Influence of terrestrial and marine air-mass on the constituents and intermixing of bioaerosols over coastal atmosphere

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Response to reviewer 2

The manuscript submitted by He et al. has a considerable and original work to clarify the interactions between bioaerosols, airborne chemicals and their sources. Although I would consider it eligible to publish, some major explanations and analyses are needed, with special attention to writing and organization of the manuscript, and some disagreement that should be discussed or clarified.

Response of the authors: We greatly appreciate the positive comments and constructive suggestions on our manuscript. According to your comments and suggestions, we have made detailed revision and incorporated them into the revised manuscript.

Major changes

1. - Introduction section. Lines 83-116 provides very specific and detailed information,

which corresponds (and can be used) for a formal discussion section. I suggest to shorten this part, keeping the main ideas to justify the work and set up the objectives but with less details of previous works.

Response of the authors: Thank you for your suggestion. We have condensed this section and addressed within the discussion section.

2. - Materials and Methods. A representative reference for chemical elements analyses would be appreciated, since no much details are provided. FAPROTAX and FUNGuild references should be specified.

Response of the authors: The reference and detailed information for chemical elements analyses, FAPROTAX and FUNGuild, have been added in the revised manuscript in Line 149-164 and Line 227-234.

The mass concentration of $PM_{2.5}$, water-soluble ions, and metal elements were quantified after sampling. The membranes were meticulously weighed utilizing a Mettler XP-6 balance with an accuracy of 10^{-6} g. Prior to the weighing, the membranes were maintained in a controlled environment with consistent temperature and humidity for a duration of 24 hours. Ion chromatography (ICS-2100, Chameleon 6. 8, AS-DV autosampler Thermo Fisher) was employed to determine the concentration of water-soluble ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}, and NH_4^+. These ions were extracted by sonication with ionized water for 45 min and then separated by anion (IonPacAS23) or cation column exchange (IonPacCS12A). Then, these ions were detected using a conductivity detector with an anion separation column of IonPacAS23, flow rate: 1.0 mL/min, an anion suppressor of AERS500, and conductivity detector. The injection volume was 25 µL, and the cation separation column was IonPacCS12A, flow rate:1.0 mL/min, cation suppressor was CERS500 (Zhang et al., 2022). Metallic elementals including Al, Fe, Ti, Mn, Co, Ni, Cu, Zn, Ga, Sr, Cd, Sn, Sb, Pb, V, Cr, and As, were extracted using microwave digestion extraction (ETHOS ONE, Milestone), with the concentrations determined by ICP-MS or ICP-OES (Thermo Fisher).

Bacterial community functional was conducted using FAPROTAX, a manually constructed database that maps prokaryotic taxa to metabolic or other ecologically functions, such as sulfur, nitrogen, hydrogen, and carbon cycling (Chen et al., 2022). FUNGuild (Fungi Functional Guild) was used to predict the fungal ecological function. This tool could classify and analyze fungal communities by the microecological guild based on current published literature or data from authoritative websites to classify fungi functionally (Nguyen et al., 2016). Three primary groups are obtained based on the nutritional mode: Pathorotroph, Symbiotroph, and Saprotroph.

Reference

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., and Kennedy, P. G.: FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild, Fungal Ecol., 20, 241-248, 10.1016/j.funeco.2015.06.006, 2016.

Chen, J., Zang, Y., Yang, Z., Qu, T., Sun, T., Liang, S., Zhu, M., Wang, Y., and Tang, X.: Composition and Functional Diversity of Epiphytic Bacterial and Fungal Communities on Marine Macrophytes in an Intertidal Zone, Front. Microbiol., 13, 10.3389/fmicb.2022.839465, 2022.

Zhang, Y., Guo, C., Ma, K., Tang, A., Goulding, K., and Liu, X.: Characteristics of airborne bacterial communities across different $PM_{2.5}$ levels in Beijing during winter and spring, Atmos. Res., 273, 10.1016/j.atmosres.2022.106179, 2022.

- Results and Discussion.

3. I think the manuscript would benefit from an appropriate Discussion section, separated from Results. Comments from some results are too long and descriptive to follow the results fluently, and also some marks are repetitive, as Deinococcus (lines 348 and 388), Comamonas (lines 367 and 394). I would suggest split Results from Discussion, making the former shorter and focused on the data, and use the information to elaborate a discussion properly, without duplicities and allowing you to explain the relationships and hypotheses in a more consistent fashion.

Response of the authors: Thank you for your suggestion. The separated "Results" and

"Discussion" section was in the revised manuscript.

4. The title "3.1 Air mass backward trajectory" does not correspond properly with the content described in this section.

Response of the authors: Thank you for your suggestion. We have revised the title.

3.1 Air masses categorization and typical pollution processes

5. Several times, "spring season" (L262, L309, L474, L491, L536) is cited in the manuscript, but the sampling was conducted mostly in winter days. It is confusing. These references are based on other works? Did one of the Pollution episodes occur in Spring? Explain it.

Response of the authors: Winter and spring in northern China are characterized by a high incidence of haze and dust pollution. During the winter months, specifically from January to February, temperatures are low. This period is the central heating period of northern China, coal burning and other heating methods will lead to the increase of air pollutant emission intensity, and haze pollution incident is more serious. As spring progresses into March, temperatures and wind speed begin to rise. Northern China enters the spring dust season. Notably, the escalating frequency of extreme dust events in recent years has facilitated the transport of regional dust aerosols, significantly impacting both the northern and southern regions of China. In this study, pollution episodes occurred in spring from Mar 9 to March 12 was typical Haze-Dust composite pollution. Pollution episodes occurred in spring from Mar 26 to March 31 was typical dust pollution.

Haze-Dust composite pollution from March 26 to March 31

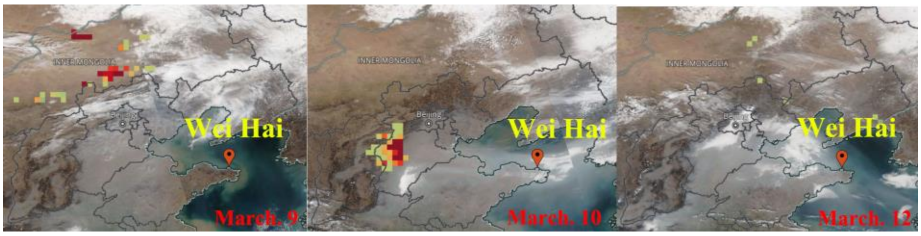

Figure 1 Satellite images of haze and dust air mass transport from March 9 to March 12

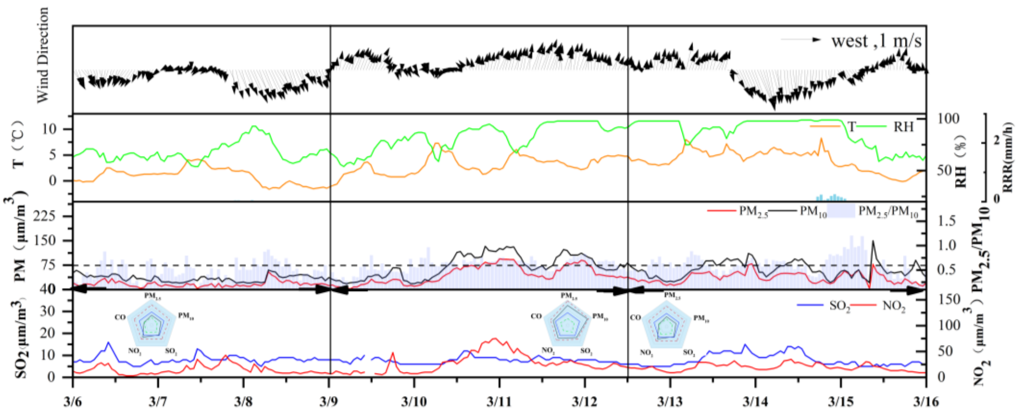

Figure 2 Air pollutants and meteorological factors of the haze-dust composite pollution

Dust pollution from March 26 to March 31

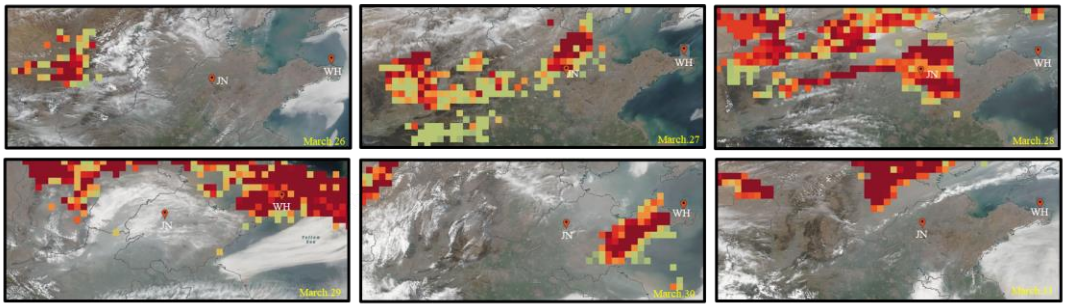

Figure 3 Satellite images of dust air masses transport (http://worldview.earthdata.nasa.gov).

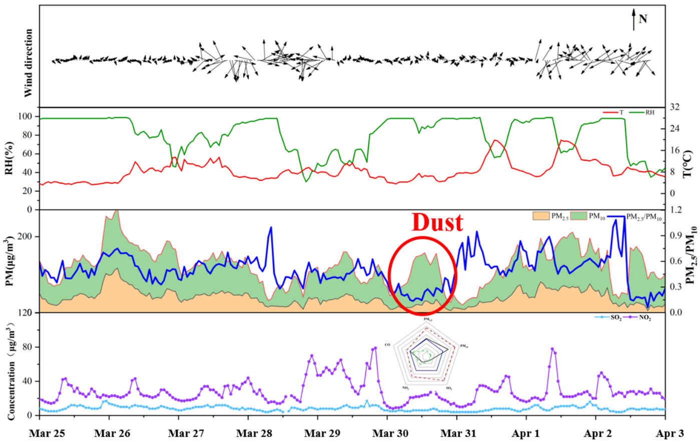

Figure 4 Air pollutants and meteorological factors of the dust pollution

6. Fig. 1. Please indicate the date of the events and the different scale for left and right axes.

Response of the authors: We have indicated the date of the events in Fig 1. The different scale for left and right axes are indicated in the figure legend. The concentration of water-soluble ions is indicated in the left axes. The concentration of metal elements (Cu, Zn, Al, Fe) is indicated in the right axes.

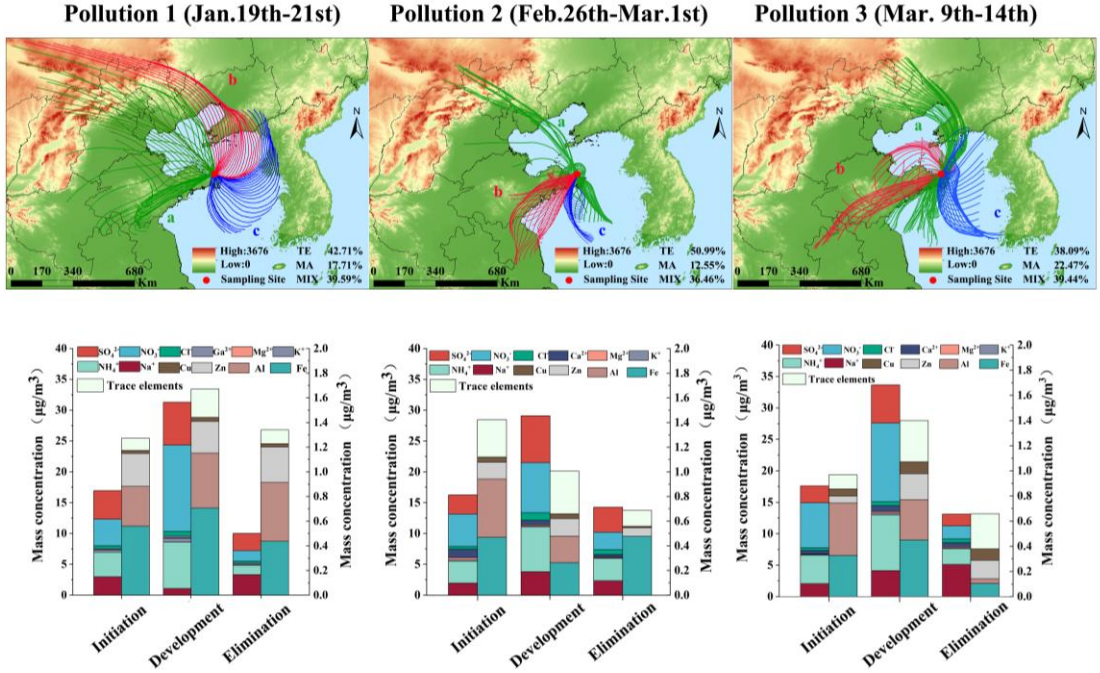

Fig. 1 Transformation of terrestrial and marine air masses, and chemical composition in $PM_{2.5}$ of three severe air pollution episodes. a, pollution initiation; b, pollution development; c, pollution elimination. TE, terrestrial air mass; MA, marine air mass; MIX, mix air mass.

7. NMDS or PCoA, and their respective ANOSIM or PERMANOVA analyses would increase the support some conclusions.

Response of the authors: We have added the Principal Coordinates Analysis (PCoA) in Fig.S4. Here, PCoA was conducted to examine the overarching differences among samples influenced by different air masses (Figure 10). PCoA is an unconstrained method of dimensionality reduction analysis that can be employed to explore similarities or disparities in the composition of microbial communities. As depicted in Figure 12, the $PM_{2.5}$ samples from each group are closely clustered, suggesting that the bacterial community structure of $PM_{2.5}$ remains consistent across groups. Conversely, the $PM_{2.5}$ samples influenced by terrestrial air masses are more dispersed, with greater distances between samples. This finding, to a certain degree, indicates variations in the airborne bacterial communities under different air mass influences.

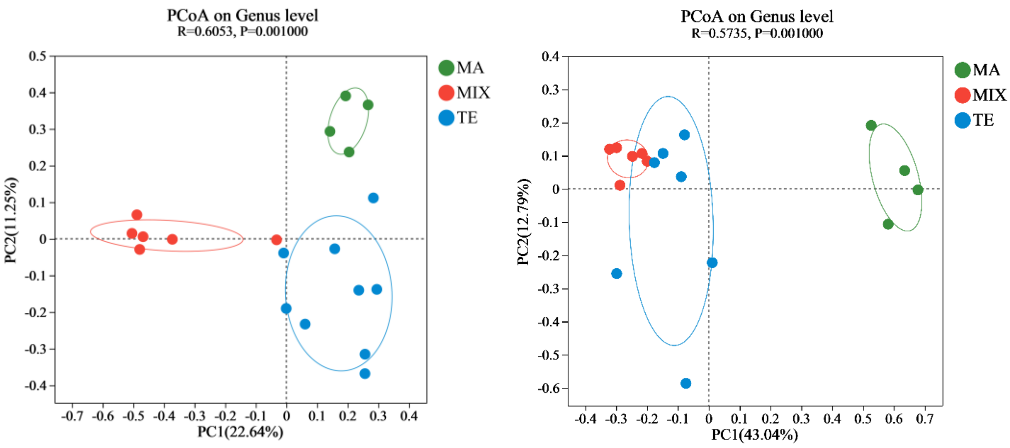

Fig.S4 PCoA analysis of bacterial and fungal community under different air masses terrestrial air masses (blue), marine air masses (green), and mixed air masses(red)

8.Fig. 2. I am guessing that left axis of the graphs are not on 100% but 1%. How the authors explain the substantial change in fungal communities? For instance, Aspergillus completely disappeared from the top abundance in MIX when it was the top one for TE and MA.

Response of the authors: We have modified Fig. 2. The X-axis represents the percentage of relative abundance, which adds up to 100%.

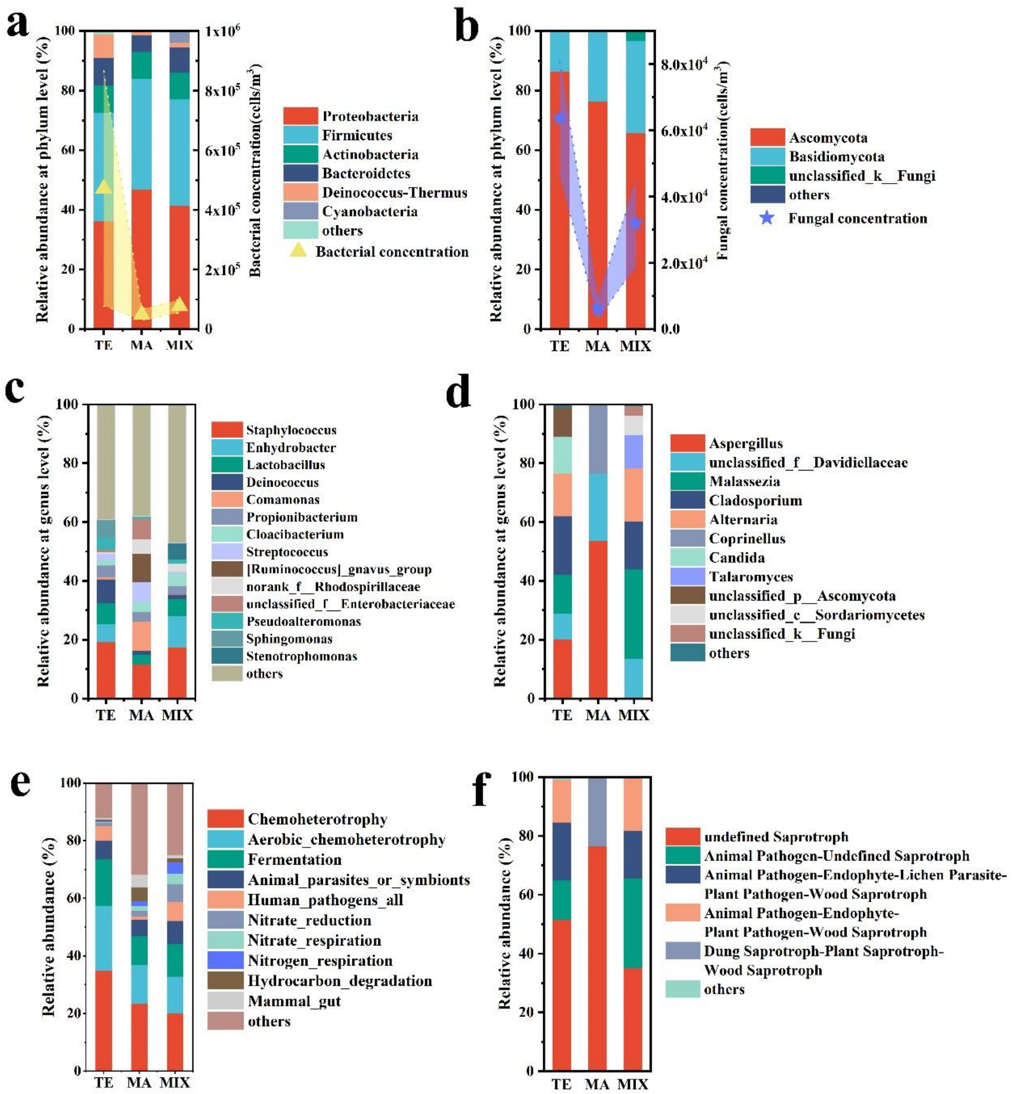

Fig. 2. Bacterial and fungal species and function influenced by different air masses. Bacterial and fungal community concentration, main phylum (a), (b), genus (c), (d), and community function (e), (f) are indicated.

Aspergillus generally has a higher abundance in cleaner samples. In the figure, it shows the highest relative abundance in marine air mass samples, followed by terrestrial air masses, and the lowest relative abundance in mixed air masses. *Aspergillus* was 53.7% and 20.1% in marine and terrestrial air-mass samples, respectively. *Aspergillus* is a dominant fungus in offshore areas such as Qingdao, China (Li et al., 2011). Moreover, the Saprophytic *Aspergillus* was also prevalent in clean samples during haze pollution episode and was commonly detected on non-Haze days (Yan et al., 2016). Prior research has established that *Aspergillus* is ubiquitously found in nature and non-polluted environments (Li and Kendrick, 1995).

For the coastal city of Weihai, we examined the relative abundance of *Aspergillus* in terrestrial and mixed air mass samples and found that the abundance was low in contaminated samples from terrestrial and contaminated samples from mixed samples (Figure 5). Moreover, we compared the abundance of *Aspergillus* in samples collected from coastal and inland cities within Shandong Province during the same sampling period, and found that in the inland city of Jinan (a highly polluted inland city), the abundance of *Aspergillus* was much higher than in the coastal city of Weihai. This indicates that *Aspergillus* has a relatively high abundance in low pollution ambient air.

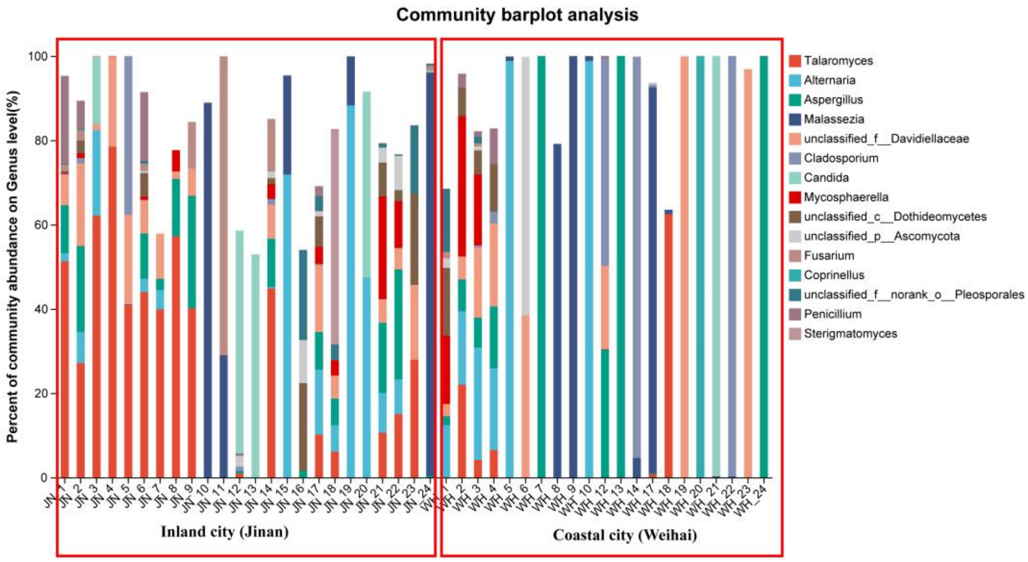

Figure 5 The top 15 abundant fungi in $PM_{2.5}$ at genus level in inland and coastal cities.

Reference

Li, D.-W. and Kendrick, B.: A year-round study on functional relationships of airborne fungi with meteorological factors, Int. J. Biometeorol., 39, 1995.

Li, M., Qi, J., Zhang, H., Huang, S., Li, L., and Gao, D.: Concentration and size distribution of bioaerosols in an outdoor environment in the Qingdao coastal region, Sci. Total Environ., 409, 3812-3819, 10.1016/j.scitotenv.2011.06.001, 2011.

Yan, D., Zhang, T., Su, J., Zhao, L.-L., Wang, H., Fang, X.-M., Zhang, Y.-Q., Liu, H.-Y., and Yu, L.-Y.: Diversity and Composition of Airborne Fungal Community Associated with Particulate Matters in Beijing during Haze and Non-haze Days, Front. Microbiol., 7, 10.3389/fmicb.2016.00487, 2016.

9. Lines 425-428 are hard to interpretate. Please revise. Are they a conclusion or supported information to your results?

Response of the authors: This section is the conclusion of the FAPROTAX analysis. Overall, the dominant airborne bacteria in the coastal city primarily inhabited anthropogenic environments such as soil, water, and terrestrial ecosystems. Additionally, marine ecosystems served as a significant source of airborne microbes.

10. Section 3.4 Community disparities influenced by terrestrial, marine and mixed air masses. The disparities mentioned in L387-388 are not supported statistically according to Fig. 3 or the p-values numbers are wrong. The same goes for the fungal graphs and text, for which the p-values do not concord (*Aspergillus*, p=0.014 or p=0.1498?; *Malassezia*, p=0.041 or 0.047??).

Response of the authors: We rechecked Fig. 3 and revised the section in Line 442-453. In the comparison of three types of air mass samples, the p value of *Aspergillus* was p=0.1498, *Malassezia* was p= 0.047. However, when comparing the oceanic and mixed air masses, the p Value of *Aspergillus* was p=0.00574, which showed significantly different between marine and mixed air masses. Comparisons between multiple groups often result in overall non-significant differences in the results (p>0.05), but with large variations in the relative abundance. In subsequent two-by-two comparisons, the results may be more significant (p<0.05). This difference maybe mainly caused by the different statistical analysis strategies used in multi group comparisons and two group comparisons.

For fungal community, Aspergillus demonstrated a significant differentiation between marine and mixed airmasses (P=0.005). The highest proportion was noted in samples from marine air masses, at 53.7%. In contrast, the values were 20.1% and 0.3% in terrestrial and mixed air masses respectively. Previous studies have confirmed that Aspergillus is widely distributed in nature and unpolluted environments (Kendrick, 1995). This fungus is predominantly found in offshore regions, such as Qingdao, China (Li et al., 2011). Furthermore, the saprophytic Aspergillus was also prevalent in clean *samples during periods of haze pollution and was frequently detected on non-haze days (Yan et al., 2016). Malassezia was higher in terrestrial and mixed air-mass samples (P=0.047), which has been found to be widespread in a variety of animals. As a parasitic fungus, Malassezia causes the majority of skin diseases, such as dandruff and seborrheic dermatitis caused by Malassezia sphericalis (Deangelis et al., 2007).*

11. Fig. 5 shows correlations with "Bacteria", "Fungi", and "Cells". Firstly, the legend shows "Pearson's r", when in Materials and Methods is specified Spearman's. Please clarify this point. Bacteria and Fungi correlations with chemical elements are referred to concentrations or community compositions? "Cells" are the direct sum of bacterial and fungal concentrations?

Response of the authors: The "Pearson's r" was used in the mantel analysis. We have corrected the expression in the Materials and Methods section in Line 246-248.

The correlation of bacteria and fungi with chemical elements refers to community composition, and the top 15 genera were screened for correlation analysis with chemical elements and Meteorological conditions. The "cells" is the abundance of bacterial and fungal concentrations based on qPCR. We have modified the expression with "microbial concentration".

The Mantel analysis was utilized to reveal the correlation between microbial community and various environmental factors. The Pearson's r coefficient at $p<0.05$ and $p<0.01$ indicates the significant correlation.

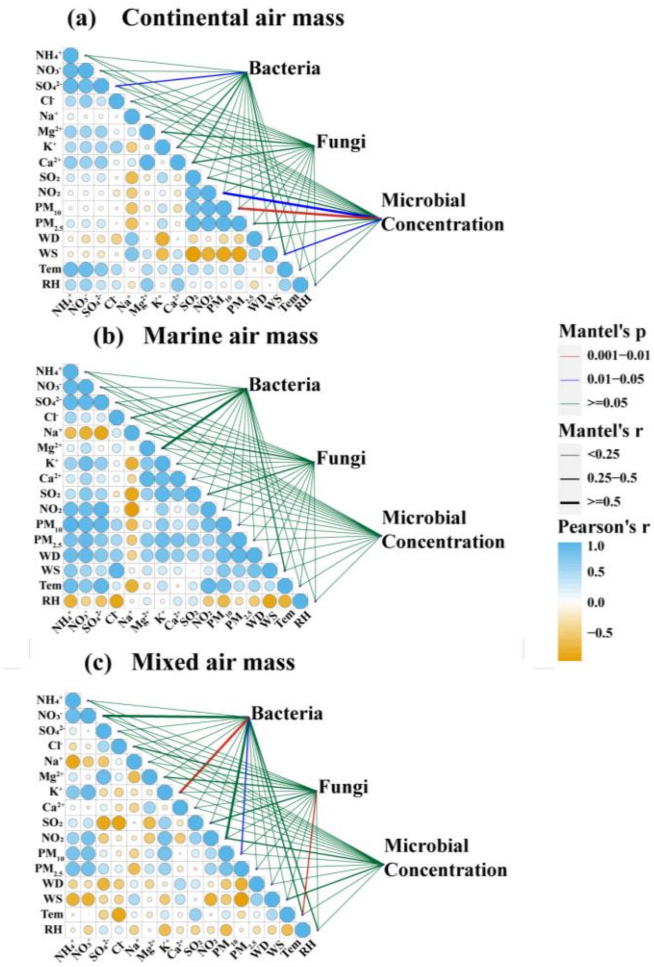

Fig. 5 Mantel analysis reveal the correlation between microbial community and various environmental factors under different air masses, terrestrial air masses (a) marine air masses (b) and mixed air masses (c). The Pearson's r correlation coefficient indicates the significant correlation at p<0.05 and p<0.01.

12. $PM_{2.5}$ and PM_{10} concentrations were positive correlated. However, the authors found a negative correlation with PM_{10} concentrations but not with $PM_{2.5}$. Moreover, several studies have shown a positive correlation between richness and microbial concentrations with PMs concentrations. The authors should elaborate a discussion about this. Are the correlations maintained by group of microorganisms (bacteria or fungi) or this is only observed when the addition of both is conducted?

Response of the authors: The positive correlation with PM_{10} is mainly due to the contribution of surface coarse particles to the atmospheric microbial community during sand dust events in spring. Under the influence of strong winds, the surface soil is the main source of coarse particles (PM_{10}). Thus, a positive correlation between airborne microorganisms and PM_{10}. During the dust period, the concentration of fine particles ($PM_{2.5}$) was lower compared to PM_{10}, thus showing a non-significant correlation between microbial concentration and $PM_{2.5}$.

Pollution episodes occurred in spring from Mar 26 to March 31 was typical dust event. The MODIS true-color images revealed a transit of highly polluted air masses originating from Northwest of the coastal city from March 26. Dust particles were then carried downwind to the eastern coastal city of Weihai on March 29 and 30. A significant increase in PM_{10} concentration was observed, with an hourly maximum value of 197 μg/m^3. The relatively low $PM_{2.5}/PM_{10}$ ratio of 0.28 indicated pronounced dust pollution. Historical radar map analysis revealed that the primary pollutants was PM_{10} on March 30.

Dust pollution from March 26 to March 31

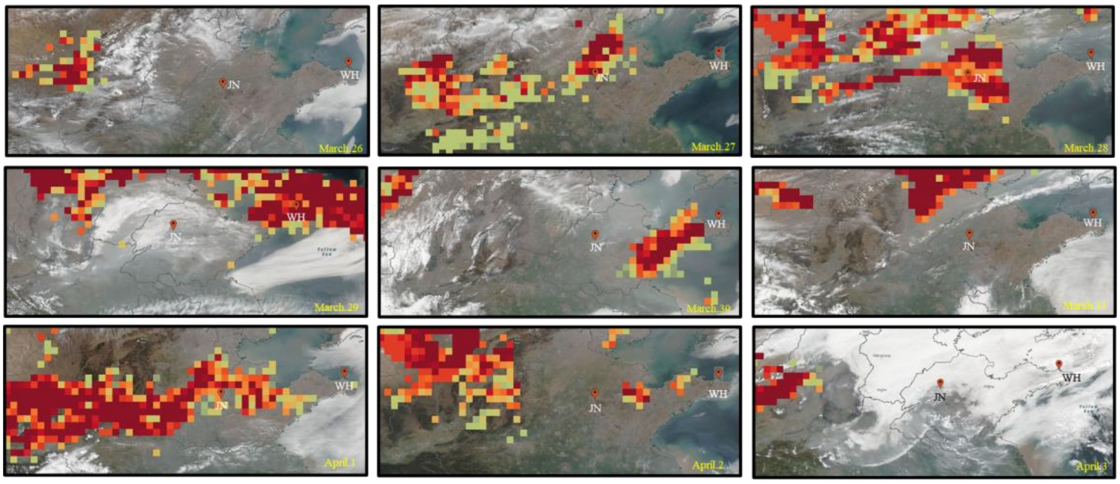

Figure 6 Satellite images of dust air masses transport (http://worldview.earthdata.nasa.gov).

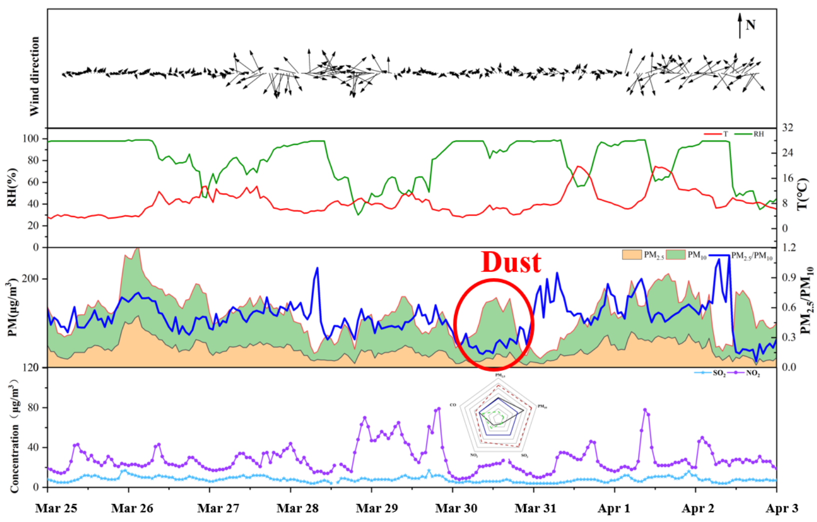

Figure 7 Air pollutants and meteorological factors of the dust pollution

13. RDA analysis with the environmental and physicochemical parameters would be complementary to correlations.

Response of the authors: We have added the RDA analysis in the supplementary materials Fig. S4. Bacterial community composition under the influence of marine air masses were positively correlated with humidity while bacteria under the influence of terrestrial air masses were positively correlated with $PM_{2.5}$ and PM_{10}, and other crustal elements, such as Mg^{2+}, Ga^{2+}, K^+.

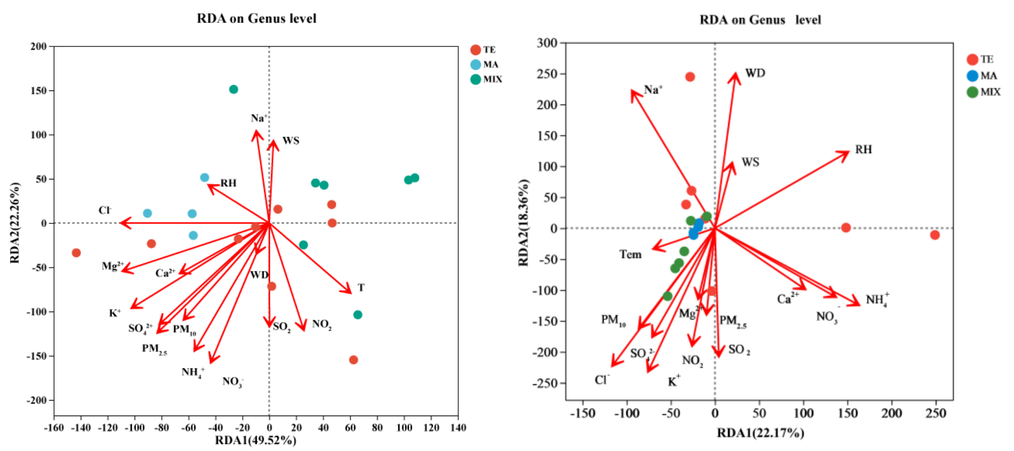

Fig. S4 RDA of bacterial and fungal community structure with environmental parameters.

14. L469: "The marine air masses are generally clean and have a strong scavenging effect on air pollutants". From Fig 5., Wind speed is positively correlated with PMs

concentrations in marine air masses (higher speed higher concentrations) and negatively with Continental ones. How does it fit with the clearance effect the authors propose?

Response of the authors: The sampling period predominantly occurs during the winter and early spring seasons in northern China, characterized by a systematic northwest wind direction. Therefore, the air masses frequently originate from terrestrial and mixed sources. The statistical examination of terrestrial and mixed air masses reveals a negative correlation between $PM_{2.5}$ concentration and wind speed, suggesting the strong scavenging effect of high winds on $PM_{2.5}$. During the study period, there were a limited number of typical marine air masses, with only 6 samples being selected for analysis. By further screening the samples, the number of samples affected by ocean air masses was increased, and a correlation analysis between wind speed and $PM_{2.5}$ was conducted. It was also found that $PM_{2.5}$ concentration was negatively correlated with wind speed.

Table 1 Correlation analysis table between $PM_{2.5}$ and wind speed

	TOTAL	TE	MA	MIX
r	-0.731	-0.770	-0.049	-0.738
P	< 0.01	0.009	0.039	0.037

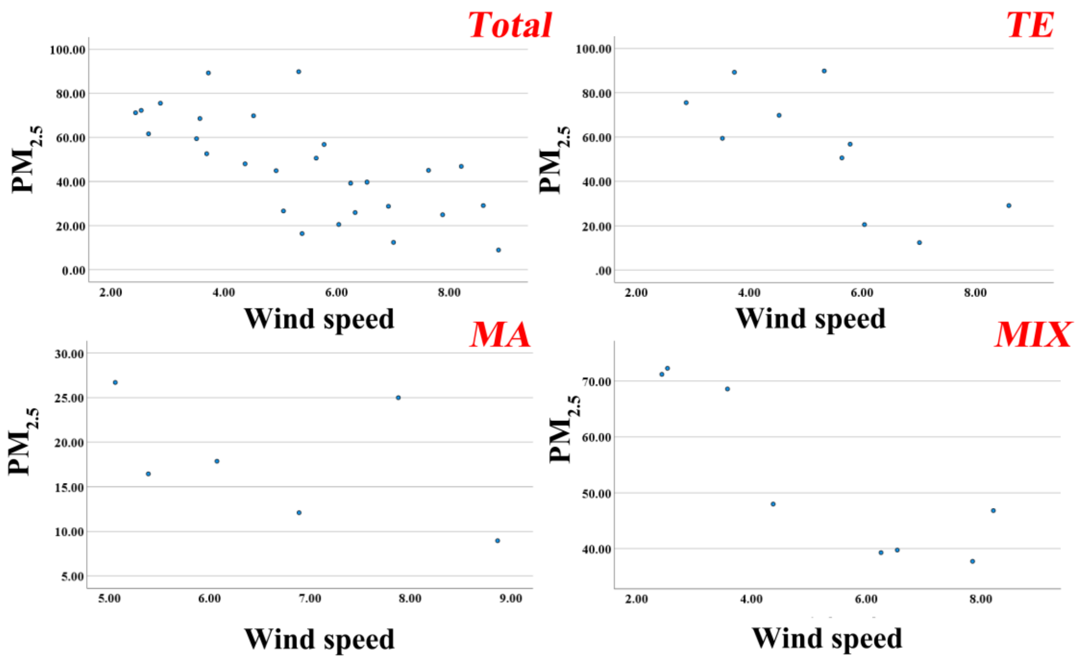

Figure 8 Scatter plot of wind speed and $PM_{2.5}$

15. Lines 480-509 reminds a mixture of Discussion and Conclusions. This would be solved by separating Results and Discussion.

Response of the authors: The separated Results and Discussion sections are provided in the revised manuscript.

16.Minor changes

Typos:

L324: a point (.) before reference.

Response of the authors: We have revised the sentence in Line 333-336.

The V/Ni ratio is employed as a measure of the influence of ship emissions. A ratio exceeding 0.7 typically indicates a significant impact from these emission sources, and is commonly used as an indicator in coastal cities (Zhang et al., 2014).

L342 Actionbacteria--> Actinobacteria

Response of the authors: We have revised the sentence in Line 349-351.

Predominantly, Proteobacteria (40.06%), Firmicutes (36.30%), Actinobacteria (8.97%), Bacteroidota (8.29%), and Deinococcus-Thermus (4.59%) were identified as the most abundant bacteria.

L448 Concertation--> Concentration

Response of the authors: We have revised the sentence in Line 478-480.

Additionally, a positive correlation was observed between bacterial and fungal concentrations and NO_2 ($P<0.05$), as well as a significant positive correlation with PM_{10} ($P<0.01$).