

Response to Referee #2 Comments on ESSD-2026-338

Dear Referee,

We sincerely thank the referee for the careful evaluation of our manuscript and for the constructive comments regarding the scientific motivation, terminology, dataset description, figure presentation, and interpretation of the results. These comments helped us identify several aspects of the manuscript that required further clarification. In response, we have revised the Introduction and Methods sections, clarified the operational definition and scope of the measured bioaerosols, improved the presentation of the figures, expanded the site-level description of the dataset, and moderated several interpretations that were not sufficiently supported by the available observations. Our point-by-point responses are provided below.

Comment 1:

The motivation for conducting a dust–bioaerosol field campaign should be explained more clearly. The Introduction emphasizes the role of dust in bioaerosol transport, but meteorological conditions (e.g., wind) likely play an equally or even more important role. More background should be provided on why dust is specifically emphasized. In addition, there is an inconsistency: The Introduction states that bioaerosols are enhanced by dust-related transport, whereas Lines 194–195 state that the data were "analysed under non-special weather conditions (e.g., dust events)." This statement seems contradictory and should be clarified.

Response:

We thank the referee for this important comment. We agree that the original manuscript did not sufficiently distinguish the specific role of dust from the broader influence of meteorological conditions on bioaerosol emissions and transport.

The DuBi field observation program was not designed on the assumption that dust is the sole or dominant factor controlling bioaerosol variability. Meteorological conditions, including wind speed and direction, atmospheric stability, boundary-layer development, temperature, humidity, and precipitation, are controls on the release, transport, and removal of biological particles. Dust was specifically emphasized because East Asia contains extensive arid and semi-arid source regions and experiences frequent long-range dust transport from continental source areas toward densely populated downwind regions. During these events, biological particles originating from soil and other terrestrial surfaces may be aerosolized and transported together with dust. Therefore, the DuBi program was designed to investigate bioaerosols within a source–transport–receptor framework that includes dust-source regions, transitional areas, and downwind receptor sites, rather than to attribute all observed bioaerosol variability exclusively to dust.

We have revised the Introduction to explain this scientific rationale more clearly and to acknowledge the important role of meteorological processes. We now describe dust transport as one important regional pathway for the mobilization and long-range dispersal of biological particles, while wind and other meteorological variables regulate whether and how such transport occurs.

We also agree that the statement at Lines 194–195 was ambiguous. The phrase “non-special weather conditions” referred to the subset of observations used to characterize general spatial and temporal patterns and was not intended to describe the overall scope of the DuBi field program. It also did not mean that dust-related observations were irrelevant to the dataset. We have rewritten this sentence to clearly distinguish the general descriptive analysis from analyses specifically targeting dust-event conditions.

Revision made:

Page 3, Line 78.

Revised:

East Asia is one of the most densely populated and environmentally diverse regions worldwide (Su et al., 2015), characterized by frequent dust events driven by vast arid source regions in Central Asia and desert (Chen et al., 2026; Yu et al., 2023), rapid urbanization and industrialization (Tseng et al., 2024), intensive agricultural activities, and strong land – ocean interactions (Zhou et al., 2025; Lang et al., 2025). The abundance, dispersal, and community composition of atmospheric bioaerosols are influenced by meteorological conditions, including wind speed and direction, atmospheric stability, temperature, humidity, and precipitation (Uetake et al., 2019; Tignat-Perrier et al., 2019; Petersson Sjögren et al., 2023; Drautz-Moses et al., 2022). Under ongoing climate change, drought conditions are projected to intensify (Araujo et al., 2025; Xu et al., 2023), with East Asia expected to be particularly affected (Qi et al., 2024). Within this broad meteorological framework, dust events are of particular relevance in East Asia because the mobilization of mineral particles can simultaneously release soil-associated microorganisms and other biological materials into the atmosphere, while strong regional winds can transport these particles over long distances (Maki et al., 2019; Zhou et al., 2024; Tang et al., 2018). Thus, dust is an important mechanism linking terrestrial source areas with downwind environments. On this basis, the Dust – Bioaerosol (DuBi) field observation program adopted a source – transport – receptor perspective, incorporating observations in source regions, along transport pathways, and at downwind receptor sites. Given the extensive arid and semi-arid regions of East Asia and the large populations residing in downwind areas (Jin et al., 2022), the regional transport of dust-associated bioaerosols warrants particular attention because of its potential environmental and public health implications.

Despite increasing bioaerosol observations in East Asia, existing studies are often fragmented in terms of sampling protocols, temporal coverage, and analytical methodologies (Safatov et al., 2022). This methodological and observational heterogeneity limits regional comparisons and large-scale assessments and makes it difficult to distinguish genuine environmental variability from differences introduced by sampling and analytical procedures (Pogner et al., 2024a). Accurate assessment of bioaerosol-related effects therefore requires broader observational networks, longer and more continuous monitoring records, and greater methodological standardization to facilitate regional and transregional data integration and sharing (Huang et al., 2024a). Accordingly, the establishment of a bioaerosol dataset with broad geographical coverage and consistent sampling and analytical methodologies is essential for advancing regional-scale research and applications (Sajjad et al., 2023; Gashi et al., 2025; Boifot et al., 2024; Jiang et al., 2022). Such a dataset would enable a more comprehensive characterization of the geographic distribution, spatiotemporal variability, and environmental and health impacts of bioaerosols, while supporting the assessment of public health risks under climate change and providing a scientific basis for mitigation strategies and policy development.

Revision made:

Page 10, Line 194.

Revised:

To characterize the general relationships between bioaerosol concentration, NDVI, and air temperature across East Asia while minimizing the influence of dust transport, this analysis was restricted to samples collected under non-dust conditions.

Comment 2:

The definition of *bioaerosol* throughout the manuscript is unclear and should be better clarified. In general, bioaerosols, often referred to as primary biological aerosol particles (PBAPs), include bacteria, fungal spores, pollen, plant debris, and viruses. However, the fluorescence analysis in this study appears to recognize only cellular particles. Viruses therefore appear to be excluded from the measurements. Furthermore, the reported significant negative correlation between bioaerosol concentration and NDVI raises additional questions. Since NDVI is positively related to vegetation cover, one might expect higher concentrations of plant-derived particles (e.g., pollen or plant fragments) in regions with higher NDVI. The manuscript also switches to the term *microbial* (e.g., Line 199). Since *microbial bioaerosols* generally refer only to microorganisms (e.g., bacteria and fungi) and exclude pollen and plant fragments, the distinction between *bioaerosol* and *microbial bioaerosol* should be clearly defined and

used consistently. Does this imply that the "bioaerosols" measured in this study do not include plant-derived particles? If so, this should be explicitly stated and the use of the term "bioaerosol" throughout the manuscript may not be appropriate.

Response:

We thank the referee for this important comment. We agree that the original manuscript did not sufficiently distinguish the broad definition of primary biological aerosol particles (PBAPs) from the operational definition applied in the present study. We have therefore clarified the scope of the fluorescence-based concentration measurements and standardized the use of the terms “bioaerosol,” “microbial bioaerosol,” and “bacterial community” throughout the manuscript.

In the broader atmospheric context, PBAPs include bacteria, fungal spores, pollen, plant debris, viruses, and other particles of biological origin. In the present study, however, bioaerosols were operationally defined as particles exhibiting cellular morphology and emitting blue-green fluorescence after DAPI staining. The reported concentrations therefore represent the subset of atmospheric biological particles that satisfied both the morphological and fluorescence-based identification criteria used in our microscopy analysis, rather than the complete spectrum of PBAPs.

The fluorescence analysis was not intended to identify only bacterial cells. Previous methodological work has shown that bacterial cells, fungal cells, and plant detritus can all exhibit detectable fluorescence after DAPI staining (Liu et al., 2023). Nevertheless, in our counting procedure, pollen and plant-derived fragments were not classified or quantified as separate particle categories. Such particles may have contributed to the reported concentrations only when they exhibited both the required morphology and characteristic fluorescence. Their specific contribution to the measured concentration cannot therefore be determined from the present data.

Viruses were not included in the reported concentration measurements because they were outside the size, optical resolution, and morphological identification range of the fluorescence microscopy procedure applied in this study. In addition, the DAPI method does not distinguish viable from non-viable biological particles. These methodological boundaries have now been stated explicitly in the revised Methods section.

We also agree that the terminology associated with the sequencing results required greater precision. The 16S rRNA gene sequencing data specifically characterize the bacterial communities associated with the collected aerosol samples. Accordingly, references to “microbial community,” “microbial diversity,” and “microbial composition” have been replaced with “bacterial community,” “bacterial diversity,” and “bacterial community composition,” respectively, wherever the discussion is based on the 16S rRNA data. The term “bioaerosol concentration” is retained for the fluorescence microscopy measurements, but it is now explicitly defined as the concentration of

particles satisfying the operational DAPI-based criteria described above.

Regarding the negative correlation between bioaerosol concentration and NDVI, we appreciate the reviewer's concern that greater vegetation cover may increase the abundance of plant-derived particles, such as pollen and plant fragments. However, the concentration reported in this study represents the total number of particles satisfying the operational DAPI-based identification criteria, rather than the concentration of any specific biological particle type. As discussed in the manuscript, regions with low NDVI are generally characterized by sparse vegetation cover and relatively dry and loose surface soils, which can facilitate the wind-driven entrainment of soil-associated microorganisms and other biological particles into the atmosphere (Qi et al., 2023). By contrast, denser vegetation in regions with high NDVI can enhance the interception of airborne particles and promote their dry deposition, thereby reducing their atmospheric concentrations (Zhai et al., 2022). These processes provide a plausible explanation for the observed regional-scale negative relationship. We acknowledge that vegetation may also act as a source of pollen and plant fragments. However, these particle types were not separately identified or quantified in the present study, and their individual contributions cannot be distinguished from those of other DAPI-defined biological particles. We have therefore revised the discussion to clarify that the negative correlation represents the overall relationship observed in this dataset and should not be interpreted as evidence that increasing vegetation cover universally reduces all categories of PBAPs. The proposed mechanisms remain an interpretation based on land-surface characteristics and require further particle-resolved observations for quantitative verification.

These revisions clarify that the fluorescence-based concentrations and the bacterial sequencing results represent two complementary but distinct components of the dataset and should not be interpreted as a complete characterization of all PBAP categories.

Revision made:

Page 7, Line 141.

Revised:

In this study, bioaerosols were operationally defined as particles exhibiting cellular morphology and emitting blue-green fluorescence after DAPI staining. Accordingly, the reported bioaerosol concentrations represent the fraction of atmospheric biological particles that met both the morphological and fluorescence-based identification criteria, rather than the full range of primary biological aerosol particles (PBAPs). Viruses were outside the detection scope of this method. Pollen and plant fragments were not separately identified or quantified and would only have been included when they met both identification criteria. Hereafter, "bioaerosol concentration" refers specifically to

the particle concentration determined using this operational definition.

Revision made:

Page 10, Line 195.

Revised:

As shown in Fig. 4a, bioaerosol concentrations were significantly negatively correlated with NDVI. In regions with low NDVI, sparse vegetation cover and dry, loose surface soils may facilitate the wind-driven entrainment of soil-associated microorganisms and other biological particles into the atmosphere (Qi et al., 2023). In contrast, denser vegetation in regions with high NDVI may enhance the interception of airborne particles and promote dry deposition, thereby contributing to lower atmospheric bioaerosol concentrations (Zhai et al., 2022). Although vegetated surfaces may also emit pollen and plant fragments, these particle categories were not separately identified in the present study. The observed correlation therefore represents the net relationship between NDVI and the operationally defined DAPI-positive particle concentration rather than the response of individual PBAP categories.

Comment 3:

Why were these observation sites selected? Most sites appear to be located in or near dust source regions. Are these regions expected to have sufficiently high bioaerosol concentrations? If the primary objective is to characterize bioaerosols, why were regions with abundant vegetation (e.g., southwestern China or the forested regions of northeastern China) not included? The current site selection seems to emphasize dust source regions rather than regions with potentially strong biological aerosol emissions.

Response:

We thank the referee for this important comment. We agree that the rationale for site selection was not sufficiently explained in the original manuscript.

The observation network was established within the Dust–Bioaerosol (DuBi) field program and was designed primarily according to a source–transport–receptor framework. Accordingly, the selected sites included arid and semi-arid source regions, locations along potential atmospheric transport pathways, and downwind receptor areas. The relatively large number of sites associated with dust-affected regions reflects the original scientific objective of the DuBi program, namely, to investigate the mobilization and regional transport of biological particles associated with east Asian dust.

The sites were not selected on the assumption that dust source regions necessarily have uniformly higher bioaerosol concentrations. Rather, these regions were included because wind and dust emission can mobilize soil-associated microorganisms and other

biological material and transport them to distant receptor regions.

This network also includes sites with surface features such as urban, coastlines and forest. Nevertheless, we acknowledge that the spatial distribution is not balanced among all ecosystem types in East Asia. In particular, highly vegetated regions, including southwestern China and the forested areas of northeastern China, are underrepresented. The current dataset should therefore not be interpreted as a statistically representative sampling of all land-cover types or potential bioaerosol source regions across East Asia. We agree that vegetation-rich regions may represent important sources of pollen, fungal spores, plant fragments, and other biological particles. Their limited representation constrains the ability of the present dataset to characterize the full range of vegetation-related bioaerosol emissions. We have revised the manuscript to clarify the scientific basis and constraints of the site selection and have added this spatial imbalance as a limitation of the dataset. Future expansion of the observation network should include additional forested and densely vegetated regions to improve ecosystem representation and enable more systematic comparisons among dust-dominated, agricultural, urban, and forest environments.

Revision made:

Page 3, Line 78.

Revised:

East Asia is one of the most densely populated and environmentally diverse regions worldwide (Su et al., 2015), characterized by frequent dust events driven by vast arid source regions in Central Asia and desert (Chen et al., 2026; Yu et al., 2023), rapid urbanization and industrialization (Tseng et al., 2024), intensive agricultural activities, and strong land – ocean interactions (Zhou et al., 2025; Lang et al., 2025). The abundance, dispersal, and community composition of atmospheric bioaerosols are influenced by meteorological conditions, including wind speed and direction, atmospheric stability, temperature, humidity, and precipitation (Uetake et al., 2019; Tignat-Perrier et al., 2019; Petersson Sjögren et al., 2023; Drautz-Moses et al., 2022). Under ongoing climate change, drought conditions are projected to intensify (Araujo et al., 2025; Xu et al., 2023), with East Asia expected to be particularly affected (Qi et al., 2024). Within this broad meteorological framework, dust events are of particular relevance in East Asia because the mobilization of mineral particles can simultaneously release soil-associated microorganisms and other biological materials into the atmosphere, while strong regional winds can transport these particles over long distances (Maki et al., 2019; Zhou et al., 2024; Tang et al., 2018). Thus, dust is an important mechanism linking terrestrial source areas with downwind environments. On this basis, the Dust – Bioaerosol (DuBi) field observation program adopted a source –

transport – receptor perspective, incorporating observations in source regions, along transport pathways, and at downwind receptor sites. Given the extensive arid and semi-arid regions of East Asia and the large populations residing in downwind areas (Jin et al., 2022), the regional transport of dust-associated bioaerosols warrants particular attention because of its potential environmental and public health implications.

Revision made:

Page 16, Line 324.

Revised:

Although significant correlations were identified between bioaerosol characteristics and environmental variables such as NDVI and temperature, the underlying mechanisms remain unresolved. The lack of year-round continuous observations at individual sites limits robust seasonal analysis, while the uneven spatial distribution of the network, particularly the underrepresentation of forested and other densely vegetated regions, constrains its ecosystem representativeness. Future studies should therefore combine longer-term monitoring, expanded coverage of vegetation-dominated environments, and more detailed process-based analyses to better resolve seasonal variability, vegetation-related emissions, and the mechanisms linking bioaerosols to environmental conditions.

Comment 4:

The descriptions of mineral particles appear to be inconsistent. Line 137 states that mineral particles appear white, whereas Line 140 states that yellow-green particles represent mineral dust enriched with water-soluble salts or organic materials. Please clarify the relationship between these two particle categories and explain why mineral particles are represented by different colors.

Response:

We thank the referee for identifying this ambiguity. The two descriptions refer to different mixing states of mineral particles. Mineral particles were identified based on their characteristic morphology and color. Under fluorescence microscopy, they generally appeared white and optically transparent, often with recognizable geometric or crystalline shapes.

The yellow-green particles described in the manuscript represent mineral particles associated with or coated by water-soluble salts or organic matter. The yellow-green fluorescence originates from these attached materials rather than from the mineral components themselves. Particle classification was therefore not based solely on fluorescence color. Mineral particles exhibiting yellow-green fluorescence could still be identified from their characteristic transparent or crystalline morphology. Thus, the

difference between the white and yellow-green appearances reflects the presence or absence of attached materials on the mineral particles, rather than an inconsistency in particle identification.

Comment 5:

Figures need to be improved:

- Please add the units of bioaerosol concentration to all figures where concentrations are presented.
- Please label all figure panels with identifiers such as (a), (b), and (c).
- Could the authors provide representative examples corresponding to each of the four particle colors shown in Figure 2? The distinction among the different particle categories in Figures 2e–g is currently not sufficiently clear.
- For the top panel of Figure 6, please clarify what the color scale of bioaerosol concentration represents.

Response:

We thank the referee for these constructive suggestions. All figures have been carefully revised to improve their clarity and consistency.

1. Units of bioaerosol concentration

The unit of bioaerosol concentration has been added to the relevant axes in all figures presenting concentration data. The concentration is now consistently reported in particles/m³ throughout the manuscript and figures.

2. Panel identifiers

(a), (b), (c) have been added to all multi-panel figures. The corresponding figure captions have also been revised to describe each panel explicitly and in the same order as presented in the figures.

3. Representative examples of the four particle-colour categories in Fig. 2

We agree that the distinction among the four particle-colour categories in the original version of Fig. 2e–g was not sufficiently clear. To address this issue, we replaced these panels with three new representative images in which the four particle types can be more clearly distinguished. In addition, the different coloured particles have now been explicitly marked in the revised figure to facilitate visual identification. The corresponding figure caption has also been revised to clarify the interpretation of each particle category.

4. Colour scale in the upper panel of Fig. 6

We thank the referee for pointing out this ambiguity. In the upper panel of Fig. 6, the different colours do not represent different bioaerosol concentration ranges. They are used only to distinguish samples collected in different months. Bioaerosol concentration is represented by the position of each data point on the

corresponding concentration axis. We have revised the figure legend and caption to clarify the meaning of the colours and avoid their interpretation as a concentration colour scale.

Revision made:

Page 8, Line 150.

Revised:

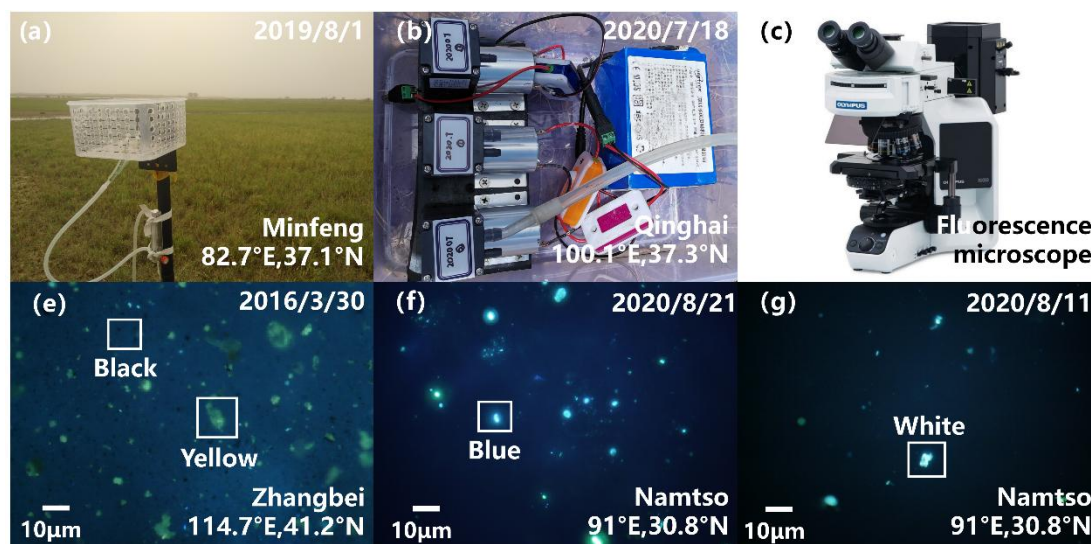


Figure 2: (a) Bioaerosols sampler used during the DuBi field sampling campaign; (b) power supply and sampling pump; (c) fluorescence microscope; (e–g) representative fluorescence micrographs of DAPI-stained samples, with the four particle-colour categories identified labels.

Revision made:

Page 11, Line 207.

Revised:

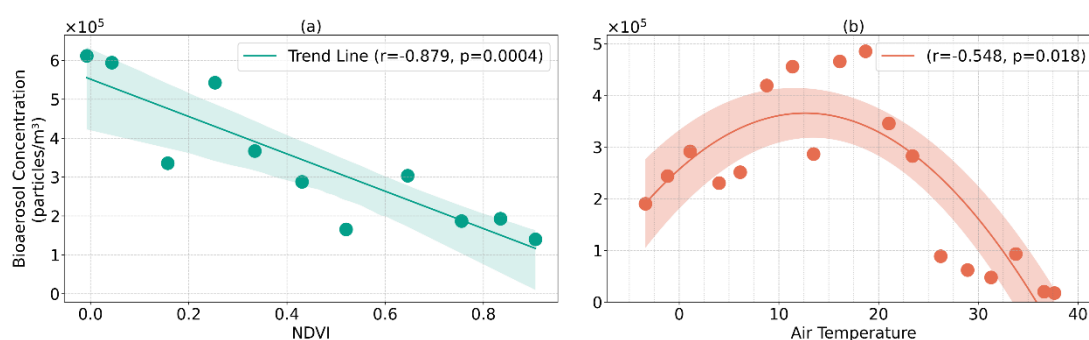


Figure 4: (a) Relationship between bioaerosol concentration and NDVI during the DuBi field sampling campaign. The green points represent mean bioaerosol concentrations calculated by binning NDVI into intervals of 0.1 and averaging all samples within each interval. (b) Relationship between bioaerosol concentration and air temperature. The orange points represent mean bioaerosol concentrations calculated by binning temperature into intervals of 2.5 °C and averaging all samples within each interval. The shaded areas indicate the 95 % confidence intervals.

Revision made:

Revised:

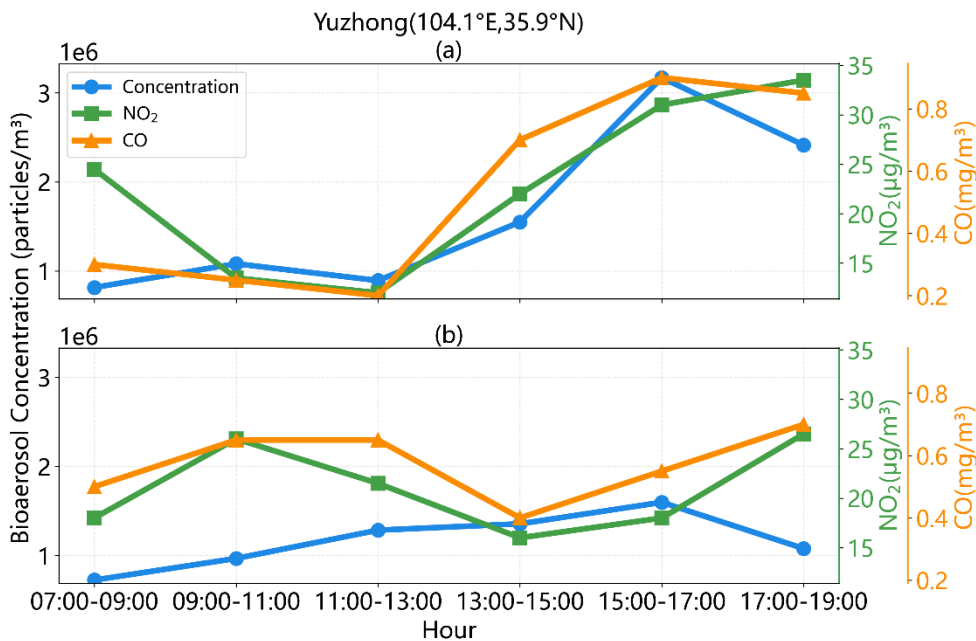


Figure 5: Temporal variations in bioaerosol, NO_2 , and CO concentrations observed in Yuzhong on (a) 6 November and (b) 15 November 2020.

Revision made:

Revised:

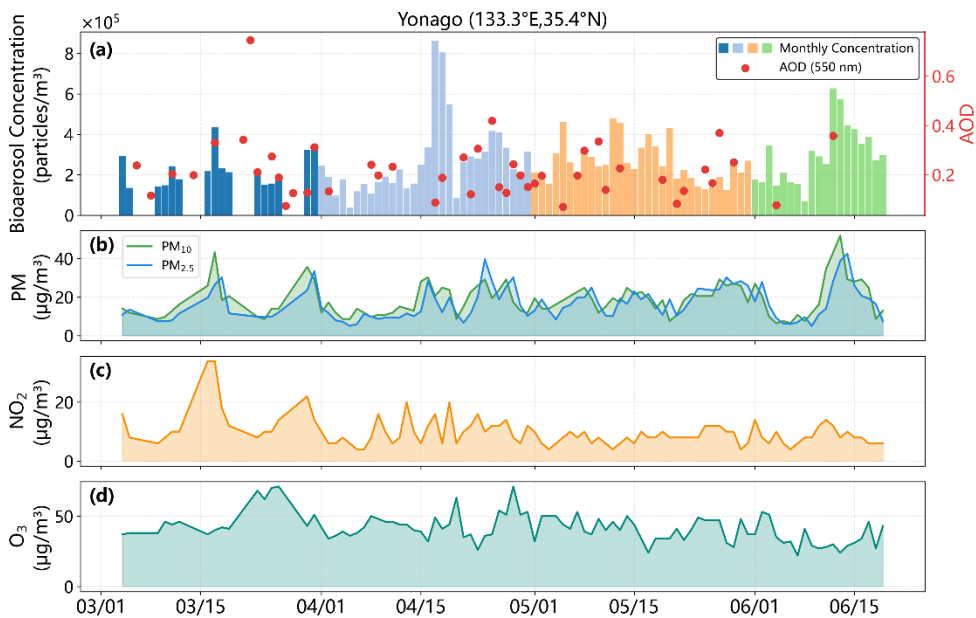


Figure 6: Daily variations in (a) bioaerosol concentration and aerosol optical depth (AOD), (b) $\text{PM}_{2.5}$ and PM_{10} concentrations, (c) NO_2 concentration, and (d) O_3 concentration in Yonago, Japan, during March–June 2015.

Comment 6:

As this is a *Scientific Data* paper, I would expect a more comprehensive description of the dataset itself before presenting downstream analyses. For example, it would be useful to first present the basic characteristics of bioaerosol concentrations and particle-type compositions at each observation site. For example, Section 3.3 and Figure 7 appear to analyze bacterial community composition using all samples combined. Would it be more informative to report the bacterial community composition separately for different sites, considering the large geographical differences among them?

Response:

We thank the referee for this important comment. We agree that, as a data paper, the manuscript should provide a more systematic description of the dataset itself before presenting subsequent analyses. In the original version, the site-level characteristics of bioaerosol concentrations, particle-type composition, and bacterial community structure were not presented in sufficient detail.

To address this concern, we have reorganized the Results section and expanded the description of the dataset at the observation-site level. The revised manuscript now first presents the distribution and variability of bioaerosol concentrations across the individual sites, together with the most abundant genera. This site-resolved presentation allows the spatial heterogeneity of the dataset to be assessed before the relationships with environmental variables are discussed.

We also agree that a site-resolved presentation can provide useful information on local bacterial community characteristics. However, the primary objective of Section 3.3 and Fig. 7 was to characterize the large-scale distribution of bacterial communities across East Asia and to examine their relationships with regional environmental gradients, rather than to establish a representative community profile for each individual site.

In particular, NDVI varies substantially among the observation sites because they encompass contrasting land-surface environments, ranging from arid and semi-arid regions to urban, coastal, and vegetated areas. By contrast, NDVI generally varies much less among samples collected at the same site over the relatively short observation periods available in this dataset. A site-by-site analysis would therefore provide insufficient within-site environmental variability for evaluating the relationship between bacterial community characteristics and NDVI. For these reasons, Fig. 7 was retained as a regional-scale overview of bacterial community composition across the dataset.

We also acknowledge that long-term, repeated observations at individual sites would be required to characterize site-specific community dynamics and evaluate their relationships with temporal changes in NDVI. This limitation and the need for future

site-resolved analyses have now been noted in the revised manuscript.

Revision made:

Page 10, Line 193.

Revised:

Table 2 reports the Mean bioaerosol concentration and genus with highest mean relative abundance at each site. Concentrations span from 3.39×10^4 at Tazhong to 6.65×10^6 at Dalanzadgad. The three highest site means occur at Dalanzadgad, Kanazawa, and Dunhuang; the three lowest occur at Tazhong, Namtso, and Minfeng. These contrasts establish pronounced spatial heterogeneity in the reported site means. *Lactococcus* is the most frequently dominant genus, ranking first at 9 of 14 sites (64.3%); *Ralstonia* is reported at 2 sites.

Table 2: Mean bioaerosol concentrations and dominant genera at the sampling sites.

Site	Mean bioaerosol concentration(particles/m ³)	Dominant genus
Ali	—	<i>Lactococcus</i>
Dalanzadgad	6.646384×10^6	—
Dunhuang	3.177384×10^6	<i>Lactococcus</i>
Erenhot	1.666249×10^6	<i>Lactococcus</i>
Hakui	1.441260×10^6	—
Inner Mongolia	5.967343×10^5	<i>Corynebacterium</i>
Jinan	—	<i>Lactococcus</i>
Kanazawa	3.828780×10^6	—
Minfeng	9.869644×10^4	<i>Ralstonia</i>
Namie	1.994137×10^5	—
Namtso	6.523244×10^4	<i>Lactococcus</i>
Qinghai	4.025444×10^5	<i>Lactococcus</i>
Tazhong-Minfeng	—	<i>Muribaculaceae</i>
Tazhong	3.391558×10^4	<i>Lactococcus</i>
Tsogt-Ovoo	1.490235×10^6	—
Xigaze	—	<i>Kocuria</i>

Site	Mean bioaerosol concentration(particles/m ³)	Dominant genus
Yadong	—	<i>Lactococcus</i>
Yonago	2.661274×10^5	—
Yongin	4.247435×10^5	—
Yuzhong	1.058873×10^6	<i>Ralstonia</i>
Zhangbei	2.067384×10^5	<i>Lactococcus</i>

Revision made:

Page 16, Line 324.

Revised:

Although significant correlations were identified between bioaerosol characteristics and environmental variables such as NDVI and temperature, the underlying mechanisms remain unresolved. The lack of year-round continuous observations at individual sites limits robust seasonal analysis, while the uneven spatial distribution of the network, particularly the underrepresentation of forested and other densely vegetated regions, constrains its ecosystem representativeness. Future studies should therefore combine longer-term monitoring, expanded coverage of vegetation-dominated environments, and more detailed process-based analyses to better resolve seasonal variability, vegetation-related emissions, and the mechanisms linking bioaerosols to environmental conditions.

We sincerely thank the referee again for the careful evaluation of our manuscript and for the constructive comments and suggestions. We have addressed all comments and revised the manuscript accordingly. We believe that these revisions have improved the clarity, completeness, and scientific value of the manuscript and have strengthened its presentation as a data paper.

The Authors