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Effects of Asian dust events on atmospheric bacterial communities at different distances downwind of the source region

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ABSTRACT

Aeolian dust particles arising from arid and semiarid zones are known to carry microbes by air currents. The effect of wind-borne bacteria on atmospheric bacterial population at various downwind distances from the dust source regions must be clarified, but has not yet been reported. This study monitored the bacterial abundance and community composition in outdoor aerosol samples in Beijing, China, which is close to the Asian dust source regions, and compared them with the results obtained in a distant region (Osaka, Japan). The Asian dust collected in Beijing contained $(4 \pm 3) \times 10^4$ bacterial cells/m³, approximately 4 times higher than in Osaka. On 15 April 2015, Beijing experienced severe Asian dust events with a 1000-fold increase in bacterial abundance, relative to non-Asian dust days. Dominant bacterial phyla and classes in Asian dust collected in Beijing were Actinobacteria, Bacilli and Acidobacteria, and the bacterial community composition varied more widely than in Osaka. The bacterial community compositions differed between the Beijing and Osaka dusts, even for the same Asian dust events. These results indicated that aerosol bacterial communities nearer the dust source are more affected by eolian dust than their distant counterparts.

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Introduction

Aeolian dust is recognized as a main vehicle of intercontinental bacterial migration by atmospheric currents. Bacteria attached to aeolian dust particles were directly observed by bio-imaging (Yamaguchi et al., 2012), and bacteria transported from arid and semi-arid regions can impact on public health and ecosystems (Griffin et al., 2001, 2003; Griffin, 2007; Lim et al., 2011). Worldwide aeolian dust occurred 0.5–5.0 billion tons in every year (Perkins, 2001), and scattered around the world. Asian dust is one of the

major aeolian dust, as Australian dust and African dust. Asian dusts affect not only East Asia (China, Korea and Japan), but can also reach North America, more than 15,000 km from the source region (Duce et al., 1980; Kellogg and Griffin, 2006; Smith et al., 2013). Several previous studies investigated the effects of Asian dust events on atmospheric microorganisms of downwind area. These studies carried out analyses of bacterial community composition (Jeon et al., 2011), abundance and viability estimates (Hara and Zhang, 2012), as well as investigations of atmospheric halotolerant bacterial communities (Maki et al., 2010). However,

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a microbiological comparative analysis of aeolian dust events at varying scales and source distances has not been reported. The Asian dust fallout is estimated as 180 g/(m²·year) in Beijing, China (500–2500 km from the dust source regions) (Nishikawa et al., 2002) and 0.005–0.05 g/(m²·year) in Osaka (3000–5000 km from the dust source regions) (Yoshinaga, 1998). In addition, because aeolian dust is lifted by updraft and transported by air current, its effect should depend on distance from the dust source region. The transport of aeolian dust is influenced by climatic conditions such as air pressure, wind direction and wind velocity, which depend on the ground conditions (Rosenfeld et al., 2001; Chkhetiani et al., 2012); moreover, larger dust particles are more difficult to transport over long distances. A previous report confirmed that 55% of the microbial cells detected on Asian dust particles were attached to larger particles (>5 µm), while 7% of them resided on smaller particles (1–2 µm) (Yamaguchi et al., 2012).

For these reasons, the microbial effect on downwind environments may depend on both the scale of the aeolian dust and the distance from the dust source regions. To assess the possible impacts by the bacteria attached to aeolian dust particles and the change of their community composition by the long-range movement, monitoring is required in downwind areas with different distance from Asian dust source regions.

The aim of study is assessment the effects of Asian dust events on atmospheric bacterial communities at different downwind distances from the source regions. To this end, we continuously monitored the bacterial abundance and community composition on various outdoor aerosol samples in Beijing collected during the 2015 Asian dust season (from April to June; on both Asian dust days and non-Asian dust days) for a general assessment of Asian dust events. We then compared the variations of bacterial abundances and community compositions in outdoor aerosol samples collected in Beijing and Osaka (which are close to and distant from the Asian dust source regions, respectively). Airborne bacterial abundances and community structures were determined by 16S rRNA gene-targeted quantitative PCR and amplicon sequencing (Yoo et al., 2017).

1. Materials and methods

1.1. Sample collection

In this study, for a general assessment of influence of bacteria transported by Asian dust event, we continuously monitored bacterial abundance and community composition of outdoor aerosols collected under different atmospheric conditions for three months in Asian dust season.

Fifty-seven outdoor aerosol samples were collected on a second-floor roof (height ca. 5 m) at the China Agricultural University in Beijing (latitude: 40°00'14.9" N, longitude: 116°21'10.8" E) using a high-volume air sampler (HV500R; SIBATA, Saitama, Japan), from 14 April to 19 June in 2015. Data in Osaka were quoted from our published data (Park et al., 2016) (Table S1). Air samples were collected onto 0.6-µm pore-size glass fiber filters at 500 L/min. During each sampling event (200 min), aerosol particles were captured from 100 m³ of ambient air.

The occurrence of atmospheric Asian dust was confirmed and their severities were assessed from the increased mass of the glass fiber filter after sampling and also the visibility at the sampling location (Fig. S1). Visibility data was obtained at China Air Dairy (<http://www.chinaairdaily.com/>). The Asian dust observed in Beijing on 15 April 2015 reached Osaka on 18 April 2015 (Fig. 1).

1.2. DNA extraction

Aerosol samples collected on the glass filter were pulverized by bead-beating (4800 r/min, 90 sec) with EZ-Beads (EZ, Tokyo, Japan). Genetic DNA (gDNA) was then extracted and purified as described by Tsai and Olson (1991). The extracted gDNA was subsequently purified using a Wizard DNA Clean-Up System kit (Promega, Madison, WI, USA) and eluted with 50 µL of TE buffer (10 mM Tris-HCl and 1 mM EDTA [pH 8.0]). No DNA contamination was detected on a blank filter by quantitative PCR and DNA gel electrophoresis.

1.3. Estimation of bacterial abundance

To determine the bacterial abundance, 16S rRNA gene was quantified by real-time PCR using a LightCycler (Roche Diagnostics, Mannheim, Germany). Real-time PCR was performed with eubacterial primer sets as described by Yamaguchi et al. (2012). To generate the standard curve for quantifying the 16S rRNA gene, PCR products of *E. coli* W3110 were used as the gDNA template (1×10^1 to 1×10^7 copies per reaction). As the copy number of the 16S rRNA gene differed among the bacterial phyla, the bacterial abundance was calibrated by a phylum-level analysis of the bacterial community composition (Park et al., 2016).

1.4. Analysis of bacterial community composition

The 16S rRNA gene was amplified for pyrosequencing by two-step PCR (Sutton et al., 2013). Two-step PCR increases the reproducibility and recovers higher genetic diversity during amplicon sequencing than one-step PCR (Berry et al., 2011; Ichijo et al., 2016). Using this approach, tags and adapters were added during a second round of PCR amplification. Second round of PCR amplification was performed with 968F (AACGCGAAGAACCTTAC) and 1401R (CGGTGTGTACAAGACCC) sets as described by Ichijo et al. (2016). Amplicons were sequenced using Ion PGM (Thermo Fisher Scientific KK, Yokohama, Japan) at the Center for Medical Research and Education, Osaka University (Osaka, Japan). The raw sequence data of the obtained amplicons were screened, trimmed, and filtered using the default settings of QIIME pipeline version 1.9.1 (<http://qiime.org/>). Over 278000 sequences were obtained across all samples (averaging 5100 sequences per sample). Total operational taxonomic units (OTUs), defined at the 97% nucleotide-sequence identity level using the UCLUST function of QIIME software (Caporaso et al., 2010), were identified in all sequences. On average, approximately 2800 OTUs per sample were recovered. The community composition differences among the samples were graphically assessed by principal coordinate analysis (PCoA) using the unweighted pair group method. Finally, the bacterial community composition was calibrated by the copy

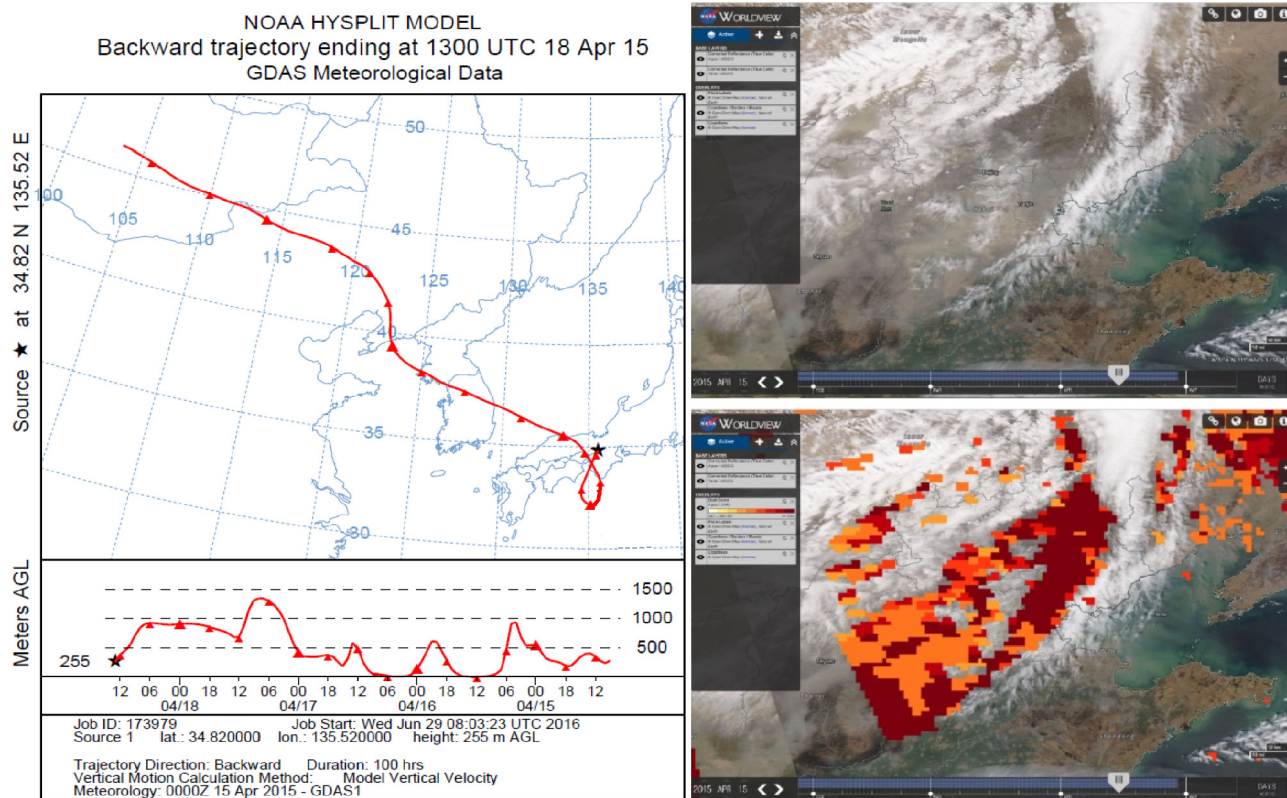


Fig. 1 – The geographic origin of Asian dust was determined by back trajectory analysis (left; <http://ready.arl.noaa.gov/HYSPLIT.php>) and was rendered in EODIS Worldview (right; <https://worldview.earthdata.nasa.gov/>).

number of the 16S rRNA gene of each phylum and class (Park et al., 2016).

1.5. Nucleotide sequence accession numbers

The sequences obtained from the amplicon sequencing were deposited in the DNA Data Bank of Japan Sequence Read Archive under the accession number DRA004472.

2. Results and discussion

2.1. Comparisons of bacterial abundance variation during an Asian dust season in Beijing and Osaka

During the Asian dust event, the bacterial abundance in Beijing's outdoor environment was approximately 40 times higher than on non-Asian dust days (Asian dust days: $(4 \pm 3) \times 10^4$ cells/m³, non-Asian dust days: $(1 \pm 0.5) \times 10^3$ cells/m³; $p = 0.01$) (Fig. 2).

The bacterial abundances on non-Asian dust days were not significantly different in Beijing and Osaka ($(1 \pm 0.5) \times 10^3$ cells/m³). The Asian dust event altered the bacterial abundance to a greater extent in Beijing than in Osaka (Beijing: $(4 \pm 3) \times 10^4$ cells/m³, Osaka: $(1 \pm 0.2) \times 10^4$ cells/m³; $p = 0.04$). Although the bacterial abundance on Asian dust days generally exceeded 10^4 cells/m³ in both areas, high bacterial concentrations ($>2 \times 10^4$ cells/m³) were confirmed in Beijing on some days (14, 15, 26, 30 April and 5 May 2015), but were

not observed in Osaka. The bacterial concentration in Beijing was especially high on 15 April 2015 (10^6 cells/m³), coinciding with the exceptional severity of the Asian dust. On that day, the concentration of the bacteria transported to Beijing was approximately 100 times higher than on other Asian dust days, and 1000 times higher than on non-Asian dust days. The same Asian dust event increased the bacterial abundance in Osaka to 10 times its level on non-Asian dust days. Previous studies reported results that atmospheric bacterial abundance in downwind areas tended to increase approximately 10–100 times by Asian dust events (Hara and Zhang, 2012; Cha et al., 2016). By severe Asian dust events like occurred on 15 April 2017, atmospheric bacterial abundance will significantly increase compared to other Asian dust days in the same region.

2.2. Comparisons of bacterial community compositions in Beijing and Osaka during the Asian dust season

To analyze the similarities in the bacterial community compositions of different samples (including those collected in Osaka), we processed the amplicon sequencing data using QIIME software (Caporaso et al., 2010), and displayed the results on a principal coordinate analysis (PCoA) plot (Fig. 3). The clustering differed between Asian and non-Asian dust days in Beijing, but not in Osaka. The bacterial community compositions on Asian dust days were more closely related in Beijing than in Osaka. The bacterial community compositions

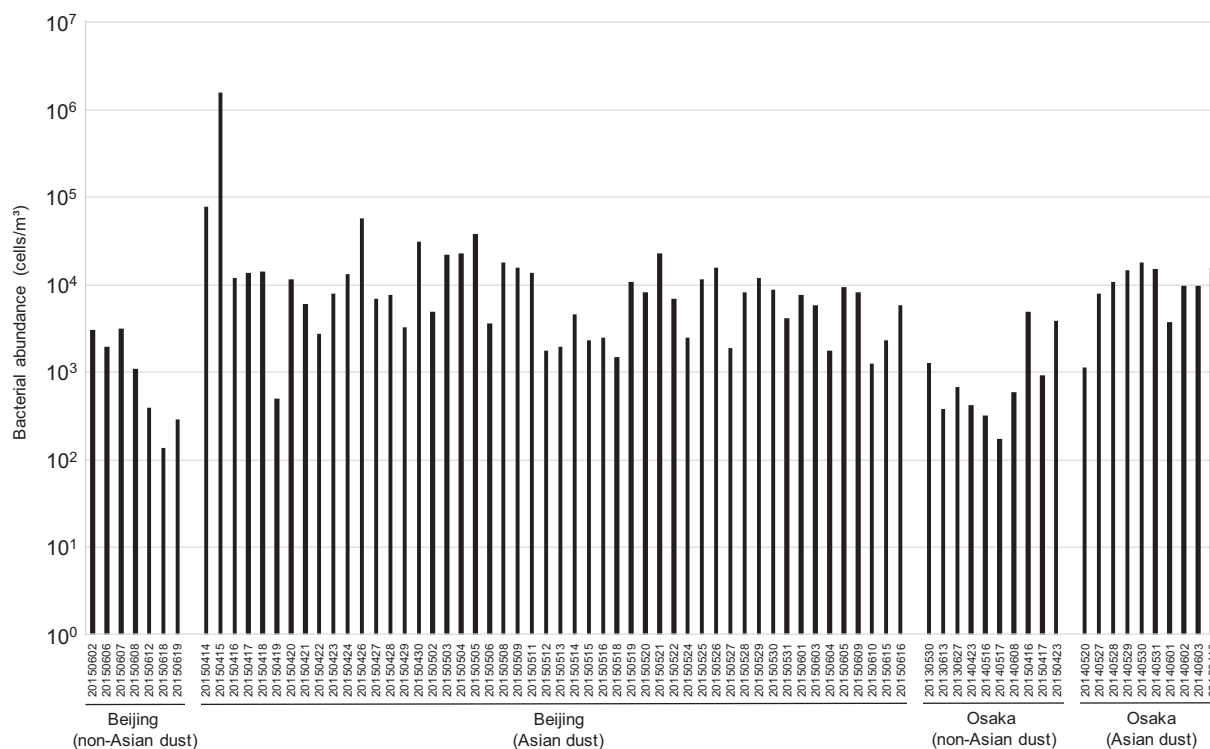


Fig. 2 – Total bacterial abundance determined by quantitative PCR targeting the 16S rRNA gene. Samples were collected during the 2015 Asian dust season in Beijing and Osaka. Included are non-Asian dust days and Asian dust days. Data in Osaka were quoted from our published data (Park et al., 2016).

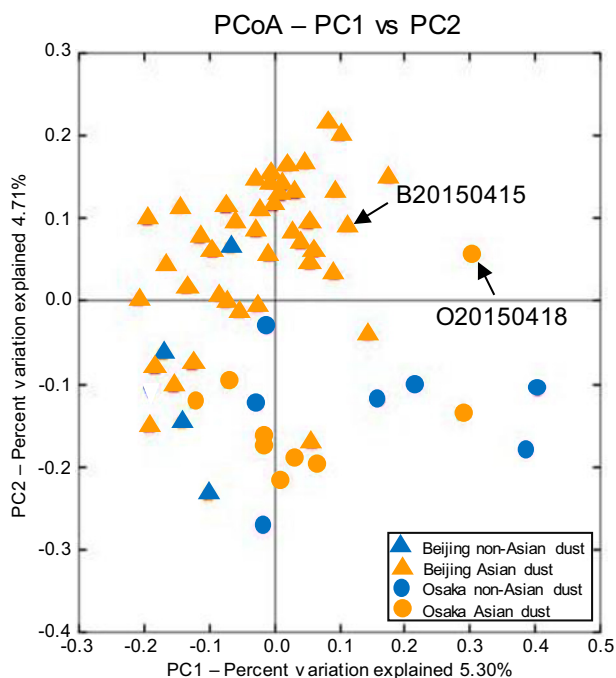


Fig. 3 – Principal coordinates analysis (PCoA) of bacterial community composition based on the 16S rRNA gene sequences obtained from aerosols collected in outdoor environments. B20150415: Asian dust collected in Beijing, 15 April 2015. O20150418: Asian dust collected in Osaka, 18 April 2015.

of these two Asian dust samples were more similar than the 15 April sample collected in Beijing and the samples collected in Osaka on days excluding 18 April 2015 (PCoA distance 15 April in Beijing with 18 April in Osaka: 0.198 ± 0.007). However, similarity of the community compositions in Beijing samples between the 15 April sample and samples collected on days excluding 15 April 2015 was higher than that between the 15 April sample collected in Beijing and the 18 April sample collected in Osaka (PCoA distance 15 April in Beijing with the other Asian dust samples in Beijing: 0.183 ± 0.016 ; distance 15 April in Beijing with 18 April in Osaka: 0.198). As a result, it was suggested that atmospheric bacterial community composition in the region near the dust source regions is rather similar during Asian dust season. On the other hands, in the long distance region from the source regions, impact of bacteria transported by Asian dust events is reduced or changed on atmospheric bacterial community.

During the Asian dust event, the bacterial phyla/classes that dominated more than 10% of the total community of aerosol samples collected in Beijing changed from *Actinobacteria* ($27 \pm 5\%$), *Cyanobacteria* ($11\% \pm 2\%$), *Bacilli* ($14\% \pm 3\%$), *Alphaproteobacteria* ($14\% \pm 3\%$), *Betaproteobacteria* ($11\% \pm 2\%$) to *Acidobacteria* ($11\% \pm 1\%$), *Actinobacteria* ($28\% \pm 1\%$) and *Bacilli* ($22\% \pm 1\%$) (Fig. 4). The *Bacilli* and *Clostridia* levels were increased by Asian dust events in Beijing (*Bacilli*: $p = 0.028$, *Clostridia*: $p = 0.00027$). *Alphaproteobacteria*, *Betaproteobacteria*, *Cyanobacteria* levels were decreased by Asian dust events in Beijing (*Alphaproteobacteria*: $p = 0.00002$, *Betaproteobacteria*: $p = 0.021$, *Cyanobacteria*: $p = 0.023$).

When the Asian dust events increased the atmospheric bacterial abundance in Beijing to over 10^4 cells/m³, *Alphaproteobacteria*, *Betaproteobacteria*, *Cyanobacteria* was occupied in non-Asian dust days (Fig. S3). Moreover, On April 15 in Beijing, where large dust storms occurred, these three phyla/classes were almost dismissed (Fig. S2). *Alphaproteobacteria* is often associated with plant body or surfaces (Fümkrantz et al., 2008), and members of *Alphaproteobacteria* cannot maintain their viabilities in the atmosphere, where environmental stressors such as temperature changes, UV irradiances, and extreme desiccation damaged bacterial cells (Maki et al., 2017). Meanwhile, *Betaproteobacteria* is well known that they require a copious amount of organic nutrients, and therefore, it is difficult to exist in low nutrient environments.

In the case of *Cyanobacteria*, their abundance was influenced by the period rather than by Asian dust events. *Cyanobacteria* increased from the end of May, regardless of Asian dust events (before 20 May: $2\% \pm 0.4\%$, after 20 May: $9\% \pm 2\%$; $p = 0.000013$) (Fig. S2). This bacterium can be found in aquatic habitats (oceans, fresh water, etc.) as well as damp soil, therefore, we inferred that *Cyanobacteria* is not dominant of Asian dust.

In Osaka, the Asian dust occurrence changed the dominant phyla/classes of aerosol samples from *Acidobacteria* ($23\% \pm 16\%$), *Actinobacteria* ($13\% \pm 6\%$) and *Bacilli* ($20\% \pm 16\%$) to *Acidobacteria* ($13\% \pm 6\%$), *Actinobacteria* ($25\% \pm 9\%$), *Cyanobacteria* ($15\% \pm 8\%$) and *Bacilli* ($11\% \pm 7\%$) (Fig. 4). That is, the Asian dust event altered the diversity of the airborne bacterial community composition more in Beijing than in Osaka. In Beijing, the Asian dust increased the levels of spore forming bacteria such as *Bacilli* (from $14\% \pm 3\%$ to $22\% \pm 1\%$) and *Clostridia* (from $2\% \pm 1\%$ to $6\% \pm 1\%$). Many studies have reported similar abundances of *Bacilli* and *Clostridia* in the source region of Asian dust and in downwind regions in China (An et al., 2013; Yuan et al., 2017).

As mentioned above, the Asian dust observed on 15 April 2015 in Beijing reached Osaka on 18 April 2015 (Fig. 1). Therefore, we conducted a comparative analysis of the bacterial community compositions in both areas (Fig. 5). The bacterial communities in Beijing and Osaka were dominated by the same groups (*Acidobacteria*, *Actinobacteria* and *Bacilli*). These phyla/classes comprised over 70% of the whole bacterial community. However, during the transport from Beijing to Osaka, *Acidobacteria* decreased from 38% to 20%, while *Actinobacteria* and *Bacilli* increased from 20% to 30% and from 14% to 23%, respectively. The other phyla/classes were not significantly changed.

Acidobacteria especially dominated the Beijing bacterial community during the severe 15 April 2015 event (Fig. 5). In addition, when the Asian dust days increased the bacterial abundance to over 10^4 cells/m³, the *Acidobacteria* concentration was higher than on non-Asian dust days (increasing from $7\% \pm 1\%$ to $16\% \pm 3\%$) (Fig. S3). *Acidobacteria* is especially distributed in soils (Janssen, 2006), and dominates in arid and semi-arid regions (Chanal et al., 2006; Yuan et al., 2014; Kutovaya et al., 2015). Bacteria attached to Asian dust particles are assumed to be affected by different environments from the source regions along the transfer route, and the bacterial community should change during the transportation. In this study, during the long-range transportation of Asian dust particles from Beijing to Osaka, the percentage of *Acidobacteria* in the total bacterial community decreased while the *Actinobacteria* and *Bacilli* levels increased. *Actinobacteria* has high tolerance to environmental stresses and inhabits extreme environments such as hypersaline lakes, thermal springs, and arid soils. *Bacilli* survive stressful environments by forming spores. Because most bacteria transported by aeolian dust experience ultraviolet exposure, reduced nutrient availability, desiccation and other stresses, the stress-tolerant groups *Actinobacteria* and *Bacilli* may persist while other phyla/classes diminish.

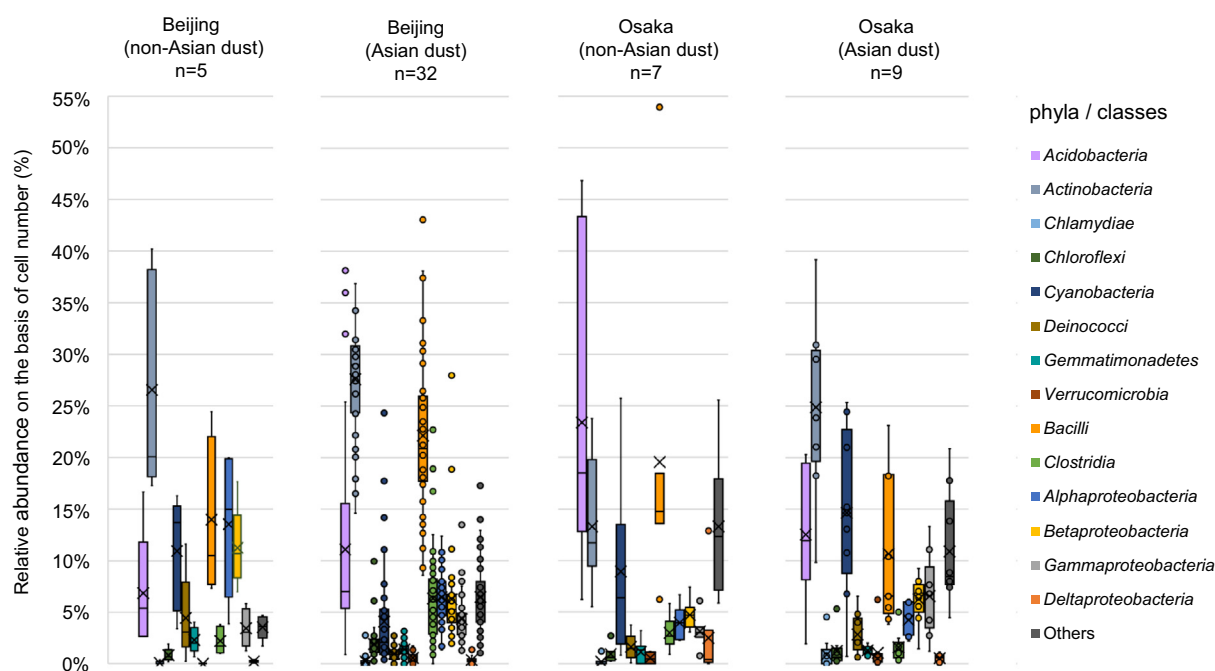


Fig. 4 – Relative abundances of the common phyla and classes in outdoor airborne samples. Data were classified by their sampling points and Asian dust events.

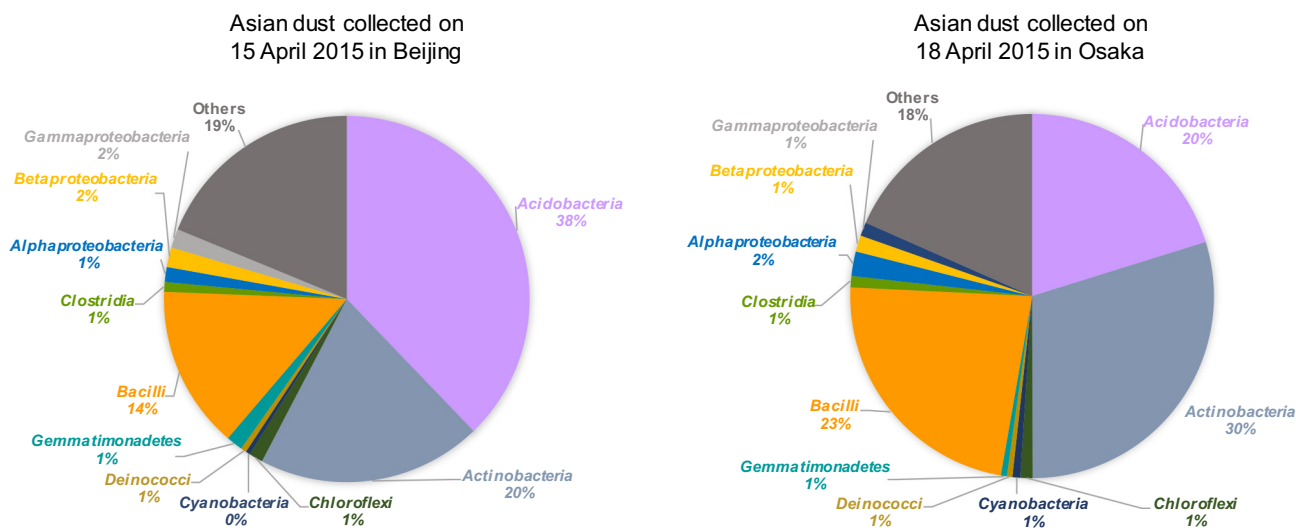


Fig. 5 – Taxonomic compositions of Asian dust samples collected in Beijing and Osaka during the same Asian dust events (15 and 18 April, 2015). Compositions were estimated from the relative abundances of bacterial 16S rRNA gene sequences assigned to the common phyla and classes.

3. Conclusions

We collected aerosol samples in Beijing (700 km from the dust source region) and Osaka, Japan (3200 km from the dust source region) and evaluated the changes in the outdoor airborne bacterial community as the dust traveled downwind to Osaka. This investigation revealed the distance-dependent effects of the Asian dust event on the atmospheric bacterial community. The results confirmed that the bacterial abundance increases near the dust source region, and that aeolian dust alters the bacterial community composition more in close regions than in regions far from the source. The bacterial effect of Asian dust on downwind environments depended on the scale of the dust event and distance from the dust source region.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jes.2017.12.019>.

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