

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

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Reviewer 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

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atmospheric abundance of different bioaerosols in only two broad size ranges (PM2.5 and PM2.5-10), 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 $>20 \mu\text{m}$. How is it relevant to show these images here as the authors are not measuring PM $> 10 \mu\text{m}$. 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 $< 2.5 \mu\text{m}$ 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

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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 PÖghilker 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 μm (D'Amato et al., 2007)."

Reviewer #2 specific remark 2: "In Fig. 2, PM2.5-10 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.

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In particular, as indicated in SI Table S4, during April 17 and 18 PM10-2.5 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 $48 \pm 11\%$ of PM10 was less than $2.5 \mu\text{m}$ on rainy days compared to $80 \pm 13\%$ on dry days". This sentence is confusing. Is the author mentioning about %contribution of PM2.5 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. PM2.5 contributed $48 \pm 11\%$ of PM10 on rainy days compared to $80 \pm 13\%$ on dry days."

Reviewer #2 specific remark 5: "Page 8, L 23: "passive release of larger pollen particles ranging $2.5\text{--}10 \mu\text{m}$ 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

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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.

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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 ($rs>0.4$, $p<0.05$)."
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 ($rs=0.7$, $p<0.001$) and the fungal cell wall component glucan and temperature ($rs=0.4$, $p=0.04$)."
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 rs ($rs = 0.7$ vs $rs = 0.4$) 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

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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 ($rs=0.01, p=1$) nor mannitol ($rs=0.2, p=0.3$). 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 ($rs=0.5, p=0.04$). Alternatively glucans may have derived from

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bacterial cells (McIntosh et al., 2005; Rylander and Lin, 2000), although their correlation was not significant ($rs=0.4, p=0.1$). 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, $92\pm5\%$ 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 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.”

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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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