



Metatranscriptomic exploration of microbial functioning in clouds

Pierre Amato, Ludovic Besaury, Muriel Joly, Benjamin Penaud, Laurent Deguillaume, Anne-Marie Delort

► To cite this version:

Pierre Amato, Ludovic Besaury, Muriel Joly, Benjamin Penaud, Laurent Deguillaume, et al.. Metatranscriptomic exploration of microbial functioning in clouds. Scientific Reports, 2019, 9 (1), pp.4383. 10.1038/s41598-019-41032-4 . hal-02098642

HAL Id: hal-02098642

<https://hal.science/hal-02098642>

Submitted on 6 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

SCIENTIFIC REPORTS

OPEN

Metatranscriptomic exploration of microbial functioning in clouds

Pierre Amato¹, Ludovic Besaury¹, Muriel Joly¹, Benjamin Penaud¹, Laurent Deguillaume² & Anne-Marie Delort¹

Received: 5 September 2018

Accepted: 27 February 2019

Published online: 13 March 2019

Clouds constitute the uppermost layer of the biosphere. They host diverse communities whose functioning remains obscure, although biological activity potentially participates to atmospheric chemical and physical processes. In order to gain information on the metabolic functioning of microbial communities in clouds, we conducted coordinated metagenomics/metatranscriptomics profiling of cloud water microbial communities. Samples were collected from a high altitude atmospheric station in France and examined for biological content after untargeted amplification of nucleic acids. Living microorganisms, essentially bacteria, maintained transcriptional and translational activities and expressed many known complementary physiological responses intended to fight oxidants, osmotic variations and cold. These included activities of oxidant detoxification and regulation, synthesis of osmoprotectants/cryoprotectants, modifications of membranes, iron uptake. Consistently these energy-demanding processes were fueled by central metabolic routes involved in oxidative stress response and redox homeostasis management, such as pentose phosphate and glyoxylate pathways. Elevated binding and transmembrane ion transports demonstrated important interactions between cells and their cloud droplet chemical environments. In addition, polysaccharides, potentially beneficial for survival like exopolysaccharides, biosurfactants and adhesins, were synthesized. Our results support a biological influence on cloud physical and chemical processes, acting notably on the oxidant capacity, iron speciation and availability, amino-acids distribution and carbon and nitrogen fates.

The outdoor atmosphere harbors diverse microbial assemblages composed of bacteria, fungi and viruses (e.g.¹) whose functioning remains largely unexplored. While the occasional presence of Human pathogens or opportunists can cause potential hazard^{2,3}, in general the vast majority of airborne microbes originate from natural environments like soil or plants, with large spatial and temporal variations of biomass and biodiversity (e.g.^{4,5}). Once ripped off and aerosolized from surfaces by mechanical disturbances such as those generated by wind, raindrop impacts or water bubbling^{6,7}, microbial cells are transported upward by turbulent fluxes^{8,9}. They remain aloft for an average of ~3 days¹⁰, a time long enough for being transported across oceans and continents^{11–13} until being finally deposited, eventually helped by water condensation and precipitation processes; microbial aerosols themselves can contribute to form clouds and trigger precipitation by serving as cloud condensation nuclei (CCN)¹⁴ and ice nuclei (IN)^{15,16}.

Living airborne microorganisms may end up concretizing aerial dispersion by colonizing their new habitat¹⁷, provided that they survive their journey from emission to deposition. Bacterial survival is indeed naturally impaired during atmospheric transport^{18,19}, but a fraction remains viable^{20,21}. At high altitude, the peculiar environments offered by cloud droplets are thus regarded in some aspects as temporary microbial habitats, providing water and nutrients to airborne living cells^{22–24}. In addition, the detection of low levels of heterotrophy²⁵ raised questions about microbial functioning in cloud water and its potential influence on the chemical reactivity of these complex and dynamic environments^{24,26}. The metabolic functioning of microbial cells in clouds is still albeit unknown, while fundamental for apprehending microbial life conditions during long distance aerial transport and their geochemical and ecological impacts.

Within the last decade, coordinated metagenomics and metatranscriptomics studies provided new insights into microbial ecosystems' functioning and the relationships that microorganisms maintain with their environment. These were pictured in soil²⁷, ocean^{28,29}, human gut³⁰ and elsewhere^{31,32}. In the atmosphere, though, microbial gene expression and metabolic functioning remain largely unexplored, in part due to low biomass and sampling difficulties. So far, metagenomics confirmed high fungal, bacterial and viral biodiversity^{33–36}, whereas

¹Université Clermont Auvergne, CNRS, SIGMA Clermont, ICCF, F-63000, Clermont-Ferrand, France. ²Université Clermont Auvergne, CNRS, LaMP, F-63000, Clermont-Ferrand, France. Correspondence and requests for materials should be addressed to P.A. (email: pierre.amato@uca.fr)

targeted genomics/transcriptomics towards ribosomal genes supported earlier findings about the maintenance of metabolic activity in aerosols^{37,38}, and in clouds⁵; Alpha- and Gamma-Proteobacteria in particular were highlighted. Consistently, in atmospheric chamber airborne bacteria were demonstrated to react to the presence of carbon substrate by regulating ribosomal gene expression³⁹.

Here we aimed at specifying microbial activity in clouds. We performed a comparative combined metatranscriptomics/metagenomics analysis to explore their metabolic and physiological functioning, their potential interactions with cloud water chemical environment, and to examine the constraints imposed by cloud environments to living microorganisms. Cloud water samples were collected from puy de Dôme Atmospheric station (1465 m a.s.l., France), and whole metagenomes and metatranscriptomes were amplified and explored for biodiversity and biological functions. Comparative analysis highlighted a diverse biological system driven by prokaryotes. Metabolism was seemingly directed for a large part toward acclimation to a demanding environment, including elevated oxidants and low temperatures. This gives an unprecedented picture of microbial life conditions in clouds and specifies possible biological impacts on the chemical reactivity.

Material and Methods

Sample collection. Cloud water was collected from the instrumental platform situated on the roof of the meteorological station at the summit of puy de Dôme Mountain (1465 m a.s.l., 45.772°N, 2.9655°E, France). Protocols similar as in Amato *et al.*⁵ were used. The volumes of water collected after sampling periods of five consecutive hours were immediately processed within the station's microbiology facility. Subsamples for total cell counts and chemical analyses were first collected and the remaining volumes were filtered through 0.22 µm porosity (MoBio 14880-50-WF). Filters were then cut in halves, transferred into ca. 5 mL of RNA Later (Sigma-Aldrich, Saint-Louis, MO, USA) and stored at −80 °C until being further processed.

Meteorological data, backward trajectories, cell counts and chemical analyses. Protocols similar as in Amato *et al.*⁵ were used for characterizing the samples. Briefly, meteorological data were provided by puy de Dôme meteorological station, backward air mass trajectories were generated using HYSPLIT⁴⁰, ion concentrations were measured by ion chromatography (Dionex, Sunnyvale, CA, USA), and cells counts were performed by flow cytometry (BD FACS Calibur, Franklin Lakes, NJ, USA) on SYBR-Green stained samples (Molecular Probes Inc., Eugene, OR, USA).

Ice nucleation assay. The concentration of ice nucleating particles (INP) in the cloud water samples were examined by droplet freezing assay, as in Joly *et al.*⁴¹. Freshly collected samples were distributed into volumes of 20 µL in 0.2 mL microtubes and exposed to decreasing temperature from 0 °C to −10 °C in a cryobath (Julabo F34-ED), and the cumulative concentration of INP at each temperature was calculated (see Joly *et al.*, 2014 for details).

Nucleic acids extraction, amplification and sequencing. DNA and RNA were extracted using MoBio PowerWater isolation kits (now Qiagen, Hilden, Germany) following manufacturer's recommendations, from dedicated filter halves kept at −80 °C in RNA Later. We attempted to generate sequencing libraries directly from DNA extracts and cDNA extracts; these could not be validated by the quality controls due to low amounts of material (0.5–0.7 ng DNA/µL). Hence, untargeted amplification was performed: whole metagenomes (MG) and whole metatranscriptomes (MT) were amplified by multiple displacement amplification (MDA) of genomic DNA and total RNAs, from volumes of 10 µL of the corresponding extracts using REPLI-g Cell WGA & WTA kit (Qiagen).

Tropospheric clouds are among the lowest biomass environments on Earth, and sampling large volumes of cloud water within a short timeframe is still not an option, so the only alternative for accessing the nucleic acid sequences was amplification. Although MDA is known to introduce biases in the relative abundance of sequences in complex samples⁴², this provided valuable information in low biomass environments such as sediments or permafrost^{43,44}. Shotgun libraries were generated using Nextera XT DNA Sample Preparation Kit. These were controlled for quality on Agilent High Sensitivity microarray, and mixed in an equimolar pool for sequencing (2 × 300 bp paired-end Illumina MiSeq; Genoscreen, Lille, France). Experimental blank controls led to exploitable library only for MT. The corresponding sequence files were deposited to NCBI's Sequence Read Archive (BioProject ID PRJEB25763) with the sample accession numbers ERS2351639 to ERS2351645.

Sequence processing and data analysis. All sequences were processed using free software and custom Perl scripts, using the regional calcul center Mesocentre Clermont Auvergne (in general 32 CPUs with 128 Go of RAM were used here), and locally under Linux Ubuntu operated computers. Sequences were first quality controlled (FastQC version 0.11.3; Babraham Bioinformatics) and trimmed for removing low quality ends, sequences < 40 bp or containing ambiguous bases using PRINSEQ-LITE⁴⁵. Mate pairs were assembled using PANDASEQ Assembler version 2.8⁴⁶; final sequence length was ~300 bp. Potential contaminant sequences were removed by alignment against the experimental control using BWA-MEM⁴⁷ and SAMtools⁴⁸.

Taxonomic annotations of prokaryotes and eukaryotes were obtained using BLASTN⁴⁹ against the ribosomal database SILVA 119.1 SSURef Nr99⁵⁰; the best hits with an alignment e-value < 0.01 were recovered. Chloroplasts and mitochondria sequences were manually removed from the analysis.

Functional annotations were performed using BLASTX⁴⁹ against the protein database UniprotKB⁵¹, restricted to cellular organisms (Archaea, Bacteria and Eukaryotes) and viruses. The best hits with an alignment e-value < 10^{−4} were collected. Data were then analyzed using gene ontologies (GO IDs terms defined by the Gene Ontology Consortium)^{52–54} associated with UniprotKB identifiers and using notably the following informatics

Sample Identifier	Cloud 20141117-1	Cloud 20141117-2	Cloud 20141117-3
Date	17 Nov 2014	17–18 Nov 2014	18 Nov 2014
Local sampling time	3:22 pm–8:22 pm	8:23 pm–1:23 am	3:50 am–8:50 am
Volume of sample processed (mL)	160	120	90
Total cell concentration in cloud water (N mL ⁻¹) ^(a)	$(2.8 \pm 0.7) \times 10^3$	$(2.8 \pm 0.7) \times 10^3$	$(2.7 \pm 0.5) \times 10^3$
Inferred total cell concentration in cloud air (N m ⁻³) ^{(a) (b)}	$(8.3 \pm 2.2) \times 10^2$	$(6.3 \pm 1.6) \times 10^2$	$(4.5 \pm 0.9) \times 10^2$
Ambient temperature during sampling (°C)	0.7	0.6	0.4
Wind speed (m s ⁻¹)	7.4	9.9	10.3
pH	5.4	5.0	4.9
Ion concentrations (μM)^(a)			
SO ₄ ²⁻	5.7 ± 1.9	7.0 ± 0.4	3.0 ± 1.1
NO ₃ ⁻	4.5 ± 0.15	9.2 ± 0.7	8.6 ± 0.1
Cl ⁻	0.7 ± 0.1	1.0 ± 0.4	1.0 ± 0.1
NH ₄ ⁺	15.4 ± 3.6	20.6 ± 2.0	50.3 ± 3.5
Na ⁺	4.8 ± 5.5	11.3 ± 14.2	13.7 ± 2.0

Table 1. Main characteristics of the cloud water samples. ^aMean of triplicate measurements ± standard error. ^bConsidering sampling at the constant air flow rate of 108 m³ of air h⁻¹.

tools and databases: Protein Information Resource PIR⁵⁵, REVIGO⁵⁶, GOSlimViewer⁵⁷, AmiGO⁵³, and KEGG^{58–60}. In total, 2670 to 3373 unique GO terms were found in each cloud water MG or MT dataset.

For comparative analysis, other sequence files were collected from literature studies selected for including both metagenomes and metatranscriptomes (Table S1), and these were reprocessed similarly as our data using our bioinformatic pipeline. When necessary, data were rarefied to ~400,000 sequences to remain consistent between datasets. Rarefaction may have decreased the sensitivity of the intercomparison, but this is acceptable in the frame of our descriptive study looking for large differences originating from multiple features (GO terms), rather than a direct comparison of the abundance of specific sequences⁶¹. GO terms represented by ≥0.05% of the sequences in at least one MT dataset were considered (823 GO terms in total; Table S2). Among those, 724 occurred in cloud MT and 764 in MG, and 699 were present in both MT and the corresponding MG: 317 of these related to a Molecular Function (MF), 313 to a Biological Process (BP), and 69 to a Cellular Component (CC). Their corresponding rank-abundance in MT and relative abundance respect to MG are shown in Figs S1 and S2. The GO were finally manually classified according to functional categories, and their relative level of expression was assessed through their relative contribution in MT (i.e. %MT), and after normalization to their relative representation in MG (i.e. %MT/%MG). This approach is commonly used in metatranscriptomics studies^{30,62}, and it has the advantage to overcome problems related to unknown gene copy numbers.

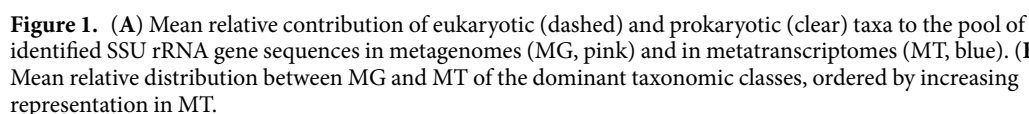
Results and Discussion

The main meteorological, chemical and biological characteristics of the cloud water samples are presented in Table 1.

Samples were collected at ambient temperatures close to freezing, under moderate to strong winds with bursts up to 14 m/s; liquid water content varied between 0.16 and 0.47 mL m⁻³. Air masses originated from West (Atlantic Ocean; Fig. S3), and the chemical signature of water samples (pH, dissolved ion concentrations) was consistently typical of “Marine” type clouds at this site⁶³. Total cell concentration was $\sim 2.8 \times 10^3$ cells mL⁻¹ of cloud water, i.e. $\sim 4 \times 10^2$ to $\sim 9 \times 10^2$ cells m⁻³ of cloud air; this is at the very lower end of microbial biomass usually observed⁶⁴ confirming the background-type situation (i.e. low influence of Human activities).

Community composition. Biodiversity was assessed from ribosomal sequences; this does not necessarily represent cell number distribution. Eukaryotes largely dominated in metagenomes (MG) (~95% of the ribosome sequences; Fig. 1), as expected from their much higher ribosome gene copy number than prokaryotes: up to thousands copies in eukaryotes *versus* ~1–15 copies in bacteria^{65,66}. Most were affiliated with Nucleomycota (equiv. Holomycota), Viridiplantae and Stramenopiles-Alveolata-Rhizaria (SAR), i.e. fungi, plants/algae and planctonic unicellular organisms. Prokaryotes consisted essentially of bacteria, dominated by Proteobacteria, over Firmicutes, Bacteroidetes, Acidobacteria, Actinobacteria, and Chloroflexi as often in atmospheric samples⁶⁷. Conversely, prokaryotes sequences were much more abundant in metatranscriptomes (MT) (50.2% ± 10.9% of the ribosome sequences) than they were in MG. Their mean relative representation in MT, with respect to MG, was thus ~20 times greater than that of eukaryotes (5.7 versus 0.26, respectively), suggesting probable higher protein synthesis and metabolic activity²⁷; this emphasized bacteria in particular as the active microbiota of clouds. Based on this ratio, the most active bacterial taxa comprised notably groups related with water environments, like Planctomycetes, Chlorobia and Cyanobacteria, along with bacteria shown previously to maintain metabolic activity in clouds, such as Alpha and BetaProteobacteria⁵.

Apart from ribosomal sequences, a number of functional genes indicated the presence of viruses. Viruses are frequently reported among aerosols, including potential pathogens^{3,68}. We observed here the presence of dsDNA and ssRNA viruses and retroviruses, known to infect with more or less specificity arthropods (Baculoviridae, Poxviridae, Picornavirales), plants (Ourmiavirus, Tymovirales), animals and Humans (Herpesviridae,



Functional analysis. In order to examine community functioning, we explored the gene ontologies (GO) associated with identified protein sequences. The relative distribution of GO terms among MG and MT datasets was variable, thus highlighting metabolic requirements within community's capabilities. Overall, metabolic processes oriented toward energy production (catabolism) and transports dominated (Fig. 2).

In the absence of reference, the information provided *per se* by metranscriptomes for examining the microbial life conditions in a particular environment is limited. Hence, since our study of cloud water microbial communities has no counterpart yet in the atmosphere, and with the objective to reveal the specificities and constraints imposed by clouds to living microorganisms, we assessed MT in regard to the corresponding MG²⁹, and we based our interpretation on a comparative analysis between clouds and other environments, obtained from reprocessed literature data selected for including both metagenomes and metatranscriptomes on same samples (see Table S1).

The main results of the functional analysis are presented in Fig. 4 and the corresponding data are shown in Table S2; these are to be consulted throughout this section. More details are presented in SI Figures, which are referenced when appropriate. These unique datasets allowed us to draw an unprecedented picture of cloud microbiota's functioning and of its relationships with its temporary cloud water habitat.

4

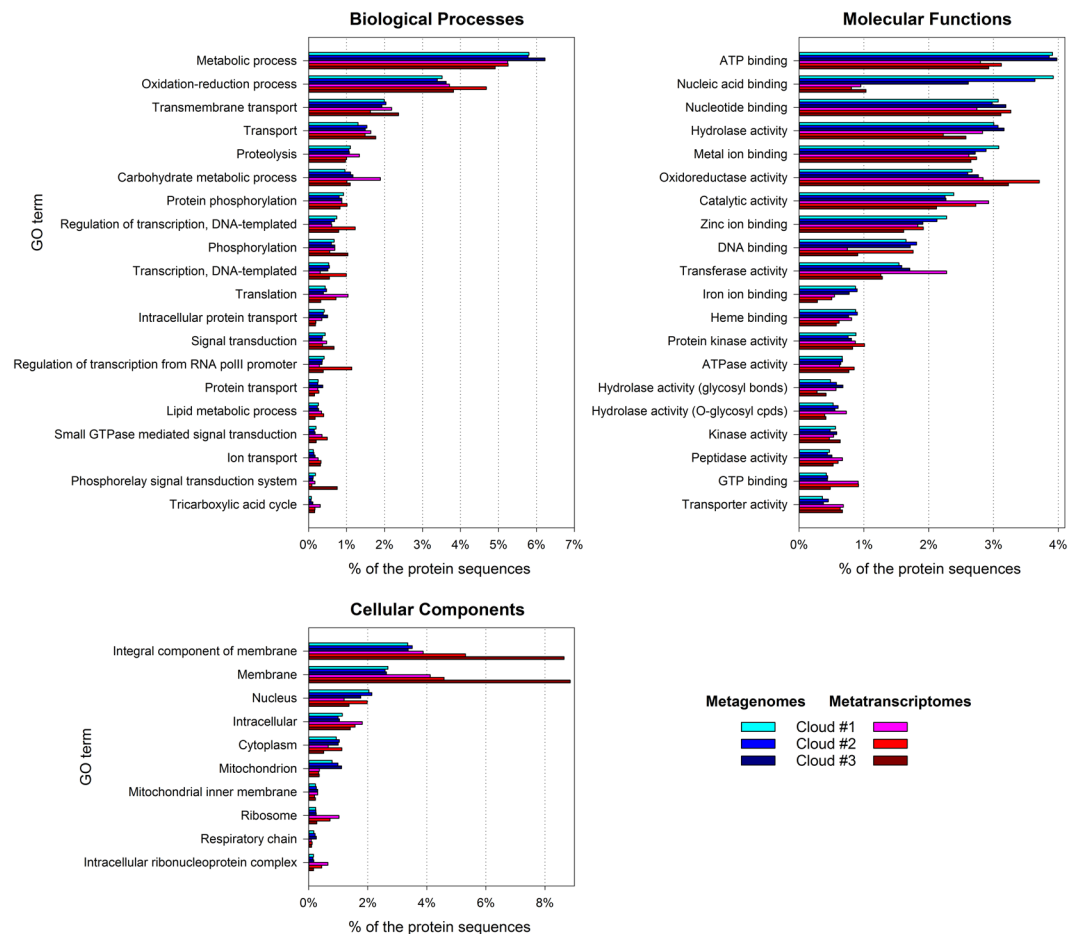


Figure 2. Main gene ontology terms related with Biological Processes, Molecular Functions and Cellular Components in cloud metatranscriptomes (blue), and in the corresponding metagenomes (red).

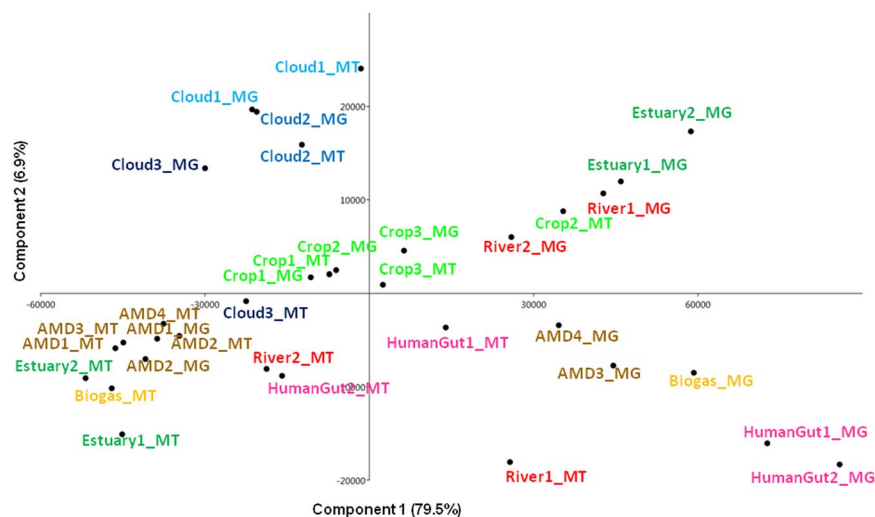


Figure 3. Principal component analysis representation of metagenomes (MG) and metatranscriptomes (MT) datasets in clouds (this study) and in other environments (from literature data, see Table S1 for references and details), based on sequence distribution among the GO terms obtained from identified protein sequences.

GO:0004096), superoxide dismutase (E.C. 1.15.1.1; GO:0004784), peroxiredoxin (E.C. 1.11.1.15; GO:0051920) and peroxidase (E.C. 3.5.2.6; GO:0004601), and antioxidant compound synthesis (glutathione; GO:0006750).

Additionally, transition metal (copper and iron) transport was notable (Fig. S5); these participate to various oxidant detoxification enzymatic reactions^{74,75}. Noteworthy, siderophore synthesis and transport processes were

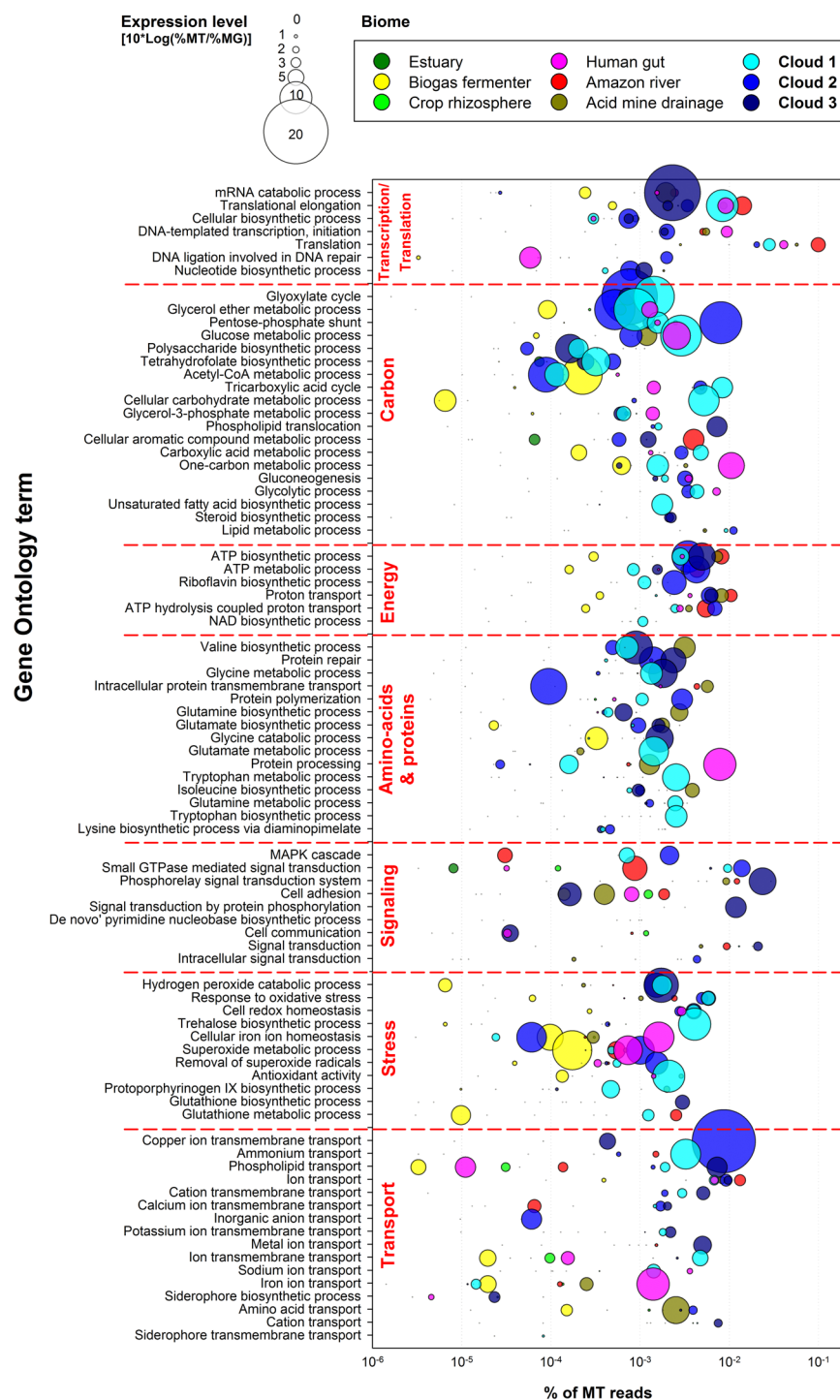


Figure 4. Biological processes expressed by cloud communities, compared with other environments (see Table S1 for details and references). Bubble size depicts function's expression level; as expressed it is equal to 0 for similar representation in MT as in MG, and >0 for greater representation in MT, so only overexpressed functions are visualized. See Table S2 for the complete GO term list and the number of corresponding sequences in each dataset.

detected; these are high affinity iron complexing compounds released by cells in their surrounding when deficient, notably by numerous *Pseudomonas* species isolated from clouds⁷⁶.

The implication of membranes, ribosomes and protein complexes is confirmed in the comparative analysis (Fig. S6). Elevated transmembrane transports and binding activities suggested important interactions between cells and their direct surroundings. The respective phosphorylation level stimuli and signals transduction systems from the environment to cell's inside, genuine cell sensing "organs"⁷⁷, indicated that mostly bacteria, but not only, were attempting to respond/acclimate to their environment (MAPK cascades in eukaryotes; GO:0000165⁷⁸; and

phosphorelay systems in bacteria; GO:0000155 and GO:0000160⁷⁹) (Fig. S4). The high calcium transport activity observed was potentially acting in signal transduction⁸⁰.

Despite temperatures close to freezing during sampling, translational activity, i.e. protein synthesis was maintained (Fig. S7). This is consistent with the reported capacity of cloud microbial communities to uptake ¹⁴C labelled leucine at 0 °C²⁵. In all likelihood ammonium, abundant in the samples (Table S1), was taken up from clouds water and fed amino-acid metabolism with nitrogen (Fig. S5)⁸¹. This was oriented toward valine, glycine, glutamate and glutamine metabolisms; in addition of permitting new proteins synthesis implied by metabolic arrangements, glycine and glutamate are constituents of glutathione, the main intracellular redox regulator. Glutamine and glutamate have a central role in nitrogen and amino-acid metabolisms, which they connect with central metabolism (TCA or glyoxylate cycles). Metabolomic profiling of the bacterium *P. syringae* isolated from clouds highlighted increased concentration of these amino-acids in cells exposed to cold⁸².

The synthesis of compatible solutes like trehalose (GO:0005992) or glycine betaine, from glycine metabolism, allowed cells to endure cold and osmotic variations^{83,84}. Functions of lipid metabolism and transport indicated other responses to cold: membrane synthesis and modifications. High activities of glycerol ether, steroid, phospholipid and unsaturated fatty acid metabolisms were indeed occurring (Fig. S8), with enzymes such as stearoyl-CoA desaturase (E.C.1.14.19.1; GO:0004768), along with branched-chain amino-acid (BCAA) biosynthetic processes (GO:0009082) and phospholipid transport (Fig. S5). Adjustments (increase) of fatty acids unsaturation level in membrane for maintaining fluidity is a well known acclimation to cold in Gram-negative bacteria⁸⁵, while Gram-positive bacteria rather adjust the branching of their branched-chain fatty acids, involving BCAA biosynthesis⁸⁶.

Laboratory investigations of cloud microbial isolates indicated that H₂O₂, at its cloud water concentration of < ~0.1 mM, was not altering microbial survival⁸⁷. When exposed to cold, bacteria such as *Pseudomonas syringae* exhibit many simultaneous metabolic regulations similar as those observed here, and imparting increased tolerance to changing environmental conditions: metabolism rerouting, compatible solutes and antioxidants synthesis, membrane modifications or again increased biochemical energy production⁸².

These defenses and acclimation mechanisms were associated with marked activities of energy transducer and redox cofactor synthesis (riboflavin, NAD and ATP, through proton translocation; Fig. S9). These attested of a substantial demand of energy for feeding the biosynthetic pathways and physiological responses ongoing here⁸⁸. Accordingly, the cell machineries were likely essentially fueled by metabolic pathways including glyoxylate, tricarboxylic acids (TCA) and pentose phosphate cycles (Fig. S8). These are known to greatly contribute to cell homeostasis and fight oxidants, consistently with the other functions observed. In clouds, indeed, ATP concentration was reported to vary in relation with that of H₂O₂ suggesting metabolic regulations linked with oxidative stress response⁸⁹.

TCA cycle is a central pathway common to all aerobic organisms and dedicated to the production of energy and reducing power from acetyl moieties (acetyl-CoA). This corresponds to the final oxydation steps of carbohydrates, lipids and amino-acids into CO₂. Numerous key enzymes of the TCA cycle were found overexpressed here, including succinate dehydrogenase, citrate synthase, phosphoenolpyruvate carboxykinase (ATP), and pyruvate dehydrogenase (Fig. S10). The glyoxylate cycle is a shortened alternative to the TCA cycle sharing several steps with it, but avoiding carbon dissimilation for allowing biomass production from C₂ substrates like acetate⁹⁰. Glyoxylate pathway is notably used for fatty acid synthesis, and it was found to be connected with the response to cold and oxidative stress in several Alpha- and Gamma-proteobacteria (*Caulobacter*, *Colwellia*, *Pseudoalteromonas*, *Pseudomonas*, *Psychrobacter*, *Rhizobium* and others)^{91–94}. Several enzymes specific of this pathway were detected at high level in MT, including malate synthase and isocitrate lyase (Fig. S11). The pentose phosphate shunt is a major pathway involved in the regulation of cell redox homeostasis, in addition to having a central role in the biosynthesis of amino acids and RNA precursors. This leads to the production and regeneration of NADPH^{95,96}, the redox cofactor required for recycling the main cell's antioxidant machinery intermediate: glutathione. Related enzymes such as ribose phosphate diphosphokinase, fructose-bisphosphate aldolase phosphogluconate dehydrogenase (NADP + dependent, decarboxylating) and again fructose 1,6-bisphosphate 1-phosphatase (Fig. S12) were found overexpressed, along with, accordingly, NADP binding activity (GO:0050661).

Other remarkable carbon metabolic pathways included glucose metabolic processes and polysaccharide synthesis (Fig. S8). This was possibly linked with the synthesis of exopolysaccharides (EPS), known to protect cells from environmental variations and dessication⁹⁷ and emphasized in cloud bacteria isolates⁹⁸, as were biosurfactants⁹⁹, and/or of adhesins, compounds involved in cell adhesion and aggregation (GO:0007155)¹⁰⁰. The production of such compounds could be beneficial for the survival of airborne living cells^{101,102}. The carbon routes detected overexpressed in clouds based on the identification of specific enzymes are summarized in Fig. S13.

Finally, this analysis can give hints about the potential carbon substrates uptaken by microorganisms in cloud water. Although the exact substrate(s) remain(s) undetermined here, biological processes involving one-carbon compounds (GO:0006730) were occurring (Fig. S8). Tetrahydrofolate (THF) synthesis (GO:0046654) notably was high; it acts as a C₁ metabolism cofactor in plants and microorganisms, allowing methylotrophy for biosynthetic processes¹⁰³. In the atmosphere, C₁ compounds are among the most abundant carbon compounds (e.g.⁶³); they are end-products of organic compounds oxydation before their complete mineralization into CO₂. Laboratory incubation of cloud water previously demonstrated a biological role in their degradation²⁶, and viable facultative methylotrophic bacteria like *Pseudomonas* spp. or *Methylobacterium* spp. are frequently detected in atmospheric samples^{5,22,24}.

Potential implications for atmospheric chemical and physical processes. Clouds are highly dynamic environments, with extremely complex chemistry and microphysics that have huge impacts on atmosphere and climate, yet far

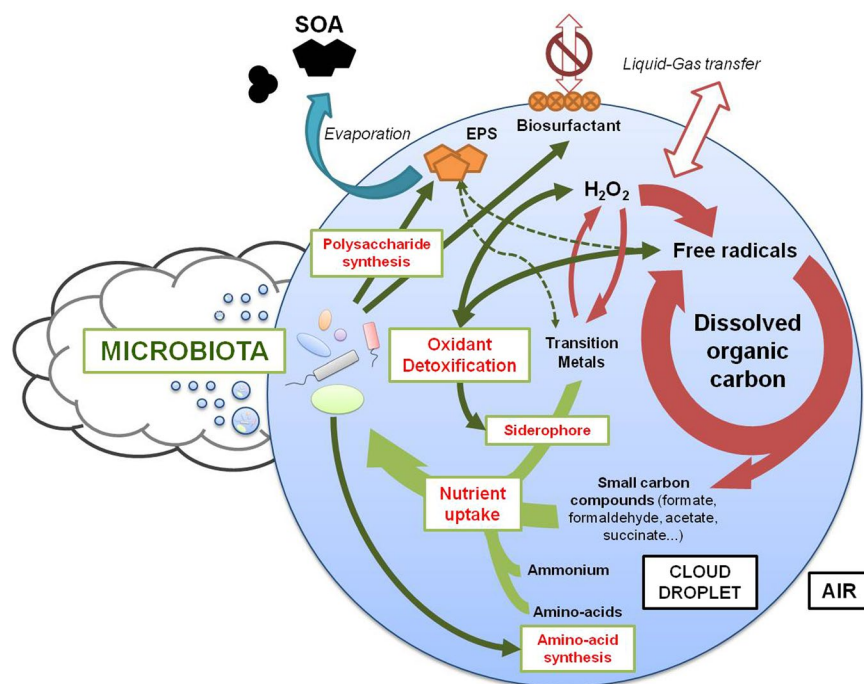


Figure 5. Schematic summary of the main probable impacts of microbial activity on cloud processes, based on coordinated metagenomics/metatranscriptomics. Biological processes and their targets are indicated by green arrows, while red arrows indicate abiotic processes. EPS: Exopolysaccharide; SOA: Secondary organic aerosol.

from being well known and understood^{104–106}. The current view in cloud water chemistry is that this is driven by free radicals, generated by sources including direct photolysis of hydrogen peroxide (H_2O_2), its dissolution from the surrounding gas phase, and its reactivity with transition metals and nitrate ions¹⁰⁷.

Biological particles and activity were regularly proposed as probably involved in processes such as cloud formation¹⁴, precipitation triggering¹⁰⁸ and chemical reactivity²⁶. Figure 5 illustrates the biological processes identified here as taking place in clouds and potentially affecting chemistry and physics. First, our results support earlier suggestions based on laboratory observations that biological activity may regulate, at least in part, the oxidant and radical concentrations in clouds²⁶ and contribute to the degradation of carbon compounds including, but not limited to, formate, formaldehyde, acetate and succinate^{109–111}. In addition, metals were shown to be largely complexed by undefined organic compounds in atmospheric water, thus limiting their chemical availability¹¹². Such strong complexants as siderophores, which the synthesis is evidenced here, are likely involved¹¹³.

Beside this, the abundance and distribution of amino-acids in cloud water were examined earlier at the same sampling site¹¹⁴. Total amino-acids amounted $\sim 3 \mu\text{M}$ ($\sim 9\%$ of the dissolved OC), and the distribution of the 16 amino-acids detected and quantified was not equimolar, but it was dominated by tryptophane, isoleucine, and phenylalanine. Here we observed overexpression of tryptophane and isoleucine biosynthetic pathways, indicating that cloudborne microorganisms themselves could be responsible for the uneven distribution of amino-acids observed.

The activity of polysaccharide synthesis possibly corresponded to biosurfactants and exopolysaccharides (EPS). The former can influence cloud formation and exchanges between liquid and gas phases¹¹⁵, whereas EPS scavenge oxidants¹¹⁶, complex metals¹¹⁷, and represent potential secondary organic aerosols once cloud dissipates⁹⁷.

Finally, ice nucleation (IN), in particular at temperatures $> -10^\circ\text{C}$, i.e. catalyzed by biological entities, is a topic of interest in atmospheric sciences as this influences precipitation¹¹⁸. There is no doubt on the presence of low numbers of ice nucleating bacteria in clouds¹¹⁹. Here, IN assays on the samples demonstrated freezing between -6°C and -7°C , with up to >200 IN particles mL^{-1} of water at -8°C (Fig. S14). This is relatively high compared with previous observations and totally compatible with bacterial IN activity⁴¹; however, no known gene coding for IN activity was observed; detecting specific signature of such rare microbial phenotypes or traits in nucleic acids requires targeted methods¹²⁰.

In conclusion, we provided here the first molecular picture of microbial living conditions in the top layer of the biosphere constituted by clouds. As samples were collected during the night, we did not detect photosynthetic activity, but the presence of diverse phototrophic organisms indicates that this potentially occurs. A different picture could have been drawn under different environmental configuration like during the day, in the case of pollution events from anthropogenic or natural emissions, under warmer/colder conditions etc, which then remain to be explored. Numerous aspects led us to affirm that cloud droplets are demanding habitats for living cells, challenged to respond or acclimate to oxidative stress, low temperatures and osmotic variations. Thus, specific central metabolic routes directed toward the management of oxidants were preferred for producing the energy required

for homeostasis, synthesis of protective compounds and physiological rearrangements. In microbial evolution, atmospheric dispersal could have promoted the maintenance and diversification of such phenotypes by exerting strong selection pressure on inapt individuals, while allowing others to spread over the planet. Furthermore, oxidants are considered as the main drivers of atmospheric chemistry. Their regulation in cloud water by the multiple ways deployed by living microorganisms clearly positions microbial cells as central actors, to some extent, of cloud chemical reactivity. The results also suggest biomass and biochemical energy production from substrates including small carbon compounds (C1 and C2, succinate), and ammonium as the source of nitrogen.

Our untargeted metatranscriptomic approach gave many insights into the functioning of microbial cells within cloud droplets, their physiological traits and potential impacts. This specified biological functions of interest, and this should help identifying specific target genes for future investigations. The low biomass imposed a step of DNA amplification by MDA. This necessarily distorted to some extent the view of the actual nucleic acid content of the samples. Significant progress to a better evaluation of biological functions expression levels will consist of absolute quantifications of genes and transcripts, i.e. without amplification step. Furthermore, as gene expression cannot be directly related with any quantification of activity, activity measurements remain necessary to determine the actual biological imprint on clouds, and in order to take into account posttranscriptional regulations, potentially caused by environmental factors.

Data Availability

The sequence files generated in this study were deposited to NCBI's Sequence Read Archive (BioProject ID PRJEB25763) with the sample accession numbers ERS2351639 to ERS2351645.

References

- Amato, P. *et al.* Main Biological Aerosols, Specificities, Abundance, and Diversity. In *Microbiology of Aerosols* (eds Delort, A.-M. & Amato, P.) 1–21, <https://doi.org/10.1002/9781119132318.ch1a> (John Wiley & Sons, Inc., 2017).
- Brodie, E. L. *et al.* Urban aerosols harbor diverse and dynamic bacterial populations. *PNAS* **104**, 299–304 (2007).
- Courault, D. *et al.* Assessment and risk modeling of airborne enteric viruses emitted from wastewater reused for irrigation. *Sci. Total Environ.* **592**, 512–526 (2017).
- Bowers, R. M., McLetchie, S., Knight, R. & Fierer, N. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J* **5**, 601–612 (2011).
- Amato, P. *et al.* Active microorganisms thrive among extremely diverse communities in cloud water. *PLOS ONE* **12**, e0182869 (2017).
- Aller, J. Y., Kuznetsova, M. R., Jahns, C. J. & Kemp, P. F. The sea surface microlayer as a source of viral and bacterial enrichment in marine aerosols. *Journal of Aerosol Science* **36**, 801–812 (2005).
- Joung, Y. S., Ge, Z. & Buie, C. R. Bioaerosol generation by raindrops on soil. *Nature Communications* **8**, 14668 (2017).
- Brunet, Y. *et al.* A relaxed-eddy accumulation system for measuring microbial emission fluxes from the vegetation. In (American Association for Aerosol Research., 2013).
- Carotenuto, F. *et al.* Measurements and modeling of surface–atmosphere exchange of microorganisms in Mediterranean grassland. *Atmos. Chem. Phys.* **17**, 14919–14936 (2017).
- Burrows, S. M. *et al.* Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems. *Atmos. Chem. Phys.* **9**, 9281–9297 (2009).
- Griffin, D. W., Gonzalez-Martin, C., Hoose, C. & Smith, D. J. Global-Scale Atmospheric Dispersion of Microorganisms. In *Microbiology of Aerosols* (eds Delort, A.-M. & Amato, P.) 155–194, <https://doi.org/10.1002/9781119132318.ch2c> (John Wiley & Sons, Inc., 2017).
- Šantl-Temkiv, T., Gosewinkel, U., Starnawski, P., Lever, M. & Finster, K. Aeolian dispersal of bacteria in southwest Greenland: their sources, abundance, diversity and physiological states. *FEMS Microbiol. Ecol.* **94** (2018).
- Barberán, A. *et al.* Continental-scale distributions of dust-associated bacteria and fungi. *PNAS* **112**, 5756–5761 (2015).
- Bauer, H. *et al.* Airborne bacteria as cloud condensation nuclei. *Journal of Geophysical Research: Atmospheres* (1984–2012) **108**, (2003).
- Morris, C. E., Georgakopoulos, D. G. & Sands, D. C. Ice nucleation active bacteria and their potential role in precipitation. *Journal de Physique IV (Proceedings)* **121**, 87–103 (2004).
- Creamean, J. M. *et al.* Dust and Biological Aerosols from the Sahara and Asia Influence Precipitation in the Western U.S. *Science* **339**, 1572–1578 (2013).
- Morris, C. E. & Sands, D. C. Impacts of microbial aerosols on natural and agro-ecosystems: immigration, invasions and their consequences. In *Microbiology of aerosols* (Delort, A. M. & Amato, P.) (2017).
- Smith, D. J., Griffin, D. W., McPeters, R. D., Ward, P. D. & Schuerger, A. C. Microbial survival in the stratosphere and implications for global dispersal. *Aerobiologia* **27**, 319–332 (2011).
- Amato, P. *et al.* Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber. *Atmos. Chem. Phys.* **15**, 6455–6465 (2015).
- Hill, K. A. *et al.* Processing of atmospheric nitrogen by clouds above a forest environment. *J. Geophys. Res.* **112**, D11301 (2007).
- Hara, K. & Zhang, D. Bacterial abundance and viability in long-range transported dust. *Atmospheric Environment* **47**, 20–25 (2012).
- Šantl-Temkiv, T., Finster, K., Hansen, B. M., Nielsen, N. W. & Karlson, U. G. The microbial diversity of a storm cloud as assessed by hailstones. *FEMS Microbiol. Ecol.* **81**, 684–695 (2012).
- Fuzzi, S., Mandrioli, P. & Perfetto, A. Fog droplets—an atmospheric source of secondary biological aerosol particles. *Atmospheric Environment* **31**, 287–290 (1997).
- Amato, P. *et al.* A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by micro-organisms. *Atmos. Chem. Phys.* **7**, 4159–4169 (2007).
- Sattler, B., Puxbaum, H. & Psenner, R. Bacterial growth in supercooled cloud droplets. *Geophysical Research Letters* **28**, 239–242 (2001).
- Vaitilingom, M. *et al.* Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. *PNAS* **110**, 559–564 (2013).
- Baldrian, P. *et al.* Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J* **6**, 248–258 (2012).
- Gifford, S. M., Sharma, S., Rinta-Kanto, J. M. & Moran, M. A. Quantitative analysis of a deeply sequenced marine microbial metatranscriptome. *The ISME Journal* **5**, 461–472 (2011).
- Gilbert, J. A. *et al.* Detection of Large Numbers of Novel Sequences in the Metatranscriptomes of Complex Marine Microbial Communities. *PLOS ONE* **3**, e3042 (2008).

30. Franzosa, E. A. *et al.* Relating the metatranscriptome and metagenome of the human gut. *Proc. Natl. Acad. Sci. USA* **111**, E2329–E2338 (2014).
31. Satinsky, B. M. *et al.* The Amazon continuum dataset: quantitative metagenomic and metatranscriptomic inventories of the Amazon River plume, June 2010. *Microbiome* **2**, 17 (2014).
32. Chen, L. *et al.* Comparative metagenomic and metatranscriptomic analyses of microbial communities in acid mine drainage. *The ISME Journal* **9**, 1579–1592 (2015).
33. Be, N. A. *et al.* Metagenomic analysis of the airborne environment in urban spaces. *Microb. Ecol.* **69**, 346–355 (2015).
34. Whon, T. W. *et al.* Metagenomic characterization of airborne viral DNA diversity in the near-surface atmosphere. *J. Virol.* **86**, 8221–8231 (2012).
35. Yoesepp, S. *et al.* A Metagenomic Framework for the Study of Airborne Microbial Communities. *PLOS ONE* **8**, e81862 (2013).
36. Xu, C. *et al.* Investigation of diverse bacteria in cloud water at Mt. Tai, China. *Science of The Total Environment* **580**, 258–265 (2017).
37. Klein, A. M., Bohannan, B. J. M., Jaffe, D. A., Levin, D. A. & Green, J. L. Molecular Evidence for Metabolically Active Bacteria in the Atmosphere. *Front. Microbiol.* **772**, <https://doi.org/10.3389/fmicb.2016.00772> (2016).
38. Womack, A. M. *et al.* Characterization of active and total fungal communities in the atmosphere over the Amazon rainforest. *Biogeosciences* **12**, 6337–6349 (2015).
39. Krumins, V., Mainelis, G., Kerkhof, L. J. & Fennell, D. E. Substrate-Dependent rRNA Production in an Airborne Bacterium. *Environ. Sci. Technol. Lett.* **1**, 376–381 (2014).
40. Draxler, R. R. & Rolph, G. D. HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model access via NOAA ARL READY Website, <http://ready.arl.noaa.gov/HYSPLIT.php>, NOAA Air Resources Laboratory, Silver Spring, MD (2013).
41. Joly, M. *et al.* Quantification of ice nuclei active at near 0°C temperatures in low-altitude clouds at the Puy de Dôme atmospheric station. *Atmospheric Chemistry and Physics* **14**, 8185–8195 (2014).
42. Yilmaz, S., Allgaier, M. & Hugenholtz, P. Multiple displacement amplification compromises quantitative analysis of metagenomes. *Nature Methods* **7**, 943–944 (2010).
43. Abulencia, C. B. *et al.* Environmental Whole-Genome Amplification To Access Microbial Populations in Contaminated Sediments. *Appl. Environ. Microbiol.* **72**, 3291–3301 (2006).
44. Yergeau, E., Hogues, H., Whyte, L. G. & Greer, C. W. The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. *The ISME Journal* **4**, 1206–1214 (2010).
45. Schmieder, R. & Edwards, R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* **27**, 863–864 (2011).
46. Masella, A. P., Bartram, A. K., Truszkowski, J. M., Brown, D. G. & Neufeld, J. D. PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics* **13**, 31 (2012).
47. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997 [q-bio] (2013).
48. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
49. Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402 (1997).
50. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596 (2013).
51. Pundir, S., Martin, M. J. & O'Donovan, C. UniProt Protein Knowledgebase. In *Protein Bioinformatics* 41–55, https://doi.org/10.1007/978-1-4939-6783-4_2 (Humana Press, New York, NY, 2017).
52. Ashburner, M. *et al.* Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* **25**, 25–29 (2000).
53. Carbon, S. *et al.* AmiGO: online access to ontology and annotation data. *Bioinformatics* **25**, 288–289 (2009).
54. The Gene Ontology Consortium. Expansion of the Gene Ontology knowledgebase and resources. *Nucleic Acids Res.* **45**, D331–D338 (2017).
55. Wu, C. H. *et al.* The Protein Information Resource. *Nucleic Acids Res.* **31**, 345–347 (2003).
56. Supek, F., Bošnjak, M., Škunca, N. & Šmuc, T. REVIGO Summarizes and Visualizes Long Lists of Gene Ontology Terms. *PLOS ONE* **6**, e21800 (2011).
57. McCarthy, F. M. *et al.* AgBase: a functional genomics resource for agriculture. *BMC Genomics* **7**, 229 (2006).
58. Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
59. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res.* **44**, D457–D462 (2016).
60. Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y. & Morishima, K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* **45**, D353–D361 (2017).
61. McMurdie, P. J. & Holmes, S. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLOS Computational Biology* **10**, e1003531 (2014).
62. Stewart, F. J., Ulloa, O. & DeLong, E. F. Microbial metatranscriptomics in a permanent marine oxygen minimum zone. *Environmental Microbiology* **14**, 23–40 (2012).
63. Deguillaume, L. *et al.* Classification of clouds sampled at the puy de Dôme (France) based on 10 yr of monitoring of their physicochemical properties. *Atmos. Chem. Phys.* **14**, 1485–1506 (2014).
64. Vaithilingom, M. *et al.* Long-term features of cloud microbiology at the puy de Dôme (France). *Atmospheric Environment* **56**, 88–100 (2012).
65. Torres-Machorro, A. L., Hernández, R., Cevallos, A. M. & López-Villaseñor, I. Ribosomal RNA genes in eukaryotic microorganisms: witnesses of phylogeny? *FEMS Microbiology Reviews* **34**, 59–86 (2010).
66. Stoddard, S. F., Smith, B. J., Hein, R., Roller, B. R. K. & Schmidt, T. M. rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. *Nucleic Acids Res.* **43**, D593–598 (2015).
67. Després, V. R. *et al.* Primary biological aerosol particles in the atmosphere: a review. *Tellus B* **64** (2012).
68. Verreault, D., Moineau, S. & Duchaine, C. Methods for Sampling of Airborne Viruses. *Microbiol. Mol. Biol. Rev.* **72**, 413–444 (2008).
69. Guo, J. Rhizosphere metagenomics of three biofuel crops. (Michigan State University, 2016).
70. Fortunato, C. S. & Crump, B. C. Microbial Gene Abundance and Expression Patterns across a River to Ocean Salinity Gradient. *PLOS ONE* **10**, e0140578 (2015).
71. Bremges, A. *et al.* Deeply sequenced metagenome and metatranscriptome of a biogas-producing microbial community from an agricultural production-scale biogas plant. *GigaScience* **4**, 33 (2015).
72. Fierer, N. *et al.* Short-Term Temporal Variability in Airborne Bacterial and Fungal Populations. *Appl Environ Microbiol* **74**, 200–207 (2008).
73. Ottesen, E. A. *et al.* Pattern and synchrony of gene expression among sympatric marine microbial populations. *PNAS* **110**, E488–E497 (2013).
74. Arredondo, M. & Núñez, M. T. Iron and copper metabolism. *Molecular Aspects of Medicine* **26**, 313–327 (2005).
75. Ma, Z., Jacobsen, F. E. & Giedroc, D. P. Coordination Chemistry of Bacterial Metal Transport and Sensing. *Chemical Reviews* **109**, 4644–4681 (2009).
76. Vinatier, V. *et al.* Siderophores in Cloud Waters and Potential Impact on Atmospheric Chemistry: Production by Microorganisms Isolated at the Puy de Dôme Station. *Environ. Sci. Technol.* **50**, 9315–9323 (2016).

77. Gunn, J. S. & Richards, S. M. Recognition and Integration of Multiple Environmental Signals by the Bacterial Sensor Kinase PhoQ. *Cell Host & Microbe* **1**, 163–165 (2007).
78. Elion, E. A. Methods for analyzing MAPK cascades. *Methods* **40**, 207–208 (2006).
79. Hoch, J. A. Two-component and phosphorelay signal transduction. *Curr. Opin. Microbiol.* **3**, 165–170 (2000).
80. Dominguez, D. C. Calcium signalling in bacteria. *Mol. Microbiol.* **54**, 291–297 (2004).
81. von Wirén, N. & Merrick, M. Regulation and function of ammonium carriers in bacteria, fungi, and plants. In *Molecular Mechanisms Controlling Transmembrane Transport* **9**, 95–120 (Springer Berlin Heidelberg, 2004).
82. Jousse, C. *et al.* Metabolomic study of the response to cold shock in a strain of *Pseudomonas syringae* isolated from cloud water. *Metabolomics* **14**, 11 (2018).
83. Ko, R., Smith, L. T. & Smith, G. M. Glycine betaine confers enhanced osmotolerance and cryotolerance on *Listeria monocytogenes*. *J Bacteriol* **176**, 426–431 (1994).
84. Kempf, B. & Bremer, E. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch. Microbiol.* **170**, 319–330 (1998).
85. Feller, G. & Gerday, C. Psychrophilic enzymes: hot topics in cold adaptation. *Nat. Rev. Microbiol.* **1**, 200–208 (2003).
86. Kaneda, T. Function, and Taxonomic Significance. *Microbiol Rev.* **55**, 15 (1991).
87. Joly, M. *et al.* Survival of microbial isolates from clouds toward simulated atmospheric stress factors. *Atmospheric Environment* **117**, 92–98 (2015).
88. Welch, W. J. How Cells Respond to Stress. *Scientific American* **268**, 56–64 (1993).
89. Wirgot, N., Vinatier, V., Deguillaume, L., Sancelme, M. & Delort, A.-M. H₂O₂ modulates the energetic metabolism of the cloud microbiome. *Atmos. Chem. Phys.* **17**, 14841–14851 (2017).
90. Cronan, J. J. & Laporte, D. Tricarboxylic Acid Cycle and Glyoxylate Bypass. *EcoSal Plus* **1** (2005).
91. Santos, J. S., da Silva, C. A. P. T., Balhesteros, H., Lourenço, R. F. & Marques, M. V. CspC regulates the expression of the glyoxylate cycle genes at stationary phase in *Caulobacter*. *BMC Genomics* **16**, 638 (2015).
92. Watanabe, S., Yamaoka, N., Fukunaga, N. & Takada, Y. Purification and characterization of a cold-adapted isocitrate lyase and expression analysis of the cold-inducible isocitrate lyase gene from the psychrophilic bacterium *Colwellia psychrerythraea*. *Extremophiles* **6**, 397–405 (2002).
93. Piette, F. *et al.* Proteomics of life at low temperatures: trigger factor is the primary chaperone in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125. *Mol. Microbiol.* **76**, 120–132 (2010).
94. Ahn, S., Jung, J., Jang, I.-A., Madsen, E. L. & Park, W. Role of Glyoxylate Shunt in Oxidative Stress Response. *J. Biol. Chem.* **291**, 11928–11938 (2016).
95. Stincone, A. *et al.* The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biol Rev Camb Philos Soc* **90**, 927–963 (2015).
96. Singh, R., Lemire, J., Mailloux, R. J. & Appanna, V. D. A Novel Strategy Involved Anti-Oxidative Defense: The Conversion of NADH into NADPH by a Metabolic Network. *PLOS ONE* **3**, e2682 (2008).
97. Kumar, A. S., Mody, K. & Jha, B. Bacterial exopolysaccharides – a perception. *Journal of Basic Microbiology* **47**, 103–117 (2007).
98. Matulová, M., Husárová, S., Capek, P., Sancelme, M. & Delort, A.-M. Biotransformation of Various Saccharides and Production of Exopolymeric Substances by Cloud-Borne *Bacillus* sp. 3B6. *Environ. Sci. Technol.* **48**, 14238–14247 (2014).
99. Renard, P. *et al.* Screening of cloud microorganisms isolated at the Puy de Dôme (France) station for the production of biosurfactants. *Atmos. Chem. Phys.* **16**, 12347–12358 (2016).
100. Berne, C., Ducret, A., Hardy, G. G. & Brun, Y. V. Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiol Spectr* **3** (2015).
101. Monier, J.-M. & Lindow, S. E. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *PNAS* **100**, 15977–15982 (2003).
102. Hoppe, H.-G. Attachment of Bacteria: Advantage or Disadvantage for Survival in the Aquatic Environment. In *Microbial Adhesion and Aggregation*, 283–301, https://doi.org/10.1007/978-3-642-70137-5_19 (Springer, Berlin, Heidelberg, 1984).
103. Vorholt, J. A. Cofactor-dependent pathways of formaldehyde oxidation in methylotrophic bacteria. *Arch Microbiol* **178**, 239–249 (2002).
104. Herrmann, H. *et al.* Tropospheric Aqueous-Phase Chemistry: Kinetics, Mechanisms, and Its Coupling to a Changing Gas Phase. *Chemical Reviews* **115**, 4259–4334 (2015).
105. Boucher, O. *et al.* Clouds and aerosols. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Stocker, T. F. *et al.*) (2013).
106. Ervens, B. Modeling the Processing of Aerosol and Trace Gases in Clouds and Fogs. *Chem. Rev.* **115**, 4157–4198 (2015).
107. Bianco, A. *et al.* A better understanding of hydroxyl radical photochemical sources in cloud waters collected at the puy de Dôme station – experimental versus modelled formation rates. *Atmos. Chem. Phys.* **15**, 9191–9202 (2015).
108. Morris, C. E. *et al.* Microbiology and atmospheric processes: research challenges concerning the impact of airborne microorganisms on the atmosphere and climate. *Biogeosciences* **8**, 17–25 (2011).
109. Herlihy, L. J., Galloway, J. N. & Mills, A. L. Bacterial utilization of formic and acetic acid in rainwater. *Atmospheric Environment* **21**, 2397–2402 (1987).
110. Šantl-Temkiv, T., Finster, K., Hansen, B. M., Pašić, L. & Karlson, U. G. Viable methanotrophic bacteria enriched from air and rain can oxidize methane at cloud-like conditions. *Aerobiologia* **29**, 373–384 (2013).
111. Amato, P. *et al.* Microbial population in cloud water at the Puy de Dôme: Implications for the chemistry of clouds. *Atmospheric Environment* **39**, 4143–4153 (2005).
112. Parazols, M. *et al.* Speciation and role of iron in cloud droplets at the puy de Dôme station. *J Atmos Chem* **54**, 267–281 (2006).
113. Passananti, M., Vinatier, V., Delort, A.-M., Mailhot, G. & Brigante, M. Siderophores in Cloud Waters and Potential Impact on Atmospheric Chemistry: Photoreactivity of Iron Complexes under Sun-Simulated Conditions. *Environ. Sci. Technol.* **50**, 9324–9332 (2016).
114. Bianco, A., Voyard, G., Deguillaume, L., Mailhot, G. & Brigante, M. Improving the characterization of dissolved organic carbon in cloud water: Amino acids and their impact on the oxidant capacity. *Scientific Reports* **6** (2016).
115. Nozière, B. Don't forget the surface. *Science* **351**, 1396–1397 (2016).
116. Kodali, V. P. & Sen, R. Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. *Biotechnology Journal* **3**, 245–251 (2008).
117. Loaec, M., Olier, R. & Guezennec, J. Uptake of lead, cadmium and zinc by a novel bacterial exopolysaccharide. *Water Research* **31**, 1171–1179 (1997).
118. Hoose, C. & Möhler, O. Heterogeneous ice nucleation on atmospheric aerosols: a review of results from laboratory experiments. *Atmos. Chem. Phys.* **12**, 9817–9854 (2012).
119. Joly, M. *et al.* Ice nucleation activity of bacteria isolated from cloud water. *Atmospheric Environment* **70**, 392–400 (2013).
120. Hill, T. C. J. *et al.* Measurement of Ice Nucleation-Active Bacteria on Plants and in Precipitation by Quantitative PCR. *Appl. Environ. Microbiol.* **80**, 1256–1267 (2014).

Acknowledgements

The authors thank in particular L. Nauton for help with informatics, J.-M. Pichon for meteorological data, F. Enault and B. Ervens for constructive comments on manuscript, and G. Lefebvre for language correction. Two anonymous reviewers are also acknowledged for their constructive remarks. This project was funded by CNRS EC2CO (MicrobiEN) program, “FONCOMIC” project attributed to P.A. MJ and LB were hired on the projects ANR-15-CE01-0002 (“INHALE”) and ANR-DFG-14-CE35-005-02 (“CHLOROFILTER”), respectively.

Author Contributions

P.A. conceptualized this study; P.A. and L.D. performed field work; P.A. analyzed the samples and did the experimental work; P.A., L.B., M.J. and B.P. analyzed the data; P.A., L.B., M.J., L.D. and A.M.D. wrote the manuscript.

Additional Information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s41598-019-41032-4>.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2019