

Response to Reviewers' comments:

Reviewer #1

The authors investigated airborne bacterial and fungal communities and their associations with sugar compounds at a typical rural site, Dingxing County, in the North China Plain. Their community structure, source, diurnal variation, possible factors, and contribution to sugar compounds were addressed in this manuscript. The results are conducive to deepening the understanding of bioaerosol and those potential influence on the enhanced sugar compounds at nighttime. This is a valuable piece of work also with a reasonable overall structure. I recommend that it can be accepted for Minor Revision.

Response:

We greatly appreciate the reviewer for the professional comments and suggestions. According to the valuable suggestions, we have made extensive corrections to the original manuscript. Please refer to the point-to-point responses to the comments below.

Comment 1

For the SourceTracker, soils, plant leaves and seawater were selected as the source tracking reference databases. But the sources of bacterial community were mostly unresolved (about 98.9%). I wondered whether the database was precise or not? Is there any seawater around the sampling site? Did the nearby river water contribute more to the bacterial community?

Response:

Previous studies reported that local sources played leading roles in shaping microbial communities (Zhai et al., 2018; Bowers et al., 2011). Hence, we only consider the contributions of local natural sources to microbial communities in this study. To ensure as much accuracy as possible, the leaf and soil samples we chose were sampled near the atmospheric sampling site. However, due to the small dataset, there is still a large uncertainty. The closest sea area to the sampling site is about 178 km away. There are rivers nearby (7.7 km), and we did not find sequencing data of nearby river waters in the NCBI database. The large unresolved sources of this study may be partially corroborated by the result of previous studies that the bacteria were more likely to be of anthropogenic origin (Jiang et al., 2022; Zhao et al., 2022).

For the large uncertainty of the SourceTracker results, we explained the possible reasons in Page 8, Line 237-242: “only the contribution of natural sources including terrestrial plants, soils and ocean to the airborne microbial community was considered in this study, which may partly explain the high unresolved sources of bacteria. It is also noticeable that in this study only local soil and vegetation samples were selected as the source samples, and the impact of long-distance transport was not considered. Therefore, unresolved sources may also come from long-range transported air masses (Gat et al., 2017) and other local sources, e.g., waters, other vegetations and human-related sources.”

Page 11, Line 332-335: “It was also found that bacterial community structure may be closely related to sucrose-traced plant origins, although the SourceTracker analysis results did not show the plant origin of bacteria. This inconsistency may be caused by single plant (maize) source samples and/or the negligence of long-distance transport in this study.”

The detailed description of SourceTracker was added as follows:

Page 5, line 141-148: “SourceTracker is a Bayesian approach to identifying sources and estimating source proportions for microbial surveys (Knights et al., 2011). The source samples used as source tracking reference databases in this study were soils, plant leaves and seawater. Maize leaves and soil samples were collected around the observation site and the samples were subjected to high-throughput sequencing as described in Section 2.2 to obtain the gene sequences, and the marine microbial sequences were obtained from the NCBI SRA database (<https://www.ncbi.nlm.nih.gov/sra/>, Table S1). The sequences of all source and sink samples were processed together by QIIME 2, and were assigned to OTUs based on the similarity of sequences \geq 97%. The output data were performed for source tracking analysis based on “SourceTracker2” package in R (<https://github.com/caporaso-lab/sourcetracker2>).”

Bowers, R. M., McLetchie, S., Knight, R., and Fierer, N.: Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments, *ISME J.*, 5, 601-612, <https://doi.org/10.1038/ismej.2010.167>, 2011.

Jiang, X. Q., Wang, C. H., Guo, J. Y., Hou, J. H., Guo, X., Zhang, H. Y., Tan, J., Li, M., Li, X., and Zhu, H. Q.: Global meta-analysis of airborne bacterial communities and associations with anthropogenic activities, *Environ. Sci. Technol.*, 56, 9891-9902, <https://doi.org/10.1021/acs.est.1c07923>, 2022.

Gat, D., Mazar, Y., Cytryn, E., and Rudich, Y.: *Origin-dependent variations in the atmospheric microbiome community in Eastern Mediterranean dust storms*, *Environ. Sci. Technol.*, 51, 6709-6718, <https://doi.org/10.1021/acs.est.7b00362>, 2017.

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., Bushman, F. D., Knight, R., and Kelley, S. T.: *Bayesian community-wide culture-independent microbial source tracking*, *Nat. Methods*, 8, 761-763, <https://doi.org/10.1038/nmeth.1650>, 2011.

Zhai, Y., Li, X., Wang, T., Wang, B., Li, C., and Zeng, G.: *A review on airborne microorganisms in particulate matters: Composition, characteristics and influence factors*, *Environ. Int.*, 113, 74-90, <https://doi.org/10.1016/j.envint.2018.01.007>, 2018.

Zhao, J., Jin, L., Wu, D., Xie, J., Li, J., Fu, X., Cong, Z., Fu, P., Zhang, Y., Luo, X., Feng, X., Zhang, G., Tiedje, J. M., and Li, X.: *Global airborne bacterial community—Interactions with Earth's microbiomes and anthropogenic activities*, *Proc. Natl. Acad. Sci. U. S. A.*, 119, e2204465119, <https://doi.org/10.1073/pnas.2204465119>, 2022.

Comment 2

It seems that the environmental factors did not explain adequate variance of bacterial community also by the RDA analysis. The cumulative explanatory variable of RDA1 and RDA2 axis were lower than 60%, hence did the RDA analysis have statistical significance or more factors should be included.

Response:

Environmental factors did not explain the bacterial community changes well in this study. We favor the view that other ecological processes played an important role in the construction of bacterial communities. We applied inferring community assembly mechanisms by phylogenetic-bin-based null model analysis (iCAMP) to unravel the drivers controlling community assembly (Ning et al., 2020) and found that deterministic processes (including environmental filtering and biological interactions) and stochastic processes (birth/death, speciation/extinction, and immigration) played nearly equal roles (53.7% and 46.3%) in shaping bacterial communities. The corresponding information was revised as follows:

Page 5, line 154-157: “Inferring community assembly mechanisms by phylogenetic-bin-based null model (iCAMP) is a general framework to quantitatively estimate community assembly

mechanisms (Ning et al., 2020; Chen et al., 2022). The assembly mechanisms of airborne microbes at Gucheng site were investigated by using the iCAMP package in R.”

Page 8-9, line 253-258: “It should be noted that environmental factors explained only 43.4% of the variation in bacterial communities, and it is hypothesized that ecological processes other than environmental filtration had impacts on shaping bacterial communities. Here, iCAMP analysis showed that the relative contributions of deterministic processes (environmental filtration and microbial interactions) and stochastic processes (birth/death, speciation/extinction, and immigration) to shaping bacterial communities were 53.7% and 46.3%, respectively, demonstrating the importance of other ecological processes on bacterial communities.”

Chen, D., Hou, H., Zhou, S., Zhang, S., Liu, D., Pang, Z., Hu, J., Xue, K., Du, J., Cui, X., Wang, Y., and Che, R.: Soil diazotrophic abundance, diversity, and community assembly mechanisms significantly differ between glacier riparian wetlands and their adjacent alpine meadows, *Front. Microbiol.*, 13, <https://doi.org/10.3389/fmicb.2022.1063027>, 2022.

Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., Yang, Y., Arkin, A. P., Firestone, M. K., and Zhou, J.: A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming, *Nature Communications*, 11, 4717, <https://doi.org/10.1038/s41467-020-18560-z>, 2020.

Comment 3

The results showed that nighttime fungal spore emitted more OC than that in daytime ($p < 0.01$, Fig 5b), however daytime samples were more affected by OC than the nighttime samples based on the redundancy analysis (Fig 4b). How to evaluate the different phenomenon?

Response:

Thank the reviewer for the valuable comment. The fungal-spore OC shown in Fig. 5b was estimated by the concentrations of mannitol based on Bauer et al. (2008), and the fungal-spore OC accounted for 3.6–19.7% (average: 7.7%) of total OC. Whereas, the parameter OC introduced into the RDA analysis (Fig 4b) was the total concentration of OC. Therefore, this phenomenon may be due to the fact that non-fungal-spore OC played role in influencing the daytime fungal community. In addition to the possible microbial origin of OC, OC may be an available nutrient for microbial development (Vařtilingom et al., 2013; Wei et al., 2019).

Vaïtilingom, M., Deguillaume, L., Vinatier, V., Sancelme, M., Amato, P., Chaumerliac, N., and Delort, A. M.: Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds, *Proc. Natl. Acad. Sci. USA*, 110, 559-564, <https://doi.org/10.1073/pnas.1205743110>, 2013.

Wei, M., Xu, C., Xu, X., Zhu, C., Li, J., and Lv, G.: Size distribution of bioaerosols from biomass burning emissions: Characteristics of bacterial and fungal communities in submicron (PM1.0) and fine (PM2.5) particles, *Ecotoxicol. Environ. Saf.*, 171, 37-46, <https://doi.org/10.1016/j.ecoenv.2018.12.026>, 2019.

Thank you very much for your comments and suggestions. Your any further comments and suggestions are appreciated.