levels increase in concentration and decrease in size (as observed on May 2, May 9, and August 22), likely due to pollen rupturing, cytoplasmic pollen allergens (Suphioglu et al., 1992; Grote et al., 2001) will be released, leading to more direct exposure of humans to aeroallergens through inhalation. In the form of smaller particles, aeroallergens penetrate deeper into the respiratory tract where they may trigger more severe allergenic responses (Taylor et al., 2002; Wilson et al., 1973). Acute asthma epidemics have been associated with rain events have been documented in Australia, Europe, Mexico and the US (D'Amato et al., 2016; Dales et al., 2003; Grundstein et al., 2008) ~~(D'Amato et al., 2007b; Dales et al., 2003; Grundstein et al., 2008; D'Amato et al., 2016)~~ earning the name “thunderstorm asthma.” Such epidemics typically occur during pollen seasons (D'Amato et al., 2007a; D'Amato et al., 2007b; D'Amato et al., 2016) and have been associated with ambient pollen counts (Marks et al., 2001). While lightning is associated with tropospheric ozone formation (Griffing, 1977; GAN, 2014) lightning alone (in the absence of rain) has not caused asthma epidemics (Grundstein et al., 2008), suggesting that rainfall plays an important role in thunderstorm asthma.

Pollen forecasting models currently do not include mechanisms for the release of pollen in response to rain and instead assume that rain serves only as a sink of pollens, by means of droplet scavenging and wet deposition (Zhang et al., 2013). This erroneous assumption leads to predictions of low atmospheric pollen levels on days with rain (e.g. May 2), when pollen tracer levels are highest and primarily in the form of fine particles. A more accurate representation of airborne pollen levels is needed to support an early-warning system to sensitive populations, but must go beyond simply the co-occurrence of elevated pollen levels and thunderstorms, which are suggested to cause too many false alarms (Newson et al., 1998). For accurate model parameterizations, a mechanistic and species-level understanding of pollen bursting is needed and should include definitions of the pollen types, seasonality, and meteorological conditions that promote the release of fine pollen

particles to the atmosphere. In the meantime, persons suffering from pollen allergies should follow the recommendations of D'Amato et al. (2007b): “when asthmatic patients realize that a thunderstorm is approaching, the best thing for them to do is to stay indoors, with windows closed.”

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 several 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 pollen particles that may be useful in monitoring conditions that precede PM_{2.5} pollen release. Because carbohydrates are not expected to undergo chemical alteration 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_{2.5} samples on filters for chemical analysis. In this way, regions and atmospheric conditions that lead to high levels of PM_{2.5} pollen particles may be better defined.

4. Conclusions

Daily concentrations of PM mass and bioaerosol tracers (including fructose, glucose, and sucrose for pollens, mannitol and glucans for fungal spores, and endotoxins from Gram-negative bacteria) demonstrated high day-to-day variability and influences from meteorology, particularly rain. Elevated bioaerosol tracer levels were observed when temperatures are warmer ~~Warmer temperatures promoted suggesting increased~~ pollen, fungal and bacterial ~~growth leading to concentrations higher ambient levels of these bioaerosols~~ during both spring and late summer periods. Rain events of spring triggered the release of pollens, with maximum levels of pollen tracers occurring on May 2 and May 9, when rain occurred following a period of elevated temperatures in spring. Airborne fungal spore tracers in coarse PM fraction, however, were suppressed by spring rain and increased in concentration following rain events. Source apportionment by CMB modelling in concert with Midwestern pollen profiles indicated significant contributions from bioaerosols to PM mass on rainy days during springtime. Importantly, the size distribution of endotoxins and pollen and fungal spore tracers shifted towards fine particles (<2.5 µm) during periods of rain. The fragmentation of pollens due to osmotic rupture, shown previously through microscopy methods, ~~is~~ For the first time, we demonstrate a shift of coarse particle pollens

(2.5-10 ~~µm-microns~~) to fine particles (2.5 ~~µm microns~~) by way of chemical tracers during a major rain event and propose that this is due to ~~osmotic~~osmotic rupture of pollens, is demonstrated in this study for the first time by way of chemical tracers. The release of finer-sized bioaerosols during rain events has important implications for human exposures, because finer particles may penetrate more deeply into the lung and be transported over longer distances.

5 | A detailed level of understanding of pollen release mechanisms, particularly as pollen fragments, is needed to improve the accuracy of allergen prediction models that erroneously forecast low airborne allergen levels during periods of rain. Future research should focus on a more precise determination of the duration of heightened pollen levels during rain events with higher time resolution measurements. Similarly, measurements with higher PM size resolution should be employed to determine the specific size range of pollen fragments during these events. Additional efforts are needed to
10 | characterize the fungal and floral species that release fine-sized bioaerosols to the atmosphere and the mechanisms that trigger such release, to allow for their accurate representation in atmospheric models to support accurate representations of environmental conditions and forewarn susceptible populations of conditions that may lead to high bioaerosol exposures.

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Table 1: Pollen diameter and mass fractions of carbohydrates and ions with standard errors. The carbohydrates arabitol, xylitol, trehalose, fucose, mannose, xylose and ribose were below detection limits.

	Red Oak	Pin Oak	Corn	Cotton ragweed^a	Cotton ragweed^b	Giant ragweed^b
n	5	5	5	5	3	3
Diameter (µm)^c	30	30	80	20	35	35
Carbohydrates (µg mg⁻¹)						
Glucose	41.1 ± 4.1	40.2 ± 3.5	15.2 ± 0.9	15.9 ± 1.6	43.3 ± 2.0	39.2 ± 2.8
Fructose	33.0 ± 1.8	33.9 ± 2.9	25.0 ± 1.1	13.5 ± 0.6	24.4 ± 1.1	22.9 ± 1.5
Sucrose	68.3 ± 4.5	55.2 ± 3.2	13.4 ± 1.7	59.4 ± 3.3	28.0 ± 1.4	27.9 ± 1.3
Erythritol	8.1 ± 3.1	8.7 ± 3.4	28.7 ± 3.2	NQ ^d	NQ ^d	NQ ^d
Mannitol	0.1 ± 0.01	0.2 ± 0.01	<0.001	<0.001	0.2 ± 0.01	0.8 ± 0.1
Rhamnose	0.1 ± 0.01	0.1 ± 0.01	<0.001	<0.001	<0.001	<0.001
Arabinose	0.3 ± 0.03	0.5 ± 0.1	0.9 ± 0.2	0.3 ± 0.03	1.2 ± 0.1	2.3 ± 0.2
Inorganic ions (µg mg⁻¹)						
Sodium	0.25 ± 0.20	0.23 ± 0.01	0.30 ± 0.10	0.03 ± 0.002		
Ammonium	1.36 ± 0.11	1.11 ± 0.90	0.89 ± 0.16	1.33 ± 0.13		
Potassium	7.56 ± 0.81	6.43 ± 0.51	11.97 ± 0.16	5.22 ± 0.48		
Magnesium	0.03 ± 0.00	0.05 ± 0.01	0.88 ± 0.01	0.74 ± 0.06		NA ^e
Calcium	0.07 ± 0.02	0.12 ± 0.01	0.37 ± 0.03	1.85 ± 0.12		
Chloride	0.40 ± 0.08	0.42 ± 0.05	2.10 ± 0.11	1.64 ± 0.18		
Nitrate	0.19 ± 0.04	0.31 ± 0.11	<0.019	<0.019		
Phosphate	3.94 ± 0.39	1.65 ± 0.41	10.5 ± 0.88	8.99 ± 0.87		
Sulfate	0.79 ± 0.29	0.46 ± 0.02	0.94 ± 0.12	0.25 ± 0.03		

^a Purchased from Ploysciences

^b Collected locally from Iowa City ~~during late~~during late summer 2

^c Approximate diameters

^d Not quantified (NQ) due to chromatographic interferences

^e Not analyzed (NA)

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Figure captions

- 5 | ~~Figure 1: Microscope images of pollens from (a) red oak, (b) pin oak, (c) corn, (d) cotton ragweed (Polysciences), (e) cotton ragweed (locally collected), and (f) giant ragweed. Images in colour were captured by fluorescence microscope, while black and white images were captured by an inverted microscope~~
- 10 | Figure 21: Temporal variation in precipitation and average temperature (a) in Iowa City, IA in the spring of 2013. Ambient concentrations of PM mass (b), glucose (c), fructose (d) and sucrose (e) in coarse and fine size fractions. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. During rain on 2 May, PM is suppressed, while pollen tracers in the fine mode substantially increased.
- 15 | Figure 32: Temporal variation in precipitation and average temperature (a) in Iowa City, IA in the late summer of 2013. Ambient concentrations of PM mass (b), glucose (c), fructose (d) and sucrose (e) in coarse and fine size fractions. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. Fungal spore tracers increased significantly in the fine mode during the 2 May rain event.
- 20 | Figure 43: Ambient concentrations of mannitol (a), glucans (b), and endotoxins (c) in coarse and fine size fractions in Iowa City, IA during spring of 2013. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. Fungal spore tracers increased significantly on 5 May, following a rainy period.
- 25 | Figure 54: Ambient concentrations of mannitol (a), glucans (b), and endotoxins (c) in coarse and fine size fractions in Iowa City, IA during late summer of 2013. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. Mannitol, the chemical tracer for fungal spores, and endotoxins from Gram-negative bacteria in fine mode increased on 22 August when it rained.
- 30 | Figure 65: Apportionment of PM₁₀ mass (a) and PM_{2.5} mass (b) during the spring of 2013 to pollens and fungal spores using CMB modeling.
- 30 | Figure 76: Distribution of pollen and fungal spore mass (apportioned by the CMB model) across fine and coarse PM during dry and rainy conditions. The size distributions of pollens and fungal spores shifted towards fine particles during rain, with a more pronounced effect for pollens compared to fungal spores.