Reply on RC2

The Technical Note, “Bioaerosol identification by wide particle size range single particle mass spectrometry” by Li, et al. describes a bioaerosol identification algorithm for a single-particle mass spectrometer SPAMS. The details of the classification are the same as those in Zawadowicz, et al. (2017) (ratios of characteristic phosphorus and organic nitrogen markers discriminated in biological and abiotic species by use of the SVM algorithm), but this is a valid contribution to the literature because it reproduces the previous study on a different single-particle instrument with a different training dataset. Particularly, it is of note that SPAMS and PALMS use ionization lasers of different wavelengths, which would be the most significant reason for this method to work with one instrument and not the other. In this way, this study is also somewhat contrary to the recent Cornwell et al. (2022) paper, which argues that the phosphate-ratio SVM technique is too sensitive to laser ionization energy to work on the ATOFMS system, which uses the same ionization wavelength as SPAMS.

Overall, support the publication of this paper after the authors address the following comments. I will rely on the editor's best judement whether this paper would fit better at ACP or AMT. My first instinct was to recommend resubmission to AMT, but I am not very familiar with the scope of the new Technical Note manuscript type, and reading ACP's guidelines it seems like it fits with "development of numerical algorithms for the interpretation of atmospheric data (such as statistical methods and machine learning)."

Overall comment: the paper would benefit from another read for syntax, grammar, etc. Some sentences are difficult to follow (some, but not all instances are noted below).

- p.2, line 64-65: Not sure I follow this sentence, “However, the research on the detection and discrimination of bioaerosols from fungi and other bioaerosols remains insufficient”. This reads like you are suggesting the need for increased speciation of bioaerosol detected by SPMS (i.e. discriminating fungal bioaerosols from other bioaerosol types), but this is not further discussed in the paper, which focuses on discriminating between bioaerosol and abiotic aerosol.

Answer: This is really a very professional question, thank you very much. We detected bacterial bioaerosols and fungal bioaerosols by SPAMS, but they could not be distinguished according to their mass spectral characteristics. The paper focuses on discriminating between bioaerosols (bacteria and fungi) and abiotic aerosols. In the process of literature review, we found that there are few studies on the distinction between fungi and abiotic aerosols. Here, the sentence was changed as "**However, there is insufficient research on the detection and differentiation of other bioaerosols such as fungi from abiotic aerosols**".

- Experimental section: can you provide some rationale for choosing these specific bacterial and fungal strains?

Answer: Thank you for your good suggestion. The 15 strains, which include bacteria, mold and yeast, are representative. The bacteria included both Gram-positive and Gram-negative bacteria, and included common shapes such as balls and rods.

- Bacterial and fungal strains that form the training dataset are discussed in the Experimental section, but road dust, exhaust and combustion products are not. Please provide some discussion of what types of abiotic phosphorus-containing species were used in the study.

Answer: Thank you for your good suggestion. The specific types of abiotic nitrogen and phosphorus species discussed in this manuscript are described in the following table.

Table 1 Summary of abiotic species samples

Sample	Description	Study
road dust	Guangzhou Accelerator Industrial Park road dust	Reported for the first time
Vehicle exhaust	Fresh exhaust collected from a light-duty diesel vehicle with the engine started and at steady state	Su et al.,2021, Journal of Hazardous Materials.
wheat stalk combustion products	Stems and leaves of mature wheat in East China	Reported for the first time
corn stalk combustion products	Stems and leaves of mature corn in East China	Reported for the first time
oblate leaf combustion products	Dried oblate leaves of eastern China	Reported for the first time

- p. 6, line 173: "Therefore, for the first time, HP-SPAMS was used to measure 10 μm coarse particulate matter under the improvement of the sampling system." Can you provide some figures of merit for your new coarse-mode sampling system? What is the transmission efficiency at 10 μm?

Answer: Thank you for your interest in the new coarse-mode sampling system. Fig. 1 shows a schematic diagram of the wide-range inlet system, which consists of four modules: a pre-focusing sampling connection, a virtual impact sampling cone, a relaxation chamber, and a seven-stage aerodynamic lens. Figs. 1b, 1c, and 1d show the detailed dimensional parameters of each module. Fig.2 shows the theoretical and experimental comparison of particle transmission efficiency provided by the wide-range aerosol inlet system. In the experiment, the pulse numbers of the two PMT beams were counted respectively. PMT_1 and PMT_2 were 12 cm and 18 cm away from the outlet of the lens system, respectively. The transmission efficiency of the lens system could be roughly estimated by the pulse number of PMT_1. In the entire particle size range of 120 nm-10 μm, the transmission efficiency calculated through PMT_1 counting was consistent with the theoretical simulation. In the particle size range of 200 nm-10 μm, the transmission efficiency of PM was close to 100%. Overall, when the wide-range aerosol inlet system was combined with the present SPAMS, it could achieve efficient focusing transmission of 120 nm-10 μm PM, and the experimental results were remarkably consistent with the theoretical predictions.

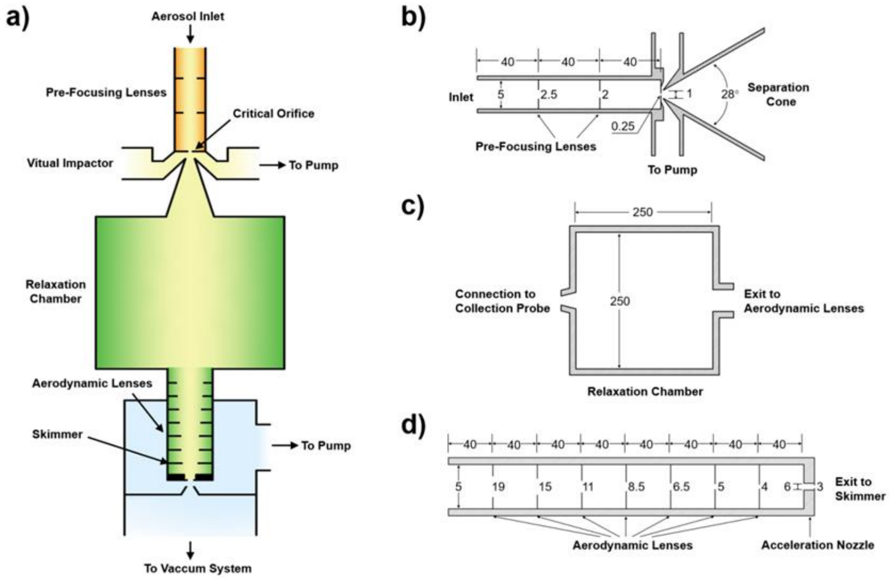

Fig. 1 Schematic diagram of the total wide range aerosol sampling system

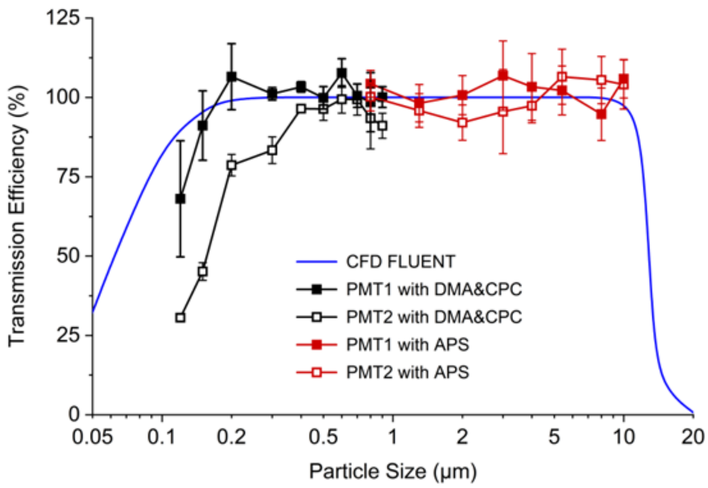

Fig. 2 Detection results of particle transmission efficiencies by SPAMS

- At these high vacuum aerodynamic diameters, what is the laser hit rate? (i.e. what proportion of optically-detected particles produce a mass spectrum?)

Answer: This is really a very professional question, thank you very much. The laser strike rate of different bacteria is different, as shown in the following table. The laser hit rate is related to the shape of the bacteria itself and the injection concentration.

Table 1 Laser hit rate of different strains

Name	Hit rate
Klebsiella pneumoniae	25.17%
Salmonella pneumoniae	33.77%
Shiga virulent Escherichia coli	45.77%
Bordetella bronchitis	21.62%
Escherichia coli	24.71%
Staphylococcus aureus	62.85%
Listeria monocytogenes	32.38%
Enterococcus faecium	54.11%
Enterobacter cloacae	40.83%
Staphylococcus epidermidis	17.73%
Candida albicans	54.26%
Candida tropicalis	13.25%
Candida glabrata	32.37%
Aspergillus brasiliensis	15.87%
Saccharomyces cerevisiae	40.57%

- p. 7, lines 178-193: It is not entirely clear if this paragraph describes previous investigators' results or your own. I found the sentence in lines 182-185 especially difficult to understand.

Answer: Lines 182-185 show the results of our study, which are compared with those of the same type of mass spectrometer in the world. We found the description unclear and have since revised it. Thank you very much for your revision.

"Czerwieniec et al. (2005) found similar peaks when detecting vegetative cells of Bacillus atrophaeus and speculated that +30, +70, +72, +74, +86, +110, and +120 were decarboxylic ionic peaks of amino acids. Srivastava et al. (2005) speculated that +59, +81, +84, and +88 ionic peaks were organic fragments containing nitrogen. Zeng et al. (2019) used SPAMS to detect 13 strains of bacteria to obtain similar bioaerosol characteristic ions; however, there were fewer characteristic peaks in the negative mass spectrum, and no negative ions with a mass charge ratio greater than 200 were detected, and the overall ionic peak signal was weak. HP-SPAMS detected the mass spectra of 15 strains of pure bacteria as shown in Fig. 3. Fig. 3 shows the stacked mass spectrograms of 14119 biological single particles with mass number of -300-300 Da ion peaks. In addition to $^{23}Na^+$ and $^{39}K^+$ metal ion peaks, there are also a large number of amino acid decarboxylic ion peaks in the positive spectrum. The positive ion peaks were mainly ^{30}[Glycine-COOH]$^+$,$^{59}[C_3NH_9]^+$,^{70}[Proline-COOH]$^+$, ^{72}[Valine-COOH]$^+$, ^{74}[Threonine-COOH]$^+$,$^{84}[C_5NH_{10}]^+$,^{86}[Leucine-COOH]$^+$,^{110}[Histidine-COOH]$^+$,and ^{120}[Phenylalanine-COOH]$^+$.The negative ionic peaks were mainly organic nitrogen $^{26}CN^-$, $^{42}CNO^-$, phosphate $^{63}PO_2^-$, $^{79}PO_3^-$, $^{97}H_2PO_4^-$, $^{159}H(PO_3)_2^-$, $^{199}NaH_2P_2O_7^-$, and other common

biological ionic peaks.”

- Figure 3: I’m not sure I understand what is indicated by the different colors in this figure.

Answer: Fig.3 shows the mass spectrograms of 14119 biological single particles stacked with ion peaks whose mass number is -300~300 Da. Each color represents the proportion of the signal intensity range of an ion peak area, which can show the mass spectral peak with strong signal more intuitively. For clearer expression, we have added the annotated information.

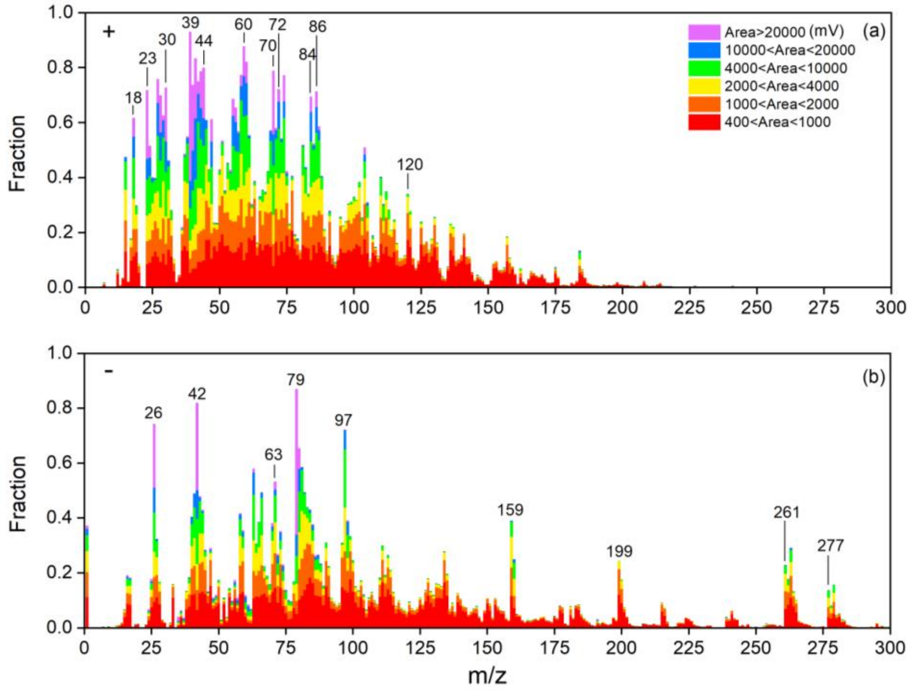

Fig. 3 Stacking diagram of the area of all bioaerosol ion peaks; (a) Positive mass spectrum; (b) Negative mass spectrum

- It would be useful to include some discussion of method uncertainties in the paper. What are the limiting factors in translating the results of this lab-based study to the atmosphere?

Answer: Thanks for your suggestions. We added this part. The classification method based on the ratio of ion peaks based on HP-SPAMS can effectively distinguish bioaerosols from abiotic aerosols. However, due to the difference in the signal intensity of the four characteristic ion peaks, not all particles contain four ion peaks at the same time, and it is impossible to distinguish 100% biological particles. In addition, the classification method using only the data measured in the laboratory cannot be directly applied to the classification of environmental particles. Bioaerosols undergo aging reactions in the atmospheric environment, and their chemical composition and morphology characteristics have different changes under the influence of a variety of natural factors. Moreover, it is necessary to consider all the mass spectrometric characteristics to identify environmental particles.