Atmos. Chem. Phys. Discuss.,
https://doi.org/10.5194/acp-2016-1095-AC1, 2017

Atmospheric Chemistry and Physics
Discussions

Interactive comment on "Variations in airborne bacterial communities at high altitudes over the Noto Peninsula (Japan) in response to Asian dust events" *by* Teruya Maki et al.

Teruya Maki et al.

makiteru@se.kanazawa-u.ac.jp

Received and published: 8 August 2017

Dear Anonymous Referee #1:

We thank for admitting the value of our manuscript very much. I take your comments into account in our revised manuscript. I revised our manuscript with paying attention to the points that you commented. The revised manuscript is attached as supplement file. I described my response for each your comment. The sections [Q] indicate your comments and the sections (A) indicate my responses. The changes introduced in the revised manuscript were indicated by the line numbers at the sections (A).

[Q] The authors should make it clearer to the readers what is dust and non-dust events. This should be emphasized in the figures (2, 3, 4, 5, 6, 7, 9); figure captions; table (I would recommend adding another column for that information); as well as in the result text. Otherwise the data presented is somehow confusing and not clear.

(A) The sampling days of dust or non-dust events have been indicated in Figures and Figure captions in the revised manuscript (Figures 2, 3, 4, 5, 6, and 7). Additional columns defining the dust event days have been inserted into Table 1.

[Q] It would be helpful to add some information on the DAPI-staining colors in the introduction part. Introducing these definitions only in the discussion (line 465) makes it hard to follow along the text beforehand.

(A) Some information on the DAPI-staining colors have been inserted in the Introduction section and the Experiment section in the revised manuscript (lines 89-1091.

[Q] line 103: It is specified that aerosol origin is from continental areas, however, trajectories and analysis shows marine contribution as well. please rephrase.

(A) As this decision, the explanations of aerosol origins over Noto Peninsula were rephrased in the revised manuscript (lines 121-122).

[Q] - line 120: How were the filter sterilized? please add either company cat. number, or sterilization technique.

(A) In the revised manuscript, we have added the information of filter and the filter -sterilization processes (lines 138-142).

[Q] - line 160: Please add the immersion oil type.

(A) The immersion oil type has been inserted in the revised manuscript (lines 181-182).

[Q] - line 174: Reference for the DNA extraction method: Authors should double check the ref., as the Maki 2008 paper refers to the Maki 2004... And - as in the 2004 paper the extraction is not from air filters, the authors should specify the extraction efficiency

Printer-friendly version

Discussion paper

from filters using this method in the current paper.

(A) Since gDNA amounts were not enough for the direct determination using light absorbance, the gDNA were determined the PCR products at the first PCR amplification. The extraction efficiency from filters were estimated by the comparison between the PCR products and the particle concentrations by DAPI count, indicating that more 90% of gDNA can be collected by this DNA extraction system. The detail explanations about the DNA extractions have been added to the section of Experiments in the revised manuscript (lines 229-235).

[Q] - section 3.3: The protease treatment is not detailed in the methodology. Although a very important examination, indicative for protein dominance is yellow particle, no documentation of such treatment and detection before and after treatment is presented. The authors should either supply such results and extend methodology, or remove this part.

(A) Although we already have possessed some results about the protease treatments of yellow particles, the data was not sufficient for demonstrating that all yellow particles are composed of protein. Moreover, I think the yellow particle fractions includes unknown organic components. Accordingly, in the revised paper, this part has been removed. The identification of yellow particles are further works.

[Q] - I find it very interesting that marine cyanobacteria contribute to the April 2013, March 2015 events etc. as was also observed by Lang-Yona et al., 2014. This could be relevant for the public health at low altitudes. Please add a discussion on the possible health effects of such species and other gram negative bacteria.

(A) Thank you for your suggestion and the information about valuable reference. We have discussed about the health effects by airborne cyanobacteria with referring to the suggested reference (lines 634-638).

[Q] - section 4.2: Organic particles might indeed represent dead bacteria and fungi, however also anthropogenic and natural SOA (especially when air transport over polluted areas, as in the current study). This should be emphasized in the discussion, as the statement (fraction of dead cells compared to total microbes) based on Fig. S4 could be misleading.

(A) Thank you for your suggestion. I agree to this comments. The anthropogenic and natural SOA were also included in the yellow fluorescent fractions. This topic has been discussed in the revised manuscript (lines 500-506).

[Q] - Line 513: I'm not convinced that cyanobacteria are significantly enriched in dust samples. As described in the result section, cyanobacteria were enriched also in non dust samples. The authors should supply arguments and statistical evidence for this statement.

(A) In the section of previous manuscript, I mistake to describe about cyanobacteria as the dust specific bacteria. Correctly, cyanobacteria are thought to be the bacterial populations in regardless of dust events and originated from marine environments. The name "cyanobacteria" has been removed at the section of dust-specific bacteria in the revised manuscript (lines 528-529).

[Q] - section 4.7: Assuming fluxes of specific bacteria as a representative for the origin of the air mass is a rough estimation and should not be made based on such a study with limited number of sampling points. For example, it is well established that the aerosolization of cyanobacteria would be dominant during bloom events. Therefore, if the authors make such statement of cyanobacteria represent marine-originated aerosols, they should supply evidence for presence of cyanobacteria in high altitudes seasonally and annually, and correlate with bloom events. In addition, one significant source of airborne cyanobacteria are the fresh water bodies. Many other factors affect the abundance of airborne microorganisms, and therefore I find it hard to accept such statement, where the presence of microbes will reflect the origin of the air mass accurately. Authors are requested to restrain their assumption.

Printer-friendly version

Discussion paper

(A) I agree to your comments. We need sufficient information obtained from more numbers of air samples and detail discussion for establishing the air-mass tracking by bacterial compositions. Then this section has been removed and the shortage description about the tracking idea was indicated in the section of Conclusion (lines 659-672).

[Q] - line 671: Please supply reference for this statement.

(A) This parts have been eliminated, because this description about bioaerosol tracking have been shortened and removed to the Conclusion section.

Technical corrections:

[Q] - Section 2.7 should be 2.5.

(A) Section 2.7 has been revised to 2.5 (line 251).

[Q] - line 361-363: Please rewrite this sentence.

(A) I have revised this sentence (lines 378-381).

[Q] - line 421: ": : :their abundance fluctuated between from: : :" please check phrasing.

(A) Sorry for mistake. I have revised this phrase (line 435).

[Q] - line 483: .."ranged from 23.3: : :" – consider rephrasing.

(A) I have rephrased this section in the revised manuscript (lines 495-496).

[Q] - line 505: Mazar et al. reported dust microbial composition over east Mediterranean areas (not European). Please correct.

(A) I'm sorry for errors. " European " has been revised to " east Mediterranean areas " (line 519).

[Q] - Line 513: Please check if "Figure 4" in the text should be corrected.

(A) Sorry for mistake. I have changed to "Figure 4" (line 529).

[Q] - Figure 2 – Caption: should be corrected for black particles denoted in grey color.

(A) The caption has been revised to indicate the matching color (line 1002).

[Q] - Figure 8b: Authors should better defined symbols. It is not clear (from both legend and caption) what are the blue circles (Are they dust samples? non-dust?) The authors should also add information on the statistics significance of the unifrac test. Consider adding dispersion ellipses with 95% standard deviation confidence interval.

(A) I agree to your comment. The definition for each sample was not clear. After the characteristics of samples have been improved to be defined, Figure 8b and its figure caption has been revised to eliminate the confusion relating to symbols (Figure 8b).

[Q] - Figure S4: Please specify in caption/legend what the black and white bars indicate.

(A) The caption of Figure S4 has been improved in the revised manuscript (Figure S4).

Please also note the supplement to this comment:
https://www.atmos-chem-phys-discuss.net/acp-2016-1095/acp-2016-1095-AC1-supplement.pdf

---

Interactive comment on Atmos. Chem. Phys. Discuss., https://doi.org/10.5194/acp-2016-1095, 2017.

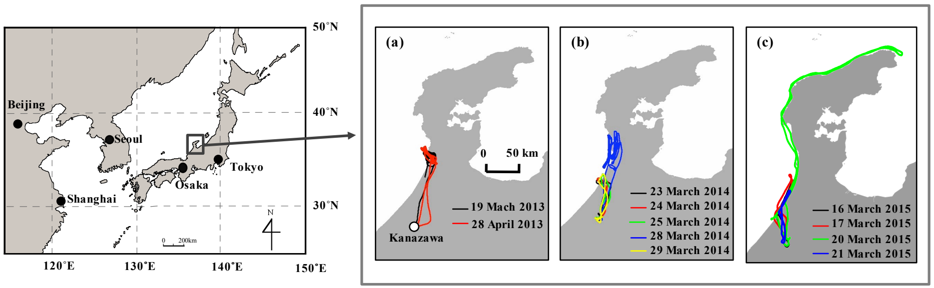

Fig. 1. Revised Figure 1

Printer-friendly version

Discussion paper

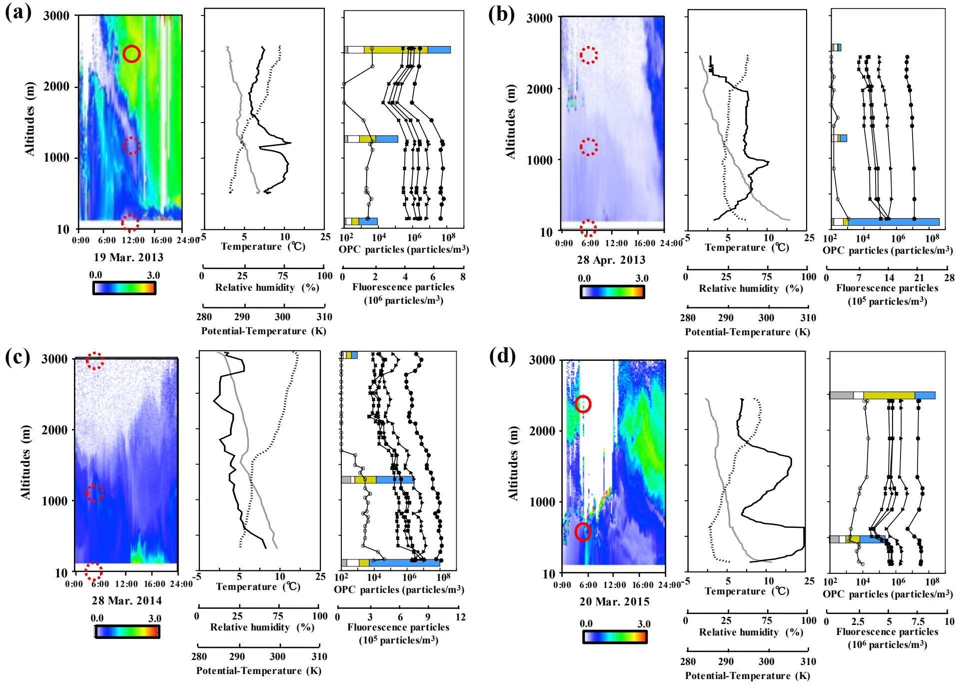

Fig. 2 T. Maki et al.

Fig. 2. Revised Figure 2

Printer-friendly version

Discussion paper

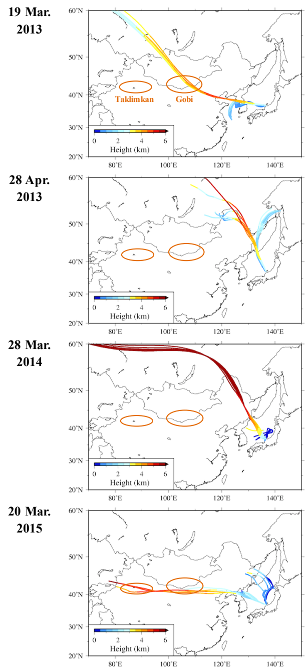

Fig. 3 T.Maki et al.

Fig. 3. Revised Figure 3

Printer-friendly version

Discussion paper

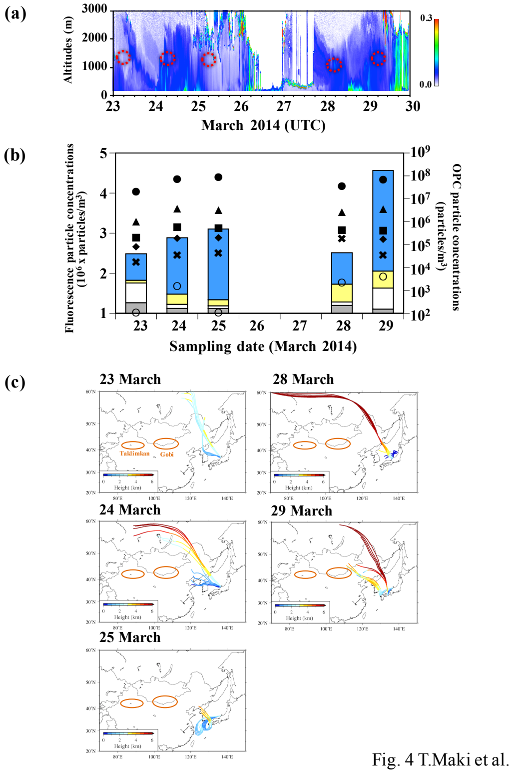

Fig. 4. Revised Figure 4

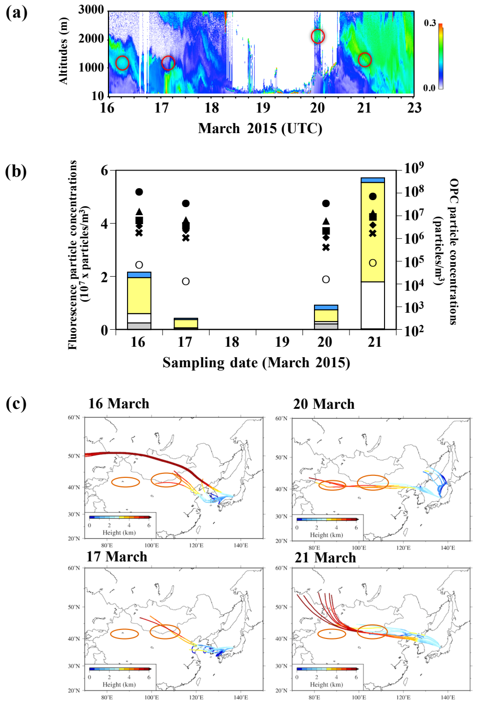

Fig. 5 T.Maki et al.

Fig. 5. Revised Figure 5

Printer-friendly version

Discussion paper

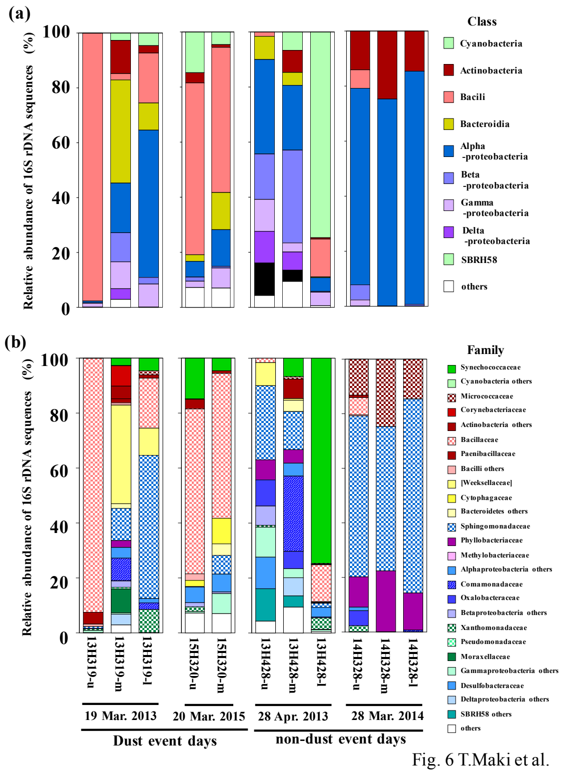

Fig. 6. Revised Figure 6

Printer-friendly version

Discussion paper

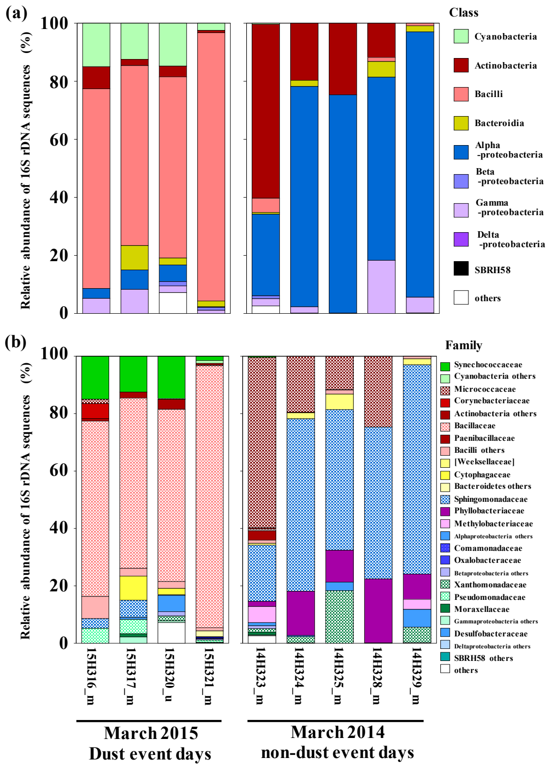

Fig. 7 T.Maki et al.

Fig. 7. Revised Figure 7

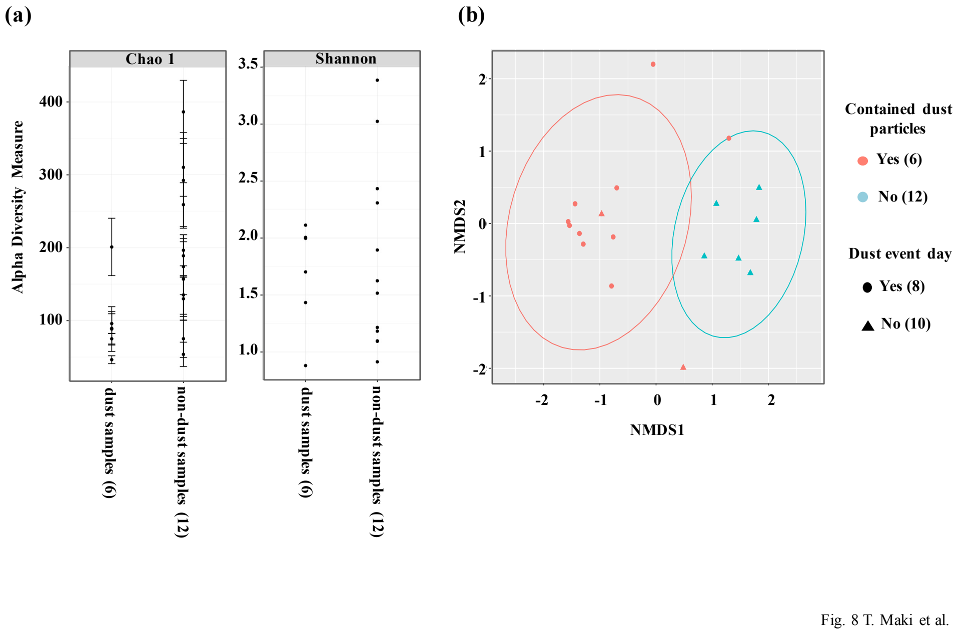

Fig. 8. Revised Figure 8

Table 1 Sampling information during the sampling periods.

Sample name	Sampling date	Collection time (JST)	Total time (min)	Air volume	Sampling method	Sampling location[*1]	Free troposphere[*2]
13H319-u	19 March 2013	14:04 – 15:04	60	700 L	helicopter	2500m	FT
13H319-m		15:19 – 16:19	60	700 L	helicopter	1200m	ABL
13H319-l		14:25 – 15:25	60	700 L	building	10m	GL
13H428-u	28 April 2013	12:10 – 13:04	56	653 L	helicopter	2500m	FT
13H428-m		13:13 – 14:03	50	583 L	helicopter	1200m	ABL
13H428-l		12:03 – 13:03	60	700 L	building	10m	GL
14H328-u	28 March 2014	12:50 – 13:50	60	700 L	helicopter	3000m	FT
14H328-m		14:04 – 15:04	60	700 L	helicopter	1200m	ABL
14H328-l		13:00 – 14:00	60	700 L	building	10m	GL
15H320-u	20 March 2015	12:26 – 13:23	47	548 L	helicopter	2500m	FT
15H320-m		13:39 – 14:40	60	711 L	helicopter	500m	ABL
14H323-m	23 March 2014	10:45 – 11:02	17	11.1 L	helicopter	1200m	ABL
14H324-m	24 March 2014	9:09 – 9:30	21	13.7 L	helicopter	1200m	ABL
14H325-m	25 March 2014	9:31 – 9:50	29	18.9 L	helicopter	1200m	ABL
14H328-m	28 March 2014	14:04 – 15:04	60	700 L	helicopter	1200m	ABL
14H329-m	29 March 2014	9:06 – 9:24	15	9.75 L	helicopter	1200m	PT
15H316-m	16 March 2015	11:21 – 11:43	22	14.3 L	helicopter	1200m	FT
15H317-m	17 March 2015	11:04 – 11:31	27	17.6 L	helicopter	1200m	FT
15H320-u	20 March 2015	12:26 – 13:23	47	548 L	helicopter	2500m	FT
15H321-m	21 March 2015	15:35 – 15:55	20	13.0 L	helicopter	1200m	FT

*1 Height above the ground.

*2 Free troposhere: FT, Atmospheric boundary layer: ABL, Phase transiens: PT, GL: Ground level

Fig. 9. Revised Table 1

Table 2. Researches targeting bacterial communities associated with Asian-dust events

Sampling area[*1]	Sample	Location	Altitudes (m)	Sampling place	Sampling method	Analytical method for microorganisms	Dominated bacteria[*2] 1st	2nd	3rd	references
Dust source area	Soil	Taklamakan Desert, China	0	Ground surface	soil sampling	clone libarary	Bacteroidetes (Sphingobacteriia)	Actinobacteria (Actinobacteria)	Proteobacteria (Alpha, Beta, Gamma)	Yamaguchi et al. 2012
Dust source area	Soil	Gobi Desert, China	0	Ground surface	soil sampling	clone libarary	Actinobacteria (Actinobacteria)	Proteobacteria (Beta)	Bacteroidetes (Sphingobacteriia)	Yamaguchi et al. 2012
Dust source area	Soil	Taklamakan Desert, China	0	Ground surface	soil sampling	pyrosequencing	Firmicutes (Bacilli)†	Actinobacteria	Proteobacteria (Gamma)	An et al. 2013
Dust source area	Soil	Gobi Desert, China	0	Ground surface	soil sampling	pyrosequencing	Firmicutes (Bacilli)†	Proteobacteria (Gamma)	Bacteroidetes	An et al. 2013
Dust source area	Soil	Taklamakan, China	0	Ground surface	soil samples	clone libarary	Actinobacteria (Actinobacteria)	Firmicutes (Bacilli)	Proteobacteria	Puspitasari et al. 2016
Dust source and deposition area	Soil	Loess plateau, China	0	Ground surface	soil sampling	clone libarary	Proteobacteria (Beta, Gamma)	Actinobacteria (Actinobacteria)	Bacteroidetes (Sphingobacteriia)	Yamaguchi et al. 2012
Dust source and deposition area	Soil	Loess plateau, China	0	Ground surface	soil sampling	PCR-DGEE	Proteobacteria	Bacteroidetes	Gemmatimonadetes	Kenzaki et al. 2010
Dust source area	Air	Tsogt-Ovoo, Mongolia	3	Ground surface	filtration	MiSeq sequencing	Proteobacteria (Alpha)	Firmicutes (Bacilli)	Actinobacteria (Actinobacteria)	Maki et al. 2017
Dust source area	Air	Dunhuang, China	10	Top of building	filtration	clone libarary	Firmicutes (Bacilli)†	Proteobacteria	Bacteroidetes	Puspitasari et al. 2016
Dust source area	Air	Dunhuang, China	800	Balloon	filtration	PCR-DGEE	Firmicutes (Bacilli)†	-	-	Maki et al. 2008
Dust source area	Air	Dunhuang, China	800	Balloon	filtration	clone libarary	Proteobacteria (Gamma)	Firmicutes (Bacilli)	-	Kakikawa et al. 2009
Dust deposition area	Air	Noto peninsula, Japan	3000	Aircraft	filtration	clone libarary	Firmicutes (Bacilli)†	Bacteroidetes (Bacteroidia)	Proteobacteria (Gamma)	Maki et al. 2013
Dust deposition area	Air	Noto peninsula, Japan	3000	Aircraft	filtration	MiSeq sequencing	Firmicutes (Bacilli)†	Actinobacteria (Actinobacteria)	Proteobacteria (Alpha&Beta)	Maki et al. 2015
Dust deposition area	Air	Mt. Bachelor Observatory, USA	2700	Mt. Bachelor	filtration	culture	Firmicutes (Bacilli)†	Actinobacteria (Actinobacteria)	Proteobacteria (Gamma)	Smith et al. 2012
Dust deposition area	Air	Mt. Bachelor Observatory, USA	2700	Mt. Bachelor	filtration	Microarray	Proteobacteria (Beta&Gamma)	Actinobacteria (Actinobacteria)	Firmicutes (Bacilli)†	Smith et al. 2013
Dust deposition area	Snow	Mt. Tateyama, Japan	2450	Mt. Tateyama	Snow sampling	PCR-DGEE	Firmicutes (Bacilli)†	Proteobacteria (Beta, Gamma)	Actinobacteria (Actinobacteria)	Tanaka et al. 2011
Dust deposition area	Snow	Mt. Tateyama, Japan	2450	Mt. Tateyama	Snow sampling	PCR-DGEE	Firmicutes (Bacilli)†	Proteobacteria (Beta)	Actinobacteria (Actinobacteria)	Maki et al. 2011
Dust deposition area	Air	Noto peninsula, Japan	1200	Helicopter	filtration	MiSeq sequencing	Firmicutes (Bacilli)†	Proteobacteria (Alpha, Gamma)	Cyanobacteria	This study
Dust deposition area	Air	Suzu, Japan	1000	Balloon	filtration	MiSeq sequencing	Firmicutes (Bacilli)†	Proteobacteria (Alpha)	Deinococcus-Thermus (Deinococci)	Maki et al. 2015
Dust deposition area	Air	Osaka, Japan	900	Air craft	filtration	clone libarary	Firmicutes (Bacilli)	Bacteroidetes (Sphingobacteriia)	Actinobacteria (Actinobacteria)	Yamaguchi et al. 2012
Dust deposition area	Air	Suzu, Japan	800	Balloon	filtration	clone libarary	Firmicutes (Bacilli)†	Bacteroidetes (Bacteroidia)	Proteobacteria (Gamma)	Maki et al. 2013
Dust deposition area	Air	Suzu, Japan	600	Balloon	filtration	PCR-DGEE	Firmicutes (Bacilli)†	-	-	Maki et al. 2010
Dust deposition area	Air	Seoul, South Korea	25	Top of building	liquid impiger	pyrosequencing	Actinobacteria (Actinobacteria)	Proteobacteria (Alpha, Gamma)	Firmicutes (Bacilli)†	Cha et al. 2017
Dust deposition area	Air	Osaka, Japan	20	Top of building	filtration	pyrosequencing	Actinobacteria (Actinobacteria)	Cyanobacteria	Acidobacteria (Acidobacteria)	Park et al. 2016
Dust deposition area	Air	Seoul, South Korea	17	Top of building	filtration	PCR-DGEE	Actinobacteria (Actinobacteria)	Firmicutes (Bacilli)†	Proteobacteria (Gamma)	Lee et al. 2011
Dust deposition area	Air	Beijing, China	15	Top of building	filtration	pyrosequencing	Firmicutes (Bacilli)	Proteobacteria (Gamma)	Bacteroidetes (Flavobacteriia)	Wei et al. 2016
Dust deposition area	Air	Beijing, China	10	Top of building	filtration	HiSeq sequencing	Actinobacteria (Actinobacteria)	Proteobacteria (Alpha, Beta, Gamma)	Chloroflexi (Thermomicrobia)	Cao et al. 2014
Dust deposition area	Air	Seoul, South Korea	10	Top of building	filtration	clone libarary	Firmicutes (Bacilli)†	Actinobacteria	Bacteroidetes	Jeon et al. 2011
Dust deposition area	Air	Suzu, Japan	10	Top of building	filtration	MiSeq sequencing	Firmicutes (Bacilli)†	Deinococcus-Thermus (Deinococci)	Proteobacteria (Alpha)	Maki et al. 2015
Dust deposition area	Air	Goyang, South Korea	-	Top of building	filtration	pyrosequencing	Actinobacteria (Actinobacteria)	Proteobacteria (Gamma)	Firmicutes (Bacilli)†	Cha et al. 2016
Dust deposition area	Air	Kanazawa, Japan	10	Roof of building	filtration	MiSeq sequencing	Firmicutes (Bacilli)†	Cyanobacteria	Proteobacteria (Alpha)	Maki et al. 2014
Dust deposition area	Air	Western Pacific Ocean	-	Ship board	filtration	pyrosequencing	Firmicutes (Bacilli)†	Proteobacteria (Beta, Gamma)	Cyanobacteria	Xia et al. 2015

*1 Dust source area: the areas providing dust mineral particles, Dust deposition area: the area where the dust mineral paticles deposit

*2 The bacterial phyla in the orders of large abundance rates in each samples.

Fig. 10. Revised Table 2

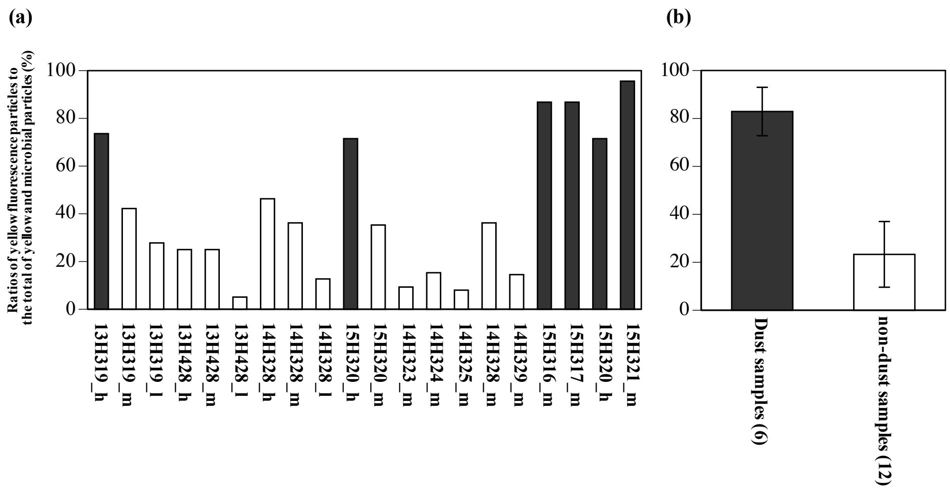

Fig. S4. Ratios of yellow fluorescence particles to the total of yellow and microbial particles. (a) The bioaerosol samples were collected at the three or two altitudes over the Noto Peninsula on 19 March 2013 (LT), 28 April 2013 (LT), 28 March 2014 (LT), and 20 March 2015 (LT) and at the altitudes of 1,200 m (except for the 500 m of 20 March 2015) over the Noto Peninsula from 16 to 23 March in 2015 (LT), and from 23 to 29 March in 2014 (LT). Dust samples and non-dust samples were indicated using black bars and white bars, respectively. (b) The average ratios of Dust samples and non-dust samples.

Fig. 11. Revised Figure S4

Printer-friendly version

Discussion paper