Editor of the journal *Biogeosciences*

Dear Prof. Carolin Löscher,

Thank you very much for your handling our manuscript and valuable and helpful comments. We have prepared the point-to-point responses to the comments and revised the manuscript accordingly. Here we submit the responses and the revised manuscript. We hope that the readability and the quality of the revised manuscript have been improved and met the requirements for the publication in the journal.

Thank you very much!

Best regards,

Daizhou Zhang

Point-to-Point Response to Editor′s Comments

Dear authors

Thank you for the thorough revision of your manuscript. I have several questions and comments, which may be beneficial to further improve the readability and impact of the paper. Generally, I was wondering in how far it is appropriate to refer to bacteria instead of microbes in general. As far as I see, no clear characterization excluding archaea, or small eukaryotes including fungi, is available. The Live-Dead staining may be more useful for bacteria but – and correct me if I am wrong- does not exclude other organisms. It would be interesting to learn if other organisms could be transported through air, and a discussion if only in some sentences would be very interesting especially given the presented hypothesis of pathogeny but also regarding cross-fertilization of different geographic regions.

Response: Thank you very much for your valuable comments.

According to the protocol of the LIVE/DEAD BacLight Bacterial Viability Kit, it provides two different nucleic acid stains—the SYTO 9 dye and propidium iodide—to rapidly distinguish live bacteria with intact plasma membranes from dead bacteria with compromised membranes. The kits enable to easily, reliably and quantitatively distinguish live and dead bacteria in minutes, even in a mixed population containing a range of bacterial types.

The LIVE/DEAD BacLight Bacterial Viability Kits have been widely used for the enumeration of bacteria, which have been applied to detect bacteria in samples of drinking water, sea water, aerosol, cloud water, and snow (Bauer et al., 2002; Boulos et al., 1999; Gasol et al., 1999; Hernlem and Ravva, 2007).

Although the LIVE/DEAD BacLight Bacterial Viability Kits are more selective for bacterial cells as implied by its name, the live/dead staining does not exclude other organisms, e.g., archaea, or small eukaryotes including fungal spores. The staining with the LIVE/DEAD BacLight Bacterial Viability Kit was shown to work not only with (eu)bacteria but also with archaea or eukaryotic cells, such as yeast (*Saccharomyces cerevisiae*) (Berney et al., 2007 and references therein, e.g., Leuko et al., 2004; Stocks, 2004; Zhang and Fang, 2004).

We used the LIVE/DEAD BacLight Bacterial Viability Kit to enumerate fluorescence particles with the size close to or smaller than 1 μm and spherical shape and attributed them to bacterial cells, which is based on two reasons. For archaea, the abundance of archaea in air is much less than that of bacteria. For instance, Fröhlich-Nowoisky et al. (2014) found that the abundance of archaea in air was only between ~1 and ~10 gene copies per cubic meter of air, while that of bacteria was ~10^4 to ~10^6 in the same air samples. For fungal spores, the dominant size range of fungal spores is 2–10 μm (Bauer et al., 2008), and the abundance of fungal spores in air could be one order of magnitude less than that of bacteria (Delort et al., 2010; Després et al., 2012). Therefore, the influence of archaea or eukaryotic cells, such as yeast on the presented results should be less than 10%. Unfortunately, it is impossible to directly distinguish these different types of microorganisms using staining enumeration only.

In the revision,

"***There are uncertainties in the bacterial cell counting caused by the LIVE/DEAD BacLight Bacterial Viability Kit because the kit could not distinguish archaea and small eukaryotes including fungi from bacteria (Berney et al., 2007). Since the abundance of archaea and fungi in air could be several (1–6) orders of magnitude less than that of bacteria (Fröhlich-Nowoisky et al., 2014, 2016; Delort et al., 2010) and the dominant size range of fungal spores is 2–10 μm (Bauer et al., 2008), the overestimation of bacteria caused by the kit we used should be less than 10% although the uncertainties could not be quantitatively evaluated.***" was added in Line 93.

References:

Bauer, H., Claeys, M., Vermeylen, R., Schueller, E., Weinke, G., Berger, A., and Puxbaum, H.: Arabitol and mannitol as tracers for the quantification of airborne fungal spores, Atmos. Environ., 42, 588-593, 10.1016/j.atmosenv.2007.10.013, 2008.

Berney, M., Hammes, F., Bosshard, F., Weilenmann, H. U., and Egli, T.: Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with flow cytometry, Appl. Environ. Microbiol., 73, 3283-3290, 10.1128/AEM.02750-06, 2007.

Boulos, L., Prevost, M., Barbeau, B., Coallier, J., and Desjardins, R.: LIVE/DEAD® BacLight™: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water, J. Microbiol. Methods, 37, 77-86, 10.1016/S0167-7012(99)00048-2, 1999.

Delort, A.-M., Vaïtilingom, M., Amato, P., Sancelme, M., Parazols, M., Mailhot, G., Laj, P., and Deguillaume, L.: A short overview of the microbial population in clouds: potential roles in atmospheric chemistry and nucleation processes, Atmos. Res., 98, 249-260, 10.1016/j.atmosres.2010.07.004, 2010.

Després, V. R., Huffman, J. A., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M. O., and Pöschl, U.: Primary biological aerosol particles in the atmosphere: a review, Tellus B Chem. Phys. Meteorol., 64, 15598, 10.3402/tellusb.v64i0.15598, 2012.

Fröhlich-Nowoisky, J., Ruzene Nespoli, C., Pickersgill, D. A., Galand, P. E., Müller-Germann, I., Nunes, T., Gomes Cardoso, J., Almeida, S. M., Pio, C., Andreae, M. O., Conrad, R., Pöschl, U., and Després, V. R.: Diversity and seasonal dynamics of airborne archaea, Biogeosciences, 11, 6067-6079, 10.5194/bg-11-6067-2014, 2014.

Gasol, J. M., Zweifel, U. L., Peters, F., Fuhrman, J. A., and Hagström, Å.: Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria, Appl. Environ. Microbiol., 65, 4475-4483, 1999.

Hernlem, B. J., and Ravva, S. V.: Application of flow cytometry and cell sorting to the bacterial analysis of environmental aerosol samples, J. Environ. Monit., 9, 1317-1322, 10.1039/b710512f, 2007.

Another question which I have is that the paper describes how fractions are detached from the

filters using vortexing and ultrasonic vibration. In how far can we be sure to not lose a significant fraction of organisms due to lysis induced by the latter method? Could this happen and therefore lead to an even more conservative result?

Response: For microbial analysis by microscopy, polycarbonate filters have been most commonly used for direct counts. Filters are often set in various kinds of air samplers during bioaerosol sampling. Microorganisms are washed from the surface of smooth-surface polycarbonate filters. The microorganisms in the wash solution are either cultured or re-filtered to uniformly distribute the microorganisms on the membrane filter. In the latter case, the microorganisms are stained and examined microscopically (Lighthart and Mohr, 1994; Jensen and Schafer, 1998; Eduard et al., 1990; Hernandez et al., 1999; Chen and Li, 2005; Maki et al., 2019; Li et al., 2011).

For the staining of microorganisms for microscopic enumeration, microorganisms on the filters are often resuspended in liquids (e.g., NaCl solution, PBS, Tween, and sterile/ultrapure water) by hand shaking, vortex and ultrasonic vibration or combined treatments. However, the time for hand shaking, vortex and ultrasonic vibration largely varies in different studies (Eduard et al., 1990; Chen and Li, 2005; Yahya et al., 2019; Li et al., 2011; Araya et al., 2019).

Sonication is concerned to cause cellular damages, especially for long sonication time. In previous studies, the sonication time in an ultrasound tank for aerosol-loaded filters in a solution ranges from 1–30 min (Yahya et al., 2019; Araya et al., 2019; Raghav et al., 2020). For a variety of environmental sample types, e.g., seawater and marine sediment, a sonification time of 30 s to 30 min has been applied to detached bacteria from other particles, gentler sonication for longer time intervals (Kepner and Pratt, 1994 and references therein). The cellular damages during the detachment procedure is generally caused by the consequent increase of temperature that cloud alter the sample by determining the bursting of prokaryotic cells with the increase of the sonication time (Danovaro, 2009).

In this study, bacteria were dislodged from the polycarbonate filters in a phosphate-buffered saline solution (PBS, pH 7.4) by vortex shaking and ultrasonic vibration in ice baths, and a gentle sonication was applied. Before we started to use this method, the dislodging time and operation conditions were confirmed in our laboratory experiments with BioSampler samples and in-line holder samples as the controls, following a number of published papers. The results using the mentioned conditions and operating procedures showed good consistency to the controls, indicating possible influence of sonication on the lysis of bacteria should be small. The filter-based techniques are not new and have been widely used before our laboratory experiments. Therefore, we don't think it is necessary to add these descriptions in the manuscript.

In the revision, "*Bacterial cells and other particles were detached from the aerosol-loaded polycarbonate membranes (47 mm in diameter) by vortex shaking and ultrasonic vibration in a phosphate-buffered saline solution (PBS, pH 7.4).*" was revised to "***Bacterial cells and other particles were detached from the aerosol-loaded polycarbonate membranes (47 mm in diameter) in a phosphate-buffered saline solution (PBS, pH 7.4) by vortex shaking and ultrasonic vibration in ice bath.***"

References:

Araya, C., Cazorla, A., and Reche, I.: Detachment Procedure of Bacteria from Atmospheric Particles

for Flow-cytometry Counting, Bio-Protocol, 9, 10.21769/BioProtoc.3273, 2019.

Chen, P.-S., and Li, C.-S.: Sampling Performance for Bioaerosols by Flow Cytometry with Fluorochrome, Aerosol Sci. Technol., 39, 231-237, 10.1080/027868290925534, 2005.

Danovaro, R.: Methods for the study of deep-sea sediments, their functioning and biodiversity, CRC Press, 2009.

Eduard, W., Lacey, J., Karlsson, K., Palmgren, U., Strom, G., and Blomquist, G.: Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated occupational environments, Am Ind Hyg Assoc J, 51, 427-436, 10.1080/15298669091369899, 1990.

Hernandez, M., Miller, S. L., Landfear, D. W., and Macher, J. M.: A Combined Fluorochrome Method for Quantitation of Metabolically Active and Inactive Airborne Bacteria, Aerosol Sci. Technol., 30, 145-160, 10.1080/027868299304741, 1999.

Jensen, P. A., and Schafer, M. P.: Sampling and characterization of bioaerosols, in: NIOSH manual of analytical methods, 15, 82-112, 1998.

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.

Lighthart, B., and Mohr, A. J.: Atmospheric microbial aerosols: theory and applications, Kluwer Academic Pub, 1994.

Maki, T., Bin, C., Kai, K., Kawai, K., Fujita, K., Ohara, K., Kobayashi, F., Davaanyam, E., Noda, J., Minamoto, Y., Shi, G., Hasegawa, H., and Iwasaka, Y.: Vertical distributions of airborne microorganisms over Asian dust source region of Taklimakan and Gobi Desert, Atmos. Environ., 214, 116848, 10.1016/j.atmosenv.2019.116848, 2019.

Raghav, N., Mamta, Shrivastava, J. N., Satsangi, G. P., and Kumar, R.: Enumeration and characterization of airborne microbial communities in an outdoor environment of the city of Taj, India, Urban Climate, 32, 10.1016/j.uclim.2020.100596, 2020.

Yahya, R. Z., Arrieta, J. M., Cusack, M., and Duarte, C. M.: Airborne Prokaryote and Virus Abundance Over the Red Sea, Front. Microbiol., 10, 1112, 10.3389/fmicb.2019.01112, 2019.

I am curious about dust transport in the context of global warming- what would be expected? How would this impact on bacteria transported by dust particles?

Response: The mentioned questions are intriguing subjects. Currently, we have only a limit number of data in short periods and hardly connect them with dust transport in the context of global warming and vice versa. We hope in near future with our data integration we can answer these questions, at least to some extent.

I also have several specific suggestions in order to improve the manuscript presentation:

L. 10 This sentence is confusing, it is unclear what is meant by ‘widespread bacteria’, it is also hard to understand what ‘both types’ refers to.

Response: The sentence '*Widespread bacteria are a major proportion of bioaerosols and their coexistence with dust enables both types of aerosols to be more active in ice cloud formation and harmful to public health.*' was revised into '***Airborne bacteria are widespread as a major proportion of atmospheric bioaerosols and their coexistence with dust particles enables both bacteria and dust particles to be more active in ice cloud formation and to be harmful to public health.***'

L. 11 'to be harmful'

Response: revised.

L. 15 'blew' could be replaced by 'transported'

Response: 'blew' was replaced by 'transported'.

L. 16 please remove 'there'; 'averagely' is used frequently throughout the manuscript- I suggest rephrasing with the more common 'on average'

Response: 'there' was removed. The word 'averagely' was changed into 'on average'.

L. 17 please replace 'in the total bacteria' with 'of the total bacteria'

Response: revised.

L. 20 I assume 'presented' would be more suitable than 'present'

Response: 'present' was replaced by 'presented'.

L. 21 'substantial amounts of bacteria'

Response: revised.

L. 22 'through the atmosphere'; I suggest replacing 'non-negligible' with 'important'

Response: 'in the atmosphere' was revised into 'through the atmosphere', and 'non-negligible' was replaced with 'important' in the revision.

L. 23 I'm not sure what is meant by 'internally mixed assemblages'

Response: The mixing state of an aerosol particle indicates whether distinct, homogeneous entities occur within the same particle (internally mixed, such as an aggregate of different phases) or whether they are separated in the air (externally mixed) (Fig. R1) (Pósfai and Buseck, 2010).

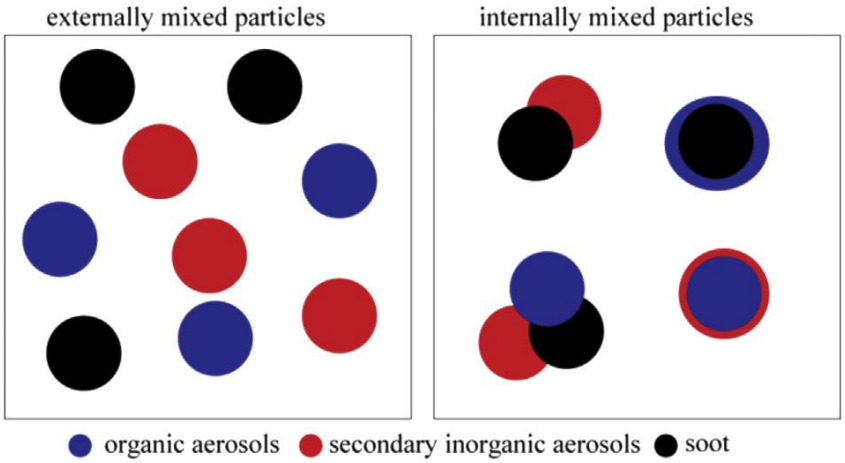

Fig. R1. Schemes of externally mixed and internally mixed particles (Li et al., 2016).

References:

Li, W., Shao, L., Zhang, D., Ro, C.-U., Hu, M., Bi, X., Geng, H., Matsuki, A., Niu, H., and Chen, J.: A review of single aerosol particle studies in the atmosphere of East Asia: morphology, mixing state, source, and heterogeneous reactions, *Journal of Cleaner Production*, 112, 1330-1349, 2016.

Pósfai, M., and Buseck, P. R.: Nature and Climate Effects of Individual Tropospheric Aerosol Particles, *Annual Review of Earth and Planetary Sciences*, 38, 17-43, 10.1146/annurev.earth.031208.100032, 2010.

L. 23 I suggest rephrasing as follows 'in cloud formation, in linking geographically isolated microbial communities, and possibly impact on human health.'

Response: '*dust and bacteria have nonnegligible roles as internally mixed assemblages in cloud formation and in linking geographically isolated microbial communities, as well as have synergistic effect on human health.*' was changed to '***dust and bacteria have important roles as internally mixed assemblages in cloud formation and in linking geographically isolated microbial communities, as well as possibly have synergistic impact on human health.***'

L. 27 'significant potential effect' is a contradiction in itself- would the effect be a potential one or is it a significant one?

Response: 'significant potential effect' was changed to 'potentially significant effect'.

Some similar expressions in geoscience studies are as follows:

Tobo, Y., Adachi, K., DeMott, P. J., Hill, T. C. J., Hamilton, D. S., Mahowald, N. M., Nagatsuka, N., Ohata, S., Uetake, J., Kondo, Y., and Koike, M.: Glacially sourced dust as a **potentially significant** source of ice nucleating particles, *Nat. Geosci.*, 12, 253-258, 2019.

Varner, R. K., Crill, P. M., and Talbot, R. W.: Wetlands: a **potentially significant** source of atmospheric methyl bromide and methyl chloride, *Geophys. Res. Lett.*, 26, 2433-2435, 1999.

Moore, R. M., and Tokarczyk, R.: Chloro-iodomethane in N. Atlantic waters: A **potentially significant** source of atmospheric iodine, Geophys. Res. Lett., 19, 1779-1782, 1992.

Clarke, G. K., Fisher, D. A., and Waddington, E. D.: Wind pumping: a **potentially significant**

heat source in ice sheets, International Association of Hydrological Sciences Publication, 170, 169-180, 1987.

L. 44 ‘are without any doubt’; this sentence, however, does not transport sensitive information, one way to solve the emptiness of the sentence would be to merge it with the following one.

Response: ‘*Quantitative data on the mutual state of airborne bacteria and dust particles in dusty air are no doubt scientifically very interesting (Schuerger et al., 2018). However, quantitative data are rare because of a lack of available and confident methods for such research, leaving unidentifiable uncertainties in both field observations and model simulations exploring the activities and roles of bacterial cells in atmospheric processes.*’ was revised to ‘*Quantitative data on the mutual state of airborne bacteria and dust particles in dusty air* ***are without any doubt*** *scientifically very interesting (Schuerger et al., 2018),* ***but are rare because of a lack of available and confident methods****, leaving unidentifiable uncertainties in both field observations and model simulations exploring the activities and roles of bacterial cells in atmospheric processes.*’

L. 46 Pleas remove ‘for such research’

Response: removed.

L. 47 ‘have previously been investigated’

Response: revised.

L. 48 ‘different survival mechanisms’- this would be clearer if phrased as ‘various survival strategies’

Response: Here we mean that the ways for airborne bacteria to survive in the atmosphere are different from the ways for bacteria to survive in soils. The sentence was rephrased. Please refer to the response to the next comment.

L. 48 ff This sentence is very long and difficult to read, please consider simplifying.

Response: The original sentence was revised into “***Whereas, the survival strategies, dispersal processes and size distribution of airborne bacteria should be different from those of bacteria in soils. The possible causes are that the aerosolization efficiency of soil bacteria from Earth surfaces varies according to bacterial species and soil types (Joung et al., 2017) and airborne bacteria suffer air turbulence and harsh atmospheric stressors (Hara and Zhang, 2012).***”

L. 59 What is an Andersen cascade impactor

Response: It is an air sampler for the collection of size-segregated aerosol samples. “**(Andersen samplers)**” was added.

L. 72 ‘in spring 2013-2016’

Response: We revised “in the spring of 2013–2016” to “during spring in the years 2013 to 2016” according to the comment of Refree#1. In fact, the editor from Wiley Editing Services previously changed “in the spring of the years 2013–2016” into “in the spring of 2013–2016”. We would like to keep the original version “in the spring of 2013–2016” that is a more common expression.

Methods: I suggest moving section 2.3 to 2.1 so that it is easier to understand how atmospheric

conditions were defined

Response: Sect. 2.3 are auxiliary information. We prefer to keep it at the end of Sect. 2.

L. 111/ 112: Please remove 'bacteria' after 'particle-attached' and after 'free-floating'

Response: removed.

L.115 ff I suggest giving a short overview of the uncertainties to provide a solid basis for the statement on the potential underestimation. Like it is now, the reader doesn't have the chance to understand what this section is about. 'presented method'?

Response: This paragraph is a short overview of the uncertainties. Since there are no available data and methods besides the present study for comparison, we do not have data to provide a solid basis for the discussion. We point out the uncertainties for awareness in any further studies on the subject.

The phrase 'present method' was changed to 'presented method'.

L. 120 'which were trapped'; What does 'with difficulty' tell us, how do we know this was difficult? If bacteria <0.43 µm were not recovered appropriately this would mean that a significant fraction of free-floating cells is probably missing?

Response: In this study, we summarize the results based on the enumeration results of eight-stage Andersen samplers. Particles with aerodynamic size smaller than 0.43 µm were not available because the smallest size range of collected particles were 0.43 µm. We used the word of 'difficulty' because 0.43 µm is the cutoff size of 50% collection efficiency for particles with size 0.43 µm and density 1 g cm^{-3}, that means some particles smaller than 0.43 µm could be trapped on the last filters but we cannot correctly quantify them. To investigate how many bacteria might be lost by the Anderson sampler, i.e., how many bacteria may pass over the last filter and are not trapped on any filters, we compared the results from Andersen sampler samples in some cases in the early-stage studies with the results from in-line filter (pore size: 0.2 µm) holder samples. As shown in Figure S4, the total bacterial concentration results of the Andersen sampler were generally consistent with those of the in-line filter holders, indicating that bacteria smaller than 0.43 µm were a minor fraction of the free-floating bacteria, that means only a minor fraction of free-floating cells was possibly missed.

In the revision, "*This result indicates that bacteria smaller than 0.43 µm, which were trapped with difficulty by the Andersen samplers, were a minor fraction of the free-floating bacteria*." was revised into "***This result indicates that bacteria smaller than 0.43 µm, which are not available by the Andersen samplers in this study, were a minor fraction of the free-floating bacteria.***"

L. 133 Please give a short overview on the categorization details

Response:

The categorization details of synoptic weather are available in Murata and Zhang (2016) as follows:

As a cyclone passed the site, the surface pressure gradually decreased, and the weather became unstable due to warm and humid air from the southwest (i.e., prefront). It frequently rained as the cold front of the cyclone approached the site, and the passage of the cold front was recognized by the surface pressure minimum. After the passage of the cold front, dry and cold air from the Asian continent blew to the site (i.e., postfront). As anticyclones approached, air descended from the upper layers, and the near-surface wind gradually weakened with the increase in surface pressure (i.e., approaching anticyclone). When anticyclones covered the site, the air was stagnant, and the weather was clear (i.e., anticyclone).

The reason for this categorization is the distinctiveness of the origins of the aerosol particles in each of the group due to the movement of the air parcels. Postfront air is usually dry and its temperature is low, in comparison with other groups. It moves fast eastward or southeastward following cold fronts, which is the most rapid and efficient route for particulate matters, such as dust from northwestern China or anthropogenic particles from the northern China, to travel from the Asian continent to the observation area. Prefront air, which usually moves slower than the postfront air and whose movement direction is usually northeastward or northward, is warm and humid in comparison with the postfront air. It may also bring air pollutants from the Asian continent but the pollutants are usually from eastern China. The movement of anticyclone air is stagnant, warm and humid. Pollutants in the anticyclone air are usually dominated by local emissions, while sometimes with significant influence of long-distance-transported ones. The approaching anticyclone air is the air in the transition stage from postfront air to anticyclone air. In general, beside the influence of aerosols from the ocean, particulate matters in the postfront, approaching anticyclone, anticyclone and prefront air are, more or less, characterized by dust particles from northwestern China, mixture of dust and locally-emitted ones, and soot particles from eastern China, respectively.

This is not the main content in this study. We prefer to avoid repeating it here.

L. 136 Some level of detail on this model would be helpful.

Response: This model is used to calculate the backward trajectory of air masses. The technical details are available at the website.

In the revision, "NOAA/HYSPLIT" was changed to "NOAA hybrid single-particle Lagrangian integrated trajectory (HYSPLIT)".

L. 151 ff: This section refers to size ranges while figure 1 uses the categories 'viable bacteria, non-viable bacteria, dust-like particles'. This should be unified; it is difficult to connect the text to the figure otherwise.

Response: Figure 1 shows the concentrations of airborne viable and nonviable bacteria in segregated size ranges, and the *x*-axes of the sub-figures refer to size ranges of airborne particles.

The description about dust-like particles is in Line 188. We think it is useful to illustrate bacteria and dust-like particles in the same figures for comparison of the size distribution.

L. 157/ L. 159: Both sentences start with 'There were', this could be avoided by merging those sentences.

Response: "*There were multiple processes, e.g., advection, deposition, local emission and local convective mixing, that could influence the size distributions.*" was changed to "***Multiple processes including advection, deposition, local emission and local convective mixing could influence the size distributions.***"

L. 168/169 Here, the standard deviations were removed, as a result the numbers show a difference of a factor of roughly two. However, this seems misleading because if the ranges are included there is no significant difference. I am aware that one reviewer recommended to remove the ranges but given the danger of misinterpretation I would recommend including them, again.

Response: We totally agree with this comment. In the revision, "T***he report of results when data are non-normal distribution should be viewed with caution, since many statistical analyses (e.g., the average and standard deviation) are only applicable to random samples from populations with a normal distribution. Aerobiological data possibly do not have a normal distribution (Kasprzyk and Walanus, 2014; Limpert et al., 2008). Whereas, in this study, to make the comparisons between the values easily understood and avoid misunderstanding, we assume the data are normally distributed.***" was added at the beginning of Sect. 3.2, and the standard deviations were added again.

L. 169 I somewhat disagree on the term 'large'. If a significant difference is claimed a statistic evaluation would be required.

Response: "(independent samples *t* test, $p<0.05$)" was added.

L. 172 Please rephrase this sentence, a suggestion would be 'During dust periods particle-attached bacteria accounted for 72 % of total bacterial counts, while during non-dust periods, they were recovered in slightly lower proportions of 56%.

Response: The original sentence was revised to "***During dust periods particle-attached bacteria accounted for 72 ±9% of total bacterial counts, while during nondust periods particle-attached bacteria occupied much lower proportions of 56 ±17% (independent samples t test, p<0.05).***"

L. 'signify' is a somewhat strong statement which I am not convinced is supported. Rephrasing to 'sugggest' could solve the problem, here. In addition, how do we know where the particle-attached bacteria are transported to?

Response: "signify" was changed to "suggest".

Here "remote downstream areas" are in comparison to "dust source areas". For example, from the Asian desert areas to the observation site.

"from dust source areas" was added.

L. 175 'was in some cases higher'

Response: revised.

L. 178 'Therefore, a substantial fraction of bacteria was free-floating.'

Response: The original sentence was revised to "Therefore, a substantial fraction of airborne bacteria were free-floating".

L. 189 – L. 192 I have difficulties understanding these two sentences- maybe this could be rephrased

Response: "In most cases, the distributions (mode sizes) of particles and bacteria in the size ranges larger than 1.1 µm showed very good consistency (Figs. 1 and S9). In some cases, the concentration of bacteria in the size ranges larger than 1.1 µm, especially nonviable bacteria, was closely correlated with the mineral dust-like particles in size-segregated samples (Fig. 2)." was revised to "*In most cases, the size distributions (mode sizes) of mineral dust-like particles and bacteria in the size ranges larger than 1.1 µm showed very good consistency (Figs. 1 and S9). In some cases, the concentration of bacteria in the size ranges larger than 1.1 µm, especially nonviable bacteria, was closely correlated with the mineral dust-like particles in the size-segregated samples (Fig. 2).*"

Section 4.1: Consider re-naming in a way that the title refers better to the content

Response: In the revision, the title was revised into "Implication from the comparison with literature data".

L. 253 'comparison'

Response: revised.

L. 258 Does 'as warm as' mean 'up to'?

Response: "as warm as" was revised to "up to".

L. 308 'substantial numbers of bacterial cells'
Response: revised.

Thank you very much for your careful review and detailed comments.

Abundance and viability of particle-attached and free-floating bacteria in dusty and nondust air

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Abstract. Airborne bacteria are widespread as a major proportion of bioaerosols and their coexistence with dust particles enables both bacteria and dust particles to be more active in ice cloud formation and to be harmful to public health. However, the abundance and viability of particle-attached and free-floating bacteria in dusty air have not been quantitatively investigated. We researched this subject based on the fact that airborne bacterial cells are approximately 1 μm or smaller in aerodynamic diameter; therefore, particle-attached bacteria should occur in aerosol samples of particles larger than 1 μm, and free-floating bacteria should occur among particles smaller than 1 μm. Our observations at a coastal site in Japan in spring, when the westerlies frequently transported dust from the Asian continent, revealed that particle-attached bacteria in dust episodes, at the concentration of 3.2 ± 2.1×10^5 cells m^{-3} on average, occupied 72 ± 9 % of the total bacteria. In contrast, the fraction was 56 ± 17 % during nondust periods and the concentration was 1.1 ± 0.7×10^5 cells m^{-3}. The viability, defined as the ratio of viable cells to total cells, of particle-attached bacteria was 69 ± 19 % in dust episodes and 60 ± 22 % during nondust periods on average, both of which were considerably lower than the viabilities of free-floating bacteria (about 87 %) under either dusty or nondust conditions. The presented cases suggest that dust particles carried substantial amounts of bacteria on their surfaces, more than half of which were viable, and spread these bacteria through the atmosphere. This implies that dust and bacteria have important roles as internally mixed assemblages in cloud formation and in linking geographically isolated microbial communities, as well as possibly have synergistic impact on human health.

1 Introduction

Biological particles in the atmosphere have a potentially significant effect on climate change (Ariya et al., 2009;Delort et al., 2010;Möhler et al., 2007;Zhang et al., 2017), efficiently link microbial communities between continents, islands and oceans (Fröhlich-Nowoisky et al., 2016;Morris et al., 2011;Caliz et al., 2018), and pose risks to human health (Polymenakou et al., 2008;Reinmuth-Selzle et al., 2017). Representing a high fraction of primary biological particles, airborne bacteria are emitted into the atmosphere from various sources, among which desert dust is a major source (Morris et al., 2011;Pöschl and Shiraiwa, 2015;Pöschl et al., 2010). The cooccurrence of dust and high concentrations of bacteria has been observed frequently in different locations, indicating the widespread nature and dissemination of bacteria with dust at local, regional and even global scales (Griffin, 2007;Hara and Zhang, 2012;Iwasaka et al., 2009). Limited available observations have revealed the coexistence of mineral and biological contents in ice crystals (Creamean et al., 2013;Pratt et al., 2009), and laboratory experiments have demonstrated that the ice nucleation ability of dust particles is enhanced by biological components, including bacteria in the particles (Boose et al., 2019;Tobo et al., 2019;Conen et al., 2011). Recent toxicological studies with mouse exposure found that the internal mixture of dust and pathogenic bacteria exacerbated pneumonia (He et al., 2012). In addition, the attachment of bacteria to dust particles is expected to largely alter the fate of bacterial cells in the air due to protection by the dust particles from harsh environmental conditions (Bowers et al., 2013) and enhanced gravitational settling (Zhang, 2008). All these results reflect that the adherence of bacterial cells to dust particles, i.e., the particle-attached state, and the viability or metabolic capability of bacterial cells are key factors affecting the roles and fate of airborne bacteria in the evolution, development and conservation of the natural environment.

Quantitative data on the mutual state of airborne bacteria and dust particles in dusty air are without any doubt scientifically very interesting (Schuerger et al., 2018), but are rare because of a lack of available and confident methods, leaving unidentifiable uncertainties in both field observations and model simulations exploring the activities and roles of bacterial cells in atmospheric processes. The cell size distributions for bacteria separated from soils have previously been investigated (Portillo et al., 2013). Whereas, the survival strategies, dispersal processes and size distribution of airborne bacteria should be different from those of bacteria in soils. The possible causes are that the aerosolization efficiency of soil bacteria from Earth surfaces varies according to bacterial species and soil types (Joung et al., 2017) and airborne bacteria suffer air turbulence and harsh atmospheric stressors (Hara and Zhang, 2012). Bacteria-associated particles in the air have an aerodynamic diameter significantly larger than the typical size (approximately 1 μm) of individual bacterial cells (Burrows et al., 2009). This is because airborne bacterial cells are favorably attached to coarse particles, such as dust particles and plant debris, or are sometimes found as assemblages of many cells (Després et al., 2012;Iwasaka et al., 2009;Maki et al., 2013;Lighthart, 1997). We quantified the fractions of particle-attached and free-floating bacterial cells in dusty and nondust air based on the fact that airborne bacterial cells are usually ~1 μm or smaller than 1 μm (Delort et al., 2010;Després et al., 2012;Pósfai et al., 2003;Burrows et al., 2009;Hara et al., 2011); thus, particle-attached bacteria should be trapped in aerosol samples of particles larger than 1 μm, and free-floating bacteria should be located among particles smaller than 1 μm.

By utilizing 8-stage Andersen cascade impactors (Andersen samplers), size-segregated aerosol samples were collected at a southwestern coastal site of Japan in the spring of 2013–2016, when the middle latitude westerly wind in the Northern Hemisphere frequently brought dust from the Asian continent to the observation site. Viable and nonviable bacteria in each sample were counted using the LIVE/DEAD BacLight bacterial viability assay to estimate bacterial concentrations (Murata and Zhang, 2013, 2016). Bacteria detected in samples of particles larger than 1.1 μm (the cutoff size of the sampler stages) were considered particle-attached bacteria, and those in the stages of particles smaller than 1.1 μm were considered free-floating bacteria. An analysis of method confidence showed that uncertainties due to the sample collection were small (Figs. S4 and S5 in the Supplement). In this study, we focus on comparisons of the quantitative results of particle-attached and free-floating bacteria in the air and the viability of these bacteria under dust and nondust conditions.

2 Methods

2.1 Sample collection and cell enumeration

Aerosol samples were collected on the platform of a building (32.324°N, 129.993°E; 15 m above ground level and 23 m above sea level) on the seaside of Amakusa Island, southwestern Japan (Fig. S1) during several observational campaigns in the spring of 2013 to 2016. Dust plumes from the Asian continent, called Asian dust, frequently pass this area in spring. There are limited fishery and agriculture activities and few anthropogenic sources of air pollutants around the area, making the site suitable for investigating airborne bacteria in the Asian continental outflow (Murata and Zhang, 2016).

Aerosol samples were collected onto 0.2 μm pore polycarbonate filters (47 mm; Merck Millipore Ltd., Cork, Ireland) with 8-stage Andersen samplers (Model AN-200; Tokyo Dylec Corp., Japan). The flow rate of the samplers was 28.3 L min^{-1}. Aerosol particles were collected onto 8 filters according to the particle aerodynamic diameter ranges of >11, 7.0–11, 4.7–7.0, 3.3–4.7, 2.1–3.3, 1.1–2.1, 0.65–1.1 and 0.43–0.65 μm. The collection time of one set of samples was from approximately 3 to 24 h. Details on the sample collection are given in Table 1 and Table S1and Fig. S2 in the Supplement.

Before the collection of each sample set, all stages of the sampler were cleaned carefully, and the plates for the filters were rinsed and wiped with 70% ethanol in a clean hood to avoid contamination. A blank control for each set of samples was prepared, i.e., a blank filter was set in the sampler without sample collection. After sample collection, the filters were sealed in Petri dishes and stored at –20°C until analysis.

The viable and nonviable bacterial cells (Fig. S3) on the filters were enumerated using the LIVE/DEAD BacLight bacterial viability assay with an epifluorescence microscope (EFM; Eclipse 80i, Nikon Corp., Tokyo, Japan) as described previously (Murata and Zhang, 2016, 2013;Hu et al., 2017). Bacterial cells and other particles were detached from the aerosol-loaded polycarbonate membranes (47 mm in diameter) in a phosphate-buffered saline solution (PBS, pH 7.4) by vortex shaking and ultrasonic vibration in ice bath. Then the suspension was treated with glutaraldehyde fixation and stained with the

LIVE/DEAD BacLight Bacterial Viability Kit (L13152, Invitrogen™, Molecular Probes Inc., Eugene, Oregon, US), followed by filtration on a 25 mm diameter and 0.2 μm pore black polycarbonate membrane for bacterial enumeration. An excitation wavelength range between 450 and 490 nm (blue) was utilized, and the microscope was operated at 1000× magnification. Fluorescent green and red/orange/yellow cells with spherical shape and size close to or smaller than 1 μm in diameter were counted as viable and nonviable bacteria, respectively. There are uncertainties in the bacterial cell counting caused by the LIVE/DEAD BacLight Bacterial Viability Kit because the kit could not distinguish archaea and small eukaryotes including fungi from bacteria (Berney et al., 2007). Since the abundance of archaea and fungi in air could be several (1–6) orders of magnitude less than that of bacteria (Fröhlich-Nowoisky et al., 2016;Fröhlich-Nowoisky et al., 2014;Delort et al., 2010) and the dominant size range of fungal spores is 2–10 μm (Bauer et al., 2008), the overestimation of bacteria caused by the kit we used should be less than 10% although the uncertainties could not be quantitatively evaluated. The cell concentrations in the size-segregated particles in the air were estimated based on cell counts and the sampling of air volumes following the subtraction of the blank controls. The viability of a group of bacterial cells was defined as the ratio of the viable bacterial cells to total bacterial cells. The procedure for the experimental operation and the formulations for the estimation of cell concentrations are given in the Supplement (Text S1 in the Supplement).

The collection efficiency of airborne bacterial cells with Andersen samplers was evaluated by comparing the results to those obtained by using BioSamplers (SKC Inc., Eighty-Four, PA, US) and in-line filter holders (47 mm, Millipore Corp., Billerica, MA, US). The comparison shows that the total bacterial concentration results of the Andersen sampler were generally consistent with those of the BioSamplers and the in-line filter holders (Fig. S4).

Table 1. Concentration and viability of total, free-floating and particle-attached bacteria. The concentration of coarse particles (>1 μm) and the ratio of particle-attached bacteria to coarse particles are also listed. The percentages of free-floating and particle-attached bacteria are given in the parentheses. The sample ID indicates the sequence number (1 to 27) of the sample, and dust condition (D, dusty; ND, nondust) and synoptic weather (Pr, prefront; Po, postfront; AA, approaching anticyclone; A, anticyclone) during the sampling period.

Sample ID	**Synoptic weather**	**Coarse particles (10^5 m^{-3})**	**Total bacteria**		**Free-floating bacteria**		**Particle-attached bacteria (PAB)**		
			Concentration (10^5 cells m^{-3})	Viability (%)	Concentration (10^5 cells m^{-3})	Viability (%)	Concentration (10^5 cells m^{-3})	Viability (%)	PAB/Coarse particles (%)
Dusty (9)									
1D-Pr	Prefront	41	7.8	84	1.7 (21)	90	6.1 (79)	82	15
2D-Po	Postfront	32	2.3	77	0.5 (23)	99	1.8 (77)	71	6
3D-AA	Approaching anticyclone	12	2.2	89	0.7 (30)	91	1.6 (70)	88	13
4D-Pr+Po	Pre-/postfront	52	7.3	61	1.8 (25)	71	5.4 (75)	58	11
5D-AA	Approaching anticyclone	21	4.7	63	0.7 (16)	79	3.9 (84)	60	19
10D-Po	Postfront	16	2.5	40	0.6 (25)	61	1.9 (75)	33	11

17D-AA	Approaching anticyclone	88	2.9	73	1.0 (36)	99	1.9 (64)	59	2
26D-Po	Postfront	10	8.2	95	2.5 (30)	97	5.7 (70)	95	59
27D-AA	Approaching anticyclone	15	1.9	87	0.9 (46)	96	1.0 (54)	78	7
Average		**32 ± 25**	**4.4 ± 2.6**	**74 ± 17**	**1.2 ± 0.7 (28 ± 9)**	**87 ± 14**	**3.2 ± 2.1 (72 ± 9)**	**69 ± 19**	**16 ± 17**
Nondust (18)									
6ND-AA	Approaching anticyclone	13	1.5	75	0.4 (27)	88	1.1 (73)	70	9
7ND-A	Anticyclone	12	1.5	74	0.6 (39)	82	0.9 (61)	69	8
8ND-A+Pr	Anticyclone+prefront	14	0.8	98	0.2 (31)	99	0.5 (69)	98	4
9ND-Pr	Prefront	26	2.7	73	1.9 (71)	84	0.8 (29)	45	3
11ND-AA	Approaching anticyclone	4	2.1	72	1.3 (64)	85	0.8 (36)	51	18
12ND-A	Anticyclone	14	2.9	83	2.1 (73)	96	0.8 (27)	48	6
13ND-A	Anticyclone	9	3.6	75	2.5 (70)	86	1.1 (30)	50	12
14ND-A	Anticyclone	13	1.9	77	0.8 (42)	99	1.1 (58)	62	9
15ND-AA	Approaching anticyclone	10	4.4	65	1.0 (24)	61	3.4 (76)	66	35
16ND-Po	Postfront	16	2.5	89	0.9 (35)	96	1.6 (65)	85	10
18ND-AA	Approaching anticyclone	15	2.9	91	0.5 (18)	86	2.4 (82)	92	16
19ND-A	Anticyclone	9	1.1	72	0.4 (35)	96	0.7 (65)	59	7
20ND-A	Anticyclone	10	1.0	77	0.4 (41)	85	0.6 (59)	72	6
21ND-A	Anticyclone	13	1.7	63	1.0 (63)	89	0.6 (37)	18	5
22ND-A	Anticyclone	8	1.2	40	0.5 (43)	56	0.7 (57)	28	9
23ND-Pr+Po	Pre-/postfront	12	1.1	59	0.5 (48)	88	0.6 (52)	32	5
24ND-Po+A	Postfront/Anticyclone	7	1.4	72	0.5 (38)	88	0.8 (62)	62	12
25ND-A	Anticyclone	6	1.5	85	0.6 (40)	95	0.9 (60)	78	15
Average		**12 ± 5**	**2.0 ± 1.0**	**75 ± 13**	**0.9 ± 0.7 (44 ± 17)**	**87 ± 12**	**1.1 ± 0.7 (56 ± 17)**	**60 ± 22**	**10 ± 7**
All (27)									
Average		**18 ± 18**	**2.8 ± 2.0**	**74 ± 14**	**1.0 ± 0.7 (39 ± 16)**	**87 ± 12**	**1.8 ± 1.7 (61 ± 16)**	**63 ± 21**	**12 ± 11**

2.2 Separation of particle-attached and free-floating bacteria

In this study, bacteria in the samples of stages with particles larger than 1.1 μm were considered particle-attached, and bacteria in the samples of stages with particles ranging from 0.43–1.1 μm were considered free-floating. The resuspension of bacteria trapped by upper stages and falling onto lower stages during sample collection may cause uncertainties in the size distribution of bacteria-associated particles and the separation of particle-attached and free-floating bacteria.

The uncertainties in the estimation of particle-attached and free-floating bacteria were investigated in the laboratory (Text S2 in the Supplement). The fractions and concentrations of particle-attached bacteria obtained by the presented method were potentially underestimated. But the underestimation did not significantly affect the size distributions of particle-attached bacteria, and, in particular, the underestimation of the concentrations of particle-attached bacterial cells was less

than 10% on average (Fig. S5). The total bacterial concentration results of the Andersen sampler were generally consistent with those of the in-line filter holders collecting total particles (Fig. S4). This result indicates that bacteria smaller than 0.43 μm, which are not available by the Andersen samplers in this study, were a minor fraction of the free-floating bacteria.

2.3 Atmospheric conditions

During the observation periods, the number concentrations of size-segregated airborne particles (>0.3, >0.5, >1.0, >2.0, and >5.0 μm in diameter) were monitored with optical particle counters (OPC, KC-01D in 2013 and KC-01E in 2014–2016, Rion Co., Ltd, Tokyo, Japan). In this study, fine particles are in the range of 0.3–1.0 μm, and those larger than 1.0 μm are referred to as coarse particles. Meteorological conditions, including temperature, pressure, relative humidity, precipitation, and wind speed and direction, were monitored with a weather transmitter (WXT520, Vaisala Inc., Helsinki, Finland). Airborne particle number concentrations and meteorological data during the observation periods are summarized in Fig. S2 and Table 1.

On the basis of surface pressure and weather charts in the days before and after sample collection (Figs. S2 and S6), the air parcels on the synoptic scales from which samples were collected were categorized into four groups: prefront, postfront, approaching anticyclone, and anticyclone (Table 1 and S1). Details of the categorization are available in Murata and Zhang (2016).

Dust episodes were identified by significant increases in coarse particle concentrations (>1 μm), the forecast for Asian dust distributions in the east Asian region (http://www-cfors.nies.go.jp/~cfors/; Fig. S7), and the backward trajectory of air masses calculated with the NOAA hybrid single-particle Lagrangian integrated trajectory (HYSPLIT) model (http://ready.arl.noaa.gov/HYSPLIT_traj.php). During dust events, the coarse particle concentration largely increased at the study site (Zhang et al., 2003). Dust particles were present in the postfront air and sometimes in the approaching anticyclone air. The results of backward trajectory analysis during dusty and nondust episodes are shown in Fig. S8.

3 Results

3.1 Concentrations of airborne bacteria in segregated size ranges

The concentrations of bacterial cells, including viable and nonviable cells, generally showed a bimodal number-size distribution during dust episodes (e.g., Fig. 1*a*, *b*, *d*, *f*). Most of the bacteria were present in particle fractions with aerodynamic size (D_p) ranges larger than 2 μm (i.e., 2.1–3.3, 3.3–4.7 and 4.7–7.0 μm; Fig. S9). These sizes are larger than the size of individual airborne bacterial cells (approximately 1 μm or smaller), indicating that the bacteria did not float individually in the air but were combined with other particles or were agglomerates of bacterial cells, i.e., the bacteria were particle-attached. The agglomerates of bacterial cells usually appear near emission sources, e.g., sea spray and leaf water (Lighthart, 1997), and

probably contributed a limited portion to particle-attached bacteria in this study. There were also many bacterial cells in the size ranges smaller than 1.1 μm, i.e., free-floating bacterial cells. Their concentration was comparable to or lower than the concentrations of bacteria in the larger size ranges (Figs. 1 and S9).

In contrast to dust episodes, during nondust periods, the number-size distribution of bacteria largely varied and did not show any trend with respect to weather conditions. In six cases during nondust periods (9ND-Pr, 11ND-AA, 12ND-A, 13ND-A, 14ND-A, and 21ND-A; Fig. S9), the bacteria appeared mainly in size ranges smaller than 1.1 μm and accumulated the most in the size range of 0.43–0.65 μm (e.g., Fig. 1*c*), indicating the predominance of free-floating bacteria. During most of the other nondust periods (6ND-AA, 7ND-A, 8ND-A+Pr, 16ND-Po, 19ND-A, 20ND-A, 22ND-A, 23ND-Pr+Po, 24ND-Po+A, and 25ND-A), the distributions of bacteria were similar to those during the dust periods, although the concentrations were much lower than or comparable to those in the dust episodes (e.g., Fig. 1*e*). There were two exceptional cases in nondust periods that had a mono-modal distribution, with peaks at 3.3–4.7 μm (15ND-AA) or larger than 11 μm (18ND-AA) (Fig. S9). Multiple processes including advection, deposition, local emission and local convective mixing could influence the size distributions. Unfortunately, we do not have enough case data to investigate statistically meaningful connections between the size distribution and those processes.

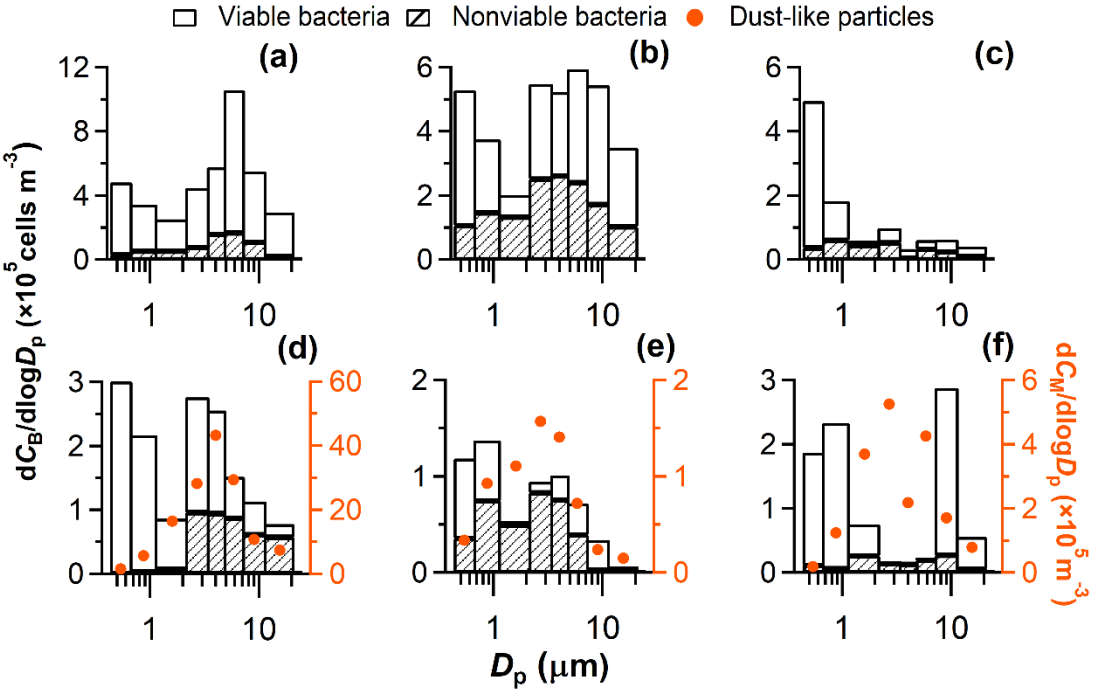

Figure 1. Concentrations of viable and nonviable bacteria (C_B) and mineral dust-like particles (C_M) in size-segregated airborne particles. Selected samples are shown as examples: (a) 1D-Pr; (b) 4D-Pr+Po; (c) 11ND-AA; (d) 17D-AA; (e) 22ND-A; (f) 27D-AA. The results of all sampling periods are depicted in Fig. S9 in the Supplement.

3.2 Concentration of particle-attached and free-floating bacteria

The report of results when data are non-normal distribution should be viewed with caution, since many statistical analyses (e.g., the average and standard deviation) are only applicable to random samples from populations with a normal distribution. Aerobiological data possibly do not have a normal distribution (Kasprzyk and Walanus, 2014;Limpert et al., 2008). Whereas, in this study, to make the comparisons between the values easily understood and avoid misunderstanding, we assume the data are normally distributed.

On average, the concentration of total bacterial cells, $4.4 \pm 2.6 \times 10^5$ cells m^{-3}, during dust episodes was more than twice that during nondust periods, $2.0 \pm 1.0 \times 10^5$ cells m^{-3} (Table 1). This large difference (independent samples *t* test, $p<0.05$) in concentration is consistent with the results of previous studies (Hara and Zhang, 2012;Yamaguchi et al., 2014). The concentrations of particle-attached bacterial cells during dust episodes and nondust periods were $3.2 \pm 2.1 \times 10^5$ and $1.1 \pm 0.7 \times 10^5$ cells m^{-3}, respectively. During dust periods particle-attached bacteria accounted for 72 ± 9% of total bacterial counts, while during nondust periods particle-attached bacteria occupied much lower proportions of 56 ± 17% (independent samples *t* test, $p<0.05$). These results suggest that dust particles carry a substantial amount of bacterial cells on their surfaces from dust source areas to remote downstream areas.

On the other hand, the percentage of free-floating bacterial cells was in some cases higher than 70% during nondust periods (Table 1). In particular, the percentage ranged from 35% to 73% (49 ± 15 % on average) under anticyclone weather conditions, when the air mass moved sluggishly and was mainly influenced by marine and local emissions and less by continental emissions (Fig. S8). Therefore, a substantial fraction of airborne bacteria were free-floating, and they were frequently the common bacteria in nondust air.

The number ratio of particle-attached bacteria to particles in the size range larger than 1.1 μm was 12 ± 11% on average (Table 1). Except for two periods when the ratios were 35% and 59%, respectively, the ratio was approximately stable (9 ± 5% on average for the other periods), regardless of dust episodes and nondust periods (Table 1). That is, assuming that a bacteria-attached coarse particle harbors at least one bacterial cell, coarse particles including mineral dust particles with attached bacteria usually made up less than 9% of the total coarse particles. Maki et al. (2008) reported that the mineral particles with attached bacteria made up approximately 10% of the total mineral particles, with the remaining mineral particles possessing few or no bacterial cells at 800-m height above the ground in an Asian dust source region, Dunhuang, China.

The number-size distributions of bacterial cells and mineral dust-like particles (insoluble and with irregular shapes; Fig. S3) in the microscope fields of some samples were compared. In most cases, the size distributions (mode sizes) of mineral dust-like particles and bacteria in the size ranges larger than 1.1 μm showed very good consistency (Figs. 1 and S9). In some cases, the concentration of bacteria in the size ranges larger than 1.1 μm, especially nonviable bacteria, was closely correlated

with the mineral dust-like particles in the size-segregated samples (Fig. 2). These results further confirm that the bacteria observed in the large size ranges were closely associated with airborne coarse particles, i.e., they were particle attached. In some cases, the mode size ranges of the bacterial cells and the dust-like particles were inconsistent (Fig. S9), likely because the number of bacteria on the surface of each coarse particle largely varied or there were less dust-like particles in the coarse size ranges (e.g., 26D-Po). Dust-like particles were rarely observed in the size ranges smaller than 1.1 μm (Fig. S9), further indicating that the bacteria observed in those size ranges were predominantly free-floating.

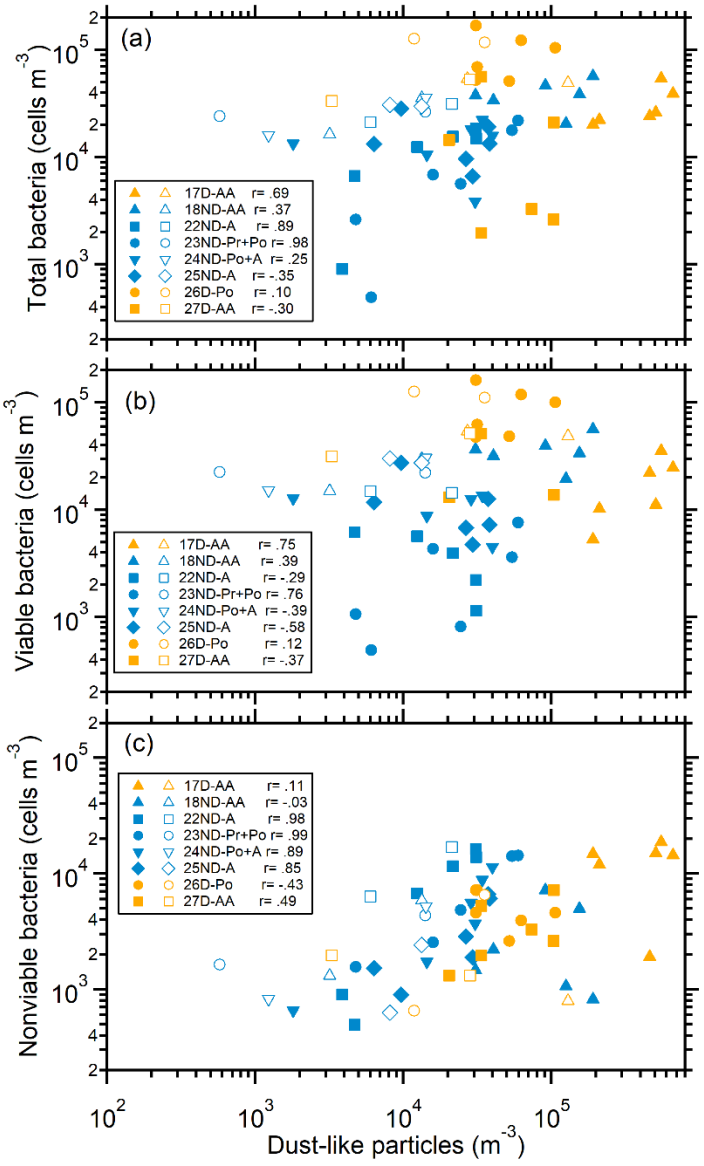

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Figure 2. Relationship between bacteria and mineral dust-like particles in size-segregated aerosols. (a) Total bacteria, (b) viable bacteria, and (c) nonviable bacteria. Solid and open circles represent particles in the size ranges larger and smaller than 1.1 μm, respectively. The Pearson correlation coefficients (*r*) between bacteria and mineral dust-like particles for particles larger than 1.1 μm are shown.

3.3 Viabilities of particle-attached and free-floating bacteria

The viability of particle-attached bacteria varied over a wide range from 18% to 98% (63 ± 21% on average), and the viability of free-floating bacteria was between 56% and 99% (87 ± 12% on average) (Table 1), much higher than the viability of particle-attached bacteria (paired samples *t* test, p=0.00). The attachment of airborne bacteria to larger particles is expected to be favorable for retaining the viability or cultivability of cells and may indirectly increase the diversity of bacterial communities because of the possible protection of bacterial cells from harsh atmospheric conditions (Bowers et al., 2013;Prospero et al., 2005;Lighthart, 2000).

However, we found that the viability of particle-attached bacteria tended to be lower than that of free-floating bacteria, regardless of weather conditions (Table 1). This result indicates that a fraction of the particle-attached bacterial cells were either nonviable when they were blown into the air with the dust or had experienced atmospheric stressors for several days during long-distance transport and changed from a viable to a nonviable state. This is also likely the reason for the poor correlation (Pearson correlation r= 0.35, p = 0.075) between the viability of particle-attached bacteria and the ratio of particle-attached bacteria to coarse particles (Table 1). In contrast, a large fraction of free-floating bacteria were viable. A fraction of these bacteria were likely from local areas, with a residence time (usually less than one day) shorter than that (2–3 days) of the particle-attached bacteria transported from the Asian continent (Fig. S8). The proportion of free-floating bacteria was higher under nondust conditions when the air masses moved slowly above the marine area. However, for special cases, such as the one of 20ND-A when the air was from the north due to the specific weather of west-high pressure versus east-low pressure in the westerly, a substantial fraction of the bacteria could be from the local and close areas due to the extremely strong wind. In terms of concentration, viable particle-attached bacteria were usually more abundant than viable free-floating bacteria in dust episodes (Figs. 1 and S9).

On average, the viability (74 ± 17%) of total bacteria in dusty episodes was close to the viability (75 ± 13%) of total bacteria during nondust periods (Table 1). The viability of particle-attached bacteria (69 ± 19%) during dust periods was slightly higher than that (60 ± 22%) during nondust periods. The majority of particle-attached bacteria were viable.

Free-floating bacteria exhibited a quite high viability, and the viabilities of the bacteria in dusty (87 ± 14% on average) and nondust (87 ± 12%) air were similar. The concentration of viable free-floating bacteria was 3.8×10^4–1.5×10^5 cells m^{-3}, which was lower than that of particle-attached bacteria (6.2×10^4–5.1×10^5 cells m^{-3}). An increase in viable free-floating bacteria on the order of 10^5 cell m^{-3} (1.1–2.2×10^5 cell m^{-3}) was observed when the weather was fine and the air masses moved slowly from marine areas (e.g., 9ND-Pr, 12ND-A, and 13ND-A**)**, favoring the accumulation of bacteria emitted from local areas (Fig. S8).

4 Discussion

4.1 Implication from the comparison with literature data

There are few data on airborne bacterial cells available for comparison with the present study. Observations in the multiphase atmosphere with culture-dependent methods revealed that approximately 60–90% or even more culturable airborne bacteria were present in the size range of particles larger than 1.1 μm (Agarwal, 2017;Burrows et al., 2009;Montero et al., 2016;Raisi et al., 2013), and the median aerodynamic diameter of particles containing culturable bacteria was approximately 2–4 μm at diverse sites (Lighthart, 2000;Raisi et al., 2013;Shaffer and Lighthart, 1997;Tong and Lighthart, 2000). These results indicate the predominance of culturable particle-attached bacteria in the air, which is approximately in line with the results under dusty and nondust conditions of this study.

Early studies with single-particle analysis frequently encountered the mode size of biological aerosol particles in the size range smaller than 1 μm (Matthias-Maser et al., 1999;Matthias-Maser and Jaenicke, 1995, 2000). In contrast, recent real-time measurements using ultraviolet aerodynamic particle sizer spectrometers and wideband integrated bioaerosol sensor techniques revealed the mode size of fluorescent biological aerosol particles (FBAP) to be approximately 2–6 μm, and the particles were mainly attributed to fungal spores (Pöschl et al., 2010;Savage et al., 2017;Yue et al., 2017;Huffman et al., 2010). However, the abundant particle-attached bacteria identified in this study in size ranges larger than 2 μm indicate dust-particle-attached bacteria should not compose small fractions of real-time FBAP results in the relevant size ranges. In addition, the mode at or smaller than 1 μm observed in real-time FBAP studies is likely consistent with the presence of free-floating bacterial cells in the present study, but the comparison and discussion on the data are not confident because of the large uncertainties caused by the low counting efficiency and accuracy in submicron size ranges of the instruments used in the studies (Yue et al., 2017;Huffman et al., 2010).

Since there are rare other equivalent data for comparison, we discuss the influences of airborne bacteria according to the results obtained in this study and relevant general understandings in the following subsections.

4.2 Ice cloud formation

Dust particles from desert areas are constantly spread at local, regional and global scales in the atmosphere. These particles transport microorganisms across continents and oceans to remote downstream areas (Griffin, 2007;Schuerger et al., 2018). It has been shown that bacteria in the air are more effective ice nuclei at temperatures up to –2°C than abiotic particles (Ariya et al., 2009;Burrows et al., 2013;Fröhlich-Nowoisky et al., 2016;Möhler et al., 2007). Biological particles coexisting with dust particles have been detected in ice residues sampled from clouds (Creamean et al., 2013;Pratt et al., 2009), and the coexistence of dust and bacterial cells increases the ability of particles to act as ice nuclei for ice crystal formation (Tobo et al., 2019). Proteins in bacteria are ice nucleation active sites and are well protected when bacteria adhere to mineral dust surfaces (Conen et al., 2011). The attachment of bacteria to dust particles possibly increases the number of sites for ice

nucleation and consequently the ice nucleation ability of dust particles (Boose et al., 2019;Conen et al., 2011;Augustin-Bauditz et al., 2016). The present results show that up to one-tenth or more dust particles could be bacteria carriers, and the concentration of particle-attached bacteria, i.e., the number of bacteria-dust contact sites in dust episodes, was on average 3 times larger than that during nondust periods (Table 1). The occurrence of dust in remote downstream areas will significantly increase not only the concentration of bacterial cells but also the concentration of dust-bacteria mixture particles and the number of ice nucleation active sites. This phenomenon could provide important sources of nuclei for ice cloud formation under saturated meteorological conditions for icing, particularly in remote elevated air, where the concentrations of aerosol particles able to act as nuclei are usually very low (Creamean et al., 2013).

4.3 Ecosystem conservation and development

More than 60% of particle-attached bacteria and approximately 87% of free-floating bacteria in the dusty air remained viable. Airborne bacteria can multiply more easily after they settle into water (lakes, rivers and oceans) and soil surfaces than in the atmosphere. As a consequence, their dissemination via the atmosphere has the potential to alter the microbial biogeography, biogeochemistry and ecosystem services of downstream areas. Moreover, a recent study on phosphorus in aerosol particles in Asian continental outflow revealed that natural dust particles supplied higher ratios of bioavailable phosphorus than other types of particles as nutrients for the primary production in marine ecosystems, and the phosphorus was presumed to be from the biological particles in dust plumes (Shi et al., 2019). The dissemination of bacteria with dust in the air is much more efficient than that via other routes, such as rivers, because dust in the atmosphere can travel globally within two weeks (Uno et al., 2009). Therefore, the wide dispersal of atmospheric dust is an efficient link between bacterial communities in geographically isolated ecosystems. This linking function is likely the key process that constantly blurs the distinctions between closely related microbial species in distant areas. Thus, the diversities of microorganisms have a geographically weak gradient at the global scale, and are functions of habitat properties but not of historical/evolutionary factors (Fenchel and Finlay, 2004).

4.4 Health effects

Allergenic and toxic bacteria inhaled and deposited on the surface of upper respiratory tracts and lungs are suggested to provoke severe adverse health effects, regardless of whether the bacteria are viable, dead or cell fragments (Fröhlich-Nowoisky et al., 2016;Després et al., 2007). Dust particles carrying biological materials, including bacteria with pathogenic, allergenic, and adjuvant activity, can cause and aggravate respiratory disorders (Reinmuth-Selzle et al., 2017). The size distribution of bacteria-related particles in the air is particularly meaningful because the movement and deposition of the particles in the airways are size-dependent. Particles larger than 0.5 μm are deposited by sedimentation and impaction mainly in the head airways, and particles smaller than 0.5 μm can reach the lower airways by diffusion (Fröhlich-Nowoisky et al., 2016). According to the size distribution of the airborne bacteria-related particles in this study (Figs. 1 and S9), the deposition fraction and abundance of particle-attached bacteria are much higher than those of individual cells in both the upper and the

lower airways. Polymenakou et al. (2008) reported that a large fraction of airborne bacteria at respiratory particle sizes (< 3.3 μm) during an intense dust event were phylogenetic neighbors to human pathogens. He et al. (2012) suggested that Asian dust caused the exacerbation of pneumonia induced by *Klebsiella pneumoniae* due to the enhanced production of pro-inflammatory mediators in alveolar macrophages. Therefore, free-floating bacterial cells are likely to more easily influence the deep parts than the upper parts of respiratory airways, while the negative influence of particle-attached bacteria, particularly under dust conditions, is expected to be more serious in the upper parts than in the deep parts of respiratory airways.

5 Conclusions

In this study, we aimed to quantify the particle-attached and free-floating bacteria in dusty and nondust air in southwestern Japan using the fluorescent enumeration of bacterial cells in size-segregated aerosol samples. The bacteria showed bimodal number-size distributions during dust episodes, while the distributions largely varied during nondust periods. Particle-attached bacteria in dust episodes, with a concentration of 3.2 ± 2.1 $\times 10^5$ cells m^{-3} on average, occupied 72 ± 9 % of the total bacteria. In contrast, this percentage was 56 ± 17 % during nondust periods, with a concentration of 1.1 ± 0.7$\times 10^5$ cells m^{-3}. The results indicate that dust particles conveyed substantial numbers of bacterial cells on their surfaces. Viable particle-attached bacteria were more abundant than viable free-floating bacteria in dusty air, which is compatible with the previous results that larger particles harbor more viable and/or culturable bacteria than smaller particles.

The viability (approximately 63 ± 21 %) of particle-attached bacteria was much lower than that (87 ± 12 %) of free-floating bacteria, likely because atmospheric stressors along with long-distance transport inhibited the survival of particle-attached bacteria and the entrainment of locally originating free-floating bacteria. High concentrations and viabilities of free-floating bacteria were observed in stagnant air, mostly under anticyclone conditions, suggesting that locally emitted bacteria accounted for the major fractions.

The present results, quantitatively showing the state of airborne bacteria in association with particles, i.e., particle-attached and free-floating bacteria, could have broad implications in the disciplines of atmospheric sciences, ecology, public health and climate. In addition, the methods used in this study are low cost and easily available but are time- and labor-intensive. Verification of the status of airborne bacteria using efficient techniques, such as *in situ* electron microscopy, and the exploration of the compositions, functions and activities of particle-attached and free-floating bacteria in the atmosphere, are necessary to deepen our understanding of the related fields.

Data availability. All data are available from the corresponding author upon request. Dataset for Figs.1 and 2 are given in Tables S2 and S3 in the supplement.

Supplement. The supplement related to this article is available online at: https://doi.org/…..

Author contributions: DZ and WH designed research; WH, KM, CF and SH performed research; WH, KM and DZ analyzed data and wrote the paper; HM and PF reviewed and commented on the paper.

Competing interests. The authors declare that they have no conflict of interest.

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