低気圧、停滞前線および台風による
降水中のバクテリアの濃度・生存状態・遺伝子組成

Bacterial concentration, viability and composition in the rainwater of cyclones, stationary fronts and typhoons

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要旨

バクテリアは、霧や雲水、雨、雹、雪などの大気中の水中に大量に存在し、降水によって地表へ撒き散らされることで、生態系の発達や進化を促進すると考えられる。一方では、環境や公衆衛生への悪影響も懸念されている。バクテリアは、大気の物理や化学的プロセスにおいても重要な役割を果たしている可能性が高い。例えば、雲凝結核および氷晶核として雲の形成および降水過程に作用することで、大気中の物質循環および放射伝達に影響を与える。

降水中のバクテリアの濃度、生存状態および遺伝子組成に関する定量的データは、バクテリアの生態系および公衆衛生への影響を評価し、また大気圏–生物圏–水圏間のリンクにおける挙動と役割を良く理解しモデル化するために不可欠である。しかし、降水中のバクテリアの濃度、生存率および遺伝子組成に関する研究は非常に限られているのが現状である。

降水中のバクテリアの存在量と生存状態を定量するために、バクテリアを測定する汎用の 4',6-diamidino-2-phenylindole (DAPI) 染色法をコントロールにして、LIVE / DEAD BacLight Bacterial Viability Kit（LIVE / DEAD BacLight 染色）を用いた落射蛍光顕微鏡法の計数の適用可能性を検証した。LIVE / DEAD BacLight 染色により計数したバクテリア細胞の総数は DAPI 染色によるものと一致し、平均検出効率は 109±29%であった。グルタルアルデヒド固定したサンプルと固定なしの同じサンプル中カウントした細胞数の比は、平均 106±5%であった。本法において、ネガティブコントロール（ブランク）におけるバクテリア濃度は、おおむね雨水試料におけるバクテリア濃度よりも一桁低かった。ただ、降水量が少ない場合、ブランク中のバクテリアの存在量は雨水試料のバクテリアより多い結果もあった。これらの結果は、グルタルアルデヒド固定の影響と慎重なネガティブコントロールの確認を行えば、LIVE / DEAD BacLight 染色による計数が、雨水中のバクテリアの濃度および生存率の測定に適用可能なアプローチであることを示している。

総観気象によって引き起こされる雨水中のバクテリアは様々な特徴を有すると考えられる。大気中のバクテリアが雲降水プロセスに果たす役割を正確に理解するためには、それらの

データが基礎的な情報となる。我々の知る限りでは、異なる総観気象における、雨水中のバクテリアの濃度、生存率および遺伝子構成についての研究はほとんどなされていない。

2014 年~2015 年の間に、熊本県立大学のキャンパス（32.806°N、130.766°E）で雨水のサンプルを採取した。上記の開発された LIVE / DEAD BacLight 染色法を用いて、低気圧（寒冷前線）、停滞前線（梅雨期および非梅雨期）および台風による雨水中のバクテリアの存在量および生存率を測定し、それらの特徴を調べた。雨水中のバクテリアの平均濃度は $2.3\pm1.5\times10^4$ cells mL^{-1} であり、バクテリアの生存率（全バクテリア濃度に対する生存可能なバクテリア濃度の比）は 80±10％であった。アジア大陸気団の侵入に伴って雲が生成された低気圧の雨水中では、バクテリア濃度はほかのタイプの雨水中より高く（$3.5\pm1.6\times10^4$ cells mL^{-1}）、生存率は低かった（75±8％）。雲が海域の影響を強く受けている梅雨前線と台風の場合、バクテリア濃度はそれぞれ $1.5\pm0.5\times10^4$、$1.2\pm0.3\times10^4$ cells mL^{-1} であり、バクテリアの生存率は 84±7％と 85±7％であった。梅雨ではない停滞前線の雨水中では、バクテリア濃度は $2.4\pm1.6\times10^4$ cells mL^{-1} であり、生存率は 78±14％であった。バクテリア濃度は雨水中の水溶性イオン nss-SO_4^{2-}、nss-Ca^{2+}および NO_3^-と関連していたが、浮遊粒子濃度と降水量とは明らかな関係がなかった。バクテリアと水溶性イオンを組み合わせた相関分析や主成分分析を行ったところ、雨水中のバクテリアは雲形成の段階で雲の中に閉じ込められている可能性が高いことが分かった。

14 雨水サンプル中のバクテリアの遺伝子組成を、16S rRNA 遺伝子配列解析を用いて同定し、低気圧（寒冷前線）、停滞前線（梅雨期および非梅雨期）および台風の雨のタイプ別にバクテリアの種類を検討した。4 タイプの雨水中に多様なバクテリアが存在し、*Proteobacteria* (37%)、*Bacteroidetes* (16%)、*Cyanobacteria* (14%)、*Actinobacteria* (9%)、*Acidobacteria* (8%)、*Firmicutes* (5%)門のバクテリアが優占していた。約半数（33 のうち 16）の門が 4 タイプの雨水中に共通して存在していた。共通の Operational Taxonomy Unit（OTU）が全配列の大部分（平均的に 74％）を構成し、雨の種類にかかわらず共通のバクテリア OTU が優占的に存在していた。一方で、雨に関連する気団の起源と総観気象の条件によって細菌組成は異なっていた。土壌起源のバクテリア指標菌の割合が高いことは土壌起源のバクテリアの寄与が大きいことを示している。おそらく、土壌起源のバクテリアは雲凝結核および氷晶核として雲中プロセスに関与していたと考えられる。台風時の雨の 1 サンプルを除いて、その他のサンプルには、*Pseudomonas*、*Xanthomonas* および *Erwinia* 属のメンバーのような氷核形成活性バクテリアがあ

った。いくつかのサンプルから海洋起源バクテリアに属する種、例えば *Pseudoalteromonas*、*Synechococcus* と *Marinobacter* が検出され、雲および雨水を介した海洋起源バクテリアの大気中拡散が示唆された。ヒトおよび動物の病気の潜在的な病原体である糞便指標バクテリアもすべてのサンプルで検出された。これらのことから、降水は大陸、海洋と島の生態系をつなぐ、自然界のバクテリア群集の普及にとって非常に効率的な経路であるといえる。

以上の結果をまとめると、降雨イベントと関連する総観気象によって、雨水中のバクテリアの濃度、生存率および群集組成には相違が見られた。雨水中には様々なバクテリア群集が検出された。総観気象が異なるにもかかわらず、共通するバクテリアが OTU を優占していた一方で、群集組成は雨水のタイプによって異なっていた。また、大陸起源のバクテリアは雨水中で大きな割合を占めていた。雨水中のバクテリアの重要なソースとして、雲の下で除去された大気中のバクテリアだけではなく、雲中凝結核および氷晶核として働いたバクテリアも含まれると考えられる。以上のことから、雨水中のバクテリアは、生態系の進化、公衆衛生上の脅威および気候変動に関与する可能性があるといえる。

キーワード : バクテリア、濃度、生存率、遺伝子組成、16S rRNA シークエンシング、降水、総観気象

Abstract

Bacteria substantially exist in atmospheric waters, e.g., fog and cloud water, rain, hail and snow. They can be disseminated by precipitation from the atmosphere to the Earth′s surfaces. On the one hand, bacteria in the atmosphere are likely a driver of the development and evolution of ecosystems; on the other hand, they are also causing great concern for their potential negative impacts on environments and public health. Bacteria likely play important roles in atmospheric physicochemical processes, such as acting as cloud condensation nuclei and ice nuclei to initiate cloud formation and precipitation, and consequently impact the mass cycle and radiation transfer in the air.

Quantitative data on the concentration, viable or nonviable status, and community composition of bacteria in precipitation are essential to assess their impacts on ecosystems and public health, as well as to better understand and model the activities of bacteria in the atmosphere–biosphere–hydrosphere links. However, studies on the concentration, viability and community composition of bacteria in rainwater, especially those dependent on different synoptic weather systems, are largely lacking.

To quantify the abundance of viable and nonviable bacterial cells in rainwater, the applicability of epifluorescence microscopy enumeration with the LIVE/DEAD BacLight Bacterial Viability Kit stain was verified, with the 4',6-diamidino-2-phenylindole (DAPI) stain for the reference of total cell counts. Results showed that the total counts of bacterial cells by LIVE/DEAD BacLight staining were consistent with those by DAPI staining, and the average detection efficiency was 109±29%. The ratio of cell count with glutaraldehyde fixation to that without fixation was 106±5% on average. The bacterial concentration in negative control was usually an order of magnitude lower than that in rainwater samples. However, in case of small precipitation, the abundance in negative control could be more than that in rainwater samples. These results indicate that the enumeration with LIVE/DEAD BacLight bacterial viability assay coupled with glutaraldehyde fixation and careful negative control investigation is an approach applicable to the measurement of the concentration and viability of bacterial cells in rainwater.

The bacteria in the rainwater of the rain caused by different synoptic systems might have distinctive characteristics. The related data are fundamental information for an accurate understanding of the roles

that airborne bacteria play in cloud and precipitation processes. To our knowledge, there have been no research studies on the dependence of abundance, viability and community composition of bacteria in rainwater on the synoptic weather.

In this study, rainwater samples were collected at a site (32.806°N, 130.766°E) in Kumamoto, southwestern Japan during 2014–2015. The abundance and viability of bacterial cells in rainwater samples were measured and their distinctiveness, according to synoptic weather systems, i.e., cyclones (cold fronts), stationary fronts (including Meiyu and non-Meiyu fronts) and typhoons, was examined. On average, the cell concentration of bacteria in the rainwater was $2.3\pm1.5\times10^4$ cells mL^{-1}, and bacterial viability, the ratio of viable cells to total cells, was 80±10%. In the rainwater of cyclones when clouds were induced by the intrusion of continental air, the bacterial concentration was higher ($3.5\pm1.6\times10^4$ cells mL^{-1}) and the viability was lower (75±8%) than in the rainwater of other types. In the rainwater of Meiyu fronts and typhoons when clouds were significantly influenced by marine air, bacterial concentrations were $1.5\pm0.5\times10^4$ and $1.2\pm0.3\times10^4$ cells mL^{-1}, and bacterial viabilities were 84±7% and 85±7%, respectively. In the rainwater of non-Meiyu stationary fronts, the bacterial concentration was $2.4\pm1.6\times10^4$ cells mL^{-1}, and the viability was 78±14%. Abundant bacteria were associated with ions nss-SO_4^{2-}, nss-Ca^{2+}, and NO_3^- in rainwater. However, the bacterial concentrations did not correlate with the ratios of airborne particle concentrations to the precipitation amounts. Further investigations with correlation analysis and principal component analysis combining bacteria and ion species revealed that bacteria in the rainwater were likely enclosed in clouds at the stage of cloud formation in addition to below-cloud scavenging, and bacteria involved in the rainwater did not show confirmable growth.

Bacterial communities in fourteen samples were identified by using 16S rRNA gene sequencing and compared according to the rain types on synoptic scales, i.e., cyclones, Meiyu and non-Meiyu stationary fronts, and typhoons. Diverse bacterial communities were present in all four types of rainwater, and were dominated by the phyla *Proteobacteria* (37%), *Bacteroidetes* (16%), *Cyanobacteria* (14%), *Actinobacteria* (9%), *Acidobacteria* (8%) and *Firmicutes* (5%). About half of the phyla (16 out of 33) were common among the rain types. The common operational taxonomic units (OTUs) comprised the majority (averagely 74%) of the sequences, indicating the predominance of the common bacterial OTUs regardless of the rain types. On the other hand, the synoptic weather systems as well as the origins of air

masses associated with the rain, likely caused distinct bacterial communities. High fractions of bacterial indicator taxa of soils, which were presumably involved in in-cloud processes as nuclei, signified the large contribution of bacteria from soils. There were ice nucleation-active bacteria, such as the members of the genera *Pseudomonas, Xanthomonas* and *Erwinia*, in all samples except one of typhoon rain. Marine bacterial taxa, e.g., *Pseudoalteromonas*, *Synechococcus* and *Marinobacter*, were detected in several samples, indicating the dispersal of marine bacteria via cloud and rainwater. Fecal indicator bacteria, which are potential pathogens of human and animal diseases, were also detected in all samples. Rain is thus a very efficient pathway for the dissemination of bacterial communities in nature to link continent, marine and island ecosystems.

In summary, there was a dependence of bacterial concentration, viability and community on the corresponding synoptic weather of rain events. Diverse bacterial communities occurred in the rainwater. Common bacteria dominated OTUs despite the synoptic weather, and distinct bacterial communities occurred in rainwater of different rain types. Bacteria from continental sources made a large contribution in the rainwater. In addition to below-cloud scavenging, bacteria as nuclei in clouds may be an important source of bacteria in rainwater. The bacteria in rainwater have a potential to involve in ecosystem evolution, public health threat and climate change.

Keywords: Bacteria; Concentration; Viability; Community composition; 16S rRNA sequencing; Rainwater; Synoptic weather

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CHAPTER 1

Bacteria in atmospheric waters: detection, characteristics and implications

1.1 Introduction

Water in the atmosphere occurs in all three phases, i.e., water vapor, liquid water in the forms of cloud water, fog and rain, and ice as cloud ice-crystals, snow, graupel and hail. Although water vapor in the atmosphere is merely about 0.2−3% of the volume, it is a very important gas and can be converted into liquid and solid states to generate clouds and produce precipitation (Zaitseva, 2009). As early as 1770s, Antoni van Leeuwenhoek discovered that there were bacteria in precipitation, but the issue was not paid attention until one hundred years later (Smit and Heniger, 1975). In this dissertation, the term "atmospheric waters" refer to atmosphere waters in aqueous and solid phases. Over the last two decades, due to the development of detection techniques, especially the progresses of metagenomics and molecular based tools, more and more studies are being carried out to study the bacteria in atmospheric waters (Delort et al., 2010, 2017).

It is well-known that atmospheric waters play an important biological role to be the habitat of microorganisms (Amato, 2012). A large number of observational studies have confirmed the substantial presence of bacteria in atmospheric waters (Delort et al., 2010; Fröhlich-Nowoisky et al., 2016; Sun and Ariya, 2006). It is considered that atmospheric microorganisms, such as bacteria, are active participants in atmospheric physical and chemical processes (Fig. 1-1), e.g., activation of cloud condensation nuclei (CCN) and ice nuclei (IN), and have potential impacts on earth ecosystems, global climate and public health (Ariya et al., 2009; Möhler et al., 2007; Morris et al., 2011; Sun and Ariya, 2006; Womack et al., 2010).

The submicron size range of bacteria makes them a long retention time in the atmosphere. Atmospheric waters are efficient means of transportation and dissemination for bacteria, which are lifted from various surfaces by wind (Fig. 1-1). Bacteria can provide surfaces for the condensation of water vapor when the air is saturated and be involved in the formation of cloud droplets as nuclei (Delort et al., 2010). They can be carried with clouds over a certain distance (Creamean et al., 2013; Zaitseva, 2009), and settle onto surfaces via wet and dry deposition (Monteil et al., 2014). Therefore, it is important to investigate bacteria in atmospheric waters for understanding their ecological, climatic and public health-related effects and controlling negative influences. However, to date, the knowledge on characteristics and activities of bacteria in atmospheric waters actually remain limited, which is a challenge for interdisciplinary studies toward understanding their impacts. More work and direct cooperation between microbiologists and atmospheric scientists and modelers are necessary to better understand and model bioaerosol–cloud–precipitation–climate interactions (Ariya et al., 2009; Burrows et al., 2009a, 2009b, 2013; Konstantinidis, 2014; Morris et al., 2011; Pöschl et al., 2010).

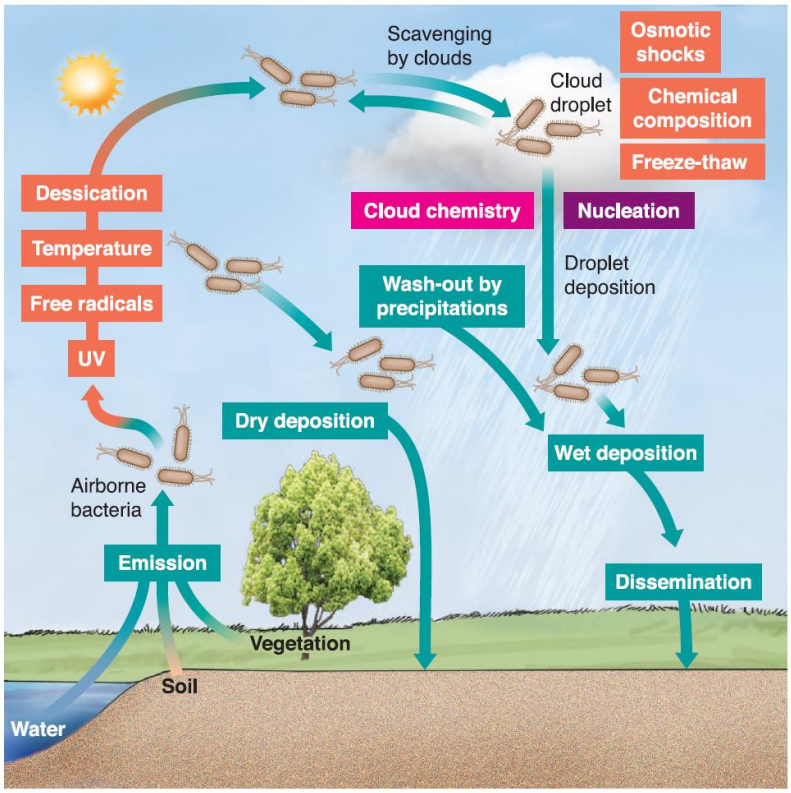

Figure 1-1. Schematic of the life cycle of bacteria in the atmosphere, of which the main steps are indicated in grey boxes. Reprinted from Amato (2012).

The cell concentration of bacteria is crucial to nuclei formation in clouds because both cells and even cell fragments can act as nuclei for icing (Christner et al., 2008b; Möhler et al., 2007; Šantl-Temkiv et al., 2015). The bacterial status (i.e., live or dead) can be a more accurate and direct indicator to address the activities and roles of bacteria in cloud formation, and assess the potential effects on environment, ecology, global climate and public health. Moreover, detailed information on community composition of bacteria in atmospheric waters is significant to elucidate the diversity and geography of bacteria in the atmosphere and give implications for their activities and roles in biogeochemical cycles and atmospheric physicochemical processes, and impacts on global climate change and public health. Therefore, it is important to study the concentration, status and composition of bacteria in atmospheric waters and their spatiotemporal variations.

1.2 Detection approaches of bacteria in atmospheric waters

1.2.1 Sampling of atmospheric waters

For effective detection of airborne bacteria, more and more robust samplers and even real-time detection systems have been developed and applied, which can be found in comprehensive reviews (Després et al., 2012; Fröhlich-Nowoisky et al., 2016; Georgakopoulos et al., 2009; Moon et al., 2012;

Xu et al., 2011). However, due to the diverse forms and existing altitudes of atmospheric waters, there have been rare commercial samplers for microorganisms in atmospheric waters.

A wide range of cloud water samplers were used to characterize the physicochemical properties in previous studies (Roman et al., 2013; Wieprecht et al., 2005). Among them, the active single-stage samplers, e. g., cloud water samplers (CWS; droplets with sizes >7 μm) and Caltech active strand cloud water collector version 2 (CASCC2; >3.5 μm), were applied often for bacterium-related analyses (Amato et al., 2005, 2007a, 2007c, 2007d; Bauer et al., 2002; Sattler et al., 2001; Vaïtilingom et al., 2012; Xu et al., 2017; Wei et al., 2017). The liquid water content (LWC) of the cloud is usually determined with a Gerber particle volume monitor PVM-100 (Amato et al., 2005; Bauer et al., 2002). Matthias-Maser et al. (2000) used three different samplers, i.e., a rotating arm collector (RAC; >7.5 μm), an isokinetic cloud probing system (ICPS; >2.5 μm) and a round-jet impactor (>2.5 μm), for cloud water sampling and microbiological particle identification. Passive string cloud collectors were also used by Ahern et al. (2007) to collect the cloud water samples for identifying bacterial community composition. In addition to ground-based collectors, a counterflow virtual impactor (CVI) and a modified Mohnen slotted rod collector installed in aircraft were reported to be deployed to collect cloud water samples for detection of biological particles (Pratt et al., 2009) and bacteria (Hill et al., 2007; Kourtev et al., 2011), respectively.

Fog is similar to clouds but they occur at lower elevations near surfaces. Ground-based collectors of cloud water for bacterial analysis are also applicable to the collection of fog water. Fuzzi et al. (1997) collected fog samples by using a string collector in sterile conditions for the pour-plate cultivation of bacteria, yeasts and moulds. Fog LWC was also measured by PVM-100. During the USNS *Hayes* 1975 cruise, fog water samples were obtained from a centrifugal collecting device at a point high above the deck near the bow of the Hayes or from a nylon mesh bow kite for IN measurements and isolation of bacteria (Schnell, 1977).

For the collection of precipitation, e.g., rainwater, snow and graupel, there have been rare well-assembled samplers applied in the studies on bacterial and microbiological properties. A variety of utensils were used to collect rainwater samples. The samplers composed of sterilized beakers, bottles or flasks equipped with sterilized funnels, or homemade rain collectors are the most often used to diminish contamination (Ahern et al., 2007; Bauer et al., 2002; Christner et al., 2008a; Du et al., 2017; Herlihy et al., 1987; Lu et al., 2016; Peter et al., 2014). Sterile plastic tubes (Monteil et al., 2014), buckets (Cho and Jang, 2014), and clean polyethylene tarpaulins (Itani and Smith, 2016) were also used to collect rainwater. Fresh snow samples were aseptically collected with a sterile spoon or shovel (Bauer et al., 2002; Sattler et al., 2001; Wunderlin et al., 2016), clean plastic tarpaulins (Monteil et al., 2014), a Teflon-coated tin (Stopelli et al., 2017), or clean plastic boxes lined with autoclaved plastic sheets (Hara et al., 2016a), etc.

Before the collection, the collectors must be carefully washed and sterilized by autoclaving, heating and flaming, or rinsed several times with autoclaved pure water. The sterility of the apparatus is tested by pouring autoclaved pure water through the apparatus and analyzing it as a blank sample. After collection, the samples need to be pretreated with multiple ways according to consequent analyses, e.g., microscopic enumeration, medium cultivation, and DNA extraction. Atmospheric water samples are often concentrated by filtration through sterile 0.2/0.22-μm-pore filters or centrifuged for metagenomic DNA extraction (Cho and Jang, 2014; Itani and Smith, 2016; Peter et al., 2014; Xu et al., 2017), and sometimes for plate cultivation (Monteil et al., 2014). However, even for similar analysis, such as microscopic enumeration, the immediate treatments (e.g., with or without fixation), stored containers, temperatures and durations are quite different (Amato et al., 2005; Bauer et al., 2002; Sattler et al., 2001). In general, the rigorousness of quality control and assurance during the sampling varied among previous studies, possibly causing a certain of uncertainties for consequent analysis. Careful verification and improvement of sampling procedures for studying microbiological properties of atmospheric waters are required.

1.2.2 Detection and analysis technologies

The common approaches to characterizing bacteria in atmospheric waters can be classified into two categories according to the objects in the measurements (Table 1-1): methods based on direct detection of entire cells, and methods based on the detection of cell components (Georgakopoulos et al., 2009).

The methods based on detection of entire cells include culture-based methods, single particle analysis, and fluorescence enumeration of stained bacterial cells. In studies using cultured samples, media with different nutrient agar have been used for the cultivation of bacteria in cloud, fog and rain water (Table 1-1). The most popular media of different nutrient agar used for cultivation of bacteria in atmospheric waters include plate count agar (PCA), tryptic soy agar (TSA) and Luriae-Bertani (LB) agar as general ones for total aerobic-heterotrophic bacteria, and Reasoner's 2A (R2A) agar for oligotrophic bacteria (Amato et al., 2005, 2007c, 2007d; Bauer et al., 2002; Cho and Jang, 2014; Fuzzi et al., 1997; Monteil et al., 2014). Selective media are also used in some studies, for instance, selective media for the isolation of specific species (Monteil et al., 2014) and marine agar (MA) for heterotrophic marine bacteria (Cho and Jang, 2014). The targets of these studies are the cultured colonies rather than total bacterial cells in the samples, and obtained results are limited to bacteria cultivable by selected media.

The microbiological particles in atmospheric waters have also been identified based on the morphology, chemical composition and behaviors of particles using single particle analysis (Table 1-1). Instruments, such as transmission/scanning electron microscopes equipped with energy dispersive X-ray detectors (TEM/SEM-EDX) and the aerosol time of flight mass spectrometry (AToFMS), have been used

in detecting microbiological particles in rainwater, cloud water and cloud ice-crystals (Casareto et al., 1996; Matthias-Maser et al., 2000; Pratt et al., 2009). However, these techniques identify biological particles mainly by the presence of certain chemical components (e.g., phosphorus, potassium, sulfur and organics) in particles, and, consequently, the obtained data are not limited to bacteria and the status of bacterial cells is unknown.

Table 1-1 Approaches applied in characterization of bacterial/microbiological particles in atmospheric waters according to the direct detection of entire cells (Category 1) and the detection of cell components (Category 2).

Method	Basic characteristics	Reference
Epifluorescence microscopy *Category 1*	Enumerating bacteria stained with fluorescent dyes Enable enumeration of all bacteria including non-cultivable fraction May misidentify of non-biological fraction High content of bacteria required	1, 2, 3, 4, 5, 6, 7, 8, 9
Flow cytometry *Category 1*	Enumerating cells labeled by DNA or RNA-binding fluorescent dyes Measuring morphological, biological and chemical properties Enable differentiation of live/dead, metabolically active/non-active cells, bio-/non-bio-particles, identifying taxa or even species Offering great speed in sample processing and identification	8, 9
Cell Cultivation *Category 1*	Enumerating and identifying visible colonies growing on media (e.g., PCA, TSA, LB, R2A agar, and selective media, e.g., MA) Underestimation of the actual number and diversity	1, 2, 3, 10, 11, 12
Characterization of biological IN *Category 1*	Immersion freezing test of lysozyme or heat (95°C) treated samples Estimating bacterial and biological INP concentrations by determining the fraction of IN susceptible to lysozyme digestion and heat treatment, respectively	8, 13, 14
Single-particle methods *Category 1*	Detection with SEM/TEM-EDX or ATOFMS Identifying PBAPs by morphology, composition and behavior Unable to provide accurate concentration and status of bacteria	15, 16, 17
General biomass measures *Category 2*	Determining organic carbon content of particles OR Estimating based on qPCR determined number of gene copies Small biomass of cloud/rain samples limits the available methods	18
Measures of ATP levels *Category 2*	Extracting and determining ATP by bioluminescence with a luciferin-luciferase assay and a luminometer	3, 10
Detection and analysis of nucleic acids *Category 2*	qPCR enables rapid quantification for the abundance of any chosen phylogenetic group and key functional genes (e.g., *inaZ* gene) in cloud/precipitation samples Gene sequencing of cultured or uncultured bacteria NGS provides a more powerful approach to uncover the diversity of bacterial communities	19, 20, 21 3, 5, 6, 18, 22

References: 1. Amato et al., 2005; 2. Bauer et al., 2002; 3. Cho and Jang, 2014; 4. Herlihy et al., 1987; 5. Kourtev et al., 2011; 6. Peter et al., 2014; 7. Sattler et al., 2001; 8. Christner et al., 2008b; 9. Liu et al., 2009; 10. Amato et al., 2007d; 11. Fuzzi et al., 1997; 12. Monteil et al., 2014; 13. Christner et al., 2008a; 14. Šantl-Temkiv et al., 2015; 15. Casareto et al., 1996; 16. Matthias-Maser et al., 2000; 17. Pratt et al., 2009; 18. Georgakopoulos, et al., 2009; 19. Hill et al., 2014; 20. Kaushik and Balasubramanian, 2012; 21. Kaushik et al., 2012; 22. Amato et al., 2007c.

Enumeration with fluorescent staining is a traditional method to detect the abundance of bacteria in the atmospheric waters. One of the most widely used fluorescent nucleic-acid dyes for enumeration of total bacterial cells is 4', 6-diamidino-2-phenylindole (DAPI) reagent (Amato et al., 2005; Bauer et al., 2002; Cho and Jang, 2014; Peter et al., 2014; Sattler et al., 2001). Acridine Orange (AO) and SYTO stains are also frequently used for direct bacterial counts (Christner et al., 2008a, 2008b; Herlihy et al., 1987; Kourtev et al., 2011). Before staining, the samples are usually fixed with formaldehyde or glutaraldehyde solution. The stained bacterial cells in samples are counted using epifluorescence techniques, such as with epifluorescence microscopy (EFM) observation and flow cytometry (FCM) measurement. Bacterial cells were recognized by fluorescent color, size and shape. Based on the determined bacterial abundance and size, the biovolume and biomass of bacteria in atmospheric waters can be estimated (Bolter et al., 2002; Posch et al., 2001; Sattler et al., 2001).

Information on the status of atmospheric bacteria can provide a scientific base for understanding the activities and roles what bacteria have and play in various atmospheric processes, as well as for assessing their environmental, ecological and climatic functions (Bolter et al., 2002; Qi et al., 2015). The simultaneous detection of live and dead bacterial cells in atmospheric waters is possible by the use of different stains, which differ in the ability to react with or penetrate intact bacterial cells, although there is still argument on the definition of dead bacteria. Hill et al. (2007) determined viable and nonviable bacterial cells by staining cloud water samples with the dyes 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) and AO. Metabolically active bacteria can take up CTC and reduce it to a fluorescent compound under the microscopy. Stopelli et al. (2017) enumerated the bacterial concentrations in snow samples, and the staining agents were SYBR green for total cell count, and both SYBR green and propidium iodide (PI) to facilitate counting cells that are viable (i.e., with intact membranes) and nonviable (with damaged membranes).

The commercially available LIVE/DEAD® BacLight™ Bacterial Viability Kit is widely used to measure bacterial viability and differentiate between alive and dead cells by detecting if their membranes are intact (Biggerstaff et al., 2006; Boulos et al., 1999). This kit is comprised by two nucleic acid probes, green-fluorescent SYTO 9 and red-fluorescent PI. Viable and nonviable bacteria and stain fluorescent green and red under blue excitation rays, respectively. This kit has been proven to accurately quantitate diverse bacterial species, but it is noted non-specific binding and autofluorescence issues could be encountered when testing complicated samples (Biggerstaff et al., 2006). In several previous studies, it has been applied to obtain the bacterial viability in atmospheric aerosols (DeLeon-Rodriguez et al., 2013; Hara and Zhang, 2012; Murata and Zhang, 2013, 2014, 2016) and atmospheric waters (Bauer et al., 2002).

Biological ice nuclei cause immersion freezing of water (Vali, 1971) at various subzero temperatures, and they can be detected in atmospheric water samples. This test cannot differentiate biotic ice nuclei from abiotic ones, although it is well-known that biological particles show ice nucleation ability at warmer temperatures than abiotic ones. However, the techniques dependent on cultivating live organisms can underestimate biological IN since even fragments of non-living organisms may retain the ice nucleation ability (DeMott and Prenni, 2010; Šantl-Temkiv et al., 2015). Christner et al. (2008a) described a strategy to detect biological ice nuclei in snow and rain by immersion freezing testing, and estimated the abundance of bacterial and biological IN by determining the fraction of ice nuclei that were susceptible to lysozyme digestion (destroying cell integrity) and heat treatment (denaturing most proteins), respectively, at each temperature interval. Hara et al. (2016a) assessed the effects of different temperature treatments (unheated, treated at 40°C and 90°C) on biological IN in snow samples under temperatures warmer than −10°C. Based on immersion freezing testing, Lu et al. (2016) and Du et al. (2017) investigated bacterial and fungal IN in rainwater samples collected at the temperate grasslands and three mountain sites in China, and the IN inactivation at temperatures above −10°C after heat and filtration treatments.

The methods based on detection of cell components include general biomass measures, measures of adenosine triphosphate (ATP) levels, and molecular bio-techniques based on detection and analysis of nucleic acids (Table 1-1).

The small biomass in atmospheric water samples limits the available methods of general biomass measures, although these measures are useful to determine the organically bound carbon content in aerosols (Georgakopoulos et al., 2009). In addition, the typical C: N: P ratios of bacterial cells fluctuate over a wide range and may thus not be used as an unambiguous indicator of living cells. Recently, a high-resolution time-of-flight aerosol mass spectrometer and positive matrix factorization (PMF) using the multilinear engine (ME-2) source apportionment was applied to characterize the mass spectral signatures of airborne bacteria in cloud simulation experiments as well as field observations (Wolf et al., 2015, 2017). This technique seems to be a new direction toward measuring the mass concentration of bacteria in atmospheric waters.

Regardless of the substrate metabolized, the ultimate energy carrier for biosynthesis in all cells is ATP. ATP is only produced when cells are active. Amato et al. (2007d) were the first to measure ATP levels in cloud water by bioluminescence (luciferine/luciferase) using a Biothema Biomass kit and a luminometer, and the measures of ATP levels were applied by their colleagues in consequent studies (Amato et al., 2007a; Vaïtilingom et al., 2012, 2013). Cho and Jang (2014) analyzed ATP in rainwater with a luciferin-luciferase assay using a ATP bioluminescence assay kit CLS II and a luminometer.

Culture-based approaches for identification of bacterial species are quite useful for understanding the physiological potential of isolated microorganisms, but they cannot provide comprehensive information on the microbial community composition (Orphan et al., 2000). These methods have been applied to investigate cultivable bacterial species in atmospheric waters in some early and recent studies (Amato et al., 2005, 2007c; Cho and Jang, 2014; Fuzzi et al., 1997; Itani and Smith, 2016; Šantl-Temkiv et al., 2015; Vaïtilingom et al., 2012). In most of these studies, bacteria were identified by 16S rRNA sequencing.

Since the 1980s (Pace et al., 1986), diverse culture-independent methods (CIMs; Fig. 1-2) have been developed to study the microbial communities in various environments, as reviewed by Su et al. (2012). Culture-independent molecular biotechniques, especially those based on gene surveys after polymerase chain reaction (PCR) amplification, have also been used for investigating bacterial communities in atmospheric waters. Alfreider et al. (1996) investigated the bacterial community structure in the snow layer of a high mountain lake by fluorescence in situ hybridization (FISH). Quantitative real-time PCR (qPCR) method enables rapid detection and quantification for the abundance of any chosen phylogenetic groups (e. g., pathogens) and key functional genes (e.g., *ina* gene), and has been applied for quantification of specific bacteria in cloud water and precipitation samples (Georgakopoulos et al., 2009; Hill et al., 2014; Kaushik and Balasubramanian, 2012; Kaushik et al., 2012; Segawa et al., 2005).

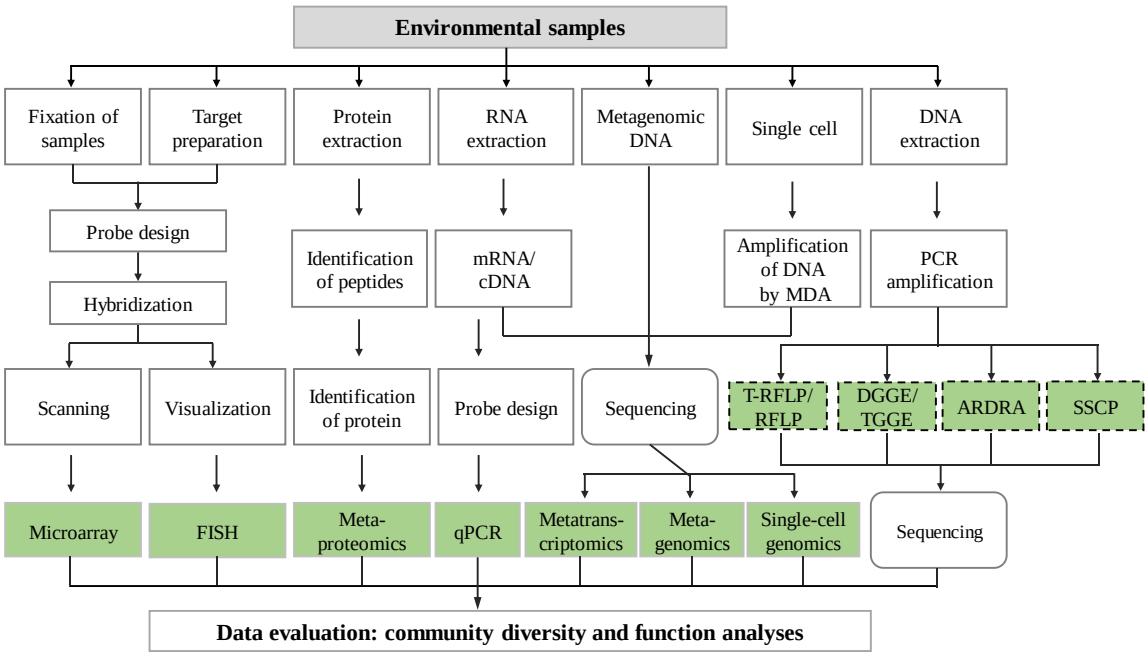

Figure 1-2. Experimental procedures of different CIMs. Different CIMs were presented as Green boxes. Adapted from Su et al. (2012).

The technique 16S rRNA gene clone library construction from directly extracted DNA of rainwater and Sanger sequencing was used to characterize bacterial communities at diverse sites, including mountain, grassland and tropical reservoir (Du et al., 2017; Kaushik et al., 2014; Lu et al., 2016). Liu et al. (2009) investigated bacterial diversity in the snow of four Tibetan Plateau glaciers through culture-independent molecular analysis of 16S rRNA gene clone library, restriction fragment length polymorphism (RFLP) screening and sequencing. Segawa et al. (2005) and Ahern et al. (2007) described the bacterial populations present in snow collected from the Tateyama Mountains (Japan), and cloud and rainwater in Scotland using a combination of amplified ribosomal DNA restriction analysis (ARDRA) and 16S rRNA gene sequencing, respectively. Itani and Smith (2016) displayed the diversity of bacteria in dust and clean rains by denaturing gradient gel electrophoresis analysis (DGGE) and sequencing of amplified 16S rDNA from cultured colonies and uncultured total DNA. Kourtev et al. (2011) identified the diversity of bacterial communities in cloud water at the upper U.S. Midwest using both PCR-DGGE and shot-gun cloning and sequencing of amplified 16S rRNA genes.

The next-generation sequencing (NGS) technologies (e. g., pyrosequencing) have stimulated the emergence of many infusive fields such as environmental (meta-)genomics (Metzker, 2010; Su et al., 2012). NGS provides a more efficient and powerful approach to uncover the diversity and richness of bacterial communities in atmospheric waters (Joly and Faure, 2015). Currently, NGS for 16S rRNA-based bacterial community profiling have been performed on communities in snow, rain, cloud and fog water (Carey et al., 2016; Cho and Jang, 2014; Wei et al., 2017; Wunderlin et al., 2016; Xu et al., 2017). Various NGS platforms produce diverse types of large 16S rRNA gene high-throughput sequencing data. Ju and Zhang (2015) discussed state-of-the-art bioinformatics and statistical analyses of these to promote their applications in exploring microbial diversity. More details of the methods based on detection and analysis of nucleic acids can refer to previous reviews on the application to atmospheric aerosols (Behzad et al., 2015; Fröhlich-Nowoisky et al., 2016; Gandolfi et al., 2013; Georgakopoulos et al., 2009; Nunez et al., 2016; Peccia and Hernandez, 2006; Xu et al., 2011; Yoo et al., 2017).

1.3 Presence of bacteria in atmospheric waters

1.3.1 Concentrations

The concentrations of bacteria in atmospheric waters at various sites across the world (Fig. 1-3), determined by direct enumeration, are shown in Fig. 1-4. It has been found the concentration of eukaryotic cells (e.g., fungi and yeasts) is generally one order of magnitude lower than that of bacteria in cloud water (Delort et al., 2010). Thus, here we assume total biological micro-particles in atmospheric waters reported by some literatures are mainly bacteria. The concentration of bacteria in atmospheric waters is very

heterogenic and depends strongly on a wide range of factors, e.g., physicochemical properties of atmospheric waters, seasonal effect, location (altitude, latitude), biogenic emission sources, meteorological conditions and the air pollution level.

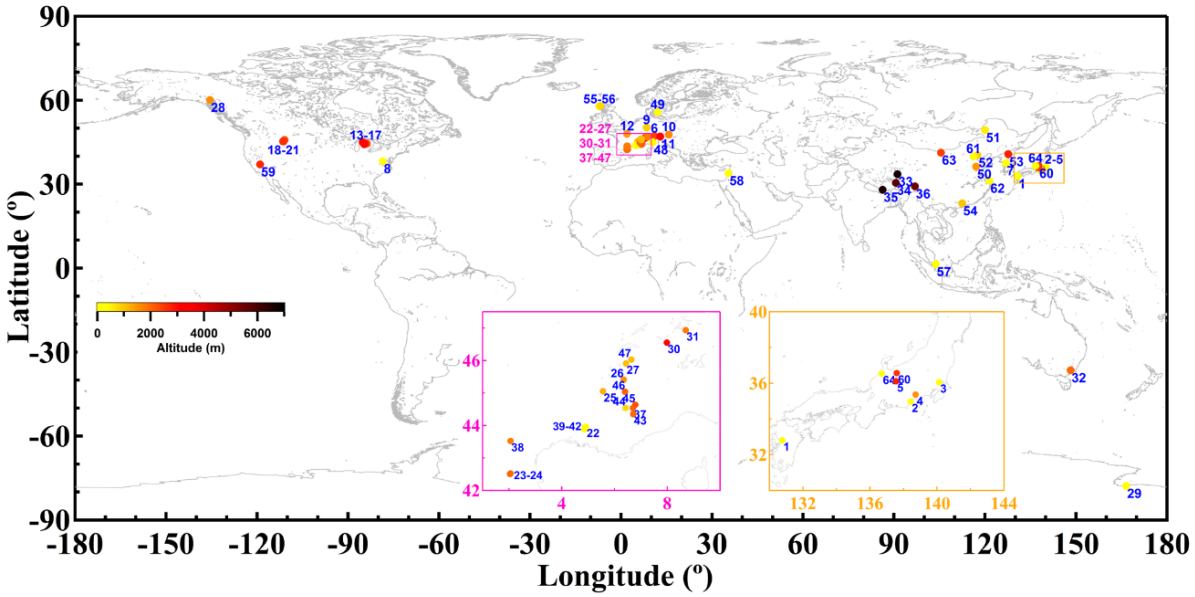

Figure 1-3. Locations conducted measurements of abundance, viability or community composition of bacteria in atmospheric waters (Table S1-1).

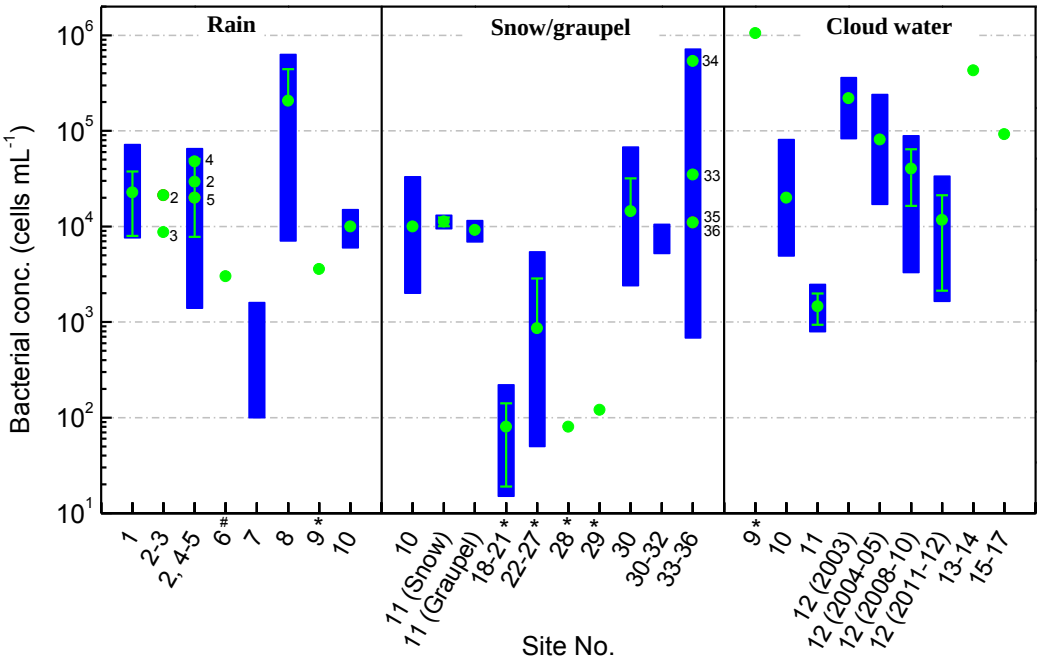

Figure 1-4. Concentrations of bacteria in atmospheric waters based on direct enumeration. The floating bar, dot and error bar mean the range, average and standard deviation, respectively. #Bulk deposition; *Total number of biological micro-particles. The details are available in Table S1-2.

Herlihy et al. (1987) measured the bacterial cells by AODC in precipitation during 1983–1984. They found the bacterial cell concentration showed a seasonal variation, which was an order of magnitude higher from April to September ($2–6\times10^5$ cells mL^{-1}) than during the rest of the year ($0.8–5\times10^4$ cells mL^{-1}). Casareto et al. (1996) first reported on the morphology and chemical identification of biological micro-particles in rain water at two sites in Japan. The concentrations of identified bacteria in rainwater at the two sites were comparable to the later reported results detected by EFM enumeration at three other sites (2, 4, 5 as shown in Fig. 1-4) in Japan (Natsume and Suzuki, 2001), and lower than the average at an urban site in Charlottesville, US (Herlihy et al., 1987). These concentrations were two times to two orders of magnitude higher than those of rainwater collected during heavy rain events at an inland site in Korea (Cho and Jang, 2014), and precipitation (e.g., rain, snow and graupel) collected at high altitude and mid- and high-latitude locations, such as at Antarctica (Bauer et al., 2002; Christner et al., 2008b; Matthias-Maser et al., 2000; Peter et al., 2014; Sattler et al., 2001; Wunderlin et al., 2016). However, the bacterial cell abundance in the snow of Tibetan glaciers was between 6.8×10^2 and 7.2×10^5 cells mL^{-1}, and the cell abundances in 17 out of 88 samples were more than 10^5 cells mL^{-1} (Liu et al., 2009).

In two previous studies (Bauer et al., 2002; Matthias-Maser et al., 2000), the bacterial concentrations in cloud water were two times and three orders higher than in precipitation at the same or close sites (Sites 9, 10), respectively. In contrast, Sattler et al. (2001) reported that the bacterial concentration in cloud water was about one order lower than in precipitation at Sonnblick Observatory (3106 m a.s.l.). In general, the average bacterial concentration in cloud water collected at the majority of sites was on the order $10^4–10^5$ cells mL^{-1}, while that in precipitation was mostly on the order $10^3–10^4$ cells mL^{-1}. Long-term observations of bacteria in cloud water were conducted at the Puy de Dôme, France from 2003 to 2012. It is noted the bacterial concentrations showed a continuous decreasing trend (Fig. 1-4).

It is often found most microbial cells are mixed with and correlated well with dust particles in cloud and precipitation residues (Casareto et al., 1996; Christner et al., 2008a; Creamean et al., 2013; Pratt et al., 2009). Biological micro-particles contribute to about 20–30% of insoluble particles or residues in cloud and precipitation. Creamean et al. (2013) suggested that the majority of residues in precipitation probably originated from the cloud particles as opposed to being below-cloud scavenged by precipitation because the precipitation residue compositions were similar to those of the cloud residues. On the other hand, Christner et al. (2008b) examined the DNA-containing cells ranging between 0.3 and 15 μm in diameter ($15–5.4\times10^3$ cells mL^{-1}) in the snow samples, which accounted for less than 1% of the total particles ($1.1\times10^4–3.9\times10^6$ particles mL^{-1}). Bauer et al. (2002) estimated that the bacterial cells contributed to merely about 0.01% of organic carbon content in cloud and snow samples due to the

submicron sizes, much lower than the contribution of fungal spores (1.5% of OC and 1.8% of TC in cloud water and snow samples, respectively).

1.3.2 Viability

Bacterial viability is primary information to assess the ecological effects because settling of live bacteria to the surfaces potentially alters the indigenous microbial diversity at the deposition regions. There have been a few research works reported the bacterial viability in atmospheric waters (Table 1-2).

Table 1-2 Concentrations of cultivable bacteria and viability of bacteria in atmospheric waters.

Site No.	Period	Sample type	Cultivable bacteria (CFU mL^{-1})	Viability (%)	Method	Reference
10	Apr. 1999 Mar. 2000	Snow/graupel	1–25	87 <0.003–1.0>	R2A cultivation (27°C)/ EFM (LIVE/DEAD)	Bauer et al., 2002
	Mar. 2000	Cloud water	135	72, 95 <2.2>		
12	Mar. 2003	Cloud water	33–1975	<0.02–0.81>	TSA/R2A cultivation (15–17°C)/ EFM (DAPI)	Amato et al., 2005
	Jan. 2004–Oct. 2005 Apr. 2008–Sept. 2010		105±108 (0–403)	<0.25±0.28 (0.00–0.90)>		Vaïtilingom et al., 2012
13–17	Jun. –Aug. 2005	Cloud water		76±12	EFM (CTC/AO)	Hill et al., 2007
30	Mar–Sept. 2013, May–Oct. 2014	Snow	[≤0.002–0.045]	60±10 (35–78)	EFM (PI/SYBR green)	Stopelli et al., 2017
24–27, 37–47	Dec. 2005 –Nov. 2011	Rain	200 [0.6]		TSA/KBC cultivation (22–25°C)	Monteil et al., 2014
		Snow	33 [4.6]			
48	Feb. –Mar. 1994	Fog water	30–150		PCA/R2A cultivation	Fuzzi et al., 1997
49	Feb. –Oct. 2009	Rain	22.9±33.0 (ND–80.8)		PCA/R2A cultivation (15°C)	Šantl-Temkiv et al., 2015
		Snow	3.3±6.4 (ND–14.7)			

Note: < > means the cultivability of bacteria in atmospheric waters (%); [ ] means *Pseudomonas syringae* only.

As mentioned in Sect. 1.2.2, some studies simultaneously identified live and dead bacterial cells in atmospheric waters by the use of fluorescence enumeration after staining with different dyes. Bauer et al. (2002) tested the LIVE/DEAD bacterial viability assay in one snow and two cloud water samples collected at Mt. Rax (1644 m a.s.l., Austria) in March 2000, and found that the viable bacteria cells (with intact membranes) accounted for about 87%, 72% and 95%, respectively. Hill et al. (2007) suggested that averagely 76±12 % of the bacteria in cloud water collected over the upper US Midwest in 2005, indicating that they were alive and actively metabolizing because they were able to reduce CTC. Stopelli et al. (2017)

found that the fraction of live cells among total bacterial cells averaged 0.6±0.1 in snow samples collected at the high-altitude observatory Jungfraujoch (3580 m a.s.l.), despite the harsh environment conditions, e. g., low temperatures (down to −25°C sometimes) and fierce solar radiation. However, the viability of bacteria in snow is likely lower than in rainwater due to the cruel environmental stresses. Monteil et al. (2014) and Šantl-Temkiv et al. (2015) found that rain samples generally contained more cultivable bacteria than snow samples (Table 1-2).

Amato et al. (2007d) first measured ATP levels (0.40 pmol mL^{-1} on average) in cloud water and, based on the available theoretical concentration for the ATP amount per viable cell, concluded that most of bacteria in their eight samples were in a viable state. Over the Long-term period 2004–2010, their colleagues detected the concentration of ATP in twenty-eight cloud water samples at the same site, the Puy de Dôme summit, which ranged from 0.01 to 2.01 pmol mL^{-1} and averaged 0.41±0.45 pmol mL^{-1} (Vaïtilingom et al., 2012). Cho and Jang (2014) measured the ATP content of the bacteria in rainwater, which ranged from 0.5 to 3.0 ng L^{-1} (0.99–5.9 fmol mL^{-1}). The per-cell ATP content (based on a fraction of 0.2–3 μm) in rainwater was in a range of 1.9–5.0 fg ATP $cell^{-1}$. The ATP amount was more strongly related to the concentration of cultivable microorganisms than to the concentration of total cells (Vaïtilingom et al., 2012). However, only a very small fraction, typically less than 1% (Table 1-2), of bacteria in atmospheric waters could be recovered by cultivation (Amato et al., 2007d; Cho and Jang, 2014; Vaïtilingom et al., 2012). This cultivability is consistent with that of airborne bacteria. The fraction of airborne bacteria that can be grown in culture is typically less than 10%, observationally ranging from 0.01% to 75%, and the estimated average is about 1% (Burrows et al., 2009b; Chi and Li, 2007; Heidelberg et al., 1997; Lighthart, 2000). The measurements mentioned above revealed that most bacteria in atmospheric waters are in a viable but non-cultivable status.

Dust particles transported through the atmosphere are likely to harbor assemblages of attached microbes (Christner et al., 2008a; Hara and Zhang, 2012; Maki et al., 2011, 2017; Yamaguchi et al., 2014). Atmospheric bacteria have to withstand a lot of stressors, such as UV radiation, extreme temperatures, desiccation and oxygen deficit, to maintain their viability (Amato, 2012). During the long-range aerial transport, a larger fraction of airborne bacteria could not remain viable due to the exposure to the stressors (Hara and Zhang, 2012; Murata and Zhang, 2014). However, atmospheric waters can protect airborne bacterial cells against desiccation, and contain organic compounds and other elements (e.g., phosphorus, iron, copper, and magnesium) that can act as nutrients and sustain metabolic activities for bacteria (Amato, 2012). Other factors, such as the origin and resistance ability of bacteria, and the physical (e.g., pH and osmotic pressure) and chemical properties of atmospheric waters, can also affect the viability of bacteria in the atmosphere (Maki et al., 2008). Delort et al. (2017) described the abilities of bacteria to survive in

clouds. For instance, bacteria developed general strategies, e.g. producing extracellular polymeric substances and pigments, and forming spores, and they can make response to specific stresses such as oxidative, osmotic and temperature stresses encountered in clouds by the activity of specific enzymes or protecting metabolites, e. g., osmo- and thermo-protectants, and anti-oxidants.

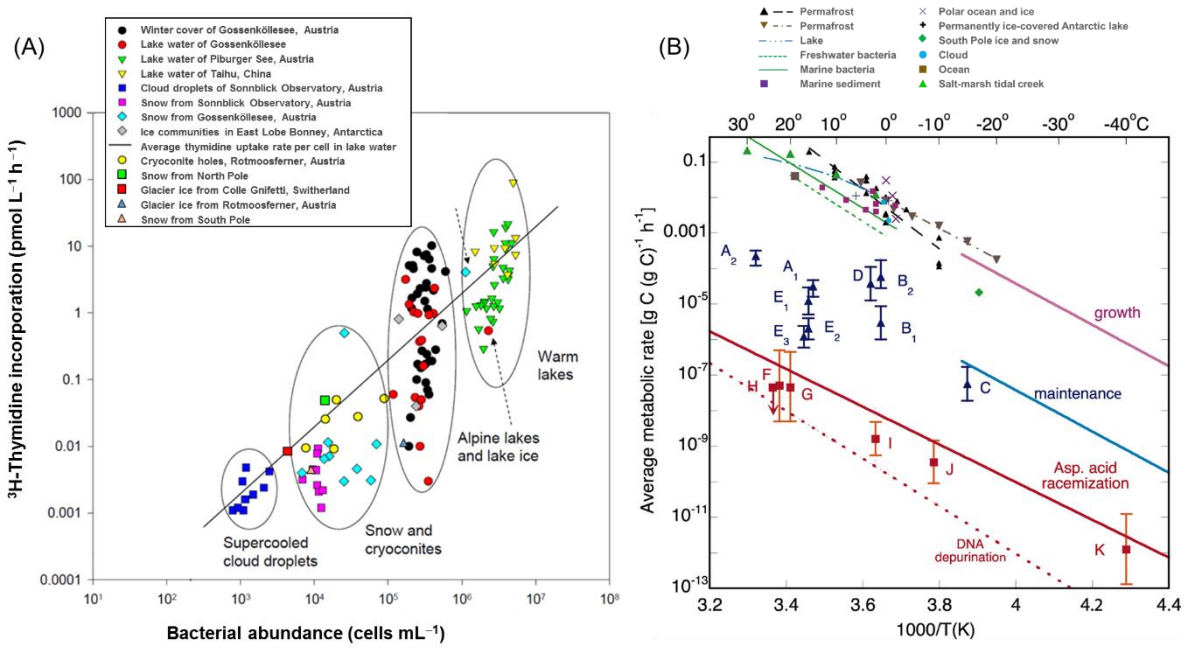

Figure 1-5. (A) Abundance and ^{3}H-thymidine uptake rates (growth rates) of bacteria in freshwater lakes, cloud water, freshly fallen snow, firn and ice. The solid line represents the average ^{3}H-thymidine uptake rate per cell in lake water at *in-situ* temperatures. Adapted from Sattler et al. (2001), Psenner et al. (2009) and Psenner (2006). (B) Comparison of metabolic rates for prokaryotes as a function of *in-situ* temperature in various ecosystems. The legend is added for the top group, labelled "growth". Lines join measurements at different temperatures in the same study. Lines at the bottom are extrapolated from rates of racemization of aspartic acid and rates of DNA depurination, both measured at high temperatures. Adapted from Price and Sowers (2004). Further details are available there.

Amato et al. (2007c) observed that about half of the isolated microorganism strains, mostly Gram-negative bacteria, have the capacity to grow at low temperatures (5°C) close to the natural atmospheric environment, and even a few of them are shown to be psychrophiles. Sattler et al. (2001) showed that bacteria can grow in supercooled cloud droplets collected at high altitudes according to ^{3}H-thymidine (Fig. 1-5 (A)) and ^{14}C-Leucine uptake rates. Other than in low-temperature clouds, psychrophilic bacteria strains have been recovered and demonstrated to be active in many cold environments, e.g. snow and ice in alpine, glacier and polar regions (Amato et al., 2007b; Carpenter et al., 2000; Christner et al., 2005, 2008a; Groudieva et al., 2004; Junge et al., 2004, 2006; Junge and Swanson, 2008; Miteva and Brenchley, 2005; Miteva et al., 2004; Segawa et al., 2005). Panikov et al. (2006) demonstrated microbial activity at

different subfreezing temperatures even down to −39°C. Price and Sowers (2004) investigated the temperature dependence of metabolic rates for microbial survival, maintenance and growth (Fig. 1-5 (B)) and found no proof for a minimum temperature for metabolism.

Although temperatures are low (below 0°C), the nutrient conditions in cloud, rainwater and freshly fallen snow can be better than in many freshwater lakes (Psenner et al., 2009; Sattler et al., 2001). It is likely that the limitation of temperature is partly compensated by nutrient availability and high abundance of microorganisms. Neither temperature nor nutrient condition but the residence time in the atmosphere is the limiting step for bacterial activity in atmospheric waters. Regarding temperature and substrate as interactional limiting factors, it is evident that the bacterial activity per cell in cold atmospheric waters is averagely comparable to that in eutrophic, temperate lakes (Fig. 1-5 (A)) (Psenner et al., 2009). Price and Sowers (2004) also found that prokaryotes in cloud water and snow have similar metabolic rates to those in freshwater, ocean, soil and sediment at the same temperature (Fig. 1-5 (B)). Therefore, the growth and processes of bacteria in the atmospheric waters cannot be ignored for assessing their impacts.

1.3.3 Community compositions

A small number of studies, mostly published recently, have provided limited information on the community structure of bacteria in atmospheric waters (Table 1-3). In rainwater, the most common bacterial phyla are *Proteobacteria* (*Alpha-*, *Beta-*, *Gamma-*), *Bacteroidetes*, *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Cyanobacteria*, etc. Kaushik et al. (2014) observed that the composite fresh rainwater in Singapore exhibited wide phylogenetic diversity: members of *Betaproteobacteria* were the most dominant, followed by *Alphaproteobacteria*, *Sphingobacteria*, *Actinobacteria* and *Gammaproteobacteria*. Lu et al. (2016) determined the bacteria community structures at the genus level were distinct in rainwater sampled at three mountain sites (900, 2740 and 1000 m a.s.l.) in China, and found that the members of *Burkholderia* were the most abundant, followed by *Massilia* and *Methylobacterium*. Cho and Jang (2014) reported that diverse bacteria appeared in rainwater collected at an inland site of Korea using both culture-dependent and independent methods, and the bacterial OTUs of the genera *Lysinibacillus*, *Bacillus*, *Escherichia*, *Acinetobacter*, *Pseudomonas* and *Sphigomonas* were detected. Marine bacteria, e.g., members of the phyla *Flavobacteria* and *Firmicutes*, were possibly dispersed via rain (Cho and Jang, 2014).

Peter et al. (2014) found that bacterial composition differed significantly between samples of rain events with and without Saharan dust intrusion arriving at a high mountain lake in the Austrian Alps. The classes *Gammaproteobacteria* and *Betaproteobacteria* were dominant in two cases, respectively, although alpha diversity indices were similar. Itani and Smith (2016) found distinct and diverse assemblages of bacteria in dusty and clean rains (Table 1-3), and the bacteria in dust rain matched, in

decreasing order of abundance, *Betaproteobacteria*, *Alphaproteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*, and those in clean rain matched only the family *Oxalobacteraceae* within *Betaproteobacteria*.

Several studies on the bacterial community structure in fresh snow or firn, mostly in surface snow in alpine and subalpine areas, were conducted. Šantl-Temkiv et al. (2015) surveyed cultivable bacterial community from snow and rainwater samples at Roskilde, Denmark, and found that Gram-negative bacteria dominated in all samples and *Pseudomonas* were predominant cultivable bacteria in rain and snow. Wunderlin et al. (2016) found that diverse bacterial communities were present in surface snow from the Swiss and Australian Alps, distributing among 25 distinct phyla, but the total relative abundance of six phyla *Proteobacteria* (*Alpha-* and *Beta-*), *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Firmicutes* was 72%–98%. Taxa associated with cold soils, such as *Acidobacteriaceae* and *Methylocystaceae*, may be atmospherically sourced, while families like *Sphingomonadaceae* were detected in each sample and are likely the common snow biome. Carey et al. (2016) studied the bacterial community structure of snow at two depths (0−15 and 15–30 cm) microbes at a subalpine site of California, US. The phylum *Proteobacteria*, consisting mostly of bacteria within the *Rhodospirillales* order and the *Acetobacteraceae* family, dominated in both layers (~72% of the sequences). *Cyanobacteria* only appeared in the upper snow layer, while *Actinobacteria* and *Firmicutes* were more abundant in deeper snow layer.

Segawa et al. (2005) identified a broad range of bacteria in the surface snow of Tateyama, including soil-derived, psychrophilic, psychrotrophic, and enteric bacteria. Most of the 16S rRNA gene clones belonged to *Betaproteobacteria*, and the clone library also contained *Alpha-* and *Betaproteobacteria*, *Bacteroidetes* (*Flexibacter*, *Cytophaga*, *Bacteroides*), and *Firmicutes* (*Bacillus/Clostridium*) and *Actinobacteria* (Table 1-3). The cultivable bacterial colonies at a low temperature in an in-situ snowmelt medium represented the *Acinetobacter*, *Aquaspirillum*, *Burkholderia*, *Corynebacterium*, *Haemophilus*, *Methylobacterium*, *Propionibacterium*, *Serratia*, *Staphylococcus*, *Streptococcus* and *Variovorax* genera (Segawa et al., 2005). Amato et al. (2007b) reported that the bacterial strains recovered from the snow cover at Spitsbergen, Svalbard belonged to *Proteobacteria (Alpha-, Beta- and Gamma-)*, *Firmicutes* and *Actinobacteria*.

Table 1-3 Dominant phylotypes of bacteria in atmospheric waters.

Site No.	Sample type	Cultivation (Y/N) [a]	Sequencing technology	Dominant phylotype	References
6	Bulk deposition	N	16S rRNA gene sequencing (clone library)	*Betaproteobacteria* (nom-dusty), *Gammaproteobacteria* (dusty), *Alphaproteobacteria*, *Bacilli*, *Sphingobacteria*	Peter et al., 2014
7	Rain	N	16S rRNA gene sequencing (NGS)	*Proteobacteria* (22–88%), *Bacteroidetes* (<5–43%)	Cho and Jang, 2014
		Y		*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria*	
52–54	Rain	N	16S rRNA gene sequencing (Sanger)	*Burkholderia* (21%), *Massilia* (17%), *Methylobacterium* (13%)	Lu et al., 2016
57	Rain	N	16S rRNA gene sequencing (clone library)	*Betaproteobacteria* (67%), *Alphaproteobacteria* (10%), *Sphingobacteria* (9%), *Actinobacteria* (7%), *Gammaproteobacteria* (7%), *Lentisphaerae* (4%)	Kaushik et al., 2014
58	Rain	N	DGGE-16S rRNA gene sequencing (clone library)	*Beta- and Alpha-proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Epsilonproteobacteria*, *Gamma- and Delta-proteobacteria* (dusty rain); *Oxalobacteraceae* (non-dusty rain)	Itani and Smith, 2016
		Y	16S rRNA gene sequencing (clone library)	*Actinobacteria*, *Firmicutes*, *Betaproteobacteria*, *Bacteroidetes*[*], *Gammaproteobacteria*, *Alphaproteobacteria* and *Deinococcus-Thermus*[*] ([*]only in dusty rain)	
61	Rain	N	16S rRNA gene sequencing (clone library)	(*Alpha-*, *Beta-*, *Gamma-*) *Proteobacteria* (75.6–100%), *Bacteroidetes*, *Actinobacteria*, *Deinococcus-Thermus*, *Cyanobacteria*, *Nitrospira* and *Firmicutes*	Liang et al., 2014a
62	Rain	N	16S rRNA gene sequencing (clone library)	*(Alpha-, Beta-, Gamma-) Proteobacteria (32.5–94.1%), Bacteroidetes, Actinobacteria, Deinococcus-Thermus, Cyanobacteria, Acidobacteria and Firmicutes*	Liang et al., 2014b
30–32	Snow	N	16S rRNA gene sequencing (NGS)	*Alpha-* and *Beta-proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Firmicutes* (72–98% in total)	Wunderlin et al., 2016

Table 1-3 (*Continued*)

Site No.	Sample type	Cultivation (Y/N) [a]	Sequencing technology	Dominant phylotype	References
33–36	Snow	N	16S rRNA gene sequencing (clone library)	*Bacteroidetes*, *Betaproteobacteria* (Guoqu Glacier); (*Alpha-*, *Beta-*, *Gamma-*) *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* (Zadang Glacier); *Gammaproteobacteria* (East Rongbuk Glacier) *Alphaproteobacteria* and *Actinobacteria* (Palong No. 4 Glacier)	Liu et al., 2009
49	Rain, snow	Y	16S rRNA gene sequencing	*Gammaproteobacteria* (49%): *Pseudomonas* and *Erwinia* (40%); *Curtobacterium*, *Arthrobacter*, *Microbacterium*, *Mcrococcus*, *Plantibacter*, *Pedobacter*, *Bacillus*	Šantl-Temkiv et al., 2015
59	Snow	N	16S rRNA gene sequencing (NGS)	*Proteobacteria* (72%): *Rhodospirillales*; *Actinobacteria* and *Firmicutes* (deep snow layer)	Carey et al., 2016
60	Snow	N	ARDRA-16S rRNA sequencing (Sanger)	*Betaproteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes* (*Flexibacter*, *Cytophaga*, *Bacteroides*), *Firmicutes* (*Bacillus*/*Clostridium*), *Actinobacteria*	Segawa et al., 2005
12	Cloud water	Y	16S rRNA sequencing (capillary electrophoresis)	(*Alpha-*, *Beta-*, *Gamma-*) *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria*	Amato et al., 2005, 2007b; Vaïtilingom et al., 2012
15–17	Cloud water	N	PCR-DGGE/ 16S rRNA gene sequencing (Shotgun)	*Cyanobacteria*, *Proteobacteria*, *Actinobacteria* and *Firmicutes*	Kourtev et al., 2011
48	Fog water	Y	Conventional and modified conventional method	*Bacillus sp.*, *Pseudomonas sp.*, *Acinetobacter lw*.	Fuzzi et al., 1997
50	Cloud water	N N	16S rRNA gene sequencing (NGS)	*Proteobacteria* (~80%), *Bacteroidetes*, *Cyanobacteria* and *Firmicutes* *Proteobacteria* (82%), *Bacteroidetes* (4%), *Firmicutes* (7%) and *Actinobacteria* (2%)	Wei et al., 2017 Xu et al., 2017
55–56	Cloud water, rain	N	16S rRNA gene sequencing (capillary electrophoresis)	*Pseudomonads sp.*, *Acinetobacter sp.*, *Comamonas testosteroni*	Ahern et al., 2007

Note: [a] Y, DNA extraction from cultivable bacteria; N, direct extraction of total DNA.

Tibetan Plateau is the highest plateau in the world, in which glaciers sensitively indicate global climatic change. The community structure of bacteria in the snow of the glaciers, e.g., East Rongbuk, Guoqu, Zadang, Palong No. 4, Laohugou and Hailuogou, located in different climatic zones in the Tibeten Plateau was described in several studies (Liu et al., 2009; Liu et al., 2006; Zhang et al., 2014; Zhang et al., 2010). The phyla, *Proteobacteria* (*Alpha-*, *Beta-* and *Gamma-*), *Firmicutes*, *Actinobacteria* and *Bacteroidetes*, were dominant in the snow of the glaciers, identified using 16S rRNA gene clone library approach and DGGE-16S rRNA sequence analysis, respectively. Some common genera, e.g., *Sphingomonas* and *Polaromonas*, existed widely among the glaciers (Liu et al., 2009; Zhang et al., 2010), and the bacterial diversity at different glaciers was influenced by the surrounding environments (Liu et al., 2009). Zhang et al. (2010) pointed that the five genera *Hylemonella*, *Delftia*, *Zoogloea*, *Blastococcus* and *Rhodococcus* were endemic in the glaciers East Rongbuk, Laohugou and Hailuogou glaciers. The class *Gammaproteobacteria* was the dominant phylotype, and *Acinetobacter* and *Leclercia* were dominant at the genus level in the snow of East Rongbuk glacier. Zhang et al. (2014) found that the bacterial diversity in the glacier Laohugou No.12 was the highest in proglacial soil, followed by that of glacial snow in the ablation zone, in the accumulation area, and in glacier terminus in order. *Flavobacterium*, *Massilia*, *Pedobacter* and *Polaromonas* were more abundant in glacial snow than in glacial soil.

Since 2000, there have been crescent studies focusing on the bacterial community and activity in Antarctic and Arctic snow (Carpenter et al., 2000; Harding et al., 2011; Hauptmann et al., 2014; Lopatina et al., 2013, 2016; Møller et al., 2017; Michaud et al., 2014b; Mortazavi et al., 2015). Recently, advance approaches, e.g., NGS, has been applied. The dominant phylotypes in polar snow are almost the same as those in other glacier snow mentioned above, although novel bacterial communities are detected at different sites. Because the bacterial metabolic activity in glaciers and polar snow is arguable (Stephen and Stephen, 2003) and the microbiological property of snow covers is probably different from that of fresh snow, here we no longer give an explicit review on the composition of bacterial community in polar snow. More details on bacteria in alpine and glacier snow are available in previous overviews (González-Toril et al., 2009; Miteva, 2008).

There are several studies reported the identification of bacteria in fog and cloud water. Based on conventional culture method, Fuzzi et al. (1997) reported the occurrence of three genera including *Pseudomonas*, *Bacillus* and *Acinetobacter* in fog droplets collected at the Po Valley, where fog droplets contained high levels of pollutants. At the Puy de Dôme summit, the cultivable bacteria communities in cloud water were more diversified. They mainly belonged to *Proteobacteria* (*Alpha-*, *Beta-* and *Gamma-*), *Bacteroidetes*, *Actinobacteria* and *Firmicutes*, and the genera *Pseudomonas*, *Sphingomonas*,

Staphylococcus, *Streptomyces* and *Arthrobacter* were dominant (Amato et al., 2005; Amato et al., 2007c; Vaïtilingom et al., 2012). Xu et al. (2017) studied the community structure of bacteria in cloud water at Mt. Tai, and found that Gram-negative bacteria including *Proteobacteria* (81.6%) and *Bacteroidetes* (3.9%) dominated the bacterial population, while Gram-positive bacteria *Firmicutes* and *Actinobacteria* accounted for 7.1% and 2.3%, respectively. Wei et al. (2017) found that the predominant phyla in cloud water collected at Mt. Tai were *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Firmicutes*, and the abundant genera were *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, and *Empedobacter*, which originated from a wide range of habitats. Ahern et al. (2007) found many strains belonging to the *Pseudomonas* and *Acinetobacter* genera were present in cloud water collected in Scotland, and noted that 80% of OTUs with two or more clones were found only in rain or only in cloud water, reflecting an intrinsic difference in bacterial compositions in rain and cloud water.

Overall, bacteria in atmospheric waters at different sites displayed highly diverse community compositions. The dominant phyla in precipitation, e.g., (*Alpha*-, *Beta*- and *Gamma*-) *Proteobacteria*, *Actinobacteria*, *Bacteriodetes*, and *Firmicutes*, were also often identified in cloud/fog water. These bacterial phyla are common in different forms of atmospheric waters, suggesting that they could be involved in precipitation at the stage of cloud nuclei formation. The fact that the Gram-negative community is dominant in atmospheric waters is consistent with that Gram-negative bacteria are much more efficient at growing at low temperatures (Amato et al., 2007c). To the extent of available data, the genus-level diversity and richness of bacteria in atmospheric waters differed substantially at various sites across the world. Diverse sources of bacteria involved in clouds likely caused the distinct bacterial community structures in atmospheric waters at different sites.

1.4 Impacts of bacteria in atmospheric waters

1.4.1 Nuclei in clouds

Bacteria could potentially have a significant role in affecting cloud formation and the hydrologic cycle by acting as nuclei for the formation of water droplets or ice crystals (Hoose et al., 2010; Konstantinidis, 2014; Vali et al., 1976). There are detailed review papers on the role of atmospheric microorganisms including bacteria in cloud formation in previous papers (e.g., Ariya et al., 2009; Delort et al., 2010; Després et al., 2012; Fröhlich-Nowoisky et al., 2016; Möhler et al., 2007; Sun and Ariya, 2006).

Ice nucleation-active (INA) bacterial species, which may function as warmer temperature (above −10°C and up to −2°C) ice-nucleating particles (INP), have been widely identified in atmospheric waters (Ariya et al., 2009; Hill et al., 2014; Morris et al., 2004). The protein product of a gene (*inaZ*) responsible

for ice nucleation by INA bacterial species, e.g., *Pseudomonas syringae*, has been identified (Green and Warren, 1985). INA bacteria may contribute effectively to atmospheric processes, i.e., potentially trigger glaciation and precipitation. INA cells are likely emitted to the atmosphere from terrestrial surfaces, either have a long-distance continental or a local origin (Šantl-Temkiv et al., 2015). Among bacterial ice nucleators, species of the genus *Pseudomonas*, e.g., *Pseudomonas syringae* and *Pseudomonas fluorescens*, are considered the most common and efficient. The members of the genera *Pseudomonas*, *Xanthomonas*, *Erwinia*, and *Pantoea* within the subclass *Gammaproteobacteria* are the most efficient INA bacteria described so far (Joly et al., 2013). In general, not all strains within one species are INA, and also within the INA strains, the fraction of ice-nucleating cells varies significantly (Hirano and Upper, 1995). Active number fractions vary between 10^{-8} and close to 1, and the onset of ice nucleation can be observed at temperatures between −2 and −10°C (Fig. 1-6).

There have been several studies on the bacterial and/or biological IN in atmospheric waters, e. g., cloud ice-crystal or water, rain and snow. Pratt et al. (2009) studied the chemistry of individual cloud ice-crystal residues, showing that biological particles and mineral dust comprised most of the ice-crystal residues (33% and 50%), suggesting that certain biological and dust particles initiated ice formation in clouds, and that biological particles can enhance the impact of desert dust storms on cloud ice formation. Creamean et al. (2013) show that long-distance transported biological and dust and aerosols appeared in glaciated high-altitude clouds coincident with elevated IN concentrations and ice-induced precipitation, suggesting that dust and biological aerosols probably act as IN and play an important role in precipitation processes. Joly et al. (2013) recovered active strains of INA bacteria as *Pseudomonas syringae*, *Xanthomonas* sp. and *Pseudoxanthomonas* sp. from cloud water, and estimated that the number of bacterial IN in clouds influenced by vegetated areas fell into 0 and 500 mL^{-1} of water between −3°C and −10°C depending on the season. Monteil et al. (2014) examined the abundance, ice nucleation activity and population structure of *Pseudomonas syringae* in rain and snow in southern France. Michaud et al. (2014a) show that hailstone embryos from three Rocky Mountain storms contained biological ice nuclei capable of freezing water at warm, subzero (°C) temperatures, indicating that biological particles can act as nucleation sites for hailstone formation.

The concentration of bacterial and biological ice nucleators is usually estimated by determining the fraction of IN that are sensitive to lysozyme digestion and heat treatment, respectively (Christner et al., 2008a; Hara et al., 2016a; Joly et al., 2014; Lu et al., 2016). Recently, it was suggested there might be potentially efficient submicron or nanoscale ice nucleator in precipitation, e.g., INA bacterial fragments (<220 nm) (Du et al., 2017; Šantl-Temkiv et al., 2015). As mentioned above, qPCR techniques are also used to detect, identify and quantify INA bacteria (Hill et al., 2014). The concentrations of

bacterial/biological ice nuclei in atmospheric waters reported in previous studies are listed in Table 1-4. Despite the difference caused by diverse approaches, the concentrations of INA bacterial/biological particles in atmospheric waters and the onset temperatures of freezing vary largely. The various detected frequencies of INA bacteria in atmospheric waters result in a large uncertainty of the numbers of bacterial IN in the atmosphere implemented in global climate models.

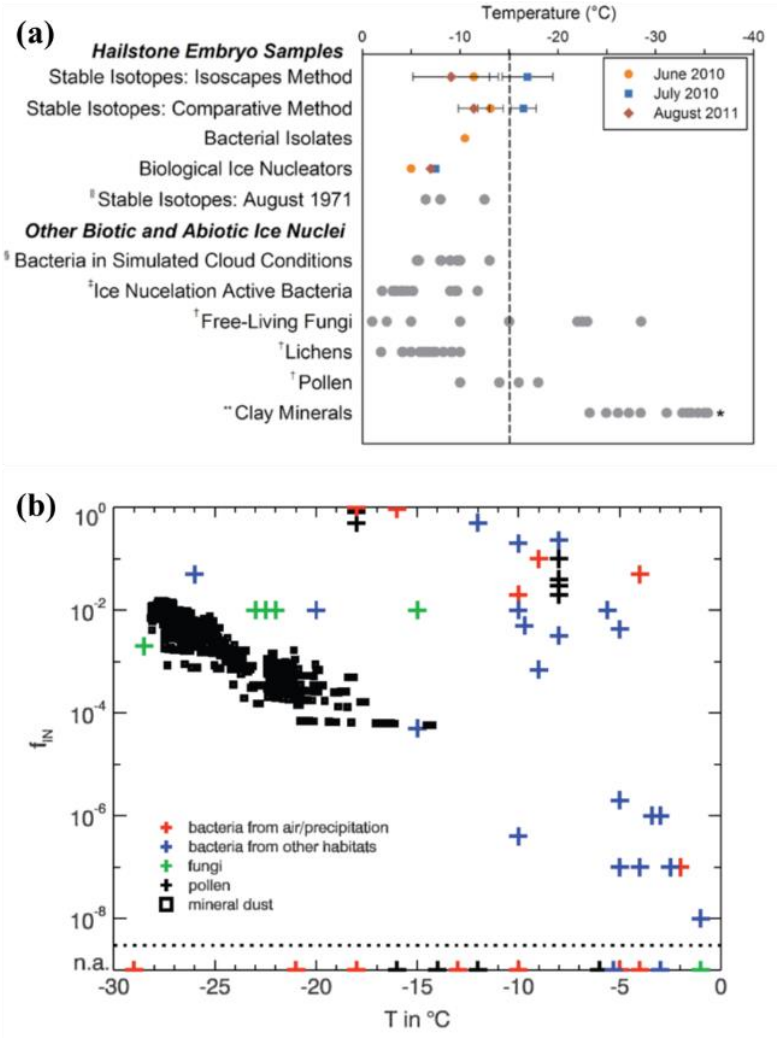

Figure 1-6. (a) Freezing point temperatures computed from four different methods for embryos from hailstorms occurring in the Rocky Mountain region. The dashed vertical line represents the warmest abiotic nucleation temperature. The single asterisk within the panel indicates that the temperature of nucleation for abiotic particles is significantly different (p<0.001) from biological ice nucleation temperatures and isotopically determined freezing temperatures presented. The right side of the figure is bounded by the warmest temperature at which water will homogenously freeze without a biotic or abiotic ice nucleator at about -40°C. Reprinted from Michaud et al. (2014a). (b) Ice nucleating number fraction f_{IN} at the observed IN onset and maximum activity temperatures from laboratory measurements. For comparison, f_{IN} data for immersion freezing on mineral dust (natural soil samples, median diameters of 0.2–1 μm) are included. Reprinted from Després et al. (2012).

Table 1-4 Concentrations of bacterial/biological ice nuclei in atmospheric waters. DFA, droplet-freezing assay.

Site No.	Number of samples	Sample type	Treatment	Measurement method	Concentration (mL^{-1})	References
12	12	Cloud water	Heat treatment	DFA (–5 – –14°C)	0 –~220 [a] [–10]	Joly et al., 2014
	25		Cultivation/isolation	DFA (–2 – –12°C)	0 –~500 [b] [–3 – –10]	Joly et al., 2013
18–30	16	Rain	Heat treatment	DFA (–2 – –13°C)	0–0.63 [a] [–11]	Christner et al., 2008a, 2008b
			Lysozyme digestion		0–0.29 [b] [–12]	
	19	Snow			0–0.12 [a] [–7]	
					ND–0.04 [b] [–7]	
Near 18-21	3	Hailstorm embryo	Heat treatment	DFA (–2 – –12°C)	3–8 [a] [–5 – –7.5]	Michaud et al., 2014a
24–27, 37–47	25	Rain	Cultivation/isolation	DFA (–2 – –8°C)	0.35 [c] [–4.03]	Monteil et al., 2014
	65	Snow			4.57 [c] [–4.07]	
49	9	Rain	Cultivation/isolation	DFA (–4, –5, –7°C)	ND–11.4 [d] [–7]	Šantl-Temkiv et al., 2015
	5	Snow			ND [d] [–7]	
			Filtrates of 0.22-μm filters/heating	DFA	0.20–0.48 [e] [–9]	
51	4	Rain	Heat treatment	DFA (–6 – –10°C)	16–78 [a] [–6– –10]	Du et al., 2017
			Filtrates of 0.22-μm filters/heating		0–61 [e] [–6– –10]	
63	17	Hail, snow	DNA extraction	qPCR	0.13±0.30 (ND–1.2) [f] [–10]	Hill et al., 2014

Note: [a] biological IN mL^{-1}; [b] bacterial IN mL^{-1}; [c] CFU of *Pseudomonas syringae* strains cultured from 1 mL samples; [d] CFU of bacterial strains cultured from 1 mL samples; [e] INA cell fragments (<220 nm) mL^{-1}; [f] *ina* gene copies mL^{-1}. Temperatures of ice nucleation activity are in square brackets. ND, not detected.

1.4.2 Atmospheric chemistry

Bacteria are significant, not only for their ability acting as CCN and IN, but for their potential role in atmospheric chemistry by actively metabolizing the organic compounds present in the air (Konstantinidis, 2014; Sun and Ariya, 2006). The chemical interactions of microorganisms in the atmosphere have been reviewed in previous literature (e.g., Deguillaume et al., 2008; Delort et al., 2010, 2017; Sun and Ariya, 2006).

Organic compounds represent a major fraction of the soluble matter in atmospheric waters, e.g., cloud and fog droplets (Delort et al., 2017). On the one hand, atmospheric waters provide good culture media for the growth of bacteria and other microorganisms (Fuzzi et al., 1997; Bauer et al., 2002). On the other hand, bacteria can adapt their metabolism because it is found that a large fraction of the community is metabolically active and that they metabolize organic compounds in atmospheric water (Delort et al., 2017).

There are some studies concerning the interactions between microbes and chemical species in atmospheric waters. For the first time, Herlihy et al. (1987) showed bacterial utilization of formic and acetic acid in rain water, suggesting a possible impact of microorganisms on atmospheric multiphase chemistry. Amato et al. (2005, 2007a) showed that using in situ ^{1}H NMR, most of the bacterial strains isolated from cloud water sampled at the Puy de Dôme were able to degrade various monoacid and diacid compounds (acetic, lactic, formic and succinic acids), methanol and formaldehyde. These compounds were present in relatively high concentrations in cloud water and play a major role in atmospheric chemistry. Preferential metabolic routes for some of those strains were indicated by intermediates. Vaïtilingom and his colleagues conducted a series of experiments to compare microbiological pathways and photochemical reaction pathways of organic compounds in cloud water (Vaïtilingom et al., 2010, 2011, 2013). Šantl-Temkiv et al. (2013) reported that viable methanotrophic bacteria in rainwater, e.g., the members of the genera *Methylocystis* and *Methylosinus*, and the bacteria could oxidize methane at atmospheric concentrations even at low pH in cloud droplets. Renard et al. (2016) identified the most efficient biosurfactant bacterial producers (σ < 45 mN m^{-1}) in cloud water sampled at the Puy de Dôme belong to a few genera (*Pseudomonas* and *Xanthomonas*) within the *Gammaproteobacteria* class (78%). *Pseudomonas*, the most frequently detected genus in clouds, was the dominant group to produce biosurfactants. The presence of biosurfactant molecules could impact atmospheric chemistry (e.g., secondary organic formation) and microphysics by modifying CCN activation owing to their exceptional scope in reducing surface tension.

In addition to the carbon cycle, bacteria likely play an important role in the nitrogen cycle in clouds (Hill et al., 2007). Nitrifying bacteria were identified in the cloud water, indicating that bacterial processing of nitrogen in the cloud water may occur. Carey et al. (2016) found that the most predominant phylotype (NCR4874) of subalpine Sierra Nevada snowpack, comprised about 22-32% of the sequences, was most closely related to the N_2-fixing bacteria *Asaia siamensis* within *Acetobacteraceae*, indicating that nitrogen fixation may be an important process in the snow layer. Christener et al. (2008b) reported that there was a negative correlation between microbial cell abundance and the concentration of ammonium and total organic carbon (TOC) in Montana snowfall. They suggested that one plausible explanation for this result is heterotrophic growth of microbes via the assimilation of carbon and nitrogen.

1.4.3 Ecosystems

Precipitation induced by bacteria acting as IN or CCN sustains the hydrological cycle and bacterial reproduction in the ecosystem from which airborne bacteria are emitted. The feedback mechanisms involved may be significant for stabilizing the ecosystems and may also be generally relevant for the evolution of ecosystems and climate on the global scale and in the Earth's history (Pöschl et al., 2010).

There have been multiple studies observed increased concentrations of bioaerosols and/or biological IN in the air during and after precipitation (Bigg et al., 2015; Hara et al., 2016b; Heo et al., 2014; Huffman et al., 2013; Kang et al., 2015; Prenni et al., 2013; Rathnayake et al., 2017). The mechanisms of such effects are complex. A fraction of the increased microbiological particles transported by falling raindrops from elevated altitude to the ground, and result from the resuspension of microbiological particles wet deposited on the surfaces. These processes significantly drive the development, evolution and conservation of the Earth ecosystems.

The impact of precipitation on the microbial properties of waters through atmospheric wet deposition of microorganisms was studied in several studies (e.g., Kaushik et al., 2014; Chen and Chang, 2014). Peter et al. (2014) found that several bacterial taxa (e.g., *Pseudomonas*, *Janthinobacterium*) in rainwater collected during Saharan dust were viable in lake freshwater. Cell numbers during the two experiments with dusty rainwater inoculated into sterile lake water rapidly increased from initially ~3×10^3 to 3.6–11.1×10^5 cells ml^{-1} within 4–5 days. Hence, microorganisms involved in atmospheric waters might be an important source for the rare bacterial biosphere in aquatic ecosystems, e.g., freshwater lakes.

Plants are a major source of airborne bacteria, including INA bacteria (Ariya et al., 2009). INA bacteria leave plant leaf surfaces and enter the troposphere during dry, warm weather and are transported. They participate in a sort of biological cycle of precipitation-whereby they are transported into clouds from plant canopies and incite precipitation thereby causing favorable conditions for their growth on plant surfaces (Constantinidou et al., 1990; Morris et al., 2004). However, the presence of INA bacteria on the surface of leaves can enhance the degree of frost damage of plants by causing water on or in plants to freeze where it might have simply supercooled. In addition, INA bacteria can play multiple roles in the plant environment, e.g., mostly as plant pathogens and as antagonists of other plant pathogens. The overall impact of INA bacteria needs to optimize the benefits for all concurrent interests (Morris et al., 2004).

1.4.4 Public health

It has been indicated that excessive rainfall is a significant contributor to historical waterborne disease outbreaks due to mobilization and transport of bacterial pathogens (Kaushik and Balasubramanian, 2012 and references therein). As mentioned above, atmospheric wet deposition (rainfall, snowfall) might thus play an important role for the entry of bacterial pathogens into aquatic systems. The spread of potential pathogens and allergens via atmospheric waters should be paid attention to as it threatens public health.

Potential bacterial pathogens in rainwater were identified in several studies. Using qPCR, Kaushik and Balasubramanian (2012) and Kaushik et al. (2012) assessed the bacterial pathogens (*Escherichia coli*,

Klebsiella pneumoniae, *Pseudomonas aeruginosa* and *Aeromonas hydrophila*) in fresh rainwater in Singapore. It was found that *E. coli* was the most prevalent potential pathogen in fresh rainwater. Cho and Jang (2014) reported the presence of potential human pathogen (i.e., *Acinetobacter johnsonii*) sequence and *E. coli*-like sequence in rainwater in Seoul, Korea, implying occasional distribution and dispersion of the pathogens in rainwater. These results suggest the need of monitoring human pathogens in rainwater for the reuse of rainwater.

The economic value of rainwater is currently highly evaluated. Rainwater harvesting (RWH) offers considerable potential as an alternative water supply, especially in water-deficient areas and developing countries. To assess the microbial quality of rainwater and treat the RWH to fulfil the standard of water supply is necessary (Evans et al., 2006; Lee et al., 2010; Dobrowsky et al., 2014). Moreover, assessing the microbiological quality of rainwater will aid in the development of effective management controls on potential pathogen contamination in surface waters as mentioned above so that they are suitable for water supply, recreation, and aquatic habitats (Kaushik et al., 2014; Lee et al., 2010; Chen and Chang, 2014; Santiago-Rodriguez et al., 2012).

1.5 Research objectives, contents and workflow

Dissemination of bacteria via atmospheric waters is considered one of crucial processes for linking bacterial communities between air, land, and water surface on the earth (Amato, 2012; Cho and Jang, 2014; Morris et al., 2014). To better understand and model the activities of bacteria in the atmosphere–biosphere–hydrosphere system, quantitative data on the concentration, status (live or dead), composition, and dynamics of bacteria in atmospheric waters are essential (Konstantinidis, 2014). In addition, information on the abundance, viability and community composition of bacteria in atmospheric waters is necessary to assess their impacts on meteorological and climatic processes, biogeographical distribution, ecosystems and public health (Fahlgren et al., 2010; Hara and Zhang, 2012).

Rain is the settling of cloud droplets to the surface when the droplets are too large to suspend in the air. Studying bacteria in rainwater is a very convenient access to understand the interactions between bacteria and cloud processes. The concentration and community composition of bacteria in rainwater have been investigated in several observational studies. However, the knowledge on the characteristics (e.g., abundance, status and community composition) of bacteria in rainwater remains largely vague. In particular, few data are available on the status of bacteria in rainwater, and most of the activities and consequences of airborne bacteria in rain remain speculative. Exploring the bacterial status can underline the versatility of bacteria in rainwater, address the activities and roles what bacteria have and play in fundamental meteorological processes (e.g., CCN and IN formation), and assess the environmental, ecological and climatic effects of bacteria in rainwater. The lack of the information is mainly because

there is not a carefully evaluated approach for the measurement of the concentration and viability of bacterial cells in rainwater, although multiple techniques have been developed to quantify bacteria in aquatic environments, e.g., river, lake and sea.

The components in rainwater are highly influenced by the nature of relevant clouds, such as cloud formation mechanisms (caused by low-level vortices or upper troughs) and origins (continental or marine). Bacteria in rainwater may originate from the air or metabolic reproduction. Since air parcels inducing the clouds and rain have respective histories of travel in the past few days, the characteristics (e.g., concentration, viability and community composition) of bacteria in the rainwater of different types of rain might be distinctive (Monteil et al., 2014). Consequently, the data on the concentration, viability and community composition of bacteria in rainwater from different types of rain events are fundamental information for an accurate understanding of the roles that airborne bacteria play in cloud and rain processes, as well as in the development and evolution of the Earth′s environments due to their dispersal via atmospheric processes (Zhang et al., 2017).

In East Asia, rain events can be classified into different types according to the processes of cloud formation in synoptic weather systems, e.g., cyclones (cold fronts), stationary fronts, and typhoons (Toyonaga and Zhang, 2016; Yu et al., 1998). In view of the circulation on synoptic scales, atmospheric processes causing these rain types are distinctive. Cold front rain results from the southeastern intrusion of cold air from the polar region and the lifting of warm air from the southwest or south, i.e., clouds are generally influenced by Asian continental outflows. Stationary fronts usually occur in early summer when cold and dry air from the west encounters warm and humid air from the south when it intends to expand northward. In such cases, clouds are continuously produced along the fronts and it rains intermittently until the warm and humid air from the south completely expands to the north. This rainy period is called the Meiyu (plum rain) season in East Asia. There are also some other types of stationary front-associated rain episodes in non-Meiyu periods. For example, there are rain episodes similar to the Meiyu rain in every autumn but the period, usually one to two weeks, is much shorter than the Meiyu period. In spring and summer, some cyclones move very slow due to blocks of high pressures in the east, which also results in short-term stationary front rain. Typhoons originate at the tropic central Pacific and move along the outskirts of subtropical anticyclones in the northwest Pacific, and usually bring short-term heavy rain. Other than the rain due to synoptic scale weather, there are also thunderstorms which are in small scales and induced by surface heating during the daytime in summer.

To our knowledge, there has been no report on the association of the characteristics of bacteria present in rainwater with the synoptic weather. The dependence of bacteria on rain types on the basis of the synoptic weather, i.e., the mechanisms causing the rain, has not been examined, which made the

reported data less referentially meaningful because it is difficult to compare the results accurately among different areas.

The main objectives of this study are listed as follows.

1. To offer a reliable method of quantifying the number concentrations of viable and non-viable bacterial cells in rainwater;

2. To study the abundance, viability and community of bacteria in rainwater according to the synoptic weather;

3. To give insights into the sources of bacteria in rainwater;

4. To support better understanding and modelling the role of bacteria playing in the biosphere-hydrosphere-atmosphere links.

The specific research contents in this study include:

1. Based on laboratory experiments and field tests, we investigated the factors influencing the results in sample preparation, LIVE/DEAD BacLight stain and enumeration, and verified the operation procedures and conditions using LIVE/DEAD BacLight stain with glutaraldehyde fixation in EFM enumeration to quantify the abundance of viable and non-viable bacteria in rainwater;

2. Using the verified method, the abundance and viability of bacteria in rainwater samples collected under different synoptic weather systems were examined, and the influences of synoptic weather on the variations of the abundance and viability of bacteria in rainwater were explored;

3. Applying the high-throughput sequencing technique, the community compositions of bacteria in rainwater associated with the synoptic weather were identified, and the association of the bacterial communities in rainwater with synoptic systems as well as the implications for cloud formation, ecosystems and public health were discussed;

4. The water-soluble ionic species in rainwater were measured. The potential sources of bacteria in rainwater were speculated based on the relationships between bacterial abundance and viability and ionic concentrations, as well as indicator bacteria of potential sources.

In specific, the method applied in this study and the workflow are show in Fig. 1-7.

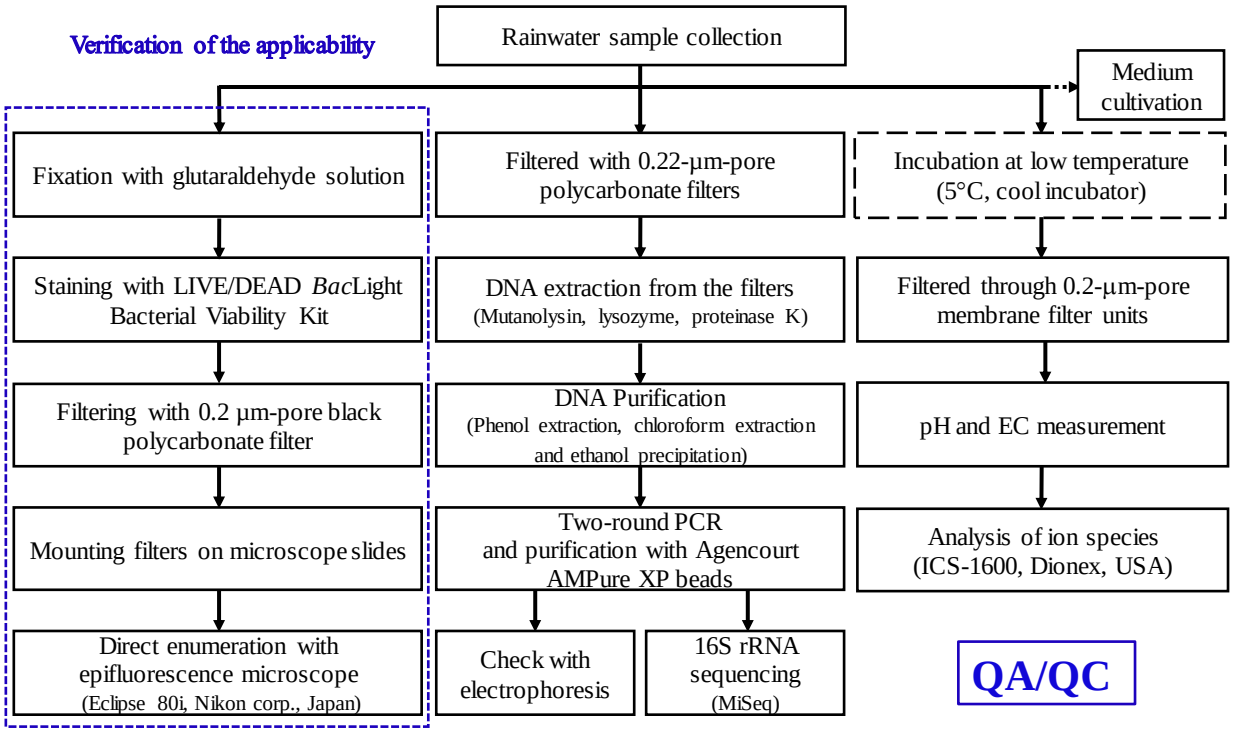

Figure 1-7. Work flowchart of this study.

Supporting information of "Bacteria in atmospheric waters: detection, characteristics and implications"

Table S1-1 Information of the sampling locations for atmospheric waters.

Site No.	Site	Altitude (m)	Latitude	Longitude
1	Kumamoto, Japan	60	32.806°N	130.766°E
2	Shizuoka, Japan	80	34.966°N	138.430°E
3	Tsukuba, Japan	45	36.055°N	140.125°E
4	Mt. Fuji, Japan	1485	35.361°N	138.728°E
5	Mt. Norikura, Japan	2770	36.108°N	137.550°E
6	Tyrolean Alps, Austria	2417	47.217°N	11.000°E
7	Seoul, Korea	102	37.460°N	126.950°E
8	Charlottesville, Virginia, US	195	38.200°N	78.508°W
9-1	Mainz, Germany		49.993°N	8.247°E
9-2	Mt. Kleiner Feldberg/Taunus, Germany	857	50.222°N	8.447°E
10	Mt. Rax, Austria	1644	47.717°N	15.767°E
11	Mt. Sonnblick, Austria	3106	47.054°N	12.957°E
12	Puy de Dôme summit, France	1465	48.000°N	2.000°E
13	Northern Michigan, US	2240	44.483°N	83.800°W
14		3090	44.483°N	84.450°W
15		2700	44.967°N	85.200°W
16		3350	44.800°N	84.900°W
17		3050	44.250°N	84.650°W
18	Bridger Bowl; Bozeman, MT, US	2265	45.817°N	110.909°W
19	Big Sky Resort; Big Sky, MT, US	2720	45.270°N	111.300°W
20	Yellowstone Club; Big Sky, MT, US	2820	45.261°N	111.413°W
21	Moonlight Basin; Bozeman, MT, US	2620	45.291°N	111.437°W
22	St. Saturnin-les-Avignon, France	83	43.959°N	4.923°E
23	Font Romeu, France	1780	42.521°N	2.063°E
24	Font Romeu, France	2000	42.505°N	2.042°E
25	Villard de Lans, France	1220/1200	45.052°N	5.564°E
26	Saint-François Longchamp, France	1416/1700	45.410°N	6.348°E
27	La Clusaz, France	1104	45.906°N	6.436°E
28	Wheaton Glacier, Yukon, Canada	1585	60.067°N	135.537°W
29	Ross Island, Antarctica	34	77.833°S	166.600°E
30	Jungfraujoch, Switzerland	3580/3450	46.548°N	7.985°E
31	Rosstock, Switzerland	1500–2366	46.931°N	8.701°E

Table S1-1 (*Continued*)

Site No.	Site	Altitude (m)	Latitude	Longitude
32	Kosciuszko/Thredbo, Australia	1956–2084	36.491°S	148.286°E
		1460–1515	36.516°S	148.280°E
33	Guoqu, Tibetan Plateau Glaciers, China	6621	33.580°N	91.180°E
34	Zadang, Tibetan Plateau Glaciers, China	5799	30.476°N	90.645°E
35	East Rongbuk, Tibetan Plateau Glaciers, China	6520	28.017°N	86.284°E
36	Palong No. 4, Tibetan Plateau Glaciers, China	5507	29.226°N	96.920°E
37	Col de Vars, Alpes de Haute Provence, France	2100	44.537°N	6.702°E
38	Font Romeu, Aveillans, Pyrénées-Orientales, France	1780	43.521°N	2.063°E
39	Châteaurenard, Bouches-du-Rhône, France	55	43.881°N	4.859°E
40	Montfavet, St Paul, Vaucluse, France	30	43.916°N	4.882°E
41	Montfavet, St Maurice, Vaucluse, France	25	43.946°N	4.864°E
42	St Saturnin-les-Avignon, Vaucluse, France	83	43.959°N	4.923°E
43	Super Sauze, Alpes de Haute Provence, France	2000	44.349°N	6.710°E
44	Savines le Lac, Alpes de Haute Provence, France	830	44.539°N	6.426°E
45	Ceillac, Hautes Alpes, France	2200	44.636°N	6.790°E
46	Col du Lautaret, Hautes Alpes, France	2100	45.036°N	6.402°E
47	Les Carroz d'Araches, Haute Savoie, France	1111	46.026°N	6.638°E
48	Po Valley, Italy	13	45.000°N	10.500°E
49	Roskilde, Denmark	~40	55.692°N	12.105°E
50	Mt. Tai, China	1534	36.250°N	117.100°E
51	Hulunber Grassland, Inner Mongolia, China	628	49.317°N	120.050°E
52	Mt. Wuling, China	900	40.383°N	117.567°E
53	Mt. Changbai, China	2740	40.700°N	127.700°E
54	Mt. Dinghu, China	1000	23.150°N	112.500°E
55	Mt. Caepabhal, Scotland, UK	365	57.836°N	7.138°W
56	Mt. An Clisean, Scotland, UK	799	57.964°N	6.817°W
57	Upper Peirce Reservoir, Singapore	42	1.3675°N	103.802°E
58	Beirut, Lebanon	20	33.902°N	35.479°E
59	Shorthair Creek, Sierra Nevada, California	2700	37.067°N	118.987°W
60	Tateyama Mountains, Japan	2450–2700	36.567°N	137.600°E
61	Beijing, China	~60	39.900°N	116.233°E
		~90	39.917°N	116.433°E
62	Shanghai, China	~25	31.183°N	121.450°E
63	Laramie and Laramie Mountains, US	2193–2676	41.250°N	105.500°W
64	Kanazawa, Japan	113	36.533°N	136.700°E

Table S1-2 Concentrations of bacteria in atmospheric water samples based on direct enumeration.

Site No.	Period	Number of samples	Atmospheric water type	Bacterial concentration (cells mL^{-1})	Method	Ref.
1	Oct. 2014–Sept. 2015	68	Rain	7.6×10^3–7.2×10^4 ($2.3\pm1.5\times10^4$)	EFM (LIVE/DEAD)	1
2–3	Jul. 1993		Rain	2.12×10^4/0.87×10^4	TEM-EDX	2
2, 4–5	Jan. 2000–Jan. 2001	9+1+3	Rain	1.4×10^3–6.5×10^4	EFM (DAPI)	3
6	Jun. 2007–Oct. 2008	6	Bulk deposition	3×10^3	EFM (DAPI)	4
7	Apr.–Jul. 2011	3	Rain	1.0×10^2–1.6×10^3	EFM (DAPI)	5
8	Nov.1983–Dec. 1984	37	Rain	7.1×10^3–6.3×10^5 ($2.1\pm2.4\times10^5$)	EFM (AO)	6
9-1	Nov. 1995		Rain	3.57×10^3 [a]	SEM-EDX	7
9-2		10	Cloud	1.05×10^6 [a]		
10	Apr.1999 and Mar.2000	4	Rain	6.0×10^3–1.5×10^4 (1.0×10^4)	EFM (DAPI)	8
		15	Snow	2.0×10^3–3.3×10^4 (1.0×10^4)		
		8	Cloud	4.9×10^3–8.1×10^4 (2.0×10^4)		
11	Apr. and May 1997	12	Cloud	7.9×10^2–2.5×10^3 ($1.5\pm0.5\times10^3$)	EFM (DAPI)	9
		7	Snow	9.5×10^3–1.3×10^4 ($1.1\pm0.1\times10^4$)		
		2	Graupel	6.9×10^3–1.2×10^4 (9.2×10^3)		
12	Mar. 2003	2	Cloud	8.3×10^4–3.6×10^5 (2.2×10^5)	EFM (DAPI)	10
	Jan. 2004–Oct. 2005	14		1.7×10^4–2.4×10^5 (8.1×10^4)		11
	Apr. 2008–Sept. 2010	17		3.3×10^3–8.9×10^4 ($4.0\pm2.4\times10^4$)		12
	Jul. 2011–Oct. 2012	10		1.6×10^3–3.4×10^4 ($1.2\pm1.0\times10^4$)		13
13–14	Aug. 2005	4	Cloud	4.3×10^5	EFM (AO)	14
15–17	Jun. 2008	4		9.2×10^4		
18–21	Oct. 2005–Apr. 2006	10	Snow	15–2.2×10^2 ($0.8\pm0.6\times10^2$) [b]	Flow cytometer (SYTO 60)	15
22–27	Dec. 2005–Mar. 2006	7		50–5.4×10^3 ($0.9\pm2.0\times10^3$) [b]		
28	Jan. 2006	1		80 [b]		
29	Jan. 2006	1		120 [b]		
30	Mar–Sept. 2013, May–Oct. 2014	56	Snow	2.4×10^3–6.8×10^4 ($1.4\pm1.7\times10^4$)	EFM (PI/SYBR green)	16
30–32	Jan.–Feb. 2015	7	Snow	5.2×10^3–1.1×10^4	EFM (DAPI)	17
	Aug. 2014	15				
33–36	2003–2006	88	Snow	6.8×10^2–7.2×10^5	Flow cytometer (SYBR Green I)	18

Note: [a] Total number of biological micro-particles; [b] DNA-containing cells between 0.3 and 15 μm. EFM, epifluorescence microscopy; TEM- and SEM-EDX, transmission and scanning electron microscope equipped with an energy dispersive X-ray detector.

References: 1. This study; 2. Casareto et al., 1996; 3. Natsume and Suzuki, 2001; 4. Peter et al., 2014; 5. Cho and Jang, 2014; 6. Herlihy et al., 1987; 7. Matthias-Maser et al., 2000; 8. Bauer et al., 2002; 9. Sattler et al., 2001; 10. Amato et al., 2005; 11. Amato et al., 2007d; 12. Vaïtilingom et al., 2012; 13. Joly et al., 2014; 14. Kourtev et al., 2011; 15. Christner et al., 2008b; 16. Stopelli et al., 2017; 17. Wunderlin et al., 2016; 18. Liu et al., 2009.

CHAPTER 2

Applicability of LIVE/DEAD BacLight stain with glutaraldehyde fixation for the measurement of bacterial abundance and viability in rainwater

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Most of the activities and consequences of airborne bacteria in rain remain speculative and the knowledge on bacteria in rainwater is very limited. This is mainly because there is not a carefully evaluated approach for the measurement of the concentration and viability of bacterial cells in rainwater, although multiple techniques have been developed to quantify bacteria in aquatic environments, such as river, lake and sea.

In this chapter, we carefully tested and verified the operation procedures and conditions using LIVE/DEAD BacLight stain with glutaraldehyde fixation in EFM enumeration to quantify the abundance of viable and non-viable bacteria in rainwater samples, investigated the factors influencing the results in sample preparation, staining and enumeration, and tried to offer a reliable method of quantifying the number concentrations of viable and non-viable bacterial cells in rainwater.

2.1 Methodology

2.1.1 Outdoor experiments

2.1.1.1 Rainwater sample preparation

The samples used for the verification of operation and procedures were prepared from rainwater collected at a suburban site (32.806°N, 130.766°E) of Kumamoto in southwestern Japan. It was on the roof of a 20-m-high building on the campus of Prefectural University of Kumamoto. Surroundings of the campus are residential buildings. No obvious anthropogenic sources of biological particles except those in nature were expected during the periods of rainwater collection.

Rainwater was collected with samplers, each of which was composed of a sterilized beaker (PTFE, 1000 mL, ϕ116 mm, ϕ109 mm × 152 mm) and a sterile funnel (PMP, ϕ210 mm). In preparation, all beakers, funnels and other utensils were washed with a 10-fold diluted detergent solution of neutral pH (Scat 20X-N, Dai-ichi Kogyo Seiyaku Co., Ltd., Japan), ultrasonically cleaned for 30 min, and then rinsed with tap water, deionized water and ultrapure water (PURELAB Ultra, ELGA Labwater, Germany; 18.2 MΩ·m, <0.1 CFU mL^{-1}), copiously three times each in sequence. The cleaned utensils were wrapped with untapped aluminum foil and then autoclaved for sterilizing. Before sample collection, the funnels and inner of beakers were rinsed with sterilized ultrapure water three times first, and then the sterility of the rainwater samplers was tested by pouring sterilized ultrapure water into the samplers and treating the water as negative control in the laboratory. The samplers were fixed at the sampling site without exposure to ambient air. They were opened to start collecting rainwater when it was raining. The sampling durations were within one day. After collection, the samplers were sealed and brought to the laboratory for treatment and analysis immediately.

2.1.1.2 Fluorescent staining and enumeration

Aldehydes, e.g., formaldehyde, formalin and glutaraldehyde, are commonly used fixatives to stabilize the fine structure of cells prior to light or electron microscopic examination, and to preserve samples for direct bacterial counts by EFM (Hopwood, 1967; Kepner and Pratt, 1994; Kiernan, 2000). Formaldehyde and formalin have been applied in fixing cells in cloud water and rainwater samples before fluorochrome staining for direct EFM enumeration (Amato et al., 2005, 2007d; Bauer et al., 2002; Cho and Jang, 2014; Sattler et al., 2001). Murata and Zhang (2013, 2014) confirmed the efficiency of EFM enumeration using LIVE/DEAD stain with glutaraldehyde fixation and conducted its application to airborne bacteria. Glutaraldehyde ($HCO\text{-}(CH_2)_3\text{-}CHO$) is a disinfectant, has a great potential for cross-linking via the -CHO groups and over variable distances, and is effective against vegetative forms of Gram-positive and Gram-negative bacteria (Gorman et al., 1980; Ross, 1966). It can thoroughly insolubilize and cross-link, but does not chemically change proteins in microbial cell membranes, and consequently can stabilize the structure of bacterial cells (Kiernan, 2000). Commercial 25% aqueous acid-potentiated glutaraldehyde solutions at approximately pH 3 contain 3% glutaraldehyde, hemiacetal and the polymers of the latter. Under neutral or slightly alkaline conditions, other glutaraldehyde polymers form, with the size of the polymers increasing with pH. As a precipitating complex form, glutaraldehyde loses its antimicrobial activity (Migneault et al., 2004). At pH 7.5–8.5, glutaraldehyde shows the best antimicrobial activity (Gorman et al., 1980). It is widely used as a rapidly penetrating fixative for electron microscopy.

For each rainwater sample, duplicate 5-mL subsamples were first treated with 0.2 mL of 0.2-μm-filtered 25% glutaraldehyde solution (Wako Pure Chemical Ind., Ltd., Japan; final concentration: approximately 1:100, *V*/*V*) to fix bacterial cells in a dark place at 4°C for 30 min, and then with 50 μL of LIVE/DEAD stain (L13152, Invitrogen™, Molecular Probes Inc., USA; final concentration: approximately 1:100, *V*/*V*) to stain bacterial cells in the samples under the same conditions for 15 min. The concentration, and the incubation conditions and the duration for the fixing and staining were determined following the product information of agents and previous tests (Boulos et al., 1999; Murata and Zhang, 2013). After staining, samples were filtered with 0.2-μm-pore 25-mm-diameter black polycarbonate filters (Advantec Toyo Kaisha Ltd., Japan), and then the filters were mounted on microscope slides.

The number of bacterial cells on the filters was determined by direct enumeration using an epifluorescence microscope (Eclipse 80i, Nikon Corp., Japan) under blue excitation rays (450-490 nm), which is equipped with an imaging system (Digital Sight DS-L, Nikon Corp., Japan). Bacterial cells stained with LIVE/DEAD stain do not always emit distinct green and red colors, and also emit yellow

and orange colors, i.e., intermediate states (weaken and damaged) of bacterial cells are also observed (Boulos et al., 1999; Berney et al., 2007). In this study, the concentrations of viable and non-viable bacteria in a sample were estimated by counting fluorescent green and red/orange/yellow cells which were spherical and close to or smaller than 1 μm in diameter (Fig. 2-1 (c-f); Hara and Zhang, 2012; DeLeon-Rodriguez et al., 2013). Counting cells on each black filter was conducted in 20 random fields of 100 μm × 100 μm area of the filter under 1000× magnification. The counting in each field was accomplished as quickly as possible, usually within 30 s. Duplicate subsamples for negative control of corresponding rainwater samples were treated with the same procedures, and bacterial cells were counted in the same way. More details of the operation of sample staining and cell counting, which are the same as the investigation of bacterial cells associated with Asian dust and ambient aerosols in the air, were described in studies of Hara and Zhang (2012) and Murata and Zhang (2013, 2014).

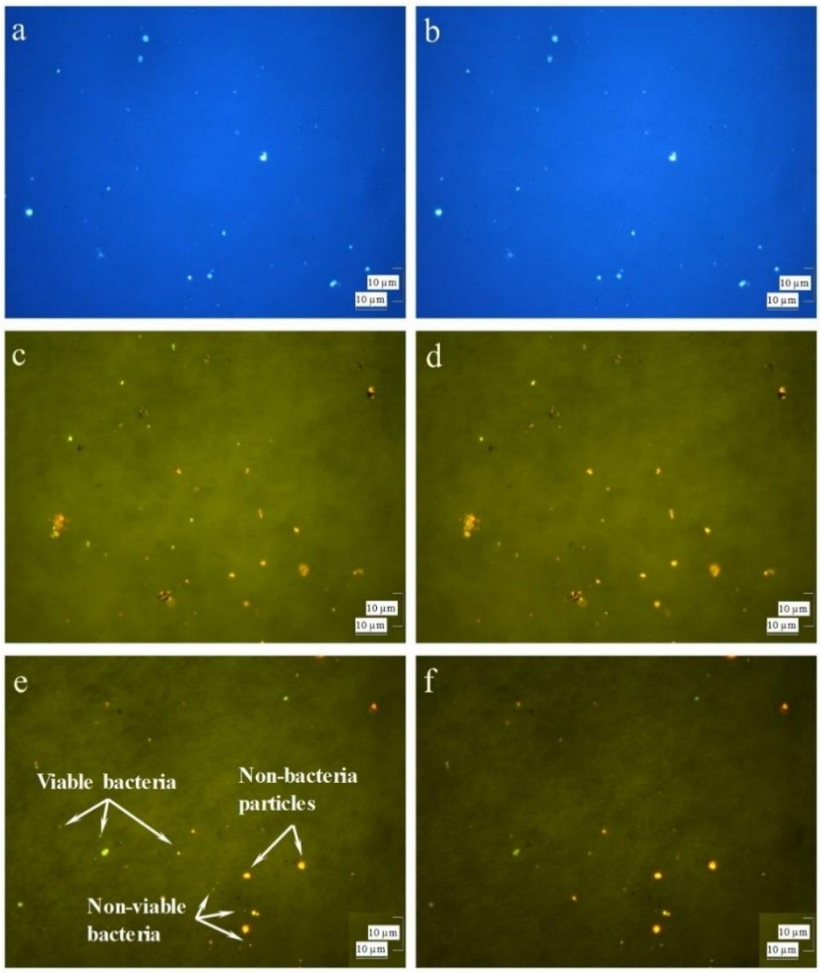

Figure 2-1. Examples of processed rainwater samples (21 February 2015) under the field of EFM: DAPI stain after the fixation (a, b); LIVE/DEAD BacLight stain without fixation (c, d); and after the fixation (e, f). The photographs at the left side were taken at the beginning of the excitation ray exposure, and those at the right side after 1 min of the exposure.

The concentrations of viable and non-viable bacterial cells in rainwater (bacterial cells per milliliter of rainwater) were calibrated with the negative control samples. The bacterial viability was defined as the ratio of viable bacterial cells to the total bacterial cells, which was used to represent the potential living ability of bacterial cells.

The efficiency of LIVE/DEAD stain to bacteria in rainwater was assessed by the comparison with the results of DAPI stain (Cellstain DAPI solution, Dojindo Lab., Japan). DAPI reagent is a common stain for the enumeration of bacteria and Protozoa in aquatic research (Bolter et al., 2002). It binds to double-stranded DNA (dsDNA) and RNA, and stains bacterial cells blue (Fig. 2-1 (a–b)) under 365 nm ultraviolet excitation. The color of stained bacteria under microscopes actually fades gradually when exposed to excitation rays, i.e., fluorescence bleaching. Counting and photographing stained bacteria have to be done quickly. Otherwise, the results may have large uncertainties. In order to test if the fixation could benefit the quantification of bacterial cells in rainwater, the results from rainwater samples with and without glutaraldehyde fixation were compared. Duplicate subsamples (5 mL) of six rainwater samples for each treatment, i.e., LIVE/DEAD staining with and without glutaraldehyde fixation, and DAPI staining with glutaraldehyde fixation, were processed and enumerated.

2.1.2 Laboratory experiments

2.1.2.1 Glutaraldehyde fixation and PBS addition

Phosphate buffer saline (PBS) can prevent possible damage of bacterial cells due to osmotic pressure change and also maintain at a relatively stable pH. In previous investigations, PBS was frequently applied in airborne bacteria enumeration coupled with LIVE/DEAD stain. Airborne bacterial cells were detached from the filters by shaking each filter in sterile filtered PBS or directly collected into the PBS liquid media, and then stained with or without glutaraldehyde fixation (DeLeon-Rodriguez et al., 2013; Hara and Zhang, 2012; Murata and Zhang, 2013). However, bacteria in aqueous samples (e.g., drinking water, cloud water, and melted snow and ice core samples) were usually treated directly without the addition of PBS (Bauer et al., 2002; Boulos et al., 1999; Cappa et al., 2014; Mitevaet al., 2009). Different from atmospheric aerosols, the rainwater is acidic in most cases. Using acid-potentiated glutaraldehyde for bacterial cell fixation can increase the acidity of rainwater samples, which can result in unsuitable living conditions for microbes.

Two filtered rainwater samples were applied to investigate possible uncertainties in cell counting due to the fixation with glutaraldehyde and the addition of PBS. Each sample were separated into three subsamples (about 30 mL). Then three laboratory-cultured bacterial strains, *Bacillus subtilis*, *Micrococcus* sp., and *Escherichia coli* (*E. coli* JM109 competent cells; Takara Bio Inc., Japan), were

respectively transferred into the subsamples. The two bacterial strains, *B. subtilis* and *Micrococcus* sp., were isolated from aerosol samples in elevated layers (Hara et al., 2015).

Four 5-mL aliquots were prepared from each of the subsamples. Two of the four were respectively treated with and without glutaraldehyde (finally about 1%, *V*/*V*), stained with the LIVE/DEAD stain, and observed with the EFM. The remained six aliquots were mixed with PBS first. Three, each with different strains transferred, were further treated with glutaraldehyde fixation. Then all the six were stained and observed with the EFM. The above treatments with and without the fixation of glutaraldehyde, and with and without the addition of PBS are summarized in Table 2-1. For the convenience of description, the treatments are named as Treatments I, II, III and VI (Table 2-1). Six actual rainwater samples were also processed with Treatments I to IV respectively.

Table 2-1 Summary of Treatment I-IV of the two filtered rainwater samples, and pH and electrical conductivity (EC) of the treated samples, rainwater, 25% glutaraldehyde and PBS.

Treatment	pH*	EC** (mS/cm)	Rainwater (mL)	PBS (mL)	25% Glutaraldehyde (mL)	LIVE/DEAD stain (mL)
I	6.4/6.2	0.28/0.26	5.0	-	-	0.05
II	3.9/3.8	0.31/0.30	5.0	-	0.2	0.05
III	7.6/7.6	7.10/7.10	5.0	5.0	-	0.10
IV	6.5/6.5	7.50/7.50	5.0	5.0	0.4	0.10
	5.3/4.5	0.01/0.02	0.5	-	-	-
	3.1	0.04	-	-	0.5	-
	7.8	14.20	-	0.5	-	-

* Measured with Laqua Twin AS-212 (Horiba Ltd., Tokyo, Japan); ** Measured with Twin Cond. B-173 (Horiba Ltd, Tokyo, Japan). "-" means no addition.

The three bacterial strains were transferred to rainwater samples in the following procedures. The collected rainwater samples were filtered through sterilized 0.20-μm mixed cellulose ester membrane filters (Advantec, Toyo Roshi Kaisha, Ltd., Japan) using sterilized 10-mL syringes (Terumo Corp., Japan). The bacterial strains of *B. subtilis*, *E. coli* and *Micrococcus* sp. grown on R2A media (BD Difco™, Becton, Dickinson and Company, USA; pH 7.3) were transferred into 3-mL liquid media, tryptic soy broth (TSB; BD Bacto™, Becton, Dickinson and Company, USA; pH 7.3), and incubated at 30°C in a shaking incubator (125 r min^{-1}) for about one day. Before inoculation into filtered rainwater samples, 100-μL media with incubated bacterial cells were re-transferred into new 3-mL TSB media, and incubated under the same condition for 12–24 h. The vegetative cells (OD $A595_{nm}$ 0.35–2.12) of the three bacterial species were first suspended respectively in filtered rainwater samples (1% *V*/*V*), and then the samples were stirred on a vortex mixer at 3000 r min^{-1} (amplitude 5 mm) for 2 min.

2.1.2.2 Quality control

To further validate the application of LIVE/DEAD stain to bacteria in rainwater, multiple preliminary tests were carried out. Unprocessed (unfixed and unstained) rainwater subsamples were filtered and then slides were prepared for the EFM observation. Five 5-mL aliquots of sterilized ultrapure water were directly treated (without rinsing) and observed as rainwater samples, to assess the number of bacterial cells which might contribute to total bacterial cells in rainwater samples due to the operations.

The influence of ambient aerosol diffusion to bacteria in rainwater was also investigated, taking no account of the deposition effect of bacteria due to their small size. A sampler prepared with the same procedures for rainwater collection was set aside of the one for rainwater collection simultaneously, but was covered with a plastic film on a higher shelf. This sampler was exposed to the ambient air with no rainwater dropping in when rainwater was collected by the rainwater sampler. After sample collection, it was rinsed with sterilized ultrapure water and the water was treated similarly to rainwater samples. This test was done in five periods of rainwater collection.

2.2 Results and discussion

2.2.1 Efficiency of bacterial cell counts

The statistics on the bacterial abundance in six rainwater samples based on LIVE/DEAD- and DAPI-staining are summarized in Fig. 2-2. The total bacterial cell counts using LIVE/DEAD stain with fixation were approximately equal to those using DAPI stain with fixation (Wilcoxon signed rank test, two tailed P=0.699), and the average ratio of LIVE/DEAD counts to DAPI counts was 109±29% (Table S2-1). Compared with the results of DAPI stain with fixation, the efficiency of the LIVE/DEAD staining without fixation was 103±25% (Wilcoxon signed rank test, two tailed P=0.937). The results of Wilcoxon signed rank test indicate that the LIVE/DEAD staining methods can provide accurate information on bacterial concentration in rainwater. The viable and non-viable bacterial concentrations quantified in subsamples duplicated from the same samples showed good consistency with the standard deviation (2.9×10^3 and 1.1×10^3 cells mL^{-1} on average) lower than the average concentration by one order of magnitude (Fig. 2-2). This result also indicates good accuracy in the measurements.

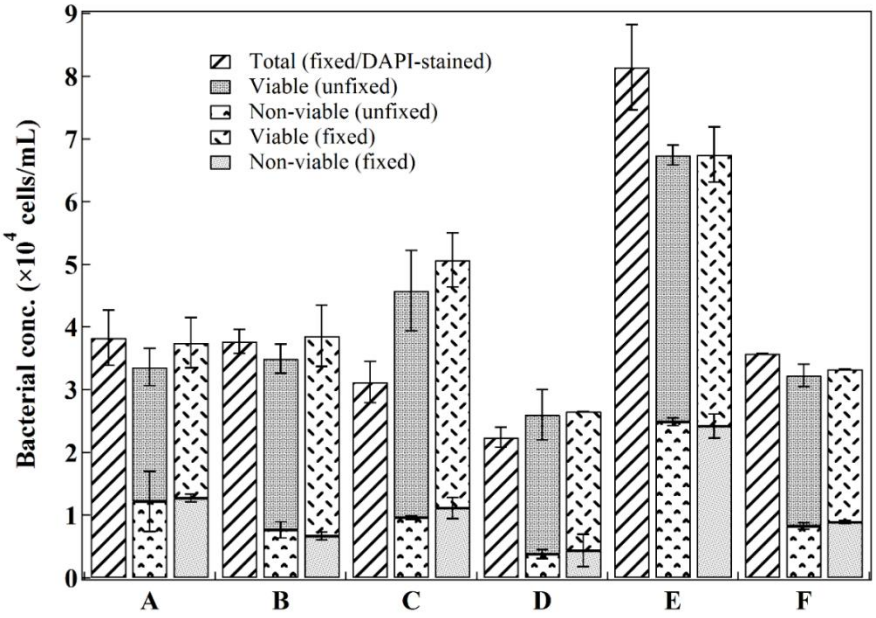

Figure 2-2. Concentrations and standard deviations of viable and non-viable bacteria in six rainwater samples (marked by A–F) identified using LIVE/DEAD BacLight stain with and without glutaraldehyde fixation, and total bacteria using DAPI stain with glutaraldehyde fixation.

In previous studies, the detection efficiency of total bacterial cells in airborne particle samples with LIVE/DEAD stain were 102±11% compared with DAPI staining (Murata and Zhang, 2013); and the total bacterial cell counts using LIVE/DEAD stain were close to those using Ethidium Bromide and DAPI stains (Hara and Zhang, 2012). In a study of bacteria in upper tropospheric aerosols, DeLeon-Rodriguez et al. (2013) compared the cell counting based on EFM using LIVE/DEAD staining with that based on qPCR. They found that the results using LIVE/DEAD staining were higher than those using qPCR by about one order of magnitude, and attributed the reason to the less small subunit ribosomal RNA copies of bacteria in the samples relative to that used in the calculations, besides the possible underestimation by qPCR due to technical limitations.

Compared with that using AO staining, the enumeration using DAPI staining was reported to underestimate the abundance and cell size of bacteria. The reason was attributed to the insufficient amount of DNA in bacterial cells for DAPI staining (Suzuki et al., 1993) and also to the lower DNA synthesis rate with DAPI stain for the visualization of bacteria (Posch et al., 2001). However, we found that it is a little difficult to differentiate between other kinds of insoluble particles (such as dust particles) in the size close to or smaller than 1 μm and bacterial cells in DAPI- and LIVE/DEAD-stained samples. In DAPI-stained samples, the mineral particles were colored greenish yellow or white (Murata and Zhang, 2013), similar to the blue bacterial cells (Fig. 2-1 (a–b)). In LIVE/DEAD-stained samples, both small mineral

particles and a few bacterial cells exhibited yellow color. Whereas, for bacteria, the consistency of total counts with LIVE/DEAD- and DAPI-staining indicates that LIVE/DEAD staining with glutaraldehyde fixation could act as an alternative approach of DAPI staining to quantify bacterial cells in rainwater.

2.2.2 Improvement by glutaraldehyde fixation

Fixation with glutaraldehyde can strengthen the membrane of bacterial cells. Murata and Zhang (2013) suggested that 1% glutaraldehyde fixation was applicable for the improvement of enumeration accuracy using LIVE/DEAD staining for aerosol samples. In this study, the total bacterial cell counts using LIVE/DEAD stain with glutaraldehyde fixation were a little higher than those without fixation (Fig. 2-2). The ratio of cell count with fixation to that without fixation was 106±5% on average (Wilcoxon signed rank test, upper tail P=0.312). The counts of viable and non-viable bacterial cells in corresponding unfixed and fixed samples had small differences (Wilcoxon signed rank test, upper tail P=0.242, 0.469). The ratios of viable and non-viable cells in fixed samples to those in unfixed ones were 108±7% and 104±11% (Table S2-1), respectively, indicating a small improvement of detection efficiency for both viable and non-viable bacterial cells with the fixation.

Murata and Zhang (2013) found that the color of viable bacterial cells in unfixed aerosol samples faded faster than in the fixed samples under the excitation ray exposure. The reduction ratio of the total cells within 1 min in unfixed samples (23%) was much higher than that in fixed samples (7%). The EFM images of LIVE/DEAD stained rainwater samples without fixation and after the fixation at the beginning and after 1 min of the excitation ray exposure are shown in Fig. 2-1 (c–f)**.** In this study, fluorescent fading was found in both unfixed and fixed samples. In rainwater samples treated with glutaraldehyde fixation, the shape of bacterial cells was clearer, and the fading of labeled color of bacteria under the excitation ray exposure was slower than in those without fixation. But the fading was not obvious for viable bacterial counts in the rainwater samples within 1 min, in comparison with the previous results in aerosol samples (Murata and Zhang, 2013).

Bacterial strains were inoculated into filtered rainwater samples to clarify the strengthening effect and uncertainties of glutaraldehyde fixation in rainwater. Fig. 2-3 shows the examples of LIVE/DEAD stain-stained *B. subtilis*, *Micrococcus* sp., and *E. coli* in filtered rainwater with and without glutaraldehyde fixation at the beginning and after 1 min of the excitation ray exposure. Glutaraldehyde induced auto-fluorescence can be observed in cells fixed for fluorescence microscopy. The wavelength of the actinic beam and pH of the environment can influence the amount of its enhancement significantly. Longer wavelengths and acid pH produce a smaller effect (Collins and Goldsmith, 1981). Herein we considered the influence of glutaraldehyde-induced fluorescence on fixed bacterial cells in rainwater was insignificant (Sect. S2.4), and the fluorescent color changes of bacteria cells reflected their actual statuses

after each treatment. To ensure the quality of enumeration results, it is better to pre-evaluate the influence of glutaraldehyde fixation by comparing the results of fixed and unfixed rainwater samples.

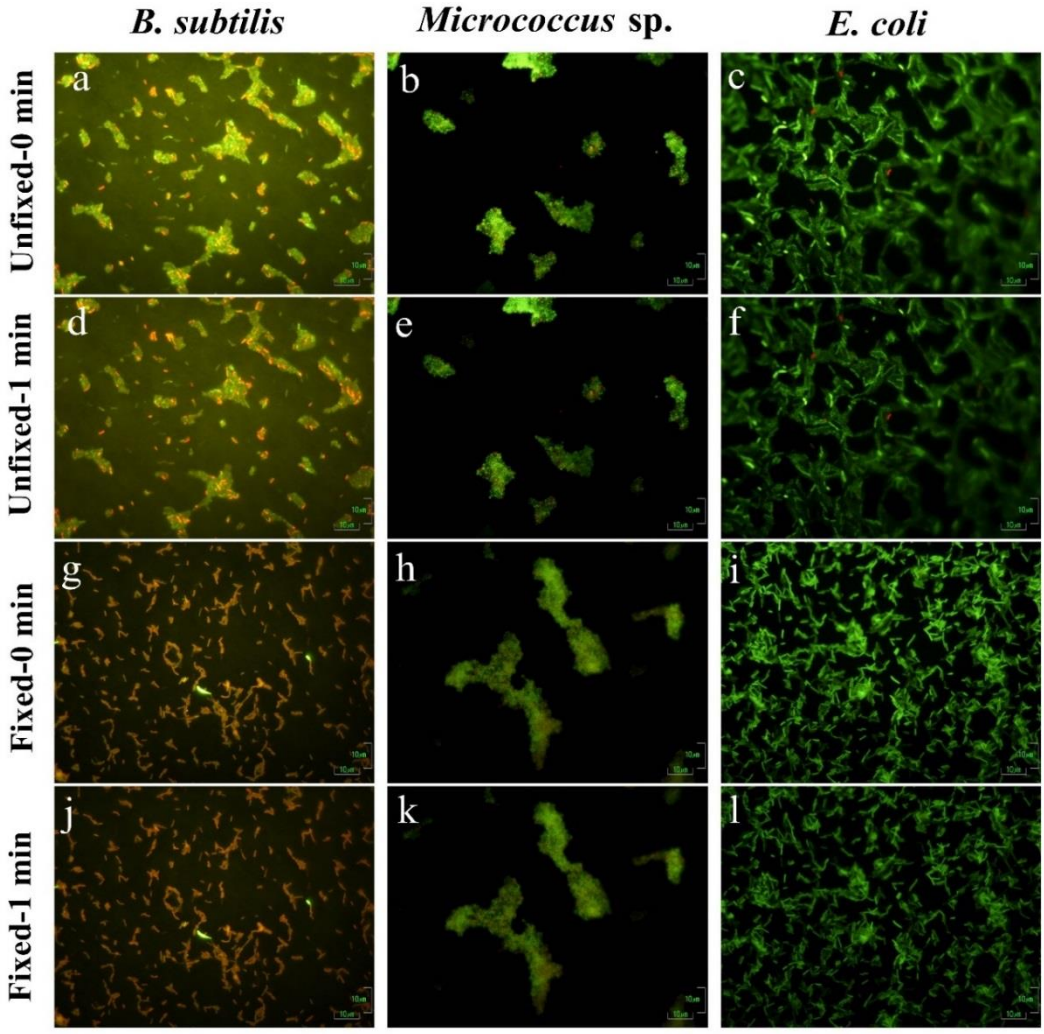

Figure 2-3. Laboratory-cultured *B. subtilis*, *Micrococcus* sp., and *E. coli* with TSB medium in filtered rainwater samples stained by LIVE/DEAD stain without (a-f) and with (g-l) glutaraldehyde fixation at the beginning (0 min, a-c and g-i) and after 1 min (d-f and j-l) of the excitation ray exposure.

Fixed Gram-positive bacteria, *B. subtilis* and *Micrococcus* sp., were of lower viability, and almost all cells of *B. subtilis* dead, while most of those unfixed cells remained alive. Almost all cells of *E. coli* (Gram-negative) remained alive, but a little more non-viable cells were detected in unfixed samples than in fixed samples (Fig. 2-3 and Table S2-3). Yamashita et al. (2012) assessed the viability of the immobilized *Magnetospirillum magneticum* (Gram-negative) cells on mica with glutaraldehyde using LIVE/DEAD stain, and also found only a few of the cells were dead for at least 1 h after immobilization. Laboratory tests with the same bacterial cells suspended in ultrapure water exhibited similar results, while experiments with bacterial strains in PBS showed that alive bacterial cells were predominant after fixation

(Sect. S2.2). In the microscopic field, fixed bacterial cells were obviously clearer in shape than unfixed ones, especially for *E. coli*, and the bacterial cells of *B. subtilis* and *E. coli* with glutaraldehyde fixation were more dispersed than those without fixation. These features were likely related to the changes of physicochemical and mechanical properties of the bacterial cell surface after glutaraldehyde fixation (Sheng et al., 2008). The color of *B. subtilis*, *Micrococcus* sp. and *E. coli* bleached under the excitation ray exposure, although the fading effect was not obvious within 1 min (Fig. 2-3).

The requirements for bacterial survival and growth include physical conditions (e.g., temperature, pH, and osmotic pressure), and chemical conditions (e.g., carbon; iron, copper, and zinc; nitrogen, sulfur, and phosphorus; and oxygen based on bacteria classifications). The pH and electric conductivity of the rainwater after the treatments and before the filtration for counting are listed in Table 2-1. Most bacteria prefer neutral conditions (pH 6.5–7.5), depending on species. Steady-state log-phase *B. subtilis* cultures could survive 60–100% when the pH changed from 6.0 to 4.5, and the cultures growing at pH 7.0 might survive less than 15% at pH 4.5 (Wilks et al., 2009). Optimal growth of two members of the genus *Micrococcus* was recorded at pH 6.0–6.2 (range 5–9) and pH 7.5 (Liu et al., 2007; Mohedano et al., 1997). However, *E. coli* strains at stationary phase can survive several hours at pH 2–3, which is considerably lower than the acid limit (about pH 4.5) for growth (Small et al., 1994). Lu et al. (2013) reported a previously uncharacterized type of acid resistance system that relies on L-glutamine in *E. coli*. It is sufficient for *E. coli* survival under extremely acidic conditions (pH~2). The alive status of three bacteria species, *B. subtilis*, *Micrococcus* sp. and *E. coli*, in filtered rainwater fixed with and without glutaraldehyde in our experiments matched these alive and dead natures of the strains under different pH conditions. *B. subtilis* and *Micrococcus* sp. trended toward lower viability in acid circumstances (Table 2-1 and Table S2-3). The pH values of rainwater samples in this study were in the range of 4.3–5.3. Hence, as species such as *B. subtilis* and *Micrococcus* sp. existed in rainwater (Amato et al., 2005, 2007c; Cho and Jang, 2014), some viable cells should possibly have been identified as non-viable ones because glutaraldehyde fixation could damage the bacterial cells. In other words, the counts of viable cells might be underestimated due to the fixation.

Phosphate groups (PO_4^{3-}) in phospholipids are a major component in bacterial cell membranes and can be modified by organic molecules (e.g., choline). The fixation with glutaraldehyde suppresses the activation of enzyme (e.g., phosphatase), because of the content of inorganic phosphates in commercial glutaraldehyde (Hopwood, 1967; Fahimi et al., 1968). Further, the phosphodiester bonds between ribitol or glycerol residues, of which the teichoic acid in cell walls of Gram-positive bacteria is composed, can be broken by hydrolysis when acid, alkali and enzyme exist. In addition, the increase of osmotic pressure (increased electric conductivity) due to glutaraldehyde fixation might also injure cell membranes

(Kiernan, 2000). For these reasons, it is generally considered that using glutaraldehyde fixation may lead to the underestimate of viable bacteria counts, which is consistent with the results of our laboratory experiments with cultured *B. subtilis* and *Micrococcus* sp. strains. We found phosphate (0.2 mg L^{-1} on average, measured by means of ion chromatography) in the fixed rainwater samples, which could have led to the breakage of Gram-positive bacterial cell walls and injure the cell membranes.

However, our measurements of bacterial cells in the collected rainwater with and without the fixation showed a small improvement of the detection efficiency of viable cells by the fixation. This was also confirmed in the previous investigation of airborne bacteria with similar fixation (Murata and Zhang, 2013, 2014). Therefore, unlike cultured bacterial strains whose viability can be reduced by fixation, bacteria of the same species living in real rainwater are likely resistant to the fixation and viable ones do not substantially lose their viability in the fixation.

2.2.3 Influence of PBS addition

PBS is isotonic and non-toxic to bacteria cells, and can be used to stabilize pH. Laboratory experiments with addition of PBS were conducted to investigate the influence of pH on fixation, staining and enumeration of rainwater bacteria. The photograph examples of *B. subtilis*, *Micrococcus* sp., and *E. coli* in filtered rainwater samples diluted by PBS and stained by LIVE/DEAD stain with and without glutaraldehyde fixation are illustrated in Fig. 2-4 and described in Table S2-3. *B. subtilis* exhibited low viability in fixed samples (pH 6.5), but significantly high viability in unfixed ones (pH 7.6). In contrast, *Micrococcus* sp. and *E. coli* bacterial cells in both fixed and unfixed samples trended toward higher viability (Fig. 2-4 and Table S2-3). The status of *E. coli* cells in fixed and unfixed samples was similar in the samples without the addition of PBS. These differences of bacterial status should be dependent on their different resistivity to the pH and osmotic pressure variations in the circumstances (Table S2-3).

Fig. 2-5 illustrates the examples of microscope photographs of six actual rainwater samples diluted by PBS and stained by LIVE/DEAD stain with and without glutaraldehyde fixation. The color of bacteria in fixed samples was much clearer, and the bleaching effect, in particular for viable bacterial cells, was apparently slower than those of unfixed samples during EFM observation. The comparison of enumeration results for Treatment I to IV is illustrated in Fig. 2-6. Compared with samples without PBS dilution, the counts of viable bacteria trended to increase in the PBS-diluted and glutaraldehyde-fixed samples. Different from single bacteria species, the bacterial viability in the PBS diluted samples was slightly higher than or comparable to that in PBS-free samples. Though artificial mistakes may be introduced due to PBS addition, overall, the abundance and viability of bacterial cells detected by the LIVE/DEAD staining in rainwater samples with Treatments I to IV were not largely different in most cases (Fig. 2-6). It is likely that the bacteria species in rainwater have adapted to keeping their viability

in the acidic ionic circumstances. Bacteria surviving in rainwater had experienced harsh conditions in the atmosphere, including acidic pH, low temperature, strong UV light intensity, cycled freeze-thaw, desiccation, and osmotic shock (Amato, 2012). Therefore, they could survive against the pH and osmotic change of solutions better, although the reasons have not been completely uncovered and should be an important subject in future studies of the viability of bacteria in rainwater.

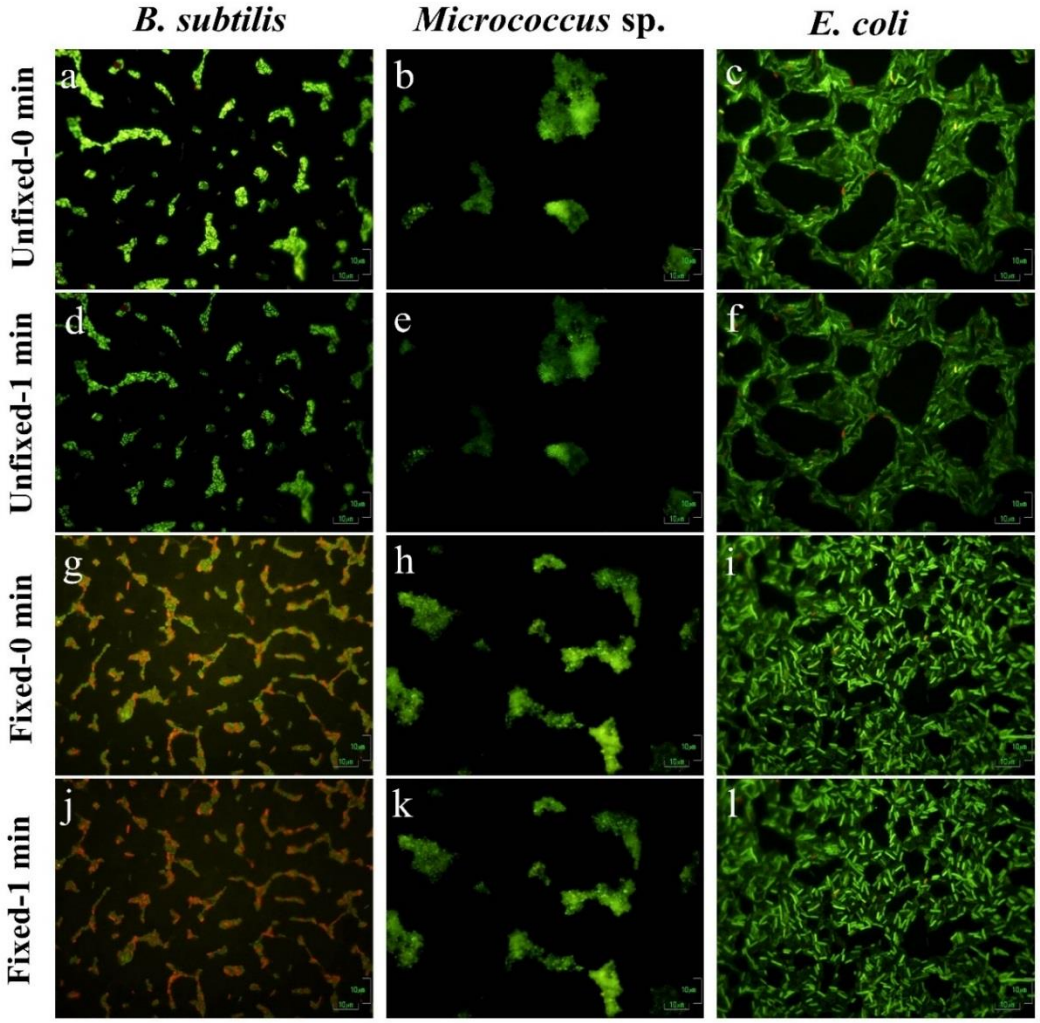

Fig. 2-4 Laboratory-cultured *B. subtilis*, *Micrococcus* sp., and *E. coli* with TSB medium in filtered rainwater samples diluted by PBS and stained by LIVE/DEAD stain without (a-f) and with (g-l) glutaraldehyde fixation at the beginning (0 min, a-c and g-i) and after 1 min (d-f and j-l) of the excitation ray exposure.

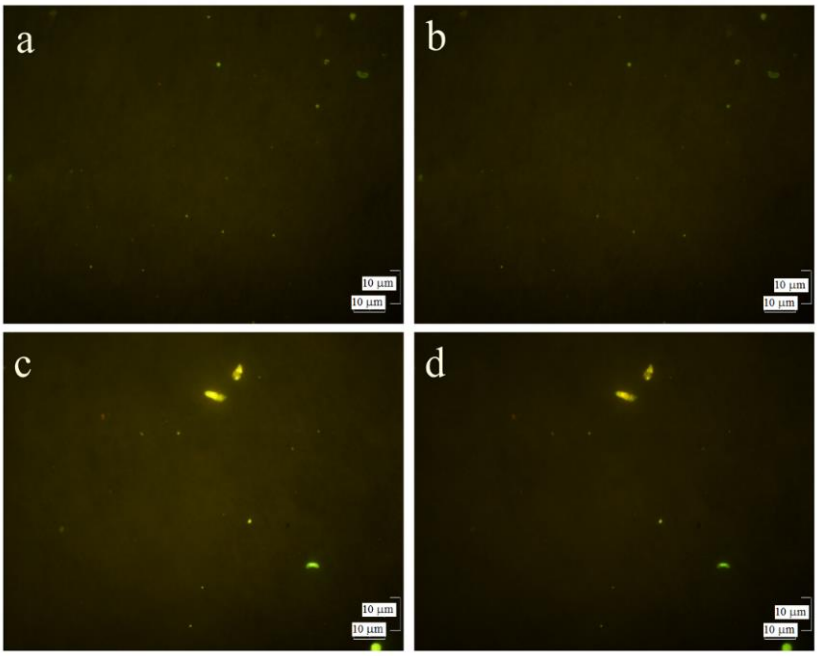

Figure 2-5 Rainwater subsamples diluted by PBS for laboratory tests and stained by LIVE/DEAD stain without (a, b) and with (c, d) glutaraldehyde fixation at the beginning (0 min, a, c) and after 1 min (b, d) of the excitation ray exposure.

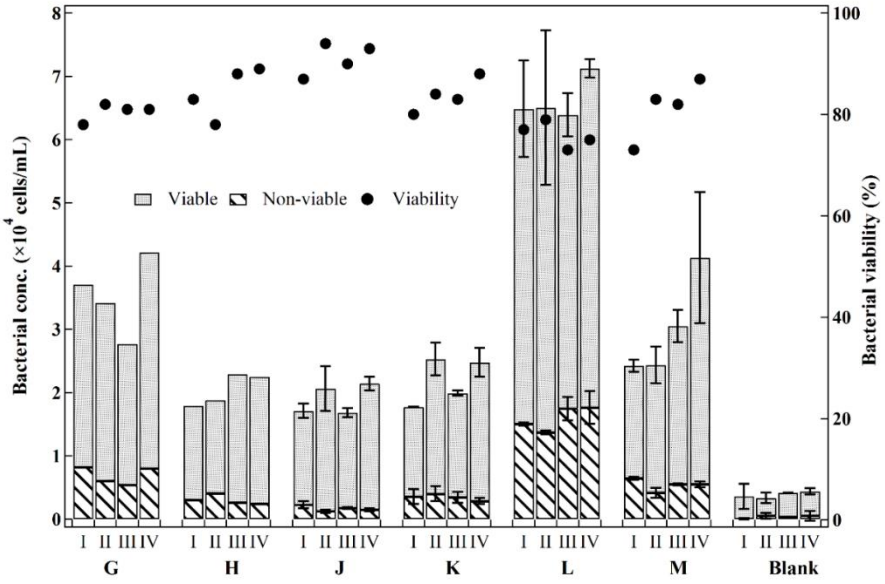

Figure 2-6 Viable and non-viable bacterial concentrations in the rainwater samples (marked by G, H, and J–M) following Treatment I to IV (Table 2-1). Duplicate or triplicate rainwater subsamples (5 mL) for each treatment were processed and enumerated. The average bacterial concentrations in negative control (blank) samples are also illustrated.

The effects of glutaraldehyde fixation differ according to bacterial species, and properties of solution (e.g., pH, chemical compositions). Peracchia and Mittler (1972) studied the improvements in

glutaraldehyde fixation by raising the pH in steps from 7 to 8, and suggested a better penetration of the fixative at neutral pH and a better cross-linking in alkaline pH. Boulos et al. (1999) tested the factors affecting the staining procedure, including addition of glutaraldehyde, staining time, and chlorine. They considered that storage after glutaraldehyde fixation decreased the total and viable cell counts of some coliform strains. The viable counts of *E. coli* in NaCl solution (0.085%) could be reduced by 5% glutaraldehyde fixation in LIVE/DEAD staining (Boulos et al., 1999), in accordance with the results of Treatments III and IV in this study. Chlorination could also cause the reduction of viable counts with the increase of chlorine concentration. In addition, glutaraldehyde can be present in at least 13 different forms depending on solution conditions such as concentration, temperature, and pH. Yet there is no agreement about the main reactive species that participates the crosslinking process because monomeric and polymeric forms are in equilibrium (Migneault et al., 2004).

In this study, we found that the combination of glutaraldehyde fixation and LIVE/DEAD staining can improve the detection accuracy of viable and total bacterial cells in rainwater slightly. However, the effects of glutaraldehyde fixation were dependent on bacterial species, physical (e.g., pH and osmotic pressure), and chemical properties of rainwater samples (solutions). The factors, such as staining time, and chemical properties of rainwater, may also affect the LIVE/DEAD staining. The bacterial abundance and viability detected in rainwater samples treated with PBS dilution and glutaraldehyde fixation trended to be a little bit higher than those detected in samples treated with glutaraldehyde fixation only, but the discrepancy was insignificant in most cases.

2.2.4 Influence of operation and atmospheric diffusion

Quality control experiments for possible contamination during the processes of sampling, storage and measurement revealed that nearly no bacterial cells were observed from the slides of unprocessed rainwater samples. The comparison of bacterial concentration and abundance in the collector-rinsing water (negative control) and the relevant six rainwater samples is shown in Fig. 2-7. The concentrations of viable and non-viable bacterial cells in the negative controls were 1.5×10^3–5.2×10^3 ($3.5\pm1.3\times10^3$) and 0–4.4×10^2 ($4.2\pm4.2\times10^2$) cells mL^{-1}. They were about 6%–14% (11±3%) and 0–10% (4±4%) of those in rainwater samples (Fig. 2-7 (a)), respectively. The total viable and non-viable bacterial concentrations were 2±2% and 0.7±0.7% of those in rainwater samples on average (Fig. 2-7 (b)). Furthermore, the total bacterial counts in the directly treated sterilized ultrapure water samples were $2.9\pm0.6\times10^3$ cells mL^{-1} ($2.8\pm0.6\times10^3$ viable cells and $1.3\pm1.4\times10^2$ non-viable cells mL^{-1}) on average, which was about 7% of the counts in rainwater samples. Therefore, the uncertainties due to the operation from the preparation to the cell counting under the microscope were small. The accuracy of the detection was largely improved in

comparison with the previous study of Natsume and Suzuki (2001), where the total bacterial concentration in negative control accounted for 13%–27% of the total concentration in rainwater with DAPI staining.

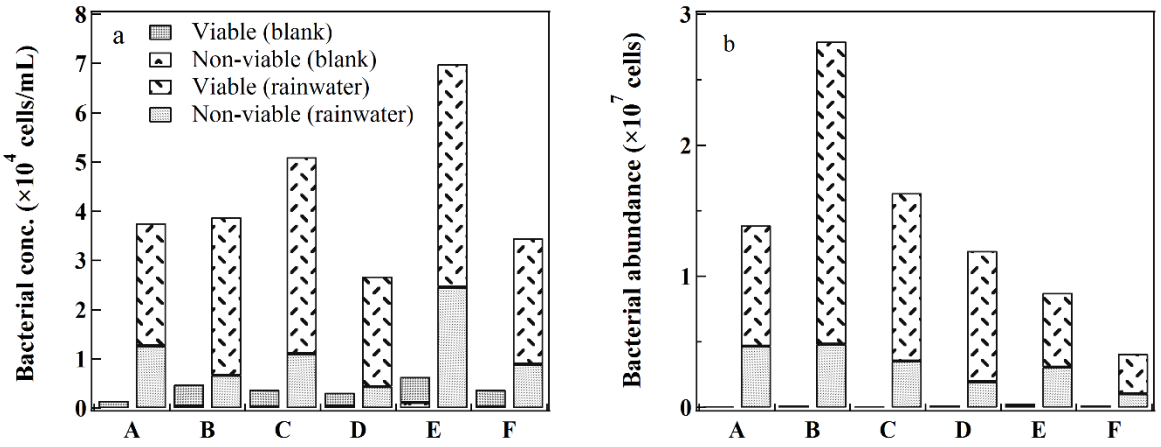

Figure 2-7 Bacterial concentrations and abundances in six rainwater samples (marked by A–F) and the relevant collector-rinsing water (blank).

The above results indicate that bacterial counts in the negative control samples for calibration could not lead to large uncertainties in the results. However, we also found that the bacterial abundance in negative control could be higher than that in some rainwater samples in cases when the amount of collected rainwater was less than that of collector rinsed water. Hence, we recommend to apply this method in the case of the amount of collected rainwater more than that of negative control (~40 mL), i.e., the precipitation during the sampling durations higher than 1.2 mm. The bacterial cell concentrations in the negative control samples showed variations, and, on occasion, the bacterial concentration in the negative control could be quite higher than that in directly treated sterilized ultrapure water samples. Therefore, the negative control experiments for the sterility of ultrapure water, collectors, and operations are critically essential to ensure the quality of counting bacterial cells in rainwater. This point is particularly important for cases when the precipitation is small.

The total bacterial concentrations in the samples used for assessing the influence of atmospheric diffusion to the bacterial counts in rainwater were in the order of magnitude 10^3 cells mL^{-1} ($4.9\pm1.5\times10^3$ cells mL^{-1} on average). All results were not much different and at the similar level to the average counts from the laboratory negative control samples (Wilcoxon signed rank test, P=0.345). The average viable and non-viable bacterial concentrations in the rainwater samples detected in this study were in the range between 2.2×10^4–4.3×10^4 cells mL^{-1} and 4.4×10^3–2.4×10^4 cells mL^{-1}, with the mean concentrations $3.1\pm0.9\times10^4$ cells mL^{-1} and $1.1\pm0.7\times10^4$ cells mL^{-1}, respectively. Therefore, the uncertainties due to the

operations and atmospheric diffusion by the approach of this study to measuring bacterial concentrations in rainwater were less than 12% on average.

2.3 Summary

An epifluorescence microscopy enumeration method using LIVE/DEAD BacLight Bacterial Viability Kit stain was tested and showed a good accuracy in counting viable and non-viable bacteria in rainwater. In comparison with DAPI stain, the LIVE/DEAD BacLight stain detected 109±29% of total bacterial cells. Bacteria in rainwater could survive better against the pH and osmotic changes due to glutaraldehyde fixation than cultured bacteria. The ratio of the total bacterial cell counts using LIVE/DEAD BacLight stain with fixation to that without fixation was 106±5% on average. The bacterial concentration in the negative controls was generally lower than that in the rainwater samples by about one order of magnitude, and the uncertainties due to the operation and air diffusion in measuring bacterial abundance were less than 12%. However, the concentration and also the abundance in the negative control could be occasionally higher than in rainwater samples in case of small precipitation.

These results indicate that, with careful verification, the LIVE/DEAD BacLight bacterial viability assay coupled with glutaraldehyde fixation is able to quantify the bacterial abundance and viability in rainwater. Careful negative control experiments considering the sterility of ultrapure water and collector, influences of glutaraldehyde and possible uncertainties from operations are critically essential for ensuring the accuracy of counting bacterial cells in rainwater.

It should be noticed that, compared with conventional methods (DNA-based and DAPI stain), LIVE/DEAD BacLight stain is usually more effective for gram-positive bacteria and cannot obtain the information on bacterial community (Murata and Zhang, 2013). The viability of bacterial cells identified with the kit is upon the status of bacterial cell membranes, i.e., injured or not injured, rather than upon metabolic activities.

Supporting information of "Applicability of LIVE/DEAD BacLight stain with glutaraldehyde fixation for the measurement of bacterial abundance and viability in rainwater"

S2.1 Efficiency of LIVE/DEAD stain and improvement by glutaraldehyde fixation

Table S2-1 Concentrations and standard deviations of viable and non-viable bacteria in rainwater samples identified using LIVE/DEAD BacLight stain with and without glutaraldehyde fixation, and total bacteria using DAPI stain with glutaraldehyde fixation. Unit, $\times 10^4$ cells mL^{-1}.

Sample No.	DAPI (fixed)	LIVE/DEAD (unfixed)		LIVE/DEAD (fixed)		Ratio [a]	Ratio [b]	Ratio [c]	Ratio [d]	Ratio [e]
	Total	Viable	Non-viable	Viable	Non-viable	(%)	(%)	(%)	(%)	(%)
A	3.83±0.44	2.14±0.30	1.22±0.48	2.48±0.40	1.27±0.06	97.6	87.8	111.2	115.6	103.6
B	3.77±0.19	2.73±0.23	0.76±0.13	3.19±0.49	0.67±0.06	102.1	92.7	110.2	116.6	87.6
C	3.12±0.33	3.62±0.64	0.96±0.03	3.96±0.43	1.11±0.17	162.5	146.9	110.6	109.4	115.4
D	2.24±0.16	2.22±0.40	0.38±0.07	2.22±0.00	0.44±0.26	118.7	116.2	102.2	100.0	115.0
E	8.14±0.68	4.25±0.16	2.49±0.06	4.33±0.44	2.42±0.19	82.8	82.8	100.0	101.9	96.8
F	3.58±0.00	2.40±0.18	0.83±0.05	2.44±0.00	0.89±0.23	93.0	89.9	103.4	101.9	107.5

[a] the ratio of total bacteria counts stained by LIVE/DEAD (fixed) to DAPI (fixed);

[b] the ratio of total bacteria counts stained by LIVE/DEAD (unfixed) to DAPI (fixed);

[c] the ratio of total bacteria counts stained by LIVE/DEAD (fixed) to LIVE/DEAD (unfixed);

[d] the ratio of viable bacteria counts stained by LIVE/DEAD (fixed) to LIVE/DEAD (unfixed);

[e] the ratio of non-viable bacteria counts stained by LIVE/DEAD (fixed) to LIVE/DEAD (unfixed).

S2.2 Laboratory experiments with liquid media-cultured bacteria in ultrapure water and PBS

Laboratory experiments with ultrapure water and phosphate buffer saline (PBS) inoculated with bacterial strains grown in tryptic soy broth (TSB) medium were conducted. The results are shown in Figs. S2-1–S2-2. It was also found that the shape of bacterial cells with glutaraldehyde fixation was clearer than those without fixation. The bacterial cells of *B. subtilis* and *E. coli* with glutaraldehyde fixation were more dispersed than those without fixation, which may be related to the changes of physicochemical and mechanical properties of the bacterial cell surface after fixation using glutaraldehyde (Sheng et al., 2008). However, the status of bacteria strains should also rely on the physiochemical properties (e.g., pH, osmotic pressure and chemical composition) of solutions. In acid circumstances, *B. subtilis* and *Micrococcus* sp. trended toward lower viability (Fig. S2-1 and Table S2-2).

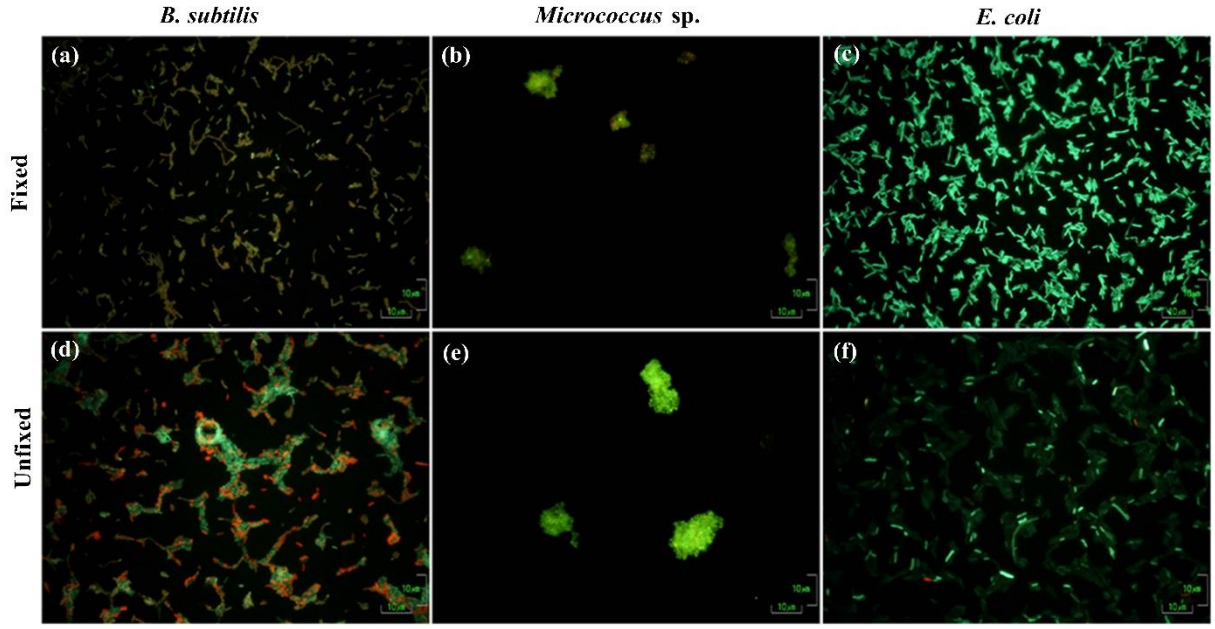

Figure S2-1. Laboratory-cultured *B. subtilis*, *Micrococcus* sp., and *E. coli* with TSB medium in ultrapure water stained by LIVE/DEAD stain with (a-c) and without (d-f) glutaraldehyde fixation.

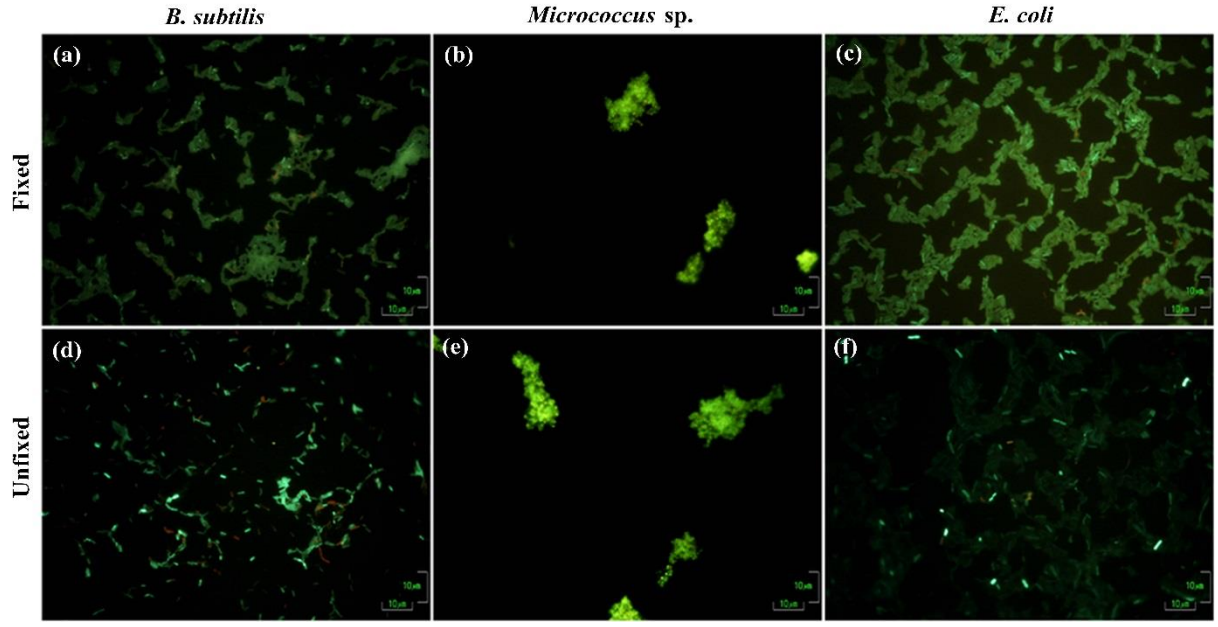

Figure S2-2. Laboratory-cultured *B. subtilis*, *Micrococcus* sp., and *E. coli* with TSB medium in PBS stained by LIVE/DEAD stain with (a-c) and without (d-f) glutaraldehyde fixation.

Table S2-2 Summary of pH and electrical conductivity (EC) of the treated ultrapure water and PBS inoculated with bacterial strains grown in TSB medium.

pH[*]	EC[**] (mS cm^{-1})	Ultrapure water[#] (mL)	PBS[#] (mL)	25% Glutaraldehyde (mL)	LIVE/DEAD stain (mL)
6.4	0.27	5.0	-	-	0.05
4.1	0.30	5.0	-	0.2	0.05
7.6	14.40	-	5.0	-	0.10
6.9	13.30	-	5.0	0.2	0.10

[*] Measured with Laqua Twin AS-212 (Horiba Ltd., Tokyo, Japan); [**] Measured with Twin Cond. B-173 (Horiba Ltd, Tokyo, Japan). [#]Bacterial strains added. "-" means no addition.

S2.3 Laboratory experiments with liquid and solid media-cultured bacteria in filtered rainwater

Table S2-3 Description of the statuses of *B. subtilis*, *Micrococcus* sp., and *E. coli* cultured with TSB medium in two filtered rainwater samples, following treatments I-IV.

Treatment	I	II	III	IV
B. subtilis	Most alive[*]	Almost all dead	Most alive or Almost all alive	Some dead or half dead[*]
Micrococcus sp.	Almost all or most alive	Some dead or most dead[*]	Almost all alive	Most alive or Almost all alive
E. coli	A few dead[*]	Almost all alive	A few dead	Almost all alive

[*] Cell shape is unclear.

Three bacterial strains cultured on R2A solid media were transferred into filtered rainwater samples for treatment and observation in the following procedures. R2A media (BD Difco™, Becton, Dickinson and Company, USA; pH 7.3) inoculated with *B. subtilis* and *Micrococcus* sp. were incubated at 30°C and that with *E. coli* at 37°C for days. The bacteria strains were pre-cultivated under the same conditions before. The collected rainwater samples were filtered through sterilized 0.20-μm mixed cellulose ester membrane filters (Advantec, Toyo Roshi Kaisha, Ltd., Japan) using sterilized 10-mL syringes (Terumo, Corp., Japan). The bacterial strains of the three species were first suspended respectively in filtered rainwater samples, and then the samples were stirred on a vortex mixer (Uzusio VTX-3000L, LMS Co., Ltd., Japan) at 3000 r min^{-1} (amplitude 5 mm) for 2 min.

Fig. S2-3 shows the examples of LIVE/DEAD stain-stained *B. subtilis*, *Micrococcus* sp., and *E. coli* in filtered rainwater with and without glutaraldehyde fixation at the beginning and after 1 min of the excitation ray exposure. Almost all fixed Gram-positive bacteria, *B. subtilis* and *Micrococcus* sp., were

dead, while almost all of those unfixed were viable. Fixed Gram-negative bacteria, *E. coli*, in the microscopic field were obviously clearer in shape than unfixed ones, especially for viable ones. Moreover, much more non-viable *E. coli* cells were detected in unfixed samples than in fixed samples (Fig. S2-3 and Table S2-4). The color of *B. subtilis*, *Micrococcus* sp. and *E. coli* bleached under the excitation ray exposure, although the fading effect was not obvious within 1 min (Fig. S2-3). The living status changes of bacteria cultured using solid R2A culture medium were a little different from those cultured using liquid TSB medium, which was caused by the weaker resistance of bacteria grown in solid media, i.e., more sensitive to pH and osmotic pressure variations.

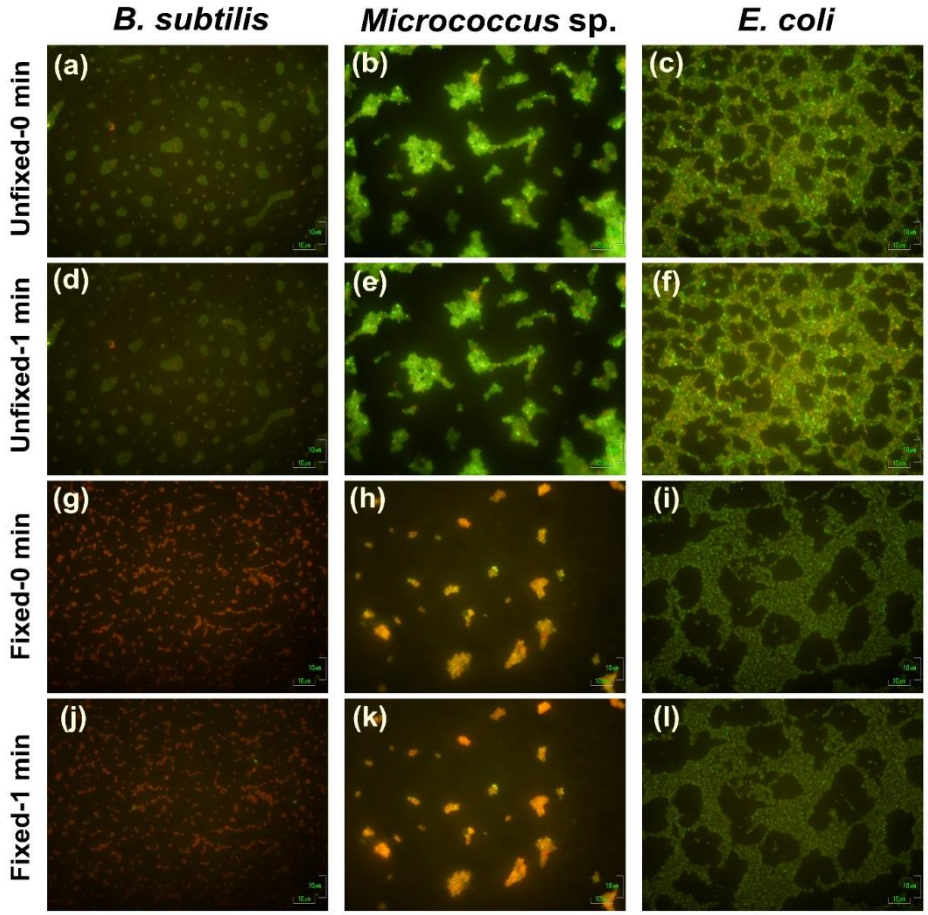

Figure S2-3. Laboratory-cultured *B. subtilis* spores, *Micrococcus* sp., and *E. coli* with R2A medium in filtered rainwater samples stained by LIVE/DEAD stain without (a-f) and with (g-l) glutaraldehyde fixation at the beginning (0 min, a-c and g-i) and after 1 min (d-f and j-l) of the excitation ray exposure.

The pH and electric conductivity of the rainwater after the treatments and before the filtration for counting are listed in Table S2-4. The living status of three bacteria species, *B. subtilis*, *Micrococcus* sp. and *E. coli*, in filtered rainwater fixed with and without glutaraldehyde in our experiments matched these alive and dead natures of the strains under different pH conditions.

The photograph examples of *B. subtilis*, *Micrococcus* sp., and *E. coli* in filtered rainwater samples diluted by PBS and stained by LIVE/DEAD stain with and without glutaraldehyde fixation are illustrated in Fig. S2-4 and described in Table S2-4. *Micrococcus* sp. exhibited extremely low viability in unfixed samples (pH 8), but significantly high viability in fixed ones (neutral). Whereas, compared with fixed *B. subtilis* and *E. coli* bacteria cells, unfixed ones trended toward higher viability and clearer shape (Fig. S2-4 and Table S2-4). These differences of bacterial status should be dependent on their different resistivity to the pH and osmotic pressure variations in the circumstances (Table S2-4 and S2-5). The viability of *Micrococcus* sp. could decrease in unfixed samples after adding PBS during the incubation time, likely because of the weaker resistivity to alkaline pH (Liu et al., 2007; Mohedano et al., 1997).

Table S2-4 Description of the states of *B. subtilis*, *Micrococcus* sp., and *E. coli* cultured with R2A medium in two filtered rainwater samples, following treatments I-IV.

Treatment	I	II	III	IV
B. subtilis	Almost all alive*	Almost all dead	Almost all alive	Almost all alive*
Micrococcus sp.	Almost all or most alive	Almost all or most dead	Almost all or half dead	Almost all or most alive
E. coli	A few or some dead	Almost all alive	A few or some dead	About or over half dead*

* Cell shape is unclear.

Table S2-5 Summary of Treatment I-IV of the two filtered rainwater samples, and pH and electric conductivity (EC) of the treated samples, 25% glutaraldehyde and PBS.

	pH*	EC** (mS cm^{-1})	Rainwater (mL)	PBS (mL)	25% Glutaraldehyde (mL)	LIVE/DEAD stain (mL)
I	4.9/4.8	0.03/0.01	5.0	-	-	0.05
II	3.7/3.7	0.14/0.13	5.0	-	0.2	0.05
III	8.0/8.0	7.90/8.20	5.0	5.0	-	0.10
IV	6.9/7.1	8.00/8.20	5.0	5.0	0.4	0.10
	3.1	0.04	-	-	0.5	-
	8.0	16.00	-	0.5	-	-

* Measured with Laqua Twin AS-212 (Horiba Ltd., Tokyo, Japan); ** Measured with Twin Cond. B-173 (Horiba Ltd, Tokyo, Japan). "-" means no addition.

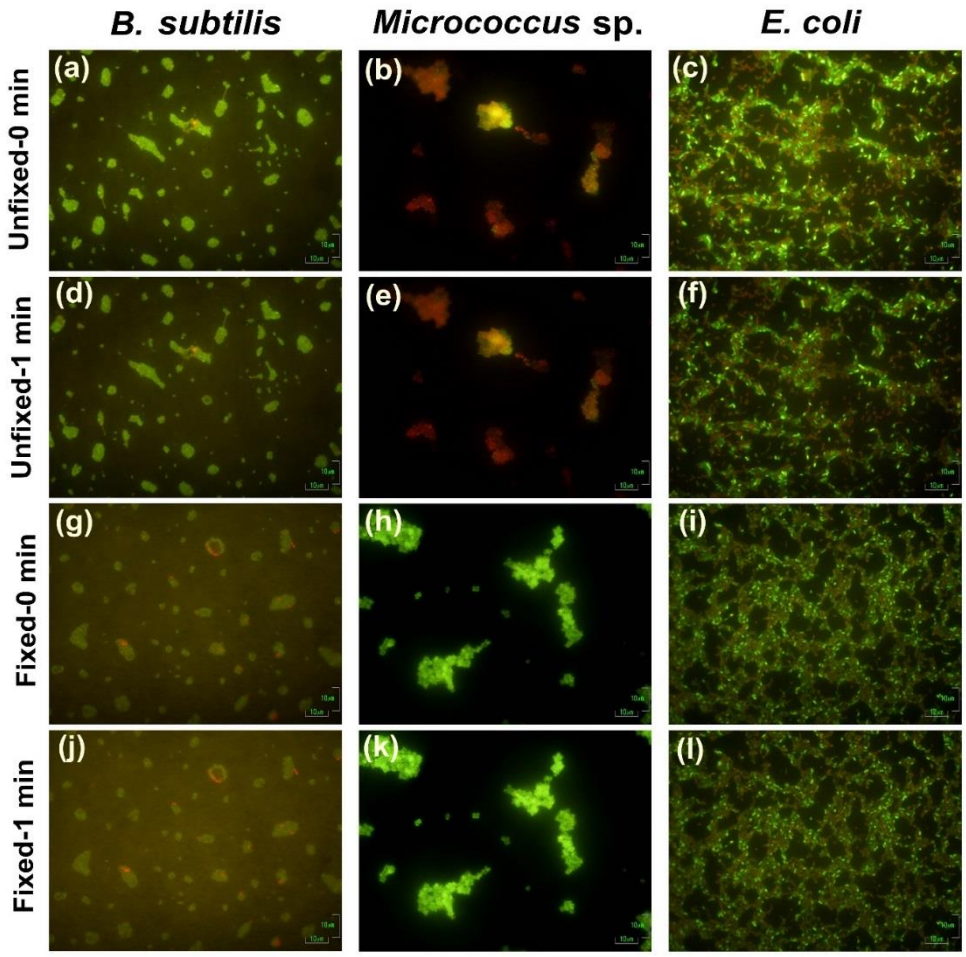

Figure S2-4. Laboratory-cultured *B. subtilis* spores, *Micrococcus* sp., and *E. coli* with R2A medium in filtered rainwater samples diluted by PBS and stained by LIVE/DEAD stain without (a-f) and with (g-l) glutaraldehyde fixation at the beginning (0 min, a-c and g-i) and after 1 min (d-f and j-l) of the excitation ray exposure.

S2.4 Fluorescence emission of glutaraldehyde

Laboratory experiments with mixed vegetative bacterial cells of *B. subtilis*, *Micrococcus* sp. and *E. coli* suspended in filtered rainwater with and without glutaraldehyde fixation and no staining were conducted. The microscopic images are shown as Fig. S2-5 (a–d). After fixation with glutaraldehyde, some of the fixed cells were indeed fluorescent green (Fig. S2-5 (c-d)). We also conducted laboratory experiments with vegetative bacterial cells suspended in two filtered rainwater samples (same subsamples with Treatment I-IV in Sect. 2.1.2.1) fixed and unfixed with glutaraldehyde, and using only PI staining. The microscopic images are shown as Fig. S2-6–S2-8. Glutaraldehyde induced auto-fluorescence can also be observed, especially for fixed *E. coli* and *Micrococcus* sp. cells. Fixed *B. subtilis* cells turned to be orange color. The glutaraldehyde-induced auto-fluorescence could fade gradually within several minutes under the excitation ray exposure (Fig. S2-6 (d-f)). However, the red color labeled cells didn't.

The numbers of red color labeled E. coli cells in fixed and unfixed subsamples were consistent with the results of Treatment I and II, III and IV. So, we think that the fluorescent color changes of *E. coli* reflected the actual statuses of cells after each treatment, and not caused by glutaraldehyde fixation. To some extent, cell damages caused by glutaraldehyde fixation and glutaraldehyde induced green fluorescence are contradictory herein. If bacterial cells were damaged after glutaraldehyde fixation, and glutaraldehyde induced green fluorescent influenced substantially, we could not observe the orange/red color labeled cells as shown in Fig. 2-3 (g, h, j and k), and Fig. 2-4 (g and j). But it was said that the wavelength of the actinic beam and pH of the environment can influence the amount of glutaraldehyde-induced fluorescence enhancement significantly. Longer wavelengths and acid pH produce a smaller effect (Collins and Goldsmith, 1981). Still, we agree that glutaraldehyde induced auto-fluorescence should be noticed in cells fixed for fluorescence microscopy. To ensure the quality of enumeration results, it is better to pre-evaluate the influence of glutaraldehyde fixation by comparing the results of fixed and unfixed rainwater samples.

Our measurement before has revealed that nearly no bacterial cells were observed from the slides of unprocessed rainwater samples. Experiments of rainwater with and without glutaraldehyde fixation and no staining were re-conducted again (Fig. S2-5 (e–h)). The microscopic fields of glutaraldehyde fixed and unfixed subsamples had little difference. Several particles can be observed in the fields, but cannot be identified what they are. The suspected bacteria cells accounted for 8% of the total cells stained by LIVE/DEAD stain (Fig. S2-9).

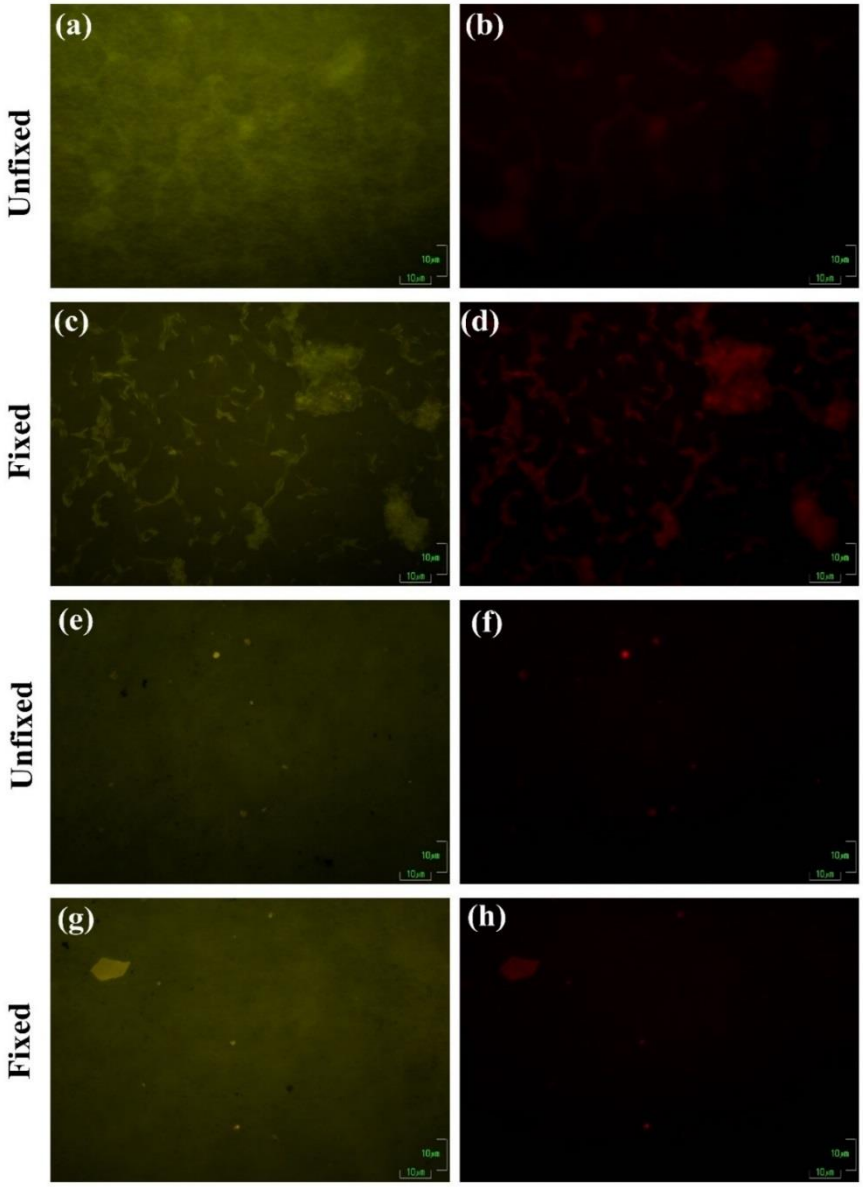

Figure S2-5. Mixed *B. subtilis*, *Micrococcus* sp. and *E. coli* cells in filtered rainwater subsamples (a-d) and actual rainwater subsamples (e-h) fixed with glutaraldehyde and without staining under blue (a, c; e, g) and green (b, d; f, h) excitation rays. The rainwater samples were collected on 22 February and 3 May 2016, respectively.

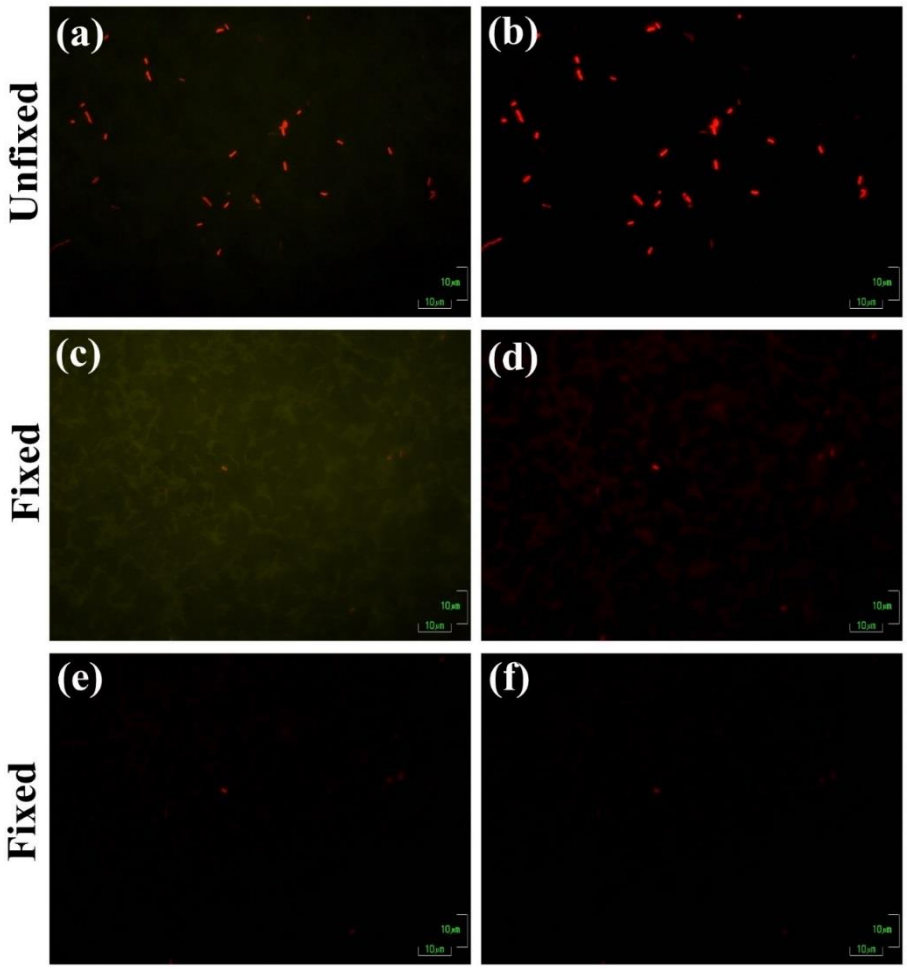

Figure S2-6. *E. coli* cells in filtered rainwater subsamples stained with PI under blue (a, c) and green (b, d-f) excitation rays. The rainwater sample was collected on 22 February 2016, the same for **Fig. S2-7–S2-8**.

Bacterial cells in three additional actual rainwater samples were stained by PI. The differences of microscopic images for fixed and unfixed rainwater subsamples, under blue and green excitation rays were not so obvious (Fig. S2-10). So, the influence of glutaraldehyde-induced fluorescence on rainwater bacterial cells was likely insignificant. Nearly no greenish bacteria cells can be observed. Non-viable bacterial cells were enumerated under blue excitation rays. The consistency of non-viable bacterial counts (Fig. S2-11) in fixed and unfixed subsamples also indicated the insignificant influence of glutaraldehyde induced fluorescence on bacteria in rainwater.

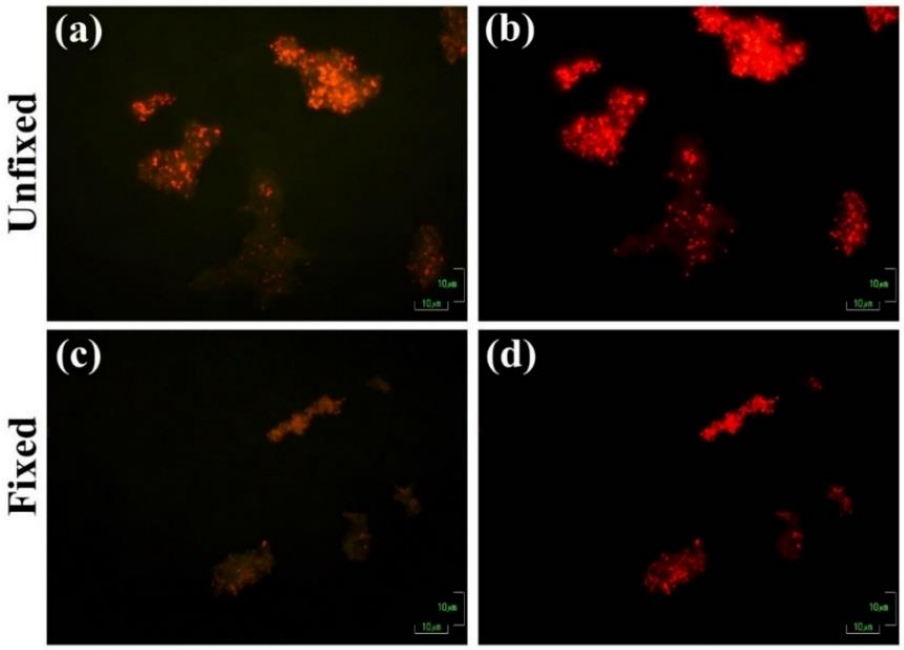

Figure S2-7. *Micrococcus* sp. cells in filtered rainwater subsamples stained with PI under blue (a, c) and green (b, d) excitation rays.

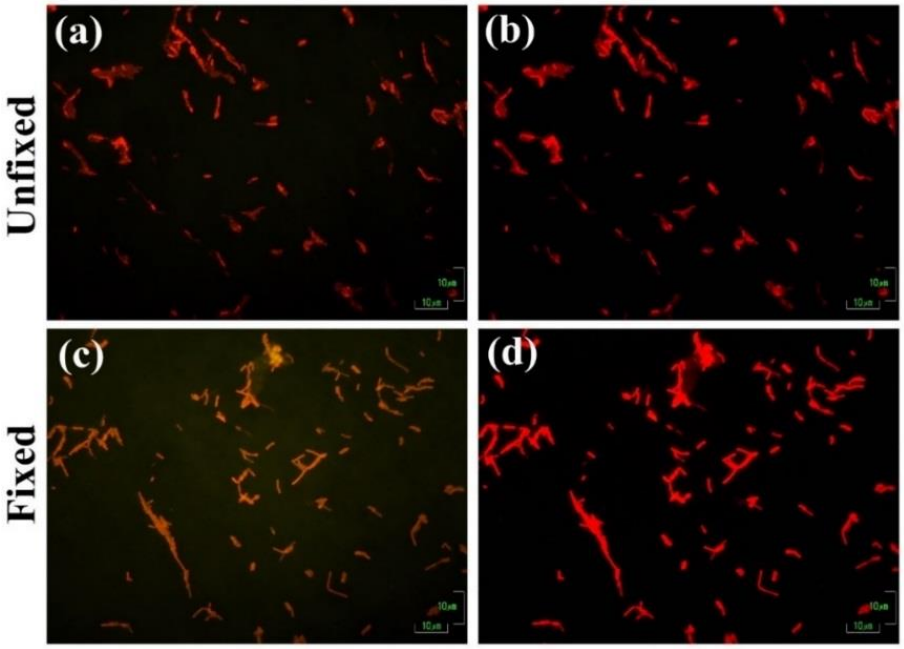

Figure S2-8. *B. subtilis* cells in filtered rainwater subsamples stained with PI under blue (a, c) and green (b, d) excitation rays.

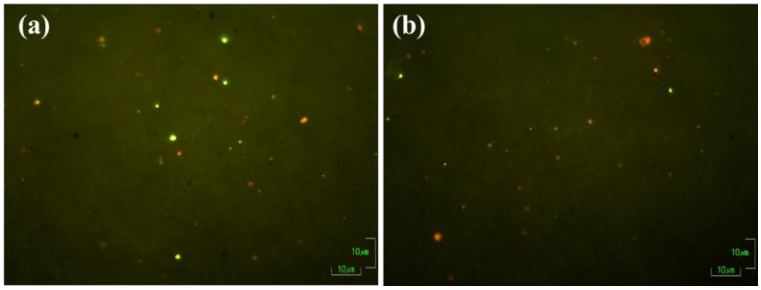

Figure S2-9. Rainwater bacterial cells stained with LIVE/DEAD stain after glutaraldehyde fixation under blue excitation rays. The rainwater sample was collected on 3 May 2016, the same for **Fig. S2-10**.

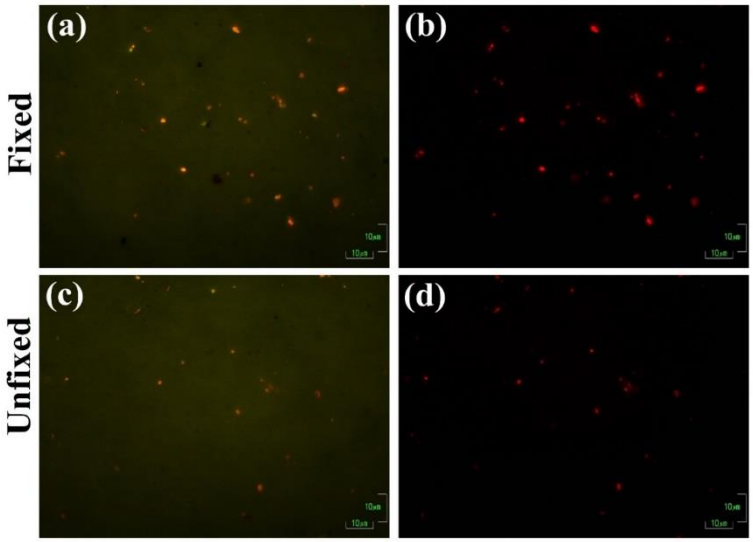

Figure S2-10. Rainwater bacterial cells stained with PI under blue (a, c) and green (b, d) excitation rays.

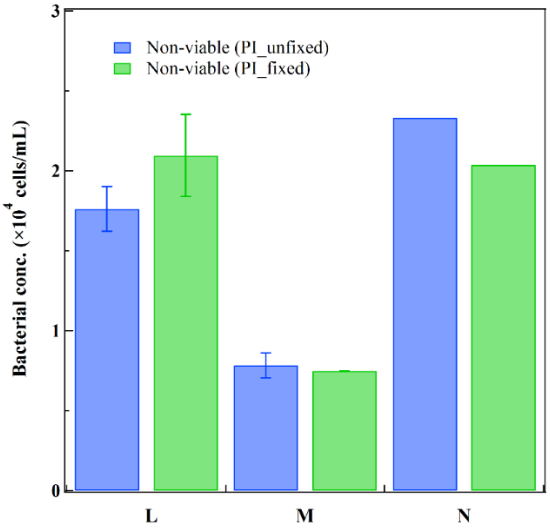

Figure S2-11. Concentrations of non-viable bacteria in three rainwater samples (marked by L–N) identified using PI stain with and without glutaraldehyde fixation.

CHAPTER 3

Bacterial abundance and viability in rainwater associated with cyclones, stationary fronts and typhoons in southwestern Japan

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The bacterial concentration and viability in the rainwater collected at a coastal site in southwestern Japan under different synoptic weather conditions were measured, using the LIVE/DEAD BacLight bacterial viability assay coupled with glutaraldehyde fixation, the effectiveness of which has been carefully verified in Chapter 2. The physical (e.g., pH and electric conductivity) and chemical properties of rainwater were also determined. The purposes of this chapter are: 1) to understand the occurrence of bacteria in rainwater under different synoptic weather conditions in aspects of abundance and viability rather than the genetic and ecological diversity of bacterial species, 2) to explore the influences of cloud forming manners on the bacteria, and 3) to gain an insight into the sources of bacteria in rainwater.

3.1 Methodology

3.1.1 Sample collection and bacterial enumeration

Rainwater was collected in Kumamoto in southwestern Japan (Fig. 3-1 (a)) between October 2014 and September 2015. The site was located on the roof of a building (about 20-m high) on the campus of Prefectural University of Kumamoto (PUK, 32.806 °N, 130.766 °E).

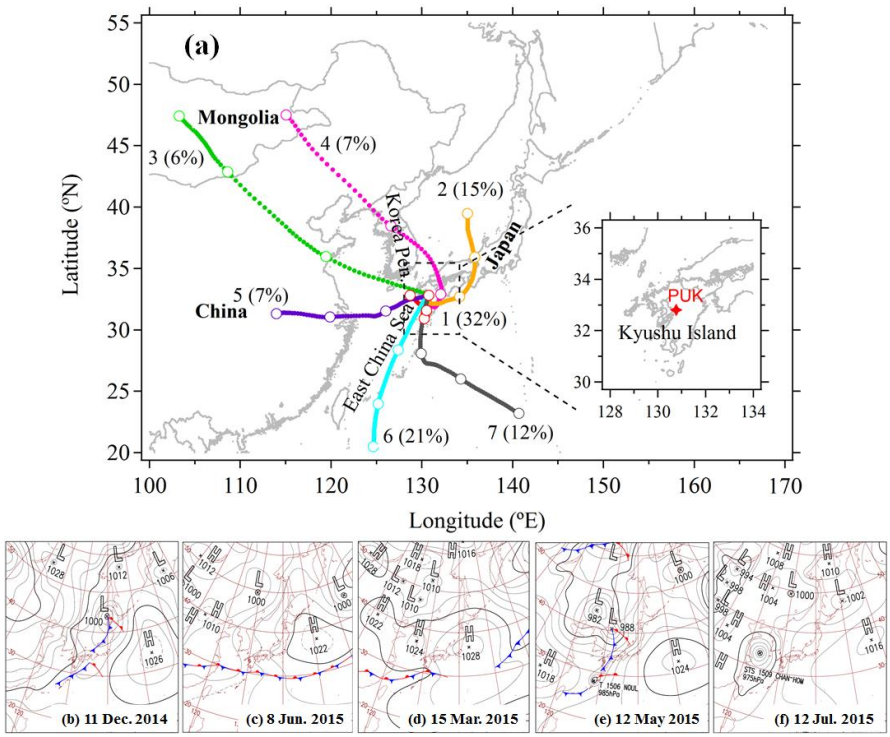

Figure 3-1. Location of the sampling site on the PUK campus (a) and typical weather charts (http://www.jma.go.jp/) during the rain events derived by cold front (b), stationary front during the Meiyu-period (c) and non-Meiyu period (d), and rain caused by both cold fronts and typhoons (e), and by typhoons only (f). The backward trajectories of air parcels (http://ready.arl.noaa.gov/HYSPLIT.php) from the sampling site are clustered into seven mean trajectories (marked as 1 to 7) according to the path, speed and source of air parcels (a). Circles on the trajectories mark one-day intervals. The percentages of each trajectory are given in parentheses, and the details can refer to Sect. S3.1.

Around the campus, there were no large and constant sources of biological particles, such as agricultural fields or factories, besides natural areas and routine human activities. Kumamoto city is located on the island of Kyushu and in the east of Ariake sound, which connects with the East China Sea. There are cyclones passing this area in all seasons causing cold front-associated rain. Meiyu occurs in every June and July. Typhoons frequently pass this area in August and September, sometimes in other months.

In total, sixty-eight rainwater samples were collected. We did not collect samples in thunderstorms because they usually occur shortly in small scales and the rain is not the target of this study. Rainwater samplers were brought to the sampling site and fixed there without exposure to ambient air. They were opened to collect rainwater when it was raining. The sampling durations were within or about one day. The collection periods and corresponding meteorological conditions are listed in supplementary Table S3-1.

The concentration of bacterial cells in a rainwater sample was estimated from the cell counts in duplicated 5-mL rainwater subsamples. After the collection of a sample, bacterial cells in its subsamples were immediately fixed with glutaraldehyde (Wako Pure Chemical Ind., Ltd., Japan) and subsequently stained with the LIVE/DEAD® BacLight™ Bacterial Viability Kit L13152 (Invitrogen™, Molecular Probes Inc., USA). The fixed and stained subsamples were filtered with 0.2-μm-pore 25-mm-diameter black polycarbonate filters (Toyo Roshi Kaisha, Ltd., Japan), and then the filters were mounted on microscope slides. The bacterial cells on the filters were examined using an epifluorescence microscope (Eclipse 80i, Nikon Corp., Japan) under blue excitation rays (450–490 nm). The concentrations of viable and nonviable bacteria were estimated by counting fluorescent green and red/orange/yellow cells which were spherical and close to or smaller than 1 μm in diameter (Fig. 2-1 (e); Hara and Zhang, 2012; DeLeon-Rodriguez et al., 2013). The bacterial cell concentrations in rainwater were calibrated with negative control samples. The negative control sample was made by pouring sterilized ultrapure water into the sampler after rinsing three times, and treating it as a rainwater sample. The viability of bacteria is defined as the ratio of viable cells to total cells, which is applied to show the alive possibility of the bacterial cells. As mentioned in Chapter 2, the uncertainty in measuring bacterial abundance due to the operation and atmospheric diffusion had been evaluated less than 12% on average. In addition, growth of bacterial cells in collected rainwater during the sample collection was not taken into account, because the collection duration was short and considerable growth was not expected (Sattler et al., 2001).

3.1.2 Analysis of ionic species in rainwater

The pH and electric conductivity (EC) of rainwater samples were measured using a pH meter (Laqua Twin AS-212, Horiba Ltd., Japan) and a conductivity meter (Twin Cond. B-173, Horiba Ltd, Japan),

respectively, under controlled room temperature (approximately 25°C). The concentration of H_3O^+ in rainwater was calculated from the determined pH value. Rainwater samples were filtered through 0.2-μm-pore membrane filter units (DISMIC-25, Toyo Roshi Kaisha, Ltd., Japan). The water-soluble species Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, and SO_4^{2-} in them were analyzed with an ion chromatograph system (ICS-1600, Dionex, USA) equipped with an automatic injector in Kumamoto Prefectural Institute of Public Health and Environmental Science. The detection limits for Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, and SO_4^{2-} were 0.003, 0.002, 0.002, 0.001, 0.003, 0.006, 0.046, and 0.021 mg L^{-1}, respectively. The positive correlations between cation and anion equivalent concentrations, and between calculated and measured total conductivities confirmed the validity of the ion analysis (Pearson correlation coefficients r=0.99, 0.97, p<0.01). The pH, EC, and concentrations of ionic species of ultrapure water and filtered blank samples were also measured and met the requirement for the Quality Assurance/Quality Control Program for Wet Deposition Monitoring in East Asia (EANET, 2000). The SO_4^{2-} and Ca^{2+} from sea salts (ss-SO_4^{2-}, ss-Ca^{2+}) and non-sea salts (nss-SO_4^{2-}, nss-Ca^{2+}) were estimated according to the guidance of EANET (2000). The pH, EC, and concentrations of major ions in rainwater samples are listed in Table S3-1.

3.1.3 Airborne particle abundance and meteorological conditions

Airborne particle number concentrations in five size ranges (>0.3, 0.5, 1.0, 2.0, and 5.0 μm in diameter) were measured with an optical particle counter (KC-01D, RION Ltd., Japan) at a 10-min time resolution during the sampling period. In this study, fine particles refer to those in the size range of 0.3–1.0 μm, and coarse particles to those larger than 1.0 μm (Zhang et al., 2006). Meteorological conditions, including surface pressure, temperature, relative humidity, wind velocity and direction, and rainfall, over each rainwater collection period were averaged from the records at the Kumamoto Meteorological Observatory (local official meteorological observatory; about 8 km from the observation site; http://www.jma.go.jp/). These data were applied to examine the scavenge effect of rain.

The movements of air parcels associated with the rain events are summarized according to the backward trajectories of air parcels from the mid-time during the samplings (Fig. 3-1 (a) and Sect. S3.1 in the supplement). The manners of cloud formation were identified according to meteorological variations based on the weather records and weather charts (http://www.jma.go.jp/). In this study, we classified the rain into five types according to the dominant pattern of cloud-forming synoptic systems. (1) Cold front rain (CF) was caused by clouds induced by cold fronts of cyclones. Air masses associated with this type of rain events come more frequently from the Asian continent (Cluster 4, 5 and 3 in Fig. 3-1 (a)). (2) Meiyu front rain (SF_MP) was caused by stationary front-induced clouds during the Meiyu period (2 June to 29 July 2015). (3) Non-Meiyu stationary front rain (SF_NMP). Air masses associated

with stationary front rain episodes in both the Meiyu and non-Meiyu periods are mostly maritime (Cluster 6 and 7), and sometimes linger over Kyushu areas (Cluster 1) because warm and cold air masses are equally matched there. (4) Rain caused by both cold fronts and typhoons (CF+T) and (5) rain caused by typhoons only (T). There were 20, 24, 16, 4, and 4 rainwater samples for the five types in order. Typical weather charts of the five rain patterns are shown in Fig. 3-1 (b–f).

3.1.4 Statistical analysis

To explore possible associations between bacterial abundance and the ionic components in the rainwater, and consequently the sources of bacteria, the correlation matrix and principal component analysis (PCA) were performed combining the bacterial concentration and the contents of major ions in rainwater using PASW Statistics 18 (formerly SPSS Statistics). For Pearson correlation analysis, a *p*-value of less than 0.01 or 0.05 indicates the correlation is significant at the 0.01 or 0.05 level (confidence level 99% or 95%, 2-tailed).

3.2 Results

3.2.1 Concentration and viability of bacterial cells in the rainwater

There are some potential factors, e.g., the addition of glutaraldehyde, staining time, bacterial species, physical (e.g., pH and osmotic pressure) and chemical properties of rainwater, could affect the enumeration result based on the LIVE/DEAD bacterial viability assay (Boulos et al., 1999). In addition, both small mineral particles and a few bacterial cells exhibited yellow color in stained samples. Whereas, the consistency of total counts of bacteria with LIVE/DEAD kit- and DAPI-staining indicates that the LIVE/DEAD bacterial viability assay could accurately quantify bacterial cells in rainwater.

The statistical results of concentrations of viable and nonviable bacteria in the rainwater samples are illustrated in Fig. 3-2. The concentrations of viable and nonviable bacteria were about $1.7\pm1.0\times10^4$ and $5.4\pm6.4\times10^3$ cells mL^{-1} on average, and varied in the ranges of 5.3×10^3–5.7×10^4 and 5.0×10^2–4.2×10^4 cells mL^{-1} (Table S3-1 and Fig. S3-2), respectively. The total number concentrations of bacterial cells varied between 7.6×10^3 and 7.2×10^4 cells mL^{-1}. High concentrations, about 7×10^4 cells mL^{-1}, were observed in cyclone-induced rain events on 4, 10 December 2014, and 15 February 2015. The cold and dry postfrontal air on these days passed the north and east Mongolia and moved fast to the observation area (Cluster 3 and 4 in Fig. 3-1 (a). The viability of the bacteria in the rainwater ranged from 39% to 96%, and the average was 80±10% (Fig. 3-2). The viability showed a negative correlation with total bacterial concentration ($r=-0.573$, $p<0.01$).

There was a dependence of bacterial concentration and viability on the synoptic weather of cloud formation (Fig. 3-2 and Table S3-2). The average concentrations of both viable and nonviable bacterial cells in the rainwater of cold-front-induced clouds were the highest (2.6×10^4 and 9.0×10^3 cells mL^{-1}). In contrast, in the rainwater caused by typhoons only, and by both cold fronts and typhoons, the average total bacterial concentrations were as low as 1.2×10^4 and 1.3×10^4 cells mL^{-1}, respectively. In the cases of stationary fronts during the Meiyu period when the rain lasted long and the rainfall was very heavy (Table S3-1), the viable and nonviable bacteria in the rainwater were also at low levels, with the average concentrations of 1.2×10^4 and 2.5×10^3 cells mL^{-1}. The average concentration of total bacterial cells in the rainwater of stationary-front-induced clouds during the non-Meiyu period were as high as 2.4×10^4 cells mL^{-1}, which was close to that in the rainwater of cold-front-induced clouds.

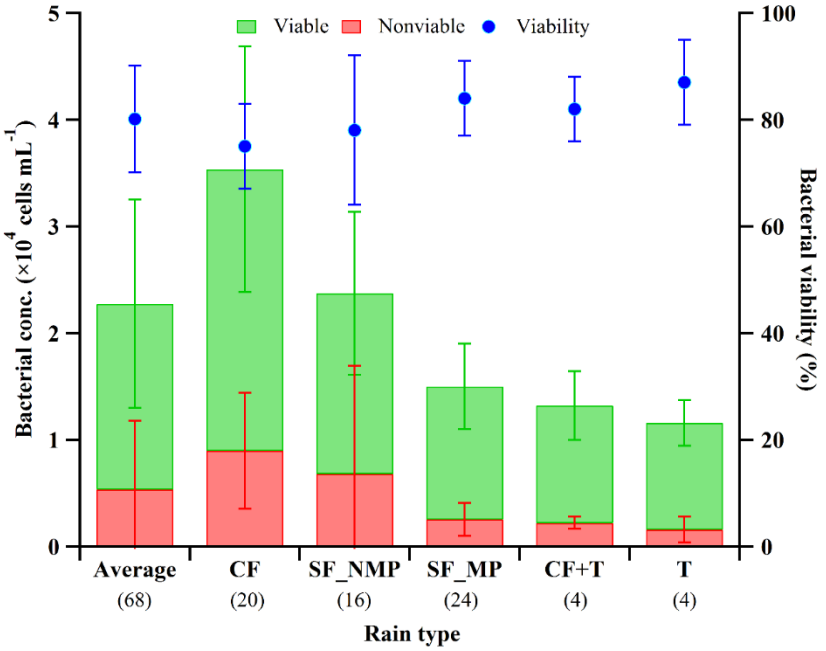

Figure 3-2. Average and standard deviation of bacterial abundance and viability in rainwater dependent on the cloud formation manners. CF, cold front; SF, stationary front; T, typhoon; MP, Meiyu period; NMP, non-Meiyu period. Sample numbers are shown in parentheses.

The viability of bacteria in the rainwater of cold-front-induced clouds was 75% on average, which was the lowest in all rain types. In the rainwater of typhoons, the bacterial viability was the highest (about 87%) among all types of rainwater. Those in the rainwater induced by stationary fronts during the Meiyu and non-Meiyu periods, and caused by both cold fronts and typhoons, were 84%, 78%, and 82%, respectively.

Multiple samples were collected during several rain periods: 10–11 December 2014, 26 January, 12 May, 2–3, 8–9, 10–11 June, 30 June–1 July, 4–6, 21–22 July, 25–26, 29–30 August, 31 August–1

September 2015, to examine the evolution of rainwater bacteria in raining. Results showed that the concentrations of bacteria in later samples were mostly equivalent with or higher than (the average ratio 115±45%) those in early samples (Fig. S3-1).

3.2.2 Relationship between rainwater bacteria and airborne particles

Average number concentrations of fine and coarse particles in the three hours before the start and in the three hours before the stop of rain (or sample collection), and their differences, were applied to investigate possible correlations between bacteria in rainwater and aerosol particles in the air associated with the rain. The concentrations of fine and coarse particles were still in high levels even during some rain events, for instance, on 30 November and 10 December 2014, and 15 February and 15 March 2015 (Fig. 3-3). The bacterial concentrations in rainwater were quite high on 10 December 2014 and 15 February 2015. On these two days, the ratios of average particle number concentrations to precipitation amount in the three hours before the sample collection (raining) were as high as 3.4×10^4 and 3.9×10^4 L^{-1} mm^{-1} for fine particles, and 3.0×10^2 and 3.4×10^2 L^{-1} mm^{-1} for coarse particles, respectively. High abundance of airborne particles could contribute some bacteria to rainwater, although the number concentrations of total airborne particles during sampling periods kept at high levels at an order 10^5 L^{-1} (Fig. 3-3 (b–c)).

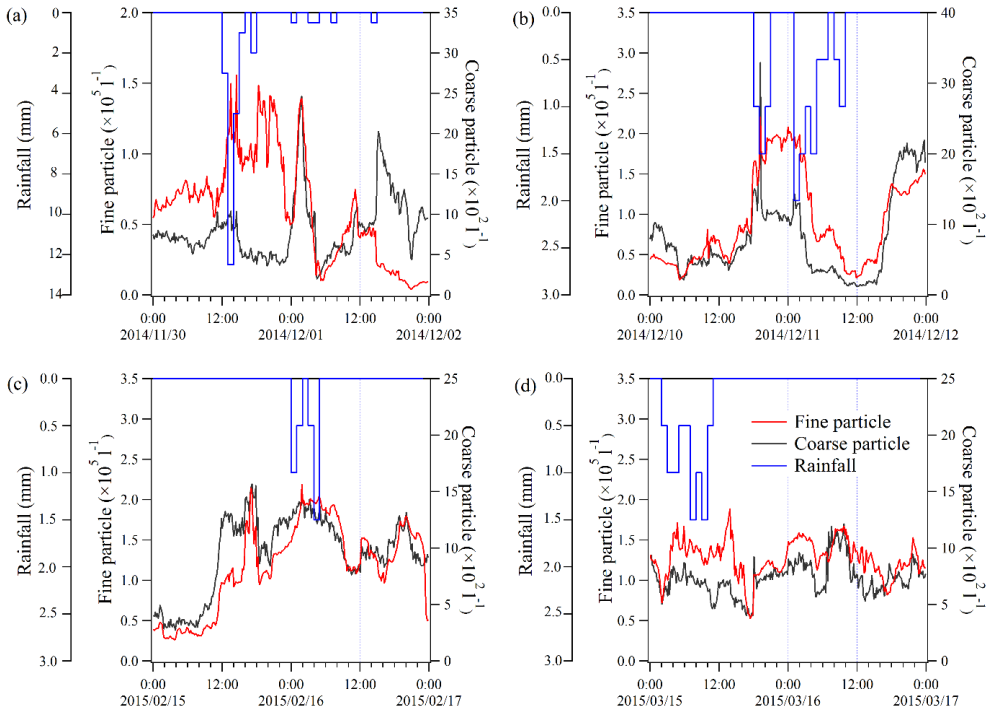

Figure 3-3. Examples of airborne particle number concentrations and rainfall during sampling periods. (a) 30 November and (b) 10 December 2014, (c) 15 February and (d) 15 March 2015.

In this study, the bacterial concentrations in rainwater did not correlate well (r=0.03–0.2, p>0.05) with the ratios of airborne particle concentrations before sample collection or raining to the precipitation amount, and also the ratios of decreased airborne particle concentrations to the precipitation amount (Table S3-3).

3.2.3 Correlation matrix and PCA combining bacteria and ion species

Relationships between bacteria and ionic species in the rainwater were examined with correlation analysis (Table 3-1). The viable, nonviable and total bacterial cell concentrations showed potential positive correlations (p<0.01) with nss-Ca^{2+}, NO_3^-, nss-SO_4^{2-}, and H_3O^+. Especially, nonviable bacteria strongly correlated with nss-Ca^{2+} (r=0.62). Nss-Ca^{2+}, NO_3^-, and nss-SO_4^{2-} accounted for 8±5%, 11±4%, and 21±9% of total determined ionic species in molar ratio, respectively. The bacterial concentration increased with pH decrease (positively correlating with H_3O^+ concentration). The viable bacterial abundance showed insignificant correlation with NH_4^+, and nonviable and total bacteria correlated weakly with NH_4^+ (Table 3-1). There was also no obvious correlation between bacterial abundance and the concentrations of ion species from marine sources (Cl^-, Na^+, K^+, Mg^{2+}, ss-SO_4^{2-}, and ss-Ca^{2+}). As listed in Table 3-1, bacterial viability showed negative correlations with H_3O^+ (r=−0.30, p<0.05), NO_3^-, nss-SO_4^{2-}, NH_4^+, and nss-Ca^{2+} (r=−0.52–−0.37, p<0.01).

In order to identify the sources of bacteria in rainwater, PCA combining independent chemical species and bacterial abundance in rainwater was also conducted. Three principal factors were retained for discussion, and these factors accounted for 85.7% of the total variance of the data set (Table 3-2). The main features in first PC (PC1) are ss-SO_4^{2-}, ss-Ca^{2+}, Na^+, Cl^-, K^+, and Mg^{2+}, and they explain 45.6% of total variance. The PC1 is associated with the marine source. The second PC (PC2) offers the highest weights for nss-SO_4^{2-}, H_3O^+, NH_4^+, and NO_3^- (22.3% of the total variance), which was mainly associated with the anthropogenic sources. The third PC (PC3) is mainly constituted by the variables nss-Ca^{2+}, nonviable bacteria, and viable bacteria (12.3% of the total variance). This PC3 is identified as crustal and microbial sources.

Table 3-1 Correlation coefficients between bacterial abundance and ionic components in rainwater.

	Cl^-	NO_3^-	Na^+	NH_4^+	K^+	Mg^{2+}	H_3O^+	nss-SO_4^{2-}	nss-Ca^{2+}	ss-SO_4^{2-}	ss-Ca^{2+}	Anion	Cation	VB	NVB	TB	Viability
N	68	68	68	68	68	68	68	68	68	68	68	68	68	67	67	67	67
Cl^-	1																
NO_3^-	**.388**	1															
Na^+	**.996**	**.361**	1														
NH_4^+	.280	**.792**	.252	1													
K^+	**.968**	**.444**	**.969**	**.377**	1												
Mg^{2+}	**.930**	**.559**	**.913**	**.373**	**.895**	1											
H_3O^+	.120	**.534**	.071	**.401**	.132	.176	1										
nss-SO_4^{2-}	.101	**.682**	.049	**.729**	.167	.209	**.851**	1									
nss-Ca^{2+}	.078	**.582**	.059	**.453**	.163	.282	.153	**.409**	1								
ss-SO_4^{2-}	**.997**	.364	1.000	.253	**.969**	**.918**	.074	.053	.062	1							
ss-Ca^{2+}	**.996**	.361	1.000	.252	**.969**	**.913**	.071	.049	.059	1.000	1						
Anion	**.806**	**.773**	**.775**	**.678**	**.826**	**.841**	**.570**	**.655**	**.354**	**.778**	**.775**	1					
Cation	**.817**	**.771**	**.790**	**.694**	**.844**	**.848**	**.543**	**.620**	**.382**	**.792**	**.790**	**.991**	1				
VB	.027	**.370**	-.003	.191	.070	.072	**.485**	**.435**	**.447**	-.002	-.003	.287	.284	1			
NVB	.172	**.561**	.142	**.331**	.199	.288	**.409**	**.472**	**.621**	.144	.142	**.443**	**.430**	**.657**	1		
TB	.093	**.488**	.060	.270	.133	.173	**.497**	**.492**	**.565**	.062	.060	**.382**	**.374**	**.944**	**.868**	1	
Viability	-.180	**-.438**	-.149	**-.366**	-.203	-.238	-.298	**-.432**	**-.517**	-.150	-.149	**-.405**	**-.391**	**-.359**	**-.829**	**-.598**	1

Note: VB, Viable bacteria; NVB, Non-viable bacteria; TB, total bacteria. Data in bold mean correlations are significant at the 0.01 level (2-tailed).

Table 3-2 Factor loadings of principal component analysis (varimax with Kaiser rotation) for anion and cation contents, and bacterial abundance in rainwater. Factor loadings greater than 0.4 are shown in bold.

Ions and bacteria	PC1	PC2	PC3
ss-SO_4^{2-}	**0.996**	0.051	0.004
ss-Ca^{2-}	**0.995**	0.048	0.002
Na^+	**0.995**	0.048	0.002
Cl^-	**0.990**	0.094	0.023
K^+	**0.965**	0.147	0.069
Mg^{2+}	**0.929**	0.172	0.158
nss-SO_4^{2-}	-0.024	**0.938**	0.246
H_3O^+	-0.002	**0.835**	0.163
NH_4^+	0.230	**0.772**	0.197
NO_3^-	0.336	**0.705**	**0.426**
Non-viable bacteria	0.122	0.262	**0.855**
nss-Ca^{2+}	0.026	0.176	**0.820**
Viable bacteria	-0.046	0.245	**0.761**
% of Variance	45.6	22.3	17.8
Cumulative %	45.6	67.9	85.7

3.3 Discussion

3.3.1 Bacterial concentration and viability according to rain types

The formation mechanisms and the movement of clouds associated with a rain event can affect the occurrence and status of bacteria in the rainwater. The rain events of cold-front-induced clouds at the observation site were the consequence of extratropical cyclones moving from the Asian continent towards the North Pacific Ocean over the East China Sea (Fig. 3-1 (b)). Intense anthropogenic and natural dust emissions caused high concentration pollutants including bacterial aerosols in the Asian continent (Burrows et al., 2009b). With the movements of cyclones, the continental outflow of anthropogenic and dust emissions from East Asia (Sect. S3.1) could result in the high abundance of bacteria in the rainwater. The low bacterial viability (75%) was comparable to the airborne bacterial viability (72%) during cyclone passage at the same site (Murata and Zhang, 2014). These results are consistent with that airborne bacteria conveyed from the Asian continent are characterized by high abundance and low viability (Murata and Zhang, 2014; 2016), and also support the hypothesis that anthropogenic emissions negatively affect the survival of the microbes transiting by the atmosphere and clouds (Amato et al., 2007d; Fuzzi et al., 1997; Vaïtilingom et al., 2012).

A nearly stationary front is usually a zone close to baroclinic state in the lower troposphere that stretches from the Eastern China coast and eastward into the Pacific (Fig. 3-1 (c–d)). Most of the stationary fronts in this study, Meiyu fronts, were present in the early summer, lasting for about two months and causing persistent heavy rainfall (Fig. S3-2). In comparison with those in cyclones, the movement and origins of air parcels associated with the Meiyu stationary fronts have mainly two differences. One is that the strength of cold and dry air in the north of the fronts is generally weaker and the movement is generally slower than the postfrontal air in cyclones. Another is that the warm and humid air is from south or southeast and is less influenced by continentally emitted pollutants than the prefrontal air in cyclones which is more or less influenced by continental emissions. These two differences make the rain of Meiyu fronts less influenced by natural and anthropogenic pollutants from the continent.

The rain of Meiyu fronts usually lasts much longer than a single cyclone rain. The airborne particle number concentrations decreased when it was raining during the Meiyu period (Fig. S3-2). Most of locally originated bacteria should have been washed out by the long-term rain. Because locally emitted airborne bacteria could be diluted by the heavy rainfall, and more importantly, the water vapor in clouds of the Meiyu fronts originated from marine air, which is usually cleaner than continental air, the bacterial abundance in the rainwater should be low. Burrows et al. (2009b) suggested the best estimate of total mean bacterial concentrations in near-surface air of seas (1×10^4 m^{-3}) was much lower than that (6.5×10^5 m^{-3}) of urban, due to less anthropogenic influences. Vaïtilingom et al. (2012) found a source effect that clouds originating from the ocean carried fewer bacteria than those from continental regions. In this study, the bacterial abundance in rainwater were also affected by the pathway of the air parcels associated with the rain events, and showed a similar source effect (Sect. S3.1). Kjelleberg et al. (1982) hypothesized that marine bacteria predominantly existed as dormant cells adapted specifically to frequently and rapidly changing environments. The higher viability during the Meiyu period was likely related to the dispersal of marine bacteria via rainwater, although the ocean was not the only source of bacteria in the rainwater.

The occurrence of non-Meiyu stationary fronts mostly from late summer to early spring is usually shorter compared with that in the Meiyu period because the southward retreat of warm tropical air cannot persist long time (Fig. 3-1 (d)). In most cases of these rainwater samples, the bacterial abundance and viability level were similar (Fig. S3-1). However, in a few cases, in addition to the maritime influence, the rain events were affected by the Asian continental outflow or emissions from local areas. For example, on 4 December 2014, the rainwater was also impacted by the long-range transport from the Asian continent (Cluster 3 in Fig. 3-1 (a)), resulting in a very high abundance and low viability.

Typhoons are cyclones with extremely low pressure in the center, and their passage usually causes short-term but very heavy rain (Fig. 3-1 (e–f)). Since typhoons originate from the central Pacific Ocean

area, and the composition in typhoon rains is mainly influenced by air near ocean surface, bacteria in the water should be primarily of marine origin. DeLeon-Rodriguez et al. (2013) found that the hurricane samples collected in the upper troposphere of the Caribbean Sea and the mid-western Atlantic Ocean were characterized by a high viability (60–100%, 90% on average) and a high abundance of marine bacteria. Similarly, the ocean surface pathway of typhoons was possibly associated with the high bacterial viability and low abundance in rainwater.

3.3.2 Origins of bacteria in the rainwater

The origins of bacteria in the rainwater were viewed from two perspectives. One is the influences of anthropogenic, terrestrial and marine sources. Another is below-cloud removal and in-cloud processes.

In polluted atmosphere, gaseous precursors (SO_2 and NO_x) of secondary inorganic aerosols (nss-SO_4^{2-} and NO_3^-) formation are mainly emitted from anthropogenic sources. Aqueous oxidation is the major pathway of the oxidation in clouds, fog and rain (Seinfeld and Pandis, 2012). Mineral particles are the major source of atmospheric Ca^{2+}. The good correlation of nss-Ca^{2+} with NO_3^- ($r=0.58$, $p<0.01$) suggested that NO_3^- formation was closely correlated with mineral particles in the rainwater. The components of mineral particles, notably $CaCO_3$, can directly react with gaseous inorganic acids such as HNO_3, to form $Ca(NO_3)_2$ (Laskin et al., 2005). Positive correlations between bacterial abundance with the ionic species nss-SO_4^{2-}, NO_3^- and nss-Ca^{2+} in rainwater suggest that anthropogenic and terrestrial emissions were significantly associated with more abundant bacteria in rainwater. Therefore, in the rainwater of cold fronts and non-Meiyu stationary fronts, which were more significantly influenced by air masses from the continent, the viable, nonviable and total bacterial concentrations correlated well ($p<0.01$ or 0.05) with nss-SO_4^{2-}, NO_3^-, and nss-Ca^{2+}. In contrast, no such correlations were found in the rainwater of Meiyu fronts (Fig. 3-4 (a–c, e–g, i–k)).

In previous studies (Amato et al., 2007d; Fuzzi et al., 1997), there was a decrease in bacterial concentrations in cloud and fog water with decreasing pH (increasing H_3O^+). The pH values of both cloud and fog water ranged from about 3 to 7, a little wider than those (~4–6) in the rainwater of this study. Ionic species SO_4^{2-}, NO_3^- and NH_4^+ accounted for over 85% of total soluble ions in fog water. Amato et al. (2007d) considered that bacteria emitted by urban areas in the direction of clouds were probably limited. Fuzzi et al. (1997) gave possible explanations that neutral pH conditions favored bacterial growth in fog water and high lead concentrations at low pH damaged metabolic enzymes in bacteria. However, in this study, the bacterial concentration increased with decreasing pH (increasing H_3O^+), also suggesting that the anthropogenic influence on bacterial abundance was positive.

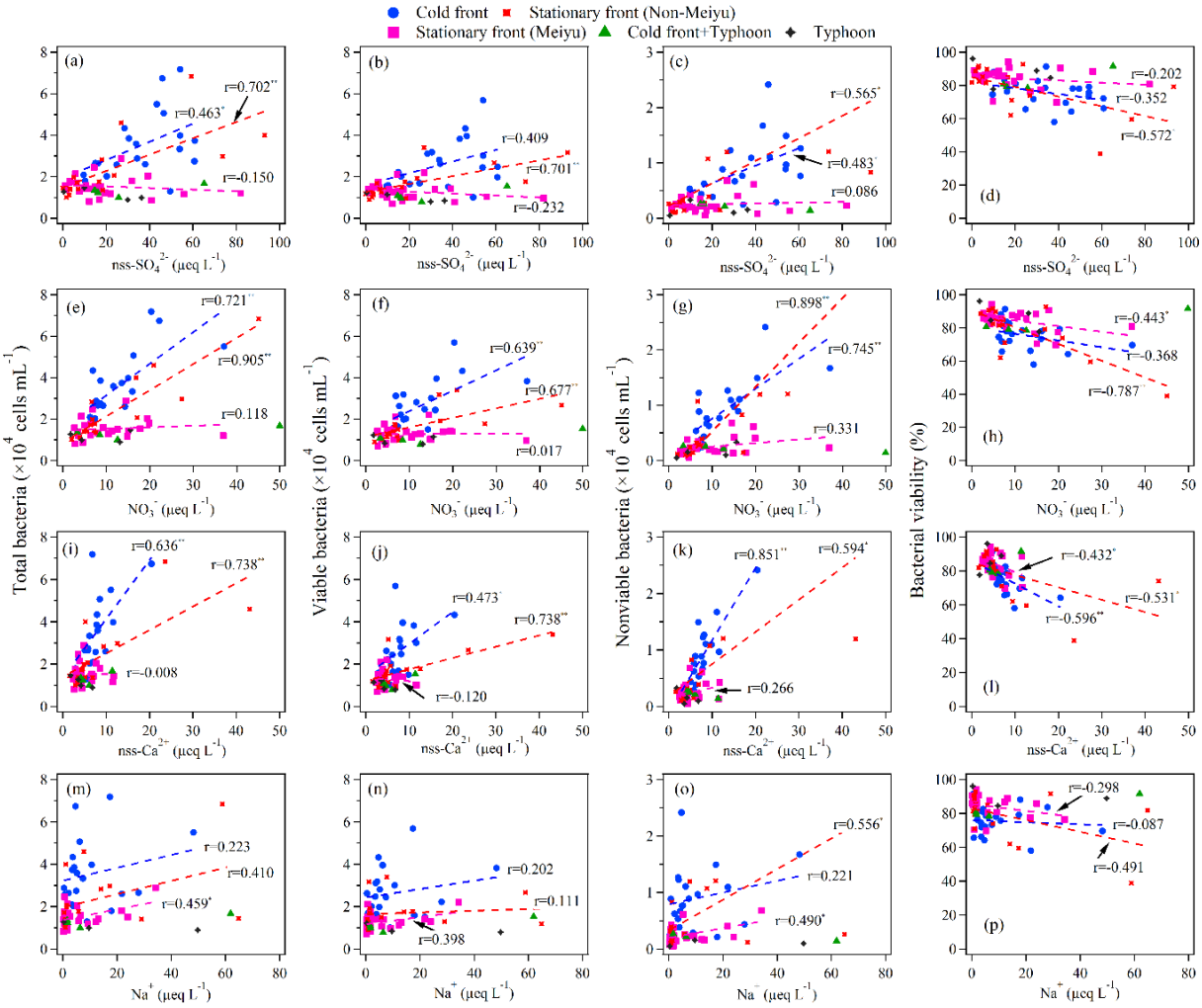

Figure 3-4. Correlations between bacterial abundance and viability with ionic species nss-SO_4^{2-} (a–d), NO_3^- (e–h), nss-Ca^{2+} (i–l) and Na^+ (m–p), according to cloud-forming synoptic systems: cold front (blue marker), stationary front during the Meiyu (violet) and non-Meiyu (red) periods, cold front and typhoon (green), and typhoon only (black). The linear fitting lines are illustrated with the same colors for the former three types. The correlation coefficients are also marked (** p<0.01; * p<0.05).

Microbial activity in agricultural and natural soils is a source of NH_3 in the atmosphere, where it is subsequently converted to NH_4^+. Nevertheless, bacterial abundance showed an insignificant/weak relationship with NH_4^+ in the rainwater in this study, implying that the contribution of agricultural and natural soils to the bacteria in the rainwater was likely insignificant. Christner et al. (2008a) found that the cell concentration negatively correlated with NH_4^+ and organics in Montana snowfall, plausibly due to heterotrophic growth via the assimilation of carbon and nitrogen, although the physiological status and metabolic capabilities of microbes were unknown.

The sea-air interactions can release abundant bacteria into the atmosphere (Blanchard, 1989). Airborne marine bacteria can be preferentially involved in atmospheric waters (e.g., cloud droplets and

rainwater) under certain conditions, and can also be transported to inland and washed out by rain (Ahern et al., 2007; Amato et al., 2007d; Cho and Jang, 2014; DeLeon-Rodriguez et al., 2013). Global model simulations and measurements of marine organic aerosols suggested that marine biogenic material may be an important source of atmospheric ice-nucleating particles (Wilson et al., 2015). Amato et al. (2007d) suggested that ocean represented a major source of microbes in cloud droplets at the Puy de Dôme summit because the concentrations of total and cultivable cells correlated well with the Na^+ content. In this study, poor correlations between bacterial abundance and the ion species from marine sources implied that the ocean was not a primary source of bacteria in rainwater due to diverse cloud formation manners. As mentioned above, marine bacteria might be disseminated via cloud or rain in the cases of stationary fronts and typhoons. In the rainwater of stationary fronts, the concentrations of total and nonviable bacteria showed better correlations with the Na^+ content than in the rainwater of cold fronts (Fig. 3-4 (m, o)).

Airborne bacteria, as a part of airborne particles, whose concentration is often highly correlated with atmospheric particle concentrations (Hara and Zhang, 2012; Murata and Zhang, 2014, 2016), can be affected by the same factors that influence airborne particles. The scavenging process of rainfall can strongly reduce airborne particle concentrations (Hu et al., 2005; Ouyang et al., 2015). Murata and Zhang (2016) reported that airborne bacterial concentration varied corresponding to the increase and decrease of coarse and fine particles before and after cold front-induced rain, although the variation was insignificant. Therefore, the below-cloud removal of bacteria by rain droplets may contribute to bacteria in rainwater.

In addition to the removal of airborne bacteria, some bacteria in the rainwater might be from in-cloud processes, e.g., bacteria acting as nuclei in clouds. Either gaseous nitrogen species other than HNO_3 can dissolve and contribute to nitrate in clouds, or substantial amounts nitrate and sulfate can be produced within cloud droplets (Hegg and Hobbs, 1981; Hegg et al., 1984). Highly hygroscopic $Ca(NO_3)_2$ particles can potentially act as efficient CCN/IN (Christner et al., 2008a; Laskin et al., 2005; Zhang et al., 2007). Christner et al. (2008a) found that the elevated microbial cell abundance correlated well with high dust content in ice cores. There was a positive correlation between the cell and biological IN concentrations at −5°C and Ca^{2+} in precipitation at diverse locations worldwide (Christner et al., 2008a). It was found that mineral dust and biological particles comprised most of the ice-crystal residues, and that biological particles can enhance the impact of dust particles on cloud ice formation (Creamean et al., 2013; Pratt et al., 2009). Creamean et al. (2013) and Cho and Jang (2014) suggest that the majority of microorganisms in precipitation probably originate from the clouds rather than being scavenged by precipitation below clouds. As mentioned above, the variations of bacterial abundance in continuous rainwater samples and the weak correlations between rainwater bacteria and airborne particles also support this inference. In this

study, the positive correlations between the bacterial concentration and ionic species nss-SO_4^{2-}, NO_3^-, and nss-Ca^{2+}, resolved by the correlation analysis and PCA, further indicate that bacteria involved in in-cloud processes, e.g., bacteria as nuclei in clouds, were more likely the major source of bacteria in rainwater.

3.3.3 Variations of bacterial abundance in atmospheric waters

The concentration of bacteria in atmospheric waters, including rain and cloud water, determined by direct enumeration at various sites over the world are summarized in Table S1-2. Data on bacteria in rainwater, especially those concerning their variations dependent on the synoptic weather of cloud formation, are largely lacking. The results of this study suggest that the classification of weather patterns will be beneficial to the model simulation of bacterial abundance in rainwater, and give hints about the roles that bacteria play in cloud and precipitation processes, and the assessment of the environmental, ecological and climatic effects of bacteria in rainwater, although difficulties remain.

It has been confirmed that the concentration of eukaryotic cells (e.g., fungi and yeasts) was one order of magnitude lower than that of bacteria in cloud water (Delort et al., 2010), suggesting that biological micro-particles in atmospheric waters mainly consisted of bacteria. In general, the total bacterial concentration in rainwater (10^3–10^4 cells mL^{-1}) collected at the majority of sites was about one order of magnitude smaller than those in cloud water (10^4 to 10^5 cells mL^{-1}). The average bacterial concentration at the Kumamoto site was comparable to the reported results of four sites in Japan (Casareto et al., 1996; Natsume and Suzuki, 2001) and an urban site in Charlottesville (Herlihy et al., 1987). These values were much higher than those in precipitation (e.g., rain, snow, and graupel) samples collected at high altitudes, and mid- and high-latitude locations, and also in rainwater collected during heavy rain events at a suburban site in Seoul, Korea (Table S1-2).

3.3.4 Survival of atmospheric bacteria

The negative correlations between bacterial viability and the ionic species H_3O^+, NO_3^-, nss-SO_4^{2-}, NH_4^+, and nss-Ca^{2+} in the rainwater samples (Table 3-1) indicate that anthropogenic pollutants and terrestrial emissions might have inhibited the liveness of bacteria in rainwater. Despite of different rain types, high concentrations of ionic species potentially resulted in a lower viability of bacteria (Fig. 3-4 (d, h, l, p)). The bacterial viability correlated negatively with total bacterial abundance in this study, while microbial activity in airborne aerosols in a coastal region showed no statistical correlation with total microbial quantity (Zhong et al., 2016). We are not sure what are the reasons for the difference, because the media (bioaerosols and rainwater) and measuring methods (fluorescein diacetate hydrolysis method and LIVE/DEAD bacterial viability assay) are different in two studies.

Dust particles transported through the atmosphere are likely to harbor assemblages of attached microbes (Christner et al., 2008a). Given the adhesion of bacteria with dust particles and both them were enclosed into in-cloud processes, the positive correlation between bacterial abundance and nss-Ca^{2+} suggests that the growth of bacterial community in the clouds and rainwater was likely quite limited. In East Asia, the residence time of cloud is usually less than two days, which is likely not long enough for bacteria to grow substantially. However, this inference is limited to the weather and environmental conditions in this study. It was observed that bacteria have the capacity to have metabolic activities and grow in cloud water at low temperatures (0–5°C) close to the natural atmospheric environment (Amato et al., 2005, 2007a; Sattler et al., 2001). In addition, there might be some other processes, such as evaporation, bubbling and scavenging, leading to the communication between bacteria in cloud water and in the air. Whether and how these processes influence bacteria in cloud water and rainwater are not clear.

A few researches reported the viability of bacteria in atmospheric waters (Table 3-3). Bauer et al. (2002) reported that about 87%, 72%, and 95% of the bacterial cells in one snow and two cloud water samples collected at Mt. Rax (1644 m a.s.l., Austria) in March 2000 were viable, using the LIVE/DEAD-staining method. In cloud water collected over the upper US Midwest in 2005, 76% of the bacteria (4.3×10^5 cells mL^{-1}) were metabolically active, as measured by the reduction of a tetrazolium dye (Kourtev et al., 2011). These values were consistent with the result that the majority of bacteria in rainwater were viable in this study. The measurements of the ATP content also revealed that a large fraction of bacteria present in cloud and rainwater were likely viable, although only a very small fraction (typically less than 1%) of the bacteria could be recovered by culture (Amato et al., 2007d; Cho and Jang, 2014; Vaïtilingom et al., 2012).

Compared to previous studies conducted at the same site (Table 3-3), the viability of bacteria in rainwater was comparable to or higher than that in the air, especially quite higher than that in the dusty air or air parcels associated with Asian continent outflows. Atmospheric bacteria have to withstand stressors, such as UV radiation, extreme temperatures, low moisture levels, and oxygen limitations, to maintain their viability (Amato, 2012). A larger fraction of airborne bacteria could not remain viable during their long-range transports due to the exposure to the stressors (Hara and Zhang, 2012; Murata and Zhang, 2014). However, cloud water and rainwater can protect airborne bacterial cells against desiccation, and also contain organic compounds and other elements (e.g., phosphorus, iron, copper, and magnesium) that can act as nutrients and sustain metabolism for bacteria (Amato, 2012). Other factors, such as the source environment and resistance ability of bacteria, and the physical (e.g., pH and osmotic pressure) and chemical properties of atmospheric waters, can also affect the viability of bacteria in the atmosphere (Maki et al., 2008).

Table 3-3 Viability of bacteria in atmospheric waters and in air as aerosols.

Site	Period	Sample type		Viability (%)	Method	Reference
Kumamoto (Japan)	Oct. 2014–Sept. 2015	Rainwater		80±10 (39–96)	EFM (LIVE/DEAD)	This study
	Oct. 2011–Apr. 2013	Aerosol		71±14 (44–100)		Murata and Zhang, 2014
	Feb. –Apr. 2010	Aerosol	Dust	25±8 (16–40)		Hara and Zhang, 2012
			Dust-end	45±22 (19–83)		
			Non-dust	82±4 (76–91)		
Upper troposphere of the Caribbean Sea and the mid-western Atlantic Ocean (US)	Aug. –Sep. 2010	Aerosol	Hurricane	90±17 (60–100)		DeLeon-Rodriguez et al., 2013
			Post-hurricane	81		
Mt. Rax (Austria) 1644 m a.s.l.	Mar. 2000	Snow		87		Bauer et al., 2002
		Cloud water		72, 95		
Northern Michigan (US)	Jun. –Aug. 2005	Cloud water		76±12	EFM (CTC/AO)	Hill et al., 2007
Puy de Dôme summit (France) 1464 m a.s.l.	Jun. 2004–Oct. 2005 Apr. 2008–Sept. 2010	Cloud water		0.25±0.28 (0.00–0.90)	R2A cultivation (15−17°C)/ EFM (DAPI)	Vaïtilingom et al., 2012

Note: CTC, 5-cyano-2, 3-ditolyl tetrazolium chloride.

3.4 Summary

The bacterial concentration and viability in rainwater collected at a suburban site, Kumamoto, in southwestern Japan during the sampling periods from October 2014 to September 2015 were investigated in this study. Several major findings are listed as follows.

(1) There was a dependence of bacterial concentration and viability on the synoptic weather of cloud formation. In the cold front rain, the average bacterial concentration was the highest, and the viability was the lowest. In the Meiyu front rain and rain influenced by typhoons, the bacterial viability was the highest (84% and 87%).

(2) Bacterial concentration positively correlated, and bacterial viability negatively correlated with nss-Ca^{2+}, NO_3^-, nss-SO_4^{2-}, and H_3O^+ in rainwater. Anthropogenic pollutants and terrestrial emissions might result in high concentration and low viability of bacteria in rainwater.

(3) The average bacterial concentration in rainwater in Kumamoto was much higher than those in the precipitation collected at high altitude and mid- and high-latitude locations. Compared with airborne bacteria, higher viability of bacteria in rainwater implied that the environmental conditions in cloud and rainwater probably favor their survival.

(4) Bacteria involved in in-cloud processes were probably an important source of bacteria in rainwater. Growth of bacteria involved in the rainwater during their life in the air was not confirmed.

This chapter provides the fundamental information on the concentration and viability of bacterial cells in the rainwater of cyclones, stationary fronts, and typhoons. However, the mechanisms for the presence of rainwater bacteria dependent on weather patterns are difficult to be clarified. We cannot conclude quantitatively on the actual contributions of anthropogenic, terrestrial and marine sources to bacteria in rainwater, and have no direct evidence for CCN/IN activation ability of bacteria. The information on the survival rates and mechanisms of atmospheric bacteria remains limited. Further bacterial community analysis, extensive observations, as well as atmospheric modelling are required to close these knowledge gaps.

Supporting information of "Bacterial abundance and viability in rainwater associated with cyclones, stationary fronts and typhoons in southwestern Japan"

S3.1 Dependence of bacterial abundance and viability on air parcels associated with the rain

The air mass transport pathways for all the rain events were performed based on air mass back-trajectories produced using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT 4.9) model (http://ready.arl.noaa.gov/HYSPLIT.php). Three-day backward trajectories from the mid-time during the rainwater collection were calculated for air parcels arriving at 500 m above ground level, which was selected as an approximation of the well-mixed boundary layer where precipitation originated (Mullaugh et al., 2013). The obtained back trajectories were clustered into seven mean trajectories according to the path, speed and source of air parcels (Fig. 3-1).

The occurrence of microorganisms in precipitation can be linked to the trajectory of the air parcels associated with the precipitation and the physical conditions (e.g., temperature, solar radiation exposure) of the air parcels (Monteil et al., 2014). The influences of the backward trajectories of the air parcels associated with each precipitation event on the bacterial abundance and viability were investigated in this study. The average bacterial abundance and viability in the rainwater samples collected at Kumamoto site associated with different air-mass trajectory clusters (Fig. 3-1 and Table S3-1) are shown in Table S3-4. The total bacterial concentrations in the rainwater samples decreased in the sequence of Cluster 4, Cluster 3, Cluster 5, Cluster 1, Cluster 2, Cluster 6 and Cluster 7, and those of Cluster 1 and 2, Cluster 6 and 7 were very approximate.

The air parcels of Cluster 4 and Cluster 3 were long-distance transported from North and East Mongolia at high altitude (over 3.5 km), which is a source area of dust particles, with the highest bacterial concentration (4.6×10^4, 3.6×10^4 cells mL^{-1}) but lowest viability (70–74%) in the rainwater. These results were consistent with those in aerosol particles influenced by dust (Hara and Zhang, 2012), the viable bacterial concentrations in dust were comparable to or higher than those in non-dusty air, while the bacterial viability in dust (lower than 40%) was much lower than that in non-dusty air (higher than 76%). Differently, those of Cluster 4 were transported across Korea Peninsula and circled around the Kyushu Island for about one day at low altitude (mostly lower than 500 m), and the highest bacterial abundance may be also resulted from the Kyushu Island surface emissions. Those of Cluster 5 were transported from

East China and crossed the East China Sea. The higher abundance and lower viability in the rainwater associated with these air parcels were more likely influenced by the outflow of anthropogenic emissions from East Asia continent (Murata and Zhang, 2014).

The air parcels of Cluster 1were circled around the Kyushu Island for over 2 days. Those of Cluster 2 were transported from Sea of Japan, across Honshu Island, and arrived at Kyushu Island. Both Cluster 1 and Cluster 2 moved with low speeds and at low altitudes around the islands. The bacterial concentrations were higher (2.1×10^4, 2.1×10^4 cells mL^{-1}), with the viability as 80% and 79%, respectively. The air parcels of Cluster 6 and Cluster 7 were long-distance transported from near surface of East China Sea or North Pacific Ocean, mainly in the marine area. The total bacterial concentrations were the lowest, with both the average as 1.5×10^4 cells mL^{-1}. The low abundance and high viability in the rainwater associated with these air parcels were probably influenced by transported marine air parcels too.

These results indicated that the occurrence of bacteria in precipitation also affected by the trajectory of the air parcels associated with the precipitation, and the abundance was consistent to that in the air to some extent.

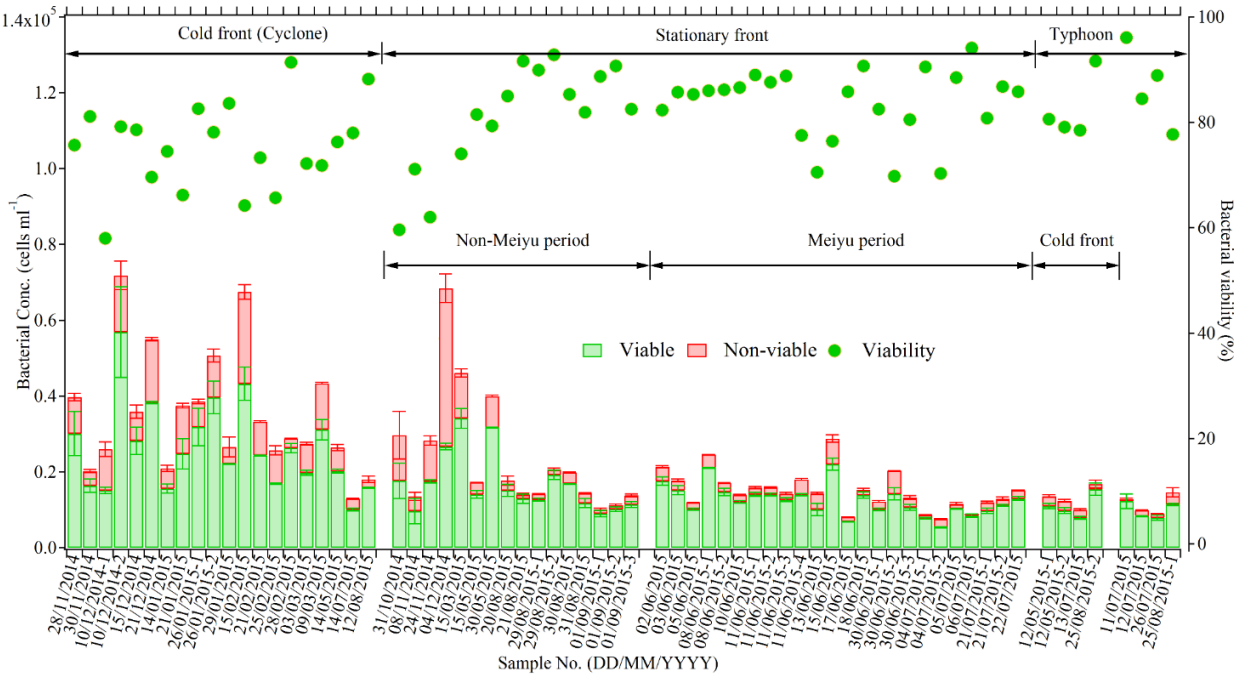

Figure S3-1. Viable and non-viable bacterial concentrations in the rainwater samples dependent on the manners of cloud formation.

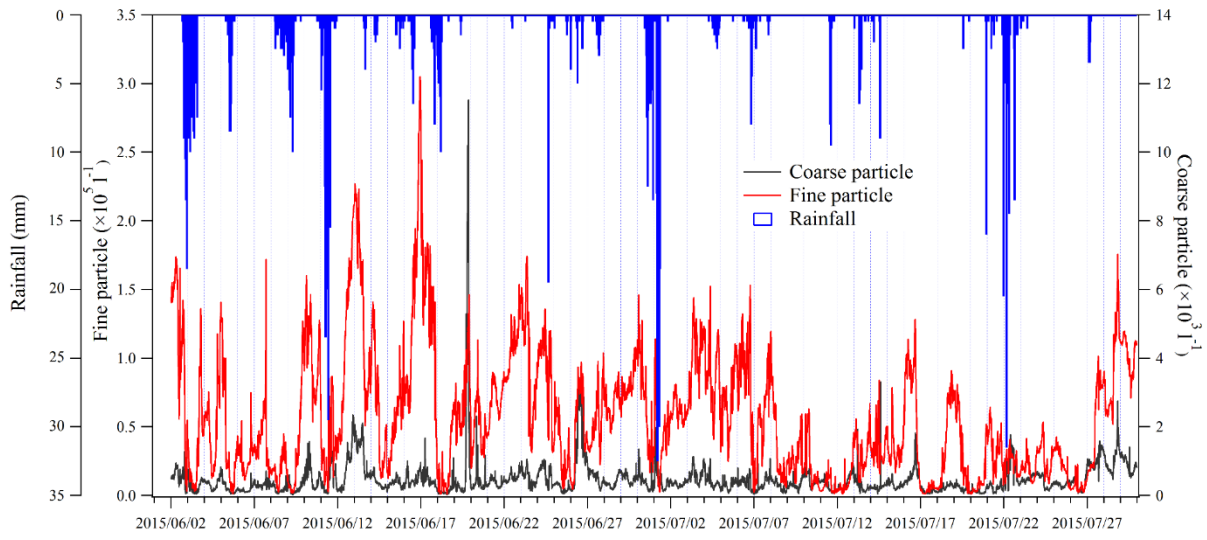

Figure S3-2. Airborne particle number concentrations and rainfall during the Meiyu season.

Table S3-1 Sampling information, abundance and viability of bacteria in rainwater samples, and pH, electrical conductivity (EC) and ionic components of rainwater samples. Meteorological conditions (precipitation amount, average air temperature (T), relative humidity (RH), pressure (P) and wind speed (WS)) and airborne particle number concentrations during samplings are also listed.

Sample No.	Sampling period			Volume 1 (mL)	Volume 2 (mL)	Avg. conc. (cells mL^{-1})			Viability (%)	Std. (cells mL^{-1})		pH	EC (μS cm^{-1})	Ionic species (μeq L^{-1})											Meteorological parameters					Cloud manner	Airborne particle number conc. (Before raining/sampling)		Airborne particle number conc. (During raining/sampling)	
	Start	End	Duration (day)			Viable	Non-viable	Total		Viable	Non-viable			Cl^-	NO_3^-	SO_4^{2-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	H_3O^+	nss-SO_4^{2-}	nss-Ca^{2+}	Precipitation (mm)	Pressure (hPa)	Temperature (℃)	RH (%)	Wind speed (m s^{-1})		Fine (L^{-1})	Coarse (L^{-1})	Fine (L^{-1})	Coarse (L^{-1})
28/11/2014	2014/11/28 20:00	2014/11/29 5:30	0.40	55.0	60.0	3.0E+04	9.7E+03	4.0E+04	76	5.8E+03	9.3E+02	4.3	26.0	17.2	15.0	55.3	10.6	28.6	0.7	3.0	12.1	50.1	54.1	11.6	3.5	1011.8	18.4	85.8	1.9	C	4.8E+04	3.9E+02	1.4E+04	1.1E+02
30/11/2014	2014/11/30 10:30	2014/11/30 22:30	0.50	800.0	800.0	1.6E+04	3.8E+03	2.0E+04	81	1.8E+03	4.7E+02	4.7	10.0	5.3	7.4	20.7	3.9	9.4	0.2	0.9	6.4	20.0	20.2	6.2	23.5	1004.9	14.2	93.6	1.7	C	7.3E+04	6.4E+02	3.1E+03	2.7E+01
10/12/2014-1	2014/12/10 18:30	2014/12/10 21:00	0.10	115.0	110.0	1.5E+04	1.1E+04	2.6E+04	58	8.6E+02	1.9E+03	5.1	14.0	25.0	14.3	40.6	21.9	50.9	0.7	4.9	10.8	7.9	38.0	9.9	3.0	1017.2	10.6	92.1	1.3	C	1.0E+05	9.1E+02	3.4E+04	3.0E+02
10/12/2014-2	2014/12/10 22:00	2014/12/11 9:50	0.49	310.0	307.0	5.7E+04	1.5E+04	7.2E+04	79	1.2E+04	3.8E+03	4.3	30.0	22.5	20.3	56.2	17.3	24.8	0.8	3.9	7.6	50.1	54.1	6.8	8.5	1014.8	10.4	94.3	1.5	C	1.9E+05	1.1E+03	2.2E+04	1.3E+02
15/12/2014	2014/12/15 22:40	2014/12/16 11:20	0.53	528.0	523.0	2.8E+04	7.7E+03	3.6E+04	79	3.6E+03	1.7E+03	4.4	18.0	10.3	11.6	34.5	5.2	12.7	0.4	1.5	8.2	39.8	33.8	8.0	16.0	1011.7	6.4	91.9	2.2	C	9.3E+04	7.8E+02	5.8E+03	4.9E+01
21/12/2014	2014/12/21 19:35	2014/12/22 5:00	0.39	60.0	62.0	3.8E+04	1.7E+04	5.5E+04	70	3.0E+02	4.7E+02	4.3	30.0	52.1	37.0	49.1	48.2	35.9	1.7	11.6	13.2	50.1	43.3	11.1	1.5	1016.8	3.2	86.8	1.5	C	3.2E+04	2.2E+02	2.1E+04	1.5E+02
14/01/2015	2015/1/14 15:10	2015/1/15 9:00	0.74	395.0	380.0	1.6E+04	5.3E+03	2.1E+04	75	1.2E+03	8.6E+02	5.1	5.0	4.6	6.2	9.9	3.4	11.5	0.3	1.0	7.1	7.9	9.5	6.9	15.0	1009.7	10.1	84.6	3.3	C	9.4E+04	1.2E+03	6.3E+03	8.2E+01
21/01/2015	2015/1/21 14:20	2015/1/22 9:30	0.80	370.0		2.5E+04	1.3E+04	3.7E+04	66	4.0E+03	6.2E+02	4.3	28.0	8.7	13.5	61.2	3.5	26.0	0.6	1.1	8.3	50.1	60.8	8.1	11.5	1010.5	8.8	97.4	1.7	C	2.1E+05	1.7E+03	1.8E+04	1.5E+02
26/01/2015-1	2015/1/26 1:00	2015/1/26 10:00	0.38	720.0		3.2E+04	6.7E+03	3.9E+04	83	4.9E+03	6.2E+02	4.3	23.0	8.2	8.6	31.0	4.2	11.6	0.3	1.0	7.9	50.1	30.5	7.7	23.5	1016.0	9.6	96.2	1.4	C	1.4E+05	6.9E+02	6.0E+03	3.0E+01
26/01/2015-2	2015/1/26 10:00	2015/1/26 20:00	0.42	320.0		4.0E+04	1.1E+04	5.1E+04	78	4.3E+03	1.7E+03	4.5	15.0	7.3	16.2	47.3	6.2	24.0	1.2	1.7	8.8	31.6	46.6	8.5	14.0	1012.4	12.3	94.9	1.7	C	1.1E+05	4.0E+02	7.9E+03	2.9E+01
29/01/2015	2015/1/29 23:00	2015/1/30 10:00	0.46	445.0		2.2E+04	4.4E+03	2.7E+04	84	0.0E+00	2.6E+03	4.7	8.0	23.6	8.1	18.4	28.0	11.2	0.9	0.2	6.3	18.6	15.0	5.1	14.0	1014.4	5.9	97.1	1.1	C	7.9E+04	3.5E+02	5.7E+03	2.5E+01
15/02/2015	2015/2/15 23:40	2015/2/16 9:00	0.39	125.0		4.3E+04	2.4E+04	6.7E+04	64	4.4E+03	1.9E+03	4.5	19.0	9.8	22.2	46.4	4.7	39.0	0.8	3.2	20.7	29.5	45.9	20.5	3.5	1012.2	8.4	94.1	1.5	C	1.4E+05	1.2E+03	3.9E+04	3.4E+02
21/02/2015	2015/2/21 20:00	2015/2/22 18:00	0.92	118.0		2.4E+04	8.9E+03	3.3E+04	73	0.0E+00	2.3E+02	4.3	21.0	9.3	16.0	54.8	7.4	42.9	0.7	2.0	6.5	46.7	53.9	6.1	3.0	1011.9	12.0	96.6	2.0	C	5.4E+04	4.4E+02	1.8E+04	1.5E+02
25/02/2015	2015/2/25 19:40	2015/2/26 15:00	0.81	780.0		1.7E+04	8.9E+03	2.6E+04	66	1.6E+02	1.2E+03	4.7	8.0	1.4	7.0	24.9	0.7	18.1	0.2	0.6	7.6	18.6	24.8	7.5	22.0	1011.8	8.3	97.4	1.1	C	8.4E+04	2.0E+03	3.8E+03	9.0E+01
28/02/2015	2015/2/28 21:15	2015/3/1 9:30	0.51	780.0		2.6E+04	2.5E+03	2.9E+04	91	1.2E+03	7.8E+01	4.3	16.0	2.7	7.7	34.4	0.4	12.3	0.4	0.3	4.7	50.1	34.3	4.7	19.0	1009.3	8.7	96.3	2.4	C	8.6E+04	7.4E+02	4.5E+03	3.9E+01
03/03/2015	2015/3/3 10:50	2015/3/3 21:00	0.42	510.0		2.0E+04	7.6E+03	2.7E+04	72	6.2E+02	3.9E+02	4.4	28.0	13.6	8.7	61.4	5.0	22.7	0.4	1.3	6.0	39.8	60.8	5.8	13.5	1004.5	8.3	95.9	2.0	C	1.1E+05	8.6E+02	7.9E+03	6.4E+01
09/03/2015	2015/3/9 0:20	2015/3/9 12:00	0.49	920.0		3.1E+04	1.2E+04	4.3E+04	72	2.7E+03	2.3E+02	4.5	11.0	5.5	6.9	28.9	3.6	13.0	0.3	1.7	8.1	29.5	28.4	7.9	27.0	1009.5	9.6	90.2	2.6	C	7.2E+04	7.4E+02	2.7E+03	2.7E+01
14/05/2015	2015/5/14 21:00	2015/5/15 9:00	0.50	145.0		2.0E+04	6.3E+03	2.6E+04	76	5.4E+02	8.6E+02	4.9	10.0	2.5	9.3	16.5	2.2	19.4	0.5	1.1	5.9	11.7	16.3	5.8	7.5	1007.7	21.7	100.0	1.3	C	1.1E+05	8.8E+02	1.5E+04	1.2E+02
14/07/2015	2015/7/14 11:30	2015/7/14 13:30	0.08	155.0		1.0E+04	2.9E+03	1.3E+04	78	3.9E+02	7.8E+01	4.3	26.3	15.0	8.0	50.6	8.9	16.7	0.4	2.1	6.2	50.1	49.6	5.8	9.5	1002.7	24.5	93.7	2.5	C	4.2E+04	1.6E+02	4.4E+03	1.7E+01
12/08/2015	2015/8/12 9:05	2015/8/12 23:30	0.60	1080.0		1.6E+04	2.1E+03	1.8E+04	88	7.8E+01	8.6E+02	4.9	9.7	18.8	6.8	12.4	17.9	10.8	0.5	4.0	3.7	13.7	10.3	2.9	55.0	999.2	25.3	93.7	2.9	C				
31/10/2014	2014/10/31 15:26	2014/11/1 18:00	1.11	50.0	50.0	1.8E+04	1.2E+04	3.0E+04	60	4.7E+03	6.3E+03	4.3	33.0	25.5	27.3	75.7	17.3	53.1	0.9	4.4	13.3	54.4	73.7	12.6	5.0	1010.0	19.1	90.9	1.9	S	8.6E+04	5.6E+02	1.7E+04	1.1E+02
08/11/2014	2014/11/8 19:30	2014/11/9 10:30	0.63	200.0	200.0	9.6E+03	3.9E+03	1.4E+04	71	3.3E+03	1.1E+03	4.8	8.0	1.8	7.6	18.5	1.0	9.4	0.2	0.3	6.9	15.8	18.4	6.9	19.0	1016.2	15.8	91.5	1.3	S	2.9E+05	1.8E+03	1.5E+04	9.5E+01
24/11/2014	2014/11/24 18:15	2014/11/25 11:00	0.70	160.0	160.0	1.8E+04	1.1E+04	2.8E+04	62	4.5E+02	1.2E+03	4.8	10.0	15.3	6.6	19.7	14.1	10.5	0.4	3.2	10.0	17.2	18.0	9.4	10.0	1007.7	17.8	87.8	1.8	S	1.4E+05	4.8E+02	1.4E+04	4.8E+01
04/12/2014	2014/12/4 9:00	2014/12/4 23:00	0.58	38.0	39.0	2.7E+04	4.2E+04	6.8E+04	39	9.0E+02	3.8E+03	4.3	36.0	61.8	45.0	66.4	58.9	41.1	2.4	15.5	26.1	50.1	59.3	23.6	2.0	1013.0	6.7	79.3	2.0	S	1.2E+04	4.5E+01	5.9E+03	2.3E+01
15/03/2015	2015/3/15 2:00	2015/3/15 12:00	0.42	300.0		3.4E+04	1.2E+04	4.6E+04	74	2.6E+03	1.1E+03	6.1	12.0	7.3	20.9	27.8	7.7	37.1	1.1	4.6	43.4	0.9	26.9	43.1	8.0	1018.3	8.9	92.8	1.6	S	1.1E+05	7.0E+02	1.3E+04	8.8E+01
15/05/2015	2015/5/15 21:00	2015/5/16 8:40	0.49	450.0		1.4E+04	3.2E+03	1.7E+04	82	1.0E+03	0.0E+00	5.1	4.7	0.5	6.3	6.5	0.6	10.5	0.3	0.3	4.3	8.6	6.5	4.3	31.5	1002.5	21.0	99.0	2.1	S	7.1E+04	5.9E+02	2.3E+03	1.9E+01
30/05/2015	2015/5/30 14:25	2015/5/31 9:00	0.77	295.0		3.2E+04	8.3E+03	4.0E+04	79	1.6E+02	2.3E+02	4.1	44.7	3.2	16.8	93.3	1.1	38.0	0.7	0.5	5.3	86.3	93.1	5.2	9.0	997.6	20.8	99.5	1.4	S	2.1E+05	1.3E+03	2.3E+04	1.4E+02
20/08/2015	2015/8/20 22:30	2015/8/21 1:00	0.10	47.5		1.5E+04	2.7E+03	1.8E+04	85	1.6E+03	1.2E+03	4.8	9.7	6.9	7.3	17.3	5.8	10.8	0.4	1.7	4.8	15.8	16.6	4.6	3.0	1004.6	25.4	92.6	1.7	S				
21/08/2015	2015/8/21 21:10	2015/8/22 0:00	0.12	215.0		1.3E+04	1.2E+03	1.4E+04	92	1.2E+03	3.1E+02	5.3	5.0	30.5	4.8	6.7	29.0	6.1	0.7	6.9	4.7	5.0	3.2	3.4	6.5	1000.7	26.2	92.8	2.5	S				
29/08/2015-1	2015/8/29 11:10	2015/8/29 21:45	0.44	1080.0		1.3E+04	1.4E+03	1.4E+04	90	3.9E+02	1.6E+02	5.3	3.0	0.7	5.5	7.1	1.0	12.2	0.1	0.3	3.5	5.4	7.0	3.5	33.0	1003.4	23.6	89.6	1.9	S	1.2E+05	7.3E+02	3.8E+03	2.2E+01
29/08/2015-2	2015/8/29 21:45	2015/8/30 7:30	0.41	87.0		1.9E+04	1.5E+03	2.1E+04	93	1.2E+03	3.1E+02	4.5	15.0	2.1	17.1	23.8	1.4	18.6	0.4	1.2	5.7	31.6	23.6	5.7	1.5	1004.9	22.5	97.8	1.4	S	3.9E+04	1.7E+02	2.6E+04	1.2E+02
30/08/2015	2015/8/30 23:00	2015/8/31 10:10	0.47	250.0		1.7E+04	2.9E+03	2.0E+04	85	7.8E+01	1.6E+02	5.6	4.0	2.3	6.8	5.1	1.4	19.0	0.2	1.1	4.9	2.3	5.0	4.9	1.5	1006.8	22.8	96.0	1.3	S	1.2E+05	4.7E+02	8.0E+04	3.1E+02
31/08/2015	2015/8/31 10:10	2015/9/1 0:25	0.59	233.0		1.2E+04	2.6E+03	1.4E+04	82	1.2E+03	1.6E+02	5.6	12.0	56.4	7.2	7.1	64.8	22.6	2.4	0.7	4.4	2.5	0.0	1.6	4.0	1005.9	24.7	93.4	1.5	S	3.5E+04	3.6E+02	8.8E+03	9.1E+01
01/09/2015-1	2015/9/1 0:25	2015/9/1 11:40	0.47	1080.0		9.0E+03	1.2E+03	1.0E+04	89	8.6E+02	2.3E+02	5.4	2.0	0.5	2.0	1.7	0.9	4.2	0.1	0.3	2.9	3.7	1.6	2.9	35.5	1005.5	23.3	96.8	1.6	S	2.1E+04	1.5E+02	6.0E+02	4.3E+00
01/09/2015-2	2015/9/1 12:40	2015/9/1 14:40	0.08	1000.0		1.0E+04	1.0E+03	1.1E+04	91	6.2E+02	2.3E+02	5.2	3.0	0.3	3.9	3.0	0.7	4.3	0.1	0.2	3.4	5.9	2.9	3.3	28.5	1005.0	22.7	95.8	2.2	S	5.5E+03	1.1E+02	1.9E+02	4.0E+00
01/09/2015-3	2015/9/1 14:40	2015/9/1 16:00	0.06	63.0		1.1E+04	2.4E+03	1.4E+04	82	7.8E+02	3.9E+02	5.2	3.0	1.1	4.9	3.6	1.1	4.7	0.5	0.6	5.2	6.3	3.4	5.1	0.5	1004.7	22.8	96.8	1.2	S	5.2E+03	1.7E+02	1.0E+04	3.4E+02
02/06/2015	2015/6/2 16:40	2015/6/3 10:15	0.73	1080.0		1.8E+04	3.8E+03	2.1E+04	82	1.1E+03	2.3E+02	5.0	5.0	1.6	4.2	6.0	1.7	6.6	0.2	0.4	4.0	9.3	5.8	3.9	128.5	1000.9	22.8	100.0	3.6	S	1.0E+05	4.9E+02	8.0E+02	3.8E+00
03/06/2015	2015/6/3 10:15	2015/6/3 14:20	0.17	495.0		1.5E+04	2.6E+03	1.8E+04	86	1.2E+03	3.9E+02	5.1	4.0	0.3	6.4	3.4	0.6	7.5	0.1	0.1	2.9	8.6	3.3	2.9	12.5	999.4	22.1	97.5	2.3	S	6.1E+03	9.1E+01	4.9E+02	7.3E+00
05/06/2015	2015/6/5 8:15	2015/6/5 16:00	0.32	980.0		1.0E+04	1.8E+03	1.2E+04	85	2.3E+02	0.0E+00	4.6	10.3	0.5	3.6	22.1	0.4	7.0	0.1	0.2	3.2	25.1	22.0	3.2	32.0	998.0	16.8	95.1	3.1	S	6.5E+04	2.9E+02	2.0E+03	9.0E+00
08/06/2015-1	2015/6/8 8:50	2015/6/8 22:00	0.55	740.0		2.1E+04	3.5E+03	2.5E+04	86	1.6E+02	7.8E+01	4.8	8.0	0.8	6.8	14.6	0.7	13.9	0.2	0.3	3.6	17.5	14.5	3.5	19.5	1002.0	21.0	98.6	1.5	S	1.3E+04	1.3E+02	6.6E+02	6.4E+00
08/06/2015-2	2015/6/8 22:00	2015/6/9 12:30	0.60	1080.0		1.5E+04	2.4E+03	1.7E+04	86	9.3E+02	7.8E+01	5.1	5.0	1.9	4.9	6.5	2.0	8.4	0.2	0.5	3.6	8.6	6.3	3.5	41.5	999.9	22.1	98.3	2.4	S	3.0E+04	2.1E+02	7.3E+02	5.1E+00
10/06/2015	2015/6/10 18:40	2015/6/11 7:30	0.53	955.0		1.2E+04	1.9E+03	1.4E+04	87	3.1E+02	1.6E+02	5.3	2.0	0.2	3.4	1.6	0.3	5.1	0.0	0.1	3.4	4.7	1.5	3.4	69.5	1002.7	21.4	99.8	1.4	S	7.3E+04	2.3E+02	1.0E+03	3.3E+00
11/06/2015-1	2015/6/11 7:30	2015/6/11 9:30	0.08	620.0		1.4E+04	1.8E+03	1.6E+04	89	5.4E+02	3.1E+02	5.4	2.0	0.2	2.7	1.2	0.4	3.5	0.1	0.1	3.0	4.0	1.2	3.0	48.0	1000.9	22.7	100.0	2.9	S	1.4E+04	1.2E+02	2.9E+02	2.4E+00
11/06/2015-2	2015/6/11 9:30	2015/6/11 12:30	0.13	430.0		1.4E+04	2.0E+03	1.6E+04	88	3.9E+02	1.6E+02	4.8	10.3	6.3	9.3	11.4	6.6	9.5	0.4	1.1	4.1	17.2	10.6	3.8	43.5	1000.5	23.6	100.0	3.3	S	1.7E+04	1.0E+02	4.0E+02	2.3E+00
11/06/2015-3	2015/6/11 12:30	2015/6/11 15:30	0.13	40.0		1.3E+04	1.6E+03	1.4E+04	89	5.4E+02	3.1E+02	4.7	12.0	13.5	11.3	14.0	13.1	10.8	0.5	3.0	7.6	20.0	12.4	7.0	1.5	1000.5	23.9	99.2	4.1	S	3.5E+04	5.2E+02	2.4E+04	3.5E+02
11/06/2015-4	2015/6/11 15:30	2015/6/11 23:00	0.31	25.0		1.4E+04	4.1E+03	1.8E+04	78	3.1E+02	2.3E+02	4.6	20.0	23.9	19.9	34.5	21.6	37.5	1.2	5.5	9.5	27.3	31.9	8.6	3.5	1000.5	23.6	99.7	2.7	S	4.2E+04	1.0E+03	1.2E+04	3.0E+02
13/06/2015	2015/6/13 15:40	2015/6/14 10:00	0.76	79.0		1.0E+04	4.2E+03	1.4E+04	70	1.6E+03	3.1E+02	5.2	12.0	3.0	15.0	9.9	0.9	22.4	0.3	1.3	11.8	6.3	9.8	11.7	7.0	1005.5	19.3	95.2	1.5	S	1.0E+05	1.1E+03	1.5E+04	1.6E+02
15/06/2015	2015/6/15 16:30	2015/6/16 18:30	1.08	153.0		2.2E+04	6.8E+03	2.9E+04	76	1.6E+03	9.3E+02	4.6	31.0	31.0	14.5	31.1	34.3	28.0	1.6	0.6	6.3	27.3	26.9	4.8	23.0	1002.4	20.8	98.1	1.3	S	6.6E+04	4.7E+02	2.9E+03	2.0E+01
17/06/2015	2015/6/17 18:40	2015/6/18 10:20	0.65	1080.0		7.0E+03	1.2E+03	8.1E+03	86	0.0E+00	7.8E+01	5.0	9.0	0.2	2.8	12.1	0.2	7.4	0.1	0.1	2.6	10.0	12.1	2.6	39.5	997.0	20.0	98.5	2.2	S	1.4E+05	3.7E+02	3.5E+03	9.4E+00
18/06/2015	2015/6/18 10:20	2015/6/18 18:35	0.34	65.0		1.4E+04	1.4E+03	1.5E+04	91	8.6E+02	3.9E+02	4.6	15.0	2.6	17.8	17.7	2.7	24.6	0.5	1.0	6.8	23.4	17.4	6.7	0.5	995.7	22.7	88.1	2.5	S	9.4E+03	8.8E+01	1.9E+04	1.8E+02
30/06/2015-1	2015/6/30 10:22	2015/6/30 20:15	0.41	1080.0		1.0E+04	2.1E+03	1.2E+04	83	3.1E+02	2.3E+02	4.6	11.0	1.7	6.4	19.7	1.5	13.5	0.3	0.4	4.3	23.4	19.5	4.3	42.0	994.9	21.7	98.8	1.1	S	7.9E+04	4.1E+02	1.9E+03	9.7E+00
30/06/2015-2	2015/6/30 20:15	2015/6/30 22:10	0.08	57.0		1.4E+04	6.1E+03	2.0E+04	70	1.6E+03	0.0E+00	4.5	18.0	4.8	19.4	39.9	5.3	40.1	1.0	1.6	7.9	31.6	39.2	7.7	19.0	992.7	23.6	100.0	3.0	S	2.8E+04	1.6E+02	1.5E+03	8.6E+00
30/06/2015-3	2015/6/30 22:10	2015/7/1 12:30	0.60	1300.0		1.1E+04	2.6E+03	1.3E+04	81	7.8E+02	5.4E+02	4.6	12.0	5.3	8.0	16.6	5.8	18.2	0.4	1.2	4.0	25.1	15.9	3.7	135.5	991.1	23.6	98.8	5.6	S	3.4E+04	2.6E+02	2.5E+02	1.9E+00
04/07/2015-1	2015/7/4 14:45	2015/7/4 21:20	0.27	345.0		7.8E+03	8.3E+02	8.7E+03	90	2.3E+02	7.8E+01	4.4	18.0	0.9	4.5	40.9	0.3	9.6	0.2	0.2	4.4	43.2	40.9	4.4	10.0	1005.8	19.8	96.7	1.9	S	6.3E+04	2.7E+02	6.3E+03	2.7E+01
04/07/2015-2	2015/7/4 21:20	2015/7/5 22:20	1.04	33.0		5.4E+03	2.3E+03	7.6E+03	70	1.6E+02	7.8E+01														1.0	1005.7	21.2	90.9	1.5	S	1.1E+05	3.9E+02	1.1E+05	3.9E+02
05/07/2015	2015/7/5 22:20	2015/7/6 10:00	0.49	33.0		1.0E+04	1.3E+03	1.2E+04	89	7.8E+01	3.9E+02	5.2	23.3	3.0	14.8	56.0	1.7	74.1	0.9	2.0	11.6	6.9	55.8	11.5	1.0	1005.7	20.6	97.6	1.1	S	1.0E+05	4.3E+02	1.0E+05	4.3E+02
06/07/2015	2015/7/6 14:50	2015/7/7 9:50	0.79	360.0		8.4E+03	5.3E+02	9.0E+03	94	4.7E+02	0.0E+00	5.0	7.0	1.9	4.6	17.0	1.7	17.2	0.3	0.5	4.4	10.0	16.8	4.3	20.0	1004.7	22.3	98.5	1.6	S	9.9E+04	4.3E+02	5.0E+03	2.2E+01
21/07/2015-1	2015/7/21 10:20	2015/7/21 20:30	0.42	115.0		9.7E+03	2.3E+03	1.2E+04	81	7.0E+02	3.1E+02	4.3	38.0	10.8	36.9	83.1	9.2	69.7	1.2	2.3	7.1	50.1	82.0	6.7	0.5	1003.9	26.1	85.3	2.3	S	2.1E+04	1.3E+02	4.2E+04	2.5E+02
21/07/2015-2	2015/7/21 20:30	2015/7/22 9:00	0.52	1080.0		1.1E+04	1.7E+03	1.3E+04	87	2.3E+02	5.4E+02	5.0	7.7	10.2	10.5	9.1	12.2	11.0	0.4	2.5	3.0	10.0	7.7	2.5	85.5	1004.9	24.9	95.0	3.1	S	4.3E+04	3.6E+02	5.1E+02	4.2E+00
22/07/2015	2015/7/22 14:00	2015/7/23 9:00	0.79	116.0		1.3E+04	2.1E+03	1.5E+04	86	5.4E+02	7.8E+01	4.8	12.0	24.6	12.5	17.7	24.0	20.7	0.9	5.4	5.3	15.8	14.8	4.3	24.0	1008.0	24.1	95.2	1.6	S	2.9E+04	1.3E+03	1.2E+03	5.3E+01
12/05/2015-1	2015/5/12 0:00	2015/5/12 9:00	0.38	760.0		1.1E+04	2.6E+03	1.4E+04	81	7.0E+02	3.1E+02	4.8	8.0	1.9	3.3	14.9	1.6	3.8	0.2	0.5	4.2	15.8	14.7	4.2	22.5	1001.8	22.7	72.8	3.0	C+T	4.2E+04	3.2E+02	1.8E+03	1.4E+01
12/05/2015-2	2015/5/12 9:00	2015/5/12 16:30	0.31	570.0		9.8E+03	2.6E+03	1.2E+04	79	7.8E+02	3.9E+02	4.8	8.0	2.3	8.5	16.4	1.8	9.4	0.2	0.7	4.7	15.8	16.2	4.6	12.5	1003.5	15.3	95.5	1.8	C+T	5.3E+03	1.3E+02	4.2E+02	1.0E+01
13/07/2015	2015/7/13 9:45	2015/7/14 9:50	1.00	37.5		7.9E+03	2.2E+03	1.0E+04	78	3.9E+02	2.3E+02	5.2	11.0	9.6	12.6	26.6	6.4	47.8	0.6	1.8	6.4	6.3	25.8	6.1	24.0	1000.5	24.6	94.9	2.3	C+T	1.6E+04	2.6E+02	6.8E+02	1.1E+01
25/08/2015-2	2015/8/25 23:10	2015/8/26 11:00	0.49	28.0		1.5E+04	1.4E+03	1.7E+04	92	1.6E+03	1.0E+03	4.3	45.0	67.2	49.8	72.6	62.0	74.0	2.2	15.2	14.1	50.1	65.1	11.4	3.5	999.5	23.0	94.5	1.5	C+T				
11/07/2015	2015/7/11 12:05	2015/7/11 22:00	0.41	330.0		1.2E+04	5.0E+02	1.3E+04	96	1.9E+03	2.3E+02	5.5	2.0	0.2	1.8	0.4	0.3	5.8	0.0	0.1	3.6	2.9	0.4	3.6	20.0	1005.0	25.8	95.1	1.8	T	1.4E+04	2.8E+02	7.2E+02	1.4E+01
12/07/2015	2015/7/12 23:50	2015/7/13 9:45	0.41	195.0		8.4E+03	1.5E+03	9.9E+03	85	7.8E+01	1.6E+02	4.5	17.0	10.6	4.3	37.4	9.6	12.4	0.6	2.3	4.6	34.4	36.3	4.2	19.5	998.6	25.7	92.3	3.7	T	2.9E+04	7.3E+02	1.5E+03	3.7E+01
26/07/2015	2015/7/26 20:00	2015/7/27 6:00	0.42	75.0		8.0E+03	9.9E+02	8.9E+03	89	7.0E+02	7.8E+01	4.5	24.7	52.5	13.1	35.9	49.8	16.2	1.4	11.2	9.0	31.6	29.9	6.8	9.5	1004.7	25.2	90.3	3.9	T	3.7E+03	1.1E+02	3.9E+02	1.2E+01
25/08/2015-1	2015/8/25 11:10	2015/8/25 23:10	0.50	135.0		1.1E+04	3.3E+03	1.5E+04	78	2.3E+02	1.2E+03	4.8	42.3	220.8	15.5	35.8	214.8	28.9	8.1	33.0	11.1	15.8	9.9	1.8	7.5	992.7	24.1	90.9	5.0	T				

Table S3-2 Average and standard deviation of bacterial abundance and viability in rainwater collected dependent on the manner of cloud formation.

Cloud formation manner	Concentrations of bacteria (cells ml^{-1})		Viability (%)
	Viable	Non-viable	
Average	$1.7 \pm 1.0\times10^4$	$5.4 \pm 6.4\times10^3$	80±10
Cold front (Cyclone)	$2.6 \pm 1.2\times10^4$	$9.0 \pm 5.4\times10^3$	75 ± 8
Stationary front (Meiyu period)	$1.2 \pm 0.4\times10^4$	$2.5 \pm 1.5\times10^3$	84 ± 7
Stationary front (Non-Meiyu period)	$1.7 \pm 0.8\times10^4$	$0.7 \pm 1.0\times10^4$	78 ± 14
Cold front + Typhoon	$1.1 \pm 0.3\times10^4$	$2.2 \pm 0.6\times10^3$	82 ± 6
Typhoon	$1.0 \pm 0.2\times10^4$	$1.6 \pm 1.2\times10^3$	87 ± 8

Table S3-3 Pearson correlation coefficients between bacterial abundances and viability with precipitation amount and airborne particle number concentrations.

	Bacteria abundance and viability				Rainfall	Particle number concentration/rainfall					
	Viable	Non-viable	Total	Viability		Fine [a]	Coarse [a]	Total [a]	ΔFine [b]	ΔCoarse [b]	ΔTotal [b]
N	68	68	68	68	68	63	63	63	39	43	39
Viable	1				-.191	.025	.084	.026	.106	.192	.107
Non-viable	.657**	1			-.232	.054	.120	.055	.080	.214	.081
Total	.945**	.867**	1		-.226	.040	.107	.041	.103	.207	.104
Viability	-.334**	-.815**	-.573**	1	.220	-.134	-.200	-.134	-.116	-.340*	-.118

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

[a] The ratio of the average airborne particle number concentration before sampling/raining over rainfall; [b] The ratio of the difference of airborne particle number concentration before and after sampling/raining over rainfall.

Table S3-4 Average and standard deviation of bacterial abundance and viability in the rainwater samples collected associated with different air-mass trajectory clusters.

Cluster of air parcels	Concentrations of bacteria (cells ml^{-1})		Viability (%)
	Viable	Non-viable	
1	$1.6 \pm 0.8\times10^4$	$4.8 \pm 3.9\times10^3$	80 ± 10
2	$1.6 \pm 0.7\times10^4$	$4.6 \pm 3.5\times10^3$	79 ± 7
3	$2.1 \pm 1.4\times10^4$	$1.5 \pm 1.9\times10^4$	70 ± 22
4	$3.4 \pm 1.6\times10^4$	$1.2 \pm 0.8\times10^4$	74 ± 13
5	$2.6 \pm 1.0\times10^4$	$8.0 \pm 4.2\times10^3$	77 ± 10
6	$1.2 \pm 0.2\times10^4$	$2.4 \pm 0.9\times10^3$	84 ± 6
7	$1.3 \pm 0.4\times10^4$	$2.1 \pm 1.0\times10^3$	86 ± 6

CHAPTER 4

Bacterial community composition in rainwater associated with synoptic weather in an area downwind of the Asian continent

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There have been a small number of studies describing the community structure of bacteria in rainwater (Ahern et al., 2007; Itani and Smith, 2016; Kaushik et al., 2014; Peter et al., 2014; Lu et al., 2016; Cho and Jang, 2014). The findings of these studies displayed various community compositions of bacteria in rainwater at different sites and under different atmospheric conditions. However, comprehensive information on bacterial communities in rainwater and the dependence on atmospheric conditions remain limited and poorly understood. Informative data from high-throughput sequencing, coupled with the analysis of atmospheric conditions, are essential to better characterize the diversity of bacteria in rainwater and to assess their potential impacts on ecosystems, public health and climate.

To our knowledge, there have been no research studies on the dependence of bacterial community structure in rainwater on the synoptic weather. It is essential to characterize the structure of bacterial communities in the rainwater according to rain types in order to accurately demonstrate the dissemination of bacteria by rain and the subsequent effects on ecosystems, human health and climate.

In this chapter, the diversity and community structure of bacteria in the rainwater samples collected at the coastal city Kumamoto were explored with high-throughput 16S rRNA gene sequencing. Based on the results, the following issues are discussed: 1) how bacterial community compositions of rainwater varied in rain associated with synoptic weather, i.e., Meiyu (plum rain), non-Meiyu stationary front rain and typhoon rain, 2) where the bacteria were probably from, and 3) whether the INA bacteria, and ecosystem and health risk-related bacteria occurred in areas downwind of the Asian continent.

4.1 Methodology

4.1.1 Sample collection and meteorological conditions

Rainwater samples were collected at a site in Kumamoto, a city in the southwestern coastal area of Japan, in 2015. The site was located on the roof of a building (approximately 20 m high) on the campus of the Prefectural University of Kumamoto (32.806°N, 130.766°E). Detailed descriptions of this site and the operation of sample collection were described Sect. 2.1.1 and Sect. 3.1.1. Rain events were classified into four groups according to the synoptic weather causing the rain. In this study, samples from four cases of cyclone (i.e., cold front) rain, four cases of Meiyu front rain, four cases of non-Meiyu stationary front rain, and two cases of typhoon rain were analyzed. Although there are only two cases of typhoon rain, the data will be meaningful as a reference for future studies of bacteria in rainwater associated with typhoons because such information is lacking. The sampling information and meteorological conditions (precipitation amount, average air temperature, relative humidity, pressure and wind speed) during these samplings are listed in Table 4-1.

Table 4-1 Sampling information, meteorological conditions including precipitation amount, average air temperature (T), relative humidity (RH), pressure (P) and wind speed (WS) during samplings, pH, electrical conductivity (EC) of rainwater, and abundance (BA) and viability (BV) of bacteria in rainwater samples.

Sample ID	Rain type	Sampling time [a]	Sample volume (mL) [b]	Rainfall (mm)	T (°C)	RH (%)	P (hPa)	WS ($m\ s^{-1}$)	pH	EC ($\mu S\ cm^{-1}$)	BA ($\times 10^4$ cells mL^{-1})	BV (%)
R022501	Cyclone	2/25 19:40 (0.81)	780 (680)	22.0	8.3	97.4	1011.8	1.1	4.7	8.0	2.58	65.7
R022801		2/28 21:15 (0.51)	780 (680)	19.0	8.7	96.3	1009.3	2.4	4.3	16.0	2.88	91.4
R030301		3/3 10:50 (0.42)	510 (410)	13.5	8.3	95.9	1004.5	2.0	4.4	28.0	2.75	72.2
R030901		3/9 0:20 (0.49)	920 (820)	27.0	9.6	90.2	1009.5	2.6	4.5	11.0	4.34	71.8
R061001	Meiyu front	6/10 18:40 (0.53)	955 (810)	69.5	21.4	99.8	1002.7	1.4	5.3	2.0	1.39	86.6
R061701		6/17 18:40 (0.65)	1080 (660)	39.5	20.0	98.5	997.0	2.2	5.0	9.0	0.81	85.8
R063003		6/30 22:10 (0.60)	1300 (1020)	135.5	23.6	98.8	991.1	5.6	4.6	12.0	1.33	80.5
R072102		7/21 20:30 (0.52)	1080 (700)	85.5	24.9	95.0	1004.9	3.1	5.0	7.7	1.29	86.8
R031501	Non-Meiyu stationary front	3/15 2:00 (0.42)	300 (200)	8.0	8.9	92.8	1018.3	1.6	6.1	12.0	4.60	74.0
R082901		8/29 11:10 (0.44)	1080 (880)	33.0	23.6	89.6	1003.4	1.9	5.3	3.0	1.42	89.9
R090101		9/1 0:25 (0.47)	1080 (965)	35.5	23.3	96.8	1005.5	1.6	5.4	2.0	1.02	88.7
R090102		9/1 12:40 (0.08)	1000 (860)	28.5	22.7	95.8	1005.0	2.2	5.2	3.0	1.12	90.7
R051201	Typhoon	5/12 0:00 (0.38)	760 (460)	22.5	22.7	72.8	1001.8	3.0	4.8	8.0	1.36	80.6
R082501		8/25 11:10 (0.50)	135 (45)	7.5	24.1	90.9	992.7	5.0	4.8	42.3	1.47	77.7

Note: [a] Starting time in Japan Standard Time. Data in the parentheses are sampling durations given in days.
[b] Data in the parentheses are the volumes for filtration.

Before a sample was collected, the sampler was rinsed with sterilized ultrapure water three times. The sampler was then washed with sterilized ultrapure water again, and the water was used as the negative control for the sample.

The backward trajectories of air parcels for these samples were calculated with the HYSPLIT model from the NOAA Air Resources Laboratory (http://www.arl.noaa.gov/ready/hysplit4.html). For each sample, 3-day backward trajectories were traced from the mid-point of the sampling period at the sampling site for three altitudes (500, 1000 and 1500 m above ground level) (Fig. S4-1).

4.1.2 DNA extraction

After a sample of rainwater was collected, a part of the rainwater was used to quantify the abundance of viable and nonviable bacterial cells with the LIVE/DEAD bacterial viability assay and to measure the physicochemical properties (pH, electrical conductivity and the contents of ionic species). The remaining rainwater was immediately filtered through polycarbonate filters (0.22-μm pore size, 47-mm diameter;

Merck Millipore Ltd., Ireland) for DNA extraction after collection. The volume of the filtered rainwater for each sample is listed in Table 4-1, corresponding to 6.6×10^5–3.6×10^7 bacterial cells. Before filtration, the membrane filters and filtrating equipment were sterilized. One sample (40 mL) of negative control was also filtered. The membranes after filtration of the rainwater and the negative control were sealed in sterile 50-mL tubes and frozen at −20°C until subsequent analyses. Considerable growth of bacterial cells during the collection and storage was not expected and not considered due to the short collection durations (Table 4-1) and low storage temperatures (Sattler et al., 2001; D'Amico et al., 2006).

Bacterial DNA was extracted from cells on the filters using mutanolysin, lysozyme and proteinase K and purified with phenol extraction, chloroform extraction and ethanol precipitation, which was modified from Maki et al. (2008). Each filter was cut into small pieces and put into a tube. The bacterial cells were re-suspended in 4 mL sterilized ultrapure water with the addition of Tris-HCl (pH 7.5, Wako Pure Chemical Ind., Ltd., Japan; final concentration: 10 mM). The sterilized ultrapure water was filtered with 0.22-μm-pore polycarbonate filters before autoclaving. After suspension, the cells were lysed with mutanolysin (Sigma Aldrich Co., USA) at 60°C for 1 h and lysozyme (Sigma Aldrich Co., USA) at 37°C for 1 h. Then, the TE buffer (10 mM Tris-HCl, pH 8.0 and 5 mM EDTA; Wako Pure Chemical Ind., Ltd., Japan) and Proteinase K (Merck, Germany) were added, and the suspensions were incubated at 37°C for 1 h.

After that, each suspension was transferred into a sterile 15-mL tube, and 4 mL TE-saturated phenol (Wako Pure Chemical Ind., Ltd., Japan) was added and mixed by a vortex mixer for 2 min. The tubes were centrifuged at 11,000 rpm for 10 min using a centrifuge (AS185, AS ONE Corp., Japan), and the supernatants were transferred into new 15-mL tubes, and isometric Sevag reagent (chloroform:isoamyl alcohol *V/V*=24:1) was added to remove free proteins. Again, the tubes were shaken by the mixer for 2 min and centrifuged at 11,000 rpm for 10 min. The supernatants were transferred into new 15-mL tubes, and 0.1 volumes of 3 M sodium acetate (pH=5.2; Wako Pure Chemical Ind., Ltd., Japan) and 2 volumes of ethanol (99.5%, Kishida Chemicals Co., Ltd, Japan) were added and mixed gently. They were centrifuged at 11,000 rpm for 15 min after standing for 30 min to 1 h at −20°C.

After centrifugation, the ethanol was discarded, and 500 μL of 70% ethanol was used to wash the tube wall where DNA was located and discarded. After the tube wall dried, the DNA fragments were re-suspended in 10 μL sterile ultrapure water, and each extraction was transferred into a 0.2-mL tube and stored at −20°C until subsequent treatments.

4.1.3 PCR amplification and 16S rRNA gene sequencing

The DNA for prokaryotic 16S rRNA genes was amplified via two rounds of PCR. The first PCR was

performed using the universal primers 515F (5′-Seq A-TGT GCC AGC MGC CGC GGT AA-3′) and 806R (5′-Seq B-GGA CTA CHV GGG TWT CTA AT-3′) for bacteria, which were developed against the V4 region of the 16S rRNA (Caporaso et al., 2011; Maki et al., 2017). The first PCR mixture contained 1 μL of template DNA solution, 1.5 μL of each primer (10 μM), 25 μL of 2× Gflex PCR buffer (Mg^{2+}, dNTP plus), 1 μL of Tks Gflex DNA polymerase (1.25 U μL^{-1}) (Takara Bio Inc., Japan), and 20 μL PCR-grade water. The PCR amplification was performed under the following conditions: denaturation at 94°C for 3 min and denaturation at 98°C for 10 s, annealing at 52°C for 15 s, and elongation at 68°C for 15 s for 30 cycles.

Each product of the first-round PCR was purified with Agencourt AMPure XP beads (Beckman Coulter Inc., USA). The second PCR was conducted using 2.5 μL of the first PCR product as a template, 1 μL of each primer (10 μM), 25 μL 2× Gflex PCR buffer, 0.5 μL Tks Gflex DNA polymerase (1.25 U μL^{-1}), and 20 μL PCR-grade water. The 5′ ends of forward (5′-adaptor C–tag sequence-Seq A-3′) and reverse (5′-adaptor D-Seq B-3′) primers for the second PCR were attached to adapters and sample-specific barcodes for the MiSeq sequencing reaction (Maki et al., 2017). The conditions for the second PCR were as follows: denaturation at 94°C for 3 min and denaturation at 98°C for 10 s, annealing at 60°C for 15 s, and elongation at 68°C for 15 s for 20 cycles.

The second-round PCR products were purified again and checked by gel electrophoresis. The remaining PCR products were sent to Fasmac Co., Ltd., Japan, for high-throughput sequencing of the 16S rRNA gene on the Illumina MiSeq platform. The obtained paired-end sequences, with read lengths of 250 bp, were grouped based on barcodes for each sample. In addition to the negative control mentioned above, a positive control (using *E. coli* strains as a template) and a negative control (sterilized ultrapure water only) were treated following all the steps of the PCR process to check for the PCR product and contamination, respectively. According to the results of gel electrophoresis, large uncertainties in the results caused by the two-round PCR amplification were not expected.

4.1.4 Phylogenetic and statistical analysis

Before the analysis of bacterial community structures, USEARCH v.8.0.1623 (Edgar, 2013) was used to process the raw Illumina sequencing reads. Anomalous sequences were removed with the workflow as described in Maki et al. (2017). After removing these sequences, the remaining sequences were clustered de novo (with a minimum identity of 97%) into 36,437 OTUs among the fourteen rainwater samples and one negative control. The OTUs appearing in the negative control and rare OTUs that contained only a single sequence were removed. In total, 6,875 OTUs remained for further analysis. The nucleotide sequences have been deposited at the NCBI Sequence Read Archive under the accession No. SRP105747.

Sample coverage estimates, estimators of community richness (Chao1 and abundance-base coverage estimator (ACE)) and diversity (Shannon and Simpson) were calculated at 97% similarity with the "summary.single" command. Rarefaction curves and Shannon-Wiener curves (Fig. S4-2 in the supplemental material) were illustrated with the "rarefaction.single" command in the Mothur v.1.35.0 (Schloss et al., 2009). To effectively compare the different samples, the number of sequences of each sample was also normalized to 13,590 reads (the lowest in the fourteen samples) to analyze the diversity. Sequencing information and alpha diversity estimators for each sample are listed in Table S4-1.

The vegan package (Oksanen et al., 2007) in R v2.12.1 was applied to calculate the Bray-Curtis distance matrix from differences at the OTU level (Table S4-2). Both principal coordinates analysis (PCoA) and the analysis of similarities (ANOSIM) of the distance matrix were executed in R to analyze whether four types of rainwater contained discrepant bacterial communities. The functions in R were also used to create Venn diagrams and heatmaps (see Figs. S4-3–S4-4), and the vegan package was used to calculate pairwise differences in heatmap clustering.

4.2 Results

Before describing the bacterial community results, we briefly introduce the physical and chemical characteristics of the rainwater. The rain caused by stationary fronts (on average 138 mm d^{-1}) was generally much heavier than that caused by cyclones (on average 38 mm d^{-1}). Air masses associated with cyclone rain were influenced by the emission of air pollutants from the Asian continent, Korean peninsula and Japanese islands. Air masses associated with stationary front rain in both Meiyu and non-Meiyu stationary front episodes were mostly maritime but were sometimes affected by Asian continent outflow (Fig. S4-1). In the case of the typhoon rain on 12 May, higher-altitude air was from the tropical central Pacific, while lower-altitude air came from the marine area and the Asian continent, indicating the influence of continental air in addition to marine air.

The pH of the rainwater ranged between 4.3 and 6.1, and the electrical conductivity (EC) ranged between 2 and 42.3 μS cm^{-1} (Table 4-1). The EC was higher in cyclone rainwater and was the highest in the typhoon rain of 25 August due to the high concentration of Na^+ (215 μeq L^{-1}) and Cl^- (221 μeq L^{-1}). The abundance of bacteria in the rainwater was 0.8–4.6×10^4 cells mL^{-1}, and the viability (the ratio of the abundance of bacteria with intact cell membranes to that of total bacteria) was 66–91% (Table 4-1).

4.2.1 Diversity of bacterial communities

At 97% similarity, Good′s coverage estimate of every sample was higher than 0.99 (Table S4-1), indicating the sequencing effort covered the major extent of the taxonomic diversity. Rarefaction curves and Shannon-Wiener curves for all samples approximately reached asymptotes (Fig. S4-2), indicating the

sequencing capacity entirely covered the bacterial communities in the samples. The bacterial community richness in the rainwater was estimated using the Chao1 and ACE indices, and the bacterial community diversity was indicated by the Shannon and Simpson indexes (Table S4-1). The typhoon rainwater collected on 12 May (R051201) had the highest bacterial species richness (ACE: 3208; Chao 1: 3051) and diversity (Shannon: 5.9; Simpson: 0.008), while the lowest community richness (ACE: 551; Chao 1: 369) and diversity (Shannon: 3.4; Simpson: 0.040) were found in the other typhoon rain sample on 25 August (R082501). The Shannon diversity index of the bacterial communities in other samples did not differ significantly, with an average of 4.6±0.7. The diversity analysis using the sequence-normalized data showed similar results to that using un-normalized data (Table S4-1).

On the basis of the Bray-Curtis distances, the rainwater collected on 10 June (Meiyu rain) and 29 August (Non-Meiyu stationary front rain) had the highest similarity (61.8%) in terms of bacterial communities (Table S4-2). Two samples collected on 1 September also showed high similarity (38.9%). Distances between typhoon rains (R051201 and R082501) and other types of rain were mostly larger than 70%, even up to 96.5% (Table S4-2), which indicates the marked difference between typhoon rain and other types of rain in bacterial community composition.

4.2.2 Community structure of bacteria

Approximately 97% of the OTUs were classified into 33 phyla. The phylum-level community structure of bacteria in the rainwater is shown in Fig. 4-1 (a). The most abundant phylum was *Proteobacteria* (averaging 37%, including *Alpha-* (29.2%)*, Beta-* (2.3%)*, Gamma-* (2.5%)*, Delta-* (3.0%) and *Epsilon-* (0.01%) subclasses), followed by *Bacteroidetes* (16%), *Cyanobacteria* (14%)*, Actinobacteria* (9%), *Acidobacteria* (8%) and *Firmicutes* (5%). The fraction of the phylum *Actinobacteria* in the non-Meiyu stationary front rain sample R031501 was 23%, dramatically higher than in other samples. The relative abundance of *Proteobacteria* in the Meiyu rain sample R061701 was the highest at 59%, and the class *Deltaproteobacteria* was remarkably abundant (16%). *Deltaproteobacteria* predominantly comprise the fruiting body-forming *Myxobacteria*, which preferentially live in soils, rotting plant materials, animal feces and tree bark (Reichenbach, 2001).

At the order level, 164 classified bacterial orders were present in 93% of the OTUs. *Cytophagales* (averaging 14% of the sequences) was the most abundant bacterial order. The orders *Chroococcales*, *Rhodospirillales*, *Rhizobiales* and *Sphingomonadales* were also predominant (Fig. 4-1 (b) and S4-3). The fraction (16%) of *Actinomycetales* in the rainwater of the non-Meiyu stationary front rain R031501 was much higher than in other samples (1−10%). The fractions of *Myxococcales* and *Rickettsiales* in the rainwater of a Meiyu rain (R061701) were higher than 10%, much higher than in other types of rain. The order *Sphingomonadales* in cyclone rain accounted for higher fractions (on average 10%) than in other

types of rain (4–6%) and represented as much as 15% of the sequences in the rainwater R022801.

At the genus level, 36% of the OTUs were assigned to 347 bacterial genera. Due to the short read-lengths, the identification of certain specific bacteria is dependent on the taxon and further confirmation is required. Among the classified genera, *Spirosoma* was the most abundant (8% of the sequences), followed by *Hymenobacter, Gemmata*, *Methylobacterium*, *Deinococcus, Sphingomonas* and *Pseudomonas* (Fig. S4-4).

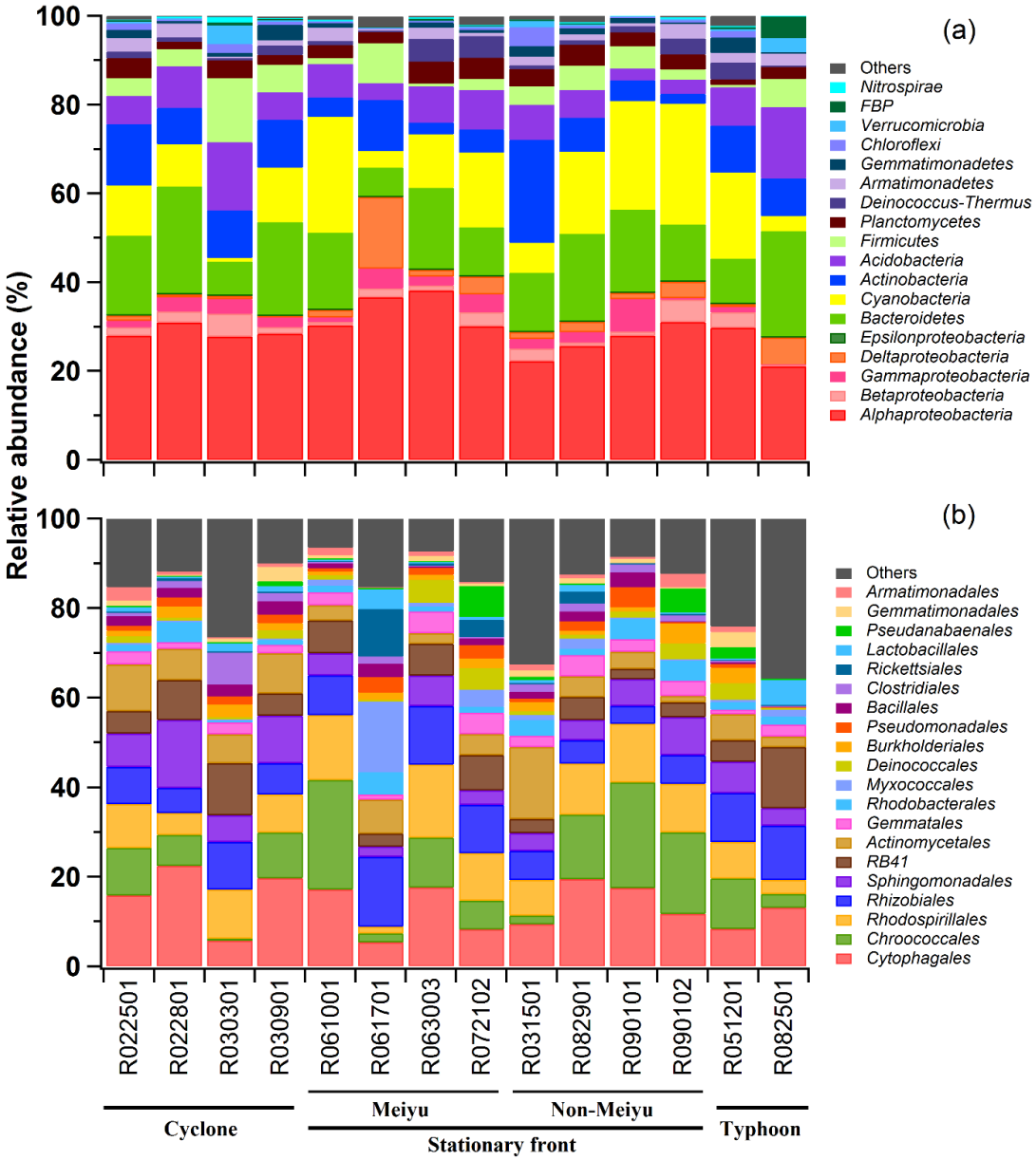


Figure 4-1. Community structure of bacteria in rainwater identified at the phylum (a) and order (b) level. The phylum *Proteobacteria* is classified into *Alpha-*, *Beta-*, *Gamma-*, *Delta-* and *Epsilon-* subclasses.

4.3 Discussion

4.3.1 Association of bacterial community composition with synoptic weather

The results of the PCoA showed that the first three axes accounted for 29.4%, 11.4% and 10.5%, respectively, of the variation in the bacterial community in the rainwater samples (Fig. 4-2). The samples of the different rain types were mostly concentrated along the first and second principal coordinate axes, indicating that the phylogenetic properties of bacterial communities in the rainwater were similar in most cases, regardless of the different synoptic weather systems causing the rain. The ANOSIM analysis (R=0.03, p=0.34) also indicates that the bacterial communities in different types of rainwater were insignificantly different.

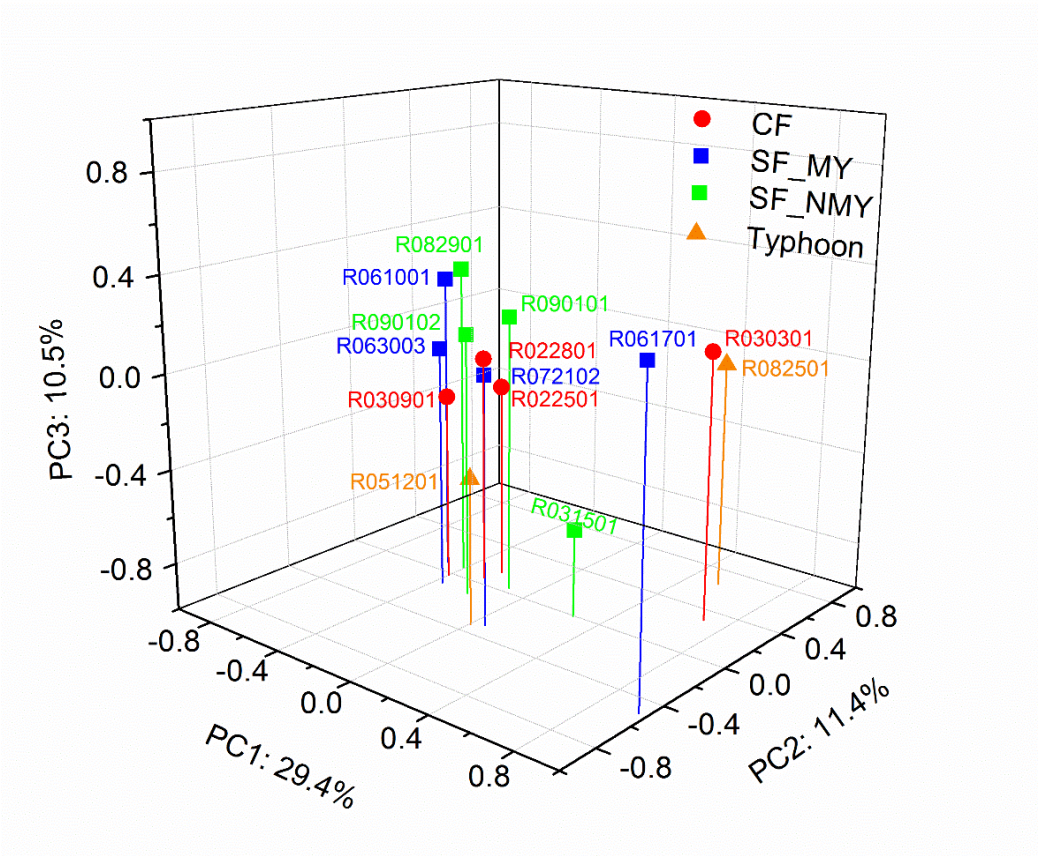


Figure 4-2. Relationships between bacterial communities in rainwater samples associated with different synoptic weather systems. Bacterial communities were clustered using principal-coordinate analysis. CF, SF_MY, SF_NMY and Typhoon in the legend represent the rain of cyclones, Meiyu fronts, non-Meiyu stationary fronts, and typhoons, respectively.

On the other hand, one sample of cyclone rain (R030301), two samples of Meiyu rain (R061701 and R072102), and two samples of typhoon rain did not overlap with most samples across the first and second principal coordinate axes. The non-Meiyu stationary front rain sample R082501 was not close to most

samples across the first principal coordinate axis. The synoptic weather and air masses associated with the rain episodes (Fig. S4-1) likely affected the structure of the bacterial community in the rainwater, i.e., distinctive bacteria in the air parcels originating from the Asian continent, islands and marine areas could be present in the rainwater. For the two cases of typhoon rain, the distinct phylogenetic properties (Fig. 4-2) could be attributed to the probable influence of air masses from different areas (Fig. S4-1).

Among the 33 bacterial phyla detected, 16 phyla appeared in all four types of rain (Fig. 4-3 (a)). As mentioned above, the most common phyla, i.e., *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Actinobacteria*, *Acidobacteria*, *Firmicutes, Planctomycetes* and *Deinococcus-Thermus* (in decreasing order of abundance), were detected in all samples. These common phyla represented the majority (94% on average) of the sequences, consistent with the similar Shannon indexes of all samples. In addition, the phyla or candidate phyla *Armatimonadetes*, *Chlorobi*, *Chloroflexi*, *Verrucomicrobia*, *Gemmatimonadetes*, TM7, FBP and WPS-2 were also detected in all types of rain. These results indicate that the bacterial communities in the rainwater were very uniform at the phylum level. The representative phyla in rainwater, such as *Proteobacteria* (including *Alpha*-, *Beta*- and *Gamma*- subclasses), *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Firmicutes*, were also often identified in cloud water (Amato et al., 2005, 2007c; Kourtev et al., 2011; Vaïtilingom et al., 2012; Xu et al., 2017), suggesting that these phyla could be involved in the rainwater in the stage of cloud formation.

At the order level, 76 out of 219 classified or unclassified orders were detected in the four types of rainwater (Fig. 4-3 (b)), accounting for 96% of the sequences on average. There were 18, 16, 20 and 10 unique orders detected in the cyclone, Meiyu, non-Meiyu stationary front and typhoon rain samples, respectively. For instance, the order *Coriobacteriales*, which is comprised of saccharolytic organisms within the phylum *Actinobacteria* and which has been found to be abundant in animal and human fecal samples (Gupta et al., 2017; Hang et al., 2012), was identified in three of the four samples of cyclone rain. The only order *Ktedonobacterales* within the class *Ktedonobacteria*, members of which are mesophilic aerobic heterotrophs and are able to grow under microaerophilic conditions, were found in the rainwater of Meiyu rain (R063003 and R072102), although similar strains were initially isolated from soils (Cavaletti et al., 2006). Unique orders detected in non-Meiyu stationary front rain and typhoon rain mostly appeared in the samples R031501 and R051201. The possible explanation is that the origins of the air parcels from which the two samples were collected were quite different from other rain episodes (Fig. S4-1).

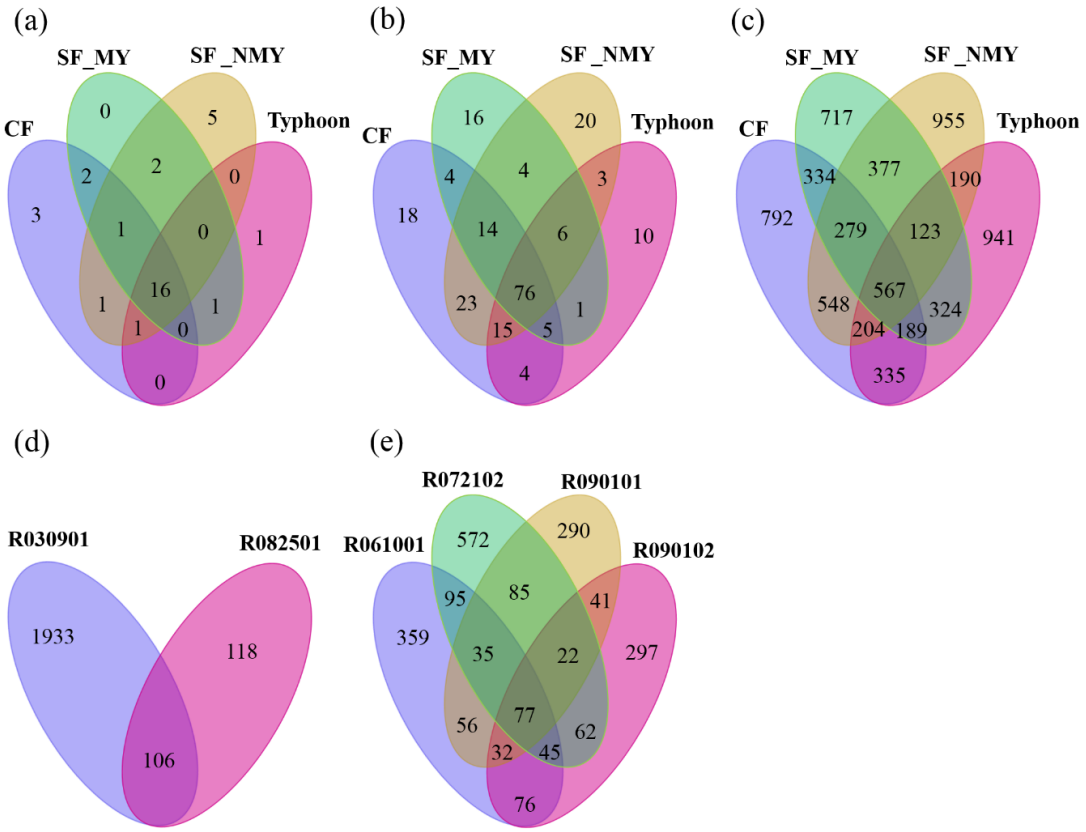


Figure 4-3. Comparisons of bacterial communities in rainwater samples. Venn diagrams showing the numbers of special and shared (a) bacterial phyla, (b) orders assigned and unassigned, and (c) OTUs in different types of rain, and Venn diagrams showing numbers of special and shared OTUs in samples associated with air masses of similar backward trajectories (d–e).

The assigned common genera included *Spirosoma*, *Hymenobacter*, *Gemmata*, *Methylobacterium*, *Deinococcus*, *Sphingomonas*, *Pseudomonas*, *Leptolyngbya*, *Rubellimicrobium*, and *Lactobacillus*, among others (Fig. S4-4). Šantl-Temkiv et al. (2015) reported that *Pseudomonas* were predominant in the communities of cultivable bacteria in rain and snow. Lu et al. (2016) found that the microbial community structures at the genus level were distinct in the rainwater collected at three mountain sites (900, 2740 and 1000 m a.s.l.) and that the members of the genus *Burkholderia* were the most abundant, followed by the genera *Massilia* and *Methylobacterium*. The bacterial OTUs of the genera, such as *Lysinibacillus*, *Bacillus*, *Escherichia*, *Acinetobacter*, *Pseudomonas* and *Sphingomonas*, were detected in the rainwater at an inland site of Korea, and the dominant OTUs also varied considerably among the samples (Cho and Jang, 2014).

Based on the available data in this and previous studies, the genus-level bacterial composition in rainwater differs substantially at various sites across the world. It is suggested by Creamean et al. (2013)

and Cho and Jang (2014) that the majority of microorganisms in precipitation probably originate from the clouds rather than being scavenged by precipitation below clouds. Bacterial populations present in clouds are highly diverse (Amato et al., 2005, 2007c; Kourtev et al., 2011; Vaïtilingom et al., 2012; Xu et al., 2017), implying that the diverse sources of bacteria in clouds likely caused the distinct bacterial community structures in rainwater at different sites.

Among the 6,875 OTUs, 567 OTUs were common to all four types of rain. The common OTUs on average accounted for 74% of the sequences, indicating the predominance of the common bacterial OTUs regardless of the synoptic weather. There were 792, 717, 955 and 941 OTUs present only in cyclone, Meiyu, non-Meiyu stationary front and typhoon rains, respectively (Fig. 4-3 (c)).

During some rain episodes, e.g., the episodes R030901 and R082501 and the episodes R061001, R072102, R090101 and R090102, the air parcels experienced similar trajectories before arriving at the sampling site, but the related synoptic weather systems were different. The shared OTUs in the two samples of the first group accounted for 31% and 75% of the sequences, respectively, and the numbers of specific OTUs were 1933 and 118 in the two samples, respectively (Fig. 4-3 (d)). On the basis of the Bray-Curtis distance matrix, the distance between the two samples was as high as 83.7% (Table S4-2). The shared OTUs in the four samples of the latter group accounted for 70%, 39%, 63% and 62% of the sequences, respectively. There were 359, 572, 290 and 297 special OTUs in the four samples, respectively (Fig. 4-3 (e)). The distances among four samples were larger than 60% (Table S4-2). These results further indicate that the synoptic weather caused distinct bacterial community structures in the rainwater, although a large portion of bacteria was common to all samples.

The unique OTUs in each type of rainwater enhanced the richness and diversity of the bacteria in the rainwater. Compared with other studies (Cho and Jang, 2014; Xu et al., 2017), the bacterial species richness in the rainwater in this study, as mentioned above, was lower than that in rainwater in Korea (ACE: 2678–13083, Chao 1: 1969–6387), but the diversity was higher than that in cloud water at Mt. Tai in China (1534 m a.s.l.) (Shannon: 3.0–3.8), despite the application of different high-throughput sequencing techniques.

4.3.2 Potential source environments of bacteria in rainwater

The bacterial indicator taxa of soils, leaf surfaces and animal feces proposed by Bowers et al. (2011b) were used to explore the contributions of possible sources to the bacteria in the rainwater (Fig. 4-4 (a)). There might be some uncertainties due to the representativeness of likely source environments and bacterial indicator taxa. The relative contribution of bacterial indicator taxa of soils, i.e., members of the phylum *Acidobacteria* and the order *Rhizobiales*, was in the range of 6.6–28.1% and averaged 16.8%.

The average contributions of these members in cyclone, Meiyu, non-Meiyu stationary front and typhoon rains were 17.2, 19.2, 10.5 and 23.8%, respectively.

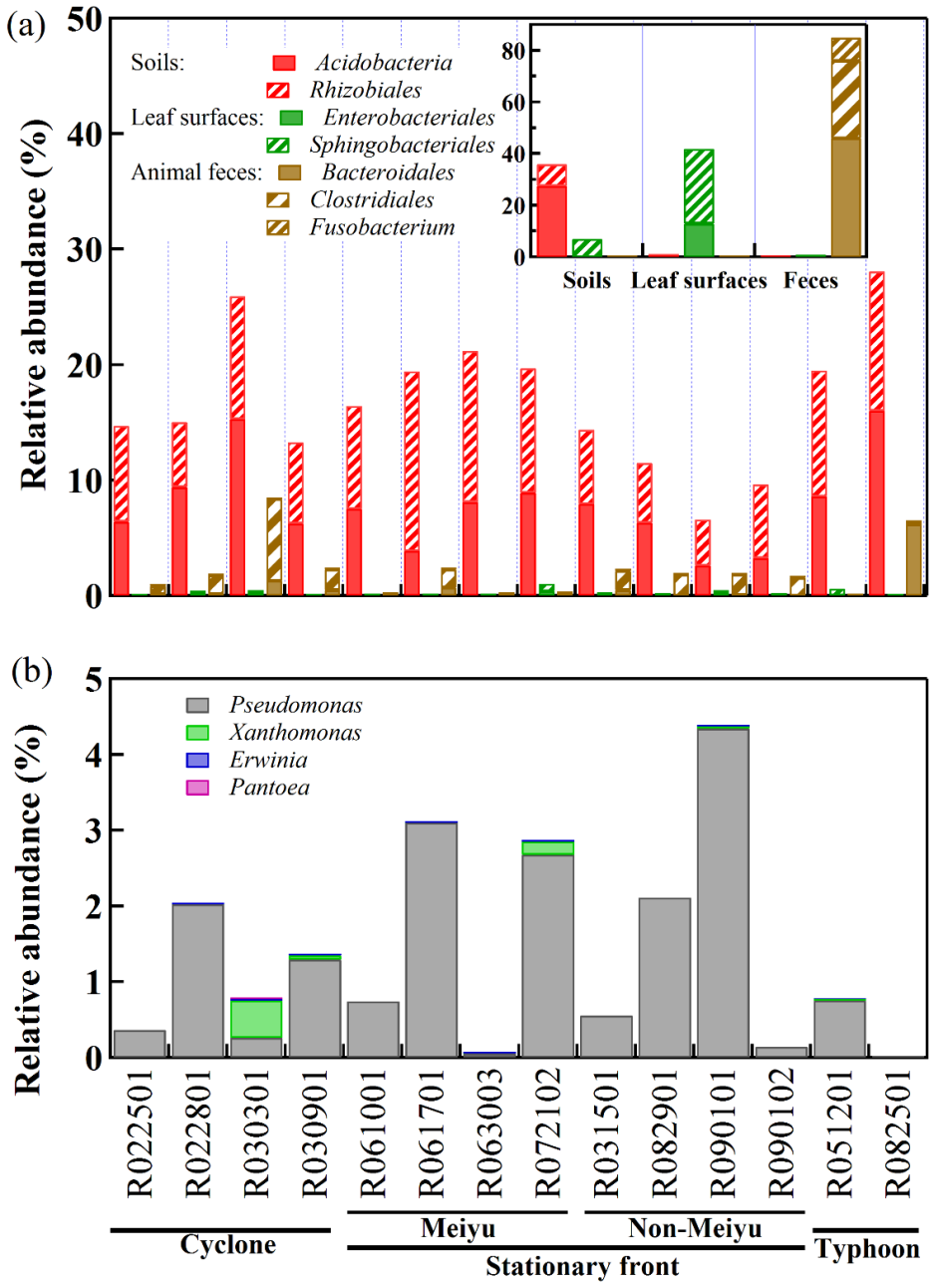


Figure 4-4. (a) Relative contributions of bacteria from potential source environments, i.e., soils, leaf surfaces, and animal/human feces. The indicator taxa of the source environments were identified by Bowers et al. (2011b), and their relative abundances are shown in the inset, copied from Bowers et al. (2011b). (b) Relative abundances of ice nucleation-active bacterial genera in the rainwater samples.

Dust particles are able to act as efficient cloud condensation nuclei (CCN) and IN in clouds (Christner et al., 2008; Laskin et al., 2005; Pratt et al., 2009). Assemblages of microbes are likely attached

to dust particles and transported through the atmosphere (Christner et al., 2008; Maki et al., 2008, 2014). Microbial cells and dust particles often coexist and correlate well in the ice-crystal residues in clouds and in precipitation (Christner et al., 2008; Creamean et al., 2013; Pratt et al., 2009). Ahern et al. (2007) found that half of the 100 detected OTUs in Hebridean rain and cloud water were related to bacteria from terrestrial psychrophilic environments. In this study, the relative abundance of indicator taxa of soils was much higher than those (<10%) in the surface air across cities in the Midwestern US (Bowers et al., 2011b), signifying the great contribution of bacterial communities from soils. These bacteria were probably involved in rainwater in the stage of in-cloud processes as nuclei in addition to the below-cloud washout.

During dust events in East Asia, members of *Proteobacteria* were primary or increased significantly in airborne bacterial communities (An et al., 2015; Maki et al., 2014, 2015), which is consistent with the dominant fraction of *Proteobacteria* in rainwater in this study. In previous studies (Kaushik et al., 2014; Itani and Smith, 2016; Peter et al., 2014), the classes *Gammaproteobacteria* and *Betaproteobacteria* were dominant in rainwater. However, in this study, the class *Alphaproteobacteria*, including the abundant orders *Rhizobiales*, *Rhodospirillales* and *Sphingomonadales*, was dominant in rainwater, and the members of this class were likely derived from soils and surface waters and transported in the Asian continent outflow.

Leaf surfaces are also a major source of airborne bacteria in the atmosphere (Ariya et al., 2009; Bowers et al., 2011a, 2011b, 2013). Vaïtilingom et al. (2012) suggested that microorganisms in clouds originated mostly from continental areas, especially from vegetation, at the Puy de Dôme, France. In this study, the bacterial indicator taxa of leaf surfaces accounted for merely 0.25% of the sequences (Fig. 4-4 (a)), which was much lower than those (~5% on average) in the surface air in cities in the Midwestern US (Bowers et al., 2011b). This result indicates a minor contribution of bacterial communities from leaf surfaces to the bacteria in the rainwater.

It is noted that the indicator taxa of animal/human feces, i.e., the members of the orders *Bacteroidales*, *Clostridiales* and the genus *Fusobacterium* (Bowers et al., 2011b), were found in all samples (Fig. 4-4 (a)). In February and March, the fraction of the indicator taxa of animal feces averaged 3.1% (with values of up to 8.4%) of the sequences and was frequently higher than in other months. Bowers et al. (2011b) found that in metropolitan areas of the Midwestern US, the fraction of these fecal indicator bacteria was much higher in winter (~30–75%) than in summer (<10%). In the samples collected on 17 June and 25 August, the fractions of the indicator taxa were 2.3% and 6.4%, respectively. *Bacteroidales* released by humans and animals have been found in the surface water of the coasts (Elmir et al., 2009; Schriewer et al., 2010). The high abundance of *Bacteroidales* in the two samples probably came from bacteria in sea

water entrained during the transport of air masses across marine areas (Fig. S4-1).

The sequences of the genera *Enterococcus*, *Escherichia* (e.g., *E. coli*), and *Streptococcus*, which are associated with human and animal feces (DeLeon-Rodriguez et al., 2013; Schriewer et al., 2010), were detected in the rainwater. In particular, members of *Enterococcus* and *Streptococcus* were identified in all samples except R022801, accounting for up to 4.1% and 0.7%, respectively, of the total sequences. The relative abundances of the fecal indicator bacterial genera identified by Barberán et al. (2015) and the indicator taxa identified by Bowers et al. (2011b) showed similar trends in the rainwater samples.

Marine bacteria emitted by the ocean could be preferentially involved in the processes of cloud formation by acting as efficient CCN/IN (Ahern et al., 2007; Amato et al., 2007d; DeLeon-Rodriguez et al., 2013; Wilson et al., 2015) or could be transported to inland areas and washed out by rain (Cho and Jang, 2014). In this study, some of the marine bacterial indicator taxa identified by Barberán et al. (2015), such as *Pseudoalteromonas*, *Synechococcus*, and *Marinobacter*, as well as gene sequences of halophilic bacterial species of *Halomonas* and *Virgibacillus* (Cho and Hwang, 2011; Cho and Jang, 2014), were detected in several rainwater samples (Fig. S4-5). However, their detected frequencies in rainwater were low (0–0.35% of the sequences), and the other presumable marine bacteria or bacteria carried from marine regions are required further confirmation.

Pelagic marine bacterial species mostly belong to *Alphaproteobacteria* and *Cyanobacteria* (Maki et al., 2015), the members of which were considerably abundant (43% on average) in the sequences. As mentioned above, the phyla *Cyanobacteria* and *Proteobacteria* were also predominant in the bacterial communities of the cloud water collected at approximately 3000 m above ground level (Kourtev et al., 2011). These results indicate that marine bacteria were presumably transported long distances in the atmosphere and were disseminated via clouds or rainwater.

4.3.3 Implications for cloud formation, ecosystems and public health

Certain bacterial species, which may function as ice-nucleating particles at relatively high temperatures (above −10°C and as warm as −2°C) in high-altitude air, have been identified as INA bacteria (Hill et al., 2014; Morris et al., 2004). INA bacteria are considered active participants in atmospheric processes, i.e., potentially triggering glaciation, cloud formation and even precipitation. The members of the genera *Pseudomonas*, *Xanthomonas*, *Erwinia*, and *Pantoea* within the subclass *Gammaproteobacteria* are the most efficient INA bacteria described so far (Joly et al., 2013).

In this study, the bacterial genera containing INA bacterial species were observed in most rainwater samples (Fig. 4-4 (b)). However, there were no species of the four genera in the typhoon rainwater collected on 25 August, coinciding with the fact that there were also no indicator taxa of leaf surfaces in

the sample (Fig. 4-4 (a)). This is consistent with the observation that INA cells are more frequently emitted to the atmosphere from terrestrial surfaces, either via long-distance transport from continental sources or via emission from local island areas (Šantl-Temkiv et al., 2015). Species of the genus *Pseudomonas* are considered the most efficient INA bacteria. Members of the genus *Pseudomonas*, such as *Pseudomonas viridiflava*, were detected in thirteen samples, and they accounted for an average of 1.3% of the sequences. The fraction of *Pseudomonas* in the sample R090101 was as high as 4.3%. The members of *Xanthomonas*, *Erwinia* and *Pantoea* were identified in five samples, nine samples and one sample, respectively.

Intact and cultivable INA bacterial cells, INA bacterial fragments, and *ina* genes are often identified in precipitation (Du et al., 2017; Hill et al., 2014; Lu et al., 2016; Šantl-Temkiv et al., 2015). The detected frequencies and concentrations of INA bacteria in precipitation varied from site to site. Regardless of the possible uncertainties due to technical limitations, the variations in INA bacteria in precipitation could result in a large uncertainty in the number of bacterial IN implemented in models. In fact, the ice nucleation ability of bacteria in precipitation, as well as the numbers of bacterial IN in the atmosphere, remains poorly constrained.

INA bacteria are abundant on crops and many nonagricultural plants, and their habitats include freshwater and associated biofilms (Hill et al., 2014; Morris et al., 2004). They can not only result in frost damage of crops because of the ability to catalyze the freezing of supercooled water at temperatures as warm as −2°C but can also lead to plant diseases (Hill et al., 2014; Joly et al., 2013; Lu et al., 2016). Similar to other bacteria, they can be dispersed in the atmosphere and disseminated to surfaces. The frequent occurrence of INA bacterial genera in the rainwater samples in the present study indicate the potential negative consequences of the settlement of INA bacteria via rain on the landscape. In contrast, members of the genus *Bacillus*, which represented 0.01–0.6% of the sequences in this study, are able to accelerate plant growth (Yadav et al., 2011).

The value of *Bacteroidales* genetic markers and fecal indicator bacteria has been applied to predict the occurrence of waterborne pathogens (Schriewer et al., 2010). In the rainwater samples, potential bacterial pathogens of human and animal diseases, such as feces-related bacteria and bacterial pathogens, including *Vibrio* sp. (0.01–0.07% of the sequences in five samples) and *Staphylococcus* sp. (0.01–1.1% in eleven samples), were detected, indicating a potential threat to public health. In general, *Bacteroides* species within the order *Bacteroidales* are resistant to a wide variety of antibiotics, e.g., β-lactams, aminoglycosides, erythromycin and tetracycline. As a result, *Bacteroides* species may become a reservoir for resistance in other more highly pathogenic bacterial strains (Löfmark et al., 2006; Salyers et al., 2004).

Acinetobacter species, which are a key source of nosocomial infections (Genitsaris et al., 2017),

were identified in 13 samples (0.003–0.5%). The genus *Sphingomonas*, some species of which may cause human infections and may be opportunistic pathogens in clinical environments (Maragakis et al., 2009; Ryan and Adley, 2010), was one of the most abundant genera in the rainwater. These bacteria can be deposited onto land and water surfaces via rainwater, can disperse into surface waters and can be emitted into the atmosphere (Huffman et al., 2013), which is a natural cycle of pathogens between the earth surface and the atmosphere.

Members of the phyla *Proteobacteria*, *Firmicutes* and *Cyanobacteria* often contribute to various geochemical processes (Maki et al., 2014) by playing roles in the conservation and evolution of microbial diversity in the ecosystems. Some species of the genera *Sphingomonas* and *Pseudomonas* within *Proteobacteria* encountered in the rainwater can degrade organic matter, such as organic acids, hydrocarbons and xenobiotics (Amato et al., 2007a; Vaïtilingom et al., 2010, 2011), and participate in chemical conversions in cloud water (Delort et al., 2010; Vaïtilingom et al., 2013). The major environmental effects of *Bacillus* sp. within *Firmicutes* are on carbon and nitrogen cycles (Stülke and Hillen, 2000; Ye and Thomas, 2001). Members of the phylum *Cyanobacteria*, including *Synechococcus* sp., have photosynthetic abilities and sometimes form blooms that drive the carbon dioxide cycles in freshwater, estuarine and marine ecosystems (O'Neil et al., 2012). The observed population of *Synechococcus* sp. could be the seed for blooms in aquatic environments.

4.4 Summary

In this chapter, the bacterial diversity and community composition in the rainwater samples associated with cyclone, Meiyu, non-Meiyu stationary front and typhoon rains were investigated. Diverse bacterial communities were present in all four types of rainwater and were dominated by the phyla *Proteobacteria* (37%), *Bacteroidetes* (16%), *Cyanobacteria* (14%), *Actinobacteria* (9%), *Acidobacteria* (8%) and *Firmicutes* (5%). Approximately half of the phyla (16 out of 33) were present in all four different types of rainwater. The common OTUs in the different types of rainwater represented the majority (74% on average) of the sequences, indicating that the common bacterial OTUs were predominant in rainwater regardless of the synoptic weather. However, the synoptic weather and the air mass history associated with the rain likely shaped the bacterial communities in the rainwater. Thus, the bacteria in the four types of rainwater were characterized by largely overlapping communities with some differences in community composition.

The high fractions of bacterial soil indicator taxa, which were presumably involved in in-cloud processes as nuclei, signified the great contribution of bacterial communities from continental emissions. INA bacteria, such as the members of the genera *Pseudomonas*, *Xanthomonas*, *Erwinia* and *Pantoea*, were identified in all samples except one typhoon rain sample, although the ice nucleation ability of the

bacteria and the numbers of bacterial ice nuclei in the rainwater remain unclear. Marine bacterial taxa, e.g., *Pseudoalteromonas*, *Synechococcus* and *Marinobacter*, were detected, suggesting that marine bacteria were dispersed via clouds and rain. Potential bacterial pathogens related to human and animal diseases, e.g., fecal indicator bacteria, were also identified in the rainwater.

These results indicate that rain is an efficient pathway for the dissemination of bacterial communities in nature and links continental, marine and island ecosystems. Due to the high diversity of bacteria in the rainwater, determining the potential influences of these bacteria on ecosystems, public health and climate change remains a considerable challenge for future studies.

Supporting information of "Bacterial community composition in rainwater associated with synoptic weather in an area downwind of the Asian continent"

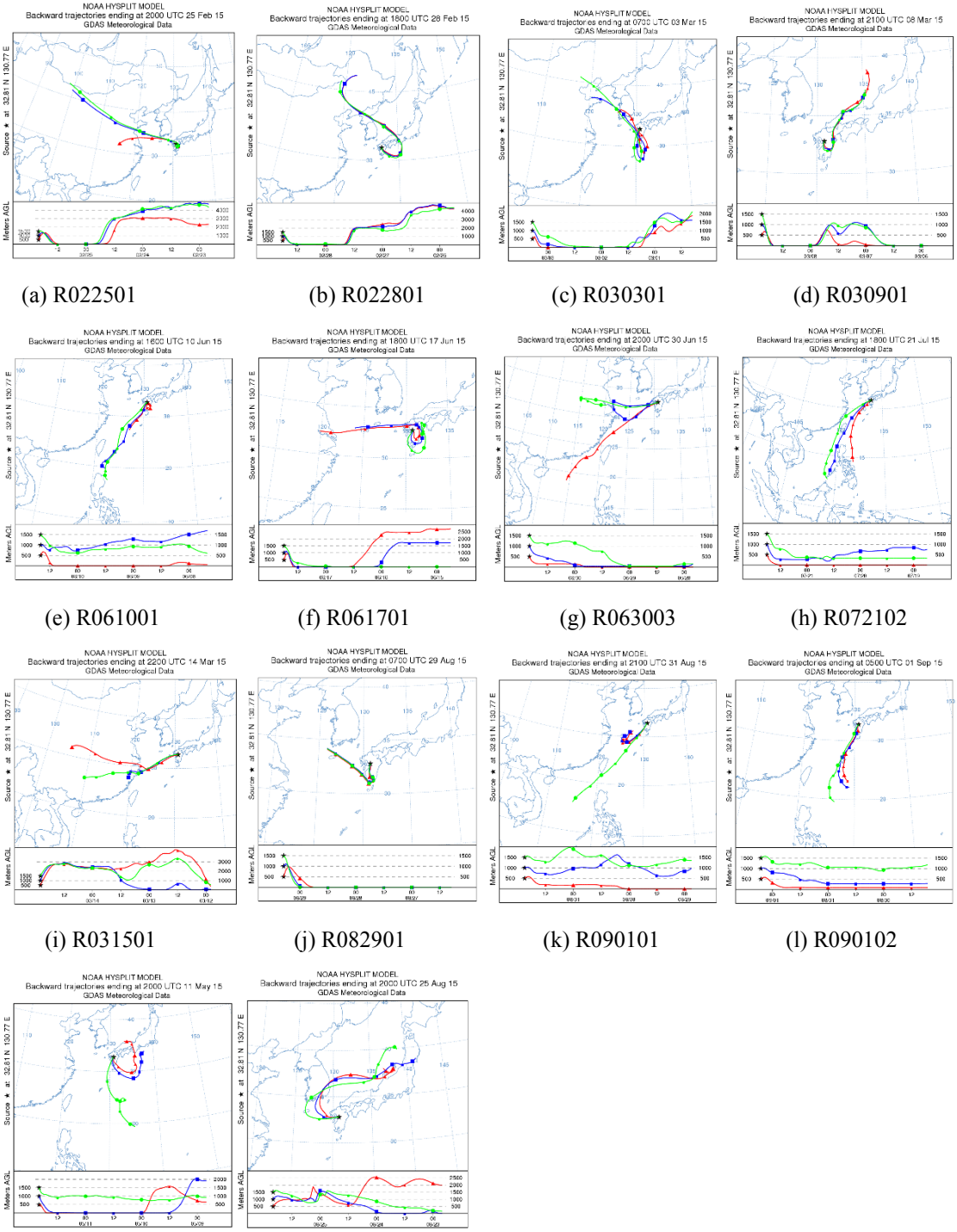
Table S4-1 Sequencing information, OTU richness and diversity for each sample.

Sample ID	High-quality sequences	OTUs	Coverage	ACE	Chao 1	Shannon	Simpson
R022501	87489	1162 (575)	0.995 (0.985)	2362 (957)	1780 (886)	4.9 (4.5)	0.019 (0.031)
R022801	81513	1041 (475)	0.995 (0.987)	2113 (880)	1576 (689)	4.3 (4.2)	0.045 (0.037)
R030301	24964	427 (371)	0.996 (0.993)	559 (488)	568 (494)	4.6 (4.6)	0.016 (0.016)
R030901	120051	2039 (818)	0.995 (0.974)	2760 (1613)	2686 (1343)	5.2 (4.4)	0.020 (0.040)
R061001	74562	789 (332)	0.996 (0.991)	1777 (720)	1264 (561)	4.1 (3.6)	0.049 (0.074)
R061701	13590	303 (303)	0.994 (0.994)	392 (392)	394 (394)	4.1 (4.1)	0.036 (0.036)
R063003	184083	1952 (477)	0.997 (0.986)	2686 (899)	2451 (715)	4.9 (3.7)	0.020 (0.063)
R072102	81549	1007 (552)	0.997 (0.986)	1357 (969)	1261 (805)	4.9 (4.7)	0.016 (0.019)
R031501	110977	1827 (966)	0.997 (0.977)	2078 (1282)	2015 (1317)	5.9 (5.4)	0.006 (0.012)
R082901	83854	1047 (409)	0.995 (0.987)	2134 (918)	1593 (679)	4.3 (4.0)	0.033 (0.042)
R090101	61643	652 (359)	0.996 (0.988)	1517 (985)	1047 (747)	4.1 (4.0)	0.033 (0.031)
R090102	77222	666 (322)	0.997 (0.991)	1502 (706)	1019 (560)	4.4 (4.2)	0.021 (0.029)
R051201	159045	2742 (994)	0.997 (0.969)	3208 (1899)	3051 (1630)	5.9 (4.8)	0.008 (0.030)
R082501	49026	224 (125)	0.998 (0.996)	551 (319)	369 (258)	3.4 (3.4)	0.040 (0.040)

Note: Data in parentheses were species richness estimates at 13590 randomly selected sequences per sample.

Table S4-2 Bray-Curtis distance matrix for OTU abundances of bacteria among different rainwater samples.

	R022501	**R022801**	**R030301**	**R030901**	**R061001**	**R061701**	**R063003**	**R072102**	**R031501**	**R082901**	**R090101**	**R090102**	**R051201**	**R082501**
R022501	0													
R022801	0.627	0												
R030301	0.836	0.845	0											
R030901	0.570	0.571	0.846	0										
R061001	0.585	0.612	0.832	0.540	0									
R061701	0.934	0.911	0.870	0.914	0.918	0								
R063003	0.670	0.627	0.890	0.566	0.567	0.953	0							
R072102	0.721	0.738	0.848	0.738	0.691	0.861	0.730	0						
R031501	0.698	0.759	0.854	0.673	0.805	0.924	0.835	0.790	0					
R082901	0.649	0.628	0.841	0.574	0.382	0.900	0.598	0.689	0.819	0				
R090101	0.684	0.667	0.826	0.698	0.630	0.914	0.722	0.735	0.806	0.676	0			
R090102	0.675	0.684	0.862	0.683	0.619	0.932	0.676	0.722	0.817	0.662	0.611	0		
R051201	0.750	0.754	0.901	0.644	0.735	0.935	0.676	0.739	0.779	0.782	0.789	0.723	0	
R082501	0.776	0.815	0.794	0.837	0.808	0.965	0.867	0.901	0.855	0.804	0.833	0.883	0.902	0



(a) R022501 (b) R022801 (c) R030301 (d) R030901

(e) R061001 (f) R061701 (g) R063003 (h) R072102

(i) R031501 (j) R082901 (k) R090101 (l) R090102

(m) R051201 (n) R082501

Figure S4-1. Three-day backward trajectories of the air masses starting at the mid-time for each sampling at the sampling location.

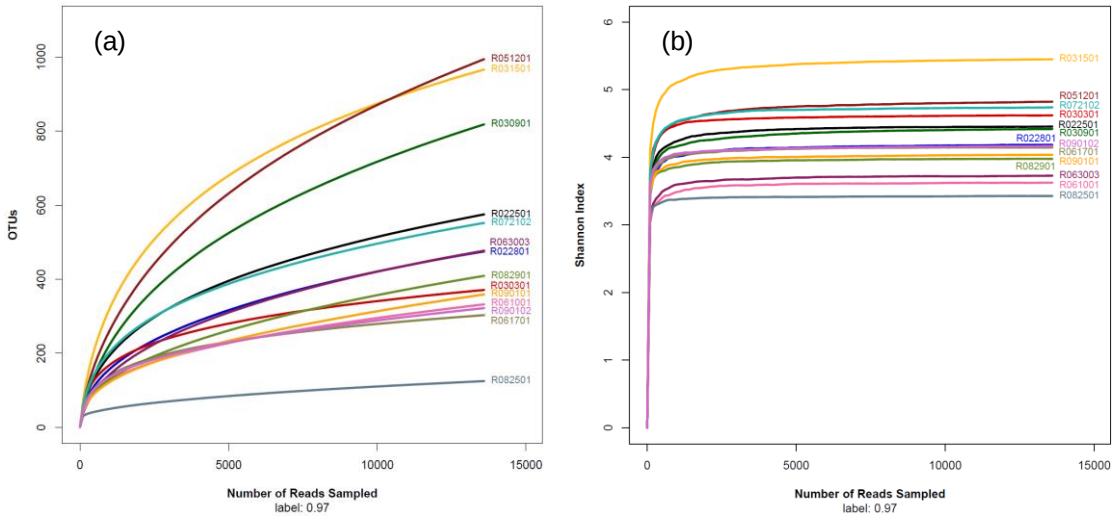


Figure S4-2. Rarefaction curves (a) and Shannon-Wiener curves (b) of each sample. All curves were calculated at the 97% similarity level with Illumina MiSeq sequencing data.

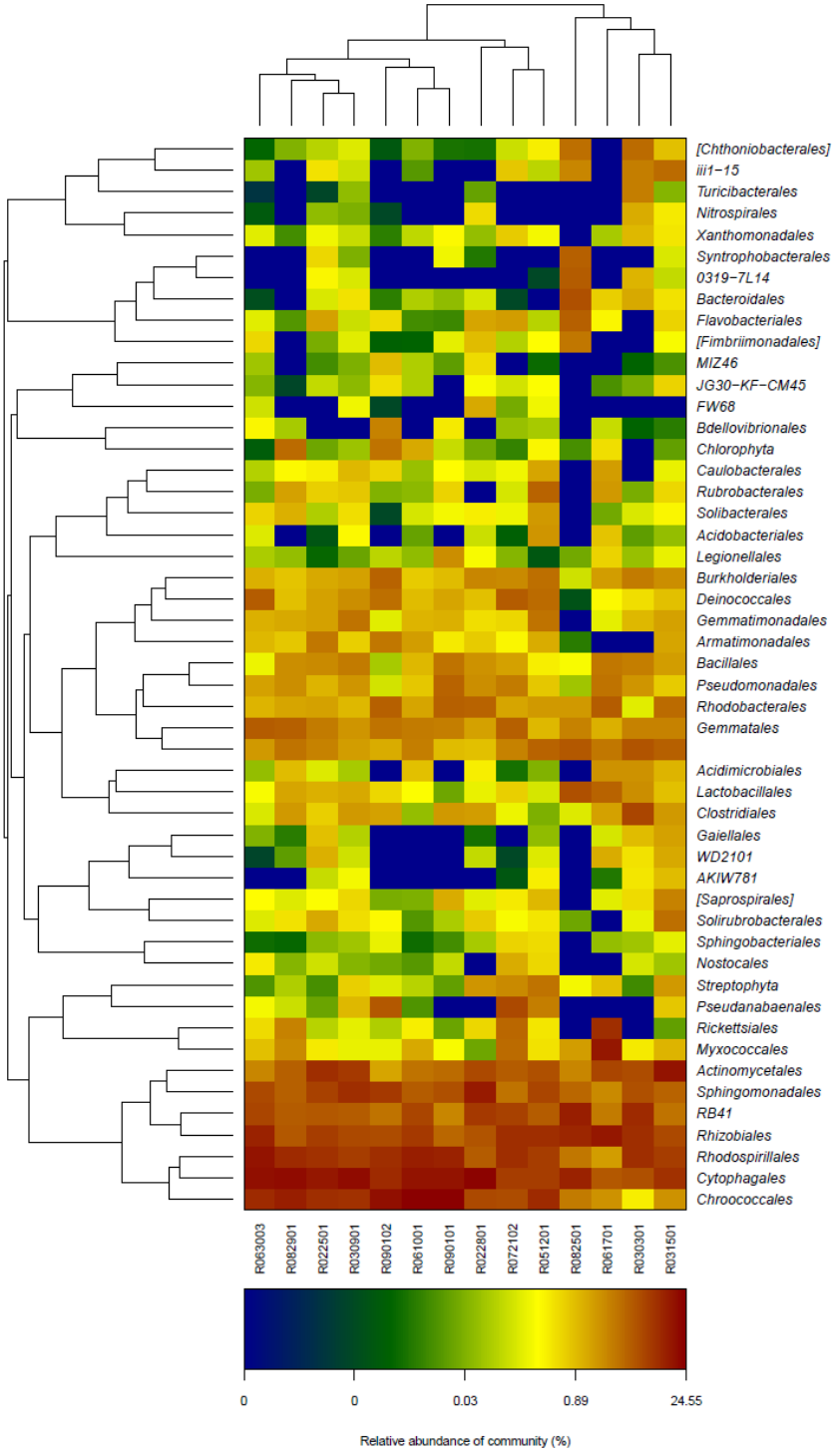


Figure S4-3. Heatmap of bacterial community structures at the order level in rainwater samples. The color of each cell represents the relative abundance of the bacteria order in each sample, as given on the right-side text. Only the most abundant 49 identified orders are shown.

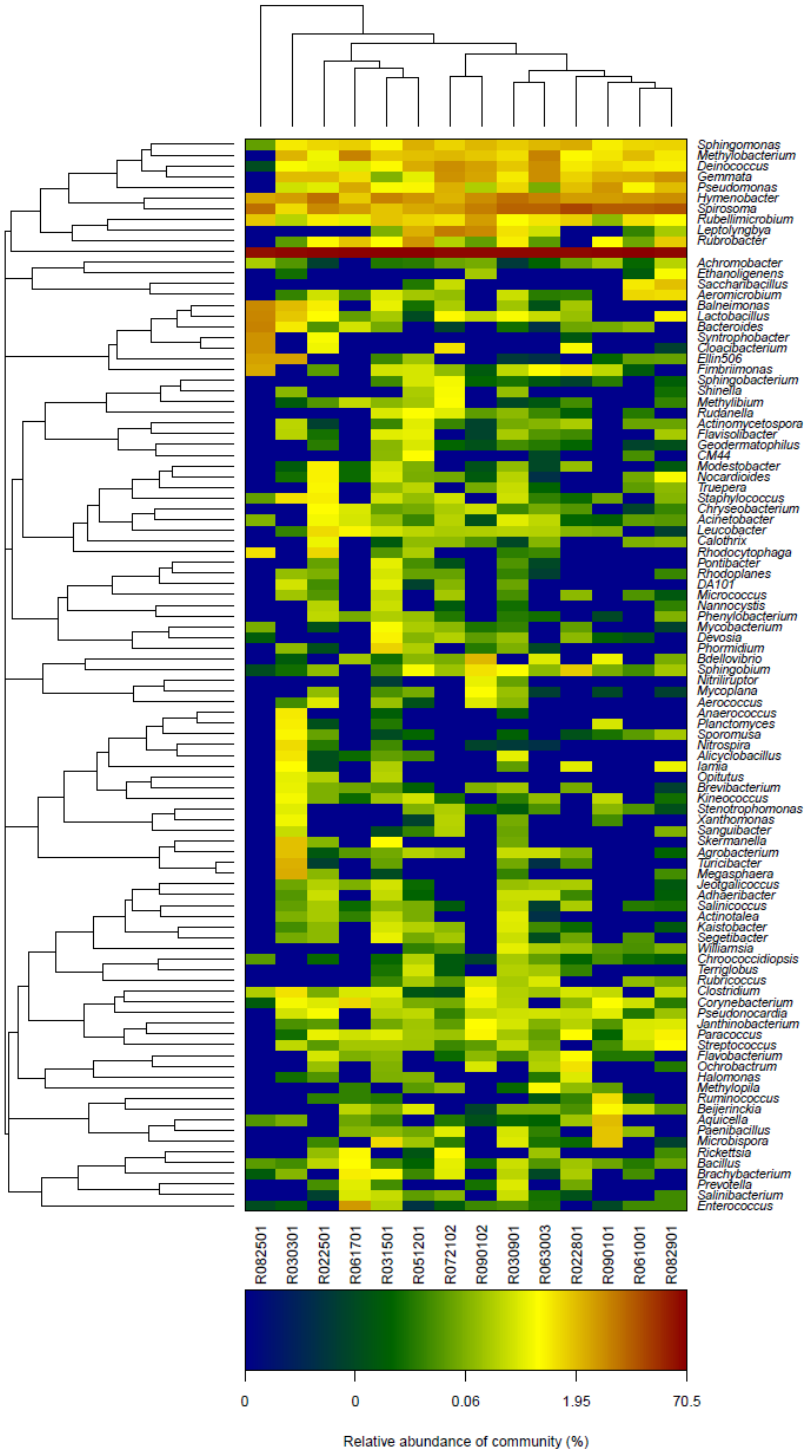


Figure S4-4. Heatmap of bacterial community structures at the genus level in rainwater samples. The color of each cell represents the relative abundance of the bacteria genera in each sample, as given on the right-side text. Only the most abundant 99 identified genera are shown.

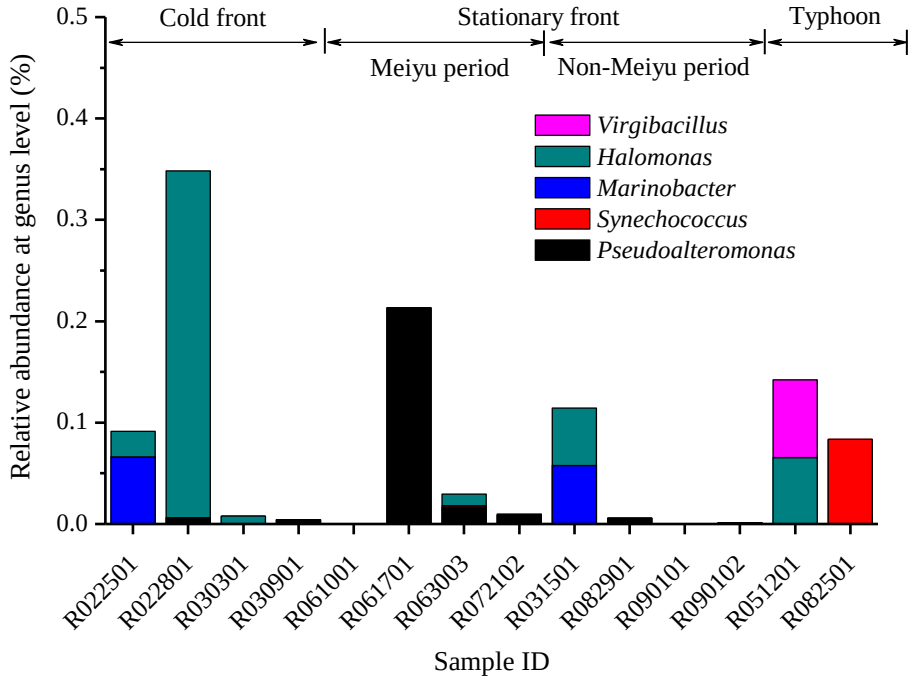


Figure S4-5. Relative abundance of bacteria genera containing marine bacterial indicator taxa.

CHAPTER 5

Conclusions and perspectives

Atmospheric bacteria are considered to play a significant role in the atmosphere–biosphere–hydrosphere links. Dissemination of bacteria via precipitation is one of the most important steps in the life cycle. It is meaningful to study bacteria in rainwater, to quantify and identify them and to explore their roles in atmospheric processes (e.g., cloud formation and atmospheric chemistry).

In this study, firstly the applicability of the LIVE/DEAD BacLight bacterial viability assay coupled with glutaraldehyde fixation in rainwater was verified. In comparison with DAPI stain, the LIVE/DEAD BacLight stain detected 109±29% of total bacterial cells. The ratio of the total bacterial cell counts using LIVE/DEAD BacLight stain with fixation to that without fixation was 106±5% on average. The bacterial concentration in the negative controls was generally lower than that in the rainwater samples by about one order of magnitude, and the uncertainties due to the operation and air diffusion in measuring bacterial abundance were less than 12% on average. With careful negative control experiments, the LIVE/DEAD BacLight bacterial viability assay coupled with glutaraldehyde fixation is suitable to quantify the bacterial abundance and viability in rainwater.

Rainwater samples under different synoptic weather systems were collected to examine the concentration and viability of bacteria in rainwater using the LIVE/DEAD bacterial viability assay, and to identify the community compositions of bacteria using high-throughput 16S rRNA gene sequencing. It was found that there was a dependence of bacterial concentration and viability on the synoptic weather of cloud formation. In future studies, the classification of weather patterns will be beneficial to the model simulation of bacterial abundance in rainwater. Diverse bacterial communities occurred in the rainwater. Common bacteria dominated OTUs despite the synoptic weather, and distinct bacterial communities occurred in rainwater of different rain types. The relationship between bacterial abundance and ionic species in rainwater, as well as the community composition of bacteria, suggest that bacteria enclosed in clouds may be an important source of bacteria in rainwater. Marine bacteria might be disseminated via cloud and rainwater. The occurrence of INA bacteria and FIB indicates the potential involvements of bacteria in rainwater in ecosystem evolution, public health and climate change.

The data obtained in this study give more information on the abundance, viability and community composition of bacteria in rainwater for understanding the roles of atmospheric bacteria in both the physics and chemistry of the atmosphere. The uncertainties, however, might exist and thus any definite

conclusions would be premature at this stage. There are multiple domains of research deserving further studies:

1. More samples representing typical environments and ecosystems and quantitative data on the abundance, viability, community composition and sources of atmospheric bacteria are still necessary to corroborate the early findings and rigorously test the hypotheses hereinbefore. Multidisciplinary approaches (e.g., atmospheric chemistry and physics, microbiology, meteorology and modelling) are required to understand the role of atmospheric bacteria in atmospheric physicochemical processes. For instance, metagenomics and metatranscriptomics could bring valuable information about the structure and function of bacterial communities in atmospheric waters. Metabolomics could also give new insights into the response and adaptation of microorganisms in atmospheric waters to stresses.

2. More work is needed to elucidate on the survival rates and mechanisms of bacteria in atmospheric waters. The viability of bacteria in the atmosphere could be altered by multiple environmental factors, e.g., oxidants such as H_2O_2, UV light, relatively low access to nutrient, osmotic shocks (condensation/evaporation) and freeze-thaw cycles (Joly et al., 2015). The information on the survival rates and mechanisms of atmospheric bacteria would help better understanding what affects the microbial diversity in the atmosphere and atmospheric waters, and better representing the spread of living microbes in the atmosphere.

3. Detailed determination of the CCN/IN activity of INA bacteria (including intact and damaged bacterial cells, as well as micrometer scale and nanoscale bacterial fragments) in atmospheric waters is needed for interpretation of the contribution of atmospheric bacteria to the formation of precipitation. It is possible that several additional proteins or cell components (similar to *inaZ* protein) that promote CCN/IN and these represent hypothetical or poorly characterized genes in the current functional databases (Konstantinidis, 2014). It is also very important to obtain a more complete understanding of which bacterial species is more efficient ice nucleators, what cell properties and proteins constitute an efficient CCN/IN, as well as the factors (such as chemical heterogeneity, surface characteristics, size, form, and environmental conditions (atmospheric pollutants, temperature, RH, pH, irradiation, etc.)) influencing the CCN/IN ability. These results will provide more accurate parameterizations of the behavior of INA bacteria in atmospheric models for investigating the potential impacts on precipitation and climate. The solutions of these issues will contribute to the search for new methods of weather modification.

4. An interdisciplinary approach is required to close these gaps in knowledge and determine whether the activities and abundances of atmospheric bacteria are important for atmospheric chemistry, and consequently influencing cloud formation and climate. There are several open issues, namely: (a) the contribution of bacterial taxa to the transformation of inorganic (e.g., trace metals and mineral particles)

and organic compounds in the atmosphere or at environmental interfaces, as well as the feedback of both oxidation and reduction reactions on the microbiological particles; (b) the importance of interactions between microbes and chemical species in the atmosphere on modification of CCN/IN ability of chemical species; (c) the types of chemical feedbacks which microbes at air/snow/water interfaces supplies to the atmosphere, and their impacts on the atmospheric physics and chemistry.

Further fundamental experiments should be performed in laboratories or atmospheric chamber facilities with a great variety of microbial isolates from atmospheric waters to get qualitative and quantitative data. These data will provide a better understanding of microbiological-chemical interactions in the atmosphere and the kinetics and mechanisms for chemical transformation by/on microbiological particles.

5. Laboratory simulation experiments or pilot tests are needed to assess the effects of bacteria deposited by precipitation on diverse ecosystems, such as ocean, freshwater, cropland and forest. Studies of the etiology and epidemiology of plant, animal and human during specific weather conditions, e.g., monsoon, typhoon and Meiyu rain, would bring into a unique interaction of scientific disciplines and shed new light on the causes and potential control measures for plant, animal and human diseases. In order to accurately assess the impacts of bacteria in atmospheric waters on the ecosystems and public health, emission, transport, and deposition mechanisms for atmospheric bacteria are needed to be further studied and an adequate transport model should be evolved.

REFERENCES

Ahern, H.E., Walsh, K.A., Hill, T.C.J., Moffett, B.F., 2007. Fluorescent pseudomonads isolated from Hebridean cloud and rain water produce biosurfactants but do not cause ice nucleation. Biogeosciences 4, 115-124.

Alfreider, A., Pernthaler, J., Amann, R., Sattler, B., Glockner, F., Wille, A., Psenner, R., 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Appl. Environ. Microbiol. 62, 2138-2144.

Amato, P., 2012. Clouds provide atmospheric oases for microbes. Microbe 7, 119-123.

Amato, P., Demeer, F., Melaouhi, A., Fontanella, S., Martin-Biesse, A.-S., Sancelme, M., Laj, P., Delort, A.-M., 2007a. A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by micro-organisms. Atmos. Chem. Phys. 7, 4159-4169.

Amato, P., Hennebelle, R., Magand, O., Sancelme, M., Delort, A.M., Barbante, C., Boutron, C., Ferrari, C., 2007b. Bacterial characterization of the snow cover at Spitzberg, Svalbard. FEMS Microbiol. Ecol. 59, 255-264.

Amato, P., Ménager, M., Sancelme, M., Laj, P., Mailhot, G., Delort, A.M., 2005. Microbial population in cloud water at the Puy de Dôme: implications for the chemistry of clouds. Atmos. Environ. 39, 4143-4153.

Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., Delort, A.M., 2007c. Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dome: major groups and growth abilities at low temperatures. FEMS Microbiol. Ecol. 59, 242-254.

Amato, P., Parazols, M., Sancelme, M., Mailhot, G., Laj, P., Delort, A.-M., 2007d. An important oceanic source of micro-organisms for cloud water at the Puy de Dôme (France). Atmos. Environ. 41, 8253-8263.

An, S., Sin, H.H., DuBow, M.S., 2015. Modification of atmospheric sand-associated bacterial communities during Asian sandstorms in China and South Korea. Heredity 114, 460-467.

Ariya, P.A., Sun, J., Eltouny, N.A., Hudson, E.D., Hayes, C.T., Kos, G., 2009. Physical and chemical characterization of bioaerosols – Implications for nucleation processes. Int. Rev. Phys. Chem. 28, 1-32.

Barberán, A., Ladau, J., Leff, J.W., Pollard, K.S., Menninger, H.L., Dunn, R.R., Fierer, N., 2015. Continental-scale distributions of dust-associated bacteria and fungi. Proc. Natl. Acad. Sci. USA 112, 5756-5761.

Bauer, H., Kasper-Giebl, A., Löflund, M., Giebl, H., Hitzenberger, R., Zibuschka, F., Puxbaum, H., 2002. The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. Atmos. Res. 64, 109-119.

Behzad, H., Gojobori, T., Mineta, K., 2015. Challenges and opportunities of airborne metagenomics. Genome Boil. Evol. 7, 1216-1226.

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

Bigg, E.K., Soubeyrand, S., Morris, C.E., 2015. Persistent after-effects of heavy rain on concentrations of ice nuclei and rainfall suggest a biological cause. Atmos. Chem. Phys. 15, 2313-2326.

Biggerstaff, J.P., Le Puil, M., Weidow, B.L., Prater, J., Glass, K., Radosevich, M., White, D.C., 2006. New methodology for viability testing in environmental samples. Mol. Cell. Probe 20, 141-146.

Blanchard, D.C., 1989. The ejection of drops from the sea and their enrichment with bacteria and other materials: A review. Estuaries 12, 127-137.

Bolter, M., Bloem, J., Meiners, K., Moller, R., 2002. Enumeration and biovolume determination of microbial cells – a methodological review and recommendations for applications in ecological research. Biol. Fertil. Soils 36, 249-259.

Boulos, L., Prevost, M., Barbeau, B., Coallier, J., Desjardins, R., 1999. LIVE/DEAD® *Bac*Light™: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. J. Microbiol. Methods 37, 77-86.

Bowers, R.M., Clements, N., Emerson, J.B., Wiedinmyer, C., Hannigan, M.P., Fierer, N., 2013. Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. Environ. Sci. Technol. 47, 12097-12106.

Bowers, R.M., McLetchie, S., Knight, R., Fierer, N., 2011a. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. ISME J. 5, 601-612.

Bowers, R.M., Sullivan, A.P., Costello, E.K., Collett, J.L., Jr., Knight, R., Fierer, N., 2011b. Sources of bacteria in outdoor air across cities in the midwestern United States. Appl. Environ. Microbiol. 77, 6350-6356.

Burrows, S., Butler, T., Jöckel, P., Tost, H., Kerkweg, A., Pöschl, U., Lawrence, M., 2009a. Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems. Atmos. Chem. Phys. 9, 9281-9297.

Burrows, S., Elbert, W., Lawrence, M., Pöschl, U., 2009. Bacteria in the global atmosphere – Part 1: Review and synthesis of literature data for different ecosystems. Atmos. Chem. Phys. 9, 9263-9280.

Burrows, S.M., Hoose, C., Pöschl, U., Lawrence, M.G., 2013. Ice nuclei in marine air: biogenic particles or dust? Atmos. Chem. Phys. 13, 245-267.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA 108, 4516-4522.

Cappa, F., Suciu, N., Trevisan, M., Ferrari, S., Puglisi, E., Cocconcelli, P.S., 2014. Bacterial diversity in a contaminated Alpine glacier as determined by culture-based and molecular approaches. Sci. Total Environ. 497-498, 50-59.

Carey, C.J., Hart, S.C., Aciego, S.M., Riebe, C.S., Blakowski, M.A., Aronson, E.L., 2016. Microbial community structure of subalpine snow in the Sierra Nevada, California. Arct. Antarct. Alp. Res. 48, 685-701.

Carpenter, E.J., Lin, S., Capone, D.G., 2000. Bacterial activity in South Pole snow. Appl. Environ. Microbiol. 66, 4514-4517.

Casareto, B.E., Suzuki, Y., Okada, K., Morita, M., 1996. Biological micro-particles in rain water. Geophys. Res. Lett. 23, 173-176.

Cavaletti, L., Monciardini, P., Bamonte, R., Schumann, P., Rohde, M., Sosio, M., Donadio, S., 2006. New lineage of filamentous, spore-forming, gram-positive bacteria from soil. Appl. Environ. Microbiol. 72, 4360-4369.

Chen, H.J., Chang, H., 2014. Response of discharge, TSS, and *E. coli* to rainfall events in urban, suburban, and rural watersheds. Environ. Sci. Proc. Impacts 16, 2313-2324.

Chi, M.-C., Li, C.-S., 2007. Fluorochrome in monitoring atmospheric bioaerosols and correlations with meteorological factors and air pollutants. Aerosol Sci. Technol. 41, 672-678.

Cho, B.C., Hwang, C.Y., 2011. Prokaryotic abundance and 16S rRNA gene sequences detected in marine aerosols on the East Sea (Korea). FEMS Microbiol. Ecol. 76, 327-341.

Cho, B.C., Jang, G.I., 2014. Active and diverse rainwater bacteria collected at an inland site in spring and summer 2011. Atmos. Environ. 94, 409-416.

Christner, B.C., Cai, R., Morris, C.E., McCarter, K.S., Foreman, C.M., Skidmore, M.L., Montross, S.N., Sands, D.C., 2008a. Geographic, seasonal, and precipitation chemistry influence on the abundance and activity of biological ice nucleators in rain and snow. Proc. Natl. Acad. Sci. USA. 105, 18854-18859.

Christner, B.C., Morris, C.E., Foreman, C.M., Cai, R., Sands, D.C., 2008b. Ubiquity of biological ice nucleators in snowfall. Science 319, 1214-1214.

Christner, B.C., Mosley-Thompson, E., Thompson, L.G., Reeve, J.N., 2005. Classification of bacteria from polar and nonpolar glacial ice, in: Castello, J.D., Rogers, S.O. (Eds.), *Life in Ancient Ice*. Princeton University Press, pp. 227-239.

Collins, J.S., Goldsmith, T.H., 1981. Spectral properties of fluorescence induced by glutaraldehyde fixation. J. Histochem. Cytochem. 29, 411-414.

Constantinidou, H., Hirano, S., Baker, L., Upper, C., 1990. Atmospheric dispersal of ice nucleation-active bacteria: the role of rain. Phytopathology 80, 934-937.

Creamean, J.M., Suski, K.J., Rosenfeld, D., Cazorla, A., DeMott, P.J., Sullivan, R.C., White, A.B., Ralph, F.M., Minnis, P., Comstock, J.M., Tomlinson, J.M., Prather, K.A., 2013. Dust and biological aerosols from the Sahara and Asia influence precipitation in the western U.S. Science 339, 1572-1578.

D'Amico, S., Collins, T., Marx, J.C., Feller, G., Gerday, C., 2006. Psychrophilic microorganisms: challenges for life. EMBO Rep. 7, 385-389.

Deguillaume, L., Leriche, M., Amato, P., Ariya, P., Delort, A.-M., Pöschl, U., Chaumerliac, N., Bauer, H., Flossmann, A., Morris, C., 2008. Microbiology and atmospheric processes: chemical interactions of primary biological aerosols. Biogeosciences, 5, 1073-1084.

DeLeon-Rodriguez, N., Lathem, T.L., Rodriguez, R.L., Barazesh, J.M., Anderson, B.E., Beyersdorf, A.J., Ziemba, L.D., Bergin, M., Nenes, A., Konstantinidis, K.T., 2013. Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. Proc. Nat. Acad. Sci. USA 110, 2575-2580.

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

Delort, A.M., Vaïtilingom, M., Joly, M., Amato, P., Wirgot, N., Lallement, A., Sancelme, M., Matulova, M., Deguillaume, L., 2017. Clouds: a transient and stressing habitat for microorganisms, in: Chénard, C., Lauro, F.M. (Eds.), *Microbial Ecology of Extreme Environments*. Springer International Publishing AG, pp. 215-245.

DeMott, P.J., Prenni, A.J., 2010. New directions: need for defining the numbers and sources of biological aerosols acting as ice nuclei. Atmos. Environ. 44, 1944-1945.

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

Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E., Khan, W., 2014. Distribution of indigenous bacterial pathogens and potential pathogens associated with roof-harvested rainwater. Appl. Environ. Microbiol. 80, 2307-2316.

Du, R., Du, P., Lu, Z., Ren, W., Liang, Z., Qin, S., Li, Z., Wang, Y., Fu, P., 2017. Evidence for a missing source of efficient ice nuclei. Sci. Rep. 7, 39673.

EANET., 2000. Quality Assurance/Quality Control (QA/QC) Program for Wet Deposition Monitoring in East Asia. The Second Interim Scientific Advisory Group Meeting of Acid Deposition Monitoring Network in East Asia (EANET).

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996-998.

Elmir, S.M., Shibata, T., Solo-Gabriele, H.M., Sinigalliano, C.D., Gidley, M.L., Miller, G., Plano, L.R., Kish, J., Withum, K., Fleming, L.E., 2009. Quantitative evaluation of enterococci and Bacteroidales released by adults and toddlers in marine water. Water Res. 43, 4610-4616.

Evans, C.A., Coombes, P.J., Dunstan, R.H., 2006. Wind, rain and bacteria: The effect of weather on the microbial composition of roof-harvested rainwater. Water Res. 40, 37-44.

Fahimi, H.D., Drochmans, P., Popowski, A., 1968. Purification of glutaraldehyde its significance for preservation of acid phosphatase activity. J. Histochem. Cytochem. 16, 199-204.

Fahlgren, C., Hagstrom, A., Nilsson, D., Zweifel, U.L., 2010. Annual variations in the diversity, viability, and origin of airborne bacteria. Appl. Environ. Microbiol. 76, 3015-3025.

Fröhlich-Nowoisky, J., Kampf, C.J., Weber, B., Huffman, J.A., Pöhlker, C., Andreae, M.O., Lang-Yona, N., Burrows, S.M., Gunthe, S.S., Elbert, W., Su, H., Hoor, P., Thines, E., Hoffmann, T., Després, V.R., Pöschl, U., 2016. Bioaerosols in the Earth system: climate, health, and ecosystem interactions. Atmos. Res. 182, 346-376.

Fuzzi, S., Mandrioli, P., Perfetto, A., 1997. Fog droplets – an atmospheric source of secondary biological aerosol particles. Atmos. Environ. 31, 287-290.

Gandolfi, I., Bertolini, V., Ambrosini, R., Bestetti, G., Franzetti, A., 2013. Unravelling the bacterial diversity in the atmosphere. Appl. Microbiol. Biotechnol. 97, 4727-4736.

Genitsaris, S., Stefanidou, N., Katsiapi, M., Kormas, K. A., Sommer, U., and Moustaka-Gouni, M., 2017. Variability of airborne bacteria in an urban Mediterranean area (Thessaloniki, Greece), Atmos. Environ., 157, 101-110.

Georgakopoulos, D.G., Després, V., Fröhlich-Nowoisky, J., Psenner, R., Ariya, P.A., Pósfai, M., Ahern, H.E., Moffett, B.F., Hill, T.C.J., 2009. Microbiology and atmospheric processes: biological, physical and chemical characterization of aerosol particles. Biogeosciences 6, 721-737.

González-Toril, E., Amils, R., Delmas, R.J., Petit, J.R., Komárek, J., Elster, J., 2009. Bacterial diversity of autotrophic enriched cultures from remote, glacial Antarctic, Alpine and Andean aerosol, snow and soil samples. Biogeosciences 6, 33-44.

Gorman, S.P., Scott, E.M., Russell, A.D., 1980. Antimicrobial activity, uses and mechanism of action of glutaraldehyde. J. Appl. Bacteriol. 48, 161-190.

Green, R.L., Warren, G.J., 1985. Physical and functional repetition in a bacterial ice nucleation gene. Nature 317, 645-648.

Groudieva, T., Kambourova, M., Yusef, H., Royter, M., Grote, R., Trinks, H., Antranikian, G., 2004. Diversity and cold-active hydrolytic bacterial characterization of an Arctic snow cover 9 enzymes of culturable bacteria associated with Artic sea ice, Spitzbergen. Extremophiles 8, 475-488.

Gupta, R.S., Nanda, A., Khadka, B., 2017. Novel molecular, structural and evolutionary characteristics of the phosphoketolases from bifidobacteria and *Coriobacteriales*. PloS One 12, e0172176.

Hang, I., Rinttila, T., Zentek, J., Kettunen, A., Alaja, S., Apajalahti, J., Harmoinen, J., de Vos, W.M., Spillmann, T., 2012. Effect of high contents of dietary animal-derived protein or carbohydrates on canine faecal microbiota. BMC Vet. Res. 8, 90.

Hara, K., Maki, T., Kakikawa, M., Kobayashi, F., Matsuki, A., 2016a. Effects of different temperature treatments on biological ice nuclei in snow samples. Atmos. Environ. 140, 415-419.

Hara, K., Maki, T., Kobayashi, F., Kakikawa, M., Wada, M., Matsuki, A., 2016b. Variations of ice nuclei concentration induced by rain and snowfall within a local forested site in Japan. Atmos. Environ. 127, 1-5.

Hara, K., Zhang, D., 2012. Bacterial abundance and viability in long-range transported dust. Atmos. Environ. 47, 20-25.

Hara, K., Zhang, D., Matsusaki, H., Sadanaga, Y., Ikeda, K., Hanaoka, S., et al., 2015. UV-tolerant culturable bacteria in an Asian dust plume transported over the East China Sea. Aerosol Air Qual. Res. 15, 591-599.

Harding, T., Jungblut, A.D., Lovejoy, C., Vincent, W.F., 2011. Microbes in high arctic snow and implications for the cold biosphere. Appl. Environ. Microbiol. 77, 3234-3243.

Hauptmann, A.L., Stibal, M., Baelum, J., Sicheritz-Ponten, T., Brunak, S., Bowman, J.S., Hansen, L.H., Jacobsen, C.S., Blom, N., 2014. Bacterial diversity in snow on North Pole ice floes. Extremophiles 18, 945-951.

Hegg, D.A., Hobbs, P.V., 1981. Cloud water chemistry and the production of sulfates in clouds. Atmos. Environ. 15, 1597-1604.

Hegg, D.A., Hobbs, P.V., Radke, L.F., 1984. Measurements of the scavenging of sulfate and nitrate in clouds. Atmos. Environ. 18, 1939-1946.

Heidelberg, J., Shahamat, M., Levin, M., Rahman, I., Stelma, G., Grim, C., Colwell, R., 1997. Effect of aerosolization on culturability and viability of gram-negative bacteria. Appl. Environ. Microbiol. 63, 3585-3588.

Heo, K.J., Kim, H.B., Lee, B.U., 2014. Concentration of environmental fungal and bacterial bioaerosols during the monsoon season. J. Aerosol Sci. 77, 31-37.

Herlihy, L.J., Galloway, J.N., Mills, A.L., 1987. Bacterial utilization of formic and acetic acid in rainwater. Atmos. Environ. 21, 2397-2402.

Hill, K.A., Shepson, P.B., Galbavy, E.S., Anastasio, C., Kourtev, P.S., Konopka, A., Stirm, B.H., 2007. Processing of atmospheric nitrogen by clouds above a forest environment. J. Geophys. Res. Atmos. 112, D11301, doi:10.1029/2006JD008002.

Hill, T.C., Moffett, B.F., Demott, P.J., Georgakopoulos, D.G., Stump, W.L., Franc, G.D., 2014. Measurement of ice nucleation-active bacteria on plants and in precipitation by quantitative PCR. Appl. Environ. Microbiol. 80, 1256-1267.

Hirano, S. S., Upper, C. D. 1995. Ecology of ice nucleation-active bacteria. In: *Biological Ice Nucleation and Its Applications* (eds Lee R. E., Warren G. J. and Gusta L. V.). American Phytopathological Society, St Paul, MN, 41–61.

Hoose, C., Kristjánsson, J.E., Burrows, S.M., 2010. How important is biological ice nucleation in clouds on a global scale? Environ. Res. Lett. 5, 024009.

Hopwood, D., 1967. Some aspects of fixation with glutaraldehyde. J. Anat. 101, 83-92.

Hu, M., Zhang, J., Wu, Z., 2005. Chemical compositions of precipitation and scavenging of particles in Beijing. Sci. China Ser. B 48, 265-272.

Huffman, J.A., Prenni, A.J., DeMott, P.J., Pöhlker, C., Mason, R.H., Robinson, N.H., Fröhlich-Nowoisky, J., Tobo, Y., Després, V.R., Garcia, E., Gochis, D.J., Harris, E., Müller-Germann, I., Ruzene, C., Schmer, B., Sinha, B., Day, D.A., Andreae, M.O., Jimenez, J.L., Gallagher, M., Kreidenweis, S.M., Bertram, A.K., Pöschl, U., 2013. High concentrations of biological aerosol particles and ice nuclei during and after rain. Atmos. Chem. Phys. 13, 6151-6164.

Itani, G.N., Smith, C.A., 2016. Dust rains deliver diverse assemblages of microorganisms to the eastern Mediterranean. Sci. Rep. 6, 22657.

Joly, D., Faure, D., 2015. Next-generation sequencing propels environmental genomics to the front line of research. Heredity 114, 429-430.

Joly, M., Amato, P., Deguillaume, L., Monier, M., Hoose, C., Delort, A.M., 2014. Quantification of ice nuclei active at near 0°C temperatures in low-altitude clouds at the Puy de Dôme atmospheric station. Atmos. Chem. Phys. 14, 8185-8195.

Joly, M., Attard, E., Sancelme, M., Deguillaume, L., Guilbaud, C., Morris, C.E., Amato, P., Delort, A.-M., 2013. Ice nucleation activity of bacteria isolated from cloud water. Atmos. Environ. 70, 392-400.

Jones, A.M., Harrison, R.M., 2004. The effects of meteorological factors on atmospheric bioaerosol concentrations – a review. Sci. Total Environ. 326, 151-180.

Ju, F., Zhang, T., 2015. 16S rRNA gene high-throughput sequencing data mining of microbial diversity and interactions. Appl. Microbiol. Biotechnol. 99, 4119-4129.

Junge, K., Eicken, H., Deming, J.W., 2004. Bacterial activity at −2 to −20 °C in Arctic wintertime sea ice. Appl. Environ. Microbiol. 70, 550-557.

Junge, K., Eicken, H., Swanson, B.D., Deming, J.W., 2006. Bacterial incorporation of leucine into protein down to −20°C with evidence for potential activity in sub-eutectic saline ice formations. Cryobiology 52, 417-429.

Junge, K., Swanson, B., 2008. High-resolution ice nucleation spectra of sea-ice bacteria: implications for cloud formation and life in frozen environments. Biogeosciences 5, 865-873.

Kang, S.M., Heo, K.J., Lee, B.U., 2015. Why does rain increase the concentrations of environmental bioaerosols during monsoon? Aerosol Air Qual. Res. 15, 2320-2324.

Kaushik, R., Balasubramanian, R., 2012. Assessment of bacterial pathogens in fresh rainwater and airborne particulate matter using real-time PCR. Atmos. Environ. 46, 131-139.

Kaushik, R., Balasubramanian, R., de la Cruz, A.A., 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. Appl. Environ. Microbiol. 78, 2813-2818.

Kaushik, R., Balasubramanian, R., Dunstan, H., 2014. Microbial quality and phylogenetic diversity of fresh rainwater and tropical freshwater reservoir. PloS One 9, e100737.

Kepner, R., Pratt, J.R., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol. Rev. 58, 603-615.

Kiernan, J.A., 2000. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. Microsc. Today 00-1, 8-12.

Kjelleberg, S., Humphrey, B. A., Marshall, K. C., 1982. Effect of interfaces on small, starved marine bacteria. Appl. Environ. Microbiol. 43, 1166-1172.

Konstantinidis, K.T., 2014. Do airborne microbes matter for atmospheric chemistry and cloud formation? Environ. Microbiol. 16, 1482-1484.

Kourtev, P.S., Hill, K.A., Shepson, P.B., Konopka, A., 2011. Atmospheric cloud water contains a diverse bacterial community. Atmos. Environ. 45, 5399-5405.

Laskin, A., Iedema, M.J., Ichkovich, A., Graber, E.R., Taraniuk, I., Rudich, Y., 2005. Direct observation of completely processed calcium carbonate dust particles. Faraday Discuss. 130, 453-468.

Lee, J.Y., Yang, J.S., Han, M., Choi, J., 2010. Comparison of the microbiological and chemical characterization of harvested rainwater and reservoir water as alternative water resources. Sci. Total Environ. 408, 896-905.

Liang, Z.., Du, R., Du, P., Wang, Y., Li, Z., 2014a. Phylogenetic diversity of bacterial aerosols in precipitation of Beijing Area. China Environ. Sci. 34, 317-323.

Liang, Z., Du, R., Du, P., Wang, Y., Li, Z., 2014b. Diversity of bacterial aerosols in precipitation of Shanghai area. Huan Jing Ke Xue 35, 2483-2489.

Lighthart, B., 2000. Mini-review of the concentration variations found in the alfresco atmospheric bacterial populations. Aerobiologia 16, 7-16.

Liu, X.Y., Wang, B.J., Jiang, C.Y., Liu, S.J., 2007. *Micrococcus flavus* sp. nov., isolated from activated sludge in a bioreactor. Int. J. Syst. Evol. Microbiol. 57, 66-69.

Liu, Y., Yao, T., Jiao, N., Kang, S., Xu, B., Zeng, Y., Huang, S., Liu, X., 2009. Bacterial diversity in the snow over Tibetan Plateau Glaciers. Extremophiles 13, 411-423.

Liu, Y., Yao, T., Kang, S., Jiao, N., Zeng, Y., Shi, Y., Luo, T., Jing, Z., Huang, S., 2006. Seasonal variation of snow microbial community structure in the East Rongbuk glacier, Mt. Everest. Chinese Sci. Bull. 51, 1476-1486.

Löfmark, S., Jernberg, C., Jansson, J.K., Edlund, C., 2006. Clindamycin-induced enrichment and long-term persistence of resistant *Bacteroides* spp. and resistance genes. J. Antimicrob. Chemother. 58, 1160-1167.

Lopatina, A., Krylenkov, V., Severinov, K., 2013. Activity and bacterial diversity of snow around Russian Antarctic stations. Res. Microbiol. 164, 949-958.

Lopatina, A., Medvedeva, S., Shmakov, S., Logacheva, M.D., Krylenkov, V., Severinov, K., 2016. Metagenomic analysis of bacterial communities of Antarctic surface snow. Front. Microbiol. 7, 398.

Lu, P., Ma, D., Chen, Y., Guo, Y., Chen, G.Q., Deng, H., et al., 2013. L-glutamine provides acid resistance for *Escherichia coli* through enzymatic release of ammonia. Cell Res. 23, 635-644.

Lu, Z., Du, P., Du, R., Liang, Z., Qin, S., Li, Z., Wang, Y., 2016. The diversity and role of bacterial ice nuclei in rainwater from mountain sites in China. Aerosol Air Qual. Res. 16, 640-652.

Maki, T., Hara, K., Kobayashi, F., Kurosaki, Y., Kakikawa, M., Matsuki, A., Chen, B., Shi, G., Hasegawa, H., Iwasaka, Y., 2015. Vertical distribution of airborne bacterial communities in an Asian-dust downwind area, Noto Peninsula. Atmos. Environ. 119, 282-293.

Maki, T., Ishikawa, A., Kobayashi, F., Kakikawa, M., Aoki, K., Mastunaga, T., Hasegawa, H., Iwasaka, Y., 2011. Effects of Asian dust (KOSA) deposition event on bacterial and microalgal communities in the Pacific Ocean. Asian J. Atmos. Environ. 5, 157-163.

Maki, T., Kurosaki, Y., Onishi, K., Lee, K.C., Pointing, S.B., Jugder, D., Yamanaka, N., Hasegawa, H., Shinoda, M., 2017. Variations in the structure of airborne bacterial communities in Tsogt-Ovoo of Gobi desert area during dust events. Air Qual. Atmos. Health 10, 249-260.

Maki, T., Puspitasari, F., Hara, K., Yamada, M., Kobayashi, F., Hasegawa, H., Iwasaka, Y., 2014. Variations in the structure of airborne bacterial communities in a downwind area during an Asian dust (Kosa) event. Sci. Total Environ. 488-489, 75–84.

Maki, T., Susuki, S., Kobayashi, F., Kakikawa, M., Yamada, M., Higashi, T., Chen, B., Shi, G., Hong, C., Tobo, Y., 2008. Phylogenetic diversity and vertical distribution of a halobacterial community in the atmosphere of an Asian dust (KOSA) source region, Dunhuang City. Air Qual. Atmos. Health 1, 81–89.

Maragakis, L.L., Chaiwarith, R., Srinivasan, A., Torriani, F.J., Avdic, E., Lee, A., Ross, T.R., Carroll, K.C., Perl, T.M., 2009. *Sphingomonas paucimobilis* bloodstream infections associated with contaminated intravenous fentanyl. Emerg. Infect. Dis. 15, 12-18.

Matthias-Maser, S., Bogs, B., Jaenicke, R., 2000. The size distribution of primary biological aerosol particles in cloud water on the mountain Kleiner Feldberg/Taunus (FRG). Atmos. Res. 54, 1-13.

Metzker, M.L., 2010. Sequencing technologies – the next generation. Nat. Rev. Genet. 11, 31-46.

Michaud, A.B., Dore, J.E., Priscu, J.C., Leslie, D., Lyons, W.B., Sands, D.C., 2014a. Biological ice nucleation initiates hailstone formation. J. Geophys. Res. Atmos., 119, 12186-12197.

Michaud, L., Lo Giudice, A., Mysara, M., Monsieurs, P., Raffa, C., Leys, N., Amalfitano, S., Van Houdt, R., 2014b. Snow surface microbiome on the High Antarctic Plateau (DOME C). PloS One 9, e104505.

Migneault, I., Dartiguenave, C., Bertrand, M.J., Waldron, K.C., 2004. Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking. BioTech. 37, 790-806.

Miteva, V., 2008. Bacteria in Snow and Glacier Ice, in: Margesin, R., Schinner, F., Marx, J.-C., Gerday, C. (Eds.), *Psychrophiles: from Biodiversity to Biotechnology*. Springer Berlin Heidelberg, pp. 31-50.

Miteva, V., Teacher, C., Sowers, T., Brenchley, J., 2009. Comparison of the microbial diversity at different depths of the GISP2 Greenland ice core in relationship to deposition climates. Environ. Microbiol. 11, 640-656.

Miteva, V.I., Brenchley, J.E., 2005. Detection and isolation of ultrasmall microorganisms from a 120,000-year-old Greenland glacier ice core. Appl. Environ. Microbiol. 71, 7806-7818.

Miteva, V.I., Sheridan, P.P., Brenchley, J.E., 2004. Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl. Environ. Microbiol. 70, 202-213.

Mohedano, A., Fernandez, J., Gaya, P., Medina, M., Nunez, M., 1997. Effect of pH, temperature and culture medium composition on the production of an extracellular cysteine proteinase by Micrococcus sp. INIA 528. J. Appl. Microbiol. 82, 81-86.

Möhler, O., DeMott, P., Vali, G., Levin, Z., 2007. Microbiology and atmospheric processes: the role of biological particles in cloud physics. Biogeosciences 4, 1059-1071.

Møller, A.K., Søborg, D.A., Abu Al-Soud, W., Sørensen, S.J., Kroer, N., 2017. Bacterial community structure in High-Arctic snow and freshwater as revealed by pyrosequencing of 16S rRNA genes and cultivation. Polar Res. 32, 17390.

Monteil, C.L., Bardin, M., Morris, C.E., 2014. Features of air masses associated with the deposition of *Pseudomonas syringae* and *Botrytis cinerea* by rain and snowfall. ISME J. 8, 2290-2304.

Moon, H.-S., Lee, J.-H., Kwon, K., Jung, H.-I., 2012. Review of recent progress in micro-systems for the detection and analysis of airborne microorganisms. Anal. Lett. 45, 113-129.

Morris, C., Sands, D., Bardin, M., Jaenicke, R., Vogel, B., Leyronas, C., Ariya, P., Psenner, R., 2011. Microbiology and atmospheric processes: research challenges concerning the impact of airborne micro-organisms on the atmosphere and climate. Biogeosciences 8, 17-25.

Morris, C.E., Conen, F., Alex Huffman, J., Phillips, V., Poschl, U., Sands, D.C., 2014. Bioprecipitation: a feedback cycle linking earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. Glob. Change Biol. 20, 341-351.

Morris, C.E., Georgakopoulos, D.G., Sands, D.C., 2004. Ice nucleation active bacteria and their potential role in precipitation. J. Phys. IV France 121, 87-103.

Morris, C.E., Sands, D.C., Bardin, M., Jaenicke, R., Vogel, B., Leyronas, C., Ariya, P.A., Psenner, R., 2011. Microbiology and atmospheric processes: research challenges concerning the impact of airborne micro-organisms on the atmosphere and climate. Biogeosciences 8, 17-25.

Mortazavi, R., Attiya, S., Ariya, P.A., 2015. Arctic microbial and next-generation sequencing approach for bacteria in snow and frost flowers: selected identification, abundance and freezing nucleation. Atmos. Chem. Phys. 15, 6183-6204.

Mullaugh, K.M., Willey, J.D., Kieber, R.J., Mead, R.N., G. B. Avery, J., 2013. Dynamics of the chemical composition of rainwater throughout Hurricane Irene. Atmos. Chem. Phys. 13, 2321-2330.

Murata, K., Zhang, D., 2013. Applicability of LIVE/DEAD BacLight stain with glutaraldehyde fixation for the measurement of bacterial cell concentration and viability in the air. Aerosol Air Qual. Res. 13, 1755-1767.

Murata, K., Zhang, D., 2014. Transport of bacterial cells toward the Pacific in Northern Hemisphere westerly winds. Atmos. Environ. 87, 138-145.

Murata, K., Zhang, D., 2016. Concentration of bacterial aerosols in response to synoptic weather and land-sea breeze at a seaside site downwind of the Asian continent. J. Geophys. Res. Atmos. 121, 11636-11647.

Natsume, T., Suzuki, Y., 2001. Abundance of biological micro-particles in rain water. Geosci. Repts. Shizuoka Univ. 28, 57-65 (In Japanese).

Núñez, A., Amo de Paz, G., Rastrojo, A., García, A.M., Alcamí, A., Gutiérrez-Bustillo, A.M., Moreno, D.A., 2016. Monitoring of airborne biological particles in outdoor atmosphere. Part 1: Importance, variability and ratios. Int. Microbiol. 19, 1-13.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14, 313-334.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M., 2007. The vegan package. Community Ecol. Package 10, 631-637.

Orphan, V., Taylor, L., Hafenbradl, D., Delong, E., 2000. Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. Appl. Environ. Microbiol. 66, 700-711.

Ouyang, W., Guo, B., Cai, G., Li, Q., Han, S., Liu, B., Liu, X., 2015. The washing effect of precipitation on particulate matter and the pollution dynamics of rainwater in downtown Beijing. Sci. Total Environ. 505, 306-314.

Pace, N.R., Stahl, D.A., Lane, D.J., Olsen, G.J., 1986. The analysis of natural microbial populations by ribosomal RNA sequences, *Advances in Microbial Ecology*. Springer, pp. 1-55.

Panikov, N.S., Flanagan, P.W., Oechel, W.C., Mastepanov, M.A., Christensen, T.R., 2006. Microbial activity in soils frozen to below −39°C. Soil Biol. Biochem. 38, 785-794.

Peccia, J., Hernandez, M., 2006. Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. Atmos. Environ. 40, 3941-3961.

Peracchia, C., Mittler, B.S., 1972. New glutaraldehyde fixation procedures. J. Ultrastruct. Res. 39, 57-64.

Peter, H., Hörtnagl, P., Reche, I., Sommaruga, R., 2014. Bacterial diversity and composition during rain events with and without Saharan dust influence reaching a high mountain lake in the Alps. Environ. Microbiol. Rep. 6, 618-624.

Posch, T., Loferer-Krossbacher, M., Gao, G., Alfreider, A., Pernthaler, J., Psenner, R., 2001. Precision of bacterioplankton biomass determination: a comparison of two fluorescent dyes, and of allometric and linear volume-to-carbon conversion factors. Aquatic Microb. Ecol. 25, 55-63.

Pöschl, U., Martin, S.T., Sinha, B., Chen, Q., Gunthe, S.S., Huffman, J.A., Borrmann, S., Farmer, D.K., Garland, R.M., Helas, G., Jimenez, J.L., King, S.M., Manzi, A., Mikhailov, E., Pauliquevis, T., Petters, M.D., Prenni, A.J., Roldin, P., Rose, D., Schneider, J., Su, H., Zorn, S.R., Artaxo, P., Andreae, M.O., 2010. Rainforest aerosols as biogenic nuclei of clouds and precipitation in the Amazon. Science 329, 1513-1516.

Pratt, K.A., DeMott, P.J., French, J.R., Wang, Z., Westphal, D.L., Heymsfield, A.J., Twohy, C.H., Prenni, A.J., Prather, K.A., 2009. In situ detection of biological particles in cloud ice-crystals. Nat. Geosci. 2, 398-401.

Prenni, A.J., Petters, M.D., Kreidenweis, S.M., Heald, C.L., Martin, S.T., Artaxo, P., Garland, R.M., Wollny, A.G., Pöschl, U., 2009. Relative roles of biogenic emissions and Saharan dust as ice nuclei in the Amazon basin. Nat. Geosci. 2, 402-405.

Prenni, A.J., Tobo, Y., Garcia, E., DeMott, P.J., Huffman, J.A., McCluskey, C.S., Kreidenweis, S.M., Prenni, J.E., Pöhlker, C., Pöschl, U., 2013. The impact of rain on ice nuclei populations at a forested site in Colorado. Geophys. Res. Lett. 40, 227-231.

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc. Natl. Acad. Sci. USA 101, 4631-4636.

Psenner, R., 2006. Bona fide microbial habitats: snow, ice, and cloud water? ESF LESC Exploratory Workshop: Microbiological Meteorology: Working at the Intersection of Biology, Physics and Meteorology to Understand and Regulate the Microbial Component of Weather, Avignon, France.

Psenner, R., Wille, A., Sattler, B., Priscu, J.C., Felip, M., Wagenbach, D., 2009. Ice ecosystems and biodiversity, in: Gerday, C., Glansdorff, N. (Eds.), *Extremophiles-Volume II*. EOLSS Publishers, pp. 33-66.

Qi, J., Zhong, X., Shao, Q., Gao, D., Wu, L., Huang, L., Ye, Y., 2015. Microbial activity levels in atmospheric bioaerosols in Qingdao. Aerobiologia 31, 353-365.

Rathnayake, C.M., Metwali, N., Jayarathne, T., Kettler, J., Huang, Y., Thorne, P.S., O'Shaughnessy, P.T., Stone, E.A., 2017. Influence of rain on the abundance of bioaerosols in fine and coarse particles. Atmos. Chem. Phys. 17, 2459-2475.

Reichenbach, H., 2001. Myxobacteria, producers of novel bioactive substances. J. Ind. Microbiol. Biotechnol. 27, 149-156.

Renard, P., Canet, I., Sancelme, M., Wirgot, N., Deguillaume, L., Delort, A.-M., 2016. Screening of cloud microorganisms isolated at the Puy de Dôme (France) station for the production of biosurfactants. Atmos. Chem. Phys. 16, 12347-12358.

Roman, P., Polkowska, Ż., Namieśnik, J., 2013. Sampling procedures in studies of cloud water composition: a review. Crit. Rev. Environ. Sci. Technol. 43, 1517-1555.

Ross, P.W., 1966. A new disinfectant. J. Clin. Pathol. 19, 318-320.

Ryan, M., Adley, C., 2010. *Sphingomonas paucimobilis*: a persistent Gram-negative nosocomial infectious organism. J. Hosp. Infect. 75, 153-157.

Salyers, A.A., Gupta, A., Wang, Y., 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. 12, 412-416.

Santiago-Rodriguez, T.M., Tremblay, R.L., Toledo-Hernandez, C., Gonzalez-Nieves, J.E., Ryu, H., Santo Domingo, J.W., Toranzos, G.A., 2012. Microbial quality of tropical inland waters and effects of rainfall events. Appl. Environ. Microbiol. 78, 5160-5169.

Šantl-Temkiv, T., Finster, K., Hansen, B.M., Pašić, L., Karlson, U.G., 2013. Viable methanotrophic bacteria enriched from air and rain can oxidize methane at cloud-like conditions. Aerobiologia 29, 373-384.

Šantl-Temkiv, T., Sahyoun, M., Finster, K., Hartmann, S., Augustin-Bauditz, S., Stratmann, F., Wex, H., Clauss, T., Nielsen, N.W., Sørensen, J.H., Korsholm, U.S., Wick, L.Y., Karlson, U.G., 2015. Characterization of airborne ice-nucleation-active bacteria and bacterial fragments. Atmos. Environ. 109, 105-117.

Sattler, B., Puxbaum, H., Psenner, R., 2001. Bacterial growth in supercooled cloud droplets. Geophys. Res. Lett. 28, 239-242.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537-7541.

Schnell, R., 1977. Ice nuclei in seawater, fog water and marine air off the coast of Nova Scotia: Summer 1975. J. Atmos. Sci. 34, 1299-1305.

Schriewer, A., Miller, W.A., Byrne, B.A., Miller, M.A., Oates, S., Conrad, P.A., Hardin, D., Yang, H.-H., Chouicha, N., Melli, A., Jessup, D., Dominik, C., Wuertz, S., 2010. Presence of *Bacteroidales* as a predictor of pathogens in surface waters of the central California coast. Appl. Environ. Microbiol. 76, 5802-5814.

Segawa, T., Miyamoto, K., Ushida, K., Agata, K., Okada, N., Kohshima, S., 2005. Seasonal change in bacterial flora and biomass in mountain snow from the Tateyama Mountains, Japan, analyzed by 16S rRNA gene sequencing and real-time PCR. Appl. Environ. Microbiol. 71, 123-130.

Seinfeld, J.H., Pandis, S.N., 2012. Atmospheric chemistry and physics: from air pollution to climate change. John Wiley & Sons.

Sheng, X., Ting, Y.P., Pehkonen, S.O., 2008. The influence of ionic strength, nutrients and pH on bacterial adhesion to metals. J. Colloid. Interface Sci. 321, 256-264.

Small, P., Blankenhorn, D., Welty, D., Zinser, E., Slonczewski, J.L., 1994. Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: role of *rpoS* and growth pH. J. Bacteriol. 176, 1729-1737.

Smit, P., Heniger, J., 1975. Antoni van Leeuwenhoek (1632–1723) and the discovery of bacteria. Antonie van Leeuwenhoek 41, 217-228.

Smith, D.J., Jaffe, D.A., Birmele, M.N., Griffin, D.W., Schuerger, A.C., Hee, J., Roberts, M.S., 2012. Free tropospheric transport of microorganisms from Asia to North America. Microb. Ecol. 64, 973-985.

Stephen, R.H., Stephen, R.H., 2003. Bacterial activity in South Pole snow is questionable. Appl. Environ. Microbiol. 69, 6340-6341

Stopelli, E., Conen, F., Guilbaud, C., Zopfi, J., Alewell, C., Morris, C.E., 2017. Ice nucleators, bacterial cells and *Pseudomonas syringae* in precipitation at Jungfraujoch. Biogeosciences 14, 1189-1196.

Stülke, J., Hillen, W., 2000. Regulation of carbon catabolism in *Bacillus* species. Annu. Rev. Microbiol. 54, 849-880.

Su, C., Lei, L., Duan, Y., Zhang, K.Q., Yang, J., 2012. Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. Appl. Microbiol. Biotechnol. 93, 993-1003.

Sun, J., Ariya, P.A., 2006. Atmospheric organic and bio-aerosols as cloud condensation nuclei (CCN): A review. Atmos. Environ. 40, 795-820.

Suzuki, M.T., Sherr, E.B., Sherr, B.F., 1993. DAPI direct counting underestimates bacterial abundances and average cell size compared to AO direct counting. Limnol. Oceanogr. 38, 1566-1570.

Toyonaga, S., Zhang, D., 2016. Wet deposition fluxes of ions contributed by cyclone-, stationary front- and typhoon-associated rains at the southwestern Japan coast. Asian J. Atmos. Environ. 10-2, 57-66.

Vaïtilingom, M., Amato, P., Sancelme, M., Laj, P., Leriche, M., Delort, A.M., 2010. Contribution of microbial activity to carbon chemistry in clouds. Appl. Environ. Microbiol. 76, 23-29.

Vaïtilingom, M., Attard, E., Gaiani, N., Sancelme, M., Deguillaume, L., Flossmann, A.I., Amato, P., Delort, A.-M., 2012. Long-term features of cloud microbiology at the puy de Dôme (France). Atmos. Environ. 56, 88-100.

Vaïtilingom, M., Charbouillot, T., Deguillaume, L., Maisonobe, R., Parazols, M., Amato, P., Sancelme, M., Delort, A.M., 2011. Atmospheric chemistry of carboxylic acids: microbial implication versus photochemistry. Atmos. Chem. Phys. 11, 8721-8733.

Vaïtilingom, M., Deguillaume, L., Vinatier, V., Sancelme, M., Amato, P., Chaumerliac, N., Delort, A.M., 2013. Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. Proc. Natl. Acad. Sci. USA 110, 559-564.

Vali, G., 1971. Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids. J. Atmos. Sci. 28, 402-409.

Vali, G., Christensen, M., Fresh, R., Galyan, E., Maki, L., Schnell, R., 1976. Biogenic ice nuclei. Part II: Bacterial sources. J. Atmos. Sci. 33, 1565-1570.

Wei, M., Xu, C., Chen, J., Zhu, C., Li, J., Lv, G., 2017. Characteristics of bacterial community in cloud water at Mt Tai: similarity and disparity under polluted and non-polluted cloud episodes. Atmos. Chem. Phys., 17, 5253-5270.

Wieprecht, W., Acker, K., Mertes, S., Collett, J., Jaeschke, W., Brüggemann, E., Möller, D., Herrmann, H., 2005. Cloud physics and cloud water sampler comparison during FEBUKO. Atmos. Environ. 39, 4267-4277.

Wilks, J.C., Kitko, R.D., Cleeton, S.H., Lee, G.E., Ugwu, C.S., Jones, B.D., et al., 2009. Acid and base stress and transcriptomic responses in *Bacillus subtilis*. Appl. Environ. Microbiol. 75, 981-990.

Wilson, T.W., Ladino, L.A., Alpert, P.A., Breckels, M.N., Brooks, I.M., Browse, J., Burrows, S.M., Carslaw, K.S., Huffman, J.A., Judd, C., Kilthau, W.P., Mason, R.H., McFiggans, G., Miller, L.A., Nájera, J.J., Polishchuk, E., Rae, S., Schiller, C.L., Si, M., Temprado, J.V., Whale, T.F., Wong, J.P.S., Wurl, O., Yakobi-Hancock, J.D., Abbatt, J.P.D., Aller, J.Y., Bertram, A.K., Knopf, D.A., Murray, B.J., 2015. A marine biogenic source of atmospheric ice-nucleating particles. Nature 525, 234-238.

Wolf, R., El-Haddad, I., Slowik, J.G., Dällenbach, K., Bruns, E., Vasilescu, J., Baltensperger, U., Prévôt, A.S.H., 2017. Contribution of bacteria-like particles to $PM_{2.5}$ aerosol in urban and rural environments. Atmos. Environ. 160, 97-106.

Wolf, R., Slowik, J.G., Schaupp, C., Amato, P., Saathoff, H., Möhler, O., Prévôt, A.S.H., Baltensperger, U., 2015. Characterization of ice-nucleating bacteria using on-line electron impact ionization aerosol mass spectrometry. J. Mass Spectrom. 50, 662-671.

Womack, A.M., Bohannan, B.J., Green, J.L., 2010. Biodiversity and biogeography of the atmosphere. Philos. Trans. R. Soc. London, Ser. B 365, 3645-3653.

Wunderlin, T., Ferrari, B., Power, M., 2016. Global and local-scale variation in bacterial community structure of snow from the Swiss and Australian Alps. FEMS Microbial. Ecol. 92, fiw132.

Xu, C., Wei, M., Chen, J., Sui, X., Zhu, C., Li, J., Zheng, L., Sui, G., Li, W., Wang, W., Zhang, Q., Mellouki, A., 2017. Investigation of diverse bacteria in cloud water at Mt. Tai, China. Sci. Total Environ. 580, 258-265.

Xu, Z., Wu, Y., Shen, F., Chen, Q., Tan, M., Yao, M., 2011. Bioaerosol science, technology, and engineering: past, present, and future. Aerosol Sci. Technol. 45, 1337-1349.

Yadav, S., Kaushik, R., Saxena, A.K., Arora, D.K., 2011. Diversity and phylogeny of plant growth-promoting bacilli from moderately acidic soil. J. Basic Microbiol. 51, 98-106.

Yakobi-Hancock, J.D., Ladino, L.A., Abbatt, J.P., 2014. Review of recent developments and shortcomings in the characterization of potential atmospheric ice nuclei: focus on the tropics. Revista de Ciencias 17, 15-34.

Yamaguchi, N., Ichijo, T., Baba, T., Nasu, M., 2014. Long-range transportation of bacterial cells by Asian dust. Genes Environ. 36, 145-151.

Yamashita, H., Taoka, A., Uchihashi, T., Asano, T., Ando, T., Fukumori, Y., 2012. Single-molecule imaging on living bacterial cell surface by high-speed AFM. J. Mol. Biol. 422, 300-309.

Ye, R.W., Thomas, S.M., 2001. Microbial nitrogen cycles: physiology, genomics and applications. Curr. Opin. Microbiol. 4, 307-312.

Yoo, K., Lee, T.K., Choi, E.J., Yang, J., Shukla, S.K., Hwang, S.I., Park, J., 2017. Molecular approaches for the detection and monitoring of microbial communities in bioaerosols: A review. J. Environ. Sci. 51, 234-247.

Yu, S., Gao, C., Cheng, Z., Cheng, X., Cheng, S., Xiao, J., Ye, W., 1998. An analysis of chemical composition of different rain types in 'Minnan Golden Triangle' region in the southeastern coast of China. Atmos. Res. 47, 245-269.

Zaitseva, N.A., 2009. Biological and microbiological properties of atmospheric water, in: Khublaryan, M.G. (Ed.), *Types and Properties of Water - Volume I*. EOLSS Publications, pp. 256-264.

Zhang, D., Iwasaka, Y., Matsuki, A., Ueno, K., Matsuzaki, T., 2006. Coarse and accumulation mode particles associated with Asian dust in southwestern Japan. Atmos. Environ. 40, 1205-1215.

Zhang, D., Murata, K., Hu, W., Yuan, H., Li, W., Matsusaki, H., Kakikawa, M., 2017. Concentration and viability of bacterial aerosols associated with weather in Asian continental outflow: current understanding. Aerosol Sci. Eng. 1, 66-77.

Zhang, M., Wang, S., Wu, F., Yuan, X., Zhang, Y., 2007. Chemical compositions of wet precipitation and anthropogenic influences at a developing urban site in southeastern China. Atmos. Res. 84, 311-322.

Zhang, S., Hou, S., Qin, X., Du, W., Liang, F., Li, Z., 2014. Preliminary study on effects of glacial retreat on the dominant glacial snow bacteria in Laohugou Glacier No. 12. Geomicrobiol. J. 32, 113-118.

Zhang, S., Yang, G., Wang, Y., Hou, S., 2010. Abundance and community of snow bacteria from three glaciers in the Tibetan Plateau. J. Environ. Sci. 22, 1418-1424.

Zhong, X., Qi, J., Li, H., Dong, L., Gao, D., 2016. Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region. Atmos. Environ. 140, 506-513.

APPENDIX Abbreviations

ACE	Abundance-base coverage estimator
ADP	Adenosine diphosphate
ANOSIM	Analysis of similarities
AO	Acridine orange
AODC	Acridine orange direct count
ARDRA	Amplified ribosomal DNA restriction analysis
AToFMS	Aerosol time of flight mass spectrometry
ATP	Adenosine triphosphate
CASCC	Caltech active strand cloud water collector
CCN	Cloud condensation nuclei
CFU	Colony-forming unit
CIMs	Culture-independent methods
CTC	5-cyano-2,3-ditolyl tetrazolium chloride
CVI	Counterflow virtual impactor
CWS	Cloud water samplers
DAPI	4', 6-diamidino-2-phenylindole
DFA	Droplet-freezing assay
DGGE	Denaturing gradient gel electrophoresis analysis
DNA	Deoxyribonucleic acid
DON	Dissoveld organic nitrogen
dsDNA	Double-stranded DNA
EC	Electrical conductivity
EDX	Energy dispersive X-ray detector
EFM	Epifluorescence microscopy
EPS	Exopolymer secretions
FCM	Flow cytometry
FIB	Fecal indicator bacteria
FISH	Fluorescence in situ hybridization
HYSPLIT	Hybrid single particle Lagrangian integrated trajectory
ICPS	Isokinetic cloud probing system
IN	Ice nuclei
INA	Ice nucleation-active
INP	Ice nucleation particle
LB	Luriae-Bertani
LWC	Liquid water content
MA	Marine agar

ME-2	Multilinear engine
NGS	Next-generation sequencing
NMR	Nuclear magnetic resonance spectroscopy
nss	Non-sea salt
OD	Optical depth
OTU	Operational taxonomic unit
PBAP	Primary biological aerosol particles
PBS	Phosphate buffer saline
PCA	Plate count agar
PCA	Principal component analysis
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
PI	Propidium iodide
PMF	Positive matrix factorization
PMP	Polymethylpentene
PTFE	Polytetrafluoroethylene
PVM	Particle volume monitor
QA	Quality assurance
QC	Quality control
qPCR	Quantitative polymerase chain reaction
R2A	Reasoner's 2A
RAC	Rotating arm collector
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RWH	Rainwater harvesting
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TGGE	Temperature gradient gel electrophoresis
TOC	Total organic carbon
T-RFLP	Terminal restriction fragment length polymorphism
TSA	Tryptic soy agar
TSB	Tryptic soy broth
USNS	United States Naval Ship
UV	Ultraviolet

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PEER-REVIEWED PUBLICATIONS

[1] **W. Hu**, K. Murata, Y. Horikawa, A. Naganuma, D. Zhang*. 2017. Bacterial community composition in rainwater associated with synoptic weather in an area downwind of the Asian continent. *Sci. Total Environ.*, 601–602, 1775–1784.

[2] **W. Hu**, K. Murata, S. Toyonaga, D. Zhang*. 2017. Bacterial abundance and viability in rainwater associated with cyclones, stationary fronts and typhoons in southwestern Japan. *Atmos. Environ.*, 167, 104–115.

[3] **W. Hu**, K. Murata, D. Zhang*. 2017. Applicability of LIVE/DEAD BacLight stain with glutaraldehyde fixation for the measurement of bacterial abundance and viability in rainwater. *J. Environ. Sci.*, 51, 202–213.

[4] **W. Hu**, K. Murata, S. Fukuyama, Y. Kawai, E. Oka, M. Uematsu, D. Zhang*. 2017. Concentration and viability of airborne bacteria over the Kuroshio extension region in the northwestern Pacific Ocean: data from three cruises. *J. Geophys. Res. Atmos.*, **under revision after peer-review**.

[5] **W. Hu**, M. Hu*, W-W. Hu, J. Zheng, C. Chen, Y. Wu, S. Guo. 2017. Seasonal variations in high time-resolved chemical compositions, sources and evolution of atmospheric submicron aerosols in the megacity Beijing. *Atmos. Chem. Phys.*, 17, 9979-10000.

[6] **W. Hu**, M. Hu*, W.W. Hu, H.Y. Niu, J. Zheng, Y.S. Wu, W.T. Chen, C. Chen, L.Y. Li, M. Shao, S.D. Xie, Y.H. Zhang. 2016. Characterization of submicron aerosols influenced by biomass burning at a site in the Sichuan Basin, southwestern China. *Atmos. Chem. Phys.*, 16, 13213–13230.

[7] **W. Hu**, H. Niu, D. Zhang, Z. Wu, C. Chen, Y. Wu, D. Shang, M. Hu*. 2016. Insights into a dust event transported through Beijing in spring 2012: Morphology, chemical composition and impact on surface aerosols. *Sci. Total Environ.*, 565, 287–298.

[8] H. Niu, W. Cheng, **W. Hu***, W. Pian. 2016. Characteristics of individual particles in a severe short-period haze episode induced by biomass burning in Beijing. *Atmos. Pollut. Res.*, 7, 1072–1081.

[9] **W. Hu**, M. Hu*, Q. Tang, S. Guo, C. Yan. 2013. Characterization of particulate pollution during Asian Games in Pearl River Delta (PRD) region. *Acta Sci. Circumst.*, 33, 1815–1823 (In Chinese).

[10] D. Zhang*, K. Murata, **W. Hu**, H. Yuan, W. Li, H. Matsusaki, M. Kakikawa. 2017. Concentration and viability of bacterial aerosols associated with weather in Asian continental outflow: current understanding. *Aerosol Sci. Eng.*, 1, 66–77.

[11] W.W. Hu, M. Hu*, **W. Hu**, J.L. Jimenez, B. Yuan, W. Chen, M. Wang, Y. Wu, C. Chen, Z. Wang, J. Peng, K. Yang, L. Zeng, M. Shao. 2016. Chemical composition, sources and aging process of sub-micron aerosols in Beijing: contrast between summer and winter. *J. Geophys. Res. Atmos.*, 121, 1955–1977.

[12] H. Niu, **W. Hu**, D. Zhang, Z. Wu, S. Guo, W. Pian, W. Cheng, M. Hu*. 2016. Variations of fine particle physiochemical properties during a heavy haze episode in the winter of Beijing. *Sci. Total Environ.*, 571, 103–109.

[13] H. Niu, **W. Hu**, W. Pian, M. Hu, D. Zhang*. 2016. Characteristics of dry deposited mineral particles associated with weather conditions in the adjacent sea areas of East China during a cruise in spring 2011. *Particuology*, 28, 86–92.

[14] H. Niu, D. Zhang, **W. Hu**, J. Shi, R. Li, H. Gao, W. Pian, M. Hu*. 2016. Size and elemental composition of dry deposited particles during a severe dust storm at a coastal site of Eastern China. *J. Environ. Sci.*, 40, 161–168.

[15] W.W. Hu, M. Hu*, **W. Hu**, C. Chen, J. Peng. 2016. Limitations of EC tracer method in areas with complicated primary sources. *Acta Sci. Circumst.* 36, 2121–2130 (In Chinese).

[16] H. Niu, W. Cheng, W. Pian, **W. Hu***. 2016. The physiochemical properties of submicron particles from emissions of industrial furnace. *World J. Eng.*, 13, 218–224.

[17] H. Niu*, **W. Hu**, W. Pian, J. Fan, J. Wang. 2015. Evolution of atmospheric aerosol particles during a pollution accumulation process: a case study, *World J. Eng.*, 12, 51–60.

[18] W.W. Hu, M. Hu*, B. Yuan, J.L. Jimenez, Q. Tang, J. Peng, **W. Hu**, M. Shao, M. Wang, L.M. Zeng, Y.S. Wu, Z.H. Gong, X.F. Huang, L.Y. He. 2013. Insights on organic aerosol aging and the influence of coal combustion at a regional receptor site of central eastern China. *Atmos. Chem. Phys.*, 13, 10095–10112.

[19] Q. Guo, M. Hu*, S. Guo, Z. Wu, W. Hu, J. Peng, **W. Hu**, Y. Wu, B. Yuan, Q. Zhang, Y. Song. 2015. The identification of source regions of black carbon at a receptor site off the eastern coast of China, *Atmos. Environ.*, 100, 78–84.

[20] M. Li, M. Hu*, Y. Wu, Y. Qin, R. Zheng, J. Peng, Q. Guo, Y. Xiao, **W. Hu**, J. Zheng, Z. Du, J. Xiao,

S. Shuai. 2016. Characteristics of particulate organic matters emissions from gasoline direct injection engine and its influence factors. *Proc. CSEE*, 36, 4443–4451 (In Chinese).

CONFERENCE PRESENTATIONS

[1] **W. Hu**, K. Murata, S. Fukuyama, D. Zhang, Y. Kawai. Abundance, viability and community of airborne bacteria over the Kuroshio Extension region, northwest Pacific Ocean. Asian Aerosol Conference (AAC) 2017, **Oral**, Jeju (Korea), Jul. 2–6, 2017.

[2] **W. Hu**, K. Murata, D. Zhang. Bacterial communities in rainwater associated with synoptic weather systems in Kumamoto, southwestern Japan. JpGU-AGU Joint Meeting 2017, **Poster**, Chiba (Japan), May 20–25, 2017.

[3] **W. Hu**, D. Zhang* (Presenter). Bacterial properties of rainwater associated with cyclones, stationary fronts and typhoons in southwestern Japan. 2016 AGU Fall Meeting. **Poster**, San Francisco (USA), Dec. 12–16, 2016.

[4] **W. Hu**, K. Murata, S. Toyonaga, D. Zhang. Abundance, viability and community of bacteria in rainwater associated with cyclones, stationary fronts and typhoons in southwestern Japan. The 3rd Environmental Forum of Nagasaki University, Prefectural University of Kumamoto and Fukuoka Institute of Technology. **Poster**, Fukuoka (Japan), Dec. 16, 2016.

[5] **W. Hu**, D. Zhang. Insights into the sources of rainwater bacteria in southwestern Japan. International Symposium on Atmospheric Haze and Advanced $PM_{2.5}$ Pollution Control Technologies, The 17th Annual Meeting of the China Association for Science and Technology. **Oral & Poster**, Xi′an (China), Sept. 23–25, 2016.

[6] **W. Hu**, K. Murata, D. Zhang. Applicability of LIVE/DEAD BacLight stain with glutaraldehyde fixation for the measurement of bacterial abundance and viability in rainwater. The 33rd Annual Meeting and Symposium on Aerosol Science and Technology. **Oral**, Sakai, Osaka (Japan), Aug. 31–Sept. 2, 2016.

[7] **W. Hu**, K. Murata, D. Zhang. Bacteria in rainwater at a suburban site in southwestern Japan: abundances, viability, communities and sources associated with different weather systems. Asia Oceania Geosciences Society 13th Annual Meeting (AOGS2016). **Oral**, Beijing (China), Jul. 31–Aug. 5, 2016.

[8] M. Hu* (Presenter), **W. Hu**, W. Hu, J. Zheng, S. Guo, Y. Wu, S. Lu, L. Zeng. Seasonal Variations of High Time-Resolved Chemical Compositions, Sources and Evolution for Atmospheric Submicron Aerosols in the Megacity of Beijing. European Geosciences Union General Assembly 2016. **Oral**, Vienna (Austria), Apr. 17–22, 2016.

[9] **W. Hu**, K. Murata, S. Toyonaga, D. Zhang. Bacterial and chemical properties of rainwater collected at a suburban site in Southwestern Japan. The 12th National Aerosol Conference and the 13th Cross-strait Workshop for Aerosol Science & Technology. **Oral**, Chongqing (China), Oct. 22–25, 2015.

[10] **W. Hu**, R. Yasutake, K. Murata, D. Zhang. Bacterial Concentration and Viability in the Rainwater Collected at a Suburban Site in Southwestern Japan in 2014. 2015 Asian Aerosol Conference (AAC). **Poster**, Kanazawa (Japan), Jun. 24–27, 2015.

[11] **W. Hu**, H. Niu, M. Hu, D. Zhang. Physiochemical characteristics of marine atmospheric particles settled on the surface of the Yellow Sea. 2015 Theory and Technique, International Aerosol Conference (T&T IAC). **Poster**, Kitakyushu (Japan), Apr. 20–21, 2015.

[12] **W. Hu**, M. Hu, H. Niu, C. Chen, Z. Gong, Y. Wu. Seasonal variations of highly time-resolved chemical compositions, sources and evolution for atmospheric submicron aerosols in Beijing. 2014 International Aerosol Conference (IAC2014). **Oral**, Busan (Korea), Aug. 28–Sep. 2, 2014.

[13] **W. Hu**, M. Hu, H. Niu, C. Chen, Z. Gong, Y. Wu. Highly time-resolved characterization of atmospheric submicron aerosols in a hazy city in the Sichuan basin, China. The 3rd International Conference on Environmental Simulation and Pollution Control. **Oral**, Beijing, Nov. 7–8, 2013.

[14] **W. Hu**, M. Hu, H. Niu, Z. Gong, Y. Wu, C. Chen, J. Zheng, W. Hu. Characterization of atmospheric submicron aerosols in two haze areas, Beijing and Ziyang, China. The 12th International Conference on Atmospheric Sciences and Applications to Air Quality (ASAAQ). **Oral**, Seoul (Korea), Jun. 3–5, 2013.

[15] **W. Hu**, H. Niu, C. Chen, D. Zhang, M. Hu. Insight into a dust event transported through Beijing in the spring, 2012. The 19th Conference on Atmospheric Environmental Sciences and Technology in China. **Oral**, Qingdao, Nov. 7–10, 2012. (In Chinese)

[16] **W. Hu**, M. Hu, Q. Tang, S. Guo. The effects of particle pollution control implementation during the Asian Games in Guangzhou, China. The 12th International Global Atmospheric Chemistry (IGAC) Science Conference. **Poster**, Beijing, Sept. 17–21, 2012.

WORKSHOP PRESENTATIONS (Since Oct. 2014)

[1] **W. Hu**, K. Murata, S. Toyonaga, D. Zhang*. Concentration, viability and community of bacteria in rainwater in Kumamoto: Dependence on synoptic weather. Meteorological Research Institute (Japan), **Oral**, 30 Mar., 2017.

[2] **W. Hu**, H. Niu, D. Zhang, Z. Wu, M. Hu. Insights into a dust event transported through Beijing in spring 2012: Morphology, chemical composition and impact on surface aerosols, Research Assembly on the Current Situation and Future Development of Chemical Transport Model over Asia, Kyushu University (Japan), **Oral**, Dec. 2, 2016.

[3] **W. Hu**, D. Zhang**.** Bacteria in rainwater at a suburban site in Southwestern Japan and insight of their sources. Sino-Japanese Workshop on Atmospheric Aerosol Study in East Asia, Prefectural University of Kumamoto (Japan), **Oral**, Nov. 14, 2015.

[4] **W. Hu**, M. Hu. Seasonal variations of highly time-resolved chemical compositions, sources and evolution for atmospheric submicron aerosols in Beijing. OUC-PUK Students' Joint Workshop on Environmental Science, Ocean University of China, **Oral**, Sept. 23, 2015.

[5] **W. Hu**, D. Zhang. Bacterial and chemical properties of rainwater collected at a suburban site in southwestern Japan. Sino-Japanese Workshop on Atmospheric Aerosol Study in East Asia, Shandong University (China), **Oral**, Sept. 19–20, 2015.