

Dear Editor and Reviewer,

We thank for your careful reading and constructive comments, which have allowed us to make further enhancements and significantly raised the quality of the manuscript. Our responses to the comments from the referee are provided below. The reviewer's comments are shown in blue, while our responses are in black. All page and line numbers mentioned in the response refer to the revised version of the manuscript. Revisions are marked in blue in the revised manuscript.

Sincerely,

HU Wei (on behalf of co-authors)

## **Response to Referee 2 and Editor:**

### **General comments:**

Comment 1: This paper has been revised in dependence on the reviewers' comments. I think this manuscript can be accepted after some revisions relating to the description about the biases of microbial isolation.

**Response:** Thank you very much for your valuable comments. We have double-checked and revised the manuscript with respect to the names and abbreviations of the isolated strains.

### **Some major comments:**

Comment 2: The compounds of few microbial isolates from aerosol samples are discussed mainly in this study to suggested the biological compounds transported in atmosphere in Asia. In contrast, almost airborne microorganisms are known to be unculturable but viable microorganisms (In general, >90% of microorganisms cannot be cultured but they are living). The authors should indicate the occupation rate of the isolate species in atmospheric microorganisms with the calculations using the previous metadata bases, which are obtained at previous investigation at China continent.

**Response:** Thank you for your valuable comments. We appreciate the important point regarding the predominance of unculturable yet viable microorganisms in the atmosphere. In our study, however, the bacterial and fungal isolates we selected are representative of high-abundance and highly active species within the atmospheric microbiome, which are believed to represent a major fraction of biological compounds transported through the atmosphere.

While we performed extensive metagenomic analysis, the samples used for metagenomics did not exactly correspond to those from which the isolates were obtained. Nevertheless, based on our metagenomic results (unpublished data), we have estimated the proportion of the genera of isolates analyzed in this study within the broader atmospheric microbiome. Specifically, we estimate that the genera of isolates selected in our study represent approximately 9.8% of the airborne microorganisms in terms of the transcript per kilobase per million mapped reads (TPM) in winter in Tianjin.

This estimation, based on metagenomic data, further underscores the relevance of the cultured isolates in representing the atmospheric microbial community. Please refer to our former response to your comment. We hope that this additional information clarifies the relative abundance of the isolated genera within the context of the overall atmospheric microbial community.

**Some minor comments:**

Comment 3: L18: What is CHON?

**Response:** According to the elemental composition of organic compounds, their molecular composition is classified into four categories: CHO, CHON, CHOS, and CHONS. This classification has been commonly used in mass spectrometry analyses. For example, CHON means that the organic compounds contain only four elements: carbon, hydrogen, oxygen, and nitrogen without any other elements. Please refer to Line 199-201 in the revised manuscript.

Comment 4: L74: This survey has been performed at Asian regions. The bioaerosols around Asian regions should be also mentioned here using relative researches.

The pioneer work performed at Asian continental areas: Tang et al. *ACP*, 18, 7131-7148, 2018, [doi.org/10.5194/acp-18-7131-2018](https://doi.org/10.5194/acp-18-7131-2018)

Asian bioaerosol review: Huang et al., *STOTEN*, 912, 168818, 2024. 168818, <https://doi.org/10.1016/j.scitotenv.2023.168818>

**Response:** Thank you for your comments. We appreciate the reviewer's recommendation to include references to previous studies on bioaerosols in the Asian region. In response, we have incorporated the relevant studies you mentioned into our revised manuscript. Specifically, we have added the following citations:

Tang, K., Huang, Z., Huang, J., Maki, T., Zhang, S., Shimizu, A., Ma, X., Shi, J., Bi, J., Zhou, T., Wang, G., and Zhang, L.: Characterization of atmospheric bioaerosols along the transport pathway of Asian dust during the Dust-Bioaerosol 2016 Campaign, *Atmospheric Chemistry and Physics*, 18, 7131-7148, <https://doi.org/10.5194/acp-18-7131-2018>, 2018. This pioneering study provides valuable insights into bioaerosols in

Asian continental areas.

Huang, Z., Yu, X., Liu, Q., Maki, T., Alam, K., Wang, Y., Xue, F., Tang, S., Du, P., Dong, Q., Wang, D., and Huang, J.: Bioaerosols in the atmosphere: A comprehensive review on detection methods, concentration and influencing factors, *Science of The Total Environment*, 912, <https://doi.org/10.1016/j.scitotenv.2023.168818>, 2024. This comprehensive review summarizes the composition and distribution characteristics of bioaerosols, providing a richer background for our study and strengthening the context for understanding bioaerosol behavior in the atmosphere, particularly in Asian regions. These additions are now included in the relevant sections of our manuscript to provide a broader context for our study and highlight the significance of bioaerosols in the Asian atmospheric environment.

Page 2, Line 44 – 46: “Atmospheric bacteria and fungi can maintain metabolic activities due to specific growth characteristics, such as spore production capacity, ultraviolet resistance, drought resistance, or through extracellular secretions (Huang et al., 2024; Matulová et al., 2014; Bryan et al., 2019).”

Page 2, Line 48 – 50: “Airborne microorganisms (especially those in Asian dust) form aggregates with organic matter, which may serve as a nutrient source, promoting microbial survival and facilitating long distance transport (Tang et al., 2018; Huang et al., 2024).”

Comment 5: L235: After the second indications of taxon name, the genera has to be abbreviated in biological research field. For example, *P. baetica*. Please correct same irregular usages at the other parts.

**Response:** Thank you for pointing out this issue. We have carefully reviewed the manuscript and corrected all instances where the genus names were not properly abbreviated after the first mention. All taxonomic names have now been revised to conform to the standard practice in biological research, such as “*P. baetica*” for *Pseudomonas baetica*, “*B. subtilis*” for *Bacillus subtilis*. We believe these corrections improve the consistency and accuracy of the manuscript. We appreciate your attention

to the detail and hope these revisions meet your expectation.

Comment 6: L276: *Aspergillus* spp. or *Aspergillus* sp. Species name is needed for matching to the forward taxon name.

**Response:** Thanks. We have carefully reviewed the manuscript and have now ensured that species names are used correctly and consistently throughout. The taxon names have been updated to ensure full consistency and completeness, with the appropriate species names included where necessary.

Page 9, Line 273 – 274: “For fungi, *Rhodotorula mucilaginosa*, *Aspergillus niger*, and *Aspergillus* sp. demonstrated higher molecular diversity of exometabolites (Fig. S4b).”

Comment 7: L448-L450: In introduction, the authors also introduce the biochemical cycle in clouds. How is this topic in conclusion? Additionally, the impact on freshwater?

**Response:** Thank you for your valuable comment. In our study, we highlight the potential role of atmospheric microorganisms in biogeochemical cycles, particularly their involvement in organic matter transformation during both transport and deposition. Atmospheric microorganisms can remain active in the air, cloud water, fog, and rainwater, where they contribute to carbon metabolism. This underscores their importance in biogeochemical processes within the atmosphere.

Regarding the conclusions, we have indeed addressed the impact of atmospheric microbial deposition on the biogeochemical cycles of freshwater ecosystems. These processes are crucial for understanding the broader ecological implications of atmospheric microorganisms. We have also ensured that the conclusion provides a clearer and more comprehensive summary of our findings and their environmental relevance.