Review for

Morphology, mixing state, and hygroscopicity of primary biological aerosol particles from a Chinese boreal forest

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The manuscript "Morphology, mixing state, and hygroscopicity of primary biological aerosol particles from Chinese boreal forest" from Li et al. presents a physical and chemical characterisation of aerosol particles collected at a boreal forest site in China. The authors (i) derive an identification of large taxonomic classes (i.e., bacteria and fungi) from the particle's morphology and chemical composition, (ii) analyse the relative abundance of large particle classes as a function of day and night cycles, and (iii) analyse the hygroscopic growth of the collected particles. These results were obtained from transmission electron microscopy (TEM) and scanning electron microscopy (SEM) with energy-dispersive x-ray spectroscopy (EDS) analyses. Ultimately, the authors derive quantitative concentrations of certain bioaerosol classes and speculate on their potential roles in clouds and in precipitation formation.

Most of the paper is based on rather established concepts of bioaerosol cycling and techniques for bioaerosol analysis (i.e., SEM and TEM, hygroscopic growth studies, etc.). In my view, the really new aspects are the analysis of bioaerosol samples from this particular Chinese boreal forest site, which may allow interesting comparisons with other (boreal) forest sites worldwide as well as the quantification of bacteria and fungal spore concentrations. Thus, the aim and focus of the study is clearly a useful one.

However, I am very concerned about the overall quality of the manuscript – formally as well as scientifically. Formally, the paper is (i) not well structured, (ii) the introduction is just a loose collection of previous literature without really motivating the present work, (iii) the summary rather lists speculations than provides rigorous conclusions, and (iv) the language should be improved. Scientifically, crucial aspects of the analysis are poorly or even not at all explained. Moreover, I am sceptical if certain key results of the study are correct. My **major points of criticism** can be summarized as follows:

- The meaning and use of "bioaerosol identification" seems very problematic in this study. The authors state for example *"As a result, P derived from the particle EDS analysis coupled with the morphological features can be used to identify the PBAPs."* First of all, it is not clear what the authors exactly mean by "identification". In some case this seems to mean discrimination of biological and non-biological particles, whereas in other cases it seems to mean taxonomic determination. Moreover, a fundamental question of this work, which remains unanswered, is to what extent SEM/TEM analysis allows an identification of certain (taxonomic) groups within the total bioaerosol population and which uncertainty this involves. I don't doubt that several aerosol particles can be recognized as biological based on their morphology, surface texture and so on. Also, certain fungal spores (the characteristic ones) can be identified taxonomically based on their appearance as shows in previous studies. However, I am sceptical if any clear discrimination between bacteria and fungal spores (as stated in this study) can be obtained. In both classes, the morphological diversity is large. Many of the "bacteria" that the authors show (e.g., Fig. 2, 4, 5) are pretty large, which rather advocates for fungal spores. In fact, I have the impression that many fungal spores are 'sold' here as bacteria (i.e., see increase of bacteria fraction towards 10 µm in Fig. 12). To point out some specific examples: (i) Some particles in Fig. 2a, which are classified as "fungi", resemble *Bacillariophyceae* (algae). Note here that the potential presences of algae and archaea is not mentioned/considered at all in the study. Moreover, the terms fungal spores and fungi are not discriminated carefully. (ii) In Fig. 2d, particles that resemble bicellular fungal spores are classified as bacteria. (iii) Also many cells in Fig. 5 resemble – in my view – fungal spores rather than bacteria. I have been in frequent contact with mycologists, who use morphological features for fungal spore taxation. Their

procedures follow very careful, iterative, and conservative guidelines for taxonomic identifications/classifications. Diametrically, the approach I see in this work does not refer transparently to any guidelines at all and further appears to be quite 'spontaneous' and suspect. Since the discrimination of bacteria and fungal spores is a core piece of the entire study, I feel that the aforementioned deficits severely challenge the experimental basis of this work.

- The experimental section is intransparent in terms of central pieces of the analysis. Examples: (i) The "identification" and quantification procedures of the taxation remain unclear. What were the exact criteria/guidelines to discriminate bacteria, fungal spores, and "other biological particles"? What are the uncertainties involved here? (ii) How exactly were the brochosoms quantified? Brochosoms tend to occur in (often quite large) clusters. Did you count clusters or individual brochosom entities to obtain the brochosom number fraction of 24 %? (iii) Relevant information in the context of the hygroscopic growth experiments are missing – e.g. uncertainty of RH measurement; how exactly D_0 (the diameter of the bioaerosol particle) was obtained, which is not trivial for a rod-shaped particle; etc. (iv) Do we expect a deformation of the cells upon impaction, which may hamper the morphological characterization?
- The caption of Fig. 14 suggest an SEM analysis was conducted prior to the hygroscopic growth experiments. Do you expect to see an authentic/representative hygroscopic response after the harsh treatment with the electron beam and the beam damage involved? I have strong reservations here.
- The study does not translate the results obtained (though questionable) into any meaningful conclusions. The conclusions section is a summary of (i) established and partly trivial statements such as *"The TEM and SEM observations both showed that the morphology of PBAPs were unique and different from that of sulfate, mineral, soot, organics, and metal particles in continental air."* or *"PBAPs from the natural source may have an important role in precipitation and cloud dynamics in the background areas."* and (ii) grotesque overstatements such as *"In this study, we establish one full database that includes the morphology and composition of bacteria, fungi, and brochosomes, and it can be used to identify primary biological particles using single particle techniques."* or *"Our results indicate that significant amounts of PBAPs are emitted from the Khingan Mountain area acting as the "green ocean" […] in Northeast Asia, and they have an important impact on clouds and climate in Northeast China and in the downwind North Pacific Ocean."*.

In my view, the paper is not publishable in the current form and needs a pretty fundamental major revision.

In the following, I listed some **more specific and/or minor comments**:

1. **Introduction:**

- In general, the introduction contains some important points. However, the structure and flow of argumentation needs improvement. The text should be more structured from general to detailed information, finally leading to the guiding research question(s) of this work. This might help to highlight the targeted knowledge gap and to emphasize the importance of the study.
- Some information and reference should be placed in more appropriate location in the text. Currently, certain statements occur redundantly. The text should be more structured in content related segments. Resulting segments should be related.
- Linking thoughts between statements/sentences is often missing. Shortening sentences will improve clarity and the flow of reading.
- p.4/l. 53 “key elements”, if this term is used, please briefly indicate in which way they are key elements in the life cycle (e.g. dispersal units).
- p.4/l. 56 “large proportion” is too imprecise. You can give some numbers here?
- p.4/l. 58-59 “Research interest in biological aerosol has been growing significantly in recent decades”. To demonstrate the relevance of PBAPs, I suggest to relate this statement to other statements like the fact that bioaerosols can act as CCN or IN like you show in l. 59-60.
- P.4/l.68-72 Better structure needed. Try to summarize information and try to avoid redundancy. E.g.: You already gave some information about the abundance at distinct sites (l.56-57).
- P.4/l.68 “significantly contribution” – can you further specify this?
- P.5/l.73 “the sampling” What does that mean? Aerosol sampling methods?
- P.5/l.76-80 The information and mentioned studies in the two sentences again appear unrelated to the present study.
- P.5/l.81 “chemical composition” is a bit too specific. In my opinion you rather try to identify present kinds of organisms, domains up to species (plant or animal debris, bacteria, fungi, viruses, etc.) by means of biochemical markers or nucleic acids.
- P.5/l.84-86 “These comprehensive and detailed studies of time- and size-resolved PBAPs and their biochemical markers do not well explain the physical properties (e.g., morphology, phase, hygroscopicity, and mixing state) of individual PBAPs in the atmosphere” The sentence is hard to understand. In this context, “studies of time- and size-resolved PBAPs” is not clear.
- P.5/l.87-88 The sentence is nebulous.
- P.5/l.90 What means “actual state”?
- P.6/ l.98-100 The information about the sodium salt in this sentence is redundant (see p.4/l. 61-63). Also, “fungal fragments sampled from Amazonia contain hygroscopic sodium salts based on an environmental scanning electron microscopy” This sentence is not smooth.
- P.6/l. 100-101 “However, whether fungal spores emitted by boreal forests are similar to the fungal spores in central Amazon forests, which contain sodium salts, has not been resolved” Here you should define why it might be important to find out if the fungal spores are similar. Furthermore, you should point out why you think they might be similar, or even not. Is that important or does that lead to the research question of the current paper? You should make clear why that leads to the required analysis (connection to sentence, l.102-103 “Therefore, the morphology, elemental composition, and mixing state of individual PBAPs (nanometre to micrometre size) collected from other global forests must be analysed”).

2. **Method:**

- If microscopic techniques are not introduced in more detail already in the introduction, it would be good to highlight the difference between the two techniques, as well as the respective advantages. For SEM you describe shortly the principle of the method (2.3). You should do that also for TEM (in 2.2) to point out the differences and the advantages. Why you are using two different methods? Also for EDS a short description would be nice. Moreover, you mentioned ESEM within the abstract, but you don’t mention it in the method section again.
- It is not easy to understand the functional principle of the IPH system. An illustration of the setup might be helpful. Moreover, your experimental steps are not described clearly. The experimental procedure is described incompletely. More information is needed - e.g.: In which steps did your increase or decrease the RH? Which time was needed?

- Moreover, it would be interesting to learn more about the functional principle of the environmental chamber, too. I am wondering if you did some calibrations for the RH measurements?
- Finally, in the method section the analysis of the quartz-fibre filters is totally missing.
- P.7/l. 123-125 “Because boreal forests play a key role in biological aerosol emissions during summer, we collected aerosol samples in August.”. What means “key role” here? The sentence states not clear enough why you chose August for sampling time. What did you expect to observe at this specific time period in contrast to other months?
- P.7/l.126-130 The first sentence is definitely too long. You can split the information for a better understanding. You are using two different types of collection substrate. What is the reason? “DKL-2 sample” Can you describe the sampler in more detail? Is it an abbreviation? The sampling times are listed in a confusing way (21:00 vs. 2:00 a.m.)! “every day” – What is the exact sampling period? How many days did you continue the sampling (dates)? Did you use both, copper grids and silicon waver, during each sample event? The size range of collected particles is missing.
- P.7/l. 132 “microscopy” Please mention the type of microscope.
- P.7/l. 133 “suitable” You should define what suitable means.
- P.7/l. 134 “guarantee” Here the information, how the procedure can guarantee the separation of the particles, is necessarily to be mentioned in the text.
- P.7/l. 137-138 Syntax.
- P.8/l. 141-142 Is the placement the same for the first sampling set (DKL-2 sampler, described on p.7) too? If yes, you should make this clearer or add the placement of DKL-2 sampler.
- P.8/l. 160 “Particles in 3-5 grids of each sample were analysed…”. It should become clearer how many samples were analysed. How many particles were roughly analyzed on every grid? This information is important to show if and in which way the results are representative (as you point out in p.9/l. 161).
- P.9/l.161-162 “TEM can determine…” here you speak only about TEM. Actually, it is EDS by means you can determine the elemental composition.

3. Results and Discussion

- P.10/l.190 Which technique was used here? TEM or SEM?
- P.10/l.200 “number fractions of size resolved aerosol particles” How was this measured/determined? Please outline in experimental part.
- P.12/l.226 “a majority” How representative is Figure 2 a and b for the whole sample set?
- P.13/l.255 “resemble parts of insects” Here is a reference missing? It would be good to describe the features you interpret here.
- P.13/l.257 You should describe in which way the SEM provides “better and more detailed information”.
- P13/l.260 “Bacterial particles range from … “. Can this be substantiated with literature?
- P.13/l. 264 “This is because certain hygrophobic secretions of insects (e.g., leafhoppers) are composed of brochosomal particles, and these secretions function in keeping the insect cuticle dry”. An explanation/definition of brochosoms should be given earlier in the text (intro or experimental part).
- P.14/l.271-277 Calculations need further clarification.
- P.14/l.286 Please explain what you mean with “differential removal”.
- p.16/l. 328-329 “was performed and it showed that bacterial and fungal spores are dominant” This should be clarified in the method section.
- P.16/l. 334 “weak”. Please put weak in a context of literature data. If different GFs are compared, the RH of the corresponding the GF should be mentioned for meaningful comparison.
- P.17/l.342-344 “We integrated the morphological, chemical composition and the low growth factor data of individual PBAPs and further concluded that certain hydrophilic organic species might enhance the PBAP size at higher RH”. Meaning of sentence nebulous.

4. Atmospheric implications and conclusion

- In this section, some aspects are explained too detailed and are therefore redundant at this point. Also here it is important to highlight the main message of the results shortly before you give your conclusion.
- P-17/l. 352-353 “one full database …”. This appears to be overstated.
- p.18/l. 360-361 “The growth factor of the bacterial and fungal spores is ~1.09 at 94%, suggesting that some hydrophilic organic species might enhance the size of PBAPs at higher RH”. Need clarification.
- P.18/l. 362-366 This statement lacks context here and seems disconnected from the conclusions.
- P.18/l.367-368 “green ocean” This term seems pretty inappropriate for the comparatively small boreal forest area.
- P18/l. 368.369 “they may have an important impact on clouds and climate in Northeast China and in the downwind North Pacific Ocean”. This sentence may be true, but seems pure speculation here as it is not related to the results/conclusions of this work.
- P.18/l.369-372 This is another long and nebulous sentence that appears quite speculative. Why speculating about “submicron” particles here?

Figures:

Figure 1: More precise information may help here to get a feeling for the size of the Khingan area.

Figure 4: Where exactly were the EDS spectra obtained?

Figure 5: The green framing seems rather confusing/distracting than helpful.

Figure 6: Where exactly was the EDS spectrum obtained?

Figure 7: Colouring micrographs in this way without any obvious reason seems to violate the widely accepted practise among microscopists to keep the images are raw as possible.

Figure 8: Where exactly was the EDS spectrum obtained?

Figure 9: See comment on Fig. 7.

Figure 12: What exactly do we learn from the ratio of PM10 and PM2.5?