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RESEARCH ARTICLE

# Aeolian dispersal of bacteria in southwest Greenland: their sources, abundance, diversity and physiological states

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**One sentence summary:** The authors find that airborne bacteria in southwest Greenland are aerosolized mostly from permafrost soils and that these bacteria include types with a high potential to be active either in the atmosphere or after settling down to new environments.

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## ABSTRACT

The Arctic is undergoing dramatic climatic changes that cause profound transformations in its terrestrial ecosystems and consequently in the microbial communities that inhabit them. The assembly of these communities is affected by aeolian deposition. However, the abundance, diversity, sources and activity of airborne microorganisms in the Arctic are poorly understood. We studied bacteria in the atmosphere over southwest Greenland and found that the diversity of bacterial communities correlated positively with air temperature and negatively with relative humidity. The communities consisted of $1.3\times10^3 \pm 1.0\times10^3$ cells $m^{-3}$, which were aerosolized from local terrestrial environments or transported from marine, glaciated and terrestrial surfaces over long distances. On average, airborne bacterial cells displayed a high activity potential, reflected in the high 16S rRNA copy number ($590 \pm 300$ rRNA $cell^{-1}$), that correlated positively with water vapor pressure. We observed that bacterial clades differed in their activity potential. For instance, a high activity potential was seen for Rubrobacteridae and Clostridiales, while a low activity potential was observed for Proteobacteria. Of those bacterial families that harbor ice-nucleation active species, which are known to facilitate freezing and may thus be involved in cloud and rain formation, cells with a high activity potential were rare in air, but were enriched in rain.

**Keywords:** arctic microbial communities; aeolian dispersal; community assembly; 16S ribosomal RNA; microbial activity; atmospheric processes

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## INTRODUCTION

The Arctic is undergoing rapid climatic changes that profoundly affect its ecosystems and coincide with major shifts in microbial communities (Bradley, Singarayer and Anesio 2014; Jansson and Taş 2014). The concomitant changes in biogeochemical processes are very difficult to predict, because different factors, i.e. dispersal, selection, drift and mutation, that affect community assembly are inadequately studied (Hanson *et al.* 2012). The introduction of new microbial cells to the environment, in particular by aeolian dispersal, can potentially influence the microbial community assembly in newly exposed and transforming terrestrial environments, resulting from dramatic ice melt (Nemergut *et al.* 2013). Thus, a study by Xiang *et al.* (2009) showed that aeolian dispersal to a large extent impacted microbial diversity and succession on de-glaciated soils. Moreover, Cameron *et al.* (2015) and Musilova *et al.* (2015) demonstrated that bacterial communities in cryoconite holes and on the surface of the Greenland ice sheet are seeded with microorganisms in snowfall and depositing dust. In Antarctica, several studies on airborne microbial communities have suggested that these microorganisms have an important role in colonizing Antarctic terrestrial environments (reviewed in Pearce *et al.* 2009). These findings have led to a recent initiative among polar scientists to address how aerial dispersal is shaping patterns of biodiversity in Antarctica (Pearce *et al.* 2016). A similar initiative is needed for the Arctic region, where colonization of newly exposed regions through atmospheric microbial dispersal and its effects on ecosystems have so far attracted only very little attention.

Studies addressing the origin, quantities, diversity, and physiological states of airborne microbes in temperate regions may offer some general insights that also apply to the Arctic. Airborne microorganisms in temperate regions were found to mainly affiliate with the phyla Proteobacteria, Firmicutes, Bacteriodetes, Actinobacteria, Acidobacteria and Cyanobacteria. (Bowers *et al.* 2009; Franzetti *et al.* 2011; Temkiv *et al.* 2012; DeLeon-Rodriguez *et al.* 2013; Barberán *et al.* 2015; Klein *et al.* 2016; Amato *et al.* 2017). The same phyla also dominated the atmospheric community in the Canadian high Arctic and over Svalbard (Harding *et al.* 2011; Cuthbertson *et al.* 2017). Both modeling and *in situ* approaches indicate that plant surfaces are an important source of atmospheric microorganisms (Bowers *et al.* 2011; Franzetti *et al.* 2011; Burrows *et al.* 2013; Šantl-Temkiv *et al.* 2013). Burrows *et al.* (2013) reported the highest cell numbers in the atmosphere over shrubs ($3.5 \times 10^5$ cells $m^{-3}$), lower cell numbers over coastal regions ($7.6 \times 10^4$ cells $m^{-3}$), and marine regions ($1.0 \times 10^4$ cells $m^{-3}$) and finally the lowest numbers over land ice ($5 \times 10^3$ cells $m^{-3}$). A large proportion of bacterial cells maintain viability during aeolian dispersal, both after emission from the ground (Bauer *et al.* 2002; Hill *et al.* 2007) and after transfer to the free troposphere and long-range dispersal (Hara and Zhang 2012; DeLeon-Rodriguez *et al.* 2013). Several studies also found evidence of metabolic activity in airborne microorganisms, based on cell-specific adenosine triphosphate (ATP) concentrations or elevated 16S rRNA copy numbers (Amato *et al.*, 2007, 2017; Delort *et al.* 2010; Vaïtilingom *et al.* 2013; Cho and Jang 2014; Krumins *et al.* 2014; Klein *et al.* 2016). It has therefore been proposed that microbial cells, which maintain their activity, take part in atmospheric element and water cycles (Möhler *et al.* 2007; Deguillaume *et al.* 2008).

Aside from some understanding of their diversity (Harding *et al.* 2011; Cuthbertson *et al.* 2017), properties of airborne microbial communities and their sources in the Arctic have yet to be investigated. Arguments have been put forward to support the view that microorganisms are not dispersal-limited, which implies that many of the characteristics of atmospheric microbial communities found in temperate regions could be generalized to atmospheric microorganisms in the Arctic. For example, studies investigating global transport of dust have suggested that microorganisms, which enter the free troposphere, can be transported over distances of several thousands of kilometers (Kellogg and Griffin 2006; Smith *et al.* 2013; Barberán *et al.* 2014). Also, models of atmospheric circulation predict that microorganisms with diameters $<3$ $\mu$m spread within the hemispheres on an annual time scale (Wilkinson *et al.* 2012). However, it is well established that latitudinal differences in air temperature result in large-scale atmospheric patterns, i.e. Hadley, mid-latitude and polar air cells, which may serve as dispersal barriers between tropical, temperate, and polar regions. Whether polar regions have biogeographically isolated atmospheric compartments, as was proposed by Womack *et al.* (2010), still needs to be determined.

The aim of this study was to investigate the Arctic atmosphere as a dynamic reservoir of active microbial cells that may colonize the terrestrial environment in this region. We use quantitative polymerase chain reaction (PCR) and high-throughput sequencing targeting 16S rRNA molecules and 16S rRNA genes combined with backward trajectory analysis in order to test two hypotheses: (i) Airborne microorganisms maintain a high activity potential. (ii) Microbial communities found in the atmospheric boundary layer assemble primarily from local sources. First, we quantified airborne microorganisms and their ribosomal content; second, we determined their likely local and distant sources and third, we identified taxa with an elevated *in-situ* activity potential.

## MATERIALS AND METHODS

### Sampling location

Air samples were collected between 25 July and 1 August, 2013, from a rock outcrop ('M500'; 64.12264 N, 51.36619 W) at 500 m above sea level near the Nuuk Basic research station (64.13 N, 51.39 W). The study area is situated in an alpine landscape in southwest low-Arctic Greenland. The surrounding area is within the extensive Godthaabsfjord complex, a part of which is the small and confined Kobbefjord, with a surface area of 25 $km^2$. The closest potential sources for locally aerosolizing particles are found in the drainage basin (32 $km^2$) of Kobbefjord. Surrounding the drainage basin, there are mountains with small glaciers that cover approximately 2 $km^2$. The research station is located in a subpolar rain tundra biome, with shrubs and non-woody plants of the low-Arctic ecosystem being the most common vegetation types.

### Collection of air and rain samples

Seventeen consecutive air samples of 130–145 $m^3$ each were collected (Table S1, Supporting Information). Three air samples (A11, A16 and A17) were collected in duplicates in order to assess the heterogeneity of the environment (Šantl-Temkiv *et al.* 2017). The samples were taken from either the north or the southeast edge of the M500 peak, always facing the current wind direction. Air was sampled for 5 h using a model DS 5600 impinger with removable vortex chamber and built-in suction pump (Alfred Kärcher GmbH & Co. KG, Germany) (Šantl-Temkiv *et al.* 2017) at a flow of 0.8–0.9 $m^3$ $min^{-1}$. Prior to use, the vortex chamber

was cleaned by operating the sampler for 5 min with the vortex chamber filled first with 1 M HCl, then with autoclaved (3 h) and filtered (0.1 $\mu$m pore size, VacuCap 90 Devices, Pall Corporation, New York) MQ water, and finally with 96% ethanol. A control for the sterility of the system was collected before each sampling. The vortex chamber was filled with 2 L of sampling liquid, the sampler was run for 5 min, so that the liquid came in contact with the entire chamber, and afterwards 0.5 L of the sampling liquid was removed, stored as a control and analyzed along with the samples. The sampling liquid was a high-salt solution (25 mM sodium citrate, 10 mM EDTA, 450 g $L^{-1}$ ammonium sulphate, pH 5.2, Lever *et al.* 2015) that was designed for real-time fixation of RNA contained in microbial cells. The importance of real-time *in situ* fixation for RNA work has been stressed by Feike *et al.* (2012). The samples and the negative controls were stored for a short period at room temperature (a few hours to up to 4 days) and up to 8 days at 4°C. Samples and negative controls where then further concentrated onto Sterivex filter units, suspended in 1 mL of RNA*later*® (Thermo Fisher Scientific Inc, *Massachusetts*), capped with Luer-locks, air-shipped to the laboratory and stored at 4°C until further analysis.

Three rain samples were collected by using an opened vortex chamber as a passive rain collection container, both at the M500 peak and next to the Nuuk Basic research station at sea level. Prior to each rain sampling, 0.5 L of the sampling solution was stored as a negative control and the remaining 0.5 L of high-salt solution were then exposed to the falling rain. All rain samples and controls were processed as described for the air samples.

## Sampling of potential sources

One liter of surface seawater was collected from the shore of Kobbefjord in proximity of the Nuuk Basic research station (64.13360 N, 51.39812 W). One-liter water samples were collected from the surface of two lakes in the Kobbefjord basin: the first lake was sampled offshore from a rubber boat (64.12948 N, 51.33448 W) and the second lake was sampled from shore (64.15666 N, 51.30993 W). The river feeding one of the lakes was also sampled (64.15449 N, 51.31860 W). Samples of surface snow and subsurface snow (after removing the surface ~5 cm of snow) were collected within 50 m of the air sampling location. All water and snow samples were collected into sterile 1 L plastic bottles and then processed as described for the air samples. A total of 15 plant samples of the dominant native plant species (Table S4, Supporting Information), 5 samples of exposed soil and a rock surface sample were collected into sterile 45 mL Falcon tubes containing 25 mL of high-salt solution (25 mM sodium citrate, 10 mM EDTA, 450 g $L^{-1}$ ammonium sulphate, pH 5.2). Plant, soil and rock surface samples were collected in the surroundings of the M500 peak and Nuuk Basic research station. Both vital plant leaves, which were green, and dead plant leaves, which had lost the green pigmentation, were collected using vinyl gloves from different plants of the same species. From *Poaceae*, the seed heads were sampled as well. Different decomposition stages of dead vegetation and soil were sampled, collecting a few grams with a sterile spoon. Rock surface was sampled directly by pipetting 10 mL of the high-salt solution up-and-down three times on ~200 $cm^2$ of a rock surface, and re-collecting the liquid with the pipette. Source samples were stored at 4°C up to 4 months before processing. Details of the samples are given in Table S4 (Supporting Information).

## Co-extraction and purification of DNA and RNA

A combination of chemical and physical lysis was used directly on the SterivexTM filter units for the air, rainwater, seawater, freshwater and snowmelt samples as described by Lever *et al.* (2015).

For bacterial cell extraction from leaf surfaces, the Falcon tubes containing leaves in the high-salt solution were first shaken at 800 rpm, 4°C, for 18 h, then ultrasonicated submersed in an ultrasonication bath for 10 min, and finally vortexed (highest speed) for 1 min. Plant fragments were removed by 11-$\mu$m-pre-filtration. Bacterial cells were concentrated by centrifugation at 20 000 × *g*, 4°C, for 20 min, after which the high-salt solution was carefully removed. The cells were then washed in 1 mL of 10 × TE buffer (300 mM TrisHCl, 100 mM EDTA, pH 10, autoclaved twice), centrifuged again at 20 000 × *g*, 4°C, for 20 min and finally suspended in 1 mL of 10 × TE buffer. After extracting bacterial cells, we measured the leaf surface areas using a model LI-COR LI-3000 leaf area meter (LI-COR, Lincoln, NE). Rock-surface bacteria were similarly concentrated by centrifugation at 20 000 × *g*, 4°C, for 20 min, washed in 1 mL of 10 × TE buffer, and finally suspended in 1 mL of 10 × TE buffer. DNA was extracted from soil, decomposing vegetation, and epiphytic and epilithic bacterial cells as described in Lever *et al.* (2015).

Each air sample had a corresponding negative control that was extracted in the same way as the sample. A blank extraction was also performed for each of the extraction series. Both types of negative controls were analyzed along with the samples for the abundance of ribosomal genes and some were also analyzed for the community composition using next generation amplicon sequencing.

## Abundance of ribosomes and ribosomal genes

The abundance of ribosomes and ribosomal genes was assessed by qPCR using universal eubacterial primers Bac908F (5´-CCCGTCAATTCMTTTGAGTT-3´) and Bac1075R (5´-CACGAGCTGACGACARCC-3) and standards as described by Lever *et al.* (2015). The PCR conditions consisted of an initial denaturation for 5 min at 95°C, 45 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 20 s, with signal acquisition for 7 s either at 80°C or 82°C, with a final melting curve step between 55°C and 95°C. The efficiencies ranged between 93.1% and 116.7% ($R^2$ minimum 0.994).

For 17 out of the 22 atmospheric samples taken, the corresponding negative controls showed that the background contamination was less than one third of the 16S rRNA gene abundance, and these were considered to be true atmospheric samples. The background contamination in these samples on average accounted for 8.2% of the abundance (Min–Max: 0.2%–32.6%). 16S rRNA gene abundances were corrected for the background contamination by subtracting the values obtained for the corresponding negative control.

## Taq-encoded amplicon sequencing

Bacterial community analysis was performed on the Ion Torrent Personal Genome Machine (PGM) platform (Life Technologies TM), with 16S rRNA genes as a phylogenetic marker. All samples that had a detectable number of ribosomal gene copies were included in the analysis, along with four sampling controls spanning the different observed levels of background contamination, and four extraction controls.

The V4 region of 16S rRNA or 16S rRNA genes was amplified with universal eubacterial primers Uni519F (Ovreås *et al.* 1997) and Uni802R (Claesson *et al.* 2009) using KAPA HiFi HotStart ReadyMix DNA polymerase (Kapa Biosystems, Wilmington, MA). PCR amplifications were performed in two steps. In the first step, the reaction was run with 1.5 $\mu$L of the cDNA or DNA template in presence 0.5 U of polymerase and 200 nM of each primer. The PCR conditions consisted of an initial denaturation for 5 min at 95°, 20 cycles of 98° for 20 s, 49° for 20 s, 72° for 30 s and the final elongation for 5 min at 72°. PCR products were purified either with Agencourt AMPure XP (Beckman CoulterInc., Brea, CA) or with E-Gel® Agarose Gel Electrophoresis (Thermo Fisher Scientific Inc., Weltham, MA) in case of unspecific amplification. A total of 5 $\mu$L of the purified PCR product was then used as a template in the second PCR reaction with 1 U of KAPA HiFi HotStart DNA polymerase and barcoded Uni519F (200 nM, final concentration of both primers). The reaction conditions were initial denaturation for 5 min at 95°, 10–15 cycles of 98° for 20 s, 61° for 20 s, 72° for 30 s and the final elongation for 5 min at 72°. The PCR products were again purified with Agencourt AMPure XP and their concentration was determined on a Qubit® Fluorometer using Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific Inc., Weltham, MA). Purified, barcoded PCR products were then mixed in the desired ratio. Normally, an equimolar ratio was kept, but in cases where the PCR product concentration was very low (e.g. in negative controls or low-abundance samples), lower concentrations were used. The samples were again purified with Agencourt AMPure XP. The libraries were prepared using the Ion Plus Fragment Library Kit (Thermo Fisher Scientific Inc, Weltham, MA) and purified with Agencourt AMPure XP. Prior to sequencing, amplicons were analyzed for fragment size distribution and DNA concentration using Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA). The samples were attached to the surface of Ion Sphere particles using an Ion OneTouch 2 System with Ion PGM Template OT2 400 Kit (Thermo Fisher Scientific Inc., Weltham, MA). This resulted in 42%–85% templated ISPs that were sequenced on 316 micro-chips on the Ion Torrent PGM (Life Technologies, Carlsbad, CA). The average read length was 249–263 bp.

### Sequence analyses

Ion Torrent PGM outputs were generated as fasq files and analyzed by Mothur version 1.31.2 (Schloss *et al.* 2009). The sequencing of 16S rRNA and 16S rRNA gene libraries resulted in 1 479 763 sequences. These were sorted by barcodes, quality trimmed for sequences that contained both primers and the barcode, had a minimum length of >210 bp and average quality scores of at least 25. This resulted in 696 802 reads of sufficient quality, which were aligned against the Silva reference alignment (release 102). A pseudo-single linkage algorithm (Huse *et al.* 2010) for removing sequences that are likely due to sequencing errors. In addition, singletons appearing in only one sample were also removed as they also likely arise due to sequencing error (Reeder and Knight 2009). Chimeric sequences were detected using the Uchime reference algorithm (Edgar *et al.* 2011) and 11.2% of all sequences (3.1% of unique sequences) were deleted as chimeras prior to subsequent analysis. The sequences were classified against Silva reference and taxonomy files. We constructed operational taxonomic units (OTUs) by calculating pairwise distances between aligned sequences and clustering the sequences at 97% similarity cut off. A biom matrix data file was generated and further analyzed in R. A total of 688 OTUs that were present in any of the eight negative controls were removed from the samples prior to the analysis.

### Statistical analyses

Data analysis was performed using R version 3.2.0 in Rstudio environment version 0.99.446. Plots were prepared using the ggplot2 version 1.0.1 package, with the RColorBrewer version 1.1.2 used for generating color palettes. For data manipulations, the packages plyr version 1.8.3 and reshape2 version 1.8.3 were used. Venn diagrams were constructed using the VennDiagram version 1.6.9 package. Distances were calculated using the Bray–Curtis method, followed by clustering using the complete linkage method without presence/absence standardization. We have analyzed sequences both on raw and rarefied data (using minimum number of sequences in a sample, N = 531) and have reported the difference in OTU numbers in Tables S2–S4 (Supporting Information). The Bray–Curtis clustering was performed on both raw and rarefied sequences with results agreeing well. Considering this agreement and in order to avoid omission of valid data (McMurdie and Holmes 2014), we present results of the analysis performed on the raw data. Branch reordering in the dendrograms was done with the dendextend package version 1.1.0. Pearson's product-moment correlation was used to assess the linear correlations. Ecological diversity indices and rarefaction species richness were calculated using vegan package version 2.0–10 and fossil version 0.3.7. The diversity indices were calculated using rarefied data, and rarefaction species richness was calculated for raw data. Multi-panel figures were combined and annotated in Adobe Illustrator CS5 version 15.0.2.

### Nucleotide sequence accession

Ion Torrent sequence data obtained from this project are available at the European Nucleotide Archive under the accession number PRJEB11167.

### Meteorological data and back trajectory analyses

All meteorological data were collected by the automated monitoring station that is collocated with our air sampling site, M500. Backward trajectories were calculated using the internet-based version of HYSPLIT, in order to pinpoint the source regions of long-range-transported air masses reaching the M500 sampling station. 3-day backward trajectories were obtained at 500 m above ground level for the middle time point in each of the sampling periods using the GDAS database and the isentropic option. For the rain samples, the backward trajectories were obtained at 1000, 1500 and 2000 m in the same way.

## RESULTS AND DISCUSSION

### The abundance, diversity and source environments of airborne microorganisms

During defined sampling periods, meteorological conditions were variable (Table S1, Supporting Information). Thus, the average air temperature ranged between 3°C and 16°C and the ground surface temperature varied between 3°C and 17°C. The incoming short wave radiation was between 2 and 565 W $m^{-2}$ depending on time of day and cloud cover. The relative humidity ranged between 36% and 99% and the water vapor pressure between 0.58 and 0.96 Pa.

By quantifying the number of 16S rRNA genes in our samples and accounting for an average of 4.02 ribosomal operons per cell (Klappenbach *et al.* 2001), we determined an average bacterial concentration of $1.3\times^{*}10^{3} \pm 1.0\times10^{3}$ cells $m^{-3}$ (Fig. 1) in the atmospheric boundary layer. The boundary layer is the lowest layer of the troposphere and is strongly affected by bacterial emissions from the surface. Typically, bacterial densities in the boundary layer of temperate regions are of the order of $10^{4}$–$10^{5}$ cells $m^{-3}$ (Burrows *et al.* 2009). Bacterial densities comparable to our study were previously recorded above the boundary layer, in the so-called free troposphere (Zweifel *et al.* 2012; DeLeon-Rodriguez *et al.* 2013). The low cell densities that we found may be explained by low local emissions, due to little physical disturbances caused by human or animal activity, compared to temperate regions. In addition, the low counts may also be due to low inputs of cells through long-range dispersal. By performing a correlation analysis, we found that the atmospheric cell densities were unlikely to be driven by any of the meteorological factors. While we can exclude that the total cell numbers were underestimated due to limited sampling efficiency (Šantl-Temkiv *et al.* 2017), we cannot exclude low DNA extraction efficiency.

The composition of the airborne bacterial community, its diversity and its sources were investigated by next generation amplicon sequencing. In total, we identified 3963 OTUs at an evolutionary distance of 3%. On the DNA level, there were 47–448 OTUs and on the RNA level, 158–950 OTUs in individual samples. Despite the low cell densities in air, the diversity inferred from rarefaction curves (Fig. S1, Supporting Information) and Shannon–Wiener indices (Tables S2 and S3, Supporting Information) was comparable to the diversity that we found in epiphytic communities. In agreement with Cuthbertson *et al.* (2017), the diversity of atmospheric microbial communities in the Arctic was similar to or higher than the diversity of atmospheric microbial communities in temperate regions. At the phylum level, airborne bacteria had a similar community composition (Fig. S2, Supporting Information). The majority of OTUs of the integrated airborne microbial community was affiliated with the phylum Proteobacteria (on average 49%), followed by the phyla Actinobacteria (13%), Acidobacteria (10%), Firmicutes (6%), Chloroflexi (5%), Planctomyces (3%), Bacteroides (4%) and Cyanobacteria (3%) (Fig. 2; Fig. S2, Supporting Information), which is comparable to atmospheric communities in temperate regions (Bowers *et al.* 2009; Attard *et al.* 2012; Temkiv *et al.* 2012; DeLeon-Rodriguez *et al.* 2013; Klein *et al.* 2016; Amato *et al.* 2017) and in the Arctic (Cuthbertson *et al.* 2017).

We performed a correlation analysis to investigate whether the meteorological conditions impacted the diversity of the total community (Shannon–Wiener index, Table S2, Supporting Information) and found that the diversity correlated positively with air temperature ($r = 0.59$, $P < 0.01$, $n = 17$) and negatively with relative humidity ($r = -0.60$, $P < 0.01$, $n = 17$). Reduced relative humidity and increased air temperatures were previously shown to increase the passive release of bioaerosols into the atmosphere, which was attributed to the reduced surface tension on drying surfaces (Jones and Harrison 2004). We suggest that the bacterial diversity observed in this study builds on contributions from source communities with diverse immanent microbial communities such as soil, that preferentially release cells under dry conditions and at relatively high air temperatures.

We investigated the relative contributions of different distant and local sources to the assembly of airborne microbial communities using non-metric multi-dimensional scaling ordination. In our study, the airborne community was stable over time and was distinct from communities in potential local source environments (Figs S2 and S3, Supporting Information). In addition, airborne communities shared about half (49%) of OTUs with potential local-source communities, while 51% of the OTUs were unique to air (Fig. S4, Supporting Information). The large fraction of OTUs being unique to air could stem from insufficient sampling size and sequencing depth of the source communities. Another reason could be dispersal of these cells from distant sources, which is supported by the differences that we found between shared and unique fractions of airborne OTUs (Fig. S4, Supporting Information). In order to pinpoint the distant source environments for the cells that came through long-range transport, we calculated backward trajectories for all the air masses, from which the samples were obtained (Table S1 and Fig. S5, Supporting Information). This approach suggested that the most important distant sources were the Greenland ice sheet, the North Atlantic Ocean and terrestrial surfaces on the west coast of Greenland.

We used a cluster dendrogram based on the Bray–Curtis dissimilarity index (Fig. 2) to investigate the major local sources for airborne cells. The average atmospheric bacterial community was most similar to communities found in exposed soils and decaying plant material. Mobilization of bacterial cells from exposed dry soils must be very efficient, as exposed soils accounted for a small fraction of local surfaces. In temperate regions, plant surfaces were pinpointed as the major source of bacterial cells in the atmosphere (Bowers *et al.* 2011; Šantl-Temkiv *et al.* 2013). In the Arctic, on the contrary, Cameron *et al.* (2015) reported distant soils as the major source of microorganisms deposited on the west Greenland ice sheet, which is in agreement with our findings. Overall, our analyses suggest that atmospheric microbial communities assemble from locally aerosolized cells originating from terrestrial environments, e.g. surface soils, with a contribution of long-range transported cells from marine, glaciated and terrestrial surfaces.

## The activity potential of airborne microorganisms at the community level

Analysis of environmental rRNA provides insights into an important housekeeping function, i.e. protein synthesis catalyzed by ribosomes (Blazewicz *et al.* 2013). In order to estimate the activity potential of atmospheric communities, we used (RT-)qPCR and determined that airborne cells on average carried 585 ± 288 16S rRNA molecules $cell^{-1}$ (Fig. 1), which we used as an approximation for the number of ribosomes in the cells. Studying laboratory cultures, it has been shown that the ribosome content of cells correlates with the growth stage and with the growth rate of the specific strains. Fast-growing (copiotrophic) species, such as *Escherichia coli* contain between 7000 and 70 000 ribosomes per cell (Dennis and Bremer 2008), while slow-growing (oligotrophic) species, such as *Sphingomonas* sp., *Rickettsia* sp., *Prochlorococcus* sp. and *Synechococcus* sp. contain only between 25 and 2000 ribosomes per cell (Pang and Winkler 1994; Fegatella *et al.* 1998; Worden and Binder 2003). In the environment, the ratio of 16S rRNA to 16S rRNA genes has been used as a proxy for bacterial activity, growth rates and dormancy of microbial communities (Jones and Lennon 2010; Campbell *et al.* 2011; Placella, Brodie and Firestone 2012; Klein *et al.* 2016). Despite the facts that both extracellular DNA and rRNA sorbed to environmental matrices can bias the *in situ* 16S rRNA:16S rRNA

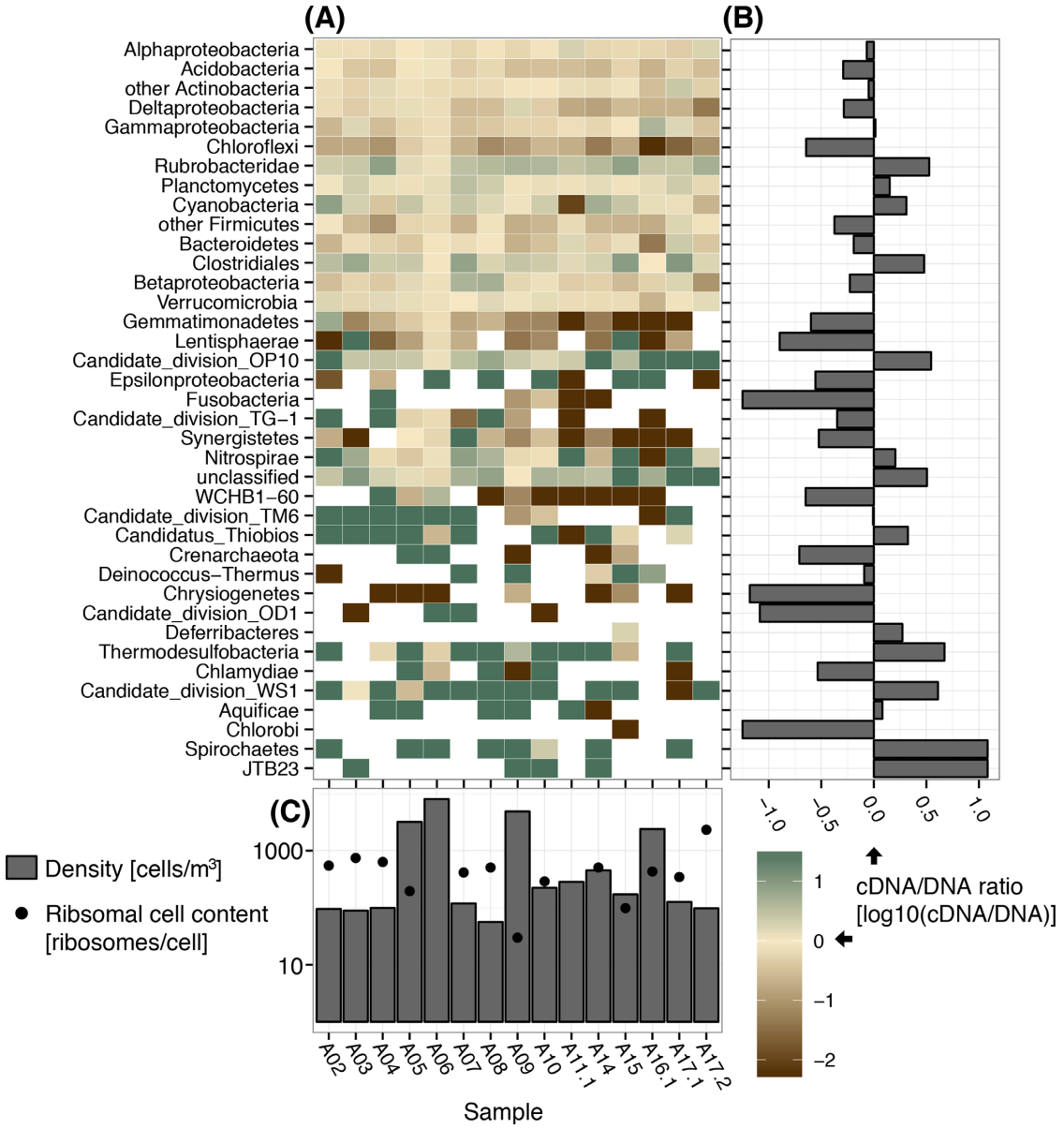


**Figure 1.** Cell concentration in air and ribosome content of cells as an indicator of *in situ* general activity potential. **A**: A heat map showing the ratio between the proportion of a clade in the active (rRNA-based) and in total (rRNA-gene-based) community for individual air samples. **B**: The ratios between the proportion of each clade in the active and in the total bacterial community, averaged for all air samples. **C**: Cell concentration in the atmosphere and cellular ribosome content at the community level in individual air samples.

gene ratio (Edgcomb *et al.* 2011; Dlott *et al.* 2015) and that dormant cell forms, such as spores, maintain a high rRNA content (Segev, Smith and Ben-Yehuda 2012), the 16S rRNA copy number per cell has been used as a measure of the *in situ* activity potential (Barnard, Osborne and Firestone 2013; Schostag *et al.* 2015; Stibal *et al.* 2015; Klein *et al.* 2016). For example, Schostag *et al.* (2015) determined the 16S rRNA:16S rRNA gene ratio of microbial communities in Arctic permafrost, which on average was 132.7 ± 33.0 16S rRNA molecules cell$^{-1}$ and was thus lower than the activity potential of the atmospheric communities in this study. Stibal *et al.* (2015) investigated cryoconite holes on the Greenland ice sheet and determined an even lower activity potential of $1.7\times10^{-5}$–57.4 16S rRNA molecules cell$^{-1}$. Placella *et al.* (2012) suggested that a high rRNA:rRNA-gene ratio was related to a rapid response of some microbial taxa to changing environmental conditions. This idea is supported by a study of Barnard *et al.* (2013) investigating the effect of water availability on ribosome content of bacterial communities in arid soil. The authors found that the rRNA content of desiccated communities (360–570 16S rRNA molecules cell$^{-1}$) doubled after rewetting (630–1130 16S rRNA molecules cell$^{-1}$). The atmospheric communities assessed in our study exhibited a range of values typical for arid soil communities during both low and high water activity (Min–Max: 30–2320, Fig. 1C) (Barnard, Osborne and Firestone 2013) and much higher values than what was reported for cryoconite holes and soil communities (Dlott *et al.* 2015; Schostag *et al.* 2015; Stibal *et al.* 2015). Overall, these comparisons clearly indicate that some airborne microorganisms in the Arctic have a strong potential for rapid protein synthesis either in the atmosphere or after their deposition to terrestrial environments.

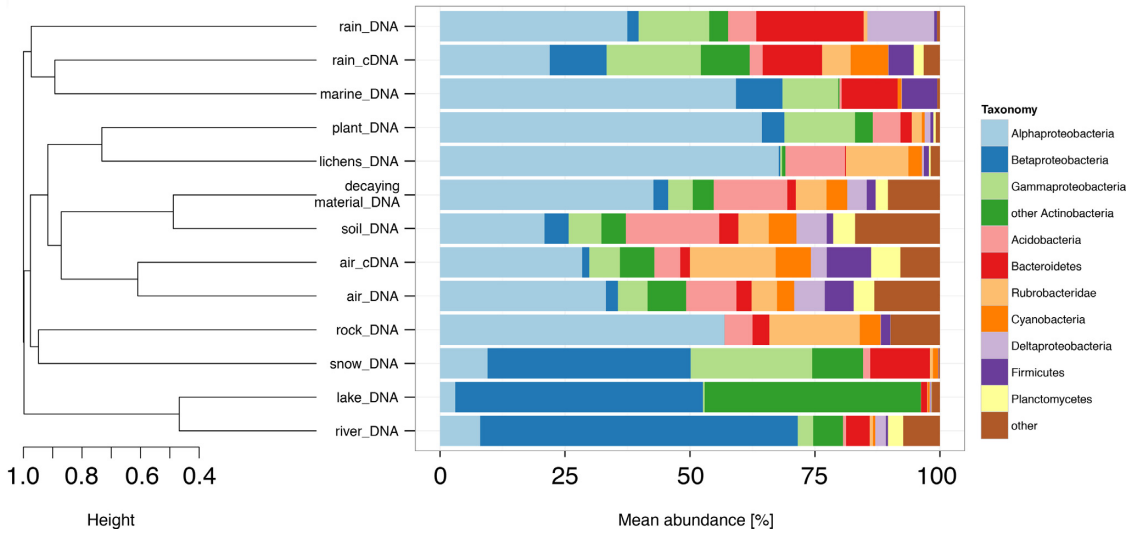


**Figure 2.** The similarity and composition of microbial communities in the atmosphere and in source environments. The cluster dendrogram (left) is based on Bray–Curtis sequence similarity of 16S rRNA molecules and 16S rRNA genes found in the microbial communities of air, rain and local source samples. Bar charts (right) show the relative abundance of microbial clades in each of the environments.

As water is a major limiting factor for microbial activity in the atmosphere (Potts 1994), we measured water vapor pressure and RH as determinants of water activity. Based on the recently established microbial activity threshold of 60% RH (Stevenson *et al.* 2015), we conclude that periods with sufficiently high water activity were sporadic during our sampling campaign (Fig. S6 and Table S1, Supporting Information). We found that the 16S rRNA content in cells of airborne communities positively correlated with water vapor pressure ($r = 0.52$, $P < 0.05$, $n = 16$). Based on this correlation, we suggest that a part of the 16S rRNA molecules detected in airborne cells could have been synthesized in the atmosphere when the water activity was sufficiently high. The synthesis of ribosomes by airborne cells has been shown in a laboratory study of cells of *Sphingomonas aerolata* that were supplied with common atmospheric volatile organic compounds (Krumins *et al.* 2014).

## The activity potential of specific microbial taxa

Microorganisms belonging to different taxa differ in capacity to engage, survive or benefit from dispersal (Hanson *et al.* 2012). Thus, the atmosphere acts as a selective barrier for microbial dispersal (Šantl-Temkiv *et al.* 2013), favoring taxa that maintain activity during their dispersal (Krumins *et al.* 2014) while killing others by e.g. UV radiation or desiccation (Joly *et al.* 2015). Taxa-related properties that are responsible for differential dispersal rates and dispersal success, i.e. their survival during aerosolization, aeolian transport and subsequent colonization, are often neglected (Nemergut *et al.* 2013). Given the high activity potential of the atmospheric communities analyzed, we examined whether specific taxa are characterized by a high activity potential or a high activity potential is a general feature of airborne microorganisms. We found evidence that supports the former. Thus, we observed that Proteobacteria, despite their high abundance in the total community, i.e. their DNA fraction, contributed less to the active community, as evidenced by their RNA fraction of 40%. In the active community, Actinobacteria (23%), Firmicutes (8%), Cyanobacteria (8%) and Planctomycetes (4%) were more prominent (Fig. S2, Supporting Information). This result was consistent across samples, in particular for Rubrobacteridae, Clostridiales and Cyanobacteria, which had a high activity potential in >80% of the samples (Fig. 1A).

We studied the relationship between the mean relative frequency of 16S rRNA and 16S rRNA genes for each OTU and found a significant positive correlation between the two ($r = 0.47$, $P << 0.001$, $n = 3802$), indicating that the activity of an individual OTU is generally related to its abundance (Fig. 3). We found that 1262 OTUs were shared between the active and the total airborne communities and OTUs in the active community represented 67% of OTUs in the total community. Among shared OTUs, the ratio between 16S rRNA molecules and 16S rRNA genes was on average 1.5 (Min-Max: 0.009–93). A couple of studies have previously addressed activity of microorganisms in air and cloud droplets using the same approach (Klein *et al.* 2016; Amato *et al.* 2017). While Klein *et al.* (2016) found a similar range of values (Min-Max: 0.002–122), Amato *et al.* (2017) identified a range mostly spanning between 0.1 and 10, but sometimes as high as 11 760. We identified 481 OTUs (35%) with a 16S rRNA:16S rRNA-gene ratio larger than 1, which is commonly used as an index of taxa with an elevated activity potential (Campbell *et al.* 2011; Placella, Brodie and Firestone 2012; Barnard, Osborne and Firestone 2013). A large proportion of these OTUs (13.5%) belonged to the deepest branching actinobacterial lineage, i.e. subclass Rubrobacteridae. In most cases, Rubrobacteridae OTUs affiliated to the genus *Conexibacter*. Members of Rubrobacteridae are common in hot and arid environments (Bull 2011) and were shown to exhibit an anticipatory life strategy when exposed to extreme desiccation stress (Barnard, Osborne and Firestone 2013), regaining metabolic activity within 15–60 min after rewetting (Placella, Brodie and Firestone 2012). In a similar way, the high ribosome content that we found typical for airborne Rubrobacteridae could allow these cells to respond fast to a sudden availability of water and nutrients.

Aside from Rubrobacteridae, OTUs belonging to the orders Rhodospirillales (Alphaproteobacteria) and Planctomycetales were commonly enriched in the portion of the community with a high activity potential. While OTUs affiliating with Rhodospirillales represented a large fraction of many source microbial communities (8%–92% on plant surfaces, 4%–13% in soil, 54%

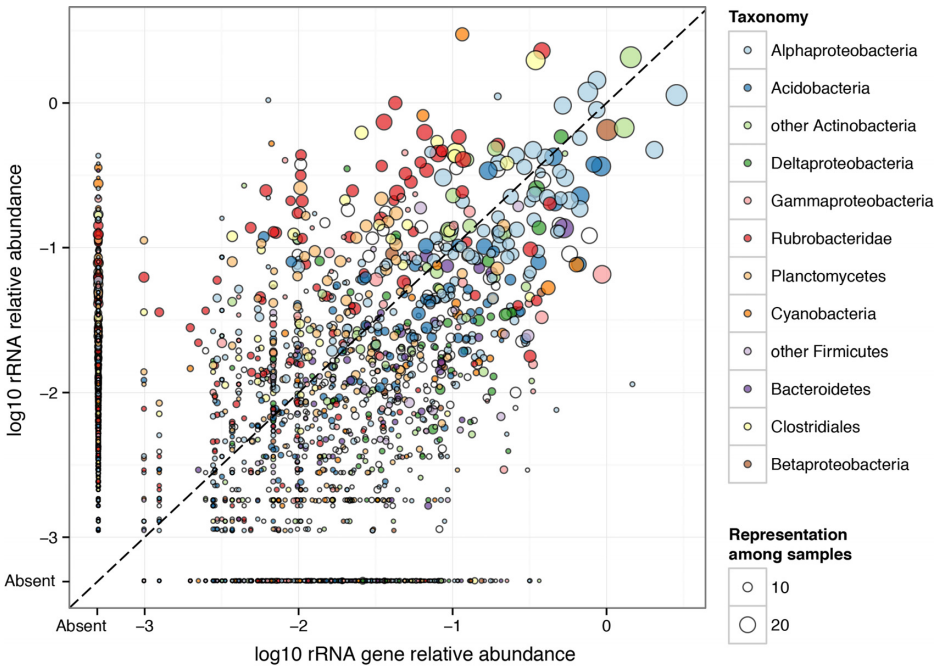


**Figure 3.** The proportion of each airborne OTU in the average active community (y-axis) as a function of its proportion in the average total community (x-axis). OTUs are considered active, if their rRNA relative abundance was higher than their rRNA gene relative abundance, hence the 1:1 line separates the OTUs that are enriched in the active (left upper part of the chart) from the OTUs that are enriched in the total community (right lower part of the chart).

on rock surface), the OTUs affiliating to Planctomycetales were most common in soil (2% of the total community). Airborne Rhodospirillales were previously found to have an elevated activity potential in the atmosphere (Klein *et al.* 2016; Amato *et al.* 2017).

Another order that was enriched in the active community (Fig. 3) was Clostridiales (Firmicutes). Its OTUs were mostly affiliated with the genus *Desulfurispora*, a thermophilic, spore-forming, sulphate-reducing obligate anaerobe, whose activity in the atmosphere is unlikely. Members of Clostridiales exhibit a variable sensitivity to oxygen. While oxygen is highly toxic for some strains, others are able to maintain their viability during short-term exposure to aerobic conditions (Brioukhanov and Netrusov 2007). It is not clear whether cells of *Desulfurispora* that we detected were present as endospores or as vegetative cells. In case that these cells remained viable in the presence of oxygen, their enrichment may suggest an anticipatory life strategy.

Finally, we looked into the activity potential of genera belonging to known ice-nucleation active (INA) bacteria, which can stimulate their own deposition from the atmosphere by ice formation (Amato *et al.* 2015) and are considered relevant for atmospheric processes including cloud and rain formation (Möhler *et al.* 2007). In the rain sample, we detected active OTUs that belonged to the families Pseudomonadacae and Xanthomonadacae, which contain most of the documented INA bacterial species. One OTU that affiliated to *Pseudomonas* sp. accounted for 3.6% of the active community in rain. OTUs belonging to the genus *Pseudomonas* were detected in four air samples as well, but always only in the total community and not in the active fraction. Thus, the cells of *Pseudomonas* sp. with a high activity potential were rare in air but were enriched in rain. This is in line with as study performed by Amato *et al.* (2017), who described a high activity potential for *Pseudomonas* sp. in cloud water. INA *Pseudomonas* sp. were shown in cloud-simulation experiments to preferentially partition from dry air into atmospheric water (Amato *et al.* 2015) and were found to associate with the water cycle both in the atmosphere and in terrestrial environments (Morris *et al.* 2008).

## CONCLUSIONS

Our findings suggest that the Arctic atmosphere harbors a low abundance of diverse microbial cells with a high activity potential. These cells most likely enter the Arctic atmospheric boundary layer from local terrestrial environments, mainly soils and decaying vegetation, or through long-range transport. Many of the taxa with an elevated activity potential were commonly affiliated with the subclass Rubrobacteridae, which is known for containing many desiccation resistant species. We suggest that by containing a high number of 16S rRNA molecules, which is a proxy for the number of ribosomes, these cells can actively participate in atmospheric processes as well as have a fast response after deposition, when they are exposed to new environmental conditions. Deciphering the extent and type of microorganisms engaging in aeolian dispersal is necessary for understanding and quantifying the impact of dispersal on community assembly and dynamics in the Arctic.

## SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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## REFERENCES

Amato P, Joly M, Besaury L *et al.* Active microorganisms thrive among extremely diverse communities in cloud water. *PLoS One* 2017;**12**:e0182869.

Amato P, Joly M, Schaupp C *et al.* Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber. *Atmos Chem Phys* 2015;**15**:6455–65.

Amato P, Parazols M, Sancelme M *et al.* Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: major groups and growth abilities at low temperatures. *FEMS Microbiol Ecol* 2007;**59**:242–54.

Attard E, Yang H, Delort A-M *et al.* Effects of atmospheric conditions on ice nucleation activity of Pseudomonas. *Atmos Chem Phys* 2012;**12**:10667–77.

Barberán A, Henley J, Fierer N *et al.* Structure, inter-annual recurrence, and global-scale connectivity of airborne microbial communities. *Sci Total Environ* 2014;**487**:187–95.

Barberán A, Ladau J, Leff JW *et al.* Continental-scale distributions of dust-associated bacteria and fungi. *Proc Natl Acad Sci* 2015;**112**:5756–61.

Barnard RL, Osborne CA, Firestone MK. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *ISME J* 2013;**7**:2229–41.

Bauer H, Kasper-giebl A, Lo M. The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. *Atmospheric Res* 2002;**64**:109–19.

Blazewicz SJ, Barnard RL, Daly RA *et al.* Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J* 2013;**7**:2061–8.

Bowers RM, Lauber CL, Wiedinmyer C *et al.* Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. *Appl Environ Microbiol* 2009;**75**:5121–30.

Bowers RM, McLetchie S, Knight R *et al.* Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J* 2011;**5**:601–12.

Bradley JA, Singarayer JS, Anesio AM. Microbial community dynamics in the forefield of glaciers. *Proc R Soc London B Biol Sci* 2014;**281**:20140882.

Brioukhanov AL, Netrusov AI. Aerotolerance of strictly anaerobic microorganisms and factors of defense against oxidative stress: A review. *Appl Biochem Microbiol* 2007;**43**:567–82.

Bull AT. *Actinobacteria of the Extremobiosphere. Extremophiles Handbook*. Tokyo Japan: Springer, 2011, 1203–40.

Burrows S, Elbert W, Lawrence M *et al.* Bacteria in the global atmosphere - Part 1: Review and synthesis of literature data for different ecosystems. *Atmospheric Chemistry and Physics* 2009;**9**:9263–9280.

Burrows SM, Rayner PJ, Butler T *et al.* Estimating bacteria emissions from inversion of atmospheric transport: sensitivity to modelled particle characteristics. *Atmos Chem Phys* 2013;**13**:5473–88.

Cameron KA, Hagedorn B, Dieser M *et al.* Diversity and potential sources of microbiota associated with snow on western portions of the Greenland Ice Sheet. *Environ Microbiol* 2015;**17**:594–609.

Campbell BJ, Yu L, Heidelberg JF *et al.* Activity of abundant and rare bacteria in a coastal ocean. *Proc Natl Acad Sci U S A* 2011;**108**:12776–81.

Cho BC, Jang GIL. Active and diverse rainwater bacteria collected at an inland site in spring and summer 2011. *Atmos Environ* 2014;**94**:409–16.

Claesson MJ, O'Sullivan O, Wang Q *et al.* Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS One* 2009;**4**:e6669.

Cuthbertson L, Amores-Arrocha H, Malard L *et al.* Characterisation of arctic bacterial communities in the air above svalbard. *Biology (Basel)* 2017;**6**:29.

DeLeon-Rodriguez N, Lathem TL, Rodriguez-R LM *et al.* Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. *Proc Natl Acad Sci U S A* 2013;**110**:2575–80.

Deguillaume L, Leriche M, Amato P *et al.* Microbiology and atmospheric processes: chemical interactions of primary biological aerosols. *Biogeosciences* 2008;**5**:1073–84.

Delort A-M, Vaïtilingom M, Amato P *et al.* A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. *Atmos Res* 2010;**98**:249–60.

Dennis PP, Bremer H. Modulation of chemical composition and other parameters of the cell at different exponential growth rates. *EcoSal Plus* 2008;**3**: DOI: 10.1128/ecosal.5.2.3

Dlott G, Maul JE, Buyer J *et al.* Microbial rRNA:rRNA gene ratios may be unexpectedly low due to extracellular DNA preservation in soils. *J Microbiol Methods* 2015;**115**:112–20.

Edgar RC, Haas BJ, Clemente JC *et al.* UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011;**27**:2194–200.

Edgcomb VP, Beaudoin D, Gast R *et al.* Marine subsurface eukaryotes: the fungal majority. *Environ Microbiol* 2011;**13**:172–83.

Fegatella F, Lim J, Kjelleberg S *et al.* Implications of rRNA operon copy number and ribosome content in the marine oligotrophic ultramicrobacterium Sphingomonas sp. strain RB2256. *Appl Envir Microbiol* 1998;**64**:4433–8.

Feike J, Jürgens K, Hollibaugh JT *et al.* Measuring unbiased metatranscriptomics in suboxic waters of the central Baltic Sea using a new in situ fixation system. *ISME J* 2012;**6**:461–70.

Franzetti A, Gandolfi I, Gaspari E *et al.* Seasonal variability of bacteria in fine and coarse urban air particulate matter. *Appl Microbiol Biotechnol* 2011;**90**:745–53.

Hanson CA, Fuhrman JA, Horner-Devine MC *et al.* Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat Rev Microbiol* 2012;**10**:497–506.

Hara K, Zhang D. Bacterial abundance and viability in long-range transported dust. *Atmos Environ* 2012;**47**:20–5.

Harding T, Jungblut AD, Lovejoy C *et al.* Microbes in high arctic snow and implications for the cold biosphere. *Appl Environ Microbiol* 2011;**77**:3234–43.

Hill KA, Shepson PB, Galbavy ES *et al.* Processing of atmospheric nitrogen by clouds above a forest environment. *Journal of Geophysical Research* 2007;**112**:D11301.

Huse SM, Welch DM, Morrison HG *et al.* Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environ Microbiol* 2010;**12**:1889–98.

Jansson JK, Taş N. The microbial ecology of permafrost. *Nat Rev Microbiol* 2014;**12**:414–25.

Joly M, Amato P, Sancelme M *et al.* Survival of microbial isolates from clouds toward simulated atmospheric stress factors. *Atmos Environ* 2015;**117**:92–8.

Jones AM, Harrison RM. The effects of meteorological factors on atmospheric bioaerosol concentrations - A review. *Sci Total Environ* 2004;**326**:151–80.

Jones SE, Lennon JT. Dormancy contributes to the maintenance of microbial diversity. *Proc Natl Acad Sci U S A* 2010;**107**:5881–6.

Kellogg CA, Griffin DW. Aerobiology and the global transport of desert dust. *Trends Ecol Evol* 2006;**21**:638–44.

Klappenbach JA., Saxman PR., Cole JR *et al.* rrndb: the Ribosomal RNA Operon Copy Number Database, *Nucleic Acids Res* 2001;**29**:181–4

Klein AM, Bohannan BJM, Jaffe DA *et al.* Molecular evidence for metabolically active bacteria in the atmosphere. *Front Microbiol* 2016;**7**, DOI: 10.3389/fmicb.2016.00772.

Krumins V, Mainelis G, Kerkhof LJ *et al.* Substrate-Dependent rRNA Production in an Airborne Bacterium. *Environ Sci Technol Lett* 2014;**1**:376–81.

Lever MA, Torti A, Eickenbusch P *et al.* A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. *Front Microbiol* 2015;**6**:476.

McMurdie PJ, Holmes S. Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* 2014;**10**:e1003531.

Morris CE, Sands DC, Vinatzer BA *et al.* The life history of the plant pathogen Pseudomonas syringae is linked to the water cycle. *ISME J* 2008;**2**:321–34.

Musilova M, Tranter M, Bennett SA *et al.* Stable microbial community composition on the Greenland Ice Sheet. *Front Microbiol* 2015;**6**:193.

Möhler O, DeMott PJ, Vali G *et al.* Microbiology and atmospheric processes: the role of biological particles in cloud physics. *Biogeosciences* 2007;**4**:1059–71.

Nemergut DR, Schmidt SK, Fukami T *et al.* Patterns and processes of microbial community assembly. *Microbiol Mol Biol Rev* 2013;**77**:342–56.

Ovreås L, Forney L, Daae FL *et al.* Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl Environ Microbiol* 1997;**63**:3367–73.

Pang H, Winkler HH. The concentrations of stable RNA and ribosomes in Rickettsia prowazekii. *Mol Microbiol* 1994;**12**:115–20.

Pearce DA, Alekhina IA, Terauds A *et al.* Aerobiology over antarctica - a new initiative for atmospheric ecology. *Front Microbiol* 2016;**7**:16.

Pearce DA, Bridge PD, Hughes KA *et al.* Microorganisms in the atmosphere over Antarctica. *FEMS Microbiol Ecol* 2009;**69**:143–57.

Placella SA, Brodie EL, Firestone MK. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proc Natl Acad Sci U S A* 2012;**109**:10931–6.

Potts M. Desiccation tolerance of prokaryotes. *Microbiol Rev* 1994;**58**:755–805.

Reeder J, Knight R. The "rare biosphere": a reality check. *Nat Methods* 2009;**6**:636–7.

Schloss PD, Westcott SL, Ryabin T *et al.* Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009;**75**:7537–41.

Schostag M, Stibal M, Jacobsen CS *et al.* Distinct summer and winter bacterial communities in the active layer of Svalbard permafrost revealed by DNA- and RNA-based analyses. *Front Microbiol* 2015;**6**:399.

Segev E, Smith Y, Ben-Yehuda S. RNA dynamics in aging bacterial spores. *Cell* 2012;**148**:139–49.

Smith DJ, Timonen HJ, Jaffe DA *et al.* Intercontinental dispersal of bacteria and archaea by transpacific winds. *Appl Environ Microbiol* 2013;**79**:1134–9.

Stevenson A, Cray JA, Williams JP *et al.* Is there a common water-activity limit for the three domains of life? *ISME J* 2015;**9**:1333–51.

Stibal M, Schostag M, Cameron KA *et al.* Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environ Microbiol Rep* 2015;**7**:293–300.

Šantl-Temkiv T, Amato P, Gosewinkel Karlson U *et al.* A high volume impinger for the study of concentration, viability, activity, and ice nucleation activity of airborne microorganisms. *Environ Sci Technol* 2017;**51**:11224–34.

Šantl-Temkiv T, Finster K, Dittmar T *et al.* Hailstones: a window into the microbial and chemical inventory of a storm cloud. *PLoS One* 2013;**8**:e53550.

Temkiv TŠ, Finster K, Hansen BM *et al.* The microbial diversity of a storm cloud as assessed by hailstones. *FEMS Microbiol Ecol* 2012;**81**:684–95.

Vaïtilingom M, Deguillaume L, Vinatier V *et al.* Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. *Proc Natl Acad Sci U S A* 2013;**110**:559–64.

Wilkinson DM, Koumoutsaris S, Mitchell EAD *et al.* Modelling the effect of size on the aerial dispersal of microorganisms. *J Biogeogr* 2012;**39**:89–97.

Womack AM, Bohannan BJM, Green JL. Biodiversity and biogeography of the atmosphere. *Philos Trans R Soc Lond B Biol Sci* 2010;**365**:3645–53.

Worden AZ, Binder BJ. Growth regulation of rRNA content in Prochlorococcus and synerchococcus (marine Cyanobacteria) measured by whole-cell hybridization of rRNA-targeted peptide nucleid acids. *J Phycology* 2003;**534**:527–34.

Xiang S-R, Shang T-C, Chen Y *et al.* Deposition and postdeposition mechanisms as possible drivers of microbial population variability in glacier ice. *FEMS Microbiol Ecol* 2009;**70**:165–76.

Zweifel UL, Hagström Å, Holmfeldt K *et al.* High bacterial 16S rRNA gene diversity above the atmospheric boundary layer. *Aerobiologia (Bologna)* 2012;**28**:481–98.