

Progresses on Metagenomic Airbiome Studies

Metagenomik Havabiyomu Çalışmalarına Dair Gelişmeler

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SUMMARY

The term microbiome refers to the collective genome of the microbial communities living in various environments/habitats, and recently, the understanding of microbiome have been significantly enhanced by metagenomic studies. The data at hand regarding marine and soil-related microbial communities is much more than that of the air, which is typically not colonized, and the number of publications sharp contrasts to those of air. Aerosols of biological origin (bioaerosols) are a subset of atmospheric particulate matter (PM), which are emitted directly from the biosphere into the atmosphere, and play a vital role in the system of the Earth, particularly in the interactions between atmosphere, biosphere, climate and public health. However, these particles could either be the direct cause of epidemics of infectious diseases or noninfectious diseases (e.g. hypersensitivity to aeroallergens). These bioparticles emitted by humans or re-suspended from surfaces in indoor environments are also challenging for infection control and safety. It is estimated that humans emit approximately 10⁶ particles per hour into the surrounding air under seated conditions. The sources, abundance, composition and effects of bioaerosols and the atmospheric microbiome are not yet well characterized; however, there is a continually increasing number of published studies. In this review, current progresses and conducted studies on airborne metagenomics were described and the health effects of aerosols to global perspective were overviewed.

Key Words: Bioaerosol; Particulate matter; Microbiome; Metagenomics; Culture independent approaches

ÖZET

Metagenomik Havabiyomu Çalışmalarına Dair Gelişmeler

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Mikrobiyom terimi çeşitli çevrelerde/habitatlarda yaşayan mikrobiyal komünitelerin kolektif genomuna karşılık gelmekte olup, anlaşılması, önemli ölçüde metagenomik çalışmalarla son zamanlarda artmaktadır. Denizel ve karasal mikrobiyal komünitelerin hakkındaki mevcut bilgi, tipik olarak kolonize edilmeyen havasal çevrelerden daha fazladır ve mevcut yayınların sayısı da oldukça belirgin bir farklılık göstermektedir. Biyolojik kökenli aerosoller (bioaerosoller) atmosferik partikül maddesi (PM) 'nin bir alt bileşeni olup, biyosferden atmosfere doğrudan yayılıp dünya sisteminde özellikle de atmosfer, biyosfer, iklim ve halk sağlığı ile ilişkili olarak hayati bir rol oynamaktadır. Bununla birlikte, bu partiküller enfeksiyöz veya enfeksiyöz olmayan hastalıkların direkt etmeni de olabilmektedir. İnsanlar tarafından da tekrar yayılan veya iç çevrelerde süspand olan bu partiküller, enfeksiyon kontrolü ve güvenlik için de zorluk çıkarmaktadır. Bir insanın oturma pozisyonunda çevresine, saat başı 10⁶ partikül yaydığı tahmin edilmektedir. Atmosferik biyomun ve bioaerosollerin kaynakları, bulunma sıklıkları, kompozisyonu ve etkileri henüz iyi bir şekilde karakterize edilememiştir ancak, sayısal olarak son yıllarda

yapılan çalışmalarda hızlı bir artış gözlenmektedir. Bu derlemede, hava kökenli metagenomik üzerine yürütülen çalışmalar ve ilerlemeler bildirilmiş ve bioaerosollerin sağlığa olan etkilerine küresel perspektifte bir bakış verilmeye çalışılmıştır.

Anahtar Kelimeler: Biyoaerosol; Partikül madde; Mikrobiyom; Metagenomik; Kültür bağımlı olmayan yaklaşım

Poor air quality is the largest environmental health risk since it has adverse effects including mortality by cardiovascular and respiratory diseases. Epidemiological studies have uncovered clear relationships of gaseous pollutants and particulate matter with adverse health outcomes^[1]. Air is a key route of global microorganisms cycling and a major source of human microbial exposure, but it remains the last of the Earth's major ecosystems (after terrestrial and aquatic) to be explored for microbial life. The atmosphere also acts as a vehicle for the dispersion of many pathogens of plant, animal and humans that sometimes travel long distances, spreading diseases across and even between continents^[2].

Bioaerosols are small particles ranging from 0.001 to 100 µm originating biologically from plants/animals and microorganisms and can cause infectious diseases and toxic responses in humans and animals upon inhalation^[3,4]. Toxic substances transported with bioaerosols include secondary metabolites of fungi (mycotoxins), cell wall components of bacteria (endotoxins), as well as endo-, neuro-, and hepatotoxins by cyanobacteria. Mycotoxins can cause acute and chronic health effects in humans and animals. Endotoxins like bacterial lipopolysaccharides (LPS), which are compounds of outer cell membrane of gram-negative bacteria, can induce strong inflammation and other adverse symptoms. In recent years, exposure to bioaerosols in both occupational and residential environments has attracted much attention since their probable impacts on human health^[3]. Global modelling combined with epidemiological exposure-response functions indicates that ambient air pollution causes more than four million premature deaths per year. Epidemiological studies usually refer to PM mass concentrations, but some health effects may relate to specific constituents such as bioaerosols, polycyclic aromatic compounds and

transition metals. PM with a diameter less than 2.5 µm (PM_{2.5}) can be deposited deep into the lungs, inducing oxidative stress and respiratory diseases. Owing to their small size and relatively high concentrations, airborne bacteria are likely to represent a significant portion of the PM_{2.5} aerosol fraction. The European Environmental Agency (EEA, 2009) estimated 510-1150 premature deaths per million inhabitants because of exposure to PM with a statistically best estimate of 830 deaths per million^[5].

Although it is often assumed that airborne microorganisms are merely passive inhabitants of the atmosphere, there is increasing evidence that they have a potential to be late atmospheric processes by serving as ice nucleating particles in atmosphere. Epidemiological studies have shown that air pollution can elevate mortality and global analyses have estimated that about 3.3 million people died due to air pollution in 2010, which has recently been updated to about 4.3 million per year^[6-9]. According to GBD, particulate air pollution is among the seventh largest health risk factors, together with high blood pressure, tobacco smoking, diabetes, childhood undernutrition, high body mass index and high cholesterol^[10]. Long term field measurements of PM_{2.5} and oxidants in urban air pollution are not only critical for evaluating air quality but also essential for conducting epidemiological studies and validating regional and global modeling^[1].

Today, it is common knowledge that many bacterial diseases are transmitted through air over short distances. In the Legionnaire's disease, there is evidence of airborne transmission up to 6 km from contaminated industrial cooling towers^[11]. Investigators have sampled microbial populations of the upper air using balloons, airplanes and rockets and have demonstrated the presence of culturable bacteria at altitudes up to nearly 80 km. Bacteria

enter the atmosphere as aerosol particles from practically all surfaces, including soil, water and plant surfaces. Once in the air, they are carried upwards by air currents and may remain in the atmosphere for many days before being removed by precipitation or direct deposition onto surfaces. Indeed, most bacteria fall into the size range of particles with the longest atmospheric residence times. Studies of aerosols in the near-surface atmosphere typically focus on non-biological particles despite increasing recognition that biological particles may represent a significant portion of the particulates suspended in the atmosphere^[12]. Any different microorganism can be in aerosol form in the atmosphere, including viruses, bacteria, fungi and protozoa. In order to survive in the atmosphere, it is important that these microbes adapt to some of the harsh climatic characteristics of the exterior world, including temperature, gases and humidity. The impact of airborne microbes and their overall contributions to global eco system is disproportionately understudied, even though microorganisms are ubiquitously present in the air. Airborne microbes have been considered passive dwellers moving with the wind; however, several studies strongly suggest that atmospheric microbes are metabolically active. Microorganisms metabolize the organic matter in cloud water and potentially contribute to the biogeochemical cycles of the Earth^[13,14].

The current understanding of airborne microorganisms mainly comes from culture-based studies; however, the majority of environmental microbes cannot be cultured in this way. The main advantage of culture-dependent studies is the ability to grow microorganisms in bulk and study them at both molecular and cellular levels. Their main disadvantage is that they disproportionately favour some microorganisms over others, providing an inaccurate representation of the microbial community as a whole^[15]. Metagenomic provides an access to the genomic potential of an environmental sample either directly or after enrichment for specific purpose. The first and foremost step in metagenomic analyses consists of isolating high molecular weight DNA from environmental samples in an unbiased manner. Metagenomic profiles of airborne microbiome and its transmission have

been analyzed from architected environments/urban to desert soils or Antarctic lands and rainforests in a wide scale, which provide information about the diversity and abundance of microbiome^[16-22].

Advances in molecular techniques have provided methods that facilitate sensitive and accurate resolution in environmental microbiological analysis overcoming the limitations of culture-dependent methods. The main challenges or difficulties of conducting metagenomic studies of airborne microbes are as follows: 1) low density of microorganisms in the air 2) efficient retrieval of microorganisms from the air 3) variability in airborne microbial community composition 4) the lack of standardized protocols and methodologies 5) DNA sequencing and bioinformatics-related challenges^[15]. Overcoming these challenges could provide groundwork for comprehensive analysis of airborne microorganisms and their potential impact on the atmosphere, global climate and our health. Metagenomic studies offer a unique opportunity to examine viral and bacterial diversity in the air and monitor their spread locally or across the globe, including threats from pathogenic microorganisms.

Molecular Characterization of the Atmospheric Microbiome

Airborne bacteria are thought to originate from different habitats on the Earth's surface (ocean, soil, freshwater, etc.). However, the full extent of microbial diversity in the atmosphere remains poorly described. This is largely the result of culture-based methods having long been the standard method for the identification of airborne microorganisms. Fairly recently, the implementation of DNA based molecular tools to the study of airborne microbiology has begun to reveal the full extent of airborne microbial communities and their spatiotemporal variabilities. The diversity and abundance of airborne microbes may be strongly influenced by atmospheric conditions. However, few comprehensive studies have described the diversity and dynamics of airborne bacteria and fungi based on culture independent techniques^[23].

Molecular-based methods can lead to a bacterial concentration of up to 3 orders of magnitude higher than culture-based methods^[24].

Most microorganisms cannot be grown readily in pure culture, and earlier studies using traditional microbiological cultivation techniques have revealed only small percentages of the species present in the investigated samples of environments; e.g., 1% of bacteria, and 17% fungi. The entire spectrum of atmospheric microbial diversity, i.e., the atmospheric microbiome has now become accessible through recent developments and applications of culture independent methods such as DNA and RNA based sequencing and or hybridization^[25]. In order to quantify the individual species, quantitative PCR has been successfully applied to air samples. A promising new method for bioaerosol quantification is the droplet digital PCR (ddPCR) technique, which utilizes a water-oil emulsion system where the sample is fractionated into thousands of nanoliter droplets to enable high-throughput digital PCR. The identification of bioaerosols are carried by traditional Sanger sequencing that provides sequences long enough to identify individual genera or species by comparison with sequences available in online databases like the National Center for Biotechnology Information. Nowadays, the Sanger sequencing-based bioaerosol analysis is being slowly replaced by modern Next Generation Sequencing (NGS) Technologies.

The quality of metagenomics analysis is directly correlated to the quality of DNA used, and several extraction procedures have been developed for environmental DNA isolation. Choosing a method of DNA isolation to be used for any particular metagenomic analysis requires consideration of both the type of sample being analyzed and the output information being generated. In determining the potential role of different microbial community members in an ecological process, it is valuable to be able to distinguish between the DNA from viable and dead cells in a sample^[26].

Preliminary estimates of total DNA concentrations of several nanogram per cubic meter in urbanair suggest that the amount of DNA inhaled by human adults may be as high as 0.1-1 μg per day, which corresponds to 10^{14} - 10^{15} bp and would be equivalent to as much biological information as 10^7 - 10^8 bacterial genomes or 10^4 - 10^5 human genomes^[27].

Two common approaches to investigate microbial diversity and/or metabolic potential in the environment are 1) the polymerase chain reaction (PCR based) rRNA (16S and 18 S) gene sequencing approach for assesment of microbial diversity, in which a single rRNA gene is used as a phylogenetic marker to compare relatedness between microorganisms and 2) the whole genome shotgun metagenomics approach for assessment of microbial diversity and function, in which the entire microbial genome is sheared into smaller fragments, sequenced and reconstructed to assess their diversity and metabolic potential^[15].

With the advent of next generation sequencing (NGS), shotgun metagenomic studies of various environments, including soil, marine and human biome, have expanded significantly as evidenced by the large data sets available for these ecosystems^[28-31]. In contrast, progress in air metagenomic research has been relatively slow^[15]. Metagenomic frameworks for the study of airborne microbial communities have been recently described in several studies^[32,33]. Relative numbers of publications related to metagenomic studies of soil, marine and air have been shown in Figure 1.

Fungal spores account for a substantial portion of air particulate matter and are biological aerosol particles ubiquitous in the Earth's atmosphere, influencing atmospheric chemistry and physics, also the biosphere, climate and public health^[34]. Recent studies have shown that airborne fungi are highly diverse and that atmospheric transport leads to efficient exchange of species among different ecosystems. The increasing number of healthcare

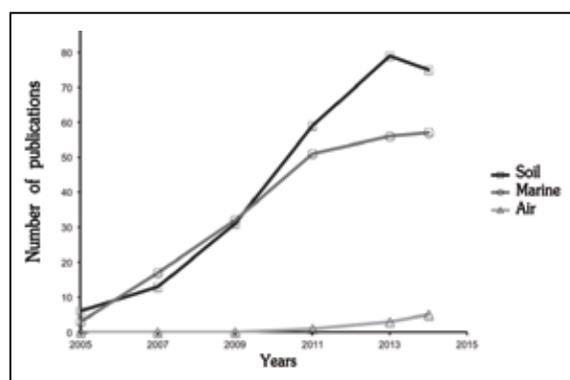


Figure 1. Relative numbers of publications related to metagenomic studies of soil, marine and air^[15].

-associated infections has received significant attention in recent decades, especially opportunistic fungal infections. Airborne infection is considered as a major route of transmission in hospitals^[16]. It is stated that surveillance of hospital infections with microbiome sequence analysis will produce higher sensitivities and shorter turnaround times than traditional methods^[17,35]. The results of the study by Reinmuth-Selzle et al. have clearly demonstrated the presence of geographic boundaries in the global distribution of microbial taxa in air and indicated that regional differences may be important for the effects of microorganisms on climate and public health^[36]. Some works have shown that microbial cells, a majority of which appear to be bacterial, constitute an important component of the total super μm -sized particles in the mid/upper tropospheric air masses sampled^[23,37].

Global atmospheric distribution of fungal phyla derived from Sanger sequencing of air samples are collected at a wide range of geographic locations. *Sordariomycetes* is the class that comprises known ice nucleation species (*Fusarium* spp.). By determining the freezing ability of fungal colonies isolated from air samples, two species of ice nucleation active fungi that were not previously known as biological ice nucleators were found. Through DNA analysis, they were identified as *Isaria farinosa* and *Acremonium implicatum*. Both fungi belong to phylum Ascomycota, produce fluorescent spores in the range of 1-4 μm in diameter, and induce freezing at -4 and -8°C. The IN seem not be bound to cells because they can be easily washed off the mycelium^[34]. Main classes of fungi obtained are shown in Figure 2.

Indoor atmosphere is an ecological unit that has an impact on public health since modern humans spend 90% of their lives indoors containing a variety of microorganisms. Comparison of air samples with each other and nearby environments suggested that indoor air microbes are not random transients from surrounding outdoor environments, but rather originate from indoor niches. It is commonly presumed that airborne microorganisms are a random assortment of aerosolized cells from nearby primary environments such as soil and water bodies as the air environment is inadequate to sustain growth. To compare the biological

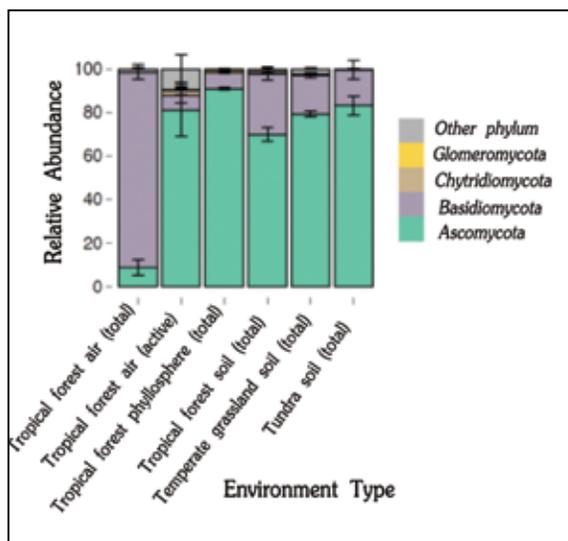


Figure 2. Relative abundances of fungal phyla in various soils and in tropical rain forest determined by DNA and RNA analysis^[22].

contents of air to other local habitats, top soil samples from locations close to the air-sampled buildings and a water sample from the Singapore River were collected and 16 srDNA clone sequencing were performed. Comparisons of these data to those from air showed the microbial diversity in the air to be substantially lower than that in the aquatic and terrestrial environments. However, the phylogenetic spectrum of organisms in the air were also very different from that of water and soil, suggesting that the organisms in the air were not those that dominate nearby terrestrial or aquatic environments. Significantly, the two air samples contained more phylotypes in common with each other than with the other environmental samples. The most abundant airborne microorganism was found to be several species including *Brevundimonas*^[19]. This species have also been cultivated from nominally sterile environments such as space station Mir and in clinical settings where they have been implicated in opportunistic infections^[38]. *Stenotrophomonas maltophilia* were also abundant in both air samples from two different locations. The other groups which were frequently observed in one sample were *Brachbacterium*, *Acinetobacter* and members of Microbacteriaceae and Micrococcaceae^[19]. Airborne communities with high relative abundances of *Actinobacteria*, *Bacteroidetes*, *Firmi-*

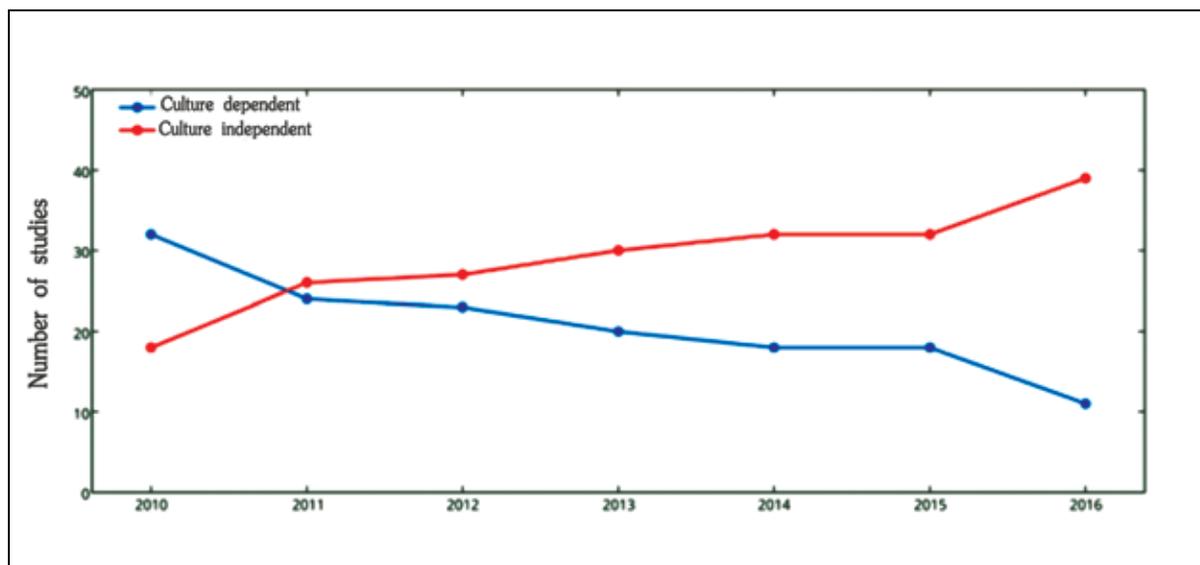


Figure 3. Distribution of the number of studies using culture dependent and culture independent approaches to determine the microbial diversity over the past 7 years on a scale of 5 randomly selected studies^[40].

cutes and *Proteobacteria* have been indicated in other surveys^[21,25].

The studies on outdoor atmospheric bacteria of various ecosystems conducted with metagenomics have been mostly concerned with urban air.

Meteorological changes associated with anthropogenic influences, such as changing land use, are also known to affect the atmospheric microbial composition, the spatial and temporal variation of the airborne microbial populations^[25,39].

The numbers of ongoing and completed investigations by culture dependent and culture independent techniques to determine the microbial diversity are shown in Figure 3.

Airborne viruses are expected to be ubiquitous in the atmosphere but they still remain poorly understood^[41]. Since viral genomes are so small that a large amount of viral particles must be obtained from air samples to be amplified, quantified and sequenced. Furthermore, the absence of conserved genes and high genetic variation also make it difficult to apply pCR assays to viral populations. Recent developments of viral metagenomics have provided a powerful tool for the exploration of viral diversity in a wide range of environments. A combination of viral metagenomics and air sampling will facilitate a new understanding of at-

mospheric viral ecology and the elucidation of new viruses^[42-44]. A metagenomic approach with high throughput sequencing was used in the extensive characterization of viral assemblages present in air samples collected from the three land use types and from rainwater collected at RD site for the first time. The identified sequences present in the four viromes were classified into DNA viruses, mostly ss DNA viruses. Sequences from the four viromes were distributed across about 12 viral families which were composed of six double-stranded DNA viral families and one ssDNA satellites. Of the identified sequences, the majority of viral sequences in the four viromes were characterized as geminivirus-related viruses. Sequences related to circoviruses were the next most abundant after geminivirus-related sequences. There were no significant differences in viral and bacterial abundances according to spatial variation studies. However, temporal variations in viral and bacterial abundances were inversely correlated with temperature and absolute humidity. UV radiation is also well known to be a primary factor influencing the survival of viruses and bacteria.

DeLeon-Rodriguez et al. reported the microbiome of low and high altitude air masses sampled onboard the National Aeronautics and Space Administration DC-8 Platform during 2010 in the

Caribbean Sea^[23]. The samples were collected in cloudy and cloud-free air masses before, during and after two major tropical hurricanes, Earl and Karl. Quantitative PCR and microscopy revealed that viable bacterial cells represented on average around 20% of the total particles in the 0.25-1 μm diameter range and were at least an order of magnitude more abundant than fungal cells, suggesting that bacteria represent an important and underestimated fraction of micrometer-sized atmospheric aerosols. Particles of similar size to that of bacterial cells tend to have greater residence times in the atmosphere compared with larger particles (such as fungal cells and spores that are typically $\geq 3 \mu\text{m}$ in diameter).

Franzetti et al. have investigated the microbial communities associated with coarse (PM_{10}) and fine ($\text{PM}_{2.5}$) airborne particulate matter, particularly in urban areas, using pyrosequencing^[5]. Particulate matter was sampled on teflon filters over 3 months in summer and 3 months in winter in

Milan (Italy) and the hypervariable region of the gene 16 srRNA amplified from the DNA extracted from the filters. Actinomycetales and Firmicutes have been abundantly found in other bioaerosol studies. They found, in all samples, a high species richness, comparable with that of soils but a low evenness. They concluded that not only can the sources of the particulate influence the presence of specific bacterial groups but also that environmental factors and stresses can shape the bacterial community.

Accumulation of bacterial information and characterization by monitoring during meteorological events should be studied in order to establish a relationship between human health and bacterial communities. Characterization of airborne bacterial communities at genus level during Asian Dust event and comparison the microbiomes with the non-Asian dust events by the pyrosequencing were also studied by Cha et al. (2017). During the non-Asian dust events, genus *Sphingomonas* be-

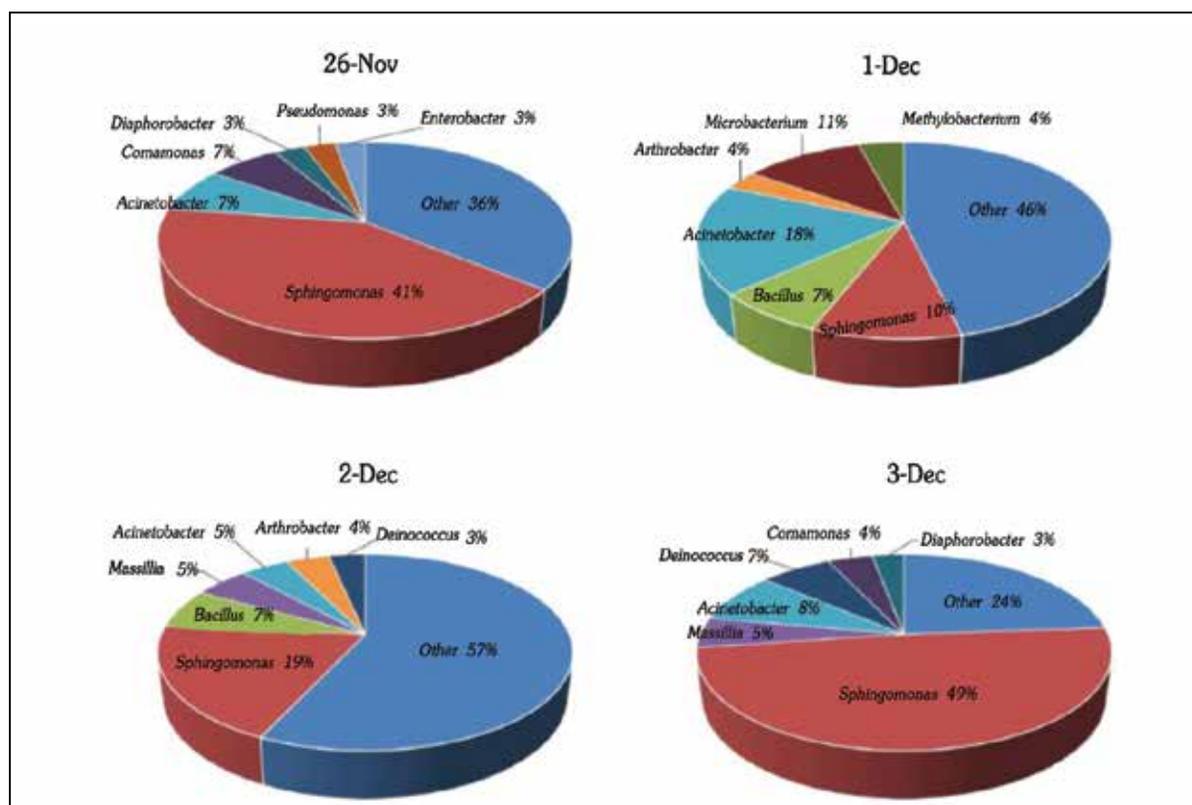


Figure 4. Bacterial communities (%) at the genus level in the Asian dust and non-Asian dust event samples^[45].

longing to Proteobacteria was presently dominant at the proportion of 45% and genera Acinetobacter, Comamonas, Deinococcus and Diaphrobacter were followed. The genus Sphingomonas was still present predominantly in the Asian dust event samples, the proportion in the total genera composition was lowered to 14.6%. The genera Bacillus (spp. circulans-fatal sepsis cause in immunocompromised patients), Arthrobacter, Microbacterium and Methylobacterium were increased as shown in Figure 4.

Conclusion

Microorganisms (including viruses) are abundant in the atmosphere and their abundances and compositions are variable across time and space. However, the atmospheric conditions responsible for driving the observed changes in microbial abundances are not well known. The diversity of airborne microorganisms and the factors influencing diversity levels also remain poorly characterized. One of the reasons for this is until recently, knowledge was based on culture-based microbiological methods. As demonstrated in a number of recent studies, advances in culture-independent techniques allow far more of the microbial diversity present in the atmosphere. Broader strategies for sequencing the genetic material of microbiome will allow investigators to describe more organisms. Therefore, metagenomics can be viewed as a useful contemporary strategy for the designing of integrative studies comprised of different scientific disciplines such as public health, agriculture, ecology, international security.

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