Editor of the journal *Biogeosciences*

Dear Prof. Carolin Löscher,

Thank you very much for your handling our manuscript. We also thank a lot the three reviewers for their valuable and helpful comments.

We have prepared the point-to-point responses to the reviewers' comments and revised the manuscript accordingly. Here we submit the responses and the revised manuscript. We hope the revised manuscript meet the quality required for the publication in the journal.

Thank you very much!

Best regards,

Daizhou Zhang

Point-to-Point Response to Reviewers' Comments

Referee #1 2

Referee #2 12

Referee #3 30

Marked revised manuscript 38

Marked revised supplement 55

Referee #1

The manuscript addresses a topic that is of interest to a range of scientific disciplines, as indicated in the Discussion sections. For their study, the authors have chosen a well-suited sampling location. They approached the topic with solid methods and patience to reveal new insights. I enjoyed reading the manuscript.

Response: We thank the reviewer very much for the encouragement and valuable comments. The manuscript was revised according to the comments, and here we give point-to-point responses to the comments as follows.

1. There are two issues, I would like the authors to think about and perhaps make according changes to the manuscript. The first issue concerns the reporting of data. Although it is common practice to report mean values and standard deviations, these metrics are not suitable when data is not normally distributed. Often with aerosol data, the value of the standard deviation is similarly large as that of the mean. In normally distributed data, about 68% of all values are within 1 standard deviation about the mean, 16% are larger and another 16% are smaller than that range. Taken seriously, a standard deviation that is as large as the mean implies that 16% of the data has a negative value, which is impossible for particle concentration values. This problem

and a solution to it are described in more detail in Limpert et al. (2008, Aerobiologia, 24:121–124, DOI: 10.1007/s10453-008-9092-4).

Response: We agree with the reviewer's opinion that the report of results when data are non-normal distribution should be viewed with caution.

The distributions of the concentration of bacterial cells and airborne particles, and the viability and proportion of free-floating and particle-attached bacterial cells in each dataset (n=27) are shown in Figures R1–R3.

As shown in Figure R1, the probability distributions of the concentration of bacterial cells and airborne particles are likely log-normally distributed. In contrast, the viabilities of total bacteria and particle-attached bacteria, and the proportion of free-floating and particle-attached bacteria in total bacteria likely exhibit normal distributions, while the viability of free-floating bacteria does not show an obvious distribution pattern (Figs. R2–R3).

The sample size range is small, in particular for the cases under dust conditions. In fact, processes that can affect the concentration of airborne particles are complicated, such as dispersion (transport), aerosolization and removal, and particularly the status of bacterial cells attaching to or isolated from other particles in the present cases. Factors influencing the variability of airborne bacterial cells are more complex because bacteria cells in general multiply in natural ecosystems and airborne bacteria suffer multiple stressors. Due to the limited available data, we currently cannot identify the processes leading to the distributions in each case and are unable to give rational interpretations for the causes of the distributions. We can use log-normal or normal distributions to selectively fit the observed ones, but we are afraid that will make the comparisons between the values difficult and easily misunderstanding.

For these reasons, **we simply give average values for the results in Table 1** and did not apply the suggested solution (using the median of log-normal data and the multiplicative standard deviation) described in Limpert et al. (2008). **Following the comment of Reviewer#2, we moved the original Table S2 to the main manuscript as Table 1 and show the results of each sample in the revision.** Please refer to the

response to Comment 1 of Reviewer #2. The descriptions relevant to these results in the whole text were modified accordingly.

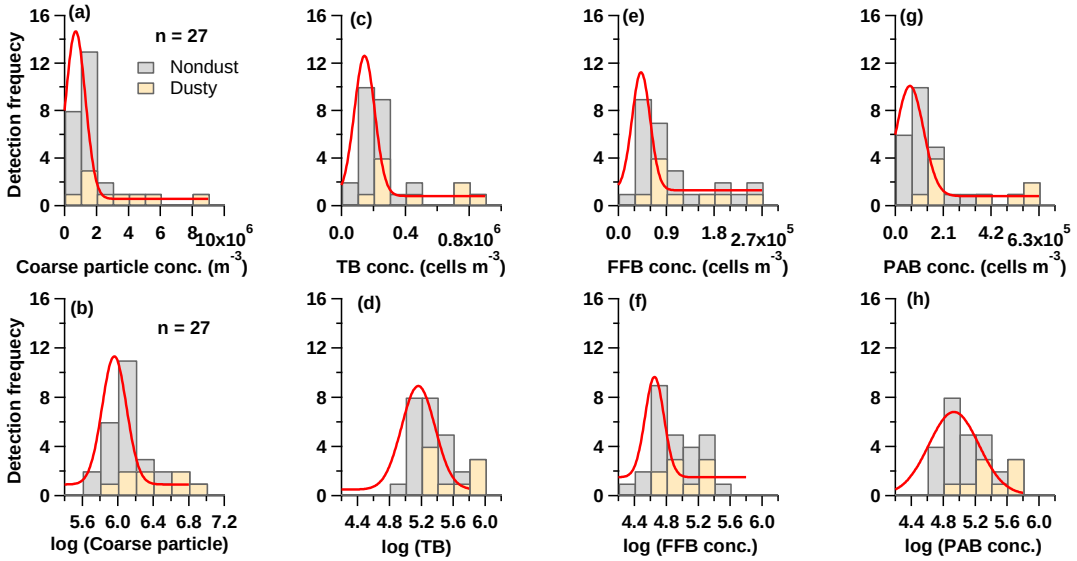

Fig. R1. The probability distribution of the concentrations of coarse particles and bacterial cells. Upper row: raw data. Lower row: logarithmically transformed data. TB, total bacteria; FFB, free-floating bacteria; PAB, particle-attached bacteria.

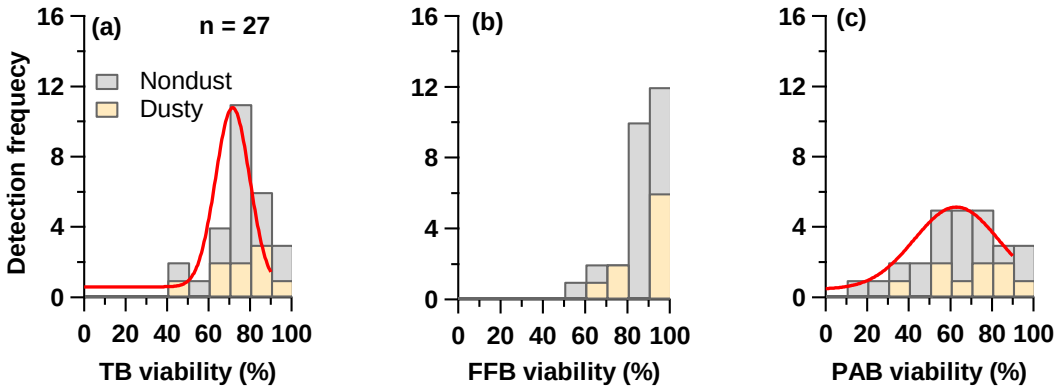

Fig. R2. The probability distribution of the viability of bacterial cells. TB, total bacteria; FFB, free-floating bacteria; PAB, particle-attached bacteria.

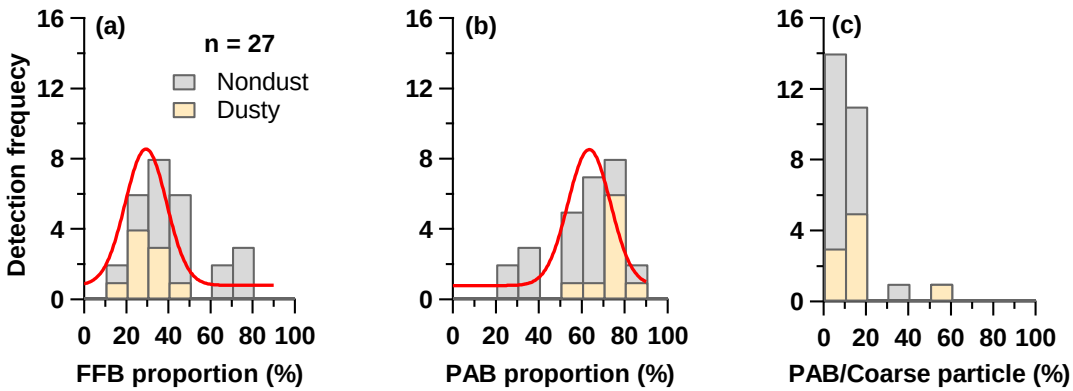

Fig. R3. The probability distribution of the proportions of free-floating bacteria (FFB)

and particle-attached bacteria (PAB) in total bacterial cells, and the ratio of PAB to coarse particles.

2. The second issue is that bacteria can attach to all sides of a particle. When looking at a particle, one sees only about half of its surface. Therefore, one also sees only about half of the bacteria attached to its surface, except the particle is transparent. Did you consider this issue? If not, maybe the number of particle-attached bacteria should be re-calculated?

Response: Thank the reviewer very much for this valuable comment. This concern is likely caused by our insufficient description of the method.

In our study, bacterial cells and other particles were detached from each of 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). After staining, each suspension was filtered on a black polycarbonate filter (25 mm diameter) and the black filter was mounted on a glass slide for fluorescent microscopic enumeration. Detailed description of the procedure of bacterial enumeration is available in the supplement material. The probability for the overlapping of non-transparent particles (e.g., mineral dust) and bacterial cells during filtration should be very small.

According to the usual orders of the concentrations of airborne dust-like particles and bacterial cells, the maximum number of mineral dust-like particles in a microscopic field of 100 μm × 100 μm is about 300 on average with 3 μm average size, and the maximum number of bacterial cells in the same field is about 100 on average with 1 μm (actually less than 1 μm) average size. In this study, the effective filter area of black polycarbonate filters (25 mm diameter) with a filter unit (Fisherbrand™ Glass Microanalysis Vacuum Filter Holders) is 1.1×10^8 μm^2. With these figures, the total area of mineral dust-like particles is estimated 2.3×10^7 μm^2 (~20% of the filtering area), and the total area of bacteria cells is estimated 8.6×10^5 μm^2 (8‰ of the filtering area). Thus during the filtration, the probability for the overlapping of mineral dust-like particles and bacterial cells is about 2‰, which is quite small.

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). 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 the filtration on a 25 mm diameter and 0.2 μm pore black polycarbonate membrane for bacterial enumeration. " was added in Line 86.

"***The probability for the overlapping of bacterial cells and mineral dust-like particles (insoluble and with irregular shapes) on the membranes for enumeration was quite small (several parts per thousand) and not considered.***" was added in Text S1 in the Supplement.

Another reference paper was also added in Line 86:

Hu, W., Murata, K., Fukuyama, S., Kawai, Y., Oka, E., Uematsu, M., and Zhang, D.: Concentration and Viability of Airborne Bacteria Over the Kuroshio Extension Region in the Northwestern Pacific Ocean: Data from Three Cruises, *J. Geophys. Res. Atmos.*, 122, 12892-12905, 10.1002/2017jd027287, 2017.

Minor issues

3. Line 11: 'aerosols more active'. Do you mean: 'aerosols to be more active'?

Response: Revised.

4. Line 13: Perhaps add that the size category is 'aerodynamic diameter'.

Response: "***in aerodynamic diameter***" was added.

5. Line 43: change 'very scientifically interesting' to 'scientifically very interesting'

Response: Revised.

6. Line 47: The sentence starting with 'Whereas. . .' seem not to be complete.

Response: "*Whereas, airborne bacteria should have different survival mechanisms,*

dispersal and size distribution from bacteria in soils because of the aerosolization process from Earth surfaces and harsh atmospheric stressors." was revised to "***Whereas, airborne bacteria should have different survival mechanisms, dispersal processes and size distribution from bacteria in soils, because 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).***"

Joung, Y. S., Ge, Z., and Buie, C. R.: Bioaerosol generation by raindrops on soil, Nat. Commun., 8, 14668, 10.1038/ncomms14668, 2017.
Hara, K., and Zhang, D.: Bacterial abundance and viability in long-range transported dust, Atmos. Environ., 47, 20-25, 10.1016/j.atmosenv.2011.11.050, 2012

7. Lines 54 and 66: Change 'in the spring of 2013−2016' to 'during spring in the years 2013 to 2016'.

Response: Revised.

8. Line 86-87: Replace 'results using BioSamplers' by 'the results to those obtained by using BioSamplers'

Response: Revised.

9. Line 89, 101: replace 'the holders' by 'the in-line filter holders'

Response: Revised.

10. Lines 126-127: 'indicating that the bacteria did not float individually in the air but were combined with other particles, i.e., the bacteria were particle-attached.' These particles could also have been other bacteria, i.e. bacteria may have been in clusters while airborne. This may affect the discussion (e.g. Line 213).

Response: We agree with the reviewer's opinion. Airborne bacterial cells are

sometimes found as assemblages of many cells, but they are usually as bacterial-slurry residue near emission sources, e.g., sea spray and leaf water (Lighthart, 1997).

In the revision, "***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).***" was added in Line 51.

Line 144, "*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, i.e., the bacteria were particle-attached.*" was revised to "***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.***"

11. Line 146: Replace 'high difference' by 'large difference'

Response: Revised.

12. Line 159: 'moved stagnantly' seems to be a contradiction, perhaps 'moved little' or 'moved sluggishly'

Response: "*moved stagnantly*" was revised to "***moved sluggishly***".

13. Figure 2 visualises a lot of information and therefore takes a little while to be understood. That is o.k., but perhaps think of removing the trendlines because they distract from the overall pattern: Concentrations of total bacteria and viable bacteria in

the size range below 1.1 um seem to increase less with increasing dust-like particle concentration, as compared to bacteria associated with particles larger than 1.1 um. In addition, why do the trendlines have different types of functions?

Response: Thank the reviewer for the valuable comment. We have modified Fig. 2 for simplicity according to the comment.

In the original figure, dashed lines represent unweighted linear fits for particles larger than 1.1 μm, but not include particles smaller than 1.1 μm.

We are not quite sure about the causes of different relationships between mineral-dust particles and bacterial cells. In the manuscript, we addressed two possible conditions that "*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).*" in Line 194.

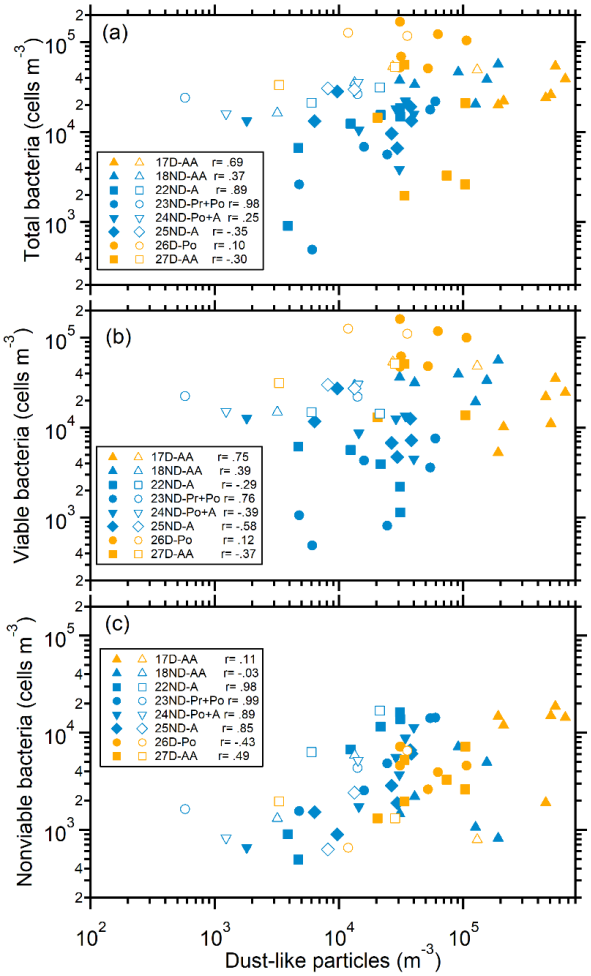

Figure 2. Relationship between bacteria and mineral dust-like particles in size-

segregated aerosols. (a) Total bacteria, (b) viable bacteria, and (c) nonviable bacteria. Solid and open circles represent particles 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.**

14. Line 195: 'with a residence time shorter than that of the particle-attached bacteria'. Could you provide a rough estimate for the atmospheric residence times of bacteria for dusty and nondusty conditions?

Response: We estimated the residence time according to the air parcels movement, i.e., the backward trajectories (Fig. S8). For particle-attached bacterial cells observed in dusty episodes, their residence time was considered as the time of the dust particles moving within the dust plumes from the desert areas to the observational site, that was about 2–3 days. For free-floating bacterial cells in non-dust periods, they were mainly from local areas, and largely influenced by local emission and thermal convective mixing near the ground surface. The time scale of local emissions and thermal convective mixing is in hours, so we estimate the residence time of free-floating bacterial cells in nondust air, at least most of them, was less than one day. Since the fluxes of emission or removal of bacterial cells under nondust conditions are unknown, it is impossible to calculate the residence time of bacterial cells directly from transport equations.

In the revision, "*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 shorter than that of the particle-attached bacteria transported from the Asian continent.*" was revised into "***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).***"

15. Line 235: A fitting reference in this context is Augustin-Bauditz et al (2016, Atmos. Chem. Phys., 16, 5531–5543, 2016 www.atmos-chem-phys.net/16/5531/2016/)

Response: The reference "Augustin-Bauditz et al., 2016" was added.

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

Referee #2

Review on manuscript https://doi.org/10.5194/bg-2020-94

This paper by Hu et al. reports the abundance and viability of particle-attached and free-floating bacteria in air samples collected at a costal site in Japan in spring during dust and nondust episodes.

The interest for bioaerosols (bacteria, fungi, yeasts, pollens, viruses...) is rather recent but growing every day, particularly because bioaerosols might have impacts on atmospheric processes (precipitation, chemistry, climate) and also on air quality (Human health, agriculture, environment). In this context, the paper presented here is quite important and interesting. Very few studies were conducted in the literature to measure the relative abundance of the bacteria attached or not to particles. In addition, the assessment of the viability of these bacteria is crucial to determine their potential impact. I am supportive of publishing this work in Biogeoscience after the following questions are addressed.

Response: We thank the reviewer very much for the encouragement and valuable comments. The manuscript was revised according to the comments, and here we give point-to-point responses to the comments as follows.

General comments on Figures and Tables

1) Nomenclature of the samples

1. The various samples are not identified in the same way depending on the figures or tables, sometimes identification is by numbers (1 to 27, see Figure 2, S7, S9), sometimes by dates (Figures 1, S2, S3, S4, S6, S8). As a consequence, it is very often hard to follow the results or comments in the text.

I suggest to adopt always **the same identification** (number is the best). In addition, the type of event (dusty, non-dust) and the metrological information (Prefront, Postfront, Approaching anticyclone and Anticyclone) should also appear in the nomenclature of the samples

For instance, the sample N°**1** collected on the 19th of march 2013 which is **D**usty **and** **Pr**efont could be named **1 D-Pr,** sample N°7 collected on the 28th of April 2013 which is **N**on-**D**ust and **A**nticyclone could be named **7ND-A…** etc These nomenclatures should be homogenous in all the Tables and Figures.

In parallel I suggest that Table S2 which contains very important results about the abundance and viability of free and attached bacteria should be moved to the main text of the manuscript (and not the Supplement). This table could be completed by the meteorological conditions (Prefront, Postfront, Approaching anticyclone and Anticyclone) and the samples named as suggested 1D-Pr, 7ND-A… etc.

Response: Thank the reviewer for the valuable comments. We have revised the text, tables, figures and supplement of the manuscript according to the suggestions.

We moved the original Table S2 to the main text of the manuscript as follows.

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**	**4.4**	**74**	**1.2 (28)**	**87**	**3.2 (72)**	**69**	**16**
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**	**2.0**	**75**	**0.9 (44)**	**87**	**1.1 (56)**	**60**	**10**
All (27)									
Average		**18**	**2.8**	**74**	**1.0 (39)**	**87**	**1.8 (61)**	**63**	**12**

Other revisions:

Figure 1: ": *(a) 19 March 2013, dusty; (b) 14 April 2013, dusty; (c) 21 March 2014, nondust; (d) 22 March 2015, dusty; (e) 22 March 2016, nondust; (f) 31 May 2016, dusty*." in the caption was revised to "***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.***"

Figure 2: The identification of samples was changed to the sequence number with dust condition and synoptic weather condition. Please refer to the response of Comment 13 of Reviewer#1.

Figure S2: The sequence numbers of sampling periods were added. The time series of meteorological conditions and airborne particle number concentrations during the observations are supportive information to identify the sampling periods, therefore the dust condition and synoptic weather conditions are omitted for brevity.

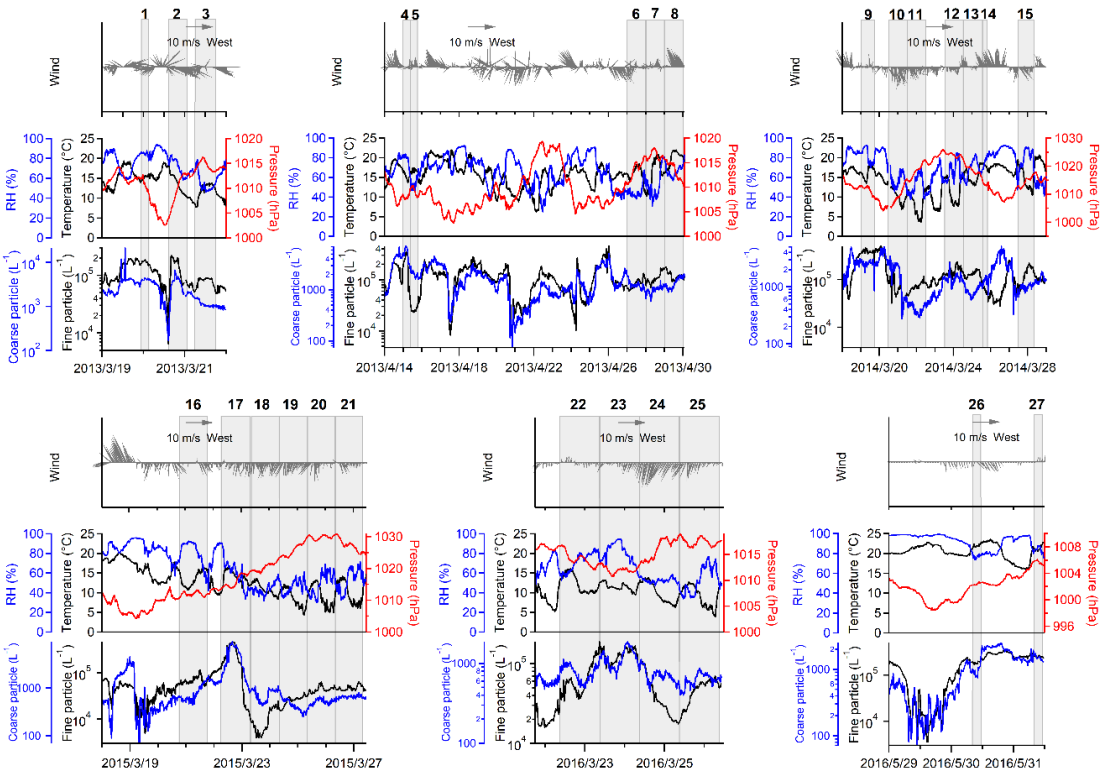

Figure S2. Time series of meteorological conditions and airborne particle number concentrations during the observations. Each sampling period is indicated with a gray shadow and the sequence number.

Figure S3: "***Sample 2D-Po***" was added in the caption.

Figure S4: This figure is not discussed according to the characteristics of sampling periods. We omitted sample identification in the figure.

Figure S6 and S7: The weather charts and dust forecasts were released by the Japan Meteorological Agency once a day and every three hours according to date, respectively.

We show them as supportive information of the synoptic weather conditions and dust conditions during the observation periods.

Figure S8 and S9: Sample ID indicating the sequence number, dust condition and synoptic condition are marked as follows.

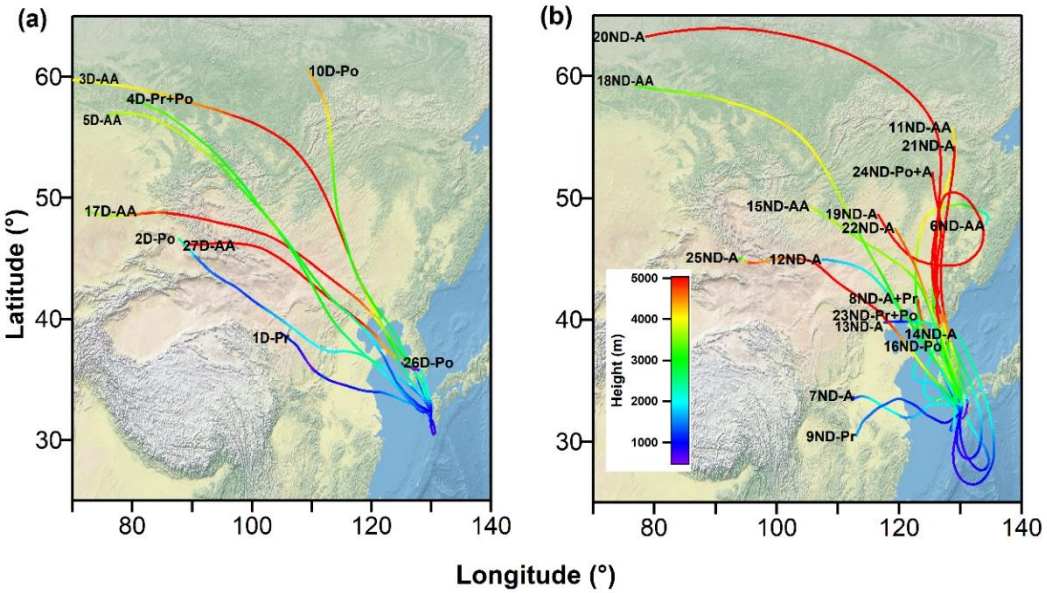

Figure S8. Seventy-two-hour backward trajectories of air parcels (http://ready.arl.noaa.gov/HYSPLIT.php) at 1000 m at the sampling site during the dusty (a) and nondust (b) periods. The sample ID of each sample is marked at the end of the corresponding trajectory. The map source is the IgorGIS package of IGOR Pro.

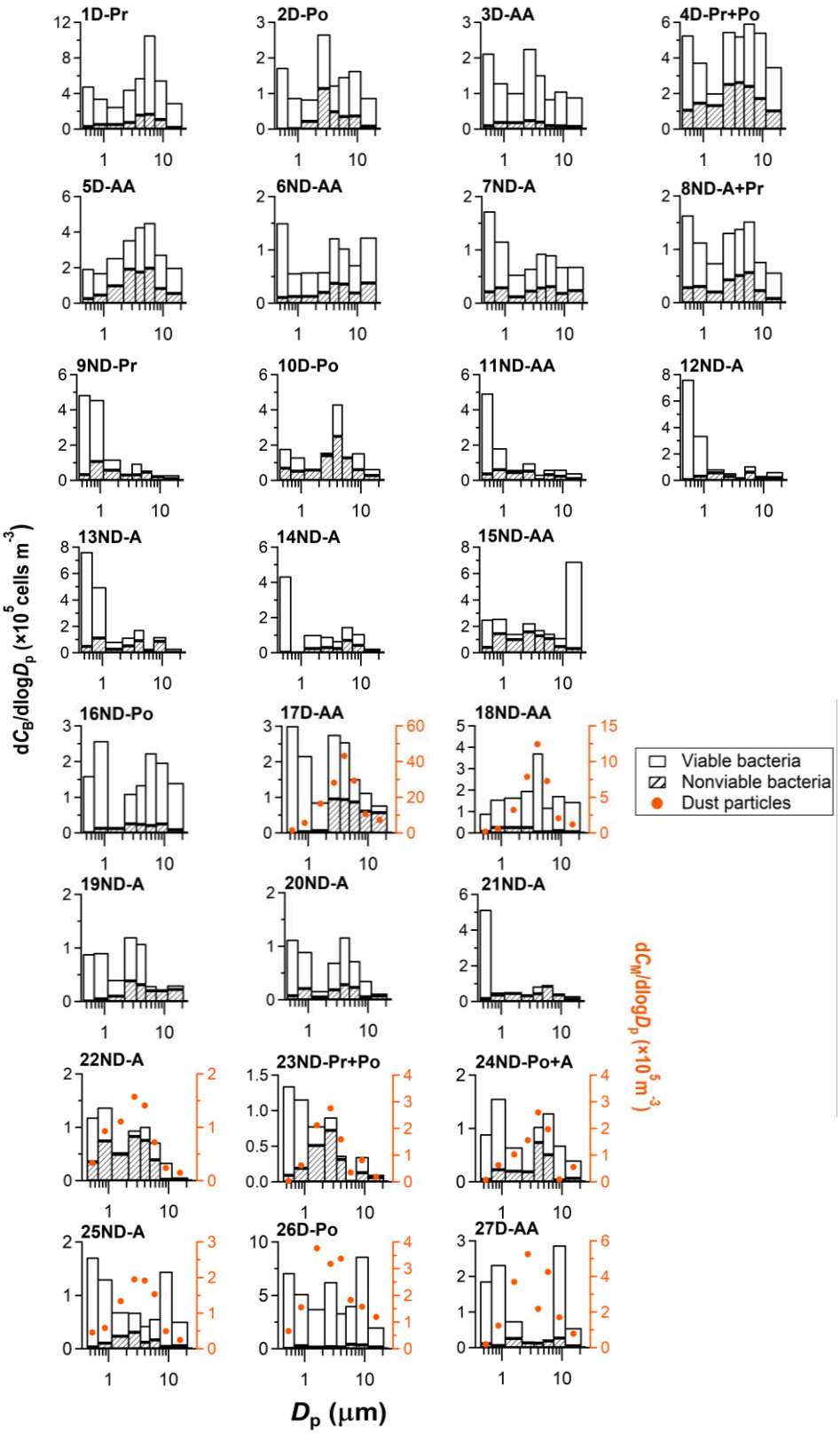

Figure S9. Concentrations of viable and nonviable bacteria (C_B) and mineral dust-like particles (C_M) in size-segregated airborne particles during the sampling periods. All *x* axes indicate particle aerodynamic diameter (D_p), left *y* axes indicate dC_B /dlog D_p

(black), and right *y* axes indicate dC_M /dlog D_p (orange). The upper limit of particle size was set to 20 μm because it is difficult for particles larger than 20 μm in aerodynamic diameter to remain airborne (Andreas et al., 1995;Mayol et al., 2014). During dust periods (1D-Pr, 2D-Po, 3D-AA, 4D-Pr+Po, 5D-AA, 10D-Po, 17D-Po, 26D-Po, and 27D-AA), the size distribution of bacteria-associated particles showed two modes. During six cases of nondust periods (9ND-Pr, 11ND-AA, 12ND-A, 13ND-A, 14ND-A, and 21ND-A), bacteria-associated particles mainly distributed in the size fraction 0.43–1.1 μm. In samples from 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), except for Samples 15ND-AA and 18ND-AA, the bacteria-associated particles showed bimodal size distributions.

2) Uncomplete presentation of the data

2. Could the authors explain why only some data on some samples are presented in many figures and not all of them (see Figures 2, S2, S6, S8).

Response: Figure 2: The study lasted four years. The designation of the experiment was modified gradually regarding the results obtained in each year. For the samples collected in 2013 and 2014, we did not count dust-like particles during bacterial enumeration. For the samples collected in 2015, we only counted the dust-like particles for samples collected during the occurrence and disappearance of a dust event. In 2016, we counted the dust-like particles in all samples.

In the revision, "***The designation of the experiment was modified gradually regarding the results obtained in each year. For the samples collected in 2013 and 2014, we counted bacterial cells only. For the samples collected in 2015, we also counted mineral dust-like particles (insoluble and with irregular shapes) in those collected during the occurrence and disappearance of dust events. In 2016, we counted the mineral dust-like particles and bacterial cells in all samples.***" was added in Text S1

in the Supplement.

Figure S2: We present all sampling periods in the figure.

Figure S6: As mentioned above, the dust forecasts are released every three hours according to date. We show them as supportive information of the synoptic weather dust conditions during the observation periods, especially during dust events. So we only show the dust load mapping of the dust events during the observation periods.

Figure S8: Sample IDs of 1D-Pr and 27D-AA are added.

Specific comments

Sample collection and cell enumeration:

3. *p3 and Figure S3:* Did you notice the presence of yeasts and fungi (spores) (>>1 μm) during your experiment based on epifluorescence microscopy? Why did not you take them into account in your study?

Response: In this study, we focus on the status of airborne bacteria and applied the LIVE/DEAD *Bac*Light Bacterial Viability Kits (L13152, Invitrogen™, Molecular Probes Inc., Eugene, Oregon, US) to quantitatively distinguish live and dead bacteria in aerosols containing a range of bacterial types. Although according to the composition (SYTO® 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide) and the principle of the staining kit, it is possible to stain yeast, fungi, and other nucleic acid-containing organisms, we did not take them into account in this study. We addressed the standard of microscopic enumeration in this study, i.e., "*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.*"

In addition, the LIVE/DEAD ® *Funga*Light™ Yeast Viability Kit is more selective for yeast enumeration combined with flow cytometry.

4. *p4 and Figure S4*: The authors note some discrepancy between the results obtains

by the Andersen sampler used in this work and the two other samplers. The bacteria concentration seems to be usually under-estimation but the main problem in my opinion is that this under- estimated is not "constant", this is the case of the sampling on the 21th of March 2013 (figure S4). How do you take this factor into account in your results?

Response: It is possible for occasional inconsistence in the comparison, because the premise of simultaneous sampling comparison is that the concentration of bacterial cells in the air is uniform. This is generally true, especially after the passage of cold fronts, air mass is more uniform. But sometimes it can be different. For example, in the air mass under high pressure or before the passage of cold fronts, local convection frequently occurs (for example, warm cells in a scale of a few meters to hundreds of meters). Besides, the air inlets of different samplers are not identical. So it is not surprising that we encountered such occasional inconsistence during the 4-year-long observation.

In the revision, we mentioned in the figure caption that "***The discrepancy between the results of the Andersen sampler and the other two samplers in some cases might have been caused by the different sampling durations and collection efficiencies of the samplers (e.g., for the Andersen sampler, there was a loss of bacteria due to bouncing, and the size fraction smaller than 0.43 μm was missing).***"

Concentrations of bacteria of airborne bacteria in segregated size ranges (p 5, Figures 1 and S9)

5. In my opinion it is quite difficult to really analyze the data presented in Figure 1 and S9 in terms of random or bimodal distributions of the bacteria ... what is the scientific basis of this analysis? Is it based on visual inspection only?

Response: Yes, it is difficult to analyze the dominant modes in size because there are too small (only 8) size-segregated bins to do statistical analysis. And yes, the results were based on visual inspection only. This is because individual airborne bacterial cells are usually ~1 μm or smaller than 1 μm (Burrows et al., 2009; Delort et al., 2010;

Després et al., 2012; Hara et al., 2011; Pósfai et al., 2003). 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. The 8-stage Andersen cascade samplers (Model AN-200; Tokyo Dylec Corp., Japan) collect aerosol particles in the segregated 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. In fact, we used 1.1 μm (the cutoff size of a sampler stage) to distinguish free-floating from attached bacteria in the atmosphere. Therefore, we classified the size distribution patterns according to the distributions of particles smaller and larger than 1.1 μm.

6. In addition, as noticed by the authors, when non-dust samples are analyzed both types of bacterial distribution are observed. Do you have any explanation about these segregations? Does it mean something linked to the physics of the system? This is not clear! I am wondering if we can really exploit these data. Could the authors comment on that?

Response: In Sect. 3.1, we mentioned that "*during nondust periods, the number-size distribution of bacteria largely varied and did not show any trend with respect to weather conditions.*" We are not sure about the causes. In fact, there are several processes, e.g., transport, local emission, and removal, that could influence the distributions. Unfortunately, we do not have further evidence by means of statistical analysis to show the connection between the distribution and the processes, because of the limited cases in each case categories.

In the revision, "***There were multiple processes, e.g., advection, deposition, local emission and local convective mixing, that 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.***" was added in Line 159.

Concentration of particle-attached and free-floating bacteria

7. *P7, Table S2, Figure S8*: The authors declare "In particular, the percentage ranged from 35% to 73% (49±15% on average) under anticyclone weather conditions, when the air parcels were from marine areas rather than from continental areas and moved stagnantly (Fig. S8). Therefore, there were a substantial amount of free-floating bacteria, and they were frequently the most common bacteria in non-dust air." This is quite interesting however when we look carefully at the data this not so true. For instance, in the event N°7 (anticyclonic, non-dust) compared to N°12 (anticyclonic, non-dust) free-floating bacteria account respectively for 39% and 73% of the total number of cells. However, N°7 (Figure 8) is clearly from marine origin with a slow motion of the air mass while N°12 moved quicker and has a continental origin. It is also true for other samples, so I do not think it is a general assumption. In addition, two events (1 and 27) are missing in Figure S8.

Also did you analyze the biodiversity of the bacteria to justify your sentence" the most common bacteria"?

Response: Thank the reviewer for the careful review. This is a misunderstanding caused by our inadequate descriptions.

Figure R4 show the one-day backward trajectories of air parcels at 1000 m at the sampling site under anticyclone weather conditions. Air parcels mainly came from marine areas and moved slowly. Only for two sampling periods of 19ND-A, 20ND-A, the air parcels passed over the Korean Peninsula. We consider that the implication is generally true.

Under anticyclonic conditions, the local wind direction was usually southwest or south and the wind was weak (airmass movement was slow). That is why we consider the bacteria was mainly from marine and local areas (in the text we forgot those from local areas). Although these phenomena are true in most anticyclone cases, in rare very strong anticyclone cases with strong cyclones to the east (e.g., cases 19ND-A and 20ND-A) moving in the westerly, the airmass movement speed could be very fast while the wind direction was usually the north.

In order to avoid the false results of backward trajectory calculation near the surface caused by thermal convective mixing, we calculated the backward trajectories with the starting point at 1000 m elevation. For the case of 12ND-A that the reviewer mentioned, the airmass movement was actually much slower than and very different from that under cyclone conditions and was a typical movement of airmass in anticyclones moving in westerlies, although the movement was somewhat fast.

In the revision, "*In particular, the percentage ranged from 35% to 73% (49±15% on average) under anticyclone weather conditions, when the air parcels were from marine areas rather than from continental areas and moved stagnantly (Fig. S8). Therefore, there were a substantial amount of free-floating bacteria, and they were frequently the most common bacteria in non-dust air*." was revised into "***In particular, the percentage ranged from 35% to 73% (49% 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, there were a substantial fraction of free-floating bacteria, and they were frequently the common bacteria in nondust air."***

In Fig. S8, the labels of Sample 1D-Pr and 27D-AA are added. Please refer to response to comment 1.

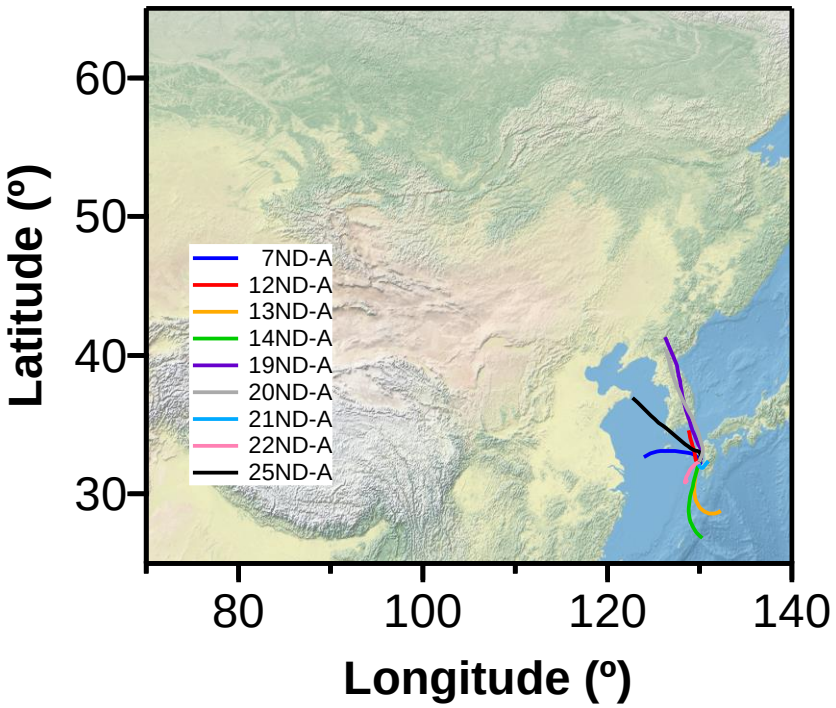

Figure R4. One-day backward trajectories of air parcels (http://ready.arl.noaa.gov/HYSPLIT.php) at 1000 m at the sampling site under anticyclone weather conditions. The sample ID of each sample is marked at the end of the corresponding trajectory. The map source is the IgorGIS package of IGOR Pro.

Here the phrase "the most common bacteria" represents the bacteria with the free-floating status rather than the bacterial species.

We tried to conduct DNA extraction from the size-segregated aerosol samples, PCR and gene sequencing to identify the bacterial community structure. However, due to the low concentration, we only obtained the results from several sets of samples. The quality control during the processes of DNA extraction and the extraction efficiency require further optimizing. Therefore, we did not discuss the results of the bacterial community structure in this study.

8. *p7, Figure 2:* The authors declare "The concentration of bacteria was usually closely correlated with the mineral dust-like particles in size-segregated samples (Fig.

2)". However, the data presented in Figure 2 are not so obvious when we look at the correlation coefficient r which are generally very low except for 2 cases in Figure 2a and 4 cases in Figure 2c. In addition, there are only 8 samples over a total number of 27. What are the results for the missing 19 samples? Finally, it would be very useful to have SEM images to confirm these conclusions. In my opinion this figure is over-interpreted and the text should be changed.

Response: Thank the reviewer for the valuable comment. In the revision, to avoid over-interpretation, the original description was revised to "***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). 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 coarse size range (e.g., 26D-Po)***".

As mentioned above, this study lasted four years, and the designation of the experiment was modified gradually. For the samples collected in 2013 and 2014, we did not count the dust-like particles during bacterial enumeration. For the samples collected in 2015, we only counted the dust-like particles for samples collected during occurrence and disappearance of a dust event. In 2016, we counted the dust-like particles for all samples collected.

In our laboratory, we have been working on airborne particles with electron microscopes (EM; both transmission and scanning electron microscopes). We collected samples for the EM analysis and analyzed them in the observations shown in this study, for the purpose of identifying individual aerosol particles. Unfortunately, there are not proper indices for confident identification of airborne bacterial cells in EM analysis. Shape and size could not be used because, in the atmosphere, there are more particles

in round shape with the size around 1 μm (some are called soot particles or tar balls or their mixture with other components) that are from vegetation burning and fossil fuel combustion. Coating of metals or carbon (sample treatments) for the visualization of particles composed of organic and biogenic components alters the shapes of particles. Some studies suggest using the occurrence of phosphor and potassium detected with element detectors (called EDX) attached to EMs to identify biological aerosol particles but the uncertainties are large because the EDX is sensitive to inorganic components. Potassium is the most constant component of vegetation burning particles, and the phosphor is rarely detected from aerosol particles, likely due to its small amount or forms insensitive to EDX detector. Metal staining is another approach, but this approach is currently not applicable to airborne biological particles. We encountered some particles that looked like the bacterial cells we focused in this study. But the confidence of the quantification is low and we are unsure they are really biological particles let alone bacterial cells.

Viabilities of particle-attached and free-floating bacteria

9. *P9, line 184, Tables 1 and S2:* The authors declare: "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%), higher than the viability of particle-attached bacteria ". Did the authors performed statistical analyzes to compare these results?

Response: Thank the reviewer for the valuable comment. We performed Wilcoxon signed ranks test to compare these results and found that there is significant difference (2-tailed $P = 0.00$) between the viabilities of particle-attached bacteria and free-floating bacteria.

In the revision, "***Wilcoxon signed rank test, 2-tailed P = 0.00***" was added.

10. *P9, line 194, Tables 1 and S2, Figure S8:* The authors declare "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 shorter than that of the particle-attached bacteria transported from the Asian continent". Although this assumption makes sense, it is less true when looking at the backward trajectories presented in Figure S8. For instance the event N°20 has a long trajectory from the Asian continent, far from the marine sampling site, while the event N°7 remains mainly over the sea, both samples present the same viability of the free bacteria (85 and 82% respectively), and also for the attached bacteria (72 and 69 % respectively). So in my opinion this reason is not so clear. Please could you modulate your conclusions.

Response: We agree with the reviewer's opinion that in some cases the viabilities of free-floating bacteria and particle-attached bacteria were similar. However, we consider that our assumption is rational. As shown in Fig. R5, a large fraction of free-floating bacteria were viable, i.e., free-floating bacteria were of higher viability. Combined with the backward trajectories of air masses (As shown in Fig. S8 and Fig. R4), those free-floating bacteria were likely from local areas because the movement of the air was slow and the proportion of free-floating bacteria was higher during nondust conditions when the air masses moved slowly.

For the case of No. 20, it is a special case that is a very strong anticyclone with a very strong cyclone to its east and very different from the usual cases of anticyclone moving in westerlies (please see the weather charts of 24-25 March 2015 in Fig. S6). The air flow arriving at the site was from the north and extremely strong because the flow was close to geostrophic wind and its strength was proportional to the pressure gradient between the high and the low. In this case, the bacteria could be from the local sea areas or even the Sea of Japan where the wind stimulated strong sea waves and from the local areas due to the extremely strong wind.

In the revision, "*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 shorter than that of the particle-attached bacteria transported from the Asian continent.*" was revised into "***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. "

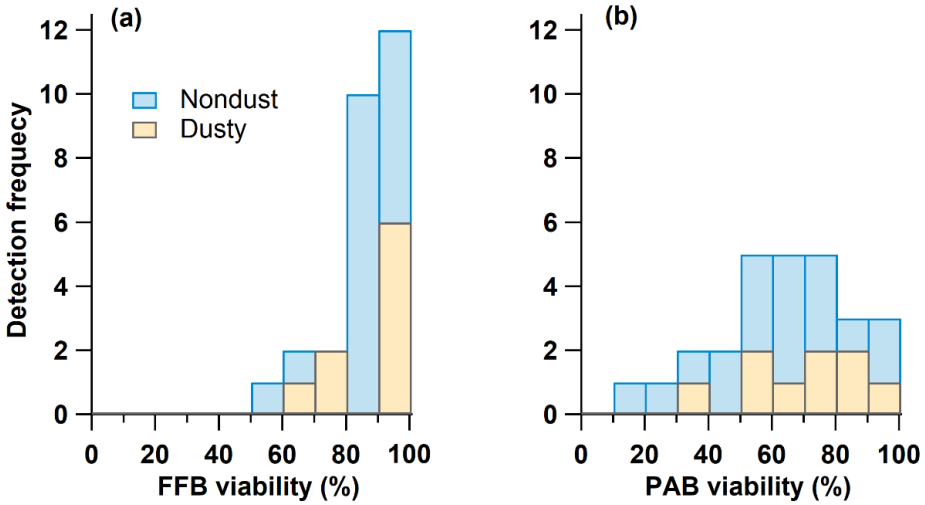

Figure R5. The probability distribution of the viability of (a) free-floating bacteria (FFB) and (b) particle-attached bacteria (PAB).

11. *P9, line 203 Tables 1 and S2, Figure S8: The authors declare* "An increase in viable free-floating bacteria on the order of 10^5 cell m^{-3} was observed when the weather was fine and the air masses moved slowly from marine areas, favoring the accumulation of bacteria emitted from local areas (Fig. S8)". Again this observation is globally true but some examples contradict it: The event N°7 (marine origin, anticyclone, 0.6×10^5 cell m−3) does not present really higher concentrations of free bacteria compared to sample N°20 (Asian continent origin, anticyclone, 0.6×10^5 cell m^{-3}). So please could you modulate your conclusions.

Response: In the revision, we make the description more accurate. "*An increase in viable free-floating bacteria on the order of 10^5 cell m^{-3} was observed when the*

weather was fine and the air masses moved slowly from marine areas, favoring the accumulation of bacteria emitted from local areas (Fig. S8)" was revised into "***An increase in viable free-floating bacteria on the order of 10^5 cell m^{-3} ($1.1–2.2 \times 10^5$ 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)***".

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

Referee #3

Summary:

This work demonstrates the abundance and viability of different existential form of bacteria in dust and non-dust periods. The manuscript fits well to the scope of Biogeosciences and presents valuable results. Thus I recommend it to be published after the following moderate/minor comments listed below have been adequately addressed.

Response: We thank the reviewer very much for the encouragement and valuable comments. The manuscript was revised according to the comments, and here we give point-to-point responses to the comments as follows.

Comments:

1. It seems that the criterion for distinguishing particle-attached bacteria and free-floating bacteria is the size of ~ 1um, which requires more explanations. I would suggest the authors discuss the uncertainty of selected critical size.

Response: In this paper, we addressed "*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 (Burrows et al., 2009; Delort et al., 2010; Després et al., 2012; Hara et al., 2011; Pósfai et al., 2003); 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.*" In fact, we used 1.1 μm (the cutoff size of a sampler stage) to distinguish free-floating from attached bacteria in the atmosphere. The reasons are explained as follows.

Based on our previous study using the LIVE/DEAD *Bac*Light Bacterial Viability Kit, most of the bacteria on airborne particles were smaller than 1.0 μm and they were close to a spherical shape with the mean size of 0.6 μm (Fig. R6; Hara et al., 2011).

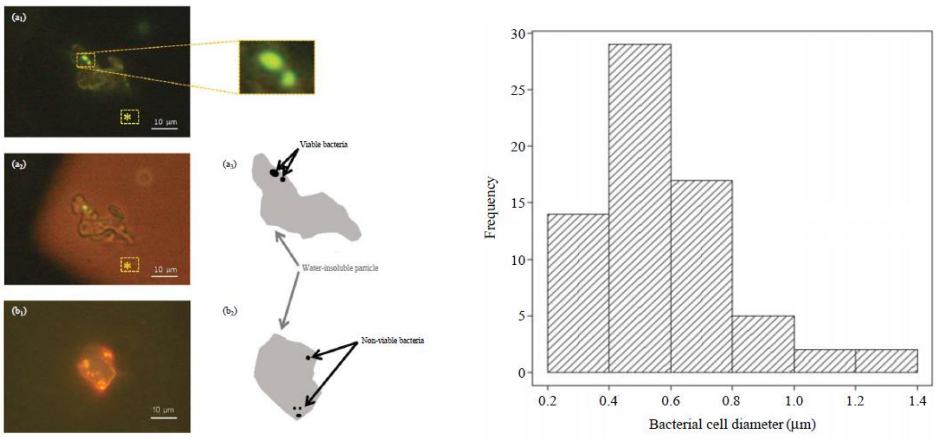

Fig. R6. Epifluorescence images of bacteria-carrying particles (left) and size distribution of bacterial cells (n=69) on airborne particles (right) collected at Kumamoto, southwestern Japan (Hara et al., 2011).

Previous studies (Maki et al., 2008, 2013; Iwasaka et al., 2009) using epifluorescence microscopy after DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride) staining also revealed that DAPI-stained bacterium-like cells located on the mineral particles were observed as small particles with bright-blue fluorescence, and coccoid-like, with a diameter of less than 1 μm (Fig. R7).

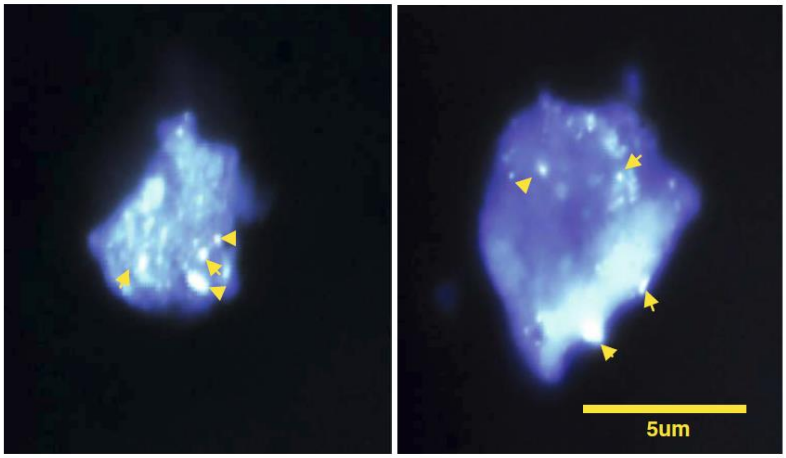

Fig. R7. Epifluorescence micrograph of biological particles on dust particles collected at an altitude of 800 m above the ground in Dunhuang, China (Maki et al., 2008).

By means of transmission electron microscopy analysis, Pósfai et al. (2003) also found almost all airborne bacteria are morphologically rod-shaped, about 1 μm long or smaller,

have one polar flagellum, and contain inclusions that are rich in P and K over the Southern Ocean (Fig. R8).

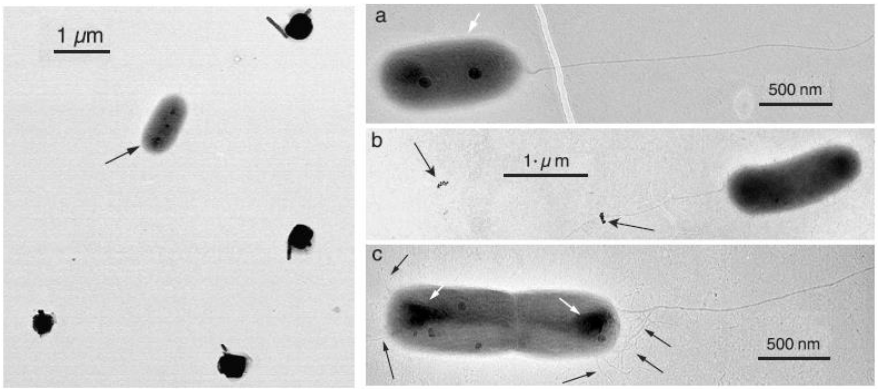

Fig. R8. Electron micrograph showing aerosol bacteria over the Southern Ocean (Pósfai et al., 2003). The bacteria possess polar flagella and phosphatic granules (the dark spots in the cells).

Many other previous papers have also addressed that airborne bacterial cells are usually ~1 μm or smaller than 1 μm (Burrows et al., 2009; Delort et al., 2010; Després et al., 2012; Wittmaack et al. 2005). Characteristic size ranges of atmospheric bioaerosols are illustrated as Fig. R9 (Fröhlich-Nowoisky et al., 2016).

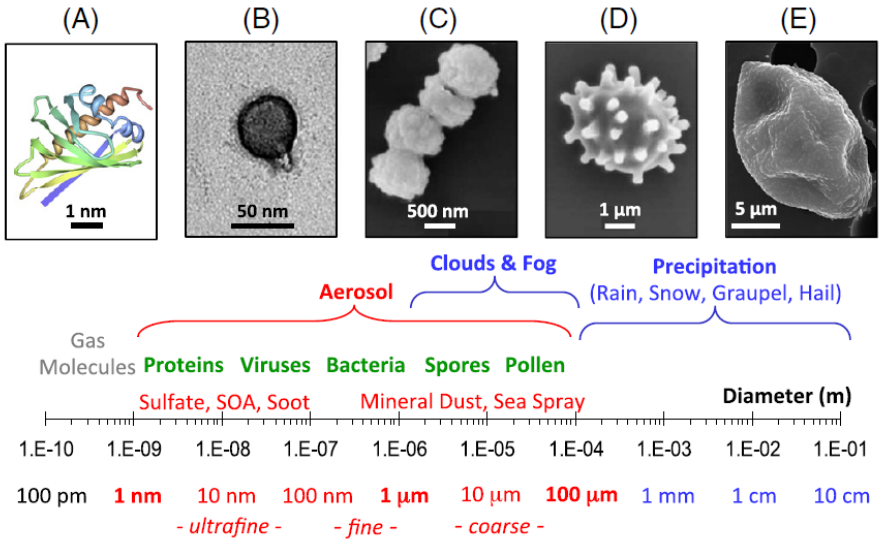

Fig. R9. Characteristic size ranges of atmospheric particles and bioaerosols with exemplary illustrations: (A) protein, (B) virus, (C) bacteria, (D) fungal spore, and (E) pollen grain (Fröhlich-Nowoisky et al., 2016).

In this study, as shown in Fig. S3, the bacterial cells on the Stages 6 and 7, i.e., the free-floating cells as we assumed, were generally in the size of close to or smaller than 1 μm in diameter, which is consistent with previous studies or literatures.

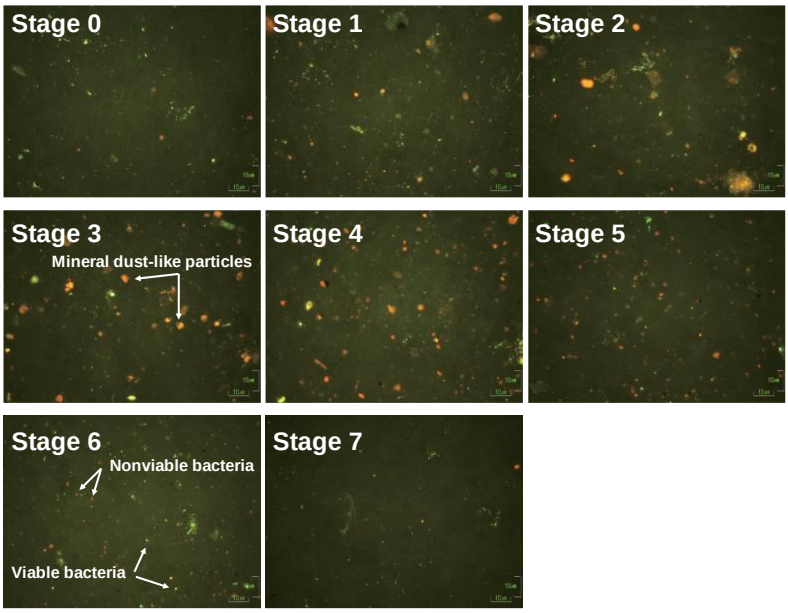

Figure S3. Images of one set of stained samples collected on 20 March 2013 (Sample 2D-Po, dust period) under the epifluorescence microscopy field.

Therefore, based on the above results of previous studies, we think the assumption that airborne particle-attached bacteria should appear, even though not exactly, at least mostly, in the samples of particles larger than 1 μm, while free-floating bacteria in samples of particles smaller than 1 μm.

Unfortunately, there are not any other quantitatively reported results on particle-attached bacteria and free-floating bacteria. Our data were actually the first estimation, to our knowledge, and we have no other approaches as controls to evaluate the uncertainties due to the usage of 1 μm as the critical size.

1. Page 3, lines 78-81: I do not understand this method. Are the bacteria identified based on the fluorescence signals? If so, how the other interferences (such as SOA,

PAH et al.) are excluded? Also, why you only count particles close or smaller than 1 um (free-floating bacteria)?

Response: Yes, the bacteria were identified based on the fluorescence signals after nucleic acid staining with the LIVE/DEAD bacterial viability kit (SYTO® 9 green-fluorescent nucleic acid stain; propidium iodide, red-fluorescent nucleic acid stain). As mentioned in the response to Comment 2 of Reviewer#1, in this study, bacterial cells and other particles were detached from each of 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). After staining, each suspension was filtered on a black polycarbonate filter (25 mm diameter) and the black filter was mounted on a glass slide for fluorescent microscopic enumeration. After staining, filtration and slide mounting, the slides were observed under the excitation wavelength range between 450 and 490 nm (blue). We addressed that "*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.*" Because the staining kit is nucleic acid-based, we counted viable and nonviable bacterial cells according to this standard. Detailed description of the procedure of bacterial enumeration is available in the supplement material.

In the revision, "***An excitation wavelength range between 450 and 490 nm (blue) was utilized, and the microscope was operated at 1000× magnification.***" was added.

Although other interferences (such as SOA and PAHs) also possibly exhibit autofluorescence, we consider the influence is minor. Firstly, we suspended the bacterial cells and other particles in the PBS and filtered the particles on PC membranes (0.2 μm pore). Therefore, the small molecules of SOA and PAHs generally passed through the PC membranes and remained litter on the PC membranes. Secondly, the excitation-emission matrixes of other autofluorescent species are distinct from that of bacterial cells (Fig. R10).

"*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". That is, based previous studies (see our response to your Comments1), particle-attached bacteria are considered as the bacterial cells in aerosol samples of particles larger than 1 μm (the nominal cutoff size of the sampler 1.1 μm), and free-floating bacteria are considered as those located among particles smaller than 1 μm.

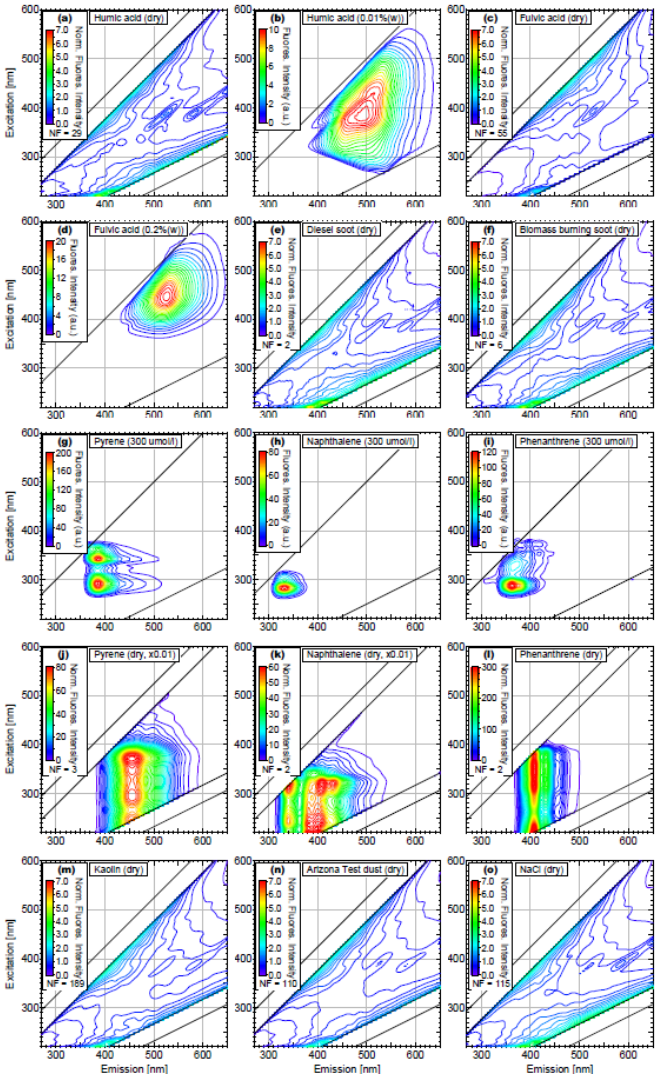

Fig. R10. Normalized EEM contour profiles for selected interferences in solid state and/or solution. Intensity color scale has been adjusted to intensity of individual components. Lower NF indicates higher fluorescence intensity (Pöhlker et al., 2013).

3. Figure 1: the label of y-axis is not right, please correct.

Response: The *y*-axes labels of Fig. 1 and Fig. S9 were corrected.

4. For the discussion part 4.2-4.4, it is important to study the influences of bioaerosols, but I would suggest the authors to discuss it with their own dataset.

Response: Thanks to the reviewer for this helpful comment. Since the available data are limited and there are no other equivalent data for comparisons, we made an effort to discuss the influences of airborne bacteria according to the results obtained in this study and some general understandings. For instance, in Sect. 4.2, we discussed the possible influence of airborne bacteria on ice cloud formation based on the results about their existing status. We mentioned that "*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).*"

In Sect. 4.3, we discussed the impact on ecosystems based on the viabilities of airborne bacteria. "*More than 60% of particle-attached bacteria and approximately 87% of free-floating bacteria in the dusty air remained viable.*"

In Sect. 4.4, we discussed the impact on public health based on the size distribution of airborne bacteria. "*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.*"

In the revision, "***Since there are rare other equivalent data for comparisons, we discuss the influences of airborne bacteria according to the results obtained in this study and relevant general understandings in the following subsections.***" was added in Line 253.

References:

Andreas, E. L., Edson, J. B., Monahan, E. C., Rouault, M. P., and Smith, S. D.: The spray contribution to net evaporation from the sea: A review of recent progress, Boundary-Layer Meteorology, 72, 3-52, https://doi.org/10.1007/BF00712389, 1995.

Joung, Y. S., Ge, Z., and Buie, C. R.: Bioaerosol generation by raindrops on soil, Nat.

Commun., 8, 14668, 10.1038/ncomms14668, 2017.
Mayol, E., Jimenez, M. A., Herndl, G. J., Duarte, C. M., and Arrieta, J. M.: Resolving the abundance and air-sea fluxes of airborne microorganisms in the North Atlantic Ocean, Front. Microbiol., 5, 557, 10.3389/fmicb.2014.00557, 2014.
Pöhlker, C., Huffman, J. A., and Pöschl, U.: Autofluorescence of atmospheric bioaerosols-fluorescent biomolecules and potential interferences, Atmos. Meas. Tech., 5, 37-71, 10.5194/amt-5-37-2012, 2013.

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

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

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Abstract. 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. 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 blew dust there from the Asian continent, revealed that particle-attached bacteria in dust episodes, averagely at the concentration of $3.2 \pm 2.1 \times 10^5$ cells m^{-3}, occupied 72±9 % in the total bacteria. In contrast, the fraction was 56±17 % during nondust periods and the concentration was $1.1 \pm 0.7 \times 10^5$ cells m^{-3}. The viability, defined as the ratio of viable cells to total cells, of particle-attached bacteria was averagely 69±19 % in dust episodes and 60±22 % during nondust periods, both of which were considerably lower than the viabilities of free-floating bacteria (about 87 %) under either dusty or nondust conditions. The present cases suggest that dust particles carried substantial bacteria on their surfaces, more than half of which were viable, and spread these bacteria in the atmosphere. This implies that dust and bacteria have nonnegligible roles as internally mixed assemblages in various atmospheric processes and in linking geographically isolated microbial communities, as well as have synergistic effect on human health.

1 Introduction

Biological particles in the atmosphere have a significant potential 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 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. The cell size distributions for bacteria separated from soils have been investigated previously (Portillo et al., 2013). Whereas, airborne bacteria should have different survival mechanisms, dispersal processes and size distribution from bacteria in soils, because 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, 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 during spring in the years 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 cascade 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) by vortex shaking and ultrasonic vibration in a phosphate-buffered saline solution (PBS, pH 7.4). 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. 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 results show 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**	**4.4**	**74**	**1.2 (28)**	**87**	**3.2 (72)**	**69**	**16**
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**	**2.0**	**75**	**0.9 (44)**	**87**	**1.1 (56)**	**60**	**10**
All (27)									
Average		**18**	**2.8**	**74**	**1.0 (39)**	**87**	**1.8 (61)**	**63**	**12**

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 bacteria, and bacteria in the samples of stages with particles ranging from 0.43–1.1 μm were considered free-floating bacteria. 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 were investigated in the laboratory (Text S2 in the Supplement). The fractions and concentrations of particle-attached bacteria obtained by the present 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). In addition, the concentrations of total bacterial cells quantified using the Andersen samplers were consistent (100±15%) with those quantified using the in-line filter holders. This result indicates that bacteria smaller than 0.43 μm, which trapped with difficulty by the Andersen samplers, 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 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/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). There were multiple processes, e.g., advection, deposition, local emission and local convective mixing, that 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.

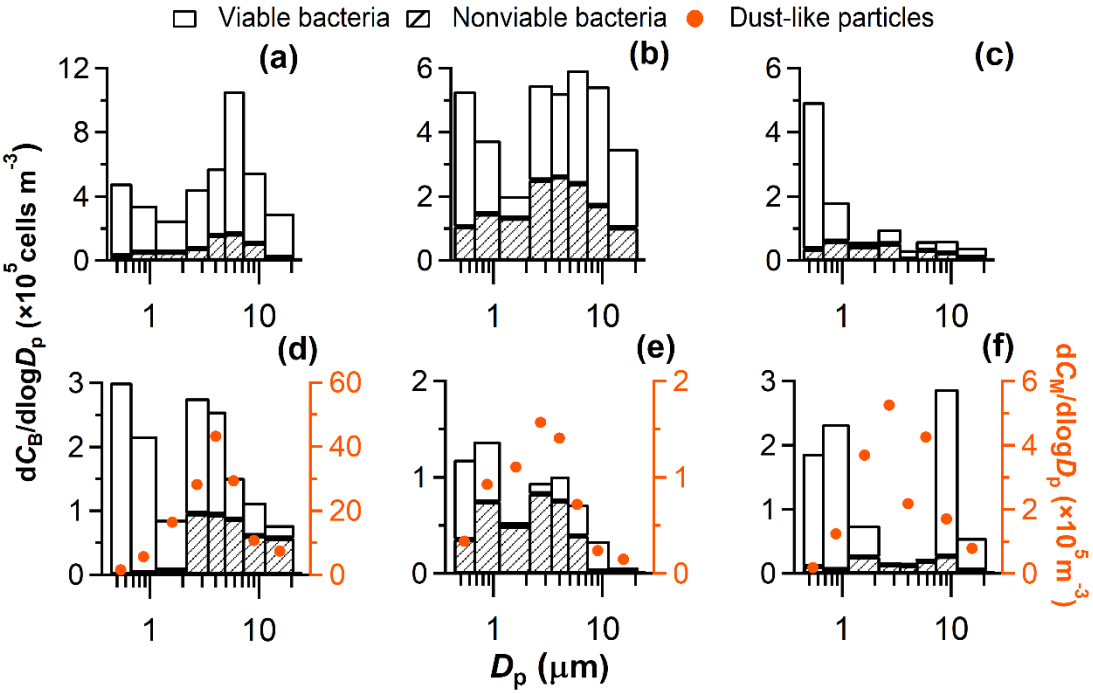


Figure 1. Concentrations of viable and nonviable bacteria (C_B) and mineral dust-like particles (C_M) in size-segregated airborne particles. Selected samples are shown as examples: (a) 1D-Pr; (b) 4D-Pr+Po; (c) 11ND-AA; (d) 17D-AA; (e) 22ND-A; (f) 27D-AA. The results of all sampling periods are depicted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

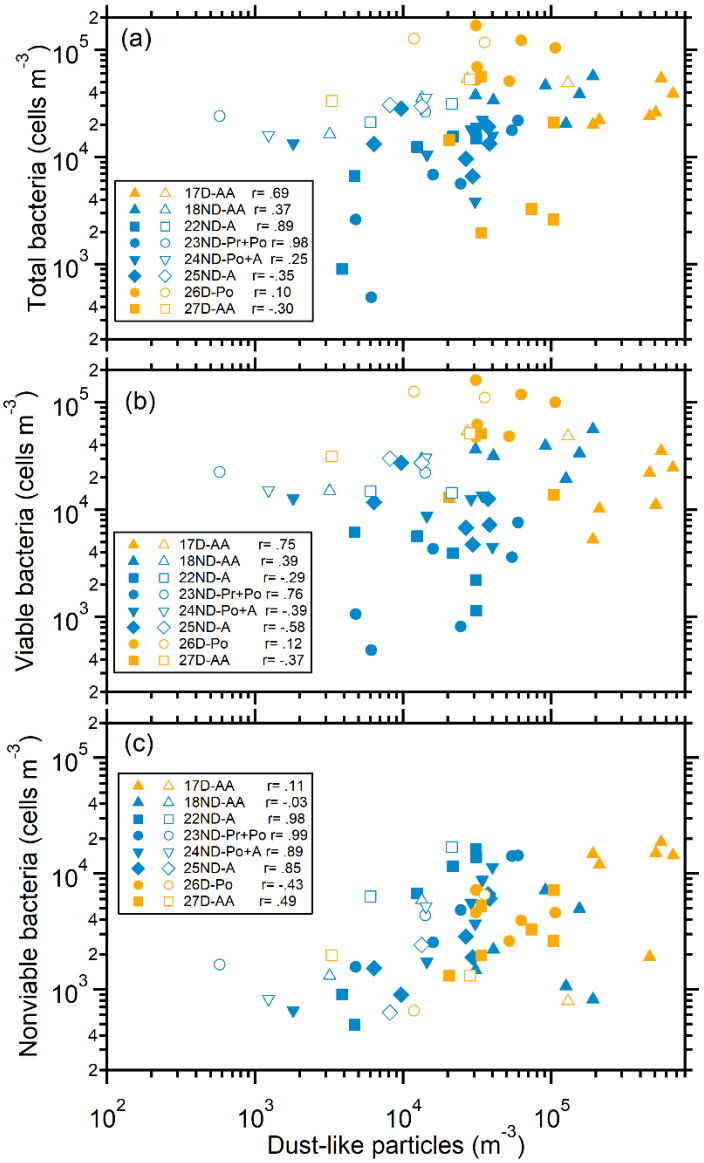
3.2 Concentration of particle-attached and free-floating bacteria

On average, the concentration of total bacterial cells, 4.4±2.6 × 10^5 cells m^{-3}, during dust episodes was more than twice that during nondust periods, 2.0±1.0 × 10^5 cells m^{-3} (Table 1). This large difference 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±2.1 × 10^5 and 1.1±0.7 × 10^5 cells m^{-3}, respectively. The percentage of particle-attached bacteria during dust periods, 72±9%, was significantly higher than that during nondust periods, 56±17% (independent samples *t* test, $p<0.05$). These results signify that dust particles carry a substantial amount of bacterial cells on their surfaces to remote downstream areas.

On the other hand, the percentage of free-floating bacterial cells was higher than 70% in some cases during nondust periods (Table 1). In particular, the percentage ranged from 35% to 73% (49% 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, there were a substantial fraction of free-floating bacteria, 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 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). 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.



200 **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 (Wilcoxon signed rank test, 2-tailed 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= 0.349, p = 0.075) between the viability of particle-attached bacteria and the ratio of particle-attached bacteria to coarse particles (Tables 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} ($1.1–2.2 \times 10^5$ 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 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 comparisons, 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 as warm as −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 $3.2 \pm 2.1 \times 10^5$ cells m^{-3} averagely, occupied 72±9 % of the total bacteria. In contrast, this percentage was 56±17 % during nondust periods, with a concentration of $1.1 \pm 0.7 \times 10^5$ cells m^{-3}. The results indicate that dust particles conveyed substantial 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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Supplement of

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

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Text S1 Procedure for bacterial enumeration

The particles collected on the filter were transferred into phosphate buffered saline (PBS) in two steps. First, the filter was cut into four pieces and placed in a tube containing 10 mL of PBS. The saline was prefiltered through a 0.025 μm pore filter and autoclaved. The tube was vigorously shaken with a vortex shaker for two minutes. Then, the suspension underwent ultrasonic treatment for 15 minutes to detach as many particles from the filter as possible.

The bacterial particles in the suspension were chemically fixed and stained for fluorescence-microscopic counting. The chemical fixation was carried out with 1/25 volume of 25% glutaraldehyde for 30 minutes at 4°C. The fluorescent staining of bacterial particles was performed with the LIVE/DEAD BacLight Bacterial Viability Kit (Invitrogen™, Molecular Probes Inc., Eugene, Oregon). This kit labels bacterial cells with different fluorescent colors according to cell membrane injury; nonviable cells (those with injured membranes) were stained red, and viable cells (those without membrane injuries) were stained green (Fig. S3). The applicability of the fluorescent staining kit to airborne bacteria was introduced in Murata and Zhang (2013).

To make a slide for microscopic counting, the particles in the treated suspension were filtrated and condensed on a black polycarbonate filter (25 mm diameter and 0.2 μm pore size, Advantec®, Toyo Toshi Kaisha, Ltd., Japan). The filter was placed on a glass slide and covered with a drop of immersion oil and a cover glass. For each slide, we counted viable and nonviable bacterial cells in 20 microscopic fields of 100 μm × 100 μm each with a fluorescence microscope (Eclipse 80i, Nikon Corp., Tokyo, Japan). The bacterial concentration (C) in each size range of the Andersen sampler was calculated using the sum of 20 fields:

$$C_{total} = \frac{(N_{viable} + N_{nonviable}) \times S_{25} \times S_{stage}}{S_{count} \times S_{47} \times V_{air}}$$

$$C_{viable} = \frac{N_{viable} \times S_{25} \times S_{stage}}{S_{count} \times S_{47} \times V_{air}}$$

$$C_{nonviable} = \frac{N_{nonviable} \times S_{25} \times S_{stage}}{S_{count} \times S_{47} \times V_{air}}$$

where N is the number of bacterial cells in 20 fields, S_{count} is the area of 20 fields, S_{25} and S_{47} represent the areas of 25 mm and 47 mm diameter filters, respectively, S_{stage} is the area of each stage plate of the Andersen sampler, and V_{air} is the volume of the sample air. The bacterial concentrations of size-segregated airborne particles were described as $dC/d\log D_p$. The upper limit of the particle size was set to 20 μm because it is difficult for particles larger than 20 μm in aerodynamic diameter to remain airborne (Andreas et al., 1995;Mayol et al., 2014).

The designation of the experiment was modified gradually regarding the results obtained in each year. For the samples collected in 2013 and 2014, we counted bacterial cells only. For the samples collected in 2015, we also counted mineral dust-like particles (insoluble and with irregular shapes) in those collected during the occurrence and disappearance of dust events. In 2016, we counted the mineral dust-like particles and

bacterial cells in all samples. In addition, the probability for the overlapping of bacterial cells and mineral dust-like particles on the membranes for enumeration was quite small (several parts per thousand) and not considered.

Text S2 Resuspension of aerosol particles in Andersen samplers

Bulk aerosol particles were collected on 0.2 μm pore polycarbonate filters (47 mm; Merck Millipore Ltd., Cork, Ireland) using in-line holders for 24 hours. Three pairs of aerosol samples were collected during 29–30 May, 16–17 October, and 2–3 November 2014. After collection, one particle-loading filter of each pair was placed on the top stage (Stage 0) plate of the Andersen sampler, and sterilized new filters were set on the plates of stages 1 to 7. The sampler was vacuumed (28.3 L min^{-1}) in a clean hood consistently for sample collection for 24 hours. Then, the filters were treated, and the concentrations of bacterial cells in each stage were enumerated based on the LIVE/DEAD BacLight bacterial viability assay to evaluate the amount of bacteria that fell from the upper stage to the next stage. The results showed that approximately 32% of the bacteria fell from the original filters in stage 0 to lower stages, after which approximately a half (48 ± 15%) of the bacteria were trapped by stage 1 and approximately one fifth (22 ± 9%) of the bacteria were trapped by stage 2 (Figure S5-1). In addition, the estimated total bacterial concentrations of the Andersen sampler were compared with those determined using another holder, and the values were consistent (100±15%) with each other, indicating that bacteria smaller than 0.43 μm only accounted for a minor fraction of the free-floating bacteria.

The transfer of bacterial cells from stages 4 and 5 (nominally just over 1 μm) to stage 6 (nominally just under 1 μm) was also assessed. Two sets of bulk aerosol particles were collected on 0.2 μm pore polycarbonate filters (47 mm; Merck Millipore Ltd., Cork, Ireland) using in-line holders for 3, 12 and 24 hours, respectively in the indoor environment. One set of three samples were put on the Stage 4 plate of the Andersen sampler, and sterilized new filters were set on the plates of stages 5 to 7. Another set of three samples were put on the Stage 5 plate of the Andersen sampler, respectively, and sterilized new filters were set on the plates of stages 6 to 7. The sampler was vacuumed (28.3 L min^{-1}) in a clean hood consistently for sample collection for 3, 12 and 24 hours correspondingly. After vacuuming, all the filters were treated, and the concentrations of bacterial cells in each stage were enumerated based on the LIVE/DEAD BacLight bacterial viability assay. The results showed that on average less than 10% of the bacteria fell from the original filters in Stage 4 or 5 to lower stages (Fig. S5-2).

To assess whether the detected single-cell bacteria had fallen from the upper stages, we calculated the ideal cell sizes of airborne bacteria collected on the 0.65 μm stage. The 50% cut-off aerodynamic diameter (D_{p50}) was calculated using the following equation:

$$D_{p50} = \sqrt{\frac{18\mu\psi N\pi \cdot Dc^3 \cdot 60}{4CQ\rho}}$$

where μ is the viscosity coefficient of air (1.8×10^{-4} g cm^{-1} s^{-1}), ψ is the inertia parameter (0.14 for 50% impaction efficiency), N is the number (216) of jet nozzles of the stage, D_c is the diameter of each jet nozzle (0.025 cm), C is the Cunningham correlation factor ($1.00 + 0.16 \times 10^{-4}/D_p$), Q is the flow rate (28300 cm^3 min^{-1}), and ρ is the density of the particle. According to the buoyant density of bacterial cells, 1.03–1.24 g cm^{-3} (Bakken and Olsen, 1983;Bratbak and Dundas, 1984), the D_{p50} of the bacterial cells collected in this study was calculated as 0.58–0.64 μm. This size range is consistent with the sizes of cells under microscopic field, although we did not measure the size of all bacterial cells. Hara et al. (2011) previously measured the size distribution of airborne bacterial cells by fluorescence microscopy and image analysis and reported a mode size of approximately 0.6 μm, which is consistent with our results. Additionally, Huffman et al. (2012) detected a peak of fluorescent biological aerosol particles at approximately 0.7 μm and 3 μm using the ultraviolet aerodynamic particle sizer (UV-APS). These results support that a single bacterial cell could be suspended in the air and collected at the lowest stage of the Andersen sampler.

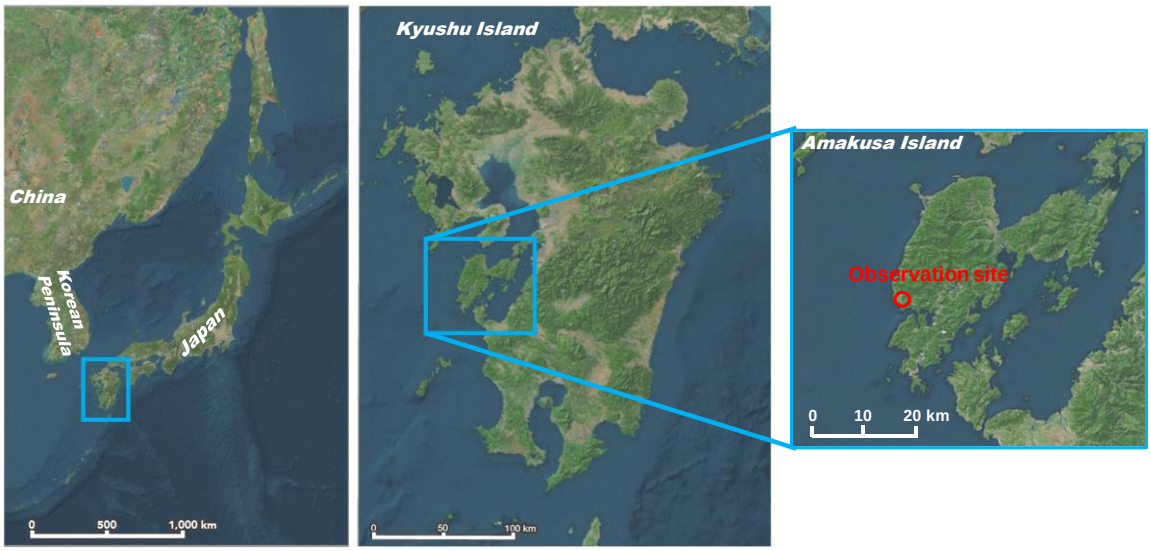

Figure S1. Location of the observation site. The map source is © Google Earth.

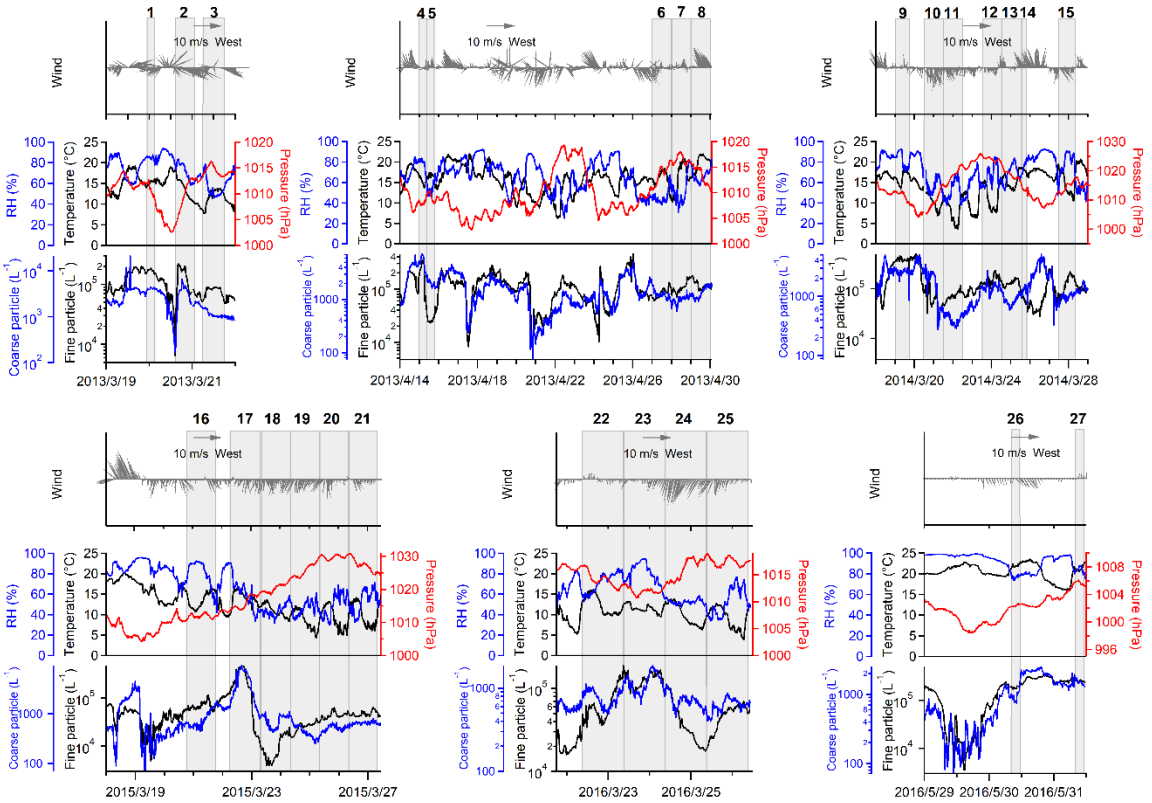

Figure S2. Time series of meteorological conditions and airborne particle number concentrations during the observations. Each sampling period is indicated with a gray shadow and the sequence number.

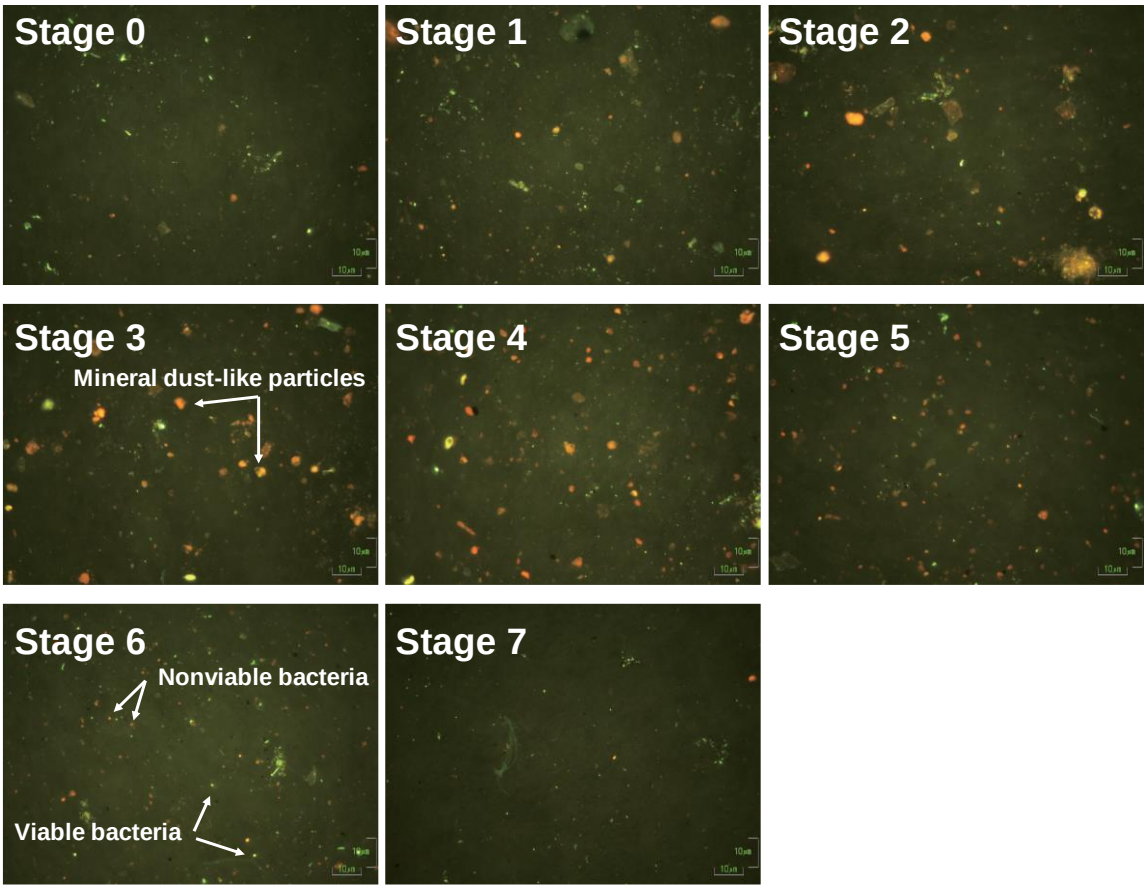

Figure S3. Images of one set of stained samples collected on 20 March 2013 (Sample 2D-Po, dust period) under epifluorescence microscopy field.

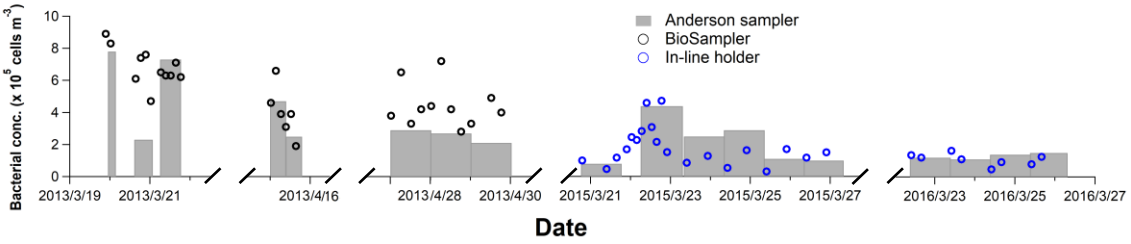

Figure S4. Comparison between the results of the Andersen sampler and the BioSampler (2013) / in-line holders (2015–2016). The flow rate for the BioSampler/holder was 10–12.5 L min^{-1}, and the collection duration was 1–2 hours. More details on sample collection with the BioSampler and the in-line holder are available in Murata and Zhang (2016) and Hu et al. (2017), respectively. The discrepancy between the results of the Andersen sampler and the other two samplers in some cases might have been caused by the different sampling durations and collection efficiencies of the samplers (e.g., for the Andersen sampler, there was a loss of bacteria due to bouncing, and the size fraction smaller than 0.43 μm was missing).

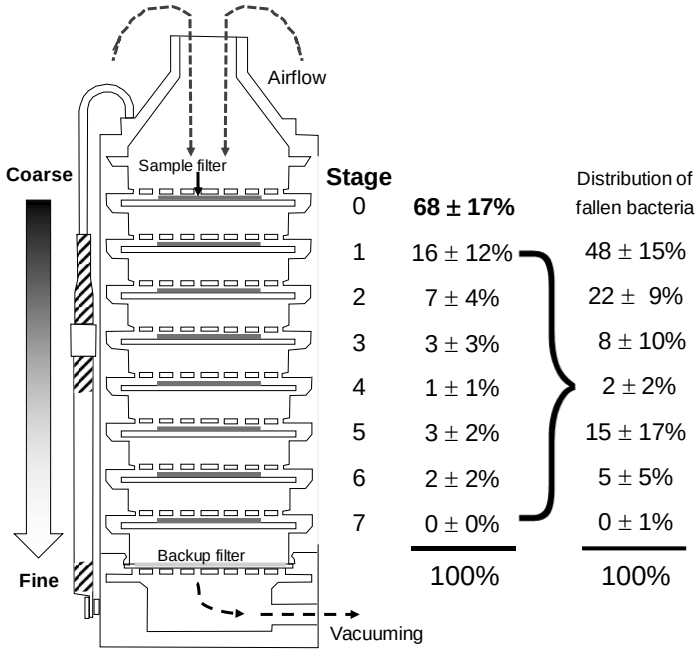

Figure S5-1. Percentages of residual bacterial cells after the sampler was run for 24 h. If no bacteria fall from the top stage, the percentage in bold should be nearly 100%. The actual percentage was 68%, indicating that 32% of the bacteria fell from the top stage. The distribution of fallen bacteria is shown in the right column. The fallen bacteria were mostly in the second and third stages and did not affect the lower stages of the sampler.

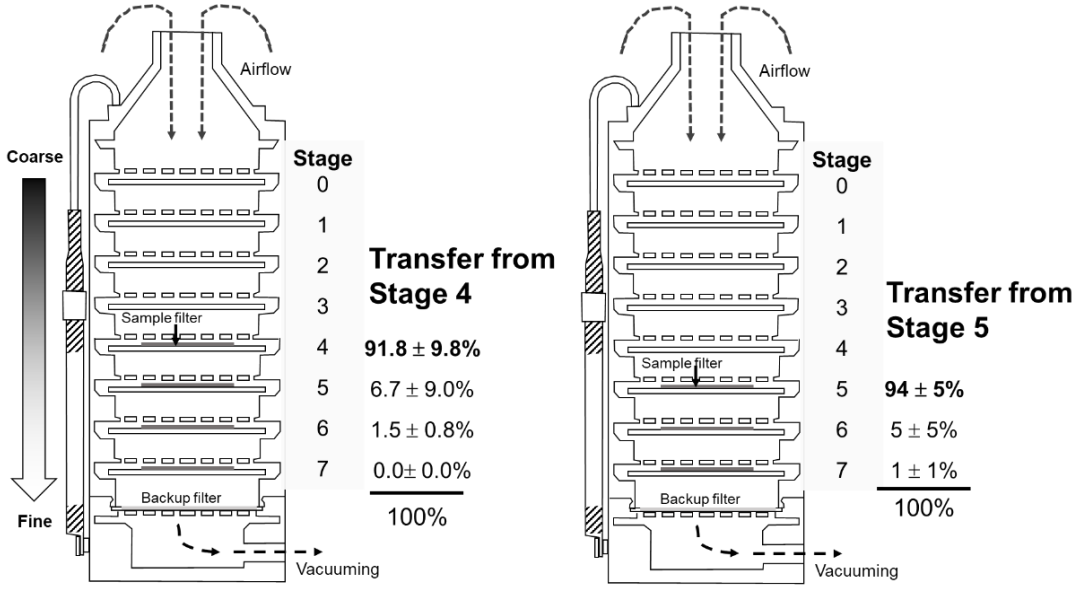

Figure S5-2. The distribution of bacterial cells fallen from Stage 4 (left) and Stage 5 (right) to lower stages. Averagely less than 10% of the bacteria fell from the original filters in Stage 4 or 5 to lower stages.

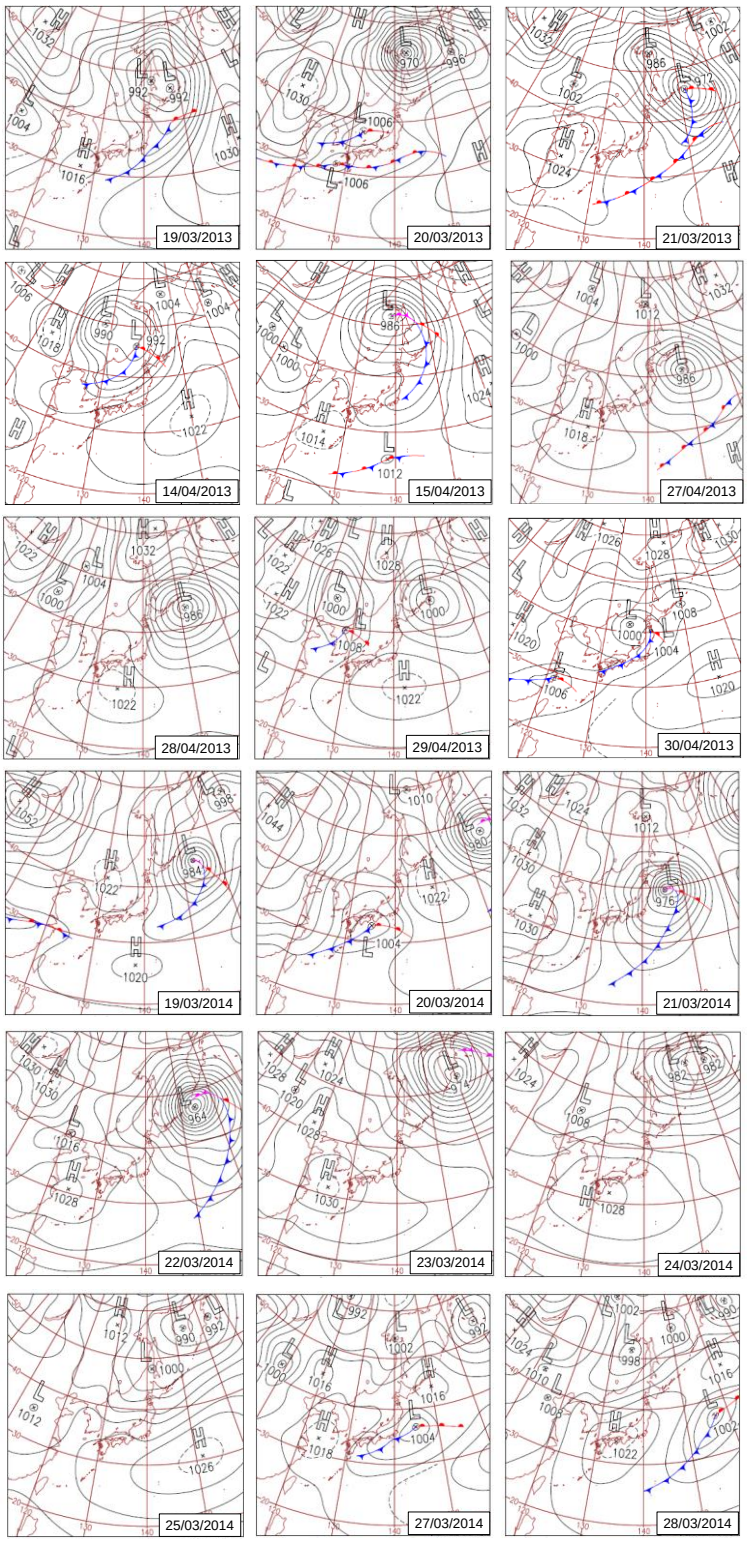

Figure S6-1. Daily weather charts (9:00 am JST every day) during the sampling periods. The data were downloaded from the Japan Meteorological Agency (http://www.data.jma.go.jp/fcd/yoho/hibiten/index.html).

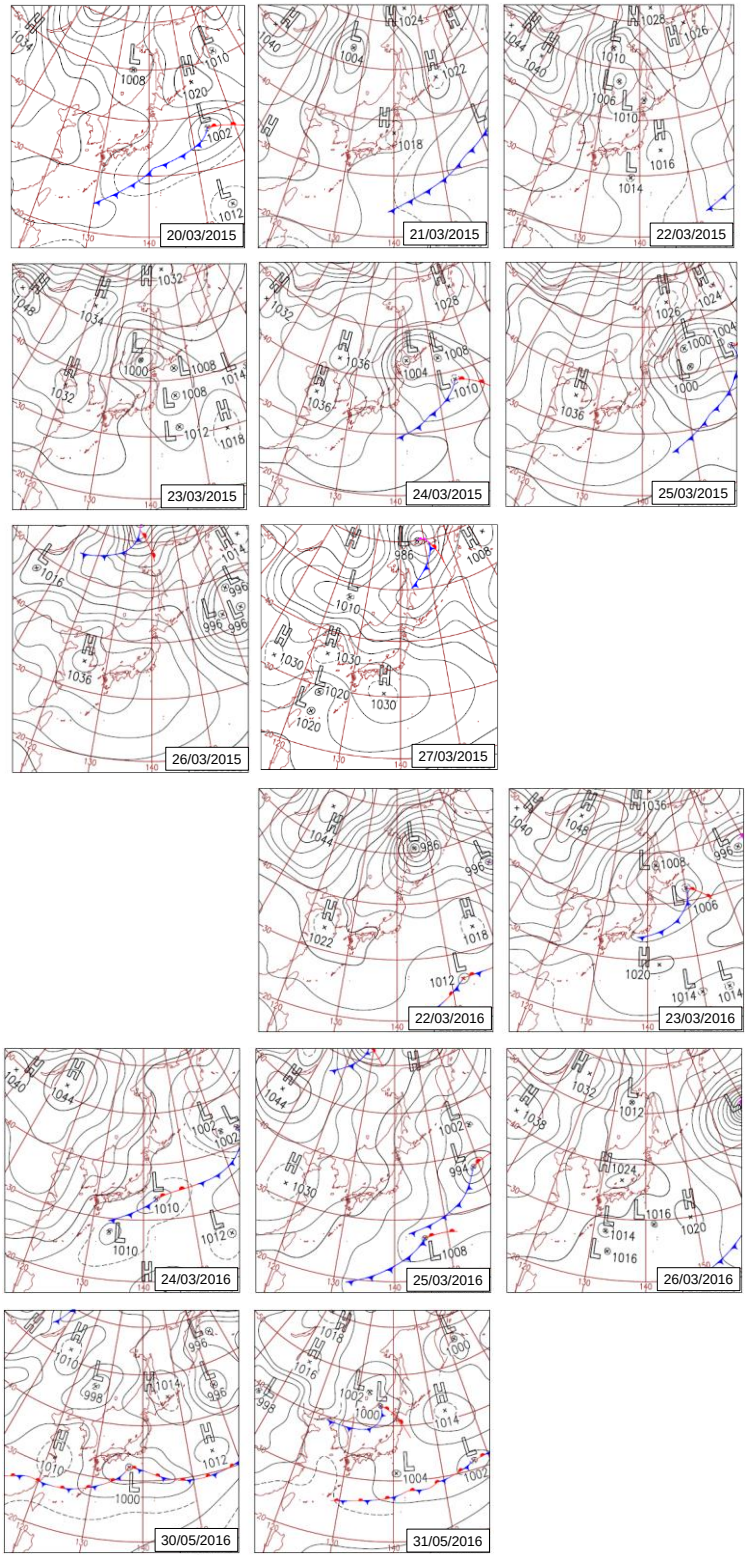

Figure S6-2. Continued.

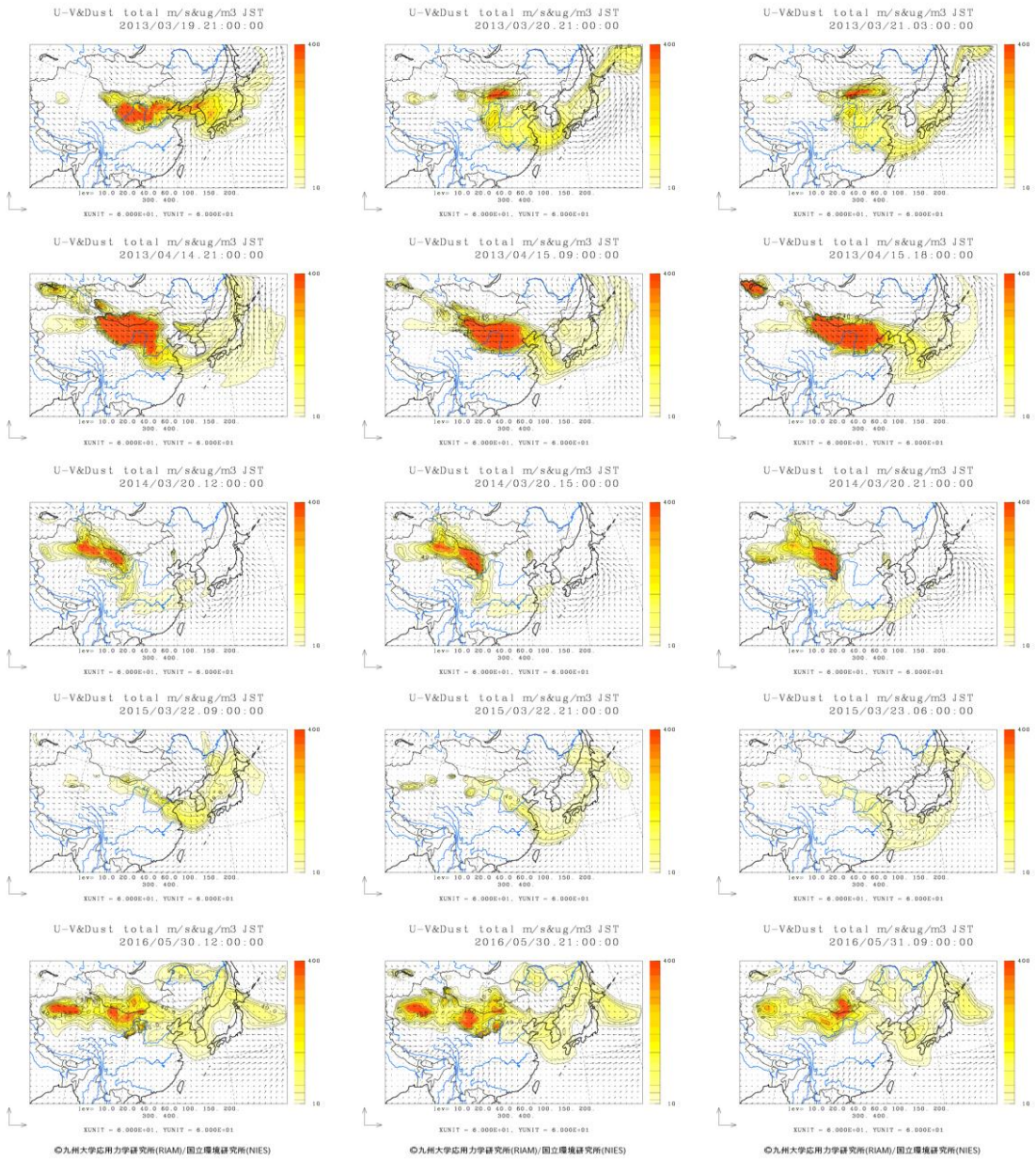

Figure S7. Spatial distributions of Asian dust in the east Asian region during the observed dust events. The data were forecasted by online RIAM/CGER/NIES-CFORS (Chemical weather FORecasting System; http://www-cfors.nies.go.jp/~cfors/).

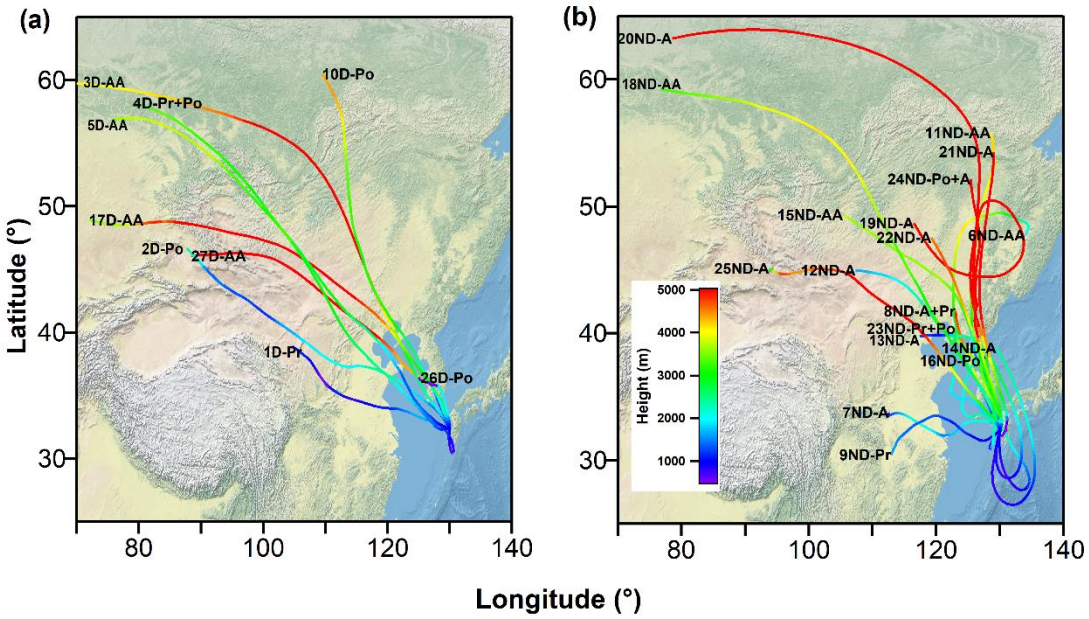

Figure S8. Seventy-two-hour backward trajectories of air parcels (http://ready.arl.noaa.gov/HYSPLIT.php) at 1000 m at the sampling site during the dusty (a) and nondust (b) periods. The sample ID of each sample is marked at the end of the corresponding trajectory. The map source is the IgorGIS package of IGOR Pro.

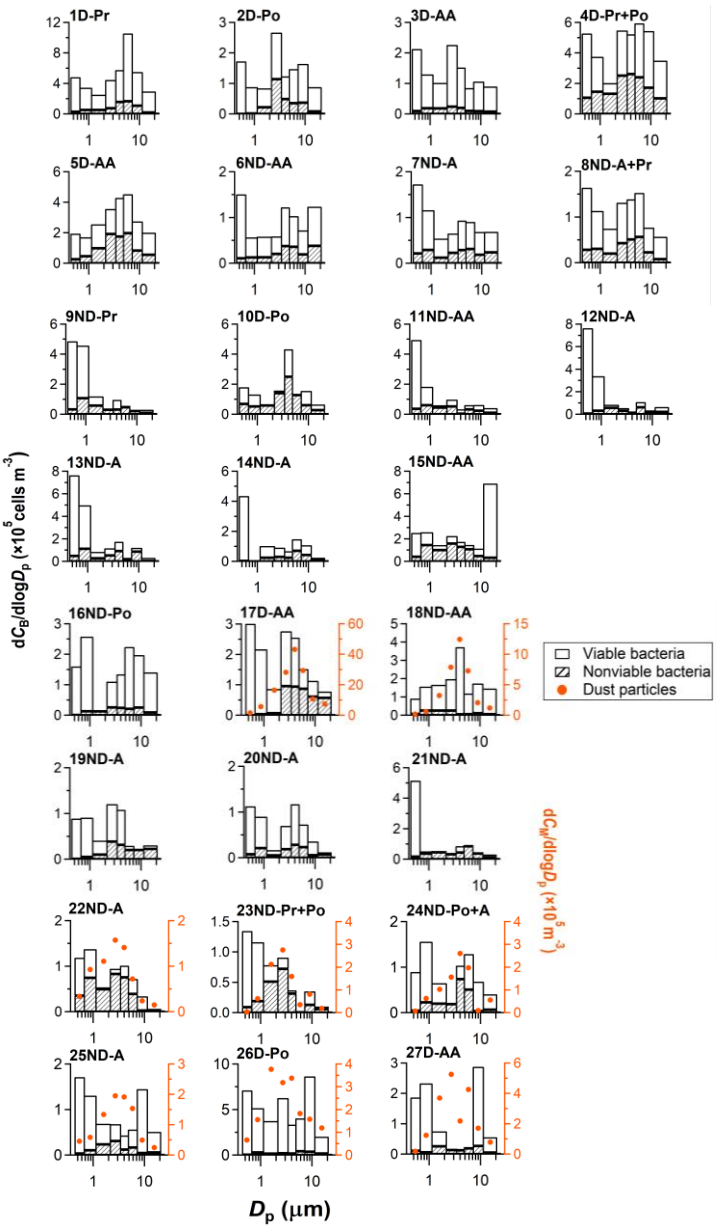

Figure S9. Concentrations of viable and nonviable bacteria (C_B) and mineral dust-like particles (C_M) in size-segregated airborne particles during the sampling periods. All *x* axes indicate particle aerodynamic diameter (D_p), left *y* axes indicate dC_B /dlog D_p (black), and right *y* axes indicate dC_M /dlog D_p (orange). The upper limit of particle size was set to 20 μm because it is difficult for particles larger than 20 μm in aerodynamic diameter to remain airborne (Andreas et al., 1995;Mayol et al., 2014). During dust periods (1D-Pr, 2D-Po, 3D-AA, 4D-Pr+Po, 5D-AA, 10D-Po, 17D-Po, 26D-Po, and 27D-AA), the size distribution of bacteria-associated particles generally showed two modes. During six cases of nondust periods (9ND-Pr, 11ND-AA, 12ND-A, 13ND-A, 14ND-A, and 21ND-A), bacteria-associated particles mainly distributed in the size fraction 0.43–1.1 μm. In samples from 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), except for Samples 15ND-AA and 18ND-AA, bacteria-associated particles showed bimodal size distributions.

Table S1. Information of samplings. 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	Year	Starting time (UTC+9:00)	Ending time (UTC+9:00)	Duration	Synoptic weather
1D-Pr	2013	19 Mar 22:50	20 Mar 03:00	04 h 10 min	Prefront
2D-Po		20 Mar 14:37	21 Mar 01:07	10 h 30 min	Postfront
3D-AA		21 Mar 06:00	21 Mar 18:00	12 h	Approaching anticyclone
4D-Pr+Po		14 Apr 23:55	15 Apr 09:02	09 h 07 min	Pre-/postfront
5D-AA		15 Apr 09:17	15 Apr 18:30	09 h 12 min	Approaching anticyclone
6ND-AA		27 Apr 00:00	28 Apr 00:00	24 h	Approaching anticyclone
7ND-A		28 Apr 00:01	29 Apr 00:01	24 h	Anticyclone
8ND-A+Pr		29 Apr 00:02	30 Apr 00:02	24 h	Anticyclone+prefront
9ND-Pr	2014	19 Mar 00:05	19 Mar 17:35	17 h 30 min	Prefront
10D-Po		20 Mar 12:00	21 Mar 12:00	24 h	Postfront
11ND-AA		21 Mar 12:00	22 Mar 12:10	24 h 10 min	Approaching anticyclone
12ND-A		23 Mar 12:40	24 Mar 12:40	24 h	Anticyclone
13ND-A		24 Mar 12:40	25 Mar 12:30	23 h 50 min	Anticyclone
14ND-A		25 Mar 12:30	25 Mar 19:45	07 h 15 min	Anticyclone
15ND-AA		27 Mar 11:23	28 Mar 08:40	21 h 27 min	Approaching anticyclone
16ND-Po	2015	20 Mar 18:10	21 Mar 18:10	24 h	Postfront
17D-AA		22 Mar 06:03	23 Mar 06:50	24 h 47 min	Approaching anticyclone
18ND-AA		23 Mar 07:58	24 Mar 08:00	24 h 02 min	Approaching anticyclone
19ND-A		24 Mar 08:03	25 Mar 08:02	23 h 59 min	Anticyclone
20ND-A		25 Mar 08:02	26 Mar 08:02	24 h	Anticyclone
21ND-A		26 Mar 08:05	27 Mar 07:30	23 h 25 min	Anticyclone
22ND-A	2016	22 Mar 09:04	23 Mar 09:02	23 h 58 min	Anticyclone
23ND-Pr+Po		23 Mar 09:00	24 Mar 09:00	24 h	Pre-/postfront
24ND-Po+A		24 Mar 09:00	25 Mar 09:00	24 h	Postfront/Anticyclone
25ND-A		25 Mar 09:00	26 Mar 07:00	22 h	Anticyclone
26D-Po		30 May 08:15	30 May 11:15	03 h	Postfront
27D-AA		31 May 07:50	31 May 10:50	03 h	Approaching anticyclone

Table S2. Data for Figures 1 and S9. Concentrations of viable (VB) and nonviable (NVB) bacteria (dC_B /dlog D_p, cells m^{-3}) and mineral dust-like particles (dC_M /dlog D_p, particles m^{-3}) in size-segregated airborne particles during the sampling periods.

Sample ID	Size range (μm) / Concentration	11-20	7-11	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	0.43-0.65
1D-Pr	130319NVB	1.9E+04	1.1E+05	1.7E+05	1.6E+05	7.5E+04	5.3E+04	5.3E+04	2.9E+04
	130319VB	2.8E+05	4.4E+05	8.9E+05	4.2E+05	3.7E+05	2.0E+05	2.9E+05	4.5E+05
2D-Po	130320NVB	8.3E+03	3.7E+04	3.5E+04	4.9E+04	1.1E+05	2.2E+04	1.6E+03	0.0E+00
	130320VB	8.0E+04	1.3E+05	1.1E+05	7.5E+04	1.5E+05	6.2E+04	8.6E+04	1.7E+05
3D-AA	130321NVB	7.6E+03	9.2E+03	1.0E+04	2.0E+04	2.4E+04	1.8E+04	1.9E+04	9.6E+03
	130321VB	8.3E+04	9.7E+04	7.5E+04	1.3E+05	2.0E+05	8.3E+04	1.1E+05	2.0E+05
4D-Pr+Po	130414NVB	1.0E+05	1.7E+05	2.4E+05	2.6E+05	2.5E+05	1.3E+05	1.5E+05	1.1E+05
	130414VB	2.5E+05	3.7E+05	3.5E+05	2.6E+05	3.0E+05	7.0E+04	2.3E+05	4.2E+05
5D-AA	130415NVB	5.6E+04	8.3E+04	2.0E+05	1.8E+05	1.9E+05	9.8E+04	4.6E+04	2.6E+04
	130415VB	1.5E+05	1.9E+05	2.6E+05	2.5E+05	1.6E+05	1.6E+05	1.2E+05	1.7E+05
6ND-AA	130427NVB	3.8E+04	1.9E+04	3.6E+04	3.7E+04	2.0E+04	1.3E+04	1.3E+04	1.1E+04
	130427VB	8.6E+04	5.3E+04	6.7E+04	8.5E+04	3.8E+04	4.5E+04	4.4E+04	1.4E+05
7ND-A	130428NVB	2.4E+04	1.8E+04	3.1E+04	2.9E+04	2.3E+04	1.2E+04	2.9E+04	2.1E+04
	130428VB	4.5E+04	5.0E+04	5.9E+04	6.4E+04	4.2E+04	4.2E+04	8.7E+04	1.5E+05
8ND-A+Pr	130429NVB	7.9E+03	2.3E+04	5.6E+04	5.1E+04	4.3E+04	2.0E+04	3.0E+04	2.8E+04
	130429VB	4.9E+04	5.4E+04	9.6E+04	8.8E+04	8.9E+04	5.4E+04	8.3E+04	1.4E+05
9ND-Pr	140319NVB	9.5E+03	2.1E+04	4.6E+04	3.2E+04	3.0E+04	5.8E+04	1.1E+05	3.2E+04
	140319VB	2.1E+04	0.0E+00	9.7E+03	6.4E+04	2.8E+03	6.1E+04	3.5E+05	4.5E+05
10D-Po	140320NVB	2.8E+04	6.0E+04	1.3E+05	2.5E+05	1.4E+05	5.9E+04	5.2E+04	6.9E+04
	140320VB	3.8E+04	9.5E+04	6.0E+02	1.8E+05	1.8E+04	5.2E+03	7.9E+04	1.1E+05
11ND-AA	140321NVB	1.0E+04	2.4E+04	3.3E+04	3.7E+03	5.2E+04	4.4E+04	6.0E+04	3.6E+04
	140321VB	3.1E+04	3.8E+04	2.8E+04	3.0E+04	4.5E+04	1.6E+04	1.2E+05	4.6E+05
12ND-A	140323NVB	2.1E+04	1.6E+04	6.3E+04	5.9E+03	3.0E+04	5.7E+04	3.1E+04	5.9E+03
	140323VB	4.3E+04	1.9E+04	4.5E+04	1.9E+04	2.5E+04	2.8E+04	3.1E+05	7.6E+05
13ND-A	140324NVB	4.1E+03	8.7E+04	2.0E+04	9.2E+04	5.3E+04	2.8E+04	1.1E+05	4.9E+04
	140324VB	2.7E+04	3.4E+04	0.0E+00	8.2E+04	6.3E+04	5.7E+04	3.9E+05	7.2E+05
14ND-A	140325NVB	1.6E+04	4.3E+04	7.1E+04	2.5E+04	3.0E+04	2.4E+04	0.0E+00	3.0E+03
	140325VB	0.0E+00	6.5E+04	7.7E+04	4.2E+04	6.1E+04	7.8E+04	0.0E+00	4.3E+05
15ND-AA	140327NVB	3.3E+04	4.7E+04	1.1E+05	1.3E+05	1.6E+05	1.0E+05	1.4E+05	4.1E+04
	140327VB	6.6E+05	6.6E+04	3.8E+04	4.4E+04	6.6E+04	4.5E+04	1.1E+05	2.1E+05
16ND-Po	150320NVB	9.2E+03	2.6E+04	2.1E+04	2.4E+04	2.6E+04	1.3E+04	1.3E+04	9.2E+02
	150320VB	1.3E+05	1.7E+05	2.0E+05	1.1E+05	8.4E+04	0.0E+00	2.5E+05	1.6E+05
17D-AA	150322Dust	7.3E+05	1.1E+06	2.9E+06	4.3E+06	2.8E+06	1.6E+06	5.7E+05	1.5E+05
	150322NVB	5.7E+04	6.1E+04	8.7E+04	9.4E+04	9.6E+04	6.8E+03	3.5E+03	0.0E+00
	150322VB	2.1E+04	5.2E+04	6.5E+04	1.6E+05	1.8E+05	7.9E+04	2.1E+05	3.0E+05
18ND-AA	150323Dust	1.2E+05	2.1E+05	7.3E+05	1.2E+06	7.9E+05	3.2E+05	5.8E+04	1.8E+04
	150323NVB	5.7E+03	1.1E+04	6.2E+03	5.3E+03	2.5E+04	2.6E+04	2.6E+04	7.3E+03
	150323VB	1.4E+05	1.6E+05	1.1E+05	3.7E+05	1.7E+05	1.4E+05	1.3E+05	8.4E+04
19ND-A	150324NVB	2.2E+04	2.0E+04	2.0E+04	3.2E+04	3.9E+04	9.9E+03	5.0E+03	9.2E+02
	150324VB	7.9E+03	0.0E+00	9.5E+03	7.7E+04	8.2E+04	3.1E+04	8.6E+04	8.8E+04
20ND-A	150325NVB	7.3E+03	5.4E+03	2.3E+04	2.8E+04	1.9E+04	5.3E+03	2.1E+04	7.8E+03
	150325VB	4.7E+03	3.0E+04	4.9E+04	8.9E+04	5.1E+04	1.1E+04	6.9E+04	1.0E+05
21ND-A	150326NVB	1.2E+04	3.7E+04	8.5E+04	4.4E+04	3.2E+04	4.5E+04	3.6E+04	1.7E+04
	150326VB	1.8E+04	0.0E+00	0.0E+00	4.2E+04	0.0E+00	0.0E+00	1.5E+04	5.0E+05
22ND-A	160322Dust	1.5E+04	2.4E+04	7.2E+04	1.4E+05	1.6E+05	1.1E+05	9.3E+04	3.3E+04
	160322NVB	3.5E+03	2.5E+03	3.9E+04	7.5E+04	8.3E+04	4.9E+04	7.4E+04	3.5E+04
	160322VB	0.0E+00	3.1E+04	3.3E+04	2.6E+04	1.1E+04	4.1E+03	6.3E+04	8.3E+04
23ND-Pr+P	160323Dust	1.8E+04	8.1E+04	3.5E+04	1.6E+05	2.8E+05	2.1E+05	6.1E+04	3.2E+03
	160323NVB	6.0E+03	1.3E+04	0.0E+00	3.2E+04	7.2E+04	5.1E+04	1.9E+04	9.2E+03
	160323VB	4.1E+03	2.2E+04	2.8E+03	5.3E+03	1.8E+04	2.7E+04	9.7E+04	1.3E+05
24ND-Po+A	160324Dust	5.6E+04	9.2E+03	2.0E+05	2.6E+05	1.6E+05	1.0E+05	6.2E+04	6.9E+03
	160324NVB	6.6E+03	3.3E+03	5.1E+04	7.4E+04	1.9E+04	2.0E+04	2.3E+04	4.6E+03
	160324VB	3.4E+04	6.5E+04	7.8E+04	2.9E+04	8.4E+02	4.5E+04	1.3E+05	8.5E+04
25ND-A	160325Dust	2.4E+04	4.9E+04	1.5E+05	1.9E+05	1.9E+05	1.3E+05	5.8E+04	4.5E+04
	160325NVB	5.9E+03	4.6E+03	1.7E+04	1.2E+04	3.1E+04	2.4E+04	1.1E+04	3.5E+03
	160325VB	4.5E+04	1.4E+05	3.9E+04	3.1E+04	3.7E+04	4.5E+04	1.2E+05	1.7E+05
26D-Po	160530Dust	1.2E+05	1.6E+05	1.8E+05	3.4E+05	3.2E+05	3.8E+05	1.6E+05	6.6E+04
	160530NVB	1.8E+04	3.7E+04	4.2E+04	1.7E+04	2.0E+04	1.6E+04	2.9E+04	3.7E+03
	160530VB	1.8E+05	8.3E+05	3.6E+05	3.2E+05	6.1E+05	3.6E+05	4.9E+05	7.1E+05
27D-AA	160531Dust	7.8E+04	1.7E+05	4.3E+05	2.2E+05	5.3E+05	3.7E+05	1.2E+05	1.8E+04
	160531NVB	5.1E+03	2.7E+04	1.9E+04	1.3E+04	1.3E+04	2.6E+04	5.8E+03	1.1E+04
	160531VB	5.1E+04	2.6E+05	0.0E+00	0.0E+00	0.0E+00	4.9E+04	2.3E+05	1.8E+05

Table S3. Data for Figure 2. Concentrations of viable (VB), nonviable (NVB), and total (TB) bacteria (C_B, cells m^{-3}) and mineral dust-like particles (C_M, particles m^{-3}) in size-segregated airborne particles.

Sample ID \ Size range (μm) / Concentration		11-20	7-11	4.7-7	3.3-4.7	2.1-3.3	1.1-2.1	0.65-1.1	0.43-0.65
17D-AA	150322Dust	1.9E+05	2.1E+05	5.1E+05	6.6E+05	5.5E+05	4.6E+05	1.3E+05	2.7E+04
	150322VB	5.3E+03	1.0E+04	1.1E+04	2.5E+04	3.5E+04	2.2E+04	4.9E+04	5.4E+04
	150322NVB	1.5E+04	1.2E+04	1.5E+04	1.4E+04	1.9E+04	1.9E+03	8.0E+02	0.0E+00
	150322TB	2.0E+04	2.2E+04	2.6E+04	3.9E+04	5.4E+04	2.4E+04	5.0E+04	5.4E+04
18ND-AA	150323Dust	3.1E+04	4.1E+04	1.3E+05	1.9E+05	1.5E+05	9.1E+04	1.3E+04	3.2E+03
	150323VB	3.6E+04	3.2E+04	2.0E+04	5.6E+04	3.4E+04	4.0E+04	3.0E+04	1.5E+04
	150323NVB	1.5E+03	2.2E+03	1.1E+03	8.2E+02	5.0E+03	7.2E+03	5.9E+03	1.3E+03
	150323TB	3.8E+04	3.4E+04	2.1E+04	5.7E+04	3.9E+04	4.7E+04	3.6E+04	1.6E+04
22ND-A	160322Dust	3.9E+03	4.7E+03	1.2E+04	2.2E+04	3.1E+04	3.1E+04	2.1E+04	6.0E+03
	160322VB	0.0E+00	6.2E+03	5.7E+03	3.9E+03	2.2E+03	1.2E+03	1.4E+04	1.5E+04
	160322NVB	9.0E+02	4.9E+02	6.7E+03	1.2E+04	1.6E+04	1.4E+04	1.7E+04	6.3E+03
	160322TB	9.0E+02	6.7E+03	1.2E+04	1.6E+04	1.9E+04	1.5E+04	3.1E+04	2.1E+04
23ND-Pr+Po	160323Dust	4.8E+03	1.6E+04	6.1E+03	2.4E+04	5.4E+04	5.9E+04	1.4E+04	5.7E+02
	160323VB	1.1E+03	4.4E+03	4.9E+02	8.2E+02	3.6E+03	7.6E+03	2.2E+04	2.2E+04
	160323NVB	1.6E+03	2.5E+03	0.0E+00	4.8E+03	1.4E+04	1.4E+04	4.4E+03	1.6E+03
	160323TB	2.6E+03	6.9E+03	4.9E+02	5.7E+03	1.8E+04	2.2E+04	2.7E+04	2.4E+04
24ND-Po+A	160324Dust	1.4E+04	1.8E+03	3.4E+04	4.0E+04	3.1E+04	2.9E+04	1.4E+04	1.2E+03
	160324VB	8.8E+03	1.3E+04	1.3E+04	4.5E+03	1.6E+02	1.3E+04	3.0E+04	1.5E+04
	160324NVB	1.7E+03	6.6E+02	8.8E+03	1.1E+04	3.7E+03	5.6E+03	5.2E+03	8.2E+02
	160324TB	1.1E+04	1.3E+04	2.2E+04	1.6E+04	3.9E+03	1.8E+04	3.6E+04	1.6E+04
25ND-A	160325Dust	6.4E+03	9.7E+03	2.7E+04	2.9E+04	3.8E+04	3.8E+04	1.3E+04	8.2E+03
	160325VB	1.2E+04	2.8E+04	6.8E+03	4.7E+03	7.3E+03	1.3E+04	2.7E+04	3.0E+04
	160325NVB	1.5E+03	9.0E+02	2.9E+03	1.9E+03	6.1E+03	6.6E+03	2.4E+03	6.3E+02
	160325TB	1.3E+04	2.8E+04	9.7E+03	6.6E+03	1.3E+04	1.9E+04	3.0E+04	3.1E+04
26D-Po	160530Dust	3.1E+04	3.1E+04	3.2E+04	5.2E+04	6.2E+04	1.1E+05	3.5E+04	1.2E+04
	160530VB	4.8E+04	1.6E+05	6.2E+04	4.9E+04	1.2E+05	1.0E+05	1.1E+05	1.3E+05
	160530NVB	4.6E+03	7.2E+03	7.2E+03	2.6E+03	3.9E+03	4.6E+03	6.6E+03	6.6E+02
	160530TB	5.3E+04	1.7E+05	7.0E+04	5.1E+04	1.2E+05	1.1E+05	1.2E+05	1.3E+05
27D-AA	160531Dust	2.0E+04	3.4E+04	7.4E+04	3.4E+04	1.0E+05	1.0E+05	2.8E+04	3.3E+03
	160531VB	1.3E+04	5.1E+04	0.0E+00	0.0E+00	0.0E+00	1.4E+04	5.2E+04	3.2E+04
	160531NVB	1.3E+03	5.3E+03	3.3E+03	2.0E+03	2.6E+03	7.2E+03	1.3E+03	2.0E+03
	160531TB	1.4E+04	5.6E+04	3.3E+03	2.0E+03	2.6E+03	2.1E+04	5.3E+04	3.4E+04

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