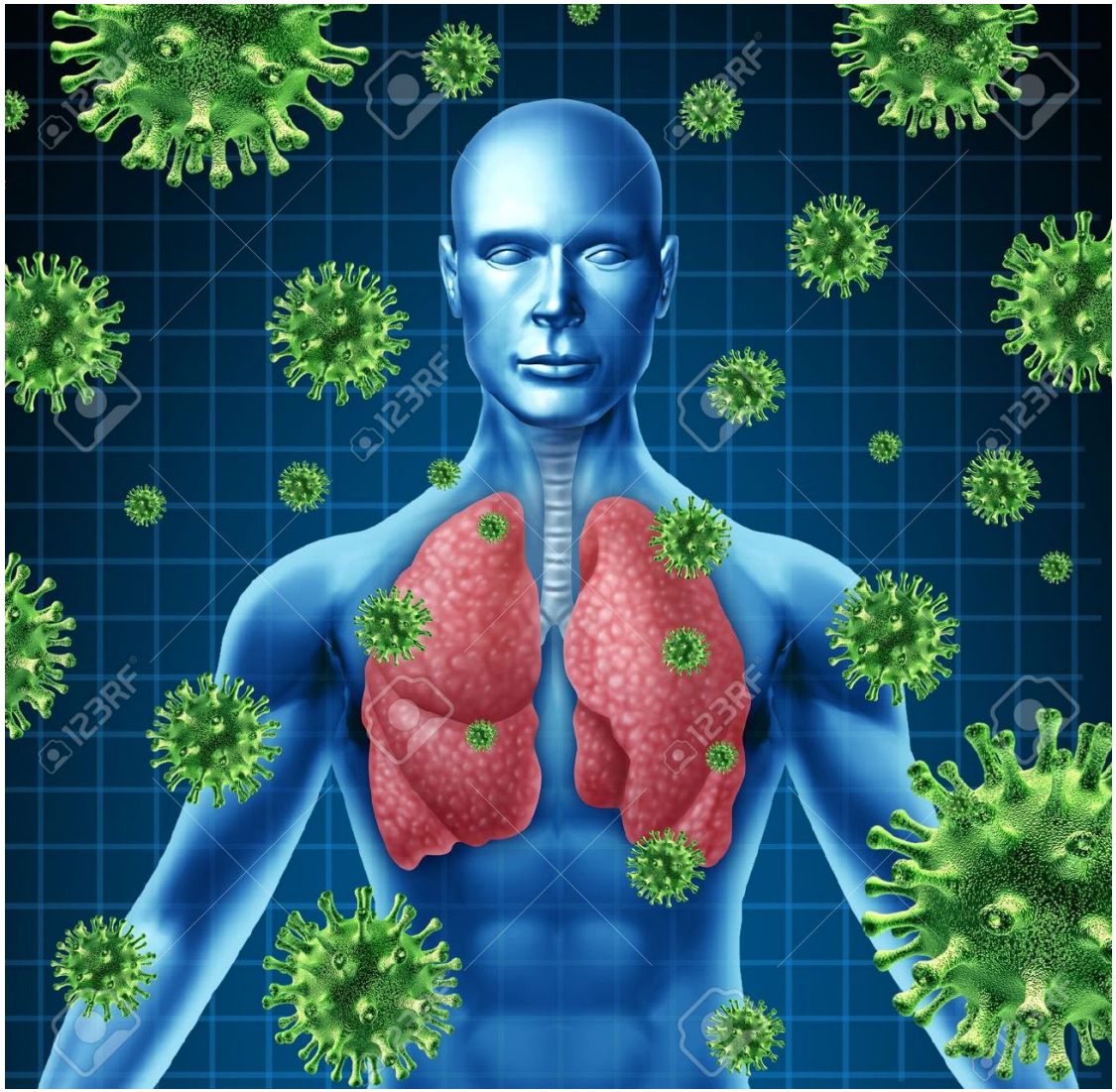
The Air We Breathe



LECTURE 3: Regional Particles

Airscape Concept: Atmosphere is habitat + conveyance

Atmospheric layering changes with seasons, weather, time of day

Major particle emitters: cities, forests, deserts & oceans

Transport: wind + cloud formation + thunderstorms + rain

20-km Tower story: its relevance for high-rise dwellings?

Air components + effect on respiratory health?

Lecture 3 Regional Particles
How rainstorm formation affects particle load

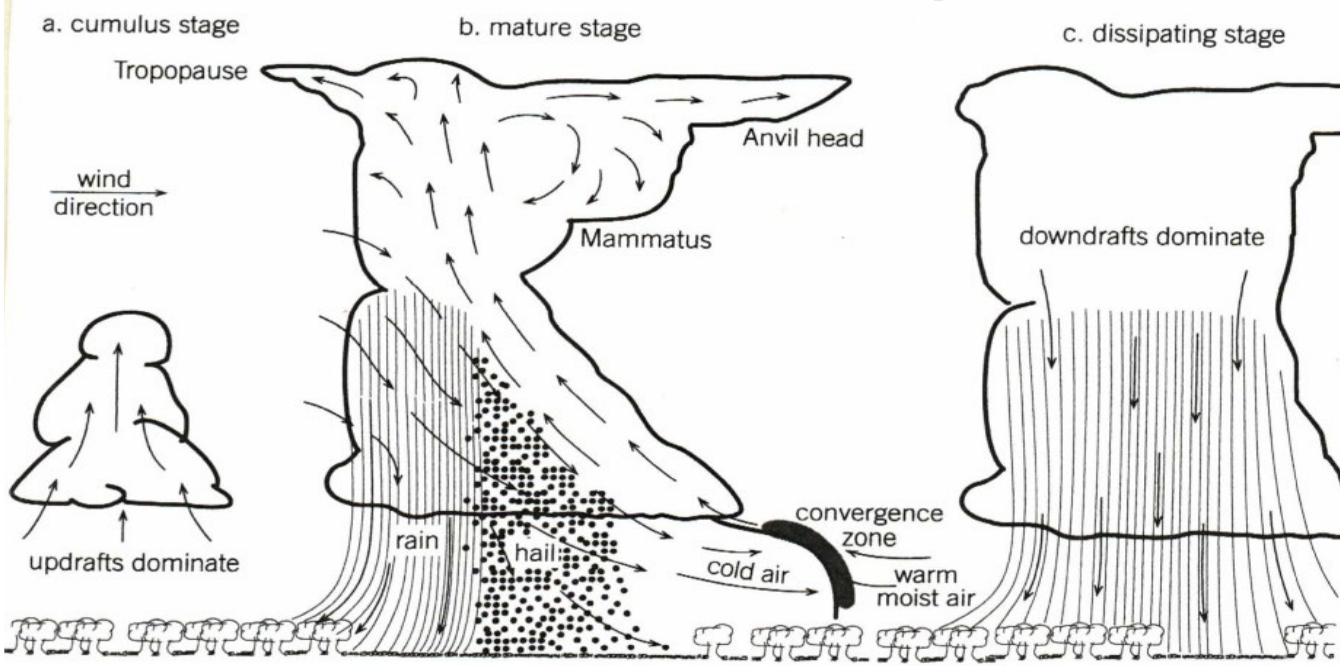


Figure 8.11. Stages of convective storm (thunderstorm) development.

Air-mass thunderstorms tend to develop when the lower atmosphere is highly unstable. Therefore, when v moist air rises from the surface, condensation quickly occurs, releasing energy and enabling vertical acc ion of the air within the cumulus cloud (a). The formation of circulation patterns within the convective cell he mature stage (b). Downdrafts of cold air originating in the upper center of the cell produce a cool c hat spreads outward above the ground surface and lifts the warm, moist air in front of the cell. A conver one develops at the outflow front where biota that resist upward movement can become concentrated. nent of the cell over warm ground helps to propagate continued activity. The dissipation stage occurs wh updrafts weaken, reducing the release of latent energy within the cell, and downdrafts prevail.

Isard SA and SH Gage 2001. *Flow of Life in the Atmosphere*. Mich State Press. p. 117.

Lecture 3: Global + regional particles

Troposphere: *mostly nitrogen with oxygen*
water vapor "we live in the bottom of an ocean of air"
carbon dioxide + other greenhouse gases
ozone
volatile organic compounds
biogeochemical cycle compounds
dust
sea salt
smoke
volcanic ash
micro-organisms, protein molecules, allergens, adjuvants
biological debris: hair, dead skin cells, cellulose, insect parts
industrial & manufacturing waste
transport's combustion engine emissions
tire wear particles

Lecture 3: Regional Particles
Scales of Inquiry

10^{4} m thunderstorm (10 km altitudinal expanse)
10^{2} m ZOTTO tall tower in Siberia (300 m ht)
10^{-1} m hailstone, large (7 cm diameter)
10^{-3} m raindrop, large (6 mm)
10^{-5} m particular matter PM (10 micron diameter)
10^{-6} m one cloud condensation nucleus (1 micron)
10^{-10} m one water molecule 3×10^{-10}

Sources: Rosinski 1966; Griffiths et al. 2012; Després et al. 2012; Jaenicke 2005; Mandrioli et al. 1973; DeMott et al. 2010.

Lecture 3: Regional Particles
U.S. E.P.A. Air Now

https://www.airnow.gov/

Compare cities: record in their daylight hours
record in their night hours

Tokyo Japan
New Delhi India
Shanghai China
Washington D.C.

U.S. EPA AirNow https://airnow.gov
5 December 2021 Covid Pandemic

U.S. Embassies and Consulates
Measure air pollution in urban areas worldwide hourly

City	AQI $PM_{2.5}$	Time
Dubai	88	1800h
Abu Dhabi	64	1700h
Jeddah KSA	125	1600h
Bagdad Iraq	147	1600h

LECTURE 3: REGIONAL PARTICLES & AVIATION CABIN STUDY

1-N	Los Angeles, USA	New York-JFK, USA	14/11/2005	757	5 h; 23 min	3960	7
1R-N	New York-JFK, USA	Los Angeles, USA	17/11/2005	757	5 h; 55 min	3960	7
2-N	Los Angeles, USA	New York-JFK, USA	5/12/2005	757	5 h; 23 min	3960	5
2R-N	New York-JFK, USA	Los Angeles, USA	8/12/2005	757	5 h; 55 min	3960	7
3-L	Los Angeles, USA	London-Heathrow, UK	15/1/2006	777	13 h; 10 min	8730	10
3R-L	London-Heathrow, UK	Los Angeles, USA	20/1/2006	777	11 h; 16 min	8730	8
4-S	Los Angeles, USA	Sydney, Australia	12/2/2006	747	14 h; 31 min	11 973	18
4R-S	Sydney, Australia	Los Angeles, USA	17/2/2006	747	13 h; 29 min	11 973	10
5-N	Los Angeles, USA	New York-JFK, USA	7/3/2006	757	5 h; 23 min	3960	4
5R-N	New York-JFK, USA	Los Angeles, USA	10/3/2006	757	5 h; 55 min	3960	5
6-J	Los Angeles, USA	Narita-Tokyo, Japan	3/4/2006	747	11 h; 18 min	8722	8
6R-J	Narita-Tokyo, Japan	Los Angeles, USA	7/4/2006	747	9 h; 57 min	8722	7
7-N	Los Angeles, USA	New York-JFK, USA	24/4/2006	757	5 h; 23 min	3960	6
7R-N	New York-JFK, USA	Los Angeles, USA	27/4/2006	757	5 h; 55 min	3960	6
8-S	Los Angeles, USA	Sydney, Australia	7/5/2006	747	14 h; 31 min	11 973	10
8R-S	Sydney, Australia	Los Angeles, USA	7/5/2006	747	13 h; 29 min	11 973	10

Null Hypothesis: Flight duration has no effect on bacterial diversity

Domestic Flights = International Flights

Osman et al. 2008 (optional reading) NASA JPL study

Old method
***Cultured* & *viable* bacteria**

Domestic Flights (n=8)

International Flights (n=8)

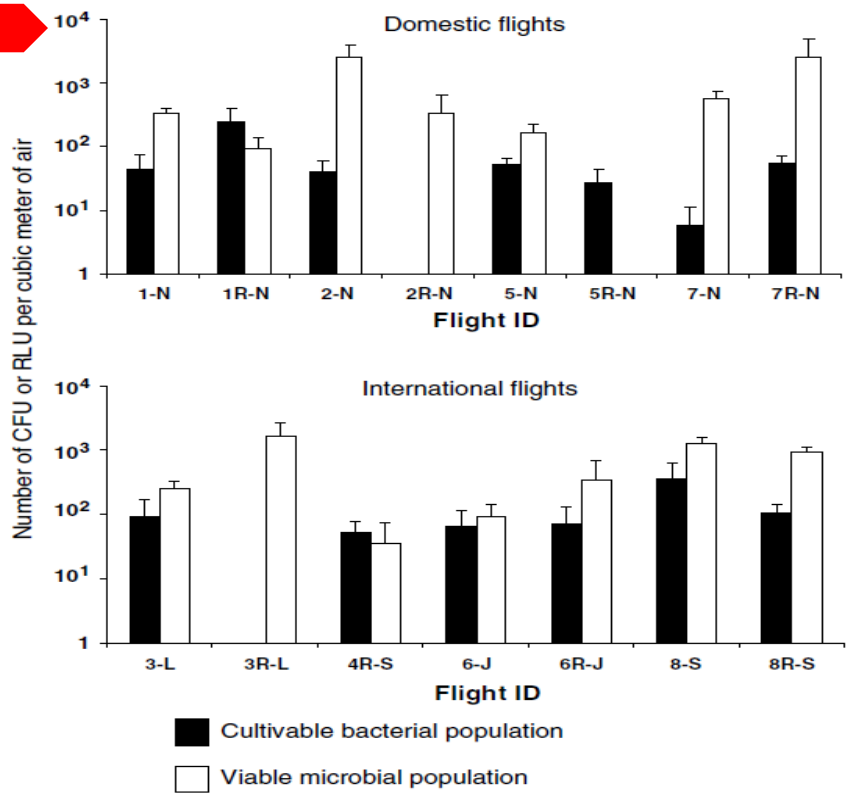


Figure 1 Microbial population of commercial airline cabin air. The solid bar represents cultivable bacterial population measured by R2A medium and viable microbial population estimated by intracellular ATP. Two measurements for cultivable bacterial counts and four replicates were carried out for total microbes. Number of samples for each flight segment is given in Table 1.

Osman et al. 2008

New method

DNA sequencing ID rRNA genes
Bacterial diversity *higher*

Domestic Flights (n=8)

International Flights (n=8)

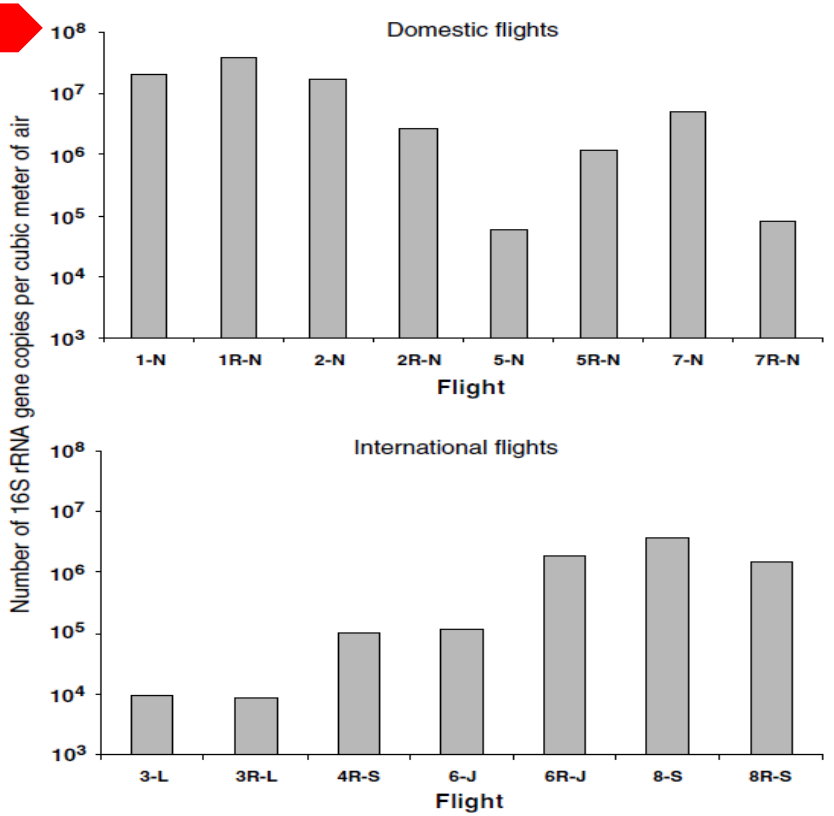
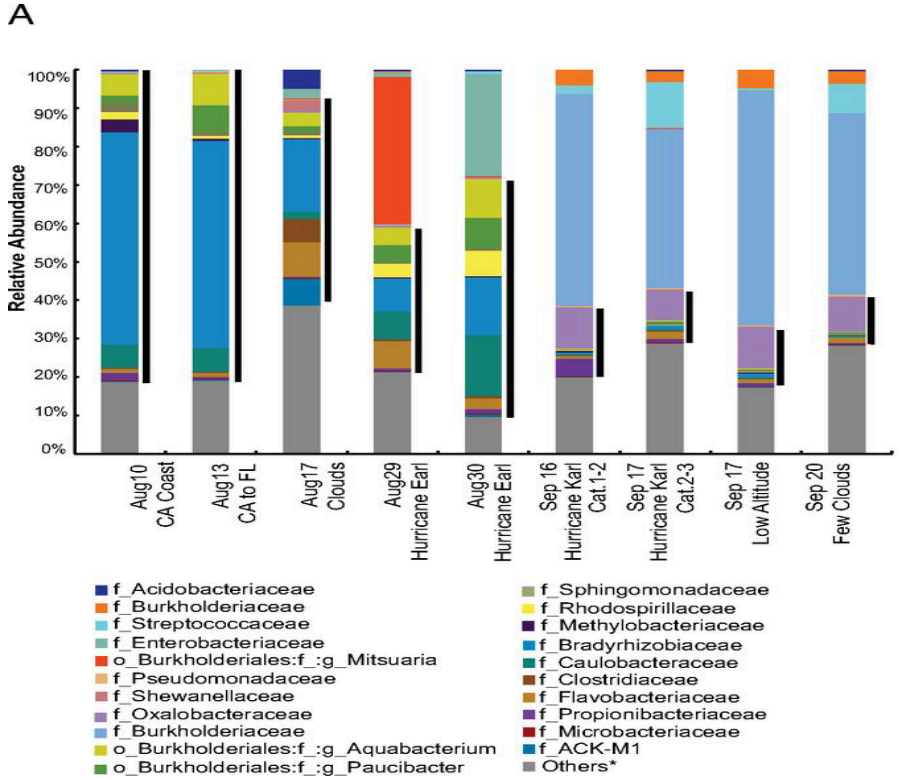


Figure 2 Total bacterial population as measured by 16S rRNA gene copy numbers of several domestic and international flight cabin air.

Osman et al. 2008

Lecture 3: Regional particles
Composition of tropospheric bacterial communities.



Natasha DeLeon-Rodriguez et al. PNAS 2013;110:7:2575-2580

PNAS

Accept the Null Hypothesis or not?

Flight duration has no effect on bacterial diversity so
Domestic Flights = International Flights

Accept the Null Hypothesis or not?

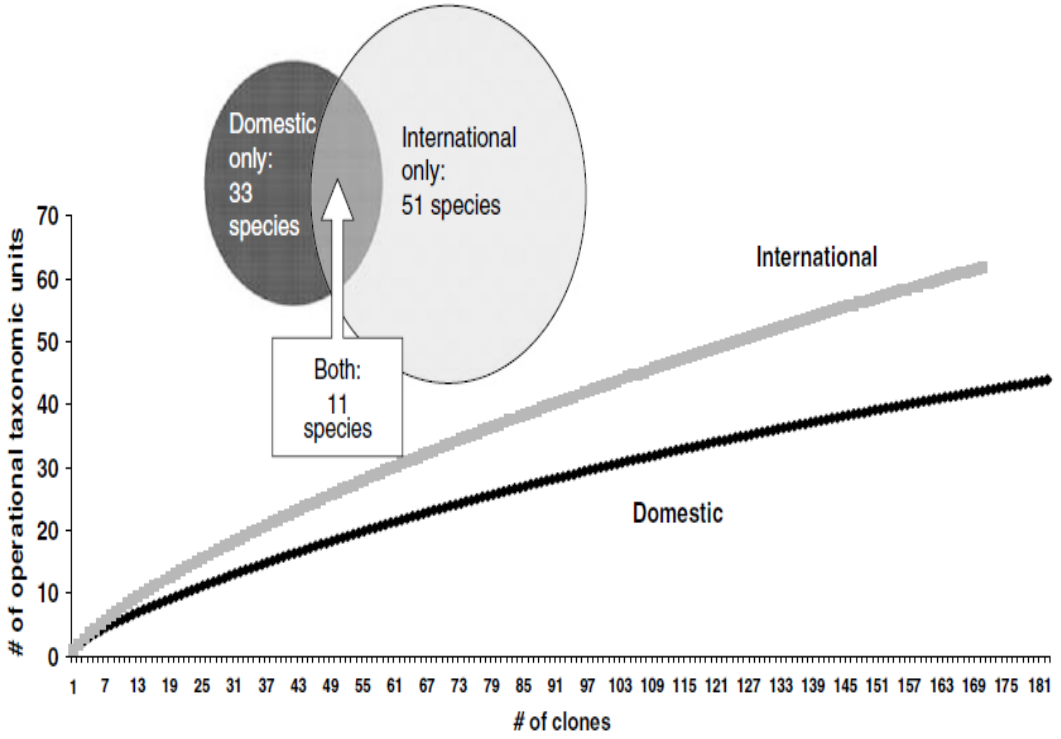


Figure 5 Rarefaction curves constructed for bacterial clone libraries from several international and domestic flight cabin air. Clones were grouped into OTUs at a level of sequence similarity of >97.5%. The overlapping OTUs between domestic and international flights are given in the inset.

U.S. Particulate Matter (PM_{10})
Air Pollution Laws + Human Health Laws

- **Environmental Protection Agency**
- **U.S. Clean Air Act 1971, 1990, 5Y Updates**
- **PM_{10} Particulate Matter = Particulate Pollution**
- **Public Health Service Act, The Childrens' Health Act**

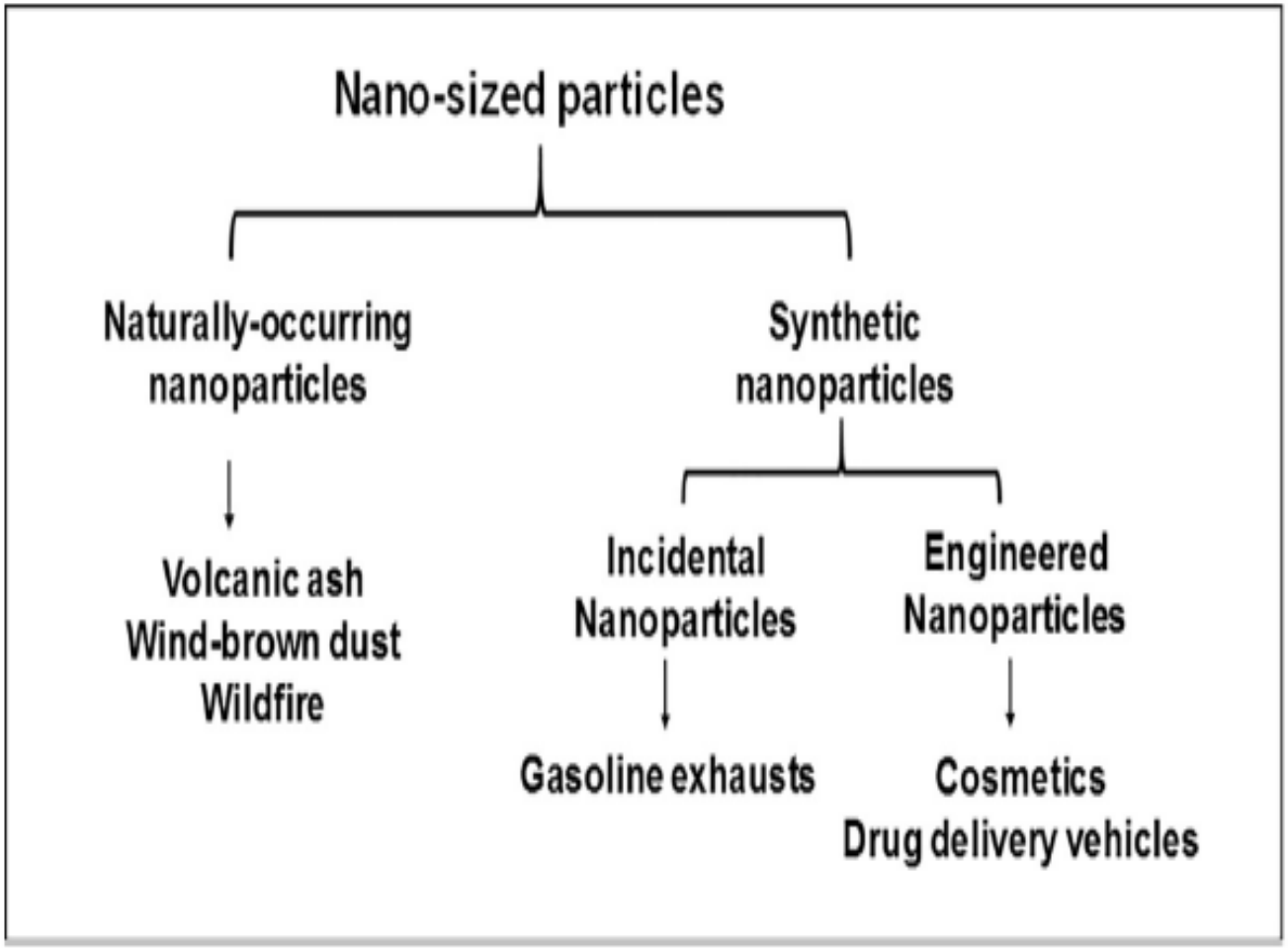
World Changes Relevant to Particulate Matter (PM_{10}) Air Pollution Laws

- Doubled human population since 1971
- 47 Mega-cities (>20 mill)
- Cities emit bioaerosols
- Precise bioaerosol measurement
- Rising allergens, respiratory illness, asthma
- Thunderstorm asthma: health + climate change

Williams & Smith (2021) Policy brief reading Atmospheric biology too small, highly fragmented in U.S. science agencies

Lecture 3: Regional particles from newer human-made sources

R. Nho / Nanomedicine: Nanotechnology, Biology, and Medicine 29 (2020) 102242



Atmospheric Bioaerosols

- **25% of atmospheric aerosols[1]**
- **4% of anthropogenic PM_{10} emissions in Europe[2]**
- **Low count + high-impact synergy**

Prof Ruprecht Jaenicke
MPI Atmospheric Chemistry Mainz DE

[1] Jaenicke (2005) Science

[2] Winiwarter et al. (2009) Atmos Env. 43: 1403-1409

Bioaerosols & Particulate Matter (PM)

Neo-Allergens form from Co-Pollutants

Negative Synergy + Agglomeration

- **Bacterial endotoxin + diesel exhaust**
- **NOx, Ozone increases permeability of airways**
- **Subpollen particles (SPP) proteins**

Rathnayake et al. (2016) J. Geophys. Res. Atmos. 121(19): 5071-5089

The ISME Journal (2008) 2, 482–497

www.nature.com/ismej

ORIGINAL ARTICLE

Microbial burden and diversity of commercial airline cabin air during short and long durations of travel

Shariff Osman[1], Myron T La Duc, Anne Dekas[2], David Newcombe[3] and Kasthuri Venkateswaran
Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, USA

Total microbial burden and diversity associated with commercial airliner cabin air was assessed by molecular methods in 125 air samples from the business-class sections of 16 domestic and international flights. Viable microbial burden within these cabin air parcels constituted only 1–10% of the total microbial population and ranged from below detection limits to 1.2×10^4 cells m^{-3} as determined with a validated ATP-based technology. Cultivable bacterial diversity was almost entirely limited to Gram-positive bacteria such as *Staphylococcus* and *Bacillus*. In contrast, cloning and sequencing 16S rRNA gene directly from the samples without cultivation indicated a significantly broader diversity, as sequences representing more than 100 species, and encompassing 12 classes of bacteria, were retrieved in varying abundance. Sequences of proteobacterial and Gram-positive lineage were retrieved most frequently (58% and 31% of all clone sequences, respectively), with Gram-positive and α-proteobacterial sequences dominating international flight samples and β- and γ-proteobacterial sequences comprising the largest portion of those retrieved from domestic flights. Significant differences in bacterial load and diversity were noted between samples obtained on domestic and international flights. The disparities observed in microbial abundance and diversity further underscore the immense value of state-of-the art molecular assays in augmenting traditional culture-based techniques.
The ISME Journal (2008) **2,** 482–497; doi:10.1038/ismej.2008.11; published online 7 February 2008
Subject Category: microbial population and community ecology
Keywords: airline; cabin air; rapid detection; 16S rDNA; bioburden; diversity

Introduction

According to the National Research Council (NRC) report (NRC, 2002), the number of air passengers worldwide has nearly quadrupled over the last 30 years: from 383 million in 1970 to 1462 million in 1998. At this rate, this percentage is likely to rise considerably with the number of international travelers expected to increase to several billions by 2020. Although the aircraft cabin is similar to other semi-contained indoor environments, such as homes and offices, in that individuals are exposed to a combination of external and recirculated air, it varies greatly from these environments with respect to occupant density, the inability of occupants to leave at will and the need for pressurization. Furthermore, while in flight, travelers encounter a combination of constantly regulated environmental factors including low humidity, reduced air pressure and potential exposure to chemical contaminants, such as ozone, carbon monoxide, various organic compounds and biological agents.

Although the Federal Aviation Administration adopted a 1986 NRC recommendation to eliminate smoking on most domestic airline flights, several other public health concerns regarding aircraft cabin air quality have yet to be adequately addressed (NRC, 2002). One concern was the microbiological burden in enclosed environments, where passengers breathe continuously recycled air. The air provided to the passengers and crew on commercial jet aircraft is typically a combination of external air brought in through the engines and air that is taken from the cabin, filtered and recirculated. The environmental control system is designed to minimize the introduction of harmful contaminants into the cabin and to control cabin pressure, ventilation, temperature and humidity. An NRC report (NRC, 2002) found that most commercial jet airlines

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provide an ample supply of air to pressurize the cabin, meeting general comfort conditions and diluting or otherwise reducing normally occurring odors, heat and contaminants. The committee noted, however, that the current design standard of a minimum of 0.55 lb of outside air per minute per occupant (Federal Aviation Regulation: 25.831) is less than one-half to two-thirds the ventilation rate recommended in American Society of Heating, Refrigerating, and Air-Conditioning Engineers Standard 62–1999, which was developed for ground structures.

The majority of published investigations of cabin air quality have focused on chemical contaminants, such as cigarette smoke, carbon dioxide, ozone and total organic carbon (Lee *et al.*, 1999; Lindgren and Norback, 2002; Lindgren *et al.*, 2002), rather than microbiological sources. However, recent concerns regarding the transmission of emerging diseases on commercial airliners, SARS and tuberculosis in particular, have brought much attention to the monitoring and control of cabin air quality aboard commercial aircraft (Olsen *et al.*, 2003). Prior to this investigation, knowledge of the microbiological composition in commercial airliner cabin air was limited to a sparse collection of cultivation-based reports (Wick and Irvine, 1995; Dechow *et al.*, 1997; Hines *et al.*, 2003; McKernan *et al.*, 2007). Although these studies have collectively reported a typical microbial burden ranging from 10 to 300 colony-forming units (CFU) per m^3, these results are fraught with bias since a majority of all bacteria are not capable of growing in well-defined conventional media (Pace *et al.*, 1985; Amann *et al.*, 1995; Pace, 1997; La Duc *et al.*, 2007a). At best, plate counts offer only relative comparisons between samples and are incapable of definitive estimations of the size or diversity of the molecular communities present.

Since the presence of viable microorganisms circulating about cabin air is of particular consequence to human health, it is critical to thoroughly and accurately assess the diversity and overall microbial burden associated with commercial cabin air systems. In a previous study, we demonstrated the application of molecular techniques to elucidate the microbial populations aboard four commercial airline flights (La Duc *et al.*, 2007b). The objective of this investigation was to greatly extend the use of these methods over a larger sampling and attempt to identify significant differences in the total microbial burden and composition among individual aircraft, and between domestic and international-bound flights.

Materials and methods

Sampling limitations on commercial airliners

The departure and destination cities, aircraft type, travel time and distance between the cities traveled are given in Table 1. At the request of the carrier that participated in this study, the name of the carrier sampled has not been provided (personal communication). The commercial aviation industry has assured the authors that standard use, positioning and maintenance of HEPA filters apply to all aircraft regardless of carrier. To gain a better understanding of the flow and engineering pertaining to aircraft cabin air systems, the authors requested designs and specifications for aircraft that were sampled, but in the interest of safety and security such information was not made available. Each flight represented a distinct aircraft of that carrier; there were no continuations of the same flight on the same aircraft. All of the flights were at or very near capacity.

Sample collection

The BioCapture BT-550 (Mesosystems Technology Inc., Kennewick, WA, USA) sampler employed in this study collects particles in the size range of 0.5–10 μm (optimum for cells and particles) from ambient air and operates at altitudes in excess of 15 000 m. Previous work has characterized the sampling efficiency of the BioCapture (BT-550) sampler using monodisperse fluorescent oleic acid particles and monodisperse fluorescent polystyrene latex particles (Kesavan *et al.*, 2003). The results showed that the BT-550 sampler had a peak sampling efficiency for 2-μm particles and the average peak sampling efficiencies for the sampler was 38% (Kesavan *et al.*, 2003). The flow rate of this light-weight (4.5 kg), field deployable sampler is 150 l min^{-1} (5.3 cubic feet per min). Each 750 l air parcel (roughly equivalent to the volume of air human lungs exchange every 2 h) was impinged into 5 ml of sterile buffered saline by running the sampler for 5 min. The particular sampler utilized in this investigation was retrofitted to operate on seat-supplied AC power in place of manufacturer standard lead-acid batteries.

A total of 125 air samples were collected from business-class seat locations aboard eight domestic and eight international flights on a single commercial carrier. All samples were obtained from Boeing aircraft on either 747, 757 or 777 models (Table 1). In addition to offering access to AC power, business class seats provided the necessary space for obtaining, storing and refrigerating samples. Samples were collected over the entire course of each flight, including preboarding and postlanding whenever possible. In each instance, the sampling device was centrally positioned within the seat row, to ensure uniformity of the aerosols, and three replicate samples were taken at each time point. Immediately following sample collection, two replicates were stored on dry ice and a third was placed in a cooler chilled with ice packs to approximately 4 °C. A total of 110 samples were processed within 24 h for viability assessment (ATP and plate counts). Sample volumes remaining after viability assays were frozen

Table 1 Sample characteristics and summary of analyses performed on air samples collected from commercial cabin air environment

Sample ID	*Departing from*	*Destination*	*Date*	*Aircraft*[a]	*Travel time*	*Travel distance (km)*	*Number of samples collected per flight*	*Estimation of*		*Bacterial*		*All samples were pooled and subjected to*		
								Total microbial population[b]	*Viable microbial population*[c]	*Enumeration of cultivable population*[d]	*Identification*	*DNA extraction*	*Total bacterial population measurement*[e]	*Phylogenetic analysis based on 16S rRNA gene sequencing*
1-N	Los Angeles, USA	New York-JFK, USA	14/11/2005	757	5 h; 23 min	3960	7	+[f]	+	+	+	+	+	+
1R-N	New York-JFK, USA	Los Angeles, USA	17/11/2005	757	5 h; 55 min	3960	7	+	+	+	+	+	+	+
2-N	Los Angeles, USA	New York-JFK, USA	5/12/2005	757	5 h; 23 min	3960	5	+	+	+	+	+	+	+
2R-N	New York-JFK, USA	Los Angeles, USA	8/12/2005	757	5 h; 55 min	3960	7	+	+	+	NA	+	+	No amplicons[g]
3-L	Los Angeles, USA	London-Heathrow, UK	15/1/2006	777	13 h; 10 min	8730	10	+	+	+	+	+	+	No amplicons[g]
3R-L	London-Heathrow, UK	Los Angeles, USA	20/1/2006	777	11 h; 16 min	8730	8	+	+	+	NA	+	+	No amplicons[g]
4-S	Los Angeles, USA	Sydney, Australia	12/2/2006	747	14 h; 31 min	11 973	18	ND	ND	ND	ND	+	+	+
4R-S	Sydney, Australia	Los Angeles, USA	17/2/2006	747	13 h; 29 min	11 973	10	+	+	+	+	+	+	+
5-N	Los Angeles, USA	New York-JFK, USA	7/3/2006	757	5 h; 23 min	3960	4	+	+	+	+	+	+	+
5R-N	New York-JFK, USA	Los Angeles, USA	10/3/2006	757	5 h; 55 min	3960	5	+	+	+	+	+	+	+
6-J	Los Angeles, USA	Narita-Tokyo, Japan	3/4/2006	747	11 h; 18 min	8722	8	+	+	+	+	+	+	+
6R-J	Narita-Tokyo, Japan	Los Angeles, USA	7/4/2006	747	9 h; 57 min	8722	7	+	+	+	+	+	+	+
7-N	Los Angeles, USA	New York-JFK, USA	24/4/2006	757	5 h; 23 min	3960	6	+	+	+	+	+	+	+
7R-N	New York-JFK, USA	Los Angeles, USA	27/4/2006	757	5 h; 55 min	3960	6	+	+	+	+	+	+	+
8-S	Los Angeles, USA	Sydney, Australia	7/5/2006	747	14 h; 31 min	11 973	10	+	+	+	NA	+	+	+
8R-S	Sydney, Australia	Los Angeles, USA	7/5/2006	747	13 h; 29 min	11 973	10	+	+	+	NA	+	+	+

Abbreviations: NA, not applicable since cultivable bacteria were not isolated; ND, not determined.
[a]Total number of passengers in 747, 757 and 777 aircraft are 524, 243 and 524, respectively.
[b]Measured by total ATP.
[c]Measured by intracellular ATP.
[d]Bacteria were cultured by spread plating onto R2A agar.
[e]Measured by quantitative PCR.
[f]+, analysis performed.
[g]Due to low DNA concentration, PCR did not amplify 1.5-kb fragment of 16S rRNA gene and hence cloning and sequencing analyses were not possible.

for subsequent DNA extraction and phylogenetic analyses. Samples from flight 4-S (Los Angeles to Sydney, Australia) were subjected to the same DNA-based analyses described below, but the portions that would otherwise have been analyzed using viability assays were instead archived for future study.

Microbiological examination

The enumeration of bacterial colonies grown on R2A medium is referred to as 'heterotrophic plate counts' in this study. Since ATP can originate from bacteria, archaea and eukaryotic cells, intracellular-ATP measurements are described as 'total viable microbial population,' while total ATP values are termed 'total microbial population.' Although the ATP assay has a very sensitive lower limit, prior validation studies of this technology have set a threshold of 10^3 relative fluorescence units (RLU) m^{-3} of air to strengthen conclusions drawn from data sets (Venkateswaran *et al.*, 2003; La Duc *et al.*, 2004). The 16S rRNA gene copy numbers measured by the quantitative PCR (Q-PCR) assay are referred to as 'total bacterial population or 16S rRNA gene copies' because bacterial-specific primer-probe sets were used and because these estimates encompass viable and nonviable bacterial cells.

Hetrotrophic plate counts. Duplicate 100 µl aliquots of each sample were spread atop R2A agar (Difco Laboratories, Detroit, MI, USA) plates and CFU were enumerated following 7 days incubation at 25 °C. Each of the plates incubated at the various destination laboratories was enumerated before shipment to the Jet Propulsion Laboratory, where counts were verified and representative colonies were selected as described below. Isolates were aseptically picked, purified and cultured, and stored at −80 °C for further processing and analysis. When fewer than 10 colonies arose on a plate, all colonies were picked. When a greater number of colonies were present, 10 representatives were picked to capture a reasonably diverse collection of isolates. Colony morphology was considered when selecting isolates for further characterization. Identification of purified strains was determined via 16S rDNA sequence analysis as described elsewhere (La Duc *et al.*, 2004).

Total and viable microbial population. A commercially available ATP-assay kit (Checklite HS Plus, Kikkoman, San Francisco, CA, USA) was used according to the manufacturer's protocols, to measure both intracellular and total ATP present in each sampled air parcel. Detailed description of the ATP assay to estimate total and viable microbial population was reported previously (Venkateswaran *et al.*, 2003; La Duc *et al.*, 2007a). It has been documented that for most Gram-negative bacteria, 1 RLU corresponds to approximately 1 CFU, as compared to Gram-positive bacteria where 1 CFU corresponds to roughly 5 RLU (La Duc *et al.*, 2007a). It should also be assumed that in regularly cleaned, low-humidity, HEPA-filtered environments, such as aircraft cabins, cells exist in a suppressed metabolic state and contain less ATP than actively growing cells (Stanley, 1989). Considering these circumstances, we conservatively estimated that a measurement of 1 RLU was approximately equal to 1 CFU (Venkateswaran *et al.*, 2003; La Duc *et al.*, 2004).

Total bacterial population. Total bacterial population regardless of viability was measured by Q-PCR as described below. All samples collected within an individual flight were pooled to increase the likelihood of obtaining sufficient quantities of nucleic acid for DNA-based analyses. DNA was extracted as described below, and ribosomal RNA gene copy numbers were quantified in triplicate using an MJ Research Chromo4 detection system. Universal bacterial primers targeting the 16S rRNA gene, 1369F (5′-CGGTGAATACGTTCYCGG-3′) and 1492R (5′-GGWTACCTTGTTACGACTT-3′), and the fluorescent-labeled probe TM1389F (5′-FAM-CTTGTA CACACCGCCCGTC-TAMRA-3′) were used in this quantitative analysis (Suzuki *et al.*, 2000, 2001). Each 50 µl reaction mixture consisted of 25 µl of 2X Taqman Universal PCR Master Mix (Applied Biosystems Inc., Foster City, CA, USA), 0.8 µM of each oligonucleotide primer, 0.5 µM of oligonucleotide probe and 1 µl of template DNA. Reaction conditions were as follows: 95 °C denaturation for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s and a combined annealing and extension at 60 °C for 1.5 min. Standards were created using known amounts of full-length *Escherichia coli* 16S rRNA genes incorporated into suitable vector plasmids (Invitrogen, Carlsbad, CA, USA) and standard curves were repeated for each Q-PCR reaction.

DNA extraction and PCR amplification. Samples collected at all time points (~5 ml sample per 750 l^{-1} air per time point) of each flight were pooled (4–10; Table 1) and concentrated in Amicon-Ultra 15 centrifugal filters (Millipore, Billerica, MA, USA) using a refrigerated centrifuge at 3000 r.p.m. for 20 min. Each filter unit has a molecular weight cutoff of 50 000 Da allowing isolation of all intact bacterial cells and naked DNA fragments greater than 100 bp. Amicon units were reusable for each sample set, making it possible for all samples from a single flight (~20–50 ml) to be concentrated to a volume of 200 µl in a single filter tube. A comparable amount of sterile, unused cartridge buffer was concentrated in a separate filter tube to act as a control for each extraction. Total DNA was extracted for each concentrated pooling of flight samples using a standard lysozyme/phenol–chloroform procedure (Johnson, 1981; Ausubel *et al.*, 2001). We have previously reported the percent DNA recovery

using this solvent-based DNA extraction method for low-biomass samples to be ~10% (Bruckner *et al.*, 2005; Bruckner and Venkateswaran, 2007). Briefly, samples were treated with lysozyme (final concentration 10 mg ml^{-1}) to degrade cell walls, followed by proteinase K and RNase treatment to remove unwanted biopolymers and finally phenol–chloroform to clean-up cellular debris. DNA was precipitated with two volumes of ice-cold ethanol, washed briefly with 70% ethanol and resuspended in TE buffer (30 µl) before being stored at −80 °C. Bacterial small subunit rRNA genes (~1.5 kb) were PCR-amplified (1–5 µl template) with a PTC-100 thermal cycler (MJ Research, Waltham, MA, USA) using eubacterially biased primers 8F and 1492R, as described previously (La Duc *et al.*, 2004).

Sequence analysis and phylogenetics. Sequences of chloroplasts and sequences that exhibited undetermined bases (*N*'s) at a frequency of >1% were removed from the calculation. An alignment of ca. 20 000 homologous full and partial sequences available in the public ARB database (Ludwig *et al.*, 2004) was used. Novel 16S sequences (ca. 1400 nucleotides) were aligned to their nearest neighbor using automated tools of the ARB software suite (Technische Universitat Munchen, Munich, Germany (http://www.mpi- 180bremen.de/molecol/arb/)). The resulting alignment was checked manually and corrected if necessary. Phylogenetic trees were reconstructed via maximum parsimony and neighbor-joining methods. GenBank accession numbers for the cultivable bacteria are EU379242–EU379312 and clones are EU341129–EU341298.

Statistical analysis. Statistical analyses were carried out using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Comparison of paired sample sets was performed using Student's *t*-test and Mann–Whitney's *U*-test. Comparisons of more than two sample sets were carried out using Bonferroni adjusted analysis of variance tests. All statistical tests were carried out at the $\alpha = 0.05$ significance level. In addition, appropriate statistical analyses were performed according to the manufacturers' instructions using software specific for each assay or with the MS Excel software package. For example, an additional measurement was taken from samples when the coefficient of variation exceeded 10% for ATP analyses, and standard deviations were calculated from four individual replicates of each sample. Likewise, standard deviations from three replicates of each Q-PCR-amplified sample were generated and linear regression models were used to determine standard curves for each Q-PCR run. The averages of two measurements were calculated for each sample that underwent plate-count analysis.

Rarefaction analysis (Heck *et al.*, 1975) and coverage calculations (Good, 1953) were applied to estimate the representation of the phylotypes in bacterial libraries. Operational taxonomic units (OTUs) were defined as clones sharing >97.5% sequence identity (Stackebrandt and Goebel, 1994; Rossello-Mora and Amann, 2001; Lawley *et al.*, 2004). The rarefaction curve was produced by plotting the number of OTUs observed against the number of clones screened using the Analytic Rarefaction 1.3 software (http://www.uga.edu/~strata/software/index.html). The coverage of clone libraries was calculated according to Good (1953) using the equation $C = (1-(n1/N))100$, where C is the homologous coverage, $n1$ is the number of OTU's appearing only once in the library and N is the total number of clones examined. For the calculations, bacterial 16S rRNA gene sequences from all samples were combined into one maximum parsimony tree using the ARB software package. Jackknifing (100 permutations) was carried out as described (Ludwig *et al.*, 2004).

Controls and lower detection limits of assays. Appropriate controls as established previously were used whenever necessary (La Duc *et al.*, 2007b). Briefly, unopened Mesosystems cartridges served as negative buffer controls in all molecular assays in addition to water blanks, free of ATP and DNA. To address problems associated with microfluidic components, which transport sample buffer throughout the sampler, a 0.2-µm sterile Millipore disc filter was placed at the mouth of the Mesosystems air sampler and the collecting fluid in the sample-retaining container was assayed for all methodologies adopted in this study. Pure ATP (Sigma, St Louis, MO, USA) was decimally diluted and served as a standard curve for ATP analyses. Purified DNA from *Bacillus pumilus* ATCC 7061 was included in the PCR amplification protocols as a positive control. To prevent false negative results in PCR reactions associated with the presence of inhibitory substances, a known amount of DNA was extracted from *B. pumilus* and spiked (1 pg per reaction mixture) as an internal standard. None of the DNA extracts in this study inhibited the PCR reaction. The lower detection limits were >30 CFU ($=2.0\times10^3$ CFU m^{-3}) for the cultivable plate-count assay, >50 RLU ($=3.4\times10^3$ RLU m^{-3}) for ATP analysis and >100 copies ($=6.7\times10^3$ copies m^{-3}) for Q-PCR analysis. Air sample collection from outside the airliner before take off and after landing was not possible due to security reasons. Sample collection in the gate area will not reflect the true environment and hence such attempts were not made.

Results

Heterotrophic plate counts

Plate counts on R2A media ranged from 0 to 10^3 CFU m^{-3}, and only 1.8% of all samples (2 of 108 samples) showed cultivable counts of 10^3 CFU m^{-3} or higher, none of which exceeded 2.7×10^3 CFU m^{-3} (Figure 1). No significant differences were detected

between plate counts on international flights ($n=62$ samples) and those on domestic flights ($n=46$ samples) when compared using Student's *t*-test and Mann–Whitney's *U*-test ($P=0.34$ and $P=0.46$; Table 2). Approximately 50% of cabin air samples collected during both domestic (25 of 46 samples) and international flights (31 of 62 samples) did not contain any cultivable bacterial counts. Average plate counts on individual flights ranged from below detection limit (2 of 15 flights) to 3.6×10^2 CFU m^{-3} (Figure 1). In general, plate counts fluctuated during each flight, and no particular trends were noticeable over the course of any single trip.

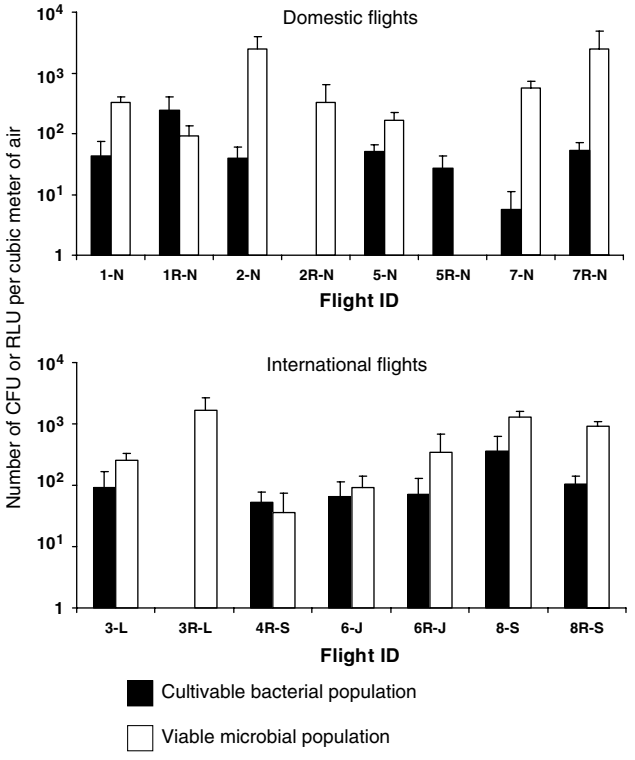


Figure 1 Microbial population of commercial airline cabin air. The solid bar represents cultivable bacterial population measured by R2A medium and viable microbial population estimated by intracellular ATP. Two measurements for cultivable bacterial counts and four replicates were carried out for total microbes. Number of samples for each flight segment is given in Table 1.

Table 2 Statistical analysis to compare microbial populations of cabin air from various domestic and international flights

Microbial population	*Number of observations* (n)		P-*values*[a]	
	Domestic	*International*	*Mean*[b]	*Median*[c]
Cultivable heterotrophic bacteria	46	62	0.340	0.460
Viable microbes[d]	46	63	0.810	0.230
Total microbes[e]	46	63	0.001	0.001

[a]$\alpha=0.05$.
[b]Student's *t*-test.
[c]Mann–Whitney's test.
[d]Measured by intracellular ATP.
[e]Measured by total ATP.

Microbial population as measured by ATP. In general, total viable population as measured by the intracellular-ATP assay was in the range of below detection limit to 10^3 RLU m^{-2} (Figure 1) and was consistently 1–2 logs lower than total ATP measurements, suggesting that approximately 1–10% of the microbial load was viable (Supplementary Table 1). No patterns in viable microbial levels were observed either during a single flight or across multiple flights. Comparison of domestic and international samples using both Student's *t*-test and Mann–Whitney's *U*-test failed to detect a significant difference in total viable microbial population ($P=0.81$ and $P=0.23$; Table 2). On comparing heterotrophic counts with ATP-derived estimates of viable microbes, two flight segments contained no detected CFU (2R-N and 3R-L) and one flight segment showed no viable microbes (5R-N; Figure 1). Approximately 2 logs higher viable but yet to be cultivated microbes were noticed in 7 out of 15 flight segments (Supplementary Table 1) than corresponding plate counts. In addition, ~99% of the viable microbial population was not cultivable in three sets of commercial airline samples, all of which were obtained on domestic segments. Two

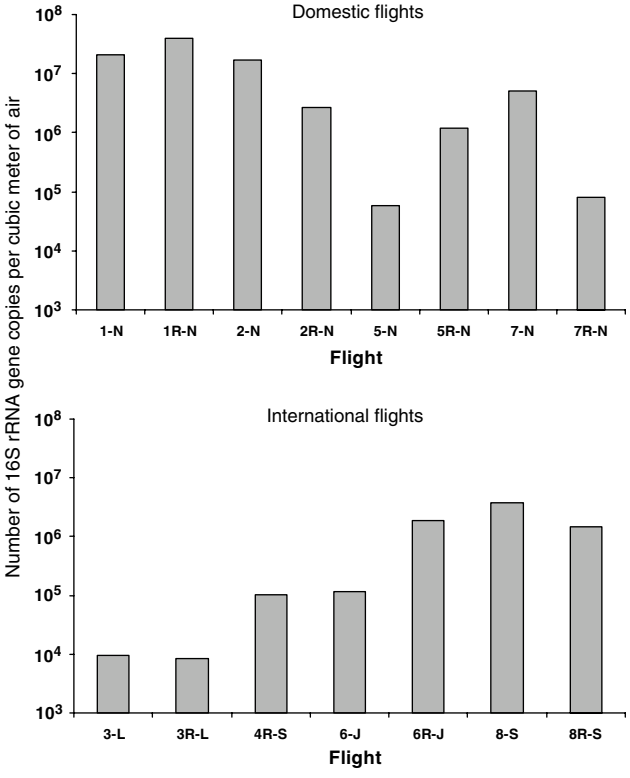


Figure 2 Total bacterial population as measured by 16S rRNA gene copy numbers of several domestic and international flight cabin air.

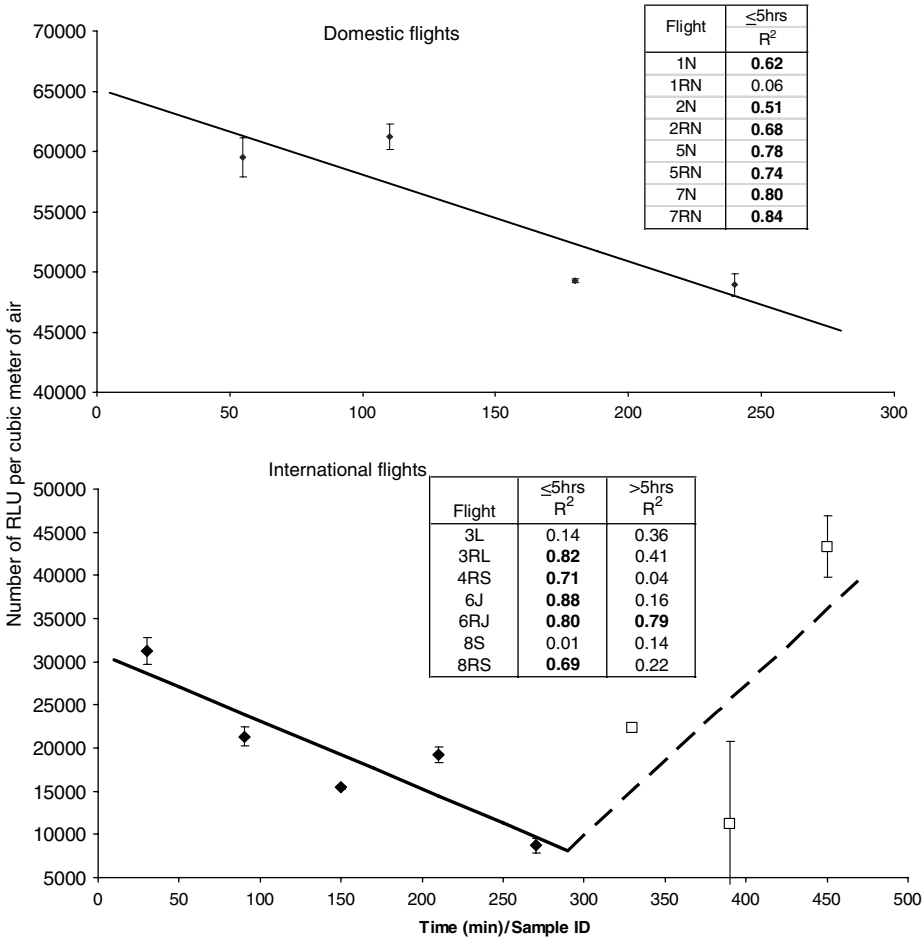
flights yielded samples with similar cultivable and viable counts.

Ranges in total microbial population were similar for both domestic (7.3×10^3–1.3×10^5 RLU m^{-3}) and international (8.7×10^3–3.7×10^5 RLU m^{-3}) cabin air samples and mean total microbes were 3.8×10^4 and 6.8×10^4 RLU m^{-3}, respectively (Supplementary Table 1). With the exception of flight 1R-N, all domestic flights (5 h flight time) showed a strong negative correlation ($R^2 = 0.51$–0.84) between total microbes and flight time (Figure 3). Five of seven international flights showed a very similar trend for the initial 5 h of travel ($R^2 = 0.69$–0.88; Figure 3). A less consistent, typically weaker positive correlation between total microbes and sampling time was noted after the 5-h mark on several international flights (Figure 3). Only 0–14% of samples collected aboard domestic segments yielded RLU values above 10^3 RLU m^{-3}, whereas 14–60% of samples collected from international flights exceeded 10^3 RLU m^{-3}.

Total bacterial population as measured by DNA. Bacterial 16S rRNA gene copies, enumerated via Q-PCR, ranged from 5.9×10^4 to 4.0×10^7 *rrn* gene copies per m^3 of air on domestic flights and 9.4×10^3 to 3.8×10^6 *rrn* gene copies per m^3 of air on international flights (Figure 2). Generally, domestic flight samples (6 of 8) typically contained from 10^6 to 10^7 *rrn* gene copies m^{-3}, while most international flight samples (5 of 7) were burdened with 10^5–10^6 *rrn* gene copies m^{-3}. Very low *rrn* gene copy number (10^3) was observed in the London/Los Angeles samples (3N and 3R-N) and coincided with difficulties in amplifying sufficient 16S rRNA gene fragments for subsequent clone library construction. Heterotrophic counts, as well as total and viable microbial population estimates (Figure 1), for these samples were similar to other flights.

Cultivable microbial diversity

Several α, β and γ-proteobacteria, as well as sporulating and nonsporulating Gram-positive bacteria, were isolated in varying abundance (Figure 4; Supplementary Table 2). Of the 112 bacterial isolates sequenced, approximately 86% were identified as Gram-positive, of which 60% were of high G/C



Flight	≤5hrs R^2
1N	**0.62**
1RN	0.06
2N	**0.51**
2RN	**0.68**
5N	**0.78**
5RN	**0.74**
7N	**0.80**
7RN	**0.84**

Flight	≤5hrs R^2	>5hrs R^2
3L	0.14	0.36
3RL	**0.82**	0.41
4RS	**0.71**	0.04
6J	**0.88**	0.16
6RJ	**0.80**	**0.79**
8S	0.01	0.14
8RS	**0.69**	0.22

Figure 3 Changes in total microbial population of several domestic and international flight cabin air. Regression analyses of all flight segments for 5 h and more than 5 h are depicted. The letters in bold are significant at 95% confidence level. The changes in total microbial population as measured by total ATP with time for one flight each for domestic (7R-N) and international flights (6R-J) are depicted. Each plot consists of four measurements.

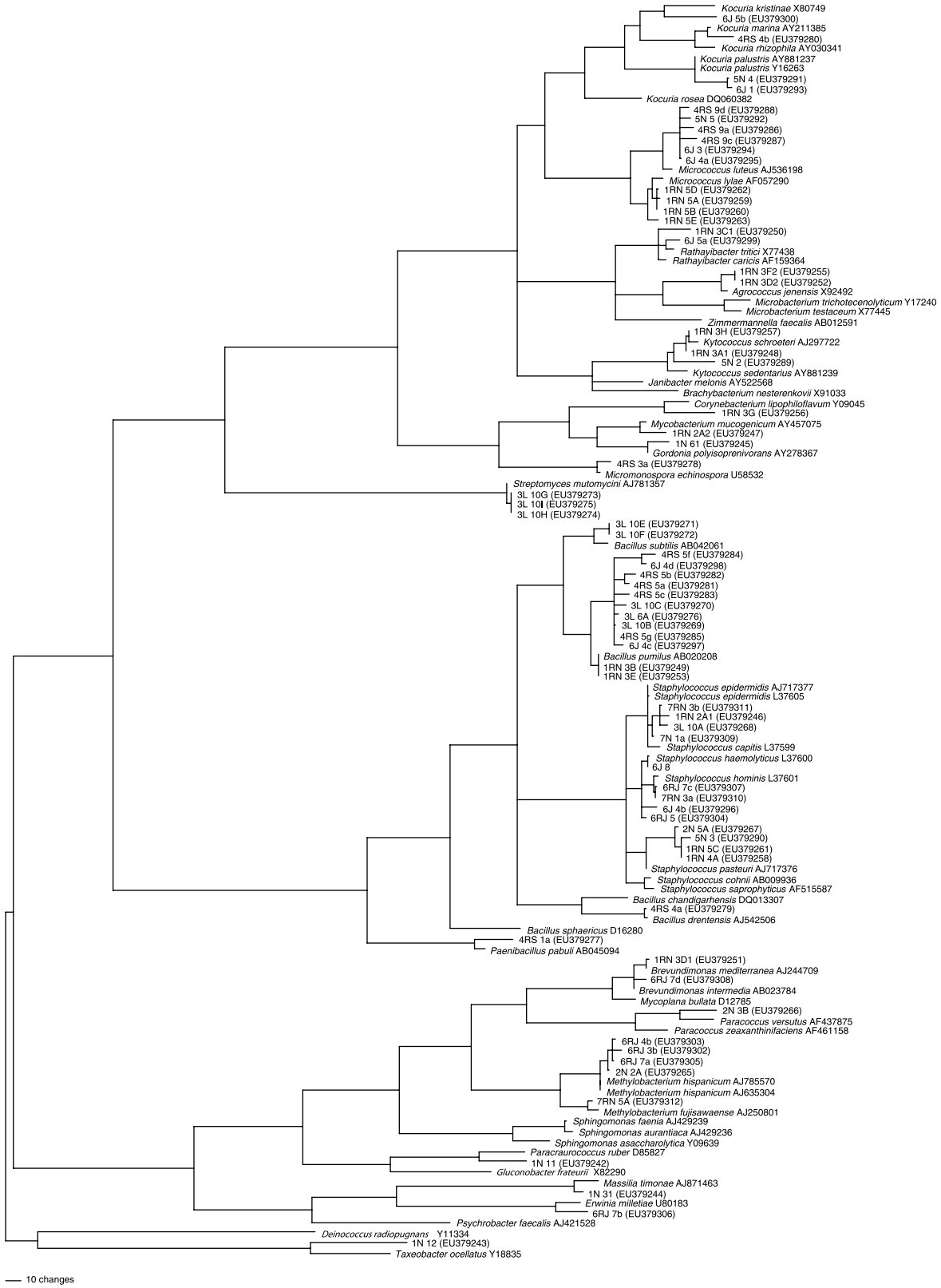


Figure 4 Cultivable bacterial diversity of commercial airline cabin air. Phylogenetic tree (maximum parsimony), showing the cultivable bacterial diversity and the phylogenetic affiliation of the sequences derived from different flights. The GenBank accession number of the closest neighbor of the reference strains is specified after the name and that of the isolated strain is given in parentheses. The strain IDs are flight numbers (1N, 1R-N and so on; refer Table 1) followed by isolate numbers (5b, 4b and so on). The scale bar shows a 10% estimated difference in nucleotide sequence positions.

content, including *Micrococcus*, *Kytococcus* and *Staphylococcus*. Staphylococci and micrococci were detected in high abundance, comprising 38 of 57 high-G/C non-spore-forming isolates. Spore-forming *Bacillus* species were also prevalent, with 19 of the 57 high-G/C Gram-positive strains belonging to this taxon. Members of the α-proteobacteria, such as *Methylobacterium* spp, comprised 10% of all bacterial strains isolated, complementary to results obtained from 16S rDNA-based clone library analyses. Five novel bacterial lineages were uncovered within the cabin air samples analyzed: two members of the α-proteobacteria (*Paracoccus* sp and *Paracraurococcus* sp), one flexibacteraceae (*Taxeobacter* sp) and two Gram-positive bacteria (*Corynebacterium* sp and *Bacillus* sp). The novelty of these strains was determined based on 16S rDNA sequence similarities of less than 97.5% with nearest neighbors in the GenBank public database (Stackebrandt and Goebel, 1994). Several bacteria implicated in human illness were also isolated, including *Janibacter melonis* (responsible for bacteremia), *Microbacterium trichotecenolyticum* –(the causative agent of neutropenia), *Massilia timonae* (a cause of cerebellar lesions), *Staphylococcus saprophyticus* (a cause of urinary tract infections) and *Corynebacterium lipophiloflavum* (the cause of bacterial vaginosis). No spatial or temporal patterns were observed in the isolation of any bacterial species.

Bacterial community analysis

The number of clones analyzed, total number of OTUs present and incidence in clone libraries are presented in Table 3. After critical examination of 2000 clone sequences (13 sample and 7 negative control libraries) to exclude chimeras and non-full-length 16S rRNA (~1.5-kb) gene sequences, 861 clones were selected for further analysis. Among these 861 quality clone sequences, 507 were removed from the calculations due to their presence in negative control libraries (Supplementary Table 3). Representatives of over 100 species, spanning 12 classes of bacteria, were identified in the remaining 354 clones. The coverage values for the domestic routes ranged from 78–96% as opposed to 57–90% for international segments. The lower coverage index for the international routes indicated that these samples possessed higher diversity than could be resolved with the number of clones sequenced. Two international routes exhibiting high numbers of singularly occurring OTUs were 4S (29 OTUs) and 8R-S (14 OTUs), yielding coverage percentages of only 56.7% and 68.9%, respectively. The two domestic clone libraries with high numbers of OTUs occurring only once were 5N (19 OTUs) and 7R-N (14 OTUs) each with ~78% coverage.

The percent incidence of all clones was determined with respect to bacterial class (Table 4). Among all bacterial classes, sequences arising from proteobacteria were collected in greatest frequency (58% of all clone sequences). The predominance of α- and γ-proteobacteria was apparent on all flights: α-proteobacteria constituted at least 10% of the detected microbial diversity and in some instances comprised 100% of the diversity on an individual flight (Table 4). A similar level of species richness was observed in the Gram-positive bacteria (57 species), which accounted for 31% of all identified clone sequences. Nearly two-thirds of these species were Firmicutes, with the remaining 20 clones representing species of actinobacterial origin.

Table 3 Molecular microbial characterization of various segments of commercial airliner travel

Flight route	*Sample ID number*	*Number of useful clones*[a]	*Number of OTUs*[b]	n1[c]	*Coverage (C) (1–((n1/N))100*
Domestic routes					
Los Angeles to New York	1N	83	8	3	96.39
New York to Los Angeles	1R-N	68	8	3	95.59
Los Angeles to New York	2N	73	13	7	90.41
Los Angeles to New York	5N	88	25	19	78.41
New York to Los Angeles	5R-N	86	13	7	91.86
Los Angeles to New York	7N	70	13	7	90.00
New York to Los Angeles	7R-N	54	20	11	79.63
International routes					
Los Angeles, USA to Narita, Japan	6J	59	22	11	81.36
Narita, Japan to Los Angeles, USA	6R-J	55	10	6	89.09
Los Angeles, USA to Sydney, Australia	4-S	67	35	29	56.72
Sydney, Australia to Los Angeles, USA	4R-S	84	22	8	90.48
Los Angeles, USA to Sydney, Australia	8S	29	9	3	89.66
Sydney, Australia to Los Angeles, USA	8R-S	45	18	14	68.89

Abbreviation: OTU, operational taxonomic unit.
[a]Number of fully sequenced bacterial clones per sample. Sequences of chloroplasts, chimera and sequences that exhibited undetermined bases (*N*) at a frequency of >1% were removed from calculation.
[b]OTU is defined when the sequence similarity was >97.5% with the type strain sequence Good (1953).
[c]Number of OUT's appearing only once in the library.

Table 4 Bacterial diversity of cabin air samples collected during various domestic and international routes

Identity of the clone	*GenBank number*	*% similarity*	*Number of clones retrieved from the air samples collected from*											
			Domestic routes							*International routes*				
			1N	*1R-N*	*2N*	*5N*	*5R-N*	*7N*	*7R-N*	*4S*	*4R-S*	*6J*	*6R-J*	*8S*	*8R-S*
Acinetobacter calcoaceticus	Z93434	97.5									1				
Acinetobacter johnsonii	X81663	99.0								1	1				
Acinetobacter junii	X81664	98.7			3	1									
Acetobacter sp	X74066	97.4								1					
Actinomyces sp	X82453	95.0								1					
Aerococcus viridans	M58797	98.6													1
Agrobacterium sp	AB021493	95.6		44								2			1
Arsenicicoccus sp	AJ558133	97.4									1				
Bacillus flexus	AB021185	98.7								2					
Bacillus licheniformis	X68416	97.9								1					
Bacillus muralis	AJ628748	99.2													1
Bacillus pallidus	Z26930	99.5							3						
Bacillus pumilus	AY456263	99.3					1								
Bacillus weihenstephanensis	AB021199	99.2								1					
Bacillus sp	AB021198	95.4									1				
Bacteriovorax sp	M34125	89.6							1						
Bacteroides sp	M58762	94.8											1		
Brevibacillus choshinensis	D78459	99.2								1					
Burkholderia cenocepacia	AF148556	98.5										2			
Burkholderia sp	U96929	94.8	1												
Caulobacter sp	AJ009957	96.9												3	15
Caulobacter sp	AJ227758	97.1												2	
Citrobacter murliniae	AF025369	98.0									1				
Corynebacterium tuberculostearicum	AJ438050	99.9									5				
Curvibacter gracilis	AB109889	99.1						1							
Deinococcus sp	Y13038	86.2													1
Deinococcus sp	Y11329	89.2												2	1
Deinococcus sp	Y13041	89.5												15	12
Diaphorobacter nitroreducens	AB064317	99.7			1										
Erythrobacter sp	AY562220	97.0		3											
Exiguobacterium oxidotolerans	AB105164	98.0									1				
Facklamia sp	Y10772	93.2													1
Fusobacterium canifelinum	AY162220	98.4								1					
Gemella haemolysans	L14326	99.4									1				
Gemmata sp	X56305	89.3							1						
Gemmata sp	AJ231191	90.3													1
Granulicatella adiacens	D50540	99.1				1									
Haemophilus sp	M75045	97.2				1									
Haemophilus sp	M75076	97.4				1									
Herbaspirillum sp	Y10146	94.7													1
Janthinobacterium lividum	Y08846	99.4										1			
Kocuria rhizophila	Y16264	99.9										1			
Lactococcus lactis subsp cremoris	M58837	99.4				1						1			
Leptothrix mobilis	X97071	97.5							1						
Massilia sp	U54470	96.9			2										

Table 4 (Continued)

Identity of the clone	*GenBank number*	*% similarity*	*Number of clones retrieved from the air samples collected from*											
			Domestic routes							*International routes*				
			1N	*1R-N*	*2N*	*5N*	*5R-N*	*7N*	*7R-N*	*4S*	*4R-S*	*6J*	*6R-J*	*8S*	*8R-S*
Meiothermus sp	X84211	84.5						1							
Methylobacterium hispanicum	AJ635304	98.9											29	2	
Methylobacterium organophilum	D32226	99.3								1					
Methylobacterium sp	AJ635304	96.8											2		
Microbacterium kitamiense	AB013907	97.9										1			
Micrococleus sp	X70770	86.9						1							
Mycobacterium chitae	M29560	98.1			2					2					
Neisseria flavescens	L06168	97.5				1									
Neisseria sp.	L10738	96.6									1				
Novosphingobium sp	X94102	91.7							1						
Novosphingobium sp	AJ416411	95.1				1									
Novosphingobium sp	AF502400	95.8							2	1					
Pantoea agglomerans	U80202	99.0													1
Paracoccus carotinifaciens	AB006899	99.5								1					
Paracoccus sp	D32238	96.5								1					
Propionibacterium sp	AJ003058	93.3				1				1					
Pseudomonas hibiscicola	AB021405	98.8				2			1		1				
Pseudomonas putida	Z76667	99.7			1	1									
Pseudomonas saccharophila	AB021407	98.6						3							
Pseudomonas trivialis	AJ492831	98.9							1						
Pseudomonas sp	AB021390	95.1								1					
Psychrobacter sp	AJ313425	93.5	64												
Psychrobacter sp	AB016057	87.9	8												
Rhizobium leguminosarum	AY509899	97.6												2	2
Rhizobium sp	AF025852	92.3			1										
Rubrobacter sp	U65647	92.7										1			
Salmonella typhi	Z47544	99.1				1		1							
Serratia entomophila	AJ233427	98.8			1										
Sphingomonas sp	AJ429239	96.9										1			
Sporanaerobacter acetigenes	AF358114	100.0		1						1					
Staphylococcus arlettae	AB009933	99.5								1					
Staphylococcus caprae	AB009935	99.6								1	2				
Staphylococcus cohnii	AB009936	99.9				1									
Staphylococcus epidermidis	D83363	99.5				1	1					3			
Staphylococcus haemolyticus	X66100	99.0									2				
Staphylococcus hominis	X66101	98.7				2	1			1		2	4		
Staphylococcus pasteuri	AB009944	98.9												1	
Staphylococcus warneri	L37603	99.4								1	1				
Stella sp	AJ535711	90.7				1									
Streptococcus australis	AY485604	98.8										2			
Streptococcus mitis	AF003929	98.9							1	1	2	1			
Streptococcus parasanguinis	AF003933	98.9								1					
Streptococcus pseudopneumoniae	AY612844	98.4													1
Streptococcus sanguinis	AF003928	99.6												1	
Streptococcus thermophilus	X68418	98.1				1				1	2				1
Streptococcus sp	AF003930	96.1							2						

Table 4 (Continued)

Identity of the clone	*GenBank number*	*% similarity*	*Number of clones retrieved from the air samples collected from*											
			Domestic routes							*International routes*				
			1N	*1R-N*	*2N*	*5N*	*5R-N*	*7N*	*7R-N*	*4S*	*4R-S*	*6J*	*6R-J*	*8S*	*8R-S*
Streptococcus sp	AF003933	97.3								1					
Wautersia paucula	AF085226	98.9						3							
Williamsia maris	AB010909	96.9					5								
Williamsia sp	AB010909	96.3							1						
Total number of sequences			73	48	11	18	8	10	15	26	23	18	36	28	40

When compared with clone libraries from domestic flights, international flight clone libraries exhibited greater biodiversity. Gram-positive and α-proteobacterial sequences were retrieved more from the international flights (Table 4), whereas β- and γ-proteobacteria are far more common in domestic cabin air parcels (Table 4). A total of 183 full-length 16S gene sequences were obtained from 7 clone libraries of domestic flights while 171 sequences were obtained from the 6 clone libraries constructed from international flight samples. Of the 95 unique OTUs obtained from these 354 clones, 51 OTUs were found only on international flights, while 31 OTUs were confined exclusively to domestic flights (Figure 5). No sequences were so ubiquitous as to appear in all sampled flights of either domestic or the international-bound aircraft. Only 11 OTUs were detected on both domestic and international flights (Figure 5). The most widely observed bacteria in the domestic clone libraries were *Acinetobacter junii*, *Pseudomonas hibiscicola*, *Pseudomonas putida*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Staphylococcus hominis*, each detected in two of the seven domestic clone libraries, whereas *S. hominis*, *Streptococcus mitis* and *Streptococcus thermophilus* sequences were retrieved in high abundance in the clone libraries of international flights.

With regard to the presence of potentially pathogenic bacterial species, it should be noted that pathogenicity is often strain specific, and the phylogenetic analyses employed may only detect OTUs with known disease-causing variants. Two species of disease-associated β-proteobacteria (*Burkholderia cepacia* and *Massilia timonae*) were retrieved at frequencies greater than 10% from at least one clone library, and 50% of all γ-proteobacterial sequences (20 species) originated in organisms with some connection to human illness (Supplementary Table 3). Six of the actinobacterial species had pathogenic activity in humans, and three of these composed over 10% of at least one clone library (Supplementary Table 3). Clones indicative of the presence of numerous firmicute pathogens (six clones) were retrieved in varying abundance, including *Staphylococcus aureus* and several streptococci.

Discussion

The efficiency of HEPA filters in retaining bacterial-sized particles and aerosols has been well documented and while specifications for modern filters vary, they typically exceed 99.97% for 0.3 μm particles and 98% for the most penetrating particle size of 0.1 μm. Viruses (0.01–0.02 μm) and larger bacteria/fungi (0.1–1.0 μm) are retained even more effectively (Aviation-Safety, 2004). Cabin air that has passed through an HEPA filter is considered comparable to the recirculated air used in operating

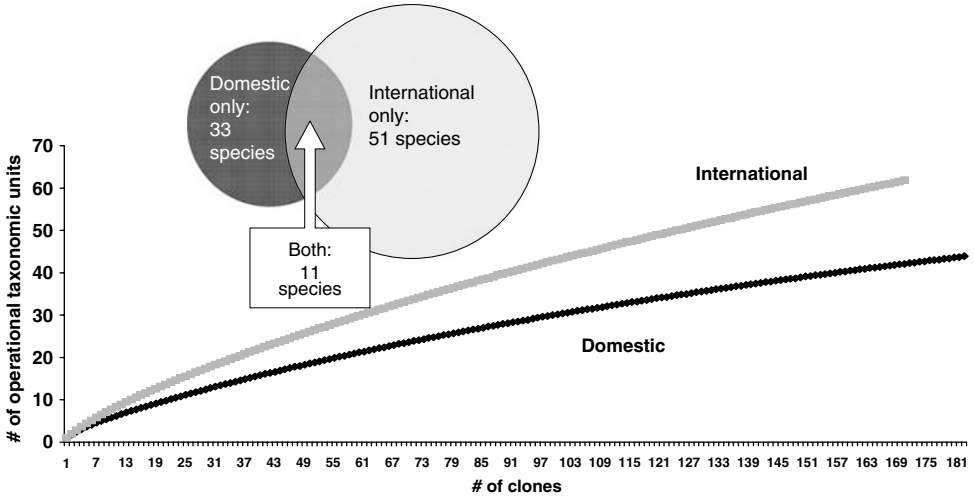


Figure 5 Rarefaction curves constructed for bacterial clone libraries from several international and domestic flight cabin air. Clones were grouped into OTUs at a level of sequence similarity of $>97.5\%$. The overlapping OTUs between domestic and international flights are given in the inset.

rooms and infectious-disease containment facilities (WHO, 1998). When compared to standard office buildings, the air in a commercial aircraft is exchanged more frequently per hour and has even been described as 'sterile' by investigations carried out using conventional plate-count assays (FSF, 1998). According to the limited reports available, it has been documented that airborne microbial levels within airline cabins are much lower than public areas on the ground (CDC, 1995; Wick and Irvine, 1995). Unfortunately, these culture-based assessments of environmental samples grossly underestimate microbial levels and are ill suited for describing the community structures of the microbial populations that are present (Pace *et al.*, 1985; Pace, 1997). Indeed, biomolecule-based examinations of these 'sterile' air samples collected directly from blowing sources, such as overhead gaspers, revealed high microbial levels and biodiversity, suggestive of biofilm formation in air ducts (La Duc *et al.*, 2007b).

The power of molecular techniques in describing the microbial burden and diversity of the HEPA-filtered airline cabins examined was apparent, as heterotrophic plate counts typically underestimated total viable microbes by at least an order of magnitude (Figure 1). Statistical analyses failed to detect significant differences between domestic and international flights with respect to both heterotrophic plate counts and total viable microbes, most likely due to constant HEPA filtration, maintaining consistently low levels of airborne microorganisms. Measurements of 16S rRNA gene copy numbers (Figure 2) and total ATP (Figure 3), however, showed large overall variation between domestic and international-bound flights. Linear regression analysis revealed repeated patterns in the levels of total ATP levels present over the course of each flight. Domestic flights (approximately 5 h in duration) and the initial 5 h of international flights typically demonstrated strong downward trends in measures of total ATP, which may be indicative of the progressive elimination of biological agents due to air exchange and continuous HEPA filtration. International flights showed a weaker upward trend in total ATP as flight time progressed beyond the five-hour point, possibly due to increase crew and passenger activity as the destination approached, or differences in air-handling protocols on longer flights. This much weaker association in the later portions of international flights indicates that a more complex set of factors may contribute to cabin cleanliness than can be correlated with the passage of time alone. Because exogenous sources (for example, food particles in the aerosols) could contribute to measurements of total ATP, the results of this assay cannot specifically be attributed to the microbial populations alone. They do, however, suggest that the length of international flight may have an impact on the aggregation of biological material that could, in the absence of HEPA filtration and air exchange, contribute to microbial persistence and proliferation.

Environmental cluster UniFrac analysis (Supplementary Figure 1; Lozupone and Knight, 2005; Lozupone *et al.*, 2006) showed that the clone library composition of different cabin air samples did not cluster by any flight segment and was confirmed by Jackknife analysis. The only exception was the clone libraries from Sydney (8S and 8R-S), which clustered together with high confidence level (91% Jackknife analysis). When four samples collected from two separate trips to Sydney (segments 4 and 8) were subjected to UniFrac analyses, these clone libraries did not form any cluster. Analysis of biodiversity for domestic and international flights

indicated vastly different population structures. Figure 5 presents rarefaction curves and the population overlap for domestic and international clone libraries. The rarefaction curve for the domestic aircraft clones ($n = 522$) suggested better coverage of the biodiversity present aboard these aircraft. Despite a smaller number ($n = 339$), clone libraries of international-bound aircraft had a steeper rarefaction curve and demonstrated much greater biodiversity, being distributed among 62 bacterial species, while clones from domestic flights comprised only 42 species. Only 11 species were detected in both sets of clones, representing 26% and 18% of species detected on domestic flights and international flights, respectively. The cultivable bacterial diversity also indicated clustering of bacterial species with flight duration or destination. The isolation of staphylococci and micrococci on both domestic and international samples was expected, since cells in these genera are found in tight association with human skin cells, which are constantly shed. Interestingly, despite expectations that the artificially dry conditions aboard these flights would promote their prevalence, *Bacillus* isolates were not widely distributed across all flights and were limited almost entirely to longer international flights. It is important to note that while differences in microbial population structures correlated with flight duration, they may also be attributed to varying cabin design and air filtration on domestic and international flights. Internationally operated 747 and 777 airplanes have much larger passenger capacity (approximately two- to threefold) than that of domestically utilized 757 equipment (http://www.boeing.com/commercial). Passenger density as well as potential differences in air handling on larger planes may have significant impact on the biodiversity of air parcels obtained from these flights.

Although distinctions were observed by clustering clones from cabin air samples by destination (international vs domestic), taxonomic groupings were also generated on an individual, flight-by-flight basis (Figure 4; Table 4). Phylogenetic analysis revealed multiple monophyletic groups containing clones from a single flight, suggesting that each plane may harbor its own unique microbial consortium. Numerous DNA-based studies have noted that microbial communities vary significantly based on the location of sampling (Brodie *et al.*, 2007; Moissl *et al.*, 2007a, b). Microbial populations aboard aircraft could easily reflect this phenomenon, particularly if planes are dedicated to particular routes of travel. Unfortunately, confirmation of this hypothesis would require disclosure from airlines on the regular deployment schedules of their aircraft.

The empirical results of this study indicated the presence of a wide range of opportunistic human-related pathogens, including causative agents of pneumonia, bacteremia, neutropenia and cerebellar lesions (US-HHS-Department, 2003), and passengers and crewmembers are thought to be their principal source (Wick and Irvine, 1995; Dechow *et al.*, 1997). It should be stressed that the retrieval of 16S rRNA gene sequences from these pathogens is not in itself evidence for public health concerns over commercial air travel. The detection of DNA arising from these pathogens does not imply viability, and the human pathogens detected in plate-count assay were overwhelmingly opportunistic, posing little threat to healthy individuals. Furthermore, the combination of engineering controls built into commercial aircraft, including frequent air exchange, HEPA filtration and directed cabin airflow (Aviation-Safety, 2004), as well as our own cultivation and viable microbial measurements (Figure 1) suggests that exposure to disease-causing microbes in doses sufficient for infection is unlikely during normal airline travel. Although high prevalence of health issues among travelers and aircraft crew members has been reported (Low and Chan, 2002; Whelan *et al.*, 2003), these reports did not account for other factors that could be responsible for this correlation, such as travel-related stress on the immune system, or transient responses to environmental conditions within the air cabin. Recent studies have shown, for example, that symptoms experienced by air travelers, especially on flights greater than 3 h, may stem from low humidity (Nagda and Hodgson, 2001; Leder and Newman, 2005), and that increasing the humidity of the air cabin by as little as 3% is sufficient to significantly alleviate physical symptoms commonly reported by air crew (Norback *et al.*, 2006).

The results of this study indicate that a much broader microbial diversity exists aboard commercial airplanes than was previously suspected. The advantages of the molecular-based approaches presented here rest in their potential for furthering the development of environmental-monitoring technologies capable of detecting premeditated bioterrorism (Hartley and Baeumner, 2003) and natural outbreaks of pandemic-causing agents (Schafer, 1999; US-HHS-Department, 2003; Sampathkumar, 2007). Biological sensors will rely on accurate and precise measurements of microbial levels and composition in public spaces, not only for equipment design, but also for validation and ongoing calibration.

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Primary biological aerosol particles in the atmosphere: a review

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ABSTRACT

Atmospheric aerosol particles of biological origin are a very diverse group of biological materials and structures, including microorganisms, dispersal units, fragments and excretions of biological organisms. In recent years, the impact of biological aerosol particles on atmospheric processes has been studied with increasing intensity, and a wealth of new information and insights has been gained. This review outlines the current knowledge on major categories of primary biological aerosol particles (PBAP): bacteria and archaea, fungal spores and fragments, pollen, viruses, algae and cyanobacteria, biological crusts and lichens and others like plant or animal fragments and detritus. We give an overview of sampling methods and physical, chemical and biological techniques for PBAP analysis (cultivation, microscopy, DNA/RNA analysis, chemical tracers, optical and mass spectrometry, etc.). Moreover, we address and summarise the current understanding and open questions concerning the influence of PBAP on the atmosphere and climate, i.e. their optical properties and their ability to act as ice nuclei (IN) or cloud condensation nuclei (CCN). We suggest that the following research activities should be pursued in future studies of atmospheric biological aerosol particles: (1) develop efficient and reliable analytical techniques for the identification and quantification of PBAP; (2) apply advanced and standardised techniques to determine the abundance and diversity of PBAP and their seasonal variation at regional and global scales (atmospheric biogeography); (3) determine the emission rates, optical properties, IN and CCN activity of PBAP in field measurements and laboratory experiments; (4) use field and laboratory data to constrain numerical models of atmospheric transport, transformation and climate effects of PBAP.

Keywords: primary biological atmospheric aerosol; climate; cloud condensation nuclei; biology; atmospheric ice nuclei

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Contents

1. Introduction

1.1. History

The occurrence and relevance of airborne biological particles have been addressed since the beginnings of scientific investigations into atmospheric aerosols (Ehrenberg, 1847; Pasteur, 1861; Carnelly et al., 1887; De Bary, 1887). For example, by the late nineteenth century, Miquel (1883) had already shown that airborne spore concentrations in France followed a seasonal cycle and were dependent on wind direction. He also showed that human mortality in Paris followed the bacteria concentration in the air. Since then, aerobiology (i.e. the study of airborne biological particles) has become well established as a multidisciplinary field of scientific research that interacts with a host of physical, biological and medical science disciplines (e.g. Gregory, 1973). The impact of aerobiology is especially notable in such diverse basic and applied sciences such as allergology, bioclimatology, palynology, biological pollution, biological warfare and terrorism, mycology, biodiversity studies, ecology, plant pathology, microbiology, indoor air quality, biological weathering, industrial aerobiology and cultural heritage.

The potential relevance of biological particles for atmospheric processes has also been recognised for many years (e.g. Dingle, 1966; Schnell and Vali, 1972; Jaenicke and Matthias, 1988; Matthias-Maser and Jaenicke, 1995; Andreae and Crutzen, 1997; Jaenicke, 2005; Pöschl, 2005). Figure 1 outlines major processes in the cycling of primary biological aerosol particles (PBAP)[1] between atmosphere and biosphere. After emission from the biosphere, PBAP undergo various physical and chemical aging processes in the atmosphere (coagulation, surface coating, reaction with photo-oxidants, etc.). Depending on their surface properties, PBAP can serve as nuclei for water droplets or ice crystals, leading to the formation of clouds and precipitation. Removal from the atmosphere proceeds via dry deposition (diffusion/sedimentation) or wet deposition with precipitation (nucleation/scavenging). After deposition, PBAP can interact with terrestrial or aquatic ecosystems and trigger biological activities leading to further PBAP emissions (growth and reproduction). However, biological particles in general have received less attention in atmospheric science than other types of aerosol particles such as sulfate, sea salt, mineral dust or volcanic ash (e.g. Junge, 1963; Hammond, 1971; Friedlander, 2000). This is primarily because the atmospheric impact of biological aerosols has been poorly understood, and because atmospherically relevant measurements have been costly and difficult to interpret. Also, global average number concentrations of biological particles have often been assumed to be insignificant compared to non-biological material and have thus not typically been considered for widespread measurements or included in global climate models. The Third Assessment Report (TAR) of the Intergovernmental Panel on Climate Change (IPCC) in 2001, for example, listed the global source strength of primary biological aerosol particles to be only 56 Tg/yr, in contrast to 3340 Tg/yr for sea salt and 2150 Tg/yr for mineral dust listed in the same report (Penner et al., 2001). Furthermore, the Fourth Assessment Report of the IPCC in 2007 stated that these estimates had not been refined, and primary biological particles were not mentioned in the contribution of Working Group I (Physical Science Basis) to the overall report (IPCC 2007b). PBAP concentrations have been estimated by other researchers (e.g. Matthias-Maser and Jaenicke, 1995) as comprising a much higher percentage of total atmospheric aerosol volume, however, and so important discrepancies exist.

[1]A list of abbreviations can be found in the appendix.

Fig 1

Fig. 1. Cycling and effects of primary biological aerosol particles in the atmosphere and biosphere (adapted from Pöschl, 2005).

Interest in biological aerosol has been growing significantly in recent decades. This is highlighted by the fact that an Institute for Scientific Information (ISI) search for the term 'biological aerosol' (including quotation marks)

results in less than a total of five publications until 1987, approximately one citation per year between 1987 and 1998, and then steadily increasing numbers with an average of ~9 citations per year between 1998 and 2011. Recently, several investigations have suggested that biological particles can have a substantial influence on clouds and precipitation and thus may influence the hydrological cycle and climate at least on regional scales (e.g. Andreae and Rosenfeld, 2008; Prenni et al., 2009; Pöschl et al., 2010). Various fields of medical research are also concerned with biological aerosols. Biological particles have been linked to many different adverse health effects spanning from infectious diseases to acute toxic effects, allergies, asthma and even cancer (Peccia et al., 2011). The negative effects that bioaerosols can play on the human respiratory system are particularly well documented (Verhoeff and Burge, 1997; Burge and Rogers, 2000; Douwes et al., 2003; Lee et al., 2005). Although medical research dealing with biological aerosols is indeed critical, this review article does not discuss medical applications of biological aerosols directly, providing instead a synthesis and overview of recent studies dealing with the observation and relevance of primary biological aerosol particles in an atmospheric context.

1.2. Definition and sources of primary biological aerosol particles

Aerosols are generally defined as colloidal systems of liquid or solid particles suspended in a gas (Hinds, 1999; Baron and Willeke, 2001; Fuzzi et al., 2006). Particle diameters are typically in the range of ~1 nm to around ~100 µm, where the lower limit is given by the size of small molecular clusters and the upper limit by high settling velocities comparable to the magnitude of atmospheric updraft velocities (~1 m s^{-1}, Hinds, 1999; Seinfeld and Pandis, 2006). Primary atmospheric aerosol particles are emitted directly into the atmosphere from a source material, whereas secondary particles are formed in the atmosphere by condensation of gaseous precursors (Pöschl, 2005; Fuzzi et al., 2006).

The term 'primary biological aerosol particles' is defined to describe solid airborne particles derived from biological organisms, including microorganisms and fragments of biological materials such as plant debris and animal dander (IGAP, 1992)[2]. The definition for PBAP used within the text, as outlined in Table 1, can thus include all sorts of intact or fragmented biological cells, dispersal units or tissues.

Table 1. Characteristic types of primary biological aerosol particles (PBAP)

Particle types	Examples
Biological organisms or dispersal units (dead or alive, isolated or aggregated)	Bacteria, fungi, protozoa, algae, spores, pollen, lichen, archaea, viruses, etc.
Solid fragments or excretions of biological organisms or dispersal units	Detritus, microbial fragments, plant debris/leaf litter, animal tissue and excrements, brochosomes, etc.

The term primary biological aerosol is more or less equivalent to the 'soft' term 'bioaerosol' (Reponen et al., 1995; Hinds, 1999). In contrast to the more rigorously defined 'biological aerosol', however, the term 'bioaerosol' is not very clearly defined and is frequently used with different meanings. In some cases, the term bioaerosol is used in a rather narrow sense, excluding biological secretion such as plant wax particles, for example (Gelencér, 2004). In other cases, it is used in a very broad sense, e.g. including any particle with biological activity/toxicity (Hirst, 1995), which would theoretically also comprise droplets of toxic chemicals such as sulphuric or nitric acid. Moreover, the term primary biological aerosol enables clear and easy distinction from biogenic secondary organic aerosols that are formed by atmospheric oxidation and gas-to-particle conversion of volatile organic compounds released from biological organisms (Hallquist et al., 2009; Jimenez et al., 2009). Therefore, we use the term biological aerosol within this review for the types of particles outlined in Table 1.

[2]Originally defined by Griffith, W. D.; Jaenicke, R.; Levin, Z.; Matthias-Maser, S; Rantio-Lehtimaki, A.; Schnell, R. C.; and Sinha, M. P.

The size of PBAP can range from several nanometers (e.g. viruses, cell fragments) to a few hundred micrometres in aerodynamic diameter (e.g. pollen, plant debris; Cox and Wathes, 1995; Hinds, 1999; Jaenicke, 2005; Pöschl, 2005). Larger particles of biological material can also be lifted into the air, but due to high settling velocities they are rapidly deposited rather than being suspended over long times. Thus, they are usually not considered to be atmospheric aerosol particles, which is also the case for self-propelled organisms.

The biosphere, or the system in which all living things interact, dominates the Earth's surface, influencing the composition of land, water and air. Thus, PBAP can be released, both actively and passively, from every region of the globe. Key PBAP-producing systems include the following.

Plants release PBAP in the course of decay processes (see Section 2.7) as well as for reproduction, including pollen from higher plants and spores from ferns and mosses (see Section 2.3).

Microorganisms inhabit most plant, soil and rock surfaces (see Sections 2.1, 2.2, 2.5, 2.6). These microorganisms can be very numerous, contributing huge number concentrations per unit surface area (10^4 – 10^8 cells cm^{-2}) in various natural environments (Morris and Kinkel, 2002; Lindow and Brandl, 2003; Yadav et al., 2004). Furthermore, the global leaf surface area is estimated to be roughly four times the terrestrial ground surface area (~6.4 10^8 km^2 vs. ~1.5 10^8 km^2) that provides a correspondingly large surface area for PBAP emission (Whittaker and Likens, 1973).

Primary biological aerosol particles originating from animals and humans include debris from skin or hair as well as, for example, excrements, brochosomes and eggs dispersed into the atmosphere by insects (see Section 2.7).

In areas of human activity, such as cities or agricultural managed areas, the numbers and composition of microorganisms such as bacteria or fungi are often increased and altered with respect to rural areas (see Sections 2.1.1, 2.2).

The cryosphere (e.g. Greenland, Antarctica, glaciers) is formed largely from precipitation, and this may be triggered by PBAP in some situations (Sands et al., 1982; Christner et al., 2008b; Pöschl et al., 2010). Bacteria have been discovered in ice cores from Antarctica at depths up to 3519 m (Raymond et al., 2008), giving possible evidence to the idea that these organisms have been introduced through precipitation. Thus, surface snow under windblown conditions could be a powerful source for PBAP via resuspension (Pomeroy and Jones, 1996).

Roughly, 70% of the globe is covered by oceans. They are full of living and decaying organisms, such as bacteria, archaea, fungi and algae, that are ejected from the ocean surface by bubble-bursting mechanisms, similar to the way other particles (e.g. sea salt) are emitted from such surfaces (Blanchard, 1983; O'Dowd et al., 2004).

In summary, the earth's biosphere provides many diverse and important sources of PBAP. We begin this review by discussing seven major categories of atmospherically relevant biological particles in detail. This is followed by a survey of available methods for PBAP detection and analysis, and overviews of the general atmospheric relevance of PBAP and their optical properties.

2. Characteristic types of primary biological aerosol particles

2.1. Bacteria and archaea

Due to their small size, bacteria have a relatively long atmospheric residence time (on the order of several days or more) compared to larger particles and can be transported over long distances (up to thousands of kilometres). Measurements show that mean concentrations in ambient air can be greater than 1×10^4 cells m^{-3} over land (Bauer et al., 2002a), whereas concentrations over the sea may be lower by a factor of ~100 – 1000 (Prospero et al., 2005; Griffin et al., 2006). Airborne bacteria may be suspended as individual cells but are more likely to be attached to other particles, such as soil or leaf fragments, or found as agglomerates of many bacterial cells (Bovallius et al., 1978; Lighthart, 1997). For this reason, whereas individual bacteria are typically on the order of ~1 μm or less in size, the median aerodynamic diameter of particles containing culturable bacteria at several continental sites has been reported to be ~4 μm, whereas at coastal sites it is ~2 μm (Shaffer and Lighthart, 1997; Tong and Lighthart, 1999; Wang et al., 2007).

The analysis of the diversity, composition and abundance of bacteria in air has experienced a recent growth in interest within the aerosol community (Morris et al., 2011). Understanding of the presence or properties of airborne bacteria is important because bacteria can influence atmospheric processes, function as human, plant

and animal pathogens and be distributed over large physical scales from their natural or anthropogenic sources. In addition, the prediction of behavioural changes in bacterial colonisation of remote environments may be linked to climatic or anthropogenic changes that influence the atmosphere and thus could be a useful marker of changes in biodiversity.

Although in recent years, research on bacteria in the atmosphere has been constantly expanding, it remains difficult to establish a clear picture of the actual abundance and composition of bacteria in the air (Mancinelli and Shulls, 1978; Grinshpun and Clark, 2005; Maki et al., 2010). The presence of bacteria in air is strongly dependent on many factors such as seasonality, meteorological factors, anthropogenic influence, variability of bacterial sources and many other complicated variables. More importantly, the analysis of airborne bacteria still suffers from a lack of standardisation in air sampling and sample processing methods (Kuske, 2006; Peccia and Hernandez, 2006; Womack et al., 2010). Thus, differences in airborne concentration estimates, as well as in composition and abundance, could either be caused by biological variations or by differences in sampling or analysis strategies. Furthermore, many airborne bacteria studies were not designed to study a broad range of species but only to detect specific, and often pathogenic, species. Thus, current understanding of airborne bacteria concentrations and properties is undoubtedly influenced by such studies, and the literature should be understood with this in mind.

One area of expanding research is work towards the use of specific bacterial species for 'source tracking', conceptually similar to other atmospheric tracer methods. For example, the relative contribution of bacteria from various source environments can be determined, thus allowing measurements at an individual measurement site to predict the history of the air arriving at that location. Bowers et al. (2010) made a contribution in this direction by identifying groups of bacteria in atmospheric samples, which are typically found primarily in the soil or on leaf-surface environments.

2.1.1. Urban airborne bacteria

The analysis and comparison of bacteria between urban and other environments has focused mostly on the comparison of bacterial concentration and not on diversity estimates. Historically, bacteria have typically been divided into Gram-positive and Gram-negative bacteria depending on their behaviour when their cell walls are treated by Gram staining. Culturing methods in general find more Gram-positive bacteria (e.g. Mancinelli and Shulls, 1978; Atlas and Bartha, 1997; Atlas and Bartha, 1997; Fuzzi et al., 1997; Kellogg and Griffin, 2006; Amato et al., 2007b; Fang et al., 2007; Fahlgren et al., 2010), whereas culture-independent techniques primarily find Gram-negative bacteria (e.g. Radosevich et al., 2002; Maron et al., 2005; Brodie et al., 2007; Després et al., 2007; Fierer et al., 2008; Fahlgren et al., 2010). Independent of the choice of detection method, several general trends can be observed from ambient measurements. A small number of existing studies suggest that both in urban and in natural areas, airborne bacterial communities are highly diverse, and variations in their species diversity are more complex than had previously been supposed (Bovallius et al., 1978; Jones and Cookson, 1983; Chihara and Someya, 1989). Bacterial diversity in rural areas is generally higher than at urban sites (Després et al., 2007). Still, the concentration of bacteria seems often to be higher in urban than in rural environments (Bovallius et al., 1978; Chihara and Someya, 1989; di Giorgio et al., 1996; Shaffer and Lighthart, 1997; Fang et al., 2007; Fahlgren et al., 2010), even when the samples are taken in the same geographic region. However, opposite trends have also been found: For example, Rosas et al. (1993) described that bacterial concentrations in rural environments are higher than in urban sites. There is also evidence that the bacterial concentrations in the urban environment are influenced by human activities (Bovallius et al., 1978; Fang et al., 2007). Different cities do vary in their bacterial composition and abundance (Brodie et al., 2007); thus, there can be no single, typical description of urban bacterial composition.

A detailed description of the composition of bacteria found in urban air is restricted to only a few studies, as most culture-based studies classify cultured bacteria only as Gram positive or Gram negative, or as cocci or rods but do not provide taxonomic identification. Other studies dealing with the analysis of urban airborne bacteria give only the number of colony forming units (CFU) or other concentration estimates and do not try to identify the bacteria at all. Still, the general trend from available reports is that bacteria found in the air often belong to groups that are also common soil bacteria: on the taxonomic level of phyla, the *Firmicutes* Table 2,[3] *Proteobacteria*, *Actinobacteria* are often present, and on the level of superphyla the *Cytophaga-Flavo-Bacteroidetes* group is often being the most commonly observed. Additionally, in some studies bacteria from the

phyla of *Verrucomicrobia*, *Cyanobacteria*, *Acidobacteria*, *Planctomycetes* and *Chloroflexi* have been detected. Within the airborne bacteria whose classes belong to *Proteobacteria*, *Gamma-* and *Betaproteobacteria* have been regularly identified, but *Alpha-*, *Delta-* and *Epsilonproteobacteria* have also been observed. Although *Bacilli* have been found often and in high numbers, drawing conclusions from these observations about their relative abundance is difficult due to high short-term variability and biases from the different detection methods.

[3]Table 2 provides an overview of all taxonomic terms used in the review

Table 2. Taxonomic information on biological particles in air mentio

Kingdom	Superphylum	Phylum	Class	Order
Bacteria		Firmicutes	Bacilli	Bacillales
Bacteria		Proteobacteria	Alphaproteobacteria	Sphingomonadales
Bacteria			Betaproteobacteria	
Bacteria			Gammaproteobacteria	Pseudomonadales
Bacteria				
Bacteria				
Bacteria				
Bacteria				
Bacteria				
Bacteria				Enterobacteriales
Bacteria				
Bacteria				
Bacteria				Xanthomonadales
Bacteria				
Bacteria			Deltaproteobacteria	
Bacteria			Epsilonproteobacteria	
Bacteria	Chlamydiae/ Verrucomicrobia group	Verrucomicrobia		
Bacteria	Bacteroidetes/Chlorophobi group	Bacteroidetes	Bacteroidia	
			Flavobacteria	Flavobacteriales
Bacteria		Cyanobacteria		Chroococcales
Bacteria		Cyanobacteria		Nostocales
Bacteria		Cyanobacteria		Oscillatoriales
Bacteria		Cyanobacteria		Oscillatoriales

Kingdom	Superphylum	Phylum	Class	Order
Bacteria		Cyanobacteria		Oscillatoriales
Bacteria	Fibrobacteres/Acidobacteria group	Acidobacteria		
Bacteria		Planctomycetes		
Bacteria		Chlorophlexi		
Bacteria		Actinobacteria	Actinobacteria	Actinomycetales
Bacteria				
Bacteria				
Bacteria				
Fungi		Basidiomycota		
Fungi		Ascomycota	Dothideomycetes	Capnodiales
Fungi				
Fungi			Eurotiomycetes	Eurotiales
Fungi				
Fungi				
Fungi				
Fungi				
Fungi				
Fungi				
Fungi				
Fungi				
Fungi				
Fungi				
Fungi			Leotiomycetes	Erysiphales
Fungi			Sordariomycetes	Hypocreales
Fungi				
Fungi				
Fungi				
Fungi				Microascales
Fungi		ZygomycotaSubphylum: Mucoromycotina	Zygomycetes	Mucorales
Plantae		Chlorophyta	Chlorophyceae	

Kingdom	Superphylum	Phylum	Class	Order
Plantae		Pinophyta	Pinopsida	Pinales
		Angiospermae		Asparagales
				Asterales
				Caryophyllales
Plantae				Ericales
Plantae				Fagales
Plantae				
Plantae				
Plantae				Lamiales
Plantae				Malpighiales
Plantae				Poales
Plantae				
Plantae		Rhodophyta	Porphyridiophyceae	Porphyridiales
Protista				
Protista /Protozoa		Dinoflagellata	Dinophyceae	Peridiniales
Chromalveolata		Heterokontophyta	Chrysophyceae	Chromulinales
Chromalveolata		Heterokontophyta	Xanthophyceae	
Archaea		Crenarchaeota		

Concentrations of bacteria in cities exhibit especially high spatial variation because they are released from strong point sources, in contrast to the more spatially homogeneous release from, for example, an agricultural field. Areas with heavy vehicular traffic or sewage pollution have much higher concentrations of airborne bacteria, with a weaker or non-existent seasonal cycle, as compared to concentrations in more naturally influenced areas such as urban parks, forests or coastal sites (e.g. Miquel, 1883; Shaffer and Lighthart, 1997; Harrison et al., 2005; Fang et al., 2007).

The concentrations and composition of bacteria undergo daily, weekly and seasonal changes. It has often been found that numbers are greatest in summer and autumn (Bovallius et al., 1978; Jones and Cookson, 1983; di Giorgio et al., 1996; Tong and Lighthart, 1999; Fang et al., 2007; Kaarkainen et al., 2008), although exceptions exist; Fahlgren et al. (2010) found that bacteria counts at a coastal site were highest in winter, which they attributed to a strong winter marine sea spray source. Over a period of 1 d, bacteria have usually been observed to exhibit a peak airborne concentration in the morning and evening (Lighthart and Shaffer, 1995; Shaffer and Lighthart, 1997; Fang et al., 2007). It has been suggested by Maron et al. (2006) that bacterial communities in cities show temporal variability, in which the daily and weekly variability is mainly influenced by anthropogenic sources, whereas seasonal variations are triggered by climate and atmospheric changes.

2.1.2. Rural airborne bacteria

Among the Gram-positive bacteria observed in rural air, *Firmicutes* and *Actinobacteria* are the prevalent groups, whereas *Proteobacteria* are the most prevalent Gram-negative bacteria (e.g. Lighthart, 1997; Maron et al.,

2005; Després et al., 2007; Fang et al., 2007). Rural and urban sampling sites usually differ with regard to bacterial genetic diversity. Studies based on cultivation often find higher counts of CFUs at urban sites compared to rural sites in the same geographic regions (Bovallius et al., 1978; Jones and Cookson, 1983; Chihara and Someya, 1989; di Giorgio et al., 1996; Fang et al., 2007; Fahlgren et al., 2010). In contrast, some studies based on cultivation or deoxyribonucleic acid (DNA) analysis have detected higher diversity at rural than at urban sites (Rosas et al., 1993; Després et al., 2007). Some studies have shown strong correlations between bacteria concentrations at rural sites and meteorological conditions. For example, Lighthart et al. (2009) found that six meteorological factors could account for 96% of the variance in culturable atmospheric bacteria concentrations measured in rural Oregon. Harrison et al. (2005) measured boundary layer concentrations of total atmospheric bacteria at sites in England and found that they increased exponentially as a function of 24-h mean temperature and, except at the coastal site, decreased logarithmically with increasing wind speed, probably due to atmospheric dilution.

Shaffer and Lighthart (1997) found that mean concentrations of culturable bacteria differed between four distinct land-use types chosen for study: urban, forest, rural and coastal. However, Bowers et al. (2010) obtained an apparently contradictory result, finding concentrations to be 10^5–10^6 m^{-3} of air in each of three distinct land-use types (agricultural fields, suburban areas and forests). The difference could be related to the specific sites chosen, or could be methodological; whereas Shaffer and Lighthart (1997) used cultivation to enumerate bacteria, Bowers et al. (2010) used fluorescent microscopy to count DNA-containing particles in the size range 0.5–10 μm in diameter.

Despite the lack of difference in total bacteria numbers between the sites, Bowers et al. (2010) found that concentrations of high-temperature ice nuclei, as determined by a droplet freezing assay, were on average two and eight times higher in the samples from agricultural areas than from the other two land-use types, which might indicate an agricultural, perhaps biological, source of ice nuclei, but the nature of the ice nuclei was not determined.

2.1.3. Airborne bacteria at marine and coastal sites

Although it has often been shown that airborne bacteria are dominated by bacterial groups that are prevalent in the soil, airborne bacteria, especially at marine and coastal sites, can also originate from marine sources. Bacteria are transmitted from water to the air by the bubble-bursting mechanism (e.g. Blanchard et al., 1981; Blanchard and Syzdek, 1982). Both, laboratory and field studies, have demonstrated that the concentration of bacteria in bubble bursting or sea spray aerosol greatly exceeds the concentration in the water from which the aerosol is produced (Blanchard and Syzdek, 1978; Blanchard et al., 1981; Blanchard and Syzdek, 1982; Blanchard, 1989; Marks et al., 2001; Aller et al., 2005). The mean atmospheric residence time of bacteria emitted from the oceans is expected to be shorter than for bacteria emitted from land surface due to their quicker removal by precipitation (Burrows et al., 2009b). In comparison with bacterial concentrations in urban and rural environments, CFU counts seem to be in general lower at coastal sites than at inland sites (Bovallius et al., 1978; Shaffer and Lighthart, 1997). Variation in bacterial concentrations over the course of a day can often be explained by considering onshore breezes that generally bring air with fewer bacteria than inland air (Lighthart, 1997).

Most of the studies dealing with the identification of airborne bacteria at marine and coastal sites have been conducted using culture-dependent techniques. However, it has also been shown in culture-independent analyses that bacteria at coastal and marine sites primarily stem from the phyla of the *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. Within the *Firmicutes*, *Bacillus* seems to be prevalent (Shaffer and Lighthart, 1997), whereas within *Proteobacteria* mainly *Alpha-*, *Beta-* and *Gammaproteobacteria* were detected (Fahlgren et al., 2010; Urbano et al., 2011).

Although in general it has been reported that the concentration of bacteria is highest in summer and autumn, while lowest in winter (Vlodavets and Mats, 1958; Pady and Gregory, 1963; Borodulin et al., 2005), local exceptions can be found to this pattern. In a study analysing the airborne bacterial community at a sampling site near the Baltic Sea with mainly marine influenced air, Fahlgren et al., (2010) detected higher CFU values during the winter compared with the summer. Because marine bacteria are ejected into the air along with sea spray aerosol particles, the source of marine bacteria to the atmosphere increases when winds become more powerful, generating more waves and surf, and thus sea spray. As a result, it is likely that marine bacteria are

transferred to the atmosphere far more effectively by stronger winter winds (Nilsson et al., 2001; Nilsson et al., 2007; Fahlgren et al., 2010).

Examination of single particles collected in the air above biologically active ocean areas (Arctic and Southern) shows that bacteria are present, but their numbers are dwarfed by the large number of particles consisting of biogenic organic aggregates and colloids (Leck and Bigg, 2005a, 2005b; Bigg, 2007; Bigg and Leck, 2008).

Methodological issues may have confounded previous measurements of bacteria concentrations in marine air using culture methods. The culturability of bacteria in seawater is estimated to be between 0.001 and 0.1%, compared to 0.25% for freshwater and 0.3% for soil (Colwell, 2000). However, Fahlgren et al. (2010) presented evidence that a majority of live airborne marine bacteria collected on the Swedish coast may be culturable on Zobell agar plates, which are based on Baltic seawater. Also, marine bacteria are smaller than land bacteria, with biovolumes often in the range 0.036 – 0.073 μm^3 (Lee and Fuhrman, 1987), corresponding to equivalent spherical diameters of 0.20 – 0.26 µm. Consistent with the smaller size of marine bacteria, the count median diameter of particles associated with culturable bacteria has been found to be smaller at coastal sites–about 2 µm–compared to about 4 µm at continental sites (Shaffer and Lighthart, 1997; Tong and Lighthart, 2000; Wang et al., 2007).

2.1.4. High altitude airborne bacteria

The presence of bacteria at high altitudes in the atmosphere had already been detected as early as 1861 (Pasteur, 1861). Metabolically active microbes have been detected in air as high as 20–70 km in elevation (Imshenetsky et al., 1978; Griffin, 2004; Wainwright et al., 2004a; Bowers et al., 2009; Womack et al., 2010). Bacteria have been shown to survive long distance transport and also to be able to live and reproduce in airborne particles (Dimmick et al., 1979). Because bacteria metabolise within cloud droplets, some authors have proposed an impact on the chemistry of cloud droplets and air (Amato et al., 2005, 2007a, 2007c; Deguillaume et al., 2008; Vaitilingom et al., 2010). Vaitilingom et al. (2010) showed that biodegradation is more likely to contribute to cloud chemistry at night than during the day because it must compete with photochemistry during the day. Bacteria collected in cloud water samples have been shown to metabolise and reproduce when those samples are incubated in the laboratory, even at supercooled conditions (Sattler et al., 2001; Amato et al., 2007a; Amato et al., 2007c). Sattler et al. (2001) found that generation times varied between 3.6 and 19.5 d, comparable to those of phytoplankton in the ocean. The mean atmospheric residence time of a bacterial cell can be up to about 1 week (Burrows et al., 2009b), but the cell will spend only a small fraction of this time inside of a cloud droplet. Lelieveld and Heintzenberg (1992) estimated that on average, tropospheric air spends about 5–6% of its time in clouds. It is, thus, unlikely that there is a significant primary production of bacteria within cloud droplets.

In addition to the effect of low temperature at high altitudes, it has been discussed repeatedly that air in general is a hostile environment for microorganisms, as they are exposed to UV light, have only small amounts of water available are subject to changing oxygen partial pressure, etc. (e.g. Womack et al., 2010 and references therein). Bacteria found at high altitudes probably either originate from high altitude habitats such as alpine sites, or they were transported to high altitudes with air currents. Rapid upwards transport of bacteria can be the result of storm activity over land and seas, volcanic activity, impact events and human activity such as weapons testing, aviation and spacecraft launches (Hall and Bruch, 1965; Bucker and Horneck, 1969; Simkin and Siebert, 1994; Kring, 2000; Griffin et al., 2002; Griffin, 2004). One evolutionary strategy for bacteria to survive in such hostile environments is the use of pigments to protect cells from harmful UV radiation. Thus, some studies on the diversity of bacteria in high altitude concentrate on pigmented bacteria (González-Toril et al., 2009).

The number of aerosol particles and PBAP decreases at high altitudes in general, and several studies could show that the diversity of airborne high-alpine bacteria is also reduced in comparison to urban and rural sites (Després et al., 2007; Bowers et al., 2009). These studies as well as others find primarily bacteria belonging to *Proteo-* and *Actinobacteria* as well as *Bacteroidetes* (González-Toril et al., 2009). Within the *Proteobacteria*, *Gamma-* and *Betaproteobacteria* are the prevalent classes detected. The presence of *Bacillus* species has also been reported from high altitude sites (Griffin, 2004; Maki et al., 2010). Between the different environmental types, bacteria phyla mainly differ in their relative proportions. Additional differences probably exist also on the species and family level.

2.1.5. Bacteria emission fluxes

Only a few studies have attempted to directly measure the surface-atmosphere flux (net rate of emission and deposition) of bacteria. Flux measurements can be made using micrometeorological methods that rely on measuring the vertical gradient of bacteria concentrations in conjunction with gradients of air velocity, temperature or other parameters. These methods require fast and very short measurements for statistical significance and can be difficult to design, carry out and interpret. Existing estimates of the flux of culturable bacteria to the atmosphere range from 4.7 CFU m^{-2} s^{-1} for a high desert chaparral in Oregon, USA (Lighthart and Shaffer, 1994) to as much as 543 CFU m^{-2} s^{-1} for undisturbed croplands (Lindemann et al., 1982) and much stronger emissions for surfaces disturbed by human behaviour. For example, during harvesting of crops, emissions may be as high as 10^9 CFU m^{-2} s^{-1} (Lighthart, 1984). A first global model study estimates that average emissions from land of 250 m^{-2} s^{-1} (range: 140–380 m^{-2} s^{-1}) would be required to reproduce observed mean concentrations of bacteria in the air (Burrows et al., 2009b; Fig. 2), note that this estimate refers to total as opposed to culturable bacteria. Major obstacles to successful flux measurements include low number concentrations of airborne bacteria and the lack of automated methods for measuring concentrations because concentration measurements must be made continuously and at high time resolution.

Fig 2

Fig. 2. Column density of bacterial tracer (10^6 m^{-2}), simulated from estimated emissions for a set of ten ecosystems estimates (Burrows et al., 2009b).

2.1.6. Archaea

All known life on earth can be categorised into one of three broad categories: archaea, bacteria and eukarya. As with bacteria, the DNA of archaea is not contained within a cell nucleus (prokaryotes). Little is known about archaea in the atmosphere. They had long been thought to occur only in very restricted, extreme environments, but by now they have been found in a wide variety of habitats (Schleper et al., 2005). They are one of the most diverse and widespread forms of life on Earth and as major players in the biogeochemical cycles of nitrogen and carbon they are involved in the production of methane, the assimilation of amino acids and the oxidation of ammonium (Schleper et al., 2005). Archaea in the atmosphere have thus far been difficult to detect and characterise. Many studies have reported the detection of archaea from samples collected from air above compost piles and biosolids (e.g. Baertsch et al., 2007; Moletta et al., 2007; Thummes et al., 2007), but these are specialised environments rich in biological material. Three aerosol studies (Després et al., 2007; Fierer et al., 2008; Bowers et al., 2009) utilised polymerase chain reaction (PCR) primers capable of amplifying archaeal DNA, but were unable to amplify and detect genetic material from archaea. This may be due to limitations of the applied PCR primers and amplification conditions as described in Section 3.3.1.3. Radosevich et al. (2002) is the only published report to successfully detect DNA sequences of three crenarchaeal clones from ambient air but no further information is provided.

2.2. Fungal spores and fragments

Fungal spores and fragments are understood to be one of the most common classes of airborne PBAP in a number of environments (Womiloju et al., 2003; Elbert et al., 2007; Bauer et al., 2008b; Crawford et al., 2009). Fungi are comprised of vegetative mycelia that consist of a large number of branched hyphae and grow in virtually every ecosystem on Earth and are also capable of efficient aerosolisation (Adhikari et al., 2009). Spores are often released by active processes such as osmotic pressure 'cannons' and surface-tension catapults (e.g. Buller's Drop; Ingold, 1971; Lacey, 1996; Ingold, 1999; Pringle et al., 2005). Spores can be released as a part of the sexual and/or asexual morph (stage) of the lifecycle of a fungus, and many species are able to produce spores from both stages. Fungi that actively release spores during the sexual morph, or telemorph, can typically produce one of three different kinds of spores: ascospores are released from an ascus (a long tube that typically holds eight spores), basidiospores from a basidium (small pedestal on fruiting bodies) or teliospores. The

asexual stage, anamorph, of some fungi produce conidia, also known as conidiospores, that are produced by hyphal portions called conidiophores. Many studies thus refer to spores as a singular class, referring to both sexual and asexual morphs together. Spores are also often subdivided for practical reasons into hyaline (colorless) and dematiaceous (colored) spores (e.g. Pady and Gregory, 1963; Adams et al., 1968). Spores most commonly observed to dominate ambient concentrations of airborne fungal material have been from the species: *Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus*, as well as *Epicoccum* and a variety of yeasts, smuts and rusts (plant pathogens) and other basidiomycetes (e.g. Madelin, 1994). Detailed reviews on fungal spores in the atmosphere are available elsewhere (e.g. Madelin, 1994; Elbert et al., 2007) and as an example Fig. 3 presents an example of fungal spore micrographs with and without coating by secondary organic aerosol. Some plants such as ferns and mosses also disperse spores that are typically larger and less numerous than fungal spores (Graham et al., 2003; Elbert et al., 2007).

Fig 3

Fig. 3. Fungal spores with and without coating by secondary organic aerosol (dark gray envelope in left panel). Electron micrographs of aerosol filter samples from pristine tropical rainforest air in the Amazon (Pöschl et al., 2010), (Reproduced with permission from AAAS).

In recent PBAP analyses, molecular genetic methods have been used that analyse the genetic substance, DNA, of biological material. As discussed by Després et al. (2007), fungal DNA detected in aerosol filter samples is most likely to originate from spores that are known to resist environmental stress and survive atmospheric transport (Madelin, 1994; Griffin, 2004; Griffin and Kellogg, 2004). But, fungal DNA may also be derived from other fungal material such as hyphae and tissue fragments. Although it is generally assumed that spores comprise the majority of airborne fungal material, this remains largely unsubstantiated and has important implications for interpretation of both, laboratory or field measurements. Hyphal fragments have been observed in ambient air in a number of studies (Gorny et al., 2002; Green et al., 2006). Sinha and Kramer (1971) suggested that airborne hyphae are most commonly unbranched conidiophores that can be 1–100 μm in length but that are more commonly 5–40 μm. Pady and Gregory (1963) summarise the observed concentrations to be within the range 10^0–10^3 m^{-3} and observed that a large fraction of ambient hyphae were viable and able to germinate resulting in fungal colony growth. As most fungal species in the biosphere are still unknown, the detection and characterisation of fungi in atmospheric aerosol samples by DNA analysis can help to elucidate the global spread and diversity of fungi. As a by-product of the active emission process, fungal spores can be coated with specific sugar (e.g. arabitol, mannitol) and sterol (e.g. ergosterol) compounds that have thus been utilised as chemical tracers for ambient fungal spore concentrations (Lau et al., 2006; Bauer et al., 2007). Spores can also be coated with hydrophobin compounds that may affect both, their ice-nucleating ability (Iannone et al., 2011) and the immune response they cause after human inhalation (Aimanianda et al., 2009).

Depending on biological species, age and ambient conditions, the diameter of fungal spores can vary (~1–50 μm); most frequently it is in the range of 2–10 μm (Elbert et al., 2007; Wang et al., 2008; Fröhlich-Nowoisky et al., 2009; Huffman et al., 2010). Furthermore, fungal spores are often observed to aggregate into long chains of spores that greatly affect their aerodynamic diameter and have implications for both, atmospheric lifetime and deposition into human tissues (Lacey, 1991; Reponen et al., 2001).

The number and mass concentrations of fungal spores are typically observed to be ~10^4 m^{-3} and ~1 μg m^{-3}, respectively, in continental boundary layer air (Tables 3 and 4). They account for up to ~10% of organic carbon (OC) and ~5% of PM_{10} at urban and suburban locations (Bauer et al., 2002a, 2000b, 2008a). In pristine tropical rainforest air, fungal spores account for up to ~45% of coarse particulate matter. Thus, the properties and effects of fungal spores may be particularly important in tropical regions where both, physico-chemical processes in the atmosphere and biological activity at the Earth's surface are particularly intense (Graham et al., 2003; Gilbert, 2005; Elbert et al., 2007; Pöschl et al., 2010; Zhang et al., 2010), but chemical tracers typical of fungal spores have also been reported in aerosols from semi-arid and arid sites (Graham et al., 2004).

Table 3. Global emission estimates for different types of PBAP and size ranges of air particulate matter (PMx, x = upper limit of particle diameter; TSP = total suspended particulates)

	Global emissions (Tg yr^{-1})	Size range	References
Bacteria	0.74 (0.4–1.8)	Diameter: 1 μm (PM_1)	Burrows et al. (2009b)
	0.7	Diameter: 1 μm (PM_1)	Hoose et al. (2010a)
	2.58	Diameter: 1.17 μm	A. Sesartic, personal communication
	28.1	Lognormally distributed with geometric mean number diameter: 2 μm, standard deviation = 1.37	Jacobson and Streets (2009)
Fungal spores	8	Diameter: 4 μm (PM_4)	Sesartic and Dallafior (2011)
	28	Two size modes: fine (<2.5 μm) and coarse (2.5–10 μm) (PM_{10})	Heald and Spracklen, (2009)
	31	Diameter: 5 μm (PM_5)	Hoose et al. (2010a)
	50	Diameter: 5 μm (PM_5)	Elbert et al. (2007)
	186	Lognormally distributed with geometric mean number diameter: 3 μm, standard deviation = 1.37	Jacobson and Streets (2009)
Pollen	47	Diameter: 30 μm (PM_{30})	Hoose et al. (2010a)
	84	Lognormally distributed with geometric mean number diameter: 30 μm, standard deviation = 1.37	Jacobson and Streets (2009)
Total PBAP	<10 (dominated by plant debris and fungal spores),	Diameter: 4 μm for fungal spores; diameter not specified for plant debris (TSP)	Winiwarter et al. (2009)
	56 (0–90)	Diameter <2.5 μm ($PM_{2.5}$)	Penner (1995)
	78 (includes only bacteria, fungal spores and pollen)	Diameters as above (PM_{30})	Hoose et al. (2010a)
	186	Split equally into the two coarse size fractions: 2.5–5 and 5–10 μm (PM_{10})	Mahowald et al. (2008)
	296 (includes only bacteria, fungal spores and pollen)	Diameters as above	Jacobson and Streets, (2009)
	~1000 (includes cellular fragments)	TSP	Jaenicke (2005)

Table 4. Characteristic magnitudes of the number and mass concentrations of PBAP in air over vegetated regions

	Number concentration [m^{-3} air]	Mass concentration [μg m^{-3}]	Size range	References
Bacteria	~10^4	~0.1	PM_{10}	Bauer et al. (2002a); Burrows et al. (2009a)
Plant debris (free cellulose)		~0.1–1	PM_{10}	Sánchez-Ochoa et al. (2007)
Viral particles	~10^4	~10^{-3}		This work, Sect. 2.4
Fungal spores	~10^3–10^4	~0.1–1	TSP	Elbert et al. (2007); Fröhlich-Nowoisky et al. (2009)
Fungal hyphal fragments	~10^3			Pady and Gregory (1963)
Pollen	~10 (up to ~10^3)	~1	TSP	Sofiev et al. (2006); Fröhlich-Nowoisky et al. (2009)

	Number concentration [m^{-3} air]	Mass concentration [$\mu g\ m^{-3}$]	Size range	References
Algae	~100 (up to ~10^3)	~10^{-3}		Reisser (2002)
Fern spores	~10 (up to ~10^3)	~1	TSP	Mücke and Lemmen (2008)

Most airborne fungi belong to the divisions of *Ascomycota* and *Basidiomycota* (Fröhlich-Nowoisky et al., 2009). Most *Ascomycota* and *Basidiomycota* actively eject their spores with liquid jets or droplets (osmotic pressure and surface tension effects), whereas others rely on dry spore detachment by wind or other external forces. Dry -discharged spore concentrations tend to be enhanced during warm, dry weather conditions, whereas actively wet discharged spores tend to be enhanced during humid conditions such as those at night and in the early morning hours (Graham et al., 2003; Elbert et al., 2007). Emission and dispersal of fungal spores can thus be selectively correlated with various meteorological parameters and usually have specific behaviours, depending on the species involved (Fitt et al., 1989; Pasanen et al., 1991; Calderon et al., 1995; Katial et al., 1997; Sabariego et al., 2000; Troutt and Levetin, 2001; Burch and Leventin, 2002; Jones and Harrison, 2004; Grinn-Gofron and Mika, 2008; Oliviera et al., 2009).

From spore counts and molecular tracers, Elbert et al. (2007) derived a global emission rate of ~50 Tg a^{-1} for fungal spores, corresponding to average mass and number emission fluxes of ~23 ng m^{-2} s^{-1} and 200 m^{-2} s^{-1}, respectively, over land. These values are in fair agreement with the global average model estimates of Heald and Spracklen (2009): ~28 Tg a^{-1} or ~6 ng m^{-2} s^{-1} over land, and 189 Tg a^{-1} calculated by Jacobson and Streets (2009) for the year 2000. For Europe, Winiwater et al. (2009) derived a value of ~0.6 ng m^{-2} s^{-1}, and recently Sesartic and Dallafior (2011) estimated average mass and number flux values of ~17 ng m^{-2} s^{-1} and 513 m^{-2} s^{-1} over land. Overall, the studies indicate that the global average emission rates of fungal spores are uncertain by about one order of magnitude, which appears comparable to many aerosol sources and less than for other types of PBAP Table 3. More field data are required to constrain better the actual emission flux of fungal spores on regional and global scales.

Studies based on DNA obtained directly from atmospheric aerosol samples offer new possibilities to identify the origin of fungal matter, independent of viability, cultivability and fragmentation (e.g. Boreson et al., 2004; Peccia and Hernandez, 2006; Fierer et al., 2008; Bowers et al., 2009; Fröhlich-Nowoisky et al., 2009). DNA-based techniques can amplify target regions of the DNA extracted directly from atmospheric aerosol samples. Amplification of the internal transcribed spacer (ITS) regions between the 18S and 28S ribosomal ribonucleic acid (rRNA) genes provides good target regions to identify fungi to genus and often to species level (O'Brien et al., 2005; Fröhlich-Nowoisky et al., 2009). DNA sequencing of this region has proven to be an efficient tool for the detection of rare and hard-to-cultivate fungi (e.g. *Blumeria graminis*) as well as highly abundant and easy-to -cultivate fungi (e.g. *Cladosporium sp.*) in aerosol samples (Fröhlich-Nowoisky et al., 2009) and other habitats (e.g. Hunt et al., 2004; O'Brien et al., 2005).

With regard to species richness, Fröhlich-Nowoisky et al. (2009) detected 64% *Basidiomycota* and 34% *Ascomycota* in a semi-urban environment in central Europe, whereas Bowers et al. (2009) found only 4% *Basidiomycota* but 82–92% *Ascomycota* at a mountain site in North America. On the class level within the *Ascomycota*, *Dothideomycetes* and *Eurotiomycetes* seem to be the prevalent groups (Bowers et al., 2009; Fröhlich-Nowoisky et al., 2009). These findings may be influenced not only by regional differences but also by measurement issues, and the actual biogeographic distribution of airborne fungal species is a subject of ongoing studies (Womack et al., 2010; Fröhlich-Nowoisky et al., 2011). As in bacteria, seasonal variation exists and differs between sampling sites also for fungi. During hot summer seasons, a decrease in the airborne microflora was reported for some fungi (Mullius et al., 1984; Filipello-Marchision et al., 1992; Fröhlich-Nowoisky et al., 2011).

The detection and apparent frequency of occurrence of different species can be affected by technical factors such as extraction efficiency of genomic DNA, varying rRNA gene copy number in the species, primer matching and performance, amplification efficiency of the target region, and cloning success. To our knowledge, from the few studies that have reported DNA analyses of fungi in atmospheric aerosol samples, some had neither found

the expected high abundance of, e.g. *Cladosporium* sp. nor a high species richness (in particular *Basidiomycota*), which may well be due to limitations of the applied PCR primers (e.g. Després et al., 2007; Fierer et al., 2008; Bowers et al., 2009), although over 1500 fungal DNA sequences from 5 urban air samples were measured by Fierer et al. (2008) and several dozens of filter samples of urban, rural and high-alpine air were analysed by Després et al. (2007). Thus, careful selection and combination of multiple PCR primer pairs and other materials for the extraction and amplification of DNA obtained from aerosol samples are key elements for achieving high coverage of species richness (>300 species), as discussed in Fröhlich-Nowoisky et al. (2009). The high number of fungal species that were detected only once indicates that a higher number of samples and clones would have to be investigated for a complete coverage of species diversity (Fröhlich-Nowoisky et al., 2009). In any case, the detected and reported numbers and frequencies of occurrence of species, families and classes can only be taken as a lower limit for the actual diversity and frequencies of occurrence.

2.3. Pollen

Among PBAP types, pollen grains can be among the largest in physical size and represent the reproductive units of plants that contain the male gamete. Pollen grains vary in size between 10 and 100 μm, have various shapes and have a hard shell that protects the sperm cells during the transportation processes. Pollen grains occur as biological aerosols not only as complete units but also as fragmented pieces. Pollen can rupture when the humidity is high, and these fragments have been shown to be in the range from 30 nm to 5 μm (Taylor et al., 2002, 2004; Miguel et al., 2006). Pollen of anemophilous plants use wind as their dispersal vector and have a typical diameter of 17–58 μm (Stanley and Linskins, 1974; Kuparinen, 2006; Nathan et al., 2008; Pope, 2010). They are usually dispersed in large amounts and over wide ranges because of their floating ability (Straka, 1975). The pollen is often ejected in clumps that stick to their neighbouring vegetation and are blown away after drying (Jones and Harrison, 2004).

The ability of pollen to disperse into the atmosphere depends on several parameters. On the one hand, dispersal depends on resuspension as described in detail by Jones and Harrison (2004). On the other hand, pollen dispersal depends on meteorological factors. For example, bonding of pollen to surfaces is affected by temperature and moisture and thus by temperature and humidity of the air (Jones and Harrison, 2004). It has also been shown that pollen counts in, for example, the cypress family show significant positive correlations with daily minimum, mean and maximum temperatures and negative correlations with precipitation (Lo and Levetin, 2007). After temperature and humidity, wind and rain are typically the most important parameters for pollen dispersal, and it has been shown that pollen emission is reduced in the presence of rain or when the wind speed was low (Ogden et al., 1969). In general, the pollen concentration decreases with height, and for birch it could be shown that at 2000 m just 40% of the ground concentration were present (Rempe, 1937). This general phenomenon can be modified by inversion layers (Linskens and Jorge, 1986). The horizontal distance over which pollen can be carried with the wind can depend on prevailing temperatures, and it has been suggested that an increase in air temperature may induce atmospheric instability and thus promote pollen dispersal (Kuparinen et al., 2009). However, pollen has been observed to be lifted into the upper layers of the atmosphere by convection (Monin and Obukhov, 1954; Tackenberg, 2003; Taylor and Jonsson, 2004; Wright et al., 2008), and this ability makes pollen grains possibly relevant as ice nuclei in many environments.

The residence time of pollen in the atmosphere depends on their settling velocities that depend on morphology (shape), density and size of the pollen and vary widely among pollen types (Digiovanni et al., 1995; Diehl et al., 2001). The time pollen stay aloft in the atmosphere also influences the horizontal distance they can travel. Long distance dispersal (LDD) is not only interesting from the atmospheric point of view but also shapes many fundamental processes in plant ecology and evolution (Ellstrand, 1992; Kawecki and Ebert, 2004; Neilson et al., 2005; Nathan et al., 2008), e.g. the ability of plants to spread into new areas (IPCC 2007a). Dust is well known to frequently travel long distances, even across oceans (Kellogg and Griffin, 2006; Ben-Ami et al., 2010). Biological particles can also be transported over similar distances and have been observed to accompany dust plumes thousands of miles from their assumed sources (Shinn et al., 2000; Kellogg and Griffin, 2006; Polymenakou et al., 2008; Hallar et al., 2011). The LDD of wind-dispersed pollen is typically promoted by turbulent vertical fluctuations in wind and by coherency in vertical eddy motion that uplifts seeds well above the vegetation canopy (Nathan et al., 2002; Tackenberg, 2003; Soons et al., 2004). For a wide range of plant types, a positive relationship has been observed between mean air temperature and the frequency of LDD by wind in a boreal forest. Thus, an increase in local air temperature increases pollen dispersal distances (Kuparinen et al., 2009).

Another characteristic of pollen is their seasonality, as the presence of pollen in the atmosphere follows a clear seasonal cycle in response to the flowering seasons of the plant sources (Tormo et al., 2010). On a local scale, the pollination season for each plant is predictable and only shifts slightly as a function of meteorological parameters. The amount of pollen disposed by individual plants can vary greatly from 1 yr to another. Pollination during a given season always has a date, at which the pollen begins to disperse but at lower numbers, followed by the main pollination season when most of the pollen is dispersed, and conclude by a date when the plant stops its pollen-producing phase. However, usage of this nomenclature varies greatly within the literature and can be confusing (Jato et al., 2006). In addition, sedimented pollen might be resuspended again from dry surfaces. Pollen grains occasionally show a diurnal cycle with concentrations rising 1–2 h after dawn, peaking a few hours later and decreasing through the afternoon (Jones and Harrison, 2004). The phenomenon of diurnal variability is also common among other biological aerosol types such as bacteria and fungal spores (Jones and Harrison, 2004; Huffman et al., 2010).

Pollen grains are large PBA particles that typically lead to short atmospheric residence times. However, they can be up-drafted to high altitudes and have large residence times, and thus pollen can reach concentrations comparable to ice nuclei in some circumstances (Scheppegrell, 1924; Pruppacher and Klett, 1997; Diehl et al., 2001). Detailed information on the behaviour of pollen in ice nucleation is given in section 4.2.

Global changes, such as increasing atmospheric CO_2 concentrations, increasing temperature, changes in the amount, distribution, and intensity of precipitation events, increases in the intensity and frequency of certain extreme weather events, changes in land use, and urbanisation, will likely have an impact on the production, distribution and dispersion of pollen (IPCC 2007b; Reid and Gamble, 2009). Climatic changes may lead to shifted or even elongated pollinations seasons. As pollen is known to cause allergies in humans, these shifts may also lead to changes in human exposure and changes in the prevalence and severity of symptoms in individuals with allergic diseases (Reid and Gamble, 2009). Alterations in the timing of aeroallergen production in response to weather variables have been clearly demonstrated for certain tree species, but less for grass, weed and mould (Katial et al., 1997; Emberlin et al., 2002; Clot, 2003).

Because pollen allergies can cause such medical problems, various pollen-monitoring programmes, networks and databases have been developed recently to provide data on pollen observation, share methods, encourage collaborations and thus create foundation for intensive pollen research. Examples include the Pollen Monitoring Program in Europe (Giesecke et al., 2010) and the Pollen Biology Research Coordination Network in the United States. The Global Pollen Database combines pollen information from Africa, the Americas and northern Asia made available by regional networks such as the Indo Pacific Database, the Latin America Pollen Database and the North American Pollen Database. These international collaborations are helping to advance knowledge of global pollen distribution.

In addition to climate-related changes in the atmospheric abundance of pollen and fungal spores, the allergenic potential of PBAP can also be enhanced by interactions with air pollutants (Taylor et al., 2002; Franze et al., 2003; Taylor and Jonsson, 2004; Franze et al., 2005; Pöschl, 2005; Reid and Gamble, 2009). For example, the reaction with ozone and nitrogen oxides leads to the formation of reactive oxygen intermediates and nitrated proteins that can influence the interaction of PBAP with the immune system and trigger or exacerbate allergic diseases (Gruijthuijsen et al., 2006; Shiraiwa et al., 2011; Zhang et al., 2011).

2.4. Viruses

Viruses are among the smallest of common PBAP classes, with physical diameter as low as 20 nm (Dongsheng, 2006). However, viruses are not commonly airborne as individuals and are more likely attached to other suspended particles (e.g. Yang et al., 2011).

Many diseases present in humans, animals, birds, fish, insects and plants are caused by viruses found in aerosols (one of possible routes of infection transmission), confirmed by numerous laboratory studies (Akers, 1969; Akers, 1973; Verreault et al., 2008). However, publications revealing the presence of viruses as PBAP are not numerous (Gloster et al., 1982; Christensen et al., 1990; Grant et al., 1994; Chen et al., 2008b). This perceived lack of research might be connected to the fact that before the development of molecular biological methods (e.g. PCR) to detect genetic material of microorganisms (Alvarez et al., 1995; Peccia and Hernandez, 2006), only viable viruses could be found in air samples. There are no universal test systems for virus detection such as nutrient media for bacteria or fungi. Instead, sensitive cell cultures, embryonated chicken eggs or

susceptible laboratory animals are required (Zhdanov and Gaudamovich, 1982a; Zhdanov and Gaudamovich, 1982b), and specific test systems allow only the detection of viruses replicating in them. No other viable viruses, which can also be present in aerosol samples, will be detected.

Another possible reason for the scarcity of publications containing information on viable viruses in atmospheric aerosol is inactivation of viruses in the atmosphere under the influence of different environmental factors (changes in temperature, relative humidity, solar radiation, etc.). Unlike bacteria, fungi and algae, viruses have no repair systems, and therefore, their inactivation rates are usually higher than those of living microorganisms.

According to Posada et al. (2010), the inactivation rate of viruses in an aerosol can be described by:

Here C is the concentration of viable viruses at the time t, C_0 is the initial concentration of viable viruses and k is the rate coefficient of inactivation. Even for the most stable viruses, k is typically of the order of 0.01 min^{-1}, corresponding to an effective half-life of about one hour (Donaldson, 1973; McDevitt et al., 2008). For most other viruses, the inactivation rate in aerosol is considerably higher (Harper, 1961; Miller et al., 1963; de Jong, 1965; Miller and Artenstein, 1967; Songer, 1967; Akers, 1969; Benbough, 1971; Barlow, 1972; Donaldson, 1972; Akers, 1973; Donaldson and Ferris, 1974). Viruses are almost completely inactivated in aerosols in the span of 1 d under such conditions.

Experimental data on the survival of viruses show that, on the whole, the inactivation rate becomes higher for all viruses with increasing temperature (Zhdanov and Gaudamovich, 1982a; Zhdanov and Gaudamovich, 1982b; Weber and Stilianakis, 2008). Also, the number of surviving viral particles in aerosol decreases with increased radiation dose that the virus aerosol is exposed to (Jensen, 1964; Zhdanov and Gaudamovich, 1982a; Zhdanov and Gaudamovich, 1982b; Sagripanti and Lytle, 2007; McDevitt et al., 2008). Relative humidity influences the survival of viruses in aerosol differently. For example, the survival rate of influenza virus in aerosol is highest at high relative humidity, whereas that of foot-and-mouth disease virus is highest at intermediate relative humidity (50–60%) (Akers, 1969; Donaldson, 1972; Schaffer et al., 1976; Weber and Stilianakis, 2008).

The use of molecular biological methods for detecting genetic material of viruses also involves certain difficulties. As genetic material of different viruses is presented by very different variants: DNA or RNA (ribonucleic acid) molecules, single-stranded genomes (with + or – strand), fragmented genomes, etc. (Zhdanov and Gaudamovich, 1982a), no universal PCR primer for detection of all viruses has been developed so far. Consequently, such methods determine only the presence of expected viruses in samples. For example, Chen et al. (2008c) presented the results of search for different subtypes of influenza-A virus in the atmosphere of Taiwan. Other viruses in atmospheric air samples were not even sought.

Virus-containing aerosols are formed in spray from water surfaces (Baylor et al., 1977a; Baylor et al., 1977b; Baylor and Baylor, 1980), from aerosolised virus-destroyed tissues of plants, insects, animals and birds; they are also shed by sick animals, birds and humans (Zhdanov and Gaudamovich, 1982b; Jones and Harrison, 2004). One should also note the intentional use of aerosols of insect viruses for plant protection (Morris, 1980; Maiorov et al., 1985; Jinn et al., 2009). The transfer of virus-containing aerosols in the atmosphere has been described for local scales, e.g. near sludge wastewater treatment plants and stock farms (Strauch and Ballarini, 1994; Carducci et al., 1995; Sigari et al., 2006; Langley and Morrow, 2010) as well as for regional scales, e.g. transfer of foot-and-mouth disease virus across the English Channel (Gloster et al., 1982) and transfer of avian influenza virus from continental China to Taiwan (Chen et al., 2008b). The hypothesis of transcontinental transfer of influenza A virus aerosol was demonstrated by Hammond et al. (1989). Consequently, virus-containing aerosols can spread worldwide.

Relatively few studies have attempted to comprehensively estimate the concentration of different viruses in ambient air and evaluate their source strength. One example of such a study was reported by Safatov et al. (2010) who collected 30 samples of atmospheric air (10–15 m^3 each) onto fibrous filters during different seasons in Southwestern Siberia. Samples were analysed using the PCR method for the presence of viruses known to cause respiratory diseases, although no virus genetic material was found. It should be noted, however, that Southwestern Siberia is not endemic to airborne viral infections largely because it is located far

from sources of viruses such as influenza (Chen et al., 2008c) and habitats of migrant birds transmitting influenza viruses (Liu et al., 2005). Electron microscopy during the same study detected bacteria, fungal spores and plant fragments (Safatov et al., 2010), but virus-like particles are impossible to confidently detect without genetic methods such as PCR, as discussed above.

As noted by Chen et al. (2008b), the total concentration of different subtypes of influenza A viruses can reach 800 m^{-3} copies of genetic material of these viruses in the ambient atmosphere of Taiwan and up to $3 \cdot 10^4$ m^{-3} in outdoor pet markets in Taiwan. It should be noted that Taiwan, like the whole of South-East Asia, is an endemic area for this virus, which explains the higher influenza virus concentrations in this region. For other viruses originating from local sources, such as excreta of infected animals and humans, sewage treatment plants, use in agriculture, etc. (Fannin et al., 1985; Carducci et al., 1995; Sigari et al., 2006), the total numbers of viruses in aerosol are not large, and their contribution to the total mass of aerosol is negligible. As for more powerful sources, such as soil, vegetation and water surfaces, data on virus aerosol in the air in natural conditions are available only for the latter (Baylor et al., 1977a, 1977b; Baylor and Baylor, 1980). Virus-like particles can also be present in the atmosphere and water, such as those described by Leck and Bigg (2005a) using electron microscopy. The concentration of virus-containing particles in the air is low, however (less than 100 cm^{-3}–total concentration of aerosol particles with diameter larger than 90 nm in marine atmosphere; data from Bigg et al. (1995)), and by these methods it is impossible to distinguish viruses from particles on which they are bounded.

Virus aerosols collected from the exhaled air of infected animals have been the subject of various research studies. For example, for pigs infected with classical swine fever virus, the concentration of viral particles may reach 10^4 m^{-3} of air (Weesendorp et al., 2008). Other infections of animals and birds (such as foot-and-mouth disease, Newcastle disease, etc.) are characterised by virus aerosol exhalation of the same order of magnitude (Downie et al., 1965; Donaldson et al., 1983; Donaldson and Alexandersen, 2002; Li et al., 2009). Taking into account that the number of infected animals is typically not large, the total number of exhaled viruses in aerosol is similarly small.

To evaluate the total mass of viruses in 1 m^3 of the atmosphere, let us consider an upper limit to the ambient concentration of viral particles to be $3 \cdot 10^4$ m^{-3}. Assuming a per virus mass of $2 \cdot 10^{-17}$ kg, one of the heaviest known viruses (vaccina virus, Zhdanov and Gaudamovich, 1982b) yields an estimated virus mass concentration of $6 \cdot 10^{-4}$ μg m^{-3}. This estimate is approximately four orders of magnitude smaller than the total concentration of biogenic substance in the atmosphere (Jaenicke, 2005; Table 4) and thus, even if the concentration of viruses in the atmosphere was underestimated by an order of magnitude, the average mass concentration of viruses in atmospheric air would be very small compared to other biogenic aerosol mass.

2.5. Algae and cyanobacteria

The occurrence of algae in fresh and sea water is well known. However, algae living outside of the aqueous environment rarely attract attention. These algae are termed terrestrial, aeroterrestrial, aerophytic or subaerial, are able to reside on almost all substrates, natural or artificial and can become airborne, constituting the aero-(phyto) plankton. Chlorophycean and xanthophycean species are common worldwide (Printz, 1921; Laundon, 1985; Dubovik, 2002; Reisser, 2002; Sharma et al., 2007; Neustupa and Skaloud, 2010) and exist free living as well as lichenised (Bubrick et al., 1984). Due to the size of the algae and their spores, many smaller than 10 μm in diameter (Printz, 1921; Dubovik, 2002; Burchardt and Dankowska, 2003; Neustupa and Skaloud, 2010), they can be easily dispersed in the atmosphere. In a recent review article, the mechanisms involved in the aerosolisation of algae were presented, and Sharma et al. (2007) stated that 'airborne algae are the least-studied organisms in both aerobiological and phycological studies'. This seems to be valid for aerosol studies, too. Quantitative measurements of algae in ambient air are very rare and it was reported (Reisser, 2002) that algal cells are present at a concentration of 300–500 cells m^{-3} of air on a dry and sunny summer day Table 4.

Cyanobacteria belong taxonomically to bacteria, although they have long been considered as algae, as they have the ability to obtain their energy through photosynthesis. Due to their colour, they have been called blue-green algae, but they still belong–as they lack a cell nucleus–to the bacteria domain. As cyanobacteria can be found in almost every environment, have habitats across all latitudes, are widespread in oceans and freshwater, terrestrial ecosystems and bare rock and soils as well as in extreme habitats such as hot springs, they have

been considered to be one of the most successful groups of microorganisms. They fulfil vital ecological functions in the world's oceans as important contributors to global carbon and nitrogen budgets (Stewart and Falconer, 2008). Cyanobacteria can not only occur as planktonic cells but also create biofilms in marine, freshwater and terrestrial environments. A few are involved in symbiosis with lichen, plants and other organisms and provide energy for their host. Although well studied in the marine environment, not much is known about their presence in the atmospheric environment.

Airborne cyanobacteria were found in Varanasi City, India, where they were more abundant than green algae and diatoms; *Phormidium fragile* as well as *Nostoc muscorum* were recorded throughout the 2 yr of sampling (Sharma and Rai, 2008). Similar observations were made in Cairo, Egypt. The species *Chroococcus limenticus*, *Lyngbya lagerheimii* and *Schizothrix purpurascens* appeared in all seasons (El-Gamal, 2008). Genitsari et al. (2011) recently published an overview of taxa of airborne algae and cyanobacteria found in aerobiological studies.

2.6. Biological crusts and lichens

Arid and semi-arid regions of the globe exhibit biological crusts (also called rock varnish, cryptobiotic, microbiotic or biological soil crust) consisting of bacteria, fungi, algae, lichens and bryophytes in variable proportions (Belnap et al., 2003). Rock varnishes are natural, thin (5 μm–l mm), brown, black or grey (lead colour) coatings on rock surfaces (Krumbein and Jens, 1981). They consist largely of iron and manganese oxides with some quartz, clays and carbonates admixed. They may contain considerable amounts of organic material that sometimes may be responsible for the gel-like or lacquer-like appearance. The grey or black films may sometimes consist predominantly of dry cyanobacteria. Rocks outside of deserts often are also covered by a varnish (Douglas, 1987). Although these rock crusts are not suspended into the air by active mechanisms, they can be eroded from their host surface and broken into fragments producing small dust particles (Grini et al., 2002; Büdel et al., 2004). Parts of biological soil crusts can also be ablated from desert surfaces, and all these particles may contribute to the content of organic material in dust transported over long distances during storms (Grini et al., 2002; Büdel et al., 2004; Prospero et al., 2005; Hua et al., 2007).

As lichens are a symbiotic form of life between a wide range of fungi, algae or cyanobacteria (Hawksworth et al., 1995; Nash III, 1996) and distribute separately or together via the air, they also can, in certain circumstances, constitute a subgroup of PBAP. As biological aerosols, they may play a role in ice nuclei activity as well as in health effects. Lichen, for example, can cause human allergenic reactions (Richardson, 1975; Fahselt, 1994; Ingolfsdottir, 2002).

Although lichens are regarded as an individual taxonomic group–lichen taxonomy is based on the taxonomy of the fungal partner, the mycobiont (Tehler, 1996)–they also must be considered separately from their host because lichens are different in behaviour and life form from their isolated partners (Nash III, 1996; Tehler, 1996). Nearly 20% of all known fungi species are lichenised (Lutzoni et al., 2001) and more than 98% of the lichenised fungi belong to *Ascomycota* and a few to *Basidiomycota* (Hawksworth et al., 1995; Tehler, 1996). Most of the lichenised fungi form symbioses with *Chlorophyta*, whereas only about 10% lichenise with cyanobacteria and 3% with both (Tschermal-Woess, 1988; Lewis and McCourt, 2004).

In general, lichens can be found in three major life forms that all can contribute to biological aerosol particles: a crust-like biofilm (crustose), a leaf-like (foliose) and a branched tree-like biofilm (fruticose) as described in Hawksworth et al. (1995) and Büdel and Scheidegger (1996). Especially for PBAP formation, the presence of lichens in extreme habitats is interesting. They can deal with extreme light, dryness or temperature that are less favourable for higher plants. They are also tolerant to extreme desiccation and UV light exposure due to their cortical pigments (Nybakken et al., 2004; Gauslaa, 2005; Vráblíkova et al., 2006). Their ability to prevent the formation or to scavenge free radicals also increases their chance of surviving during their transport in air. Lichens face considerable problems in colonising new sites and maintaining existing populations. Mycobionts can distribute separately via Asco- or Basidiospores, or asexual by singular lichen structures, that contain both, mycobiont and phycobiont, such as insidia (protuberances from the thallus of corticated algae and medullary tissue), soredia (several algal cells encased by a hyphae) or hormocrusts (fragments if filamentous cyanophyte *Nostoc spp* with hypha penetrating the thallus fragments (Marshall, 1996; Oksanen, 2006)).

Although lichen species have been detected in the atmosphere, estimates of lichens biomass and consequently estimates of the number of lichen-derived aerosol particles are difficult. Lichen species are adapted to almost

every temperate to extreme environment on Earth, such as arctic tundra, hot deserts, rocky coasts, or toxic slag heaps, but the spread of lichen population within each environment type is different. They are abundant on plant leaves or branches in rain forests or temperate woodlands, as well as on bare rock including walls, and on exposed soil surfaces. Henderson-Begg et al. (2009) suggested that canopy lichen biomass in temperate forest is similar to leaf biomass. Lichen detection directly in air has been pursued by Marshall (1996) in an aerobiological monitoring programme, where he found lichen soredia to be the most abundant airborne propagule with a size range of 30–100 μm on Signy Island in Maritime Antarctica.

Lichens are not only adapted to survive hot and dry environmental conditions but are also often frost tolerant (Kershaw, 1985). In ice nucleation studies pursued by Kieft (1988), nearly all lichens tested showed ice nucleation activity at temperatures above −8 °C or even above −5 °C (see also Table 6). In theory, the IN could be the bacterium or the fungal partner, but the failure to culture IN bacteria from lichen and also the high density of IN in active lichen carrying only few numbers of bacteria suggests a non-bacterial source of lichen IN. Studies by Kieft and Ahmadjian (1989) show that in their tests, always the mycobiont was responsible for IN activity. IN activity in lichen might enhance the uptake of atmospheric moisture by enhancing the condensation or causing deposition of ice from water vapour. Lichen-associated IN might even contribute to atmospheric IN (Kieft, 1988).

2.7. Others

Apart from the PBAP defined above, there exist a variety of other primary aerosol particles from the biosphere. Plant fragments, in particular, are one of the largest mass fractions of atmospheric PBAP and comprise a wide spectrum of decaying matter. However, drawing a consistent definition between PBAP and other organic matter can be difficult because plant materials can eventually be broken down into humic-like substances (HULIS, Fuzzi et al., 2006; Graber and Rudich, 2006) by oxidative modification and degradation of biopolymers, and are therefore, important components of soil. Cellulose, a homopolymer of D-glucose (Pöhlker et al., 2011), is the most frequently occurring biopolymer in the terrestrial environment (Butler and Bailey, 1973; Sánchez-Ochoa et al., 2007), although other biopolymers and structural components (e.g. lignin, chitin) may also be atmospherically relevant (Pöhlker et al., 2011). Cellulose is a major component of plant tissue and pollen and can be produced by some bacteria (Cannon and Anderson, 1991) but is not present in insects and animalian tissue (Winiwarter et al., 2009). Atmospheric concentrations of cellulose thus exhibit a strongly seasonal cycle and have been shown to have mass concentrations at least in the order of ~0.5 μg m^{-3} (Puxbaum and Tenze-Kunit, 2003). Presence of cellulose in atmospheric aerosols has also been frequently used as a proxy for total concentrations of plant debris (e.g. Sánchez-Ochoa et al., 2007; Winiwarter et al., 2009). Due to large particles of biological material being lifted into the atmosphere (e.g. air-dispersed seeds), the actual concentration of cellulose and related materials in total air particulate matter may be substantially higher under windy conditions.

Emissions of liquid and solid secretions from organisms are also found in the atmosphere. Brochosomes and epicuticular wax (Wittmaack, 2005; Wittmaack et al., 2005) are expelled from leafhoppers and do not contain cells. Brochosomes serve as a highly water-repellent body coating of leafhoppers. They usually become airborne not as individuals but in the form of large clusters containing up to 10 000 individuals or even more, but then usually separate into individual brochosomes or small clusters. They are extremely widespread and can be found in aerosol samples from all continental regions (Bigg, 2003). Exopolymer secretions (EPS) of microalgae and bacteria in water become airborne with the bubble- bursting mechanism (Leck and Bigg, 2005a, 2005b). EPS might deteriorate the ability of sea spray particles acting as Cloud condensation nuclei (CCN, see studies in Section 4.1).

Other sources of PBAP are fur fibres, dandruffs and skin fragments of animals (including humans) that are shed in large amounts per day and can also be airborne (Cox and Clark, 1973). Insect fragments have also been observed in a number of studies and thus may represent an important class of physically large PBAP in some areas (e.g. Wittmaack, 2005). Whole macroscopic organisms, such as spiders lofted to air by silk 'ballooning' lines, have been observed to travel extremely long distances (>several 100 km) and to high altitudes (stratosphere) (e.g. Thomas et al., 2003). These organisms may contribute to PBAP dispersal efficiently through body or silk fragments.

2.8. Characteristic concentrations and emission estimates

Biological particles cover an extremely broad range of sizes and are morphological very diverse. Thus, measurements of ambient PBAP concentrations are extremely challenging and reports from literature have been very few. PBAP have unique properties, such as large particle size, cell viability concerns and physical changes associated with environmental variables (see Section 3.1). In addition, biological material suspended in the atmosphere undergoes drastic changes as a function of manifold biological variables such as species and ecosystem health, seasonal and episodic cycles and meteorology. As a result, average concentrations for PBAP are extremely difficult to estimate without rigorously taking all of these variables into account. However, PBAP concentrations have been estimated to be up to 25% of total aerosol mass on a global basis (Jaenicke, 2005), and so more detailed measurements are desperately needed.

As a first overview of global importance, Table 3 present a survey of global emission estimates and characteristic magnitudes for the number and mass concentration of major PBAP classes, respectively. Such synthesis provides means for discussion and motivation of future measurements.

3. Techniques for PBAP collection and analysis

3.1. PBAP sampling methods

The sampling and collection of atmospheric aerosols requires a sound understanding of the physical principles that govern interactions with suspended particles (<100 μm). As such, particle size is by far the most important characteristic for choosing a sampling procedure for both PBAP and all other classes of airborne particles (Nicholson, 1995). Losses of particles within inlets, inlet lines and instruments can cause huge biases in both quantitative and qualitative understanding, and therefore sampling procedures must be designed properly (Zimmermann et al., 1987). While small particles (<0.1 μm) are most susceptible to Brownian diffusion and electrostatic forces, large particles (>5 μm) are more influenced by losses due to gravitational settling and inertial surface impaction. In addition, losses of particles to surfaces due to air turbulence can affect particles of all sizes. These issues are dealt with in detail elsewhere (e.g. Hinds, 1999; Baron and Willeke, 2001).

However, the sampling of biological aerosols often requires special handling procedures as compared to the sampling of inert particles and these are introduced briefly here. Although sampling issues due to particle size effects are not unique to biological aerosol sampling, PBAP size and mass are often hard to predict based on complicated relationships between the biological tissue and environmental variables such as temperature and relative humidity (e.g. Madelin and Johnson, 1992; Reponen et al., 1996). In addition, many biological particles (e.g. chain agglomerates of fungal spores) can have very elongated morphologies that can create large differences between physical and aerodynamic diameter measurements (Lacey, 1991; Reponen et al., 2001). Collection of living, or viable, material requires care in order not only to sample microorganisms appropriately but also to keep them alive until the desired analysis or cultivation can be performed. Certain species of bacteria and fungal spores are more easily cultivated than others, and in many cases this relates to the ability of sampled particles to survive harsh conditions related to sample collection (i.e. sun exposure, extended period of high airflow). Bacteria, in particular, are susceptible to damage or death when impacted too forcefully onto collection substrates (e.g. Stewart et al., 1995). Samples also must often be immediately frozen after collection to preserve DNA for later analysis. This can complicate sampling of PBAP in remote environments and add both uncertainty and bias to measurements. Small biological particles (e.g. viruses, bacteria) may also be attached to larger, non-biological particles such as mineral dust and thus be sized much larger than the microorganism by itself. This fact must be kept in mind when analysing size-resolved PBAP measurements. Due to the additional complexities in determining the biological nature of a collected set of particles compared to that of non-biological particles, PBAP measurements should be carefully scrutinised and in many circumstances taken as lower limit values.

As a result of the issues specific to biological aerosol sampling, a number of sampling methods have been developed primarily for PBAP collection. Again, a review of these topics can be found elsewhere (e.g. Henningson and Ahlberg, 1994; Madelin, 1994; Crook and Sherwood-Higham, 1997; Levetin, 2004; Xu et al., 2011) and so only a very brief overview of key classes of PBAP samplers will be given here. Devices that collect aerosol based on inertial forces have been particularly well utilised for PBAP measurement. The most common single-stage impactor used for microbiological sampling has been the relatively simple slit-to-agar sampler

(Henningson and Ahlberg, 1994), first developed by Bourdillon et al. (1941) and capable of providing CFU counts with high time resolution of approximately 1 h. Many different cascade impactors (multiple stages) have also been developed and extensively used for PBAP sampling (Mitchel, 1995). Probably, the most widely used of these has been the Andersen sampler (Andersen, 1958). This device has been frequently used as a reference method for sampling of culturable microorganisms, providing six or eight stages of particle sizing but due to physical constraints, the inner jets on each plate have a different sampling efficiency than the outer jets. An alternative in which this phenomenon is considered is the Berner-Impactor (Hillamo et al., 1991). The Marple 8-stage impactor is a personal cascade impactor design that provides size-resolved PBAP information directly from the breathing zone of an individual who wears it (Rubow et al., 1987). The Burkhardt spore trap has been an important sampler for collecting large PBAP such as fungal spores and pollen (Hirst, 1952). In it, air is brought in through a small slit in a housing designed to face the prevailing wind, and material collected continuously for up to a week can later be analysed via microscopy. Impingers collect airborne particles into liquid media and offer the possibilities of extended sampling times and increased collection efficiency with respect to many agar samplers (Crook, 1995a). Greenburg and Smith (1922) developed the first liquid impinger, and many types have since been designed for PBAP sampling (e.g. Henderson, 1952.) . The AGI-30 has been particularly important and has been used as a reference sampler for the collection of viable organisms (Brachman et al., 1964; Henningson and Ahlberg, 1994). Other inertial samplers that have been commonly applied to PBAP collection include rotary arm samplers and both single- and multiple-stage centrifugal cyclones (e.g. IPCC 2007b; Crook, 1995a).

Non-inertial sampling methods such as passive settling and filtration have also both been widely used for PBAP collection. Many types of filter substrates have been used, including fibrous, flat (e.g. Nucleopore™) and membrane (Crook, 1995b). Each have their own advantages and disadvantages and these should be investigated thoroughly by the individual investigator. Electrostatic (Mainelis et al., 1999, 2002; Tan et al., 2011) and thermal precipitators (Kethley et al., 1952; Orr et al., 1956) have been developed in an attempt to collect viable aerosols with less damaging force. Recently, methods for online detection of biological aerosols have been developed using a variety of optical and additional techniques. These will be discussed in detail in Section 3.3.

3.2. Traditional analysis methods

Before the invention of molecular and other physicochemical methods, the diversity, identity and concentration of airborne microorganisms were primarily studied via microscopic analysis and cultivation methods on both selective and non-selective media. These methods have long and rich histories within aerobiological studies and still remain useful tools for PBAP analysis. Thus, the terms provided here ('traditional' and 'modern') are historical and somewhat arbitrary, but helpful for rough categorisation. A detailed discussion of these methods is beyond the scope of this text and can be found elsewhere (e.g. Cox and Wathes, 1995).

3.2.1. Cultivation

Cultivation methods are only capable of collecting and detecting certain viable bacteria, fungi and algae, whereas they are incapable of detecting all other biological aerosol particles from these and other organism groups (e.g. dead bacteria and fungi, tissue fragments such as cell walls or cytoplasmic material). Although not capable of directly infecting a host, non-viable PBAP classes are still extremely important because they can provoke deleterious health effects (Gorny et al., 2002; Green et al., 2006) and may still be relevant for cloud formation.

As mentioned, studies investigating only the culturable fraction of PBAP have historically comprised a large portion of reported measurements. However, studies investigating cell viability suggest that the vast majority of environmental microbiota is non-culturable, even when viable (Staley and Konopka, 1985; Roszak and Colwell, 1987; Amann et al., 1995; Colwell, 2000; Rappé and Giovannoni, 2003; Wainwright et al., 2004b). Thus, studies based on culturing alone usually drastically underestimate microbial diversity (Fierer et al., 2008) and concentration. Only ~17% of known fungal species can be grown in culture (Bridge and Spooner, 2001) and for bacteria, the fraction is typically less than 10%, with an observed range of 0.01%–75%, and average values estimated at ~1% (Heidelberg et al., 1997; Lighthart, 2000; Chi and Li, 2007). In addition, the culturability of bacteria decreases rapidly following aerosolisation, (Heidelberg et al., 1997) and bacteria can be easily damaged when impacted via traditional sampling devices (Stewart et al., 1995). Within the culturing processes, additional

biases may occur. For example, the sampling efficiency and culturability of viable bacteria depend strongly not only on the bacterial strain but also on experimental and environmental factors, including the growth medium used (ZoBell and Mathews, 1936; Kelly and Pady, 1954; Shahamat et al., 1997; Griffin et al., 2006), the choice of impaction versus filtration as a collection method (Stewart et al., 1995), the incubation temperature and length of incubation time (Amato et al., 2007c; Wang et al., 2007; Wang et al., 2008), the air sampler volume (Griffin et al., 2006), relative humidity (Wang et al., 2001), time of day (Tong and Lighthart, 1999) and even the collection season (Amato et al., 2007c). Finally, single particles containing bacteria may represent a colony consisting of many cells, leading to a further underestimation of their abundance (Tong and Lighthart, 2000).

Despite these limitations, cultivation methods have the advantage that they are far less expensive than molecular techniques and can give an indication of the number of viable cells in the air, for species that respond well to the culturing method used. Cultivation is particularly useful for targeting individual species or groups of microorganisms and for creating culture collections. Culture studies can also characterise certain bacterial strains by various biochemical methods and give qualitative information about relative changes in the concentration over the course of days or season.

3.2.2. Light microscopy

In addition to cultivation, microscopic techniques have been extremely important to the history and development of PBAP analysis and continue to be invaluable tools. Light microscopy of various types has been applied to the characterisation of collected PBAP (Spurny, 1994; Cox and Wathes, 1995 and references therein). For pollen, it still remains the most common analysis method. Simple optical microscopy was the first technique to be applied to PBAP analysis in the seventeenth century (e.g. Miquel, 1883) and continues to be utilised extensively. But, it has to be taken into consideration that particles <2 μm are only visible as dots under an optical microscope and thus cannot be analysed in detail according to their size and shape. Aerosol samples collected by sedimentation and impaction devices can be directly visualised and counted by light microscopy. If the sampling device used is volumetric, the concentration of particles in the air can be quantified. However, the identification of biological particles via direct counting is both very tedious and somewhat subjective, as the particles must be counted by eye. Various traditional stains can be used in combination with light microscopy, including methylene blue for simple examination, and Gram staining and other differential staining techniques that aid classification into groups of species. Protein staining gives an estimate of all (total) PBAP (Matthias-Maser and Jaenicke, 1995). For the analysis of the diversity and composition of bacteria and fungi, microscopic analyses are not usually reliable, as small non-descript spores and hyphae or fragments of fungal tissues cannot be classified (Pitt and Hocking, 1997). Some fungi or bacteria may remain morphologically undistinguishable or may only be identifiable to a class or family level.

3.2.3. Fluorescence microscopy

Fluorescence microscopy has been applied both to look at the autofluorescence of PBAP (Pöhlker et al., 2011), and especially with the use of fluorescent dye labelling (Karlsson and Malmberg, 1989; Hernandez et al., 1999). The most common traditional method for determining the total count of environmental microorganism is direct fluorescence microscopy, either by taking advantage of the autofluorescence of certain biological compounds or by using samples that have been treated with a fluorescent dye, most commonly 4.6-diamidino-2-phenylindole (DAPI) or acridine orange (Francisco et al., 1973; Hobbie et al., 1977; Kepner and Pratt, 1994; Matthias-Maser and Jaenicke, 1995; Harrison et al., 2005). Acridine orange binds to both DNA and RNA, fluorescing green when bound to DNA and red when bound to RNA and some mucins. DAPI fluoresces blue when bound to DNA and yellow when unbound or bound to a non-DNA material. Traditionally, particles have been classified and counted by a human investigator, taking into account the size and morphology of stained particles. Epifluorescence microscopy permits counting of the total number of unlysed cells containing DNA; a number that includes both viable and non-viable microorganisms. It is not always possible to unambiguously distinguish different types of biological particles, such as bacterial and fungal spores, via fluorescence microscopy.

However, the colour of autofluorescence combined with the application of fluorescent stains can be used to distinguish some broad groups of microorganisms. Fluorescence microscopy is both tedious and labour intensive, although the use of fluorescence aids the identification of biological particles compared to light

microscopy. Recently, more automatic techniques have been attempted, including computer analysis of microscopic images (Carrera et al., 2005) and fluorescence spectroscopy (Reyes et al., 1999; Courvoisier et al., 2008). Other modern fluorescence-based techniques will be described in the next section.

3.3. Modern analysis methods

3.3.1. Molecular techniques

3.3.1.1. Chemical tracers

Chemical tracers, such as the sugar alcohols mannitol and arabitol, can be used to not only assess the abundance of PBAP, especially fungal spores in air particulate matter, but also characterise other aerosol types (Hensel and Petzhold, 1995; Graham et al., 2003; Graham et al., 2004; Lau et al., 2006; Elbert et al., 2007; Yttri et al., 2007; Bauer et al., 2008a; Engling et al., 2009; IInuma et al., 2009; Burshtein et al., 2011). A number of other chemical tracers have been used as proxies for various types of biological aerosol particles, such as endotoxins, mycotoxins, glucan, ergosterol, extracellular polymeric substances, carbohydrates, proteins, peptides, sugars and adenosine triphospate (ATP). These can be analysed by a wide range of instrumental and bioanalytical techniques such as chromatography coupled to mass spectrometry, spectrophotometry, fluorescence spectroscopy, immunoassays, dye assays, etc. (Griffith and DeCosemo, 1994; Reponen et al., 1995; Franze et al., 2005; Pöschl, 2005; Demirev and Fenselau, 2008).

Chemical tracer analysis has the advantage of providing quantitative information, but it does usually not provide information about the identity and biodiversity of PBAP on the species level.

3.3.1.2. Nucleic acid sampling and extraction

For the study of biological aerosols molecular techniques, e.g. those based on molecular genetic analyses, have some advantages compared to traditional methods. The results can enable the identification and quantification of culturable as well as uncultivable microorganisms, of viable and dead cells, and of plant and animal fragments (Després et al., 2007).

To analyse biological aerosols with molecular genetic tools, biological aerosols need to be collected and the nucleic acids extracted. Biological aerosols are either collected in culturing media (liquid or plates) or water (Boreson et al., 2004), special biological aerosol particle collectors (e.g. the Wetted-wall Cyclone sampler (Biotrace) in Maron et al. (2006)) or on appropriate air filter samples. Several different filter types have already successfully been used for the DNA analysis but not been compared quantitatively to each other (e.g. glass fibre filters (Després et al., 2007; Fröhlich-Nowoisky et al., 2009); quartz fibre filters and polypropylene filtres (Després et al., 2007); cellulose nitrate filtres (Després et al., 2007; Bowers et al., 2010); Celanex polyethylene terephthalate filtres (Brodie et al., 2007); borosilicate filtres (DeSantis et al., 2005) etc.).

The basis for most molecular analyses techniques is the successful extraction of DNA. Every cell carries this molecule: eukaryotes carry DNA in the form of chromosomes within the cell nucleus, whereas prokaryotes and archaea lack a nucleus and carry DNA in genophores directly within their cell body. During the DNA extraction processes, proteins are denatured and separated together with lipids, pigments, cell wall fragments and organelle structures.

DNA extraction protocols vary according to the tissue for which they are used. Plant cells, with strong cell walls, present different extraction challenges than animal tissue cells. Endospores of bacterial and fungal spores have very strong cell walls that need to be broken down before DNA extraction. Thus, some compromise is always required in selecting a DNA extraction method for ambient air filter samples that contain a mixture of various organisms and tissue types. Either the DNA is extracted using a generic method that does not exclude any tissue type but is also not ideally matched to any specific type such that some organisms and tissue types are undersampled, or the DNA is extracted using a method specific to a particular organism or tissue type, and other types are undersampled. Thus, for ambient samples that include a mixture of many types of biological material, PBAP that contain thick membranes or cell walls could elude extraction of DNA by a generic method, leading to an underestimation of such species. Usually, commercial kits can be used for the extraction (e.g. soil DNA extraction kit (Després et al., 2007; Fröhlich-Nowoisky et al., 2009)); Fast Prep 120 agitator together with

Spin column of MolBio Laboratories (DeSantis et al., 2005); alternatively, some investigators develop their own extraction methods (e.g. Boreson et al., 2004; Maron et al., 2005).

3.3.1.3. Amplification of genomic DNA

The DNA extract of an air filter sample still contains the genomic DNA of all sorts of organisms; thus, it is a mixture of DNA from living and dead material of fungi, bacteria, archaea, plants, animals or viruses. To be able to identify single genera or species, it is necessary to enrich the DNA of the organism of interest relative to other genomes. The PCR efficiently amplifies characteristic regions of the DNA of a species, or a group of species, for detailed analyses. The method is based on thermal cycling in which the DNA is heated, thereby separating the double strands. Short DNA sequences, called primers, attach to matching sequences on the single stranded DNA that mark the borders of the region of interest on the genome. In the final step of PCR, the DNA polymerase enzymatically assembles a new matching DNA strand in the target region using single nucleotides. PCR, followed by genetic sequencing of the amplified DNA, can be used to identify organisms, to understand phylogenetic relationships or to study specific genes. Thus, it is an essential technique when studying the identity, diversity and composition of biological aerosols.

Within every genomic DNA, there are areas that are highly conserved between organisms such as housekeeping genes, whereas other areas vary enormously. The primers used in PCR should ideally attach to highly conserved areas, whereas the sequence between the primers should be diverse and thus uniquely present in the targeted organisms or group of organisms. Areas such as ribosomal RNA genes or the ITS-regions with a huge variability are often used in taxonomic identification studies, as they enable to differentiate between genera or even species and thus can be used as a fingerprint of a species. For bacteria, e.g. often the 16S rRNA genes are amplified (Moffett et al., 2000).

While amplification followed by taxonomic analysis allows the identification of PBAP species within aerosol samples, it gives no information about the quantity of particular species in the air. Quantitative PCR allows the calculation of the number of DNA template molecules in the DNA extract. Thus, when studying, e.g. a single copy gene within a single bacterial species, it is possible to estimate the number of individuals of this particular species that was present in the air filter sample. This could be an especially valuable tool for studying pathogenic biological aerosol particles.

Although molecular techniques are thought to detect biological aerosols unambiguously, they may not amplify all airborne bacteria in a sample (Peccia and Hernandez, 2006; Fierer et al., 2008). Theoretically, the DNA polymerase can amplify a single DNA copy and thus detect even organisms that are present in minor quantities. But, the sensitivity of the PCR depends on several factors. One major issue is the choice of the primer pair used for the amplification process. Different primer pairs have different specificity and sensitivity (Alvarez et al., 1995; Polz and Cavanaugh, 1998). Thus, while one primer pair might start the amplification from just a single DNA strand, a less sensitive primer pair might need 100 molecules to get started. It is also possible that a primer pair is more sensitive to some species than to others within a targeted group. Although a primer pair might have been designed to perfectly match the target organism, it might also co-amplify other organisms. This is especially important for biological aerosol DNA extracts where many competing DNA molecules may be present, possibly even in higher concentrations than the organism of interest. Literature research can help to find suitable specific primers, but often the specificity of primers is overstated in the literature (e.g. Fröhlich-Nowoisky et al., 2009).

Another important point that must be considered is the possible presence of PCR inhibitors. Substances such as humic acids inhibit the DNA amplification process either by hindering the attachment of polymerase to the primers to initiate amplification, or by binding to the DNA and thereby preventing primers or enzymes from attaching. Different PCR primers as well as DNA polymerases vary in their ability to overcome inhibitory factors. In addition, filter materials on which biological aerosol particles are collected can inhibit PCR (Després et al., 2007).

Finally, although DNA is a stable molecule, which under cool, dark and dry conditions can sometimes be preserved for several thousand years (Pääbo et al., 2004), DNA starts to degrade, breaks into smaller pieces and is chemically modified as soon as an organism dies (Pääbo, 1989; Lindahl, 1993; Höss et al., 1996; Smith et al., 2001). UV light, ozone and melting-freezing processes speed up these degradation processes, and thus a long residence time of biological material in air leads to deterioration of DNA and loss of genetic information.

Pollen grains, as well as many spores of fungi and bacteria, are encased by thick cell walls to protect their DNA from DNA-destroying environmental processes.

3.3.1.4. Restriction fragment length polymorphism techniques

A standard molecular genetic technique is the fragmentation of DNA by a restriction enzyme that cuts the DNA wherever a specific target sequence occurs. The resulting restriction fragments are separated according to their lengths by gel electrophoresis. It can be applied following colony PCR (confirmation of plasmid insertion) to select as many different clones as possible for the sequencing reaction (Fröhlich-Nowoisky et al., 2009). For a broad characterisation of the community structure and diversity of a PBAP sample, the terminal restriction fragment length polymorphism (T-RFLP) technique can be used to get a rough estimate of the diversity and relative abundances (Després et al., 2007; Georgakopoulos et al., 2009). The first step of a T-RFLP is a PCR amplification in which one primer is fluorescently labelled. The PCR products are then digested with a restriction enzyme that cuts each DNA strand at the target sequence, resulting in shorter, labelled fragments. Because the position of the target sequence on the genome varies among bacterial strains, the length of the labelled fragments also varies. The fluorescently labelled end fragments are separated by electrophoresis, and the strand lengths and fluorescence intensities are calculated. The fluorescence intensity of strands of a given length indicates the frequency of the corresponding bacterial strains in the original sample. The genetic diversity indicated by a T-RFLP profile (as measured by the number of different strand lengths) is highly dependent on the choice of the restriction enzyme and the part of the gene used to generate the terminal fragments. If the PCR products are simultaneously cloned and sequenced, the size of the terminal fragment that each will produce can be calculated, and so each sequence can be attributed to a T-RFLP peak (Després et al., 2007; Georgakopoulos et al., 2009).

3.3.1.5. Sequencing methods

To determine the identity of the genomes obtained from atmospheric aerosol samples, the PCR products are often cloned and sequenced (e.g. Boreson et al., 2004; Maron et al., 2005; Després et al., 2007; Fierer et al., 2008; Bowers et al., 2009; Fröhlich-Nowoisky et al., 2009; Georgakopoulos et al., 2009). Species can often be identified by comparing the obtained sequences with those that are already available in online databases, e.g. that of the National Center for Biotechnology Information (NCBI). The BLAST search[4] is the easiest way to search for matching sequences. If a new organism (e.g. new bacterial strain) is found, it is possible to identify the most closely matching sequences already available in the database. For bacteria, the generally accepted levels of discrimination are 97–99% similarity for species and 95–97% for genera; for fungi, clustering sequences with 97% or greater sequence similarity are accepted (O'Brien et al., 2005; Bowers et al., 2009; Fröhlich-Nowoisky et al., 2009; Georgakopoulos et al., 2009). The traditional sequencing technique is using a chain termination (Sanger and Coulson, 1975; Sanger, 1981). The sequencing process is essentially another PCR but only one primer instead of two is used. The reaction mix contains not only the standard DNA bases (deoxynucleotide-triphosphate, dNTP) but also chain terminator dideoxynucleotide-triphosphates (ddNTPs) that are labelled with fluorescent dyes that emit light at different wavelengths (dye-terminator sequencing). The incorporation of a ddNTP results in a chain termination, and the fluorescence peaks are detected after capillary electrophoresis resulting in the DNA sequence.

The high demand for low-cost sequencing in recent years has led to the development of high-throughput sequencing technologies. In these technologies, the sequencing process is parallelised for several samples, and thus in a short time thousands or millions of sequences are produced. One high-throughput technique, 454 pyrosequencing, has been applied successfully several times in biological aerosol research (Bowers et al., 2009, 2011). The DNA is amplified within an oil droplet by the so-called emulsion PCR. Each oil droplet contains a single DNA template attached to a single primer-coated bead that then forms a clonal colony. The sequencing machine contains many picolitre-volume reactions areas, each containing a single bead and sequencing enzyme. Pyrosequencing uses luciferase to generate light for detection of the individual nucleotides added to the nascent DNA, and the combined data are used to generate sequence readouts. For studies in which the taxonomic identification of biological aerosols is anticipated, the amplified sequences must have a minimum length to enable differentiation at the genus or species level. Often, this minimum length is around 600–1000 bp (base pairs). In traditional sequencing, this length can be reached easily, whereas in high throughput sequencing the length of the different sequences is usually only around 300–400 bp. Thus, with current high-throughput techniques, it is often not possible to identify biological aerosol particles on a species or genus level.

[4]Provided by NCBI http://blast.ncbi.nlm.nih.gov/Blast.cgi, 28 March 2011.

3.3.1.6. Hybrid and chip technology

Microarrays can be used for the characterization of PBAP (Wilson et al., 2002; Brodie et al., 2007; Georgakopoulos et al., 2009). Species or group specific probes (e.g. bacterial 16S rRNA genes of DNA; Loy and Bodrossy, 2006) are fixed on glass slides. DNA from atmospheric samples is fluorescently labelled and can hybridise with the DNA on the microarray if complementary sequences exist in the microarray. The sequence is determined from the position of the fluorescence on the chip. This technology was recently used on atmospheric aerosol samples for bacterial 16S rRNA genes (Wilson et al., 2002; Brodie et al., 2007). Both studies compared the cloning and sequencing method with microarray technology that was found to be more sensitive in detecting bacterial taxa.

3.3.2. Optical methods

Most of the above-mentioned methods for detecting PBAP are limited to offline analyses that can be time consuming, costly and which often provide measurements that suffer from poor time resolution (hours/days). However, much effort has been invested in the last decades, largely by military research facilities interested in quick detection of bio-warfare agents, to be able to detect biological aerosols in real time and with high time resolution. However, a comprehensive review of all modern methods for PBAP detection is well beyond the scope of this text and the following section is intended to present an overview of the most important classes of techniques. It focuses on field-based techniques, although some techniques that are primarily laboratory based are also discussed, as they may have important relevance to ambient detection.

3.3.2.1. On-line autofluorescence methods

All biological materials contain fluorophores that may be helpful for identification. Among most existing instruments that utilise biological auto-fluorescence for online determination of biological aerosol particle concentration, the choices of wavelength for the excitation source can generally be classified into one of two regions. Sources that provide light in the region of approximately 350–370 nm enable detection of reduced pyridine nucleotides (e.g. NAD(P)H) and riboflavin that are biological molecules linked to cellular metabolism (Harrison and Chance, 1970; Eng et al., 1989; Kell et al., 1991; Li et al., 1991; Iwami et al., 2001). Detection of auto-fluorescence under these conditions may indicate the presence of viable biological material in the aerosol particles, although other biological molecules (e.g. chlorophyll, cellulose) can also auto-fluoresce under many environmental conditions. The second region of fluorescence excitation commonly utilised by biological aerosol instrumentation is approximately 260–280 nm and highlights amino acids, such as tryptophan, tyrosine and phenylalanine that are present in all proteins (e.g. Teale and Weber, 1957; Pöhlker et al., 2011).

Thus, it can be broadly stated that detection of fluorescence by this class of instruments allows determination of fluorescent biological aerosol particles (FBAP). However, different instrument designs measure and report different fractions of the PBAP and comparisons across instruments should be conducted with this in mind. Among the uncertainties involved is the possibility that material of non-biological origin will also fluoresce within a given particle, causing a positive artifact. However, non-biological particles producing such artefacts are unlikely to contribute significantly to the coarse (>1 μm) fraction of ambient particulate mass (Huffman et al., 2010) and may also exhibit much weaker fluorescence than biological particles. For example, results of a study performed in remote Amazonia indicated that coarse FBAP closely approximated PBAP concentrations and size distributions (Pöschl et al., 2010). It is also known that not all biological microorganisms or fractions will fluoresce under the experimental conditions of such instruments, and opaque or absorbing PBAP are likely to fluoresce only very weak at best (Pöhlker et al., 2011). The weak fluorescence of some PBAP suggests that fluorescence measurements underestimate the total biological material present. Further investigation will be required to achieve full understanding of the response of fluorescence-based biological aerosol particle detectors to all types of biogenic aerosol particles, and to quantify potential interferences by fluorescence of non-biological particles. However, our present understanding is that FBAP can generally be regarded as an approximate lower limit for the actual abundance of PBAP (Huffman et al., 2010).

The Ultraviolet Aerodynamic Particle Sizer[5] (UV-APS) was the first commercially available, fluorescence-based instrument for real-time analysis of biological aerosols (Hairston et al., 1997; Brosseau et al., 2000). The UV-

APS measures the aerodynamic diameter and side scatter parameter (analogous to the optical diameter) of incoming particles by measuring their time-of-flight between two lasers (633 nm) and then detects fluorescence in the wavelength range of 420–575 nm after excitation by a pulsed ultraviolet laser (Nd:YAG, 355 nm). An example of UV-APS measurement data is given in Fig. 4. This shows time series and size distributions of FBAPs detected with a UV-APS at a site in central Europe (Mainz, Germany, October 2006). The figure highlights the ability of the UV-APS to measure FBAP size distributions of discrete particle events with much higher time and size resolution than is achievable by most traditional sampling and detection methods (Huffman et al., 2010).

[5]UV-APS; TSI Inc., Model 3314, St. Paul, MN

Fig 4

Fig. 4. Characteristic time series and number size distribution of fluorescent biological aerosol particles (FBAPs) measured with an ultraviolet aerodynamic particle sizer (UV-APS) in central Europe (Mainz, Germany, October 2006). The peaks at ~1.5, ~3 and ~13 μm can be attributed to bacteria, fungal spores, and pollen. $N_{F,c}$ is the number concentration of FBAPs, and $N_{T,c}$ is the number concentration of total aerosol particles with aerodynamic diameters D_a>1 μm; $dN_F/d\log D_a$ is the number size distribution function of FBAPs (Huffman et al., 2010).

The Wide Issue Bioaerosol Spectrometer (WIBS) is also available in limited commercial production and not only provides conceptually similar information to the UV-APS but also provides a rough estimate of particle sphericity (Kaye et al., 2000, 2005). Incoming particles are first optically sized by measurement of scattered laser light. UV pulses at ~280 and ~370 nm from a xenon flash lamp excite each particle, and multiple bands of fluorescent emission in the range of 310–400 nm (for 280 nm excitation) and 420–650 nm (for 280 and 370 nm excitation) are recorded.

A number of other instruments have been developed for military research use and have not been commercialised or widely used for measurements published within peer-reviewed literature. However, these technical and scientific developments have been significant contributions to the scientific community (e.g. Hill et al., 1995; Cheng et al., 1999; Reyes et al., 1999; Seaver et al., 1999; Kopczynski et al., 2005; Cabredo et al., 2007; Campbell et al., 2007; Manninen et al., 2008; Pan et al., 2009; Sivaprakasam et al., 2009).

Although the development of such instruments has left a detailed trail in publically accessible military reports and peer-reviewed literature, ambient measurements by such techniques have been less frequently documented or de-classified. An early generation UV-APS (Ho, 2002) was used for short periods to measure background FBAP concentrations at several military locations within Canada and Sweden (Ho and Spence, 1998; Ho et al., 2004), and a different LIF spectrometer design was utilised for detection of FBAP in ambient air in several US locations (Pinnick et al., 2004; Pan et al., 2007; Pan et al., 2009). Recently, fluorescence measurements of biological aerosols have also been reported in more detail for tropical rainforest air (Prenni et al., 2009; Gabey et al., 2010; Pöschl et al., 2010) as well as in two urban European locations (Huffman et al., 2010; Gabey et al., 2011).

3.3.2.2. Flow cytometry

Flow cytometry has also long been an important tool in the investigation of ambient PBAP. Among the papers that report successful applications of flow cytometry to real-time investigation of biological aerosol are those by Sincock et al. (1999), Chen and Li (2005) and Chen and Li (2007). Fluorescent in situ hybridization (FISH) flow cytometry has been used by a number of groups to characterise airborne bacteria and aerosolised byproducts (e.g. Lange et al., 1997). Using a flow, cytometric system (Ho and Fisher, 1993) investigated *Bacillus subtilis* bacterial spores, and Prigione et al. (2004) developed a flow cytometer that could be selectively applied to the investigation of airborne fungi.

3.3.2.3. LIght Detection And Ranging (LIDAR) and remote sensing

The LIght Detection And Ranging (LIDAR) technique has been utilised to quickly and remotely monitor the presence of PBAP over a larger spatial range. A LIDAR system (Evans et al., 1994) was operated to determine its sensitivity to aerosolised *Bacillus subtilis* spores, and subsequent efforts have investigated LIDAR

performance for PBAP detection in more detail (e.g. Simard et al., 2004; Glennon et al., 2009). The detection of fluorescent ambient aerosol was reported for the first time using a LIDAR system (Immler et al., 2005), attributing the signals to a combination of PAH-containing particles and/or PBAP. Furthermore, it was shown (Sassen, 2008) that pollen can generate strong laser depolarisation in LIDAR backscatter during Alaskan springtime measurements and suggested that pollen plumes may be mistaken for upper cirrus clouds and therefore introduce important errors into identifying aerosols in the atmosphere. Atmospheric optical phenomena caused by pollen also have been reported,[6] and will be discussed briefly in Section 5.

3.3.2.4. Fluorescent and Raman spectroscopy

Fluorescent properties of collected biological aerosols have been widely used for assessment of concentrations and properties of ambient PBAP. Fisar et al. (1990) showed that fluorescence detected from dyed biological aerosol particles could be effectively scaled to more traditionally measured CFUs. Raman spectroscopy has been used for investigation and characterisation of individual pollen grains in a number of different manners by various groups (Laucks et al., 2000; Boyain-Goitia et al., 2003; Kano and Hamaguchi, 2006; Schulte et al., 2008). A particularly interesting technique utilised fluorescence measured from particles impacted on a surface as a pre-selector, before Raman spectroscopy enabled bacterial identification in more detail (Rosch et al., 2006). Surface-enhanced Raman spectroscopy has also been employed to sensitively detect and characterise pollen and bacteria after injection into a silver suspension (Sengupta et al., 2005, 2006, 2007). Raman microscopy has been utilised by Ivleva et al. (2005) for the chemical investigation and discrimination of ambient PBAP. They used a combination of Raman microscopy with multivariate analyses to characterise sampled pollen with the goal of differentiating between allergenic species.

3.3.2.5. Additional optical methods

While the classes of optical techniques mentioned have been most commonly utilised for biological aerosol analysis, a review of the literature highlights the virtually endless list of techniques that have been and could be applied. Among notable additional efforts include the use of X-ray fluorescence (Pepponi et al., 2004) and total internal reflection fluorescence microscopy (TIRFM) (Axelrod, 2008) for the investigation of biological aerosol properties. Infrared vibrational spectroscopy has also been widely used for pollen identification (Pappas et al., 2003; Gottardini et al., 2007; Dell'Anna et al., 2009; Zimmermann, 2010). Scanning electron microscopy (SEM) has been particularly useful at the investigation of PBAP, allowing a close look at the morphology and surface of particles (Karlsson and Malmberg, 1989; Wittmaack et al., 2005; Coz et al., 2010; Pöschl et al., 2010; Gilardoni et al., 2011).

[6] http://www.atoptics.co.uk/droplets/pollen1.htm, 28 March 2011

A recent study has applied Scanning Transmission X-ray Microscopy with Near-Edge X-ray Absorption Fine Structure (STXM-NEXAFS) spectroscopy to the study of PBAP (Pöhlker, personal communication). This technique allows the structural examination of particles with a resolution down to about 30 nm, combined with spatially resolved chemical characterisation. It is suitable for the determination of the major elements, carbon, nitrogen and oxygen, as well as some minor elements, e.g. iron and chlorine. In addition to the measurement of elemental abundances, it can also yield information on the bonding state of the various elements. Using STXM-NEXAFS, Pöhlker (personal communication) could show that the coarse fraction of the Amazonian aerosol consisted predominantly of intact PBAP.

3.3.3. Non-optical methods

Several reviews of modern techniques of biological aerosol detection and analysis have been published (Spurny, 1994; Ho, 2002; Douwes et al., 2003; Lim et al., 2005; Kuske, 2006) and so, again, only highlights of important classes of non-optical modern techniques will be discussed here.

3.3.3.1. Mass spectrometry

Advances in mass spectrometry (MS) have been utilised in many areas of physical and biological science in the last decades to provide detailed information about chemical composition. The analysis of biological aerosols has been no different, and many different MS techniques have been employed for PBAP characterisation. Matrix-

Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) has been successfully applied in several PBAP studies (Kim et al., 2005; van Wuijckhuijse et al., 2005; Kleefsman et al., 2008; Russell, 2009). Parker et al. (2000) developed an instrument that utilised ion-trap mass spectrometry for detection and analysis of pollen, bacteria and other aerosol types. Stowers et al. (2006) demonstrated the utility of laser-induced fluorescence as a pre-selector before more detailed MS identification. Lawrence Berkeley National Lab has also utilised a similar concept for its biological aerosol mass spectrometry (BAMS) instrument (e.g. Fergenson et al., 2004) and has deployed the instrument in a number of field locations. Other real-time MS instruments designed primarily for other aerosol-related detection purposes have also been applied to PBAP characterisation. For example, an Aerodyne Aerosol Mass Spectrometer (AMS) was deployed in Amazonia, Brazil and was able to detect molecular ion markers in submicron aerosol consistent with the presence of biological aerosols (Chen et al., 2009; Pöschl et al., 2010; Schneider et al., 2011). Pratt et al. (2009) utilised an Aerosol Time-of-Flight Mass Spectrometer (ATOFMS) to detect the presence of biological aerosols as ice nuclei during some flights of an aircraft campaign in the Western United States. While not technically a mass spectrometer, both Dworszanski (1997) and Snyder et al. (2001) report development of automated instruments that utilise pyrolysis-gas chromatography in front of an ion mobility spectrometer for rapid detection of biological aerosol particles in the field.

3.3.3.2. Breakdown spectroscopy

Many groups have also utilised different types of breakdown spectroscopy (BS) to identify elemental composition as means of biological aerosol detection and analysis, although these have not been as widely applied to ambient measurements. Laser-induced breakdown spectroscopy (LIBS) has easily been the most common form of BS method and has been employed to characterise pollen (Boyain-Goitia et al., 2003), and fungal spores (Hybl et al., 2003), bacteria (Morel et al., 2003) and a variety of biological aerosol particle types (Samuels et al., 2003). Other forms of elemental analysis such as spark-induced breakdown spectroscopy (SIBS), particle-induced X-ray emission (PIXE) and various forms of combustion analysis have also been utilised for laboratory study of biological aerosol (Sarantaridis and Caruana, 2010; Schmidt and Bauer, 2010).

3.3.3.3. Miscellaneous non-optical-methods

As has been the case for recently developed optical methods of biological aerosol analysis, a large variety of non-optical techniques of chemical and physical analysis have been employed. Bioluminescence and chemiluminescence have been extensively explored for potential analytical benefits, and much of this effort has focused on the detection of ATP within collected aerosol (e.g. Lee et al., 2008; Seshadri et al., 2009; Yoon et al., 2010). Electrochemical, immunochemical and immune biological methods have also been investigated for ambient PBAP characterisation (Rishpon et al., 1992; Spurny, 1994; Sarantaridis and Caruana, 2010; Schmidt and Bauer, 2010). It should be noted here that complementary instruments such as biological aerosol particle concentrators have also helped enable analyses by a variety of instruments (e.g. Pan et al., 2004).

4. Atmospheric relevance

4.1. Atmospheric transport of biological particles

Particles in the atmosphere, both biological and non-biological, are transported primarily together with air currents, as well as vertically downwards by gravitational sedimentation and inside of airborne water droplets and ice crystals. Particles are removed from the air either by sedimentation and deposition onto the ground and plant or other surfaces, or through washout by precipitation. Depending on their size and aerodynamic properties, the average residence time of various biological particles in the atmosphere can range from less than a day to a few weeks. We do not attempt a complete review of atmospheric transport processes and their particular impact on biological aerosols here, but only give a brief overview of some key research areas. Existing reviews cover aspects of this topic in more detail (Gregory, 1973; Niklas, 1985; Aylor, 1986; Nagarajan and Singh, 1990; Brown and Hovmøller, 2002).

Dry deposition (sedimentation and interception/impaction onto surfaces) is the most important removal mechanism for particles tens of microns in diameter or larger. Dry deposition rates are characterised primarily by the particle's aerodynamic diameter (diameter of a spherical particle of unit density with the same terminal velocity in air as the particle in question (e.g. Hinds, 1999)). Because biological particles often have complex structure (rough surfaces, internal pores and asymmetric shape), their physical and aerodynamic sizes can differ significantly. In particular, certain fungal spores, pollen and seeds are adapted to be aerodynamically buoyant,

to promote long-range airborne transport. Observations show that the aerodynamic diameter may be either larger or smaller than the physical diameter, with considerable variation between species (Gregory, 1973; Fig. 3, p. 17; Madelin and Johnson, 1992; Reponen et al., 1998; Reponen et al., 2001), and microorganisms may sometimes be present in the atmosphere in clumps or attached to other particles (Lacey, 1991; Tong and Lighthart, 2000).

For particles ~0.1–10 μm in diameter, washout by falling precipitation is the most efficient removal mechanism. A single rain event (even a slight drizzle) can efficiently remove a large percentage of particles from the air in many circumstances (McDonald, 1962).

In spite of limited differences related to buoyancy and IN activity, biological particles can be expected, to a good first approximation, to be transported in the atmosphere similarly to mineral dust and are treated similarly to dust in most model studies of atmospheric dispersion. Desert dust particles of a similar size to bacteria-carrying particles are well known to be transported over long distances, particularly during intermittent dust storms that are visible to satellites. Biological particles, undistinguishable to satellites, can be transported over long distance in a similar fashion, as is borne out by case studies showing marked changes in the concentration and composition of airborne microorganisms during some dust transport events (e.g. Bovallius et al., 1978; Prospero et al., 2005; Jeon et al., 2011); global atmospheric model simulations produce similar results (Burrows et al., 2009b; Wilkinson et al., 2012).

A wide variety of approaches have been used for modelling the transport of biological particles on different spatial scales: Gaussian plume models (distances up to ~100 m; Ganio et al., 1995; Skelsey et al., 2008), Lagrangian stochastic models (Jarosz et al., 2004), regional-scale models (Helbig et al., 2004; Sofiev et al., 2006) and global climate and transport models (Burrows et al., 2009b; Wilkinson et al., 2012). Most studies of the airborne dispersal of biological particles have focused on the spread of specific human, animal and plant diseases on the local or regional scale (Donaldson et al., 1982; Gloster et al., 1982; Aylor et al., 2003; Isard et al., 2005). Others have addressed the potential for dispersal and cross-fertilization of GM organisms (Aylor et al., 2003; Jarosz et al. 2004) and seed dispersal (Nathan et al., 2002).

4.2. PBAP as cloud condensation nuclei

Aerosol particles form the nucleus for the condensation of cloud droplets, and their number and properties influence cloud microphysical properties. How active particles are as cloud condensation nuclei (CCN) depends on their size and hygroscopicity (Petters and Kreidenweis, 2007). Primary biological aerosol particles are generally assumed to be efficient CCN, provided that their surfaces are wettable (Andreae and Rosenfeld, 2008; Ariya et al., 2009). It has been suggested that the largest PBAP (e.g. pollens grains) may act as the so-called 'giant CCN', i.e. they may form cloud droplets at lower supersaturations than most other aerosol particles and quickly grow to large droplet sizes, thereby facilitating rain formation (Dingle, 1966; Möhler et al., 2007; Pope, 2010).

Table 5 lists measurements of hygroscopic growth of bacteria, fungal spores, pollen and algal exudate below water saturation and shows critical supersaturations that have been determined for some bacterial species and algal exudate (exopolymer secretions, EPS). Below the saturation point of water, hygroscopicity is quantified by the diameter increase relative to the dry diameter ('diameter growth factor') or by the mass of the water taken up at a certain relative humidity (RH) compared to the dry mass. The diameter growth factors (GF) observed for bacteria and fungal spores are usually modest (GF ~1.05 to 1.3 at RH = 98%) compared to those of inorganic salt particles such as sodium chloride (GF ~1.65 at RH = 98%) (Lee et al., 2002). Pollen grains usually show no or only little increase in geometric size at increasing relative humidities, but can take up substantial amounts of water, e.g. up to three times their dry weight at RH = 95% (Diehl et al., 2001). Critical supersaturations for bacteria have been reported over a wide range between 0.07 and more than 2% (Franc and DeMott, 1998; Bauer et al., 2003). Algal exudate, which is mixed into sea spray particles, reduces the hygroscopicity and CCN activity relative to artificial seawater devoid of exudate (Wex et al., 2010; Fuentes et al., 2011).

Table 5. Compilation of laboratory measurements of the hygroscopic properties of biological particles (n.a.=data not available)

		Measurements at subsaturation		Measurements at supersaturation		
Species	Diameter, D_{ve}–volume equivalent, D_{gma}. geometric mass aerodynamic, D_{mma}–mass median aerodynamic, D_a–aerodynamic	Maximum growth factor at RH	RH at which hygroscopic growth was measured	Critical supersaturation	CCN/CN ratio	References
Bacteria						
Pseudomonas syringae, *Erwinia herbicola*	n.a.	–	–	activation observed at 0.5%	n.a.	Snider et al. (1985)
Erwinia carotovora	3 μm (maximum cellular dimension)	–	–	0.2% to 2.2%	≤ 0.5	Franc and DeMott (1998)
Arthrobacter agilis	1.1 μm D_{ve}	–	–	0.11%	1.03±0.7	Bauer et al. (2003)
"new species"	1.1 μm D_{ve}	–	–	0.11%	0.88±0.5	Bauer et al. (2003)
Sphingomonas echinoides	1.2 μm D_{ve}	–	–	0.09%	0.92±0.6	Bauer et al. (2003)
Sphingomonas echinoides–fixed	1.2 μm D_{ve}	–	–	0.07%	0.99±0.4	Bauer et al. (2003)
Saccharomonospora viridis	1.15 μm D_{gma}	1.3 at 95%	95%	–	–	Madelin and Johnson (1992)
Streptomyces albus	1.15 μm D_{gma}	1.09 at 95%	95%	–	–	Madelin and Johnson (1992)
Bacillus subtilis	0.94 μm D_{mma}	~1.22 at 90%	10–90%	–	–	Johnson et al. (1999)
Pseudomonas syringae	0.89 μm D_{mma}	~1.15 at 90%	10–90%	–	–	Johnson et al. (1999)
Escherichia coli	0.63 μm D_a	1.34 at 98%	20–98%	–	–	Lee et al. (2002)
Bacillus subtilis	0.75 μm D_a	1.16 at 98%	20–98%	–	–	Lee et al. (2002)
Fungal spores						
Aspergillus flavus	3.3 μm D_{gma}	1.15 at 95%	95%	–	–	Madelin and Johnson (1992)
Aspergillus fumigatus	1.9 μm D_{gma}	1.16 at 98%	95 and 98%	–	–	Madelin and Johnson (1992)
Cladosporium cladosporioides	2.3 μm D_{gma}	1.12 at 98%	95 and 98%	–	–	Madelin and Johnson (1992)
Paecilomyces variotii	2.5 μm D_{gma}	1.06 at 98%	95 and 98%	–	–	Madelin and Johnson (1992)

		Measurements at subsaturation		Measurements at supersaturation		
Species	Diameter, D_{ve}–volume equivalent, D_{gma}. geometric mass aerodynamic, D_{mma}–mass median aerodynamic, D_a–aerodynamic	Maximum growth factor at RH	RH at which hygroscopic growth was measured	Critical supersaturation	CCN/CN ratio	References
Penicillium chrysogenum	2.6 μm D_{gma}	1.07 at 98%	95 and 98%	–	–	Madelin and Johnson (1992)
Penicillium minioluteum	1.6 μm D_{gma}	1.12 at 98%	95 and 98%	–	–	Madelin and Johnson (1992)
Scopulariopsis brevicaulis	5.1 μm D_{gma}	1.08 at 95%	95%	–	–	Madelin and Johnson (1992)
Penicillium brevicompactum	2.9 μm D_{gma}	~1.05 at 90%	30–100%	–	–	Reponen et al. (1996)
Penicillium melinii	2.4 μm D_{gma}	~1.08 at 90%	30–100%	–	–	Reponen et al. (1996)
Aspergillus versicolor	2.1 μm D_{gma}	~1.07 at 90%	30–100%	–	–	Reponen et al. (1996)
Aspergillus fumigatus	2.1 μm D_{gma}	~1.06 at 90%	30–100%	–	–	Reponen et al. (1996)
Cladosporium cladosporioides	1.8 μm D_{gma}	~1.04 at 90%	30–100%	–	–	Reponen et al. (1996)
Pollen						
various ragweed, amaranth-chenopod and grass pollens	n.a.	Mass increase by up to a factor of 2	'very dry' to 'moist'	–	–	Durham (1943)
Ambrosia artemisiifolia	20 μm	Effective density increase: 1.52 at 93–100%, no geometric growth	11–100%	–	–	Harrington and Metzger (1963)
various pollens (deciduous trees, conifers and grasses)	22 to 115 μm	Mass increase by up to a factor of 4 at 95%	73 and 95%	–	–	Diehl et al. (2001)
Daffodil, water birch and pussy willow pollens	25 μm (birch pollens)	Mass increase by up to a factor of 1.3 at 85%	2–~85%	≤0.002% (calculated)		Pope (2010)
Algal exudates (extracellular polymeric substances, EPS)						
Artificial seawater with diatomaceaous and	40–105 nm	~2.5 at 92%, lower than for	45–92%	0.1 to 0.5% for sizes between 40 and 105 nm	–	Fuentes et al. (2011)

		Measurements at subsaturation		Measurements at supersaturation		
Species	Diameter, D_{ve}–volume equivalent, D_{gma}. geometric mass aerodynamic, D_{mma}–mass median aerodynamic, D_a–aerodynamic	Maximum growth factor at RH	RH at which hygroscopic growth was measured	Critical supersaturation	CCN/CN ratio	References
nanoplancton exudates		artificial seawater devoid of exudates				
Artificial seawater with exudate of four different algal species	25–500 nm	~4 at 99%, lower than for artificial seawater devoid of exudates	75–99%	0.1 to 0.4% for diameters between 40 and 100 nm	–	Wex et al. (2010)

4.3. PBAP as ice nuclei

Cloud water droplets do not freeze directly at 0°C but can remain in a supercooled liquid state down to temperatures of approximately −38°C. At higher temperatures, aerosol particles are required as ice nuclei to initiate ice formation via heterogeneous freezing, i.e. the formation of ice germs by the aid of crystal-like structure elements or other the so-called active sites on the particle surface. If an ice nucleus (IN) is contained inside a liquid droplet when initiating freezing, the process is termed 'immersion freezing'. 'Condensation freezing' is a special case of immersion freezing, in which the CCN of a cloud droplet acts as an IN during the condensational growth phase. If a supercooled droplet collides with an aerosol particle and freezes as a result of the collision, one speaks of 'contact nucleation'. On dry particles ('deposition nucleation'), ice can also form directly from the vapour phase (Pruppacher and Klett, 1997). Freezing of a relatively small number of cloud droplets can trigger glaciation, i.e. turning a whole cloud or a region within a cloud into pure ice. Glaciation is driven by the different values of the saturation vapour pressure over supercooled liquid water and over ice, which leads to depositional growth of the ice crystals at the expense of evaporating droplets once the relative humidity falls below water saturation (Wegener-Bergeron-Findeisen process, Findeisen, 1938). This process is connected to the formation of large crystals that tend to fall out as precipitation. Furthermore, ice-multiplication processes can occur, in which additional ice crystals are produced from existing ice crystals, for example due to the formation of ice splinters during riming of ice particles (Hallett and Mossop, 1974).

In numerous studies, it has been established that mineral dust particles are relatively efficient ice nuclei. At lower temperatures, soot particles also can nucleate ice. Interestingly, the most active IN (those nucleating ice at the highest subzero temperatures) discovered so far are of biological origin. Overviews of biological ice nucleation measurements and discussions of their possible implications are given in previous reviews (Lee et al., 1995; Szyrmer and Zawadzki, 1997; Möhler et al., 2007; Delort et al., 2010; DeMott and Prenni, 2010). Here, we summarise the observational basis of biological ice nucleation seen in laboratory experiments and, as far as available, in the atmosphere, including both, historical and recent measurements. With the help of modelling studies, the observations are put into the context of typical atmospheric concentrations and atmospherically relevant processes.

4.3.1. Laboratory studies of ice nucleation active biological particles

Since the first ice-nucleating biological particles were discovered, numerous microorganisms have been screened for ice nucleation activity. A selection of results is summarised in Table 6. Listed are the highest

temperatures at which ice nucleation was observed, which are often cited as key findings of the experiments. Equally important is the fraction of ice-nucleating particles to total particles because often the apparent freezing onset depends on the particle concentration (Yankofsky et al., 1981). For many biological species, only a small fraction of the total particles (i.e. in the case of bacteria: cells) actually nucleates ice, even at relatively low temperatures. Nevertheless, the entire species or strain is referred to as 'ice nucleation active' (INA). The IN number fractions at different temperatures, as far as they are available from the experiments in Table 6, are displayed in Fig. 5.

Fig 5

Fig. 5. Ice nucleating number fraction f_{IN} at the observed IN onset and maximum activity temperatures from the experiments listed in Table 6. For comparison, f_{IN} data for immersion freezing on mineral dust (natural soil samples, median diameters of 0.2–1 μm) are included (M. Niemand, personal communication).

Table 6. Compilation of laboratory measurements of the IN properties of biological particles (n.a.=data not available)

Species	Highest T,°C where INA observed	Active number fraction or active IN per unit mass at highest INA temperature	Highest observed active fraction and corresponding temperature	Freezing mode (Immersion freezing = If, Contact freezing = Ctf, Condensation freezing = Cdf)	References
Bacteria isolated from air or precipitation					
Pseudomonas fluorescens isolated from leaves, lake/stream water and/or snow	−10	0.02	0.94 (T=−16°C)	If	Maki and Willoughby (1978)
Unidentified microbacterium isolated from air above the Arctic Ocean	−4	0.05	1 (T=−18°C)	If	Jayaweera and Flanagan (1982)
Pseudomonas sp. isolated from air above the Arctic Ocean	−9	0.1	n.a.	If	Jayaweera and Flanagan (1982)
Pseudomonas syringae isolated from rain and hail	−4	n.a.	n.a.	If	Sands et al. (1982)
Pseudomonas syringae and *Erwinia herbicola* isolated from air above plant canopies and bare soil	−10	n.a.	n.a.	If	Lindemann et al. (1982)
Pseudomonas syringae isolated from air and rainwater sampled over a soybean field	−5	n.a.	n.a.	If	Constantinidou et al. (1990)
Pseudomonas sp. isolated from cloud and rain water	−21 to −29	n.a.	n.a.	If	Ahern et al. (2007)
Pseudomonas syringae isolated from rain, snow, alpine streams, lakes and wild plants	−2 to −6	10^{-7}	n.a.	If	Morris et al. (2008)
Microbacterium, *Xanthomonas*, *Bacillus*, *Acinetobacter*, *Luteimonas*, *Stenotrophomonas* and	−13 to −18	n.a.	n.a.	If	Mortazavi et al. (2008)

Species	Highest T,°C where INA observed	Active number fraction or active IN per unit mass at highest INA temperature	Highest observed active fraction and corresponding temperature	Freezing mode (Immersion freezing = If, Contact freezing = Ctf, Condensation freezing = Cdf)	References
unspecified bacteria isolated from snow					
Bacteria isolated from air other habitats (list not exhaustive)					
Pseudomonas syringae	−5	2×10^{-6}	5×10^{-5} (T = −15°C)	If	Vali et al. (1976)
Pseudomonas syringae, different strains	−5	0.0043 to 10^{-7}	n.a.	If	Gross et al. (1983)
Pseudomonas syringae strain 31R1	−1	10^{-8}	0.5 (T = −12°C)	If	Lindow et al. (1989)
Pseudomonas syringae	−8±1	0.0032	n.a.	If/Cdf	Möhler et al. (2008)
Pseudomonas viridiflava/*Pseudomonas syringae* mixture	−9.7	0.005	n.a.	If/Cdf	Möhler et al. (2008)
Pseudomonas syringae isolated from decaying alder leaves (Alnus tenuifolia)	−3	10^{-6}	0.01(T = −20°C)	If	Maki et al. (1974)
Pseudomonas sp. isolated from the guts of sub-Antartic beetles	−3.4	10^{-6}	n.a.	If	Worland and Block (1999)
Pseudomonas Antarctica	−4	10^{-7}	0.2 (T = −10°C)	If	Obata et al. (1999)
Erwinia herbicola	−9±1	0.0007	n.a.	If/Cdf	Möhler et al. (2008)
Erwinia herbicola, cell-free centrifuged suspensions	−3	n.a.	n.a.	If	Phelps et al. (1986)
M1	−3	10^{-6}	0.01 (T = −10°C)	If	Yankofsky et al. (1981)
M1	−3	n.a.	n.a.	Ctf	Levin and Yankofsky (1983)
M1	−3	n.a.	n.a.	If	Levin and Yankofsky (1983)
Flavobacterium sp., *Psychrobacter* sp., and *Sphingomonas* sp. isolated from permafrost soil	n.a.	n.a.	4×10^{-7} (T = −10°C)	If	Ponder et al. (2005)
Snomax	−5.6	0.01	0.23 (T = −8±1°C)	If/Cdf	Möhler et al. (2008)
Snomax	−4	1.3×10^{12} g^{-1}	5.5×10^{12} g^{-1} (T = −12°C)	Cdf	Ward and DeMott (1989)
Snomax	−5.3	n.a.	n.a.	If	Wood et al. (2002)

Species	Highest T,°C where INA observed	Active number fraction or active IN per unit mass at highest INA temperature	Highest observed active fraction and corresponding temperature	Freezing mode (Immersion freezing = If, Contact freezing = Ctf, Condensation freezing = Cdf)	References
Snomax	−26, Rhi = 116±6%	0.001–0.01	n.a.	Deposition nucleation (no experiments at warmer T)	Chernoff and Bertram (2010)
INA bacteria on oat leaves	−2.5	10^{-7}	0.008 (T < −4°C)	If	Hirano et al. (1985)
several representative Arctic and Antarctic sea-ice bacterial isolates	−40 to −42	n.a.	n.a.	If	Junge and Swanson (2008)
Lichens					
Rhizoplaca chrysoleuca (the most active of 15 investigated lichen species)	−2.3	10^3 g^{-1}, grinded material	10^8 g^{-1} (T = −3C), grinded material	If	Kieft (1988)
Psora decipiens (the least active of 15 investigated lichen species)	−8	10^3 g^{-1}, grinded material	10^5 g^{-1} (T = −12 °C), grinded material	If	Kieft (1988)
18 lichen mycobionts	−4.1 to −10	n.a.	n.a.	If	Kieft and Ahmadjian (1989)
Lecanora dispersa (lichen fungus)	−4.2	~10^4 g^{-1}	~7$\times 10^7$ g^{-1} (T = −8 °C)	If	Kieft and Ahmadjian (1989)
Cladonia cristatella (lichen fungus)	−6.3	~10^6 g^{-1}	~5$\times 10^6$ g^{-1} ((T = −12 °C)	If	Kieft and Ahmadjian (1989)
Ascospora fuscata (lichen fungus)	−9.1	~2$\times 10^4$ g^{-1}	~2$\times 10^5$ g^{-1} (T = −12°C)	If	Kieft and Ahmadjian (1989)
Rhizoplaca chrysoleuca (lichen fungus), different clones	−4.6 to −4.8	10^4 to 2$\times 10^5$ g^{-1}	~2$\times 10^7$ g^{-1} (T = −12°C)	If	Kieft and Ahmadjian (1989)
13 lichen photobionts	−5.1 to −16	n.a.	n.a.	If	Kieft and Ahmadjian (1989)
Trebouxia incrustata (lichen photobiont)	−9.1	~2$\times 10^4$ g^{-1}	~6$\times 10^4$ g^{-1} (T = −12°C)	If	Kieft and Ahmadjian (1989)
Trebouxia erici (lichen photobiont)	−9.2	~6$\times 10^5$ g^{-1}	~10^6 g^{-1} (T = −12°C)	If	Kieft and Ahmadjian (1989)
Trebouxia sp. (lichen photobiont)	−6	~6$\times 10^4$ g^{-1}	~10^6 g^{-1} (T = −12°C)	If	Kieft and Ahmadjian (1989)
Unspecified lichen fragments from Norway, Faroe Islands, Ethiopia, UK, Australia, Antartica	−5.1	n.a.	n.a.	If	Henderson-Begg et al. (2009)
Fungi					

Species	Highest T,°C where INA observed	Active number fraction or active IN per unit mass at highest INA temperature	Highest observed active fraction and corresponding temperature	Freezing mode (Immersion freezing = If, Contact freezing = Ctf, Condensation freezing = Cdf)	References
Penicillium digitatum spores isolated from air	−10	0.01	n.a.	If	Jayaweera and Flanagan (1982)
Cladosporium herbarum spores isolated from air	−15	0.01	n.a.	If	Jayaweera and Flanagan (1982)
Penicillium notatum spores isolated from air	−22	0.01	n.a.	If	Jayaweera and Flanagan (1982)
Penicillium frequentes spores isolated from air	−22.5	0.01	n.a.	If	Jayaweera and Flanagan (1982)
Rhizopus stolonifera spores isolated from air	−23	0.01	n.a.	If	Jayaweera and Flanagan (1982)
Fusarium avanaceum	−2.5	10^5 g^{-1}	10^{11} g^{-1} (T = −10°C)	If	Pouleur et al. (1992)
Fusarium acuminatum	−5	n.a.	n.a.	If	Pouleur et al. (1992)
Fusarium sp. isolated from the guts of insect larvae	−5	n.a.	n.a.	If	Tsumuki et al. (1992)
Fusarium oxysporum (12 out of 42 isolates, from plants)	−1	n.a.	n.a.	If	Richard et al. (1996)
Fusarium tricinctum (8 out of 14 isolates, from plants and soil)	−1	n.a.	n.a.	If	Richard et al. (1996)
Cladosporium spores	−28.5	~0.002	0.2 to 1 (T = −35°C)	If	Iannone et al. (2011)
Pollen					
Pine pollen	−8	0.1	0.9 (T = −18°C)	Cdf	Diehl et al. (2001)
Pine pollen	−16	n.a.	n.a.	If	Diehl et al. (2002)
Pine pollen	−12	n.a.	n.a.	Ctf	Diehl et al. (2002)
Birch pollen	−8	0.04	0.98 (T = −18°C)	Cdf	Diehl et al. (2001)
Birch pollen	−10	n.a.	n.a.	If	Diehl et al. (2002)
Birch pollen	−6	n.a.	n.a.	Ctf	Diehl et al. (2002)
Oak pollen	−8	0.03	0.5 (T = −18°C)	Cdf	Diehl et al. (2001)
Oak pollen	−14	n.a.	n.a.	If	Diehl et al. (2002)

Species	Highest T,°C where INA observed	Active number fraction or active IN per unit mass at highest INA temperature	Highest observed active fraction and corresponding temperature	Freezing mode (Immersion freezing = If, Contact freezing = Ctf, Condensation freezing = Cdf)	References
Oak pollen	−10	n.a.	n.a.	Ctf	Diehl et al. (2002)
Grass pollen	−8	0.02	0.8 (T = −18°C)	Cdf	Diehl et al. (2001)
Grass pollen	−14	n.a.	n.a.	If	Diehl et al. (2002)
Grass pollen	−10	n.a.	n.a.	Ctf	Diehl et al. (2002)
Alder pollen	−10	n.a.	n.a.	If	von Blohn et al. (2005)
Alder pollen	−10	n.a.	n.a.	Ctf	von Blohn et al. (2005)
Lombardy poplar pollen	−18	n.a.	n.a.	If	von Blohn et al. (2005)
Lombardy poplar pollen	−14	n.a.	n.a.	Ctf	von Blohn et al. (2005)
Redtop grass pollen	−16	n.a.	n.a.	If	von Blohn et al. (2005)
Redtop grass pollen	−16	n.a.	n.a.	Ctf	von Blohn et al. (2005)
Kentucky blue pollen	−14	n.a.	n.a.	If	von Blohn et al. (2005)
Kentucky blue pollen	−10	n.a.	n.a.	Ctf	von Blohn et al. (2005)
Various pollen, including crushed pollen	no IN observed	–	–	Deposition nucleation	Diehl et al. (2001)
Algae					
25 algae species isolated from Antarctic soils	−5 (>-8 for 4 out of 25 species)	n.a.	n.a.	If	Worland and Lukesova (2000)
Seaweed (8 species)	−7	n.a.	n.a.	If	Lundheim (1997)
Leaf litter					
Poplar mulch	−5	$\sim 10^5$ g^{-1}	$\sim 5 \cdot 10^9$ g^{-1} (T = −15°C)	If	Schnell and Vali (1972)
Sage leaf litter	−6	$\sim 10^3$ g^{-1}	$\sim 10^7$ g^{-1} (T = −17°C)	If	Schnell and Vali (1972)
Green poplar leaves	−9	$\sim 2 \cdot 10^2$ g^{-1}	$\sim 2 \cdot 10^4$ g^{-1} (T = −17°C)	If	Schnell and Vali (1972)
Leaf litter of several trees and grasses in tropical climate zones	−7	$\sim 10^2$ g^{-1}	$\sim 4 \cdot 10^4$ g^{-1} (T = −18°C)	If	Schnell and Vali (1976)

Species	Highest T,°C where INA observed	Active number fraction or active IN per unit mass at highest INA temperature	Highest observed active fraction and corresponding temperature	Freezing mode (Immersion freezing = If, Contact freezing = Ctf, Condensation freezing = Cdf)	References
Leaf litter of several trees and grasses in humid mesothermal climate zones	−6	~10^2 g^{-1}	~4 10^8 g^{-1} (T = −23°C)	If	Schnell and Vali (1976)
Leaf litter of several trees and grasses in humid microthermal climate zones	−4	~10^2 g^{-1}	~4 10^{10} g^{-1} (T = −22°C)	If	Schnell and Vali (1976)
Tea leaf litter	−5	10^2 g^{-1}	~5 10^4 g^{-1} (T = −12°C)	If	Schnell and Tan-Schnell (1982)
Plankton					
Cachonina Niei	−3	10^2 g^{-1}	~1 (T = −14°C); 10^6 g^{-1} (T = −10°C)	If	Schnell (1975)
Ochromonus danica and *Porphyridium aerugineum*	>−15	n.a.	n.a.	If	Schnell (1975)
Unspecified mixture of 95% phytoplankton, 5% zooplankton and associated debris	−3.5	10^2 g^{-1}	10^6 g^{-1} (T = −10°C)	If	Schnell and Vali (1975)

Many studies listed in Table 6 used biological particles sampled not from the atmosphere, but from plants, lichens, soils, ocean water and insect guts. Some of them were cultivated under favourable conditions being tested for IN activity. It can thus be questioned whether the investigated samples are representative of biological aerosols. Most studies employ the so-called droplet freezing assay with rather large volumes (several µl) of particle suspensions (thus testing for immersion freezing). Only a few experiments employ atmospherically relevant droplet sizes (e.g. Möhler et al., 2008) or test for other nucleation modes (e.g. Levin and Yankofsky, 1983).

Among bacterial ice nucleators, *Pseudomonas syringae* is the most common. Other INA species include *Pseudomonas fluorescens* and *Erwinia herbicola*. In general, not all strains within a species are INA, and also within the INA strains, the fraction of ice-nucleating cells varies significantly (Hirano and Upper, 1995). Active number fractions between 10^{-8} and close to 1 are reported, and the onset of ice nucleation can be seen at temperatures between −2 and −10°C Table 6 and Fig. 5. Species with intermediate IN activity begin ice nucleation only at lower temperatures (e.g. Mortazavi et al., 2008).

A number of fungi (both free living and lichen fungi) were found to nucleate ice at temperatures comparable to INA bacteria Table 6, some even at −1°C. Fungal spores, which are more likely to become airborne than other parts of the fungal thallus, have been observed to nucleate ice at lower temperatures (−10 to −28.5°C, Jayaweera and Flanagan, 1982; Iannone et al., 2011). Lichen photobionts (algae or cyanobacteria) are in general less efficient IN than the corresponding lichen fungi (Kieft and Ahmadjian, 1989).

Pollen of various plant species have been shown by a number of studies (Diehl et al., 2001, 2002; von Blohn et al., 2005) to nucleate ice starting at temperatures around −10°C Table 6. Very high active fractions (up to 1.0) can be reached at −18°C (Diehl et al., 2001). Other biological materials, such as algae, leaf litter and plankton initiate freezing at temperatures comparable to bacteria and fungi. IN numbers are usually given in relation to the mass of the bulk material, which is of only limited relevance for atmospheric applications, where particle surface area and number fractions matter.

Also shown in Fig. 5 are ice nucleating number fractions for mineral dust particles of atmospherically relevant sizes (median diameters of 0.2–1 µm) in the immersion freezing mode (Niemand, personal communication). The

observed ice nucleating fractions for dust range from about 10^{-4} to 10^{-2} at temperatures between −15 and −28°C. Larger dust particles (not shown) exhibit larger ice-nucleating number fractions because ice nucleation is proportional to the particle surface area. Conen et al. (2011) showed that soil with high organic content exhibits higher IN activity than montmorillonite and postulate that ice active proteins in the soil from fragments of decaying biological material are likely the cause. The atmospheric importance of different INA materials depends not only on their ice-nucleating fractions that are shown in Fig. 5 but also on their number concentrations at cloud altitudes and the prevailing environmental conditions (e.g., temperature and humidity).

4.3.2. Observation of biological ice nuclei in the atmosphere

The plant pathogenic bacteria *Pseudomonas* are frequent in the biosphere, and *Pseudomonas* bacteria are also frequently found as airborne particles or in cloud droplets (e.g. Fuzzi et al., 1997; Amato et al., 2005, 2007a, 2007c). However, in these studies, the ice nucleation activity of the isolated *Pseudomonas* strains was not determined. Little is known about the atmospheric abundance of INA lichen and fungal spores. Pollen, for which ice nucleation activity seems to be a common property of many species, can reach peak concentrations on the order of several per litre during the pollination season (Vogel et al., 2008).

Direct observations of the involvement of biological particles in cloud ice and precipitation formation are difficult to obtain. The presence (or absence) of INA biological particles in the air, cloud condensate or precipitation can give some indications on their role in the atmosphere. Such observations are discussed in the following section.

Kumai (1961) found indirect evidence of the possible involvement of biological particles in atmospheric ice nucleation by microscopically identifying bacteria at the centre of 3 out of 307 examined snow crystals. In the pioneering studies by Maki and Willoughby (1978) and Sands et al. (1982), ice-nucleating bacteria were isolated from rain and snow. Some were even sampled at altitudes up to 2500 m above ground (Sands et al., 1982). Jayaweera and Flanagan (1982) and Lindemann et al. (1982) were the first to determine the ice nucleation activity of biological particles sampled directly from the atmosphere. In the Arctic, two types of bacteria (an unidentified strain and a *Pseudomonas* strain) and five fungal spores were observed with moderate to high ice nucleation activity (Jayaweera and Flanagan, 1982). Lindemann et al. (1982) found significant concentrations of INA bacteria at 10°C above different plant canopies and bare soil and identified them as *Pseudomonas syringae* and *Erwinia herbicola*. The INA bacteria accounted for 0–4% of the total bacteria. Constantinidou et al. (1990) isolated *Pseudomonas syringae* from rainwater and aerosol samples over a soy bean field and tested them as INA at −5°C. Recently, Morris et al. (2008) studied *Pseudomonas syringae* strains isolated from rain, snow, alpine streams, lakes and wild plants and found that all the strains isolated from snow showed ice nucleation activity at −2 to −5°C, while this property was rare among the other strains. However, other samples of bacteria isolated from precipitation exhibited only moderate ice nucleation activity (Ahern et al., 2007; Mortazavi et al., 2008).

Christner et al. (2008a, b) demonstrated that snow and rain samples from around the world contain ice nuclei active at temperatures above −10°C. A majority of these ice nuclei was found to be inactivated through heat treatment (at 95°C) and/or lysozyme digestion, indicating a probable bacterial and/or proteinaceous origin. The observed concentrations of warm-temperature ice nuclei ranged between below 10 and several hundred per litre of snow/rain water, which is much lower than the estimated numbers of hydrometers per litre precipitation (Diehl and Wurzler, 2010; Hoose et al., 2010a). Ice nuclei sampled from the urban atmosphere (Henderson-Begg et al., 2009), which were also active at temperatures above −10°C, were not deactivated by lysozyme treatment and heating to 60 °C (but by heating to 90 °C). This was interpreted as an indication that these ice nuclei were not bacterial, but rather from lichen or fungi. Also, ice nuclei concentrations at 10°C in air and snow at a high-elevation mountain site did not correlate with the relative abundances of well-known INA bacteria species such as *Pseudomonas syringae* and *Erwinia herbicola* (Bowers et al., 2009). It was suggested that ice nucleation at this site was due to other bacterial species, varying expression of the bacterial ice-nucleation capacities, or other biological particles.

It can be expected that biological ice nuclei are particularly abundant in regions with active vegetation. Prenni et al. (2009) investigated aerosol particles sampled above the canopy in the Amazon forest and inferred that the majority of the ice nuclei at −25°C and warmer were of biological origin (but absolute concentrations of ice nuclei at these temperatures were low, on the order of 1–2 l^{-1}). Ice nuclei at colder temperatures were in large

part contributed by mineral dust. At cirrus cloud altitude, Pratt et al. (2009) observed a case of co-occurrence of biological material with mineral dust after long-range transport: From mass-spectroscopic measurements of ice crystal residues in wave clouds, 50% mineral dust particles and 33% biological particles were identified. But, only in one out of many research flights was a measurable fraction of biological material observed.

In summary, the presently available observations do not give a consistent picture. The strongest indications of ice-nucleation activity in clouds are available for bacterial or fungal ice nucleators. Quantitative measurements are unfortunately rare and cover only anecdotal events. In particular, more studies quantifying the atmospheric concentrations of INA biological particles in comparison to other ice-nucleating agents, such as mineral dust and soot, would be desirable.

4.3.3. Modelling of biological ice nuclei

Numerical simulations of the effects biological ice nuclei in clouds began with Levin et al. (1987), who studied the efficiency of cloud seeding with INA bacteria for precipitation enhancement in a 1.5-dimensional cloud model. Later, Diehl and Wurzler (2004); Diehl et al. (2006) parameterised freezing of droplets containing or colliding with unknown amounts of different biological ice nucleators for a parcel model with detailed microphysics. In a follow-up study (Diehl and Wurzler, 2010), the freezing rates were scaled with typical fractions of droplets containing bacteria, mineral dust and soot particles, respectively, and it was shown that typical bacteria concentrations yielded much lower ice crystal concentrations than typical mineral dust concentrations. Ariya et al. (2009), in studies with a 1.5-dimensional model of a convective cloud, and employing INA biological aerosol concentrations of less than 1 l^{-1}, pointed out that even such low IN concentrations can trigger cloud glaciation through ice-multiplication processes. The comprehensive empirical ice nucleation parameterisation by Phillips et al. (2008) includes biological material as part of the 'insoluble organic' particles. In a cloud-system resolving model, cloud micro- and macrophysical properties, including precipitation, were sensitive to variations of the 'insoluble organic' aerosol by a factor of 100 (Phillips et al., 2009). The first global model studies of the impact of biological particles by Hoose et al. (2010a), Hoose et al. (2010b) used simple emission parameterisations for bacteria, fungal spores and pollens to simulate their concentration in the atmosphere. The freezing rates were derived from classical nucleation theory and laboratory data (Chen et al., 2008a). The average contribution of biological particles to heterogeneous ice nucleation in mixed-phase clouds (integrated over all temperature regions in the troposphere) was calculated to be only 0.00001%, with an uppermost estimate of 0.6%. Instead, atmospheric ice nucleation was found to be dominated by mineral dust, which is also in agreement with recent observations (Choi et al., 2010; 2010; DeMott et al., 2010; Klein et al., 2010). The contribution of biological particles to ice nucleation in relatively warm cloud layers (above −10°C) might be higher but was not quantified in these studies.

In summary, the effect of biological particles as ice nuclei on clouds and precipitation is strongly dependent on the atmospheric concentrations of INA species. Most likely, these concentrations exhibit strong temporal and spatial variations, and any effect on clouds is therefore likely seasonal and local in nature. However, more quantitative observations of INA biological particles in the atmosphere are necessary to allow better estimates of their effects on clouds.

4.4. Optical properties

The absorption and scattering of radiation by aerosol particles are important physical properties that influence regional and global radiation budgets. Better understanding of the effect that natural aerosols have on the atmosphere is necessary to constrain effects that anthropogenic influence may have on global climate. Because PBAP can be a major fraction of aerosol number and surface area in certain locations, it is possible that they may also affect climate forcing both directly (by absorbing or scattering radiation) and indirectly (through cloud processes). Certain fungal spores and other PBAP classes can be highly coloured and absorbing, which may increase their direct influence on the surrounding atmosphere (e.g. Adams et al., 1968; Troutt and Levetin, 2001). However, there have been very few studies estimating the direct effect of PBAP on climate, in part because geographically or temporally comprehensive PBAP measurements are not yet available. A theoretical description of the interaction between electromagnetic radiation and PBAP is difficult because the Mie theory is only valid for spheres, and thus many biological aerosol particles cannot be well described (Bohren and Huffman, 1983).

Some work has been done to directly measure certain optical properties of PBAP that could be relevant to the investigation of atmospheric aerosol. Spankuch et al. (2000) showed that down-welling infrared flux was significantly increased when pine pollen concentrations were higher, suggesting that emissions of certain pollens may cause localised atmospheric warming events. Several groups have shown that pollen can cause visible coronae around the sun and moon and could therefore influence local solar radiation properties (Parviainen et al., 1994; Trankle and Mielke, 1994; Mims, 1998; Schneider and Vollmer, 2005).

Most work involving the optical properties of PBAP has used detailed physical measurements in the laboratory and, as such, has only limited obvious application to the atmosphere at large. Surbek et al. (2009) characterised the elastic light scattering of a number of pollen species. Several groups have made detailed measurements of the polarisation and scattering properties specific to bacteria and other biological aerosol types (Bickel et al., 1976 and references therein; Bohren and Huffman, 1983; Vandemerwe et al., 1989). Gurton et al. (2001) measured infrared extinction by bacterial spores. Harding and Johnson (1984) measured quasi-elastic light scattering, and Gittins et al. (1999) measured infrared absorption of *Bacillus subtilis* bacteria. Yabushita and Wada (1985) performed IR and UV absorption measurements of *E. coli* and yeast organisms.

In view of the much higher abundance of mineral dust discussed by Hoose et al. (2010b), the influence of biological material on aerosol optical properties may be relatively small on global scales. However, on regional scales PBAP may sometimes have a substantial influence on the total scattering and absorption of light by aerosols. Using chemical tracers and multivariate statistical analysis, Guyon et al. (2004) showed that up to 66% of aerosol mass and 47% of the light absorption in air over the Amazon Rainforest were attributable to biogenic particles during the wet season. During the wet-to-dry transition period, biogenic particles still accounted for up to 35% of light absorption, even though there was already a substantial amount of biomass burning aerosol present. These results are consistent with many earlier measurements (e.g. Artaxo et al., 1988, 1998), which showed that biogenic aerosol makes up a large fraction of the Amazonian aerosol mass burden, especially in the coarse fraction. In the absence of definitive identification, earlier studies assumed that it consisted mostly of PBAP (Andreae and Crutzen, 1997). This was confirmed with less uncertainty using multiple techniques by Pöschl et al. (2010), who showed that PBAP accounted for ~67% of the aerosol mass (or volume) of all particle sizes sampled above the rainforest in a remote section of Amazonia, Brazil during a time period of very low influence from airborne mineral dust or anthropogenic pollution. In addition, biological organisms or material may often be attached to mineral dust particles and might also influence the optical properties of these combined particles. Thus, PBAP need to be considered when modelling local and regional optical properties above and downwind of biologically active regions. Additional direct atmospheric measurements of light absorption and scattering by biogenic particles will likely be necessary to help provide suitable model inputs and such measurements are strongly recommended.

5. Conclusions and outlook

Scientific investigations of biological aerosol particles in the atmosphere have a long history going back to the nineteenth century. Since then, the topic has attracted attention in various different research areas. In the last decade, there has been a surge in interest within the atmospheric community based partly on the development of new measurement techniques and on studies indicating that PBAP may play an important role as ice nuclei influencing the formation of clouds and precipitation.

Depending on the character of the biological particle of interest, as well as on the scope of the scientific questions asked, manifold methods are available to study PBAP. Thus, we have included an overview of current methods for sampling and analysis of biological aerosols including traditional and modern techniques.

Further studies of biological aerosols will lead to a better understanding of their role in climate and atmospheric processes and will also help to improve understanding of their impacts on humans. Recently developed and emerging techniques for sampling and analysing airborne biological particles have bolstered efforts to understand the properties of ambient PBAP. However, for better comparison among different datasets, sampling and analysis techniques have to be standardised. Efficient and reliable analytical techniques must be developed for the identification and quantification of PBAP as well as for the determination of the abundance and diversity of PBAP and their seasonal variation on regional and global scales (atmospheric biogeography).

One of the main influences of PBAP on climate and atmosphere is through the capability of certain PBAP to function as excellent ice nuclei. Future research should thus concentrate on the determination of actual emission

rates and optical properties of PBAP and to link results from laboratory experiments concerning the IN ability of PBAP to atmospheric measurements.

Global modelling is one of the rising methods to track and understand the worldwide global distribution of PBAP. Atmospheric models can estimate the atmospheric effects of IN active biological aerosol particles on clouds and help identify targets for experimental research. However, the models need to be constrained and confirmed by experimental data. Future research should use field and laboratory data to better constrain numerical models of PBAP sources, their transformation in the atmosphere and effects on climate. An important task is the delivery of larger and more comprehensive datasets from sampling sites worldwide and their incorporation into atmospheric models, to ground model efforts in a solid empirical foundation.

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7. Appendix

AMS	Aerodyne Mass Spectrometer
ATOFMS	TSI Aerosol Time-of-Flight Mass Spectrometer
ATP	Adenosine Triphosphate
BAMS	Bioaerosol Mass Spectrometry
Bp	Base Pairs
BS	Break-Down Spectroscopy
CCN	Cloud Condensation Nuclei
CFU	Colony Forming Units
DAPI	4.6-diamidino-2-phenylindole
ddNTP	dideoxynucleotidetriphophate
DNA	Deoxyribonucleic Acid
dNTP	deoxynucleotidetriphosphate
EPS	Exopolymer Secretions
FBAP	Fluorescent Biological Aerosol Particles
FISH	Fluorescent in-situ hybridization
GF	Growth Factor
Hulis	Humic Like Substances
IN	Ice Nuclei
INA	Ice Nucleation Active
IPCC	Intergovernmental panel on climate change
ISI	Institute for Scientific Information
ITS	Internal Transcribed Spacer
LDD	Long Distance Dispersal
LIBS	Laser-Induced Breakdown Spectroscopy
LIDAR	LIght Detection And Ranging
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization Time-of-Flight
MS	Mass Spectrometry
NCBI	National Center for Biotechnology Information
OC	Organic Carbon
PBAP	Primary Biological Aerosol Particles

PCR	Polymerase Chain Reaction
PIXE	Particle-Induced x-ray Emission
PM	Patriculate Matter
RH	Relative Humidity
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
SEM	Scanning Electron Microscopy
SIBS	Spark-Induced Breakdown Spectroscopy
SOA	Secondary Organic Aerosol
STXM	Scanning Transmission X-ray Microscopy with Near-Edge X-ray Absorption Fine Structure
TIRFM	Total Internal Reflection Fluorescence Microscopy
T-RFLP	Terminal Restriction Fragment Length Polymorphism
TSP	Total Suspended Particles
UV	Ultraviolet Light
UV-APS	Ultraviolet Aerodynamic Particle Sizer
WIBS	Wide Issue Bioaerosol Spectrometer

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Review

Biodiversity and biogeography of the atmosphere

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The variation of life has predominantly been studied on land and in water, but this focus is changing. There is a resurging interest in the distribution of life in the atmosphere and the processes that underlie patterns in this distribution. Here, we review our current state of knowledge about the biodiversity and biogeography of the atmosphere, with an emphasis on micro-organisms, the numerically dominant forms of aerial life. We present evidence to suggest that the atmosphere is a habitat for micro-organisms, and not purely a conduit for terrestrial and aquatic life. Building on a rich history of research in terrestrial and aquatic systems, we explore biodiversity patterns that are likely to play an important role in the emerging field of air biogeography. We discuss the possibility of a more unified understanding of the biosphere, one that links knowledge about biodiversity and biogeography in the lithosphere, hydrosphere and atmosphere.

Keywords: biogeography; biodiversity; micro-organism; air; atmosphere

> *Noi viviamo sommersi nel fondo d'un pelago d'aria*. We live submerged at the bottom of an ocean of air.
> (Evangelista Torricelli 1644 quoted in Middleton 1963)

1. INTRODUCTION

As humans, we have an intimate relationship with the air around us. This relationship is by and large unconscious; we breathe in without thinking, move through the eddies and tides of air often without notice. This largely unconscious relationship has led to a delayed appreciation of the air as a biological entity. But air is as alive as soil or water. Not only does it host large macroscopic organisms such as a soaring hawk or a drifting wildflower seed, but it also hosts a wide variety of micro-organisms. Hundreds of thousands of individual microbial cells can exist in a cubic metre of air (Burrows *et al.* 2009*b*), representing perhaps hundreds of unique taxa (Brodie *et al.* 2007; Fierer *et al.* 2008; Bowers *et al.* 2009). The ecology of these organisms—their diversity, distribution and interactions—is poorly understood. Given our intimate relationship with air, this lack of knowledge comes at a great cost. The life of the air, especially the microbial life, is in constant interaction with human life, both directly as a source of pathogenic and beneficial microbes (Kellogg & Griffin 2006) and indirectly through biological effects on atmospheric processes (Deguillaume *et al.* 2008). The atmosphere—the layers of air surrounding the Earth—has been described as 'one of the last frontiers of biological exploration on Earth' (Rothschild & Mancinelli 2001).

In this paper, we summarize our current state of knowledge of the ecology of the atmosphere, with an emphasis on the atmosphere's *biogeography*. Biogeography is the study of patterns in the distribution of life and the processes that underlie these patterns (Lomolino *et al.* 2006). The air has long been recognized as an important conduit for the movement of organisms from one geographical location to another, and thus is important for the biogeography of land and water. However, it is commonly assumed that the atmosphere is not a habitat in its own right but merely a conveyance for terrestrial and aquatic life. We review evidence that challenges this assumption and suggests the existence of metabolically active and actively reproducing organisms in the atmosphere. We argue that the atmosphere has a biogeography of its own. Our discussion will focus on micro-organisms, the numerically dominant forms of life in the atmosphere.

2. A BRIEF HISTORY OF AEROBIOLOGY

Aerobiology has captivated scientists for centuries. Antoni van Leeuwenhoek—commonly known as the founder of microbiology—was one of the first to ask whether the air could be a habitat for micro-organisms (Gregory 1971), observing that 'there may be living creatures in the air, which are so small as to escape our sight' (van Leeuwenhoek 1941). Charles Darwin collected airborne dust on the HMS Beagle; this dust was found to contain 17 'different organic forms' of micro-organisms (Darwin 1846). Micro-organisms have recently been isolated from these samples, demonstrating the ability of some airborne

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microbes to remain viable after long periods of time (Gorbushina *et al.* 2007). Darwin's contemporary Louis Pasteur, one of the first to systemically study airborne micro-organisms, showed that there are viable bacteria and moulds in the air, and that the densities of these organisms vary from location to location (Pasteur 1861).

When flight in fixed-wing aircraft became possible in the early 1900s, interest in aerobiology took wing as well. Phytopathlogist Fred C. Meier was perhaps the most enthusiastic proponent of studying microbes at high altitudes. Meier was adept at creating sampling devices and recruiting others to participate in his studies. Charles and Anne Lindbergh collected fungal spore samples for Meier using his 'sky-hook' device during a flight over the Arctic from North Haven, Maine to Copenhagen, Denmark (Meier & Lindbergh 1935). Amelia Earhart took Meier's collection device with her during her attempt to circumnavigate the globe. According to Meier, Earhart's collections, had she not perished during her voyage, would have been an 'invaluable' sample set that spanned the circumference of the globe over massive bodies of water where little sampling had been previously conducted (Montague 1937).

Despite this long and rich history of study, we know very little about the biology of the atmosphere relative to aquatic and terrestrial habitats. Technical limitations have hindered the study of the air. Low densities of micro-organisms in the air can make even sensitive molecular analysis difficult because of the small amount of biological material present in the air. Additionally, the lack of standardization in air collection and sample-processing methods complicate comparisons across studies (Kuske 2006; Peccia & Hernandez 2006). Owing to this lack of methodological standardization, it is unclear whether large differences in density estimates among studies can be attributed to biological variation (reviewed in Peccia & Hernandez 2006; Burrows *et al.* 2009*b*). Conceptual limitations also continue to impede the advancement of our understanding of life in the atmosphere. Most of what is known about airborne micro-organisms is based on the assumption that the atmosphere is a conduit for the dispersal of microbes rather than a dynamic habitat where micro-organisms actively metabolize and reproduce. Characterizing the role of biological processes in the atmosphere has enormous implications for furthering our understanding in a number of disciplines, from atmospheric chemistry and meteorology to biodiversity and biogeography.

3. AN ATMOSPHERIC HABITAT FOR MICRO-ORGANISMS

In the atmosphere, micro-organisms may belong to one of three groups—those that are not metabolically active, those that are metabolically active but rarely reproduce and those that are both metabolically active and actively reproducing. Microbes can form inactive propagules (e.g. spores) that disseminate through the atmosphere; however, for these organisms, the atmosphere would not be a 'habitat' in the conventional sense. We suggest that microbes that remain metabolically active in the atmosphere but rarely reproduce are organisms for which the atmosphere serves only as an accidental dispersal mechanism. The last group—both metabolically active and reproducing—can be thought of as 'residents' of the atmosphere. We argue below that, despite past assumptions, residents of the atmosphere are likely to exist, and that the atmosphere can act as a habitat for microbial life. We rely on four sources of information to make these arguments: that large portions of the atmosphere have environmental characteristics consistent with other microbial habitats; that biogeochemical cycling (probably mediated by microbes) occurs in the atmosphere; that at least some microbes found in the atmosphere are metabolically active; and that residence times of microbes in the atmosphere are long enough that actively reproducing residents could exist.

(a) *The atmosphere is not the most extreme microbial habitat*

By several measures (pH, temperature, ultraviolet (UV) radiation, resource and water availability), the atmosphere appears to be less extreme than many other microbial habitats. The pH of clouds and rainwater ranges from 3 to 7 (Warneck 1988), a narrower range than that found in many microbial habitats. Microbes have adapted to a much wider range of pH conditions that occur in air, from highly acidic conditions near pH 0 (Schleper *et al.* 1995) to extremely alkaline conditions up to pH 11 (Jones *et al.* 1998).

Temperature can vary widely throughout the atmosphere, but includes ranges that are suitable for microbial life. In the lower atmosphere (up to 20 km above the Earth's surface), average temperatures decrease with altitude and range from an average of 15°C (at sea level) to −56°C (at 20 km) (NOAA NASA US Air Force 1976). Many micro-organisms are capable of growth at temperatures near and below 0°C (Morita 1975), with some communities reported to be metabolically active at temperatures as low as −18°C (Rothschild & Mancinelli 2001).

As with temperature, UV radiation, including DNA-damaging UVB, increases with altitude (Blumthaler *et al.* 1992). Increased UV radiation at higher altitudes does not necessarily mean that airborne micro-organisms are exposed to more UV radiation than their terrestrial counterparts, especially those terrestrial organisms that live at high elevations. Micro-organisms in the atmosphere may have a variety of methods for protection from UV radiation in addition to the suite of DNA-repair mechanisms found in all micro-organisms (Witkin 1976). It has been suggested that airborne microbes may mitigate levels of UV exposure by being embedded within larger particles with UV-attenuating properties, such as dust, pollen or water droplets (Lighthart 1997; Pearce *et al.* 2009). Pigments may also protect microbes from UV; the occurrence of pigmented micro-organisms in the atmosphere has been correlated with the presence of high levels of solar radiation (Tong & Lighthart 1997). These protective

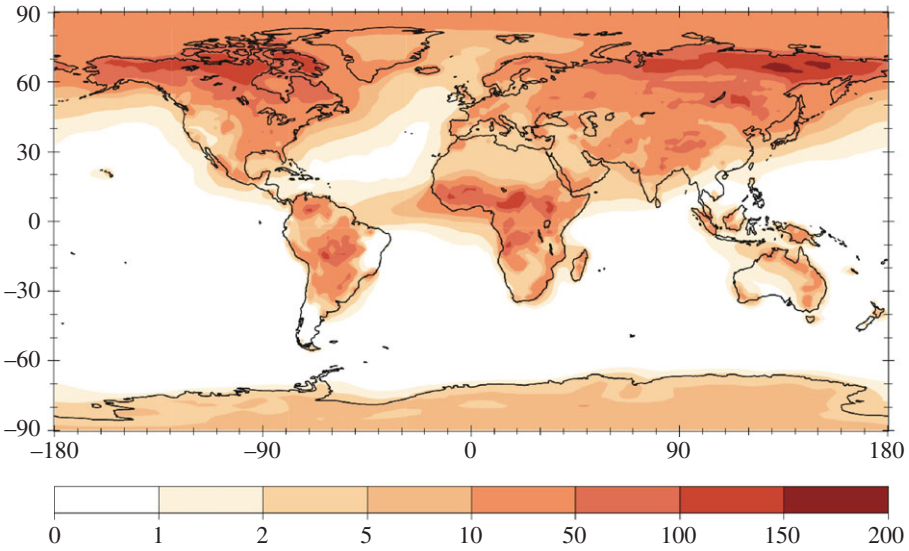

Figure 1. Simulated concentration ($10^3\ m^{-3}$) of 1 μm bacteria in near-surface air based on an adjusted general circulation model (Burrows *et al.* 2009*a*).

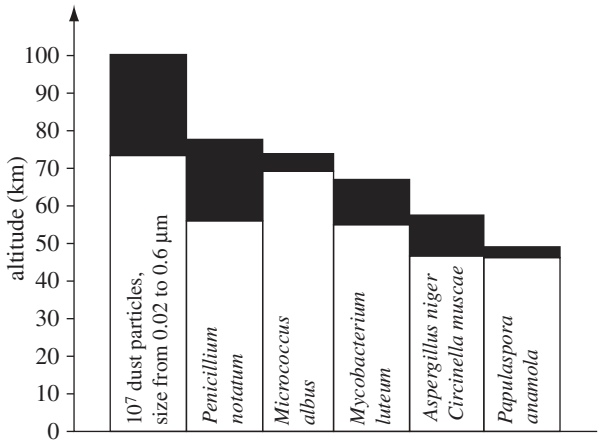

Figure 2. Isolation of microbes from the atmosphere. Shaded portions of the columns correspond to the altitude from which the organisms were sampled by a meteorological rocket and isolated in the laboratory. The first column depicts the altitude at which dust particles were sampled and detected (adapted from Imshenetsky *et al.* (1978)).

mechanisms are especially important for the survival of organisms at the upper level of the stratosphere, where levels of mutagenic UVB and UVC are not attenuated by the ozone layer (Smith *et al.* 1992).

Resource availability in the atmosphere is not necessarily lower than that of many terrestrial or aquatic environments. In clouds and rainwater, concentrations of nutrients (e.g. sulphate and nitrate) reach levels typical of oligotrophic lakes (Pearce *et al.* 2009). Numerous potential carbon sources are found in both clouds and the atmosphere, including carboxylic acids and alcohols (at concentrations up to $1\ mg\ l^{-1}$; Pearce *et al.* 2009) as well as a variety of hydrocarbons (at concentrations up to $4\ ng\ l^{-1}$; Warneck 1988). In addition to available resources for supporting heterotrophic metabolisms, the air provides a suitable habitat for phototrophs. Pigmented micro-organisms found in the atmosphere could be using pigments for photosynthesis. Gene sequences from putative photoautotrophs have been amplified from air samples (Brodie *et al.* 2007), although to our knowledge, no photoautotrophs have been isolated from the atmosphere.

(b) *Microbes in air are metabolically active*

Direct *in situ* evidence of microbial metabolic activity in the atmosphere is rare and limited primarily to approaches that require culturing of microbes in the laboratory. For example, bacteria aerosolized in the laboratory have been shown to be capable of metabolizing glucose (Dimmick *et al.* 1975) and dividing (Dimmick *et al.* 1979), suggesting that aerosolization is not a barrier to metabolic activity and reproduction. Sattler *et al.* (2001) showed that micro-organisms incubated in cloud water at 0° have generation times of 3.6–19.5 days and take up labelled substrates at rates typical of bacteria in lake water. Micro-organisms isolated from cloud water degrade organic acids when cultured in artificial cloud water at 5°C and 17°C (Vaïtilingom *et al.* 2010).

These approaches have significant limitations. The environmental conditions microbes are exposed to in clouds (e.g. temperatures of −15°C in super-cooled droplets) cannot be easily reproduced in the laboratory. Culturing aerosolized microbes in the laboratory is likely to impose a bias and may not be representative of the airborne community (Pace 1997). A few studies have avoided these biases by using culture-independent methods for detecting metabolic activity. For example, Hill *et al.* (2007) observed that 76 per cent of cells in cloud water reduced the dye CTC (5-cyano-2,3-ditolyl tetrazolium), suggesting that this proportion was metabolically active. Measurements of ATP concentrations in cloud water approximate what would be expected for metabolically active cells at the cell density at which they are found in clouds (Amato *et al.* 2007*b*), suggesting that microbes can be metabolically active in the atmosphere.

(c) *Biogeochemical cycling may occur in the atmosphere*

If metabolically active microbes are present in the atmosphere, they should leave chemical 'footprints' of their metabolisms. For example, microbes are intimately involved in biogeochemical transformations, and evidence for such transformations in the atmosphere would support the hypothesis of a resident microbiota. Nitrogen cycling in clouds (including mineralization and nitrification) has been demonstrated (Hill *et al.* 2007), suggesting the presence of metabolically active microbes. There is some evidence for carbon cycling in clouds, although it is not as clear-cut as the case for nitrogen. For example, bacteria have been isolated from clouds that are able to use organic compounds commonly found in cloud water, including acetate, formate, succinate, L-lactate, formaldehyde and methanol as carbon sources (Amato *et al.* 2007*a*; Vaïtilingom *et al.* 2010). Bacterial end products of these metabolic reactions are also commonly found in cloud water (Amato *et al.* 2007*a*), suggesting that these microbes are actively transforming these compounds in clouds. Microbial degradation of organic compounds in the atmosphere may not be limited to cloud aerosols. Bacteria have been collected outside of clouds that can degrade a variety of dicarboxylic acids, producing end products that can be further transformed in the atmosphere (Ariya *et al.* 2002).

(d) *Microbes likely go through multiple generations of growth in the atmosphere*

The studies described above support the idea that the atmosphere is an environment capable of supporting resident (i.e. metabolically active and actively reproducing) microbial communities. The environmental stressors imposed by an aerial habitat are not unique, and there are multiple examples of micro-organisms that have adapted to live under conditions more extreme than those found in the atmosphere. If environmental conditions are not likely to prevent the presence of resident microbes in the atmosphere (at least in the lower atmosphere), what else might prevent their presence? It has been suggested (Sattler *et al.* 2001; Burrows *et al.* 2009*b*) that residence time may be the largest limiting factor for resident microbial communities in the atmosphere.

Residence times of microbes probably vary as a function of the size of the particles they are associated with, and as a function of air temperature and relative humidity, among other factors (Williams *et al.* 2002; Burrows *et al.* 2009*b*; Pearce *et al.* 2009). There have been no direct estimates of microbial residence times in the atmosphere. Currently, the best estimates of residence times are derived from mathematical models of particle transport (Williams *et al.* 2002; Burrows *et al.* 2009*a*). The most recent estimates of residence times for bacteria-sized particles range from 2.2 to 188.1 days (Burrows *et al.* 2009*a*). The shorter estimates assume efficient removal of bacteria by rain, ice and snow; the longer estimates do not assume this. Is this sufficient time for a resident microbiota to develop (i.e. to complete one or more generations)? Microbes have been shown to have generation times as short as 20 min, under ideal conditions, but under the conditions present in the atmosphere (cold and nutrient-poor), microbial generation times are likely to be substantially longer. As discussed above, Sattler *et al.* (2001) measured generation times of microbes in cloud water, and reported generation times of 3.6–19.5 days, similar to the generation times of microbes in cold, oligotrophic Arctic lakes (Panzenboeck *et al.* 2000). These rough estimates of residence and generation times suggest that at least some microbes could be undergoing more than 50 generations of growth while in the atmosphere.

4. WHAT WE KNOW ABOUT AIR BIOGEOGRAPHY

Viewing the air as a microbial habitat has the potential to radically expand the scope of biodiversity and biogeography research. Biogeography has historically focused on understanding biological variation across the surface of the Earth, and has thus been primarily limited to the study of aquatic and terrestrial ecosystems. Understanding biological variation in aerial ecosystems opens the possibility for a truly unified view of biogeography, one that links biodiversity across each component of the biosphere: the lithosphere, hydrosphere and atmosphere. A preliminary picture of microbial life in aerial ecosystems is just beginning to emerge.

(a) *Density patterns for airborne microbes*

The vast majority of aerobiology studies report patterns in the density (i.e. concentration) of micro-organisms (reviewed in Burrows *et al.* 2009*b*). Although the quantification of total, community-level abundance has a rich history in microbiology (Whitman *et al.* 1998), plant and animal surveys rarely report patterns in community-level abundance. This difference may reflect the reality that researchers commonly document what is most tractable to measure.

Aerobiologists have historically measured the density of culturable micro-organisms, reporting the number of colony-forming units per volume of air sampled (CFU m^{-3}). Culture-based studies suggest that, as in terrestrial and aquatic systems, microbial densities vary with space, time and environmental conditions in the air. For example, the density of culturable microbes has been shown to decrease with increasing altitude (Fulton 1966), and numerous studies have documented seasonal and diurnal temporal variation in the density of culturable micro-organisms in the atmosphere (Bovallius *et al.* 1978; Lindemann & Upper 1985; Lighthart & Shaffer 1995; Tong & Lighthart 1999, 2000; Fang *et al.* 2007). Culture techniques, however, reveal only a fraction of microbial life. More recent studies use epifluorescent microscopy and report the total count of microbial cells per volume of air sampled (cells m^{-3}). There is some evidence that total cell density counts from microscopy parallel culture-based counts (Tong & Lighthart 1999, 2000); however, few studies have enumerated airborne microbial densities using both approaches, making comparative inferences problematic. The density of micro-organisms in the atmosphere has also been

estimated using particle transport models (Burrows *et al.* 2009*a*). Modelling approaches suggest that atmospheric cell density varies spatially, and that patterns in airborne cell density can occur on a global scale (figure 1).

(b) *Patterns of species distribution in the atmosphere*

Although the majority of aerobiology has focused on community-level abundance patterns, culture-based research has provided a foundation for exploring taxa-level patterns. The study of taxa-level distributional patterns, such as a species' geographical range, is central to biogeography. Culture-based work has begun to address fundamental questions about the upper boundary of microbial geographical ranges in the atmosphere. Isolated cultures of the common mould, *Penicillium notatum*, have been collected at an altitude of 77 km, and the bacteria *Micrococcus albus* and *Mycobacterium luteum* at an altitude of 70 km (Imshenetsky *et al.* 1978; figure 2). Culture-based studies have also been used to understand the link between atmospheric environmental conditions and the occurrence of particular microbial species. For example, the occurrence of *Micrococcus* has been shown to correlate with the concentration of airborne particulate matter (Mancinelli & Shulls 1978); this might explain why airborne *Micrococcus* species are commonly dominant in urban environments (Fang *et al.* 2007). Finally, culture-based studies can help identify ubiquitous species that are likely to have large geographical range sizes. Spore-forming organisms, such as *Bacillus* species and other Gram-positives, tend to dominate culture-dependent surveys of airborne microbial diversity and thus may have large geographical ranges (Mancinelli & Shulls 1978; Lighthart 1997; Fang *et al.* 2007).

(c) *Airborne microbial community composition*

Species do not exist in isolation, they occur together in complex ecological communities. To understand the mechanisms that shape biological variation on Earth, biogeographers study patterns in the composition and diversity of ecological communities in space and time. The development of environmental molecular biology has led to an explosion of investigations on the biodiversity and biogeography of microbial communities in terrestrial and aquatic environments (Green & Bohannan 2006). However, we currently know very little about microbial diversity in the atmosphere. Most studies demonstrating spatio-temporal variability of airborne microbial communities have been limited to culture-dependent methods. Recently, culture-independent molecular approaches have begun to be applied to airborne microbial communities. In contrast to culture-based studies, these molecular-based studies have revealed that airborne microbial assemblages can be as diverse as those in terrestrial environments, including soils (Maron *et al.* 2005; Brodie *et al.* 2007).

The first applications of molecular techniques in aerobiology typically analysed a single environmental sample (Hughes *et al.* 2004). More recently, investigators have begun to explore the biogeography of the atmosphere, by comparing microbial communities across multiple samples. Most comparative studies have focused on temporal variation in community structure at the same spatial location, with results ranging from pronounced differences in the daily (Fierer *et al.* 2008) and seasonal (Fröhlich-Nowoisky *et al.* 2009) cycles of airborne microbial community structure, to relatively static community structure across time (Bowers *et al.* 2009; Pearce *et al.* 2010).

There has been little research on the spatial variation of microbial communities in the atmosphere. Because microbial community composition can shift dramatically over short time scales (Fierer *et al.* 2008), comparative analyses between spatial locations require statistically controlling for time. Brodie *et al.* (2007) published the most definitive evidence for spatial variation of airborne microbial communities. In two Texas cities, these investigators pooled air filters in a manner that resulted in a random air sample per city, collected on a weekly basis for 17 weeks. In any given week, the data showed significant differences in community composition between cities; however, temporal and meteorological influences proved to be a greater factor in explaining variability of aerosol bacterial composition. Future research that explores spatial variability in microbial diversity, while accounting for temporal variability, will significantly expand our understanding of atmospheric biogeography.

5. COMPARATIVE BIOGEOGRAPHY OF AIR, WATER AND LAND

The studies reviewed above indicate that microorganisms vary in abundance, distribution and diversity in the atmosphere. Yet, the air remains the least understood environment from a biogeographic perspective. Patterns in the variation of microorganisms in the atmosphere have not been well documented, nor have the processes that underlie these patterns been identified. What might these patterns and processes look like in the atmosphere? Here, we consider defining attributes of land, water and air environments, and how these attributes may contribute to similar and different biogeography patterns across these domains. Building on a rich history of research in terrestrial and aquatic systems, we explore two patterns that are likely to play an important role in shaping the emerging field of air biogeography: environmental diversity gradients and the existence of biogeographic regions. Ultimately, a more unified understanding of the biosphere will entail comparing and contrasting these patterns across the lithosphere, hydrosphere and atmosphere.

(a) *An ocean of air*

The vast majority of biogeographic studies to date have focused on terrestrial environments. However, there is increasing interest in the biogeography of marine environments (e.g. DeLong 2009; Tittensor *et al.* 2010), and marine biogeography may be the best model for what a biogeography of the atmosphere might look like. Landscape-scale analyses of terrestrial

environments have often been reduced to two spatial dimensions (with soil depth ignored), simplifying both the measurement of biogeographic patterns and the development of theory to explain these patterns. But marine environments, much like the atmosphere, are unavoidably three dimensional. A given terrestrial environment (a particular forest, for example) is relatively long-lived and stationary; a given marine environment (e.g. a particular mass of ocean water) can be ephemeral and under constant motion, much like the atmosphere. We suggest that the major environmental gradients in marine environments (light/UV, temperature, nutrients etc.) vary in space and time at rates and scales more similar to the atmosphere than those of terrestrial systems. Given our assumption that atmospheric biogeography may be most similar to marine biogeography, we primarily focus our discussion below on the biogeographic patterns and processes shown to be important in marine systems.

(b) *Environmental diversity gradients in the atmosphere*

Environmental gradients—geographical gradients in the abiotic and biotic environment—have been used for centuries as a tool to understand the ecological and evolutionary forces that shape biological diversity. Environmental gradients have inspired some of the earliest hypotheses about the origin and spread of life on Earth (Linnaeus 1781). Since Linnaeus, hundreds of studies of community structure along gradients of elevation, latitude and depth have contributed to the foundations of modern ecology and biogeography (Lomolino *et al.* 2006).

Despite the wealth of plant and animal environmental gradient research, there have been relatively few studies of microbial diversity gradients. The resounding message from recent microbial depth gradient research is that, as with macro-organisms, the structure and composition of microbial communities are significantly influenced by environmental variability. For example, in the ocean, temperature, pressure, light and nutrients vary from sea level to the sea floor. Recent culture-independent studies clearly demonstrate that this environmental variation influences the vertical distribution of oceanic microbial diversity, for example, patterns of taxonomic richness, RNA/DNA ratios, gene copy number and metabolic pathways (DeLong *et al.* 2006; Johnson *et al.* 2006; Wilms *et al.* 2006; De Corte *et al.* 2008; Brown *et al.* 2009; Treusch *et al.* 2009). In the atmosphere, temperature, pressure and moisture vary from sea level to the outermost layer of the atmosphere. Given the strong evidence for shifts in community structure along similar types of environmental gradients, it is parsimonious to assume that microbial biodiversity changes in predictable ways with altitude in aerial systems.

Another widely studied environmental diversity pattern is the increase in numbers of animal and plant species as one travels from the poles towards the tropics. This latitudinal diversity gradient has been recognized for centuries (Mittelbach *et al.* 2007), and in recent years, this pattern has received heightened attention in the microbial biogeography literature. Although the generality of latitudinal diversity gradients remains equivocal, both molecular-based studies (Fuhrman *et al.* 2008) and biodiversity models (Barton *et al.* 2010) have revealed a decrease in species richness with latitude for marine microbes. The most parsimonious explanation for an aerial latitudinal diversity gradient is that the diversity of the atmosphere reflects the diversity of terrestrial and marine systems (i.e. aerial communities are a random sample of metacommunities on the surface of the Earth). However, the possibility exists that the atmosphere has a unique latitudinal diversity pattern. Numerous mechanisms have been proposed to explain latitudinal diversity gradients that may be relevant in the atmosphere, including gradients in energy, temperature and moisture. To our knowledge, there have been no published studies on a latitudinal diversity gradient for micro-organisms in the atmosphere.

(c) *Biogeographic regions in the atmosphere*

One of the most striking biogeographic patterns at a global scale is the existence of biogeographic regions. The globe is divided into six unique biogeographic regions, areas of the Earth's land surface that contain unique plants and animals (Wallace 1876; Lomolino *et al.* 2006). These unique biotas are hypothesized to exist because of vicariance, the evolutionary separation of species owing to historic barriers to dispersal. More recently, attempts have been made to define marine biogeographic regions (Lomolino *et al.* 2006). This is more challenging for several reasons: marine systems have fewer dispersal barriers, are more dynamic in space and time, have a more complicated geological history and are more obviously three dimensional in nature. Nonetheless, there are large-scale differences in marine biotas, even among pelagic organisms. These patterns are believed to be driven by environmental barriers (e.g. warm tropical oceans act as barriers to cold-adapted organisms) and differences in the biogeography of the underlying benthos and/or adjacent coastal regions, differences believed to reflect tectonic and oceanographic history.

Could there be biogeographic regions in the air? The short answer is that we do not know. No studies have attempted to ask whether there are large-scale patterns in the distribution of airborne micro-organisms. However, there are reasons to believe that such patterns are possible. There are large-scale patterns in the distribution of masses of air that could conceivably drive large-scale patterns in air organisms. At the largest scale, differential heating of air at the tropics and poles combines with the Earth's rotation to produce six 'cells' of air blanketing the globe (figure 3). Mixing of air is more frequent within cells than between them, resulting in barriers to air movement, and the potential for vicariance. These mixing barriers often coincide with strong differences in temperature between adjacent cells; thus, environmental barriers could augment physical barriers to dispersal. The major cells of air are relatively stable geographically, and rest on areas of the

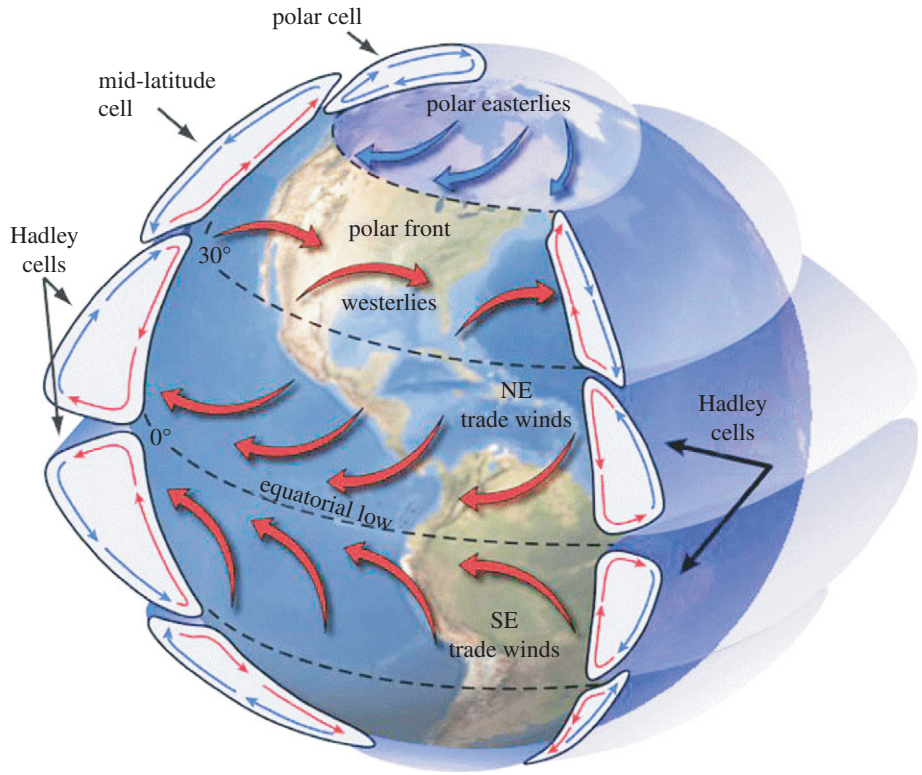

Figure 3. The six major air cells of the Earth's atmosphere (source: NASA).

Earth's surface that are often biogeographically distinct. Thus the input of organisms to each cell could be distinct, and reflect tectonic and/or oceanographic history, much like the influence of benthic or coastal biogeography on marine pelagic distributions. Together, this suggests that large-scale patterns in the distribution of air organisms are possible. We suggest that a logical starting place for such studies is to ask whether airborne communities are more similar within the six major air cells than among them.

These major cells of air are restricted latitudinally (i.e. they occur within particular bands of latitude; figure 3). If these cells do represent biogeographic regions, differences in microbial community structure among these cells could reinforce latitudinal patterns in airborne microbial communities (if such patterns exist; see above). There is also the potential for biogeographic patterns within these major cells. Individual air masses (volumes of air with particular environmental characteristics) continually form, move and disperse within the major air cells. If these masses are sufficiently long-lived to allow for multiple generations of microbial growth, they could harbour unique resident microbial communities, analogous to patterns in the distribution of plant and animal communities in biogeographic 'provinces' within regions. However, to date, no study has attempted to determine whether microbial communities are more similar within particular air masses than among them.

6. CONCLUSION

Despite the potential importance of understanding the distribution of life in the air, there are major gaps in our current understanding of the air's biogeography. These gaps include a lack of accurate and comprehensive estimates of many important attributes of life in the air such as estimates of microbial densities and residence times, the proportion of organisms that are metabolically active, generation times of airborne organisms and the structure of airborne microbial communities. The use of new technology and standardization of techniques across studies will allow for a more complete understanding of the distribution of life in the atmosphere.

Most importantly, to move our understanding of life in the air forward, air biologists must learn to think like biogeographers. This includes designing studies that allow the disentangling of spatial and temporal effects on the distribution of life in the air, as well as using our knowledge of atmospheric dynamics to develop testable hypotheses regarding the biogeography of air. We feel that it would be especially fruitful to ask whether airborne communities are more similar within air cells and/or air masses than among them. Finally, studies of the biogeography of land and sea suggest that there are a number of biogeographic patterns that may be universal (Green & Bohannan 2006; Martiny *et al.* 2006). These include: (i) the distance–decay relationship (how similarity in community composition varies with the spatial, temporal or environmental distance that separates them), (ii) the taxa–area (or taxa–volume) relationship (how taxa richness increases with spatial scale), and (iii) latitudinal diversity patterns. These patterns are a promising starting point for developing a biogeography of the air.

The study of the biogeography of the air is in its infancy, but it has the potential to greatly alter how we think of the distribution of life on Earth. Given the important role the air plays in the dispersal of surface organisms, a more detailed understanding of the distribution of life in the atmosphere will allow us to

better understand the distribution of life throughout the globe. It will also allow us to determine whether there are common patterns (and ultimately processes) underlying the distribution of life in the lithosphere, hydrosphere and atmosphere, bringing biologists a step closer to a comprehensive understanding of the distribution of all life.

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Water Vapor

The most variable as well as the most remarkable of the atmospheric trace gases is water vapor. Much of this book describes the ways in which water vapor, in its various forms, controls the behavior of the air surrounding our planet. Unlike the other trace gases, water exists in the atmosphere in gas, liquid, and solid form, and adds and extracts heat from the air whenever it changes from one to another.

Water vapor and airborne particles are essential for the stability of the global ecosystem. Their variations and interactions, combined with the global circulation of the atmosphere, produce the world's weather (including its clouds and precipitation) and are responsible for the blue-green-white appearance of the earth as seen from space.

How We Perceive the Atmosphere

The natural atmosphere has many features that everyone knows and enjoys: the blue of the sky, the clarity of the air, the brilliance of the stars at night, the color of the rainbow, the smell of the sea, the fresh air following a thunderstorm, the red of the sunset, and the symmetry of a snow crystal. In the same category as the deep blue of the clear sky is the blue color of glacial ice and the soft turquoise of the shadows in holes in freshly fallen snow.

But the atmosphere has another face. The terrible destructiveness of a tornado, a hailstorm, or a hurricane, the wearying monotony of the persistent gusty Foehn or Mistral winds, the roar of a wildfire sweeping up a mountain slope — these are some of the terrifying examples of the disturbed atmosphere.

Cities in general are poor places to survey the most attractive features of the atmosphere. Apart from the pollution that rises from the city to mask the stars and make the visible sky a dirty brown or at best a pale blue, the buildings of the city obstruct the view. Except for the panorama that can be enjoyed from the top of a skyscraper, the observable atmosphere is not very available to the city dweller. This *Field Guide* is intended to encourage the reader to seek the natural surroundings of the countryside; by doing so, the reader will be better able to understand the beliefs and intentions of those who would preserve the environment through intelligent land-use practices and control of all forms of pollution.

Fortunately for the survival of all earth's organisms, the atmosphere has many self-healing properties. Clouds are its air-cleaning agents, and the remarkable global circulation systems constantly purge the air of its foreign matter; it is only when air contamination overloads the system that living organisms are faced with serious trouble.

The Range in Size of Atmospheric Components

One of the intriguing aspects of the atmosphere is the way its components vary in size; at one end of the scale are molecules, and at the other are gigantic storm systems and the entire atmosphere itself, so that sizes may range from 0.0001 micron to 10,000 km. This range of 17 orders of magnitude may be more easily imagined if you refer to the front endpaper (very conveniently, human beings happen to be about midway in size between these extremes).

Reduced Visibility in the Atmosphere

The reduction in visibility of distant objects is caused by a variety of factors and often the combination of several. The most effective particle shape and size for scattering visible light is a spherical particle with a diameter of 0.6 micron. These particles scatter light effectively in nearly all directions, but the maximum scattering occurs on a line between the observer and the light source. This accounts for our being able to look directly at the sun without hurting our eyes under the right conditions — when the air contains enough particles to restrict visibility significantly, and the sun is 10° or less above the horizon. In clean air, as soon as the sun rises it becomes extremely hazardous to view directly.

Types of Airborne Particles

The atmosphere contains a wide variety of airborne objects and substances. The largest of these range in size from the debris of tornadoes, waterspouts, volcanoes, burning embers from forest fires, and tumbleweeds, to spider webs, seed parachutes, soil particles, pollen grains, and other living microscopic forms.

The smaller light-scattering particles include fragments of rock made airborne by dust devils and gusty winds, salt and spray from the breaking of bubbles at sea, forest-fire smoke, and particles that produce the blue haze often seen over forested mountains. The greatest number of airborne particles are so small as to be invisible. These submicroscopic particles are formed by the condensation of vapors, the chemical combination of reacting molecules, the photochemical effects produced by the ultraviolet radiation from the sun, and the electrical and other ionizing forces that come from thunderstorms, cosmic rays, and radioactivity.

The Formation of Airborne Particles

Airborne particles are produced by 2 very different mechanisms. The larger particles are fragments of still larger ones which, through weathering, mechanical breakage, solution, or some other attrition process, finally become small enough to float in the at-

Modification of Clouds." (Today, "modification" would be better expressed as "classification.") As part of this new classification system for clouds, Howard developed a set of Latin names for the main and secondary cloud types.

Howard's paper was so perceptive that now, nearly 2 centuries later, the classification system and cloud names are still the same as his in some respects and closely resemble them in others.

Luke Howard's practiced and keen eye discerned the presence of 2 primary categories of cloud, *heap clouds* and *layer clouds,* and these are still the basis of most classification schemes. They also will be the 2 primary classes discussed here, identified as Group I and Group II. There are 3 additional groups, as shown in Fig. 8. Four groups are discussed in detail here.

Group I. Heap Clouds — The Cumulus Family

These are flattish-based clouds of domed cauliflower shape, with sharply defined edges and the tops of towers rising to different

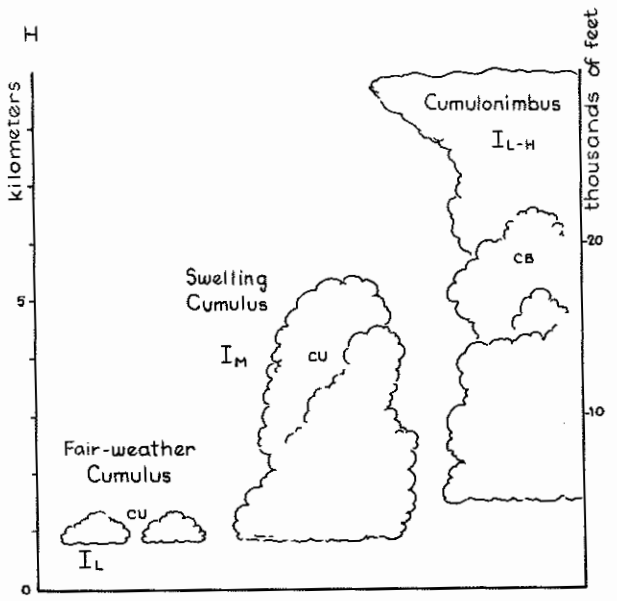
Fig. 8 Cloud Classification.

GROUP (av.)	Tier or Layer		
	Low (0-3 km)	Middle (3-7 km)	High (> 7 km)
I (HEAP)	cumulus fair-weather	swelling cumulus	cumulo-nimbus (non-precip.)
II (LAYER)	stratus	alto-stratus	cirro-stratus
III (LAYERS & HEAPS)	strato-cumulus	alto-cumulus	cirro-cumulus
IV (PRECIPITATING)	nimbostratus cirrus cumulonimbus		
V (UNUSUAL)	separate listing (note: 1 km = 3280 ft.)		

heights (see Fig. 9). The vertical and horizontal dimensions of the heap clouds are approximately the same. On some occasions, the vertical dimension is only a few hundred meters while on other occasions the cloud tops rise to heights of 20 km (65,600 ft.). The horizontal diameter ranges from that of a city block to a few kilometers. Individual clouds are well separated from each other. Cloud color is usually white, though it can change to dark, threatening gray depending on form of development, illumination, and age of cloud. The cumulus, or heap cloud, is a convective cloud whose essential character is that of many rising bubbles of relatively warm air. If moisture is evenly distributed horizontally through the local air mass, the ascending air generally reaches its saturation point at the same level. The onset of condensation produces the flattish base (which is reported by glider pilots to be slightly higher at the *center* than around the edge). Descending air in the surrounding region experiences heating and drying and causes the cloudless space.

Plate 26 shows a sky of fair-weather cumulus (Group I_L) in vary-

Fig. 9 Group I. Heap Clouds—The Cumulus Family.



Pl.36 There are many further combinations of heaps and layers, resulting from the attempt by the individual heap cloud to grow vertically and yet remain separate from others, and the restraint put upon this attempt by the presence of the stable layer that says, in effect, "You can't go through but you can spread out." The factors that determine the appearance of a particular sky are: (a) height of the lifting condensation level, (b) strength of convective activity, (c) height and strength of the stable layers.

These possibilities are illustrated here. There is strong inversion, **1,** at about 2 km (6500 ft.) with the lifting condensation level, **2,** at 1 km (3300 ft.). In this unusual instance the limiting of upward cloud motion is shown in convenient cross section. Small cumulus towers, **3,** grow as at **4** and on reaching the inversion level, **1,** can only spread out. The sky then becomes a grouping of small cumulus and stratocumulus.

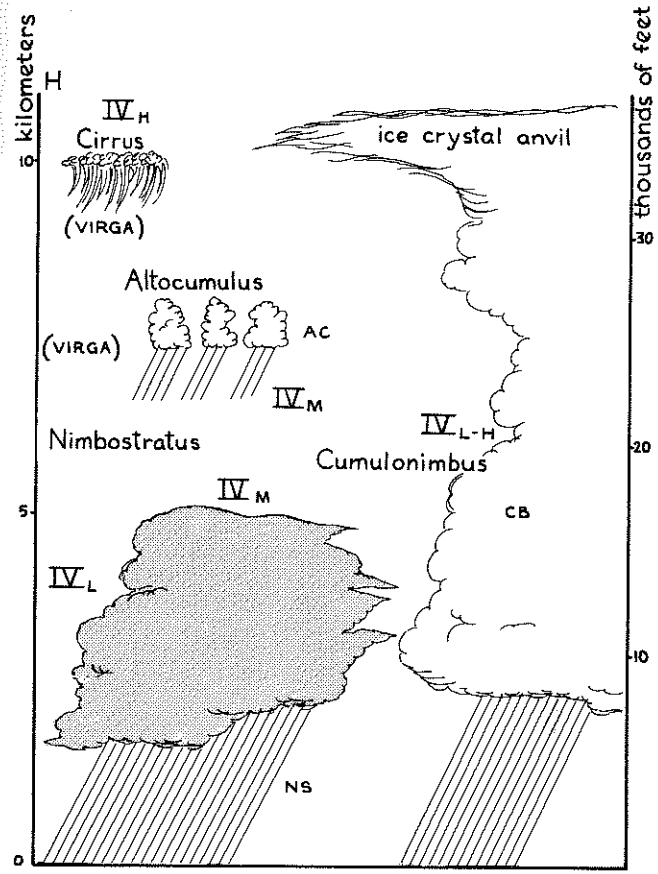
Group IV. Clouds that Generate Precipitation

Nimbus is the traditional term for clouds of this type. The most common precipitating cloud found in low and middle layers is the *nimbostratus.* In general, it has a darkish gray appearance, ragged edges, and rain or snow falling continuously from its base.

In the winter season, at middle and high latitudes, the freezing level often comes down to the surface, with cirrus-type clouds found at all elevations. It is not uncommon under such circumstances for a cirrostratus layer to produce a gentle "rain" of columnar ice crystals that fall to the ground before they have a chance to evaporate.

One important factor affecting all clouds is the amount of water vapor the atmosphere contains. The standard way of specifying water content of clouds is in terms of water-vapor density measured in grams per cubic meter (g/m^3). A gram of liquid water occupies very nearly the volume of 1 cubic centimeter, which is conveniently remembered as the size of a small sugar cube; a cubic meter is about the volume of an ordinary desk. The average values of water-vapor density in clouds range from about 0.1 g/m^3 to 3 g/m^3.

Fig. 12 Group IV. Clouds Generating Precipitation.



6

Severe Storms

Severe storms are impressive and terrifying natural phenomena. They are unusual manifestations of the organized energy from the sun received by the earth, oceans, and atmosphere.

They range in size from the large cyclonic storm that affects many thousands of square kilometers to the savage tornado that devastates part of a town. Most severe storms are only moderately intense, but from time to time (from a few times a year to a few times a century) the supply of moisture, the convection patterns, the convergence flow patterns, and the instability of the atmosphere are just right to allow a "giant" storm, in size or intensity or both, to develop. When this occurs the storm and its effects overwhelm the normal precautions taken for the protection of life and property, and a disaster occurs. A few such cataclysmic storms are likely to be the cause of many of the scars of land erosion or widespread timber blowdown to be seen in various parts of the world.

Considerable progress has been made in recent years toward improving the forecasting of severe storms and providing storm alerts, watches, and warnings by radio and television. With reliable forecasts and adequate warning, protective steps and safety measures have reduced to a remarkable degree the human death toll from severe storms. Nature, however, is very capricious; there are times when even the most elaborate of plans and warnings are inadequate or become inoperative. But the weatherwise individual can often provide valuable information to his or her family and neighbors by understanding weather signs and taking proper precautions.

The cumulonimbus cloud is a veritable weather factory. Out of individual cumulonimbus clouds can come at least 5 distinct severe atmospheric effects: (1) thunderstorm with associated heavy winds, (2) cloudburst, (3) hail, (4) tornado, and (5) waterspout. Cumulonimbi that produce severe weather are awesome giants of clouds, extending sometimes to the top of the troposphere — roughly 12 km (40,000 ft.) — and occasionally into the lower stratosphere. Clouds of this size do not occur unless there is an adequate supply of energy, and this means the air mass must be loaded with copious amounts of water vapor distributed through a deep layer. Furthermore, the right set of conditions must exist to trigger the cloud's development, and there must be no inhibiting

factors that prevent its growth to full maturity, such as inversion layers in the middle levels of the atmosphere.

The explanations that follow can provide only a general account of what is going on during severe storms. The reader should refer to the numerous texts in meteorology for more details.

Thunderstorm

As it forms, a cumulonimbus thunderstorm cloud develops a separation of electrical charge, with a center of positive charge in the frozen upper portion of the cloud and center of negative charge near its base. Precisely how this happens is still not known with certainty, mainly because of the extreme complexity of the problem and the difficulties of making enough measurements on the same storm so that reasonable conclusions can be drawn. Although it has been done, it is very difficult and dangerous to make measurements inside the cloud because of inaccessibility, extreme turbulence, and the strong electrical fields themselves. It is known from laboratory work that breakup of raindrops, freezing, ice splintering, frosting, and rubbing effects — crystal on crystal and drop on drop — are some of the events producing charge separation.

The earth is a negatively charged body, but under a thunderstorm the negative charge is pushed away by electrostatic repulsion of the underside of the cloud (charged negatively), and a positive charge is induced that becomes stronger as the cloud's negative charge center strengthens. Positively charged ions move up to the top of elevated objects and attempt to establish a flow of current, but are prevented from doing so by the air, which is a poor conductor of electricity.

Lightning occurs when the electrical field becomes so strong — as high as 1 million volts per meter — that the insulating capability of the air breaks down. The lightning stroke is a sudden flow, primarily of negative charge, from the cloud to the ground through a channel initiated by a complex series of "leader strokes" followed in rapid succession by a ground-to-cloud "streamer" carrying a positive charge. These continue until the strength of the electric field is reduced — in perhaps 1 second.

The path of the lightning stroke carries peak current reaching 200,000 amperes or more, and the associated temperatures may rise to millions of degrees. As a consequence of this heating, there is explosive expansion and contraction of the air, producing the pressure pulses that are heard as the crash and rumble of thunder.

Because of the speed of sound, the distance in miles to a lightning stroke can be quickly estimated by counting the number of seconds between the sight of the lightning flash and the sound of the thunder, and dividing by 5 (if flash and sound are simultaneous, it was close).

Lightning comes in many forms. *Streak lightning* is the kind most frequently seen — a single or multiple line from cloud to ground; *forked lightning* shows multiple channels; *ball lightning* (a controversial form) appears as a luminous globe that maneuvers like a flying saucer; *heat lightning*, seen along horizons in hot weather, is the reflection of lightning occurring beyond the horizon. St. Elmo's Fire is a phenomenon that occurs when the electrical potential produces coronae from grounded objects, most typically metal ones.

There are about 1800 thunderstorms in progress over the earth's surface at any moment, and strokes hit the earth 100 times each second. The thunderstorm performs the vital function of returning to earth much, if not all, of the negative charge it continually loses by leakage to the atmosphere.

According to data assembled by the National Center for Health Statistics, the annual death toll from lightning is greater than for tornadoes or hurricanes. About 150 Americans are killed by lightning per year, and 250 treated for injuries. Property damage is high.

Note: Persons struck by lightning receive a severe electrical shock and may be burned, but they *carry* no electrical charge and can be handled safely. A person "killed" by lightning can often be revived by prompt mouth-to-mouth resuscitation, cardiac massage, and prolonged artificial respiration. In a group struck by lightning, those apparently dead should be treated first; those who show vital signs will probably recover spontaneously, although burns and other injuries may require treatment. Recovery from lightning strikes is usually complete except for possible impairment or loss of sight or hearing.

Cloudburst

This is a sudden burst of excessively heavy rain from an extremely vigorous cumulonimbus cloud. Such storms are characteristic of summer conditions. When they occur over high country, such as the Rocky Mountains or the neighboring terrain, the rain may be suddenly channeled down an otherwise dry streambed and produce a flashflood. This can present real danger to campers or picnickers, and to small communities whose houses are built too close to the stream channel.

Hikers and campers should be very careful about choosing an overnight camping site, particularly if any cumulonimbus activity is noted.

Every community should have some sort of warning system for flashfloods (5 minutes notice may save many lives) especially if it is located downstream from a dam that might become endangered by sudden and excessive upstream rainfall.

Hail

Every so often a severe cumulonimbus generates hail. There is evidence that hail forms rapidly. An ice pellet gets caught in a powerful updraft, grows as it collides with supercooled water drops, falls, is caught again, and still again, and finally gets into a downdraft where it and other hailstones fall from cloud to ground. There is new evidence that some hailstones grow by "sitting still" in the updraft and gathering the supercooled drops that strike them.

Perhaps the average hailstone in a midwest storm is marble-sized; in the more severe storms the stones are the size of golf balls. Excessively severe storms generate some stones the size of baseballs. Hail can and does cause considerable structural and crop damage; occasionally livestock and humans are also killed.

Substantial efforts are being made to study the hailstorm in hopes of altering growth patterns by weather modification techniques to prevent large hail from falling from the clouds, but much more work needs to be done. The major effort is to produce many smaller hailstones instead of a few large ones. This is accomplished by seeding the storm in its early stages with many more ice nuclei than are present under natural conditions. This action may also reduce the size of the storm.

Tornado

The cause of tornadoes is known generally but not precisely. The ingredients for a tornado-spawning cumulonimbus are: a southerly flow of warm, very moist air, topped by a westerly flow of cool, dry air, and a squall line whose convergence sets off convective clouds. Tornadoes usually, but not always, move along in a southwest-to-northeast direction. The funnel dips down from cloud to ground, becoming visible as moist air moves into the region of sharply lowered pressure and condenses, and as the vortex sucks in debris from the ground.

Tornadoes are difficult to forecast with precision. However, most communities in tornado-prone regions are covered by the Tornado Watch of the National Weather Service and receive advisories by radio and television.

People caught in the open should try to find refuge in a ditch or culvert, and keep down to avoid injury from flying debris. Those interested in scientific observation might try to make a written and photographic record of the behavior of the funnel.

In the home, a basement wall, or preferably storm shelter, will provide the best protection; crouching under a heavy table will help some; do not go upstairs.

Windows must be opened to prevent the house from exploding as a result of the suddenly lowered outside barometric pressure near the funnel. Gas and electricity mains should be turned off,

and flashlights and a transistor radio readily available. Refer to Appendix 19.

Waterspout

The waterspout is a first cousin of the tornado, forming from an active cumulonimbus cloud over the water, and developing a long trunk-shaped funnel that dips down to the water. Because of associated strong winds and resulting disturbance of the water surface, waterspouts should be avoided if at all possible by ships and particularly by small boats.

Tropical Storm

Tropical storms (known by the local names of hurricanes, typhoons, cyclones) breed in the low-latitude belts over the oceans, primarily from 5° to 15° north or south latitude. Westward-moving regions of unstable air produce areas of intense vertical growth. Colonies of cumulonimbus clouds generate heavy rain squalls. In a way not fully understood these colonies start to become organized as a circulating wind system, counterclockwise in the northern hemisphere and clockwise in the southern hemisphere. As growth and organization proceed, the storm develops a ring of intense convective clouds surrounding a central, relatively cloudless region at the storm center known as the eye of the storm (the relative absence of clouds at the eye is accounted for by the sinking of the air). Surrounding the central core are radiating bands of large cumulus that take on a pinwheel effect when viewed from high above.

Northern hemisphere storms move out of the central tropical Atlantic and Pacific toward the west, generally guided by the upper wind stream. As they reach the western end of the ocean basin, they tend to recurve to the north and then to the northeast. However, the guidance is a critically balanced matter and some storms' paths are erratic in the extreme, making northward starts and retreats several times, most of the time moving on to the west.

Severe tropical storms can generate surface winds up to 160 km/hr. (100 mi./hr.) and are therefore dangerous if only because of the winds. However, the strong winds also produce tremendous seas in advance. If the impact of a hurricane on the land takes place at a time of extreme high tides, then there can be massive flooding. Furthermore, the circulation of the storm pumps great amounts of warm, moist air ahead of it, and the result of the condensation of this water vapor and consequent precipitation can produce flooding as well.

A hurricane, or typhoon, involves a massive release of atmospheric potential energy. At this stage in history, little can be done to modify such a storm system, though this is a subject of current study, and beginnings have been made.

The generation, development, and movement of these storms are today kept under continuous surveillance by weather satellites and by weather radar stations that are particularly concentrated at the southeast perimeter of the U.S.

An effective warning system has made it possible to order wholesale evacuation well in advance from land regions the storm is to strike. Though such storms still produce extensive property damage, loss of life is now reduced to low levels.

A note of precaution: Follow the instructions of the local Severe Storms Warning Service or other competent authority.

Blizzard

Following a winter frontal storm, which may have deposited from 2–6 in. or more of snow, the airflow swings from the south or southwest to the west or northwest and often the wind produces blizzard conditions. A blizzard consists of strong winds at ground level sometimes exceeding 80 km/hr. (50 mi./hr.), containing airborne snow and ice particles in blinding proportions. The winds are gusty and persistent, producing massive snow drifts and cornices in the lee of mountaintops, road cuts, houses, and other obstacles. Under some conditions a snow structure known as a "sastrugi" forms. It resembles a sand dune but is a compacted, rippled, icy substance, the bane of polar travelers. Although a sastrugi resembles a permanent structure, it, like a sand dune, is in the constant process of transformation as long as the wind blows.

Whiteout

One aspect of a blizzard is the "whiteout" which, because of the high density of airborne particles, restricts visibility to a few meters or less. A whiteout can also be produced by much smaller particles such as a liquid-droplet or an ice-crystal fog. Under whiteout conditions the sense of gravity can nearly disappear, as mountain skiers learn to their dismay.

A special form of whiteout is the ground blizzard, in which the restricted visibility has a vertical thickness often of less than 1.8 m (6 ft.). Polar explorers protected by tent or igloo quickly learn to occasionally check the depth of the whiteout swirling around their temporary habitation. Ground blizzards sometimes persist for many days.

Ice Storm (sometimes called silver thaw)

In the middle latitudes of the continents, the ice storm often develops in winter when warm moist air encounters a cold air mass and slides up over the colder, denser air. When this happens precipitation often forms, generally by coalescence, although the raindrops may be mixed with ice crystals falling into the warmer air from