Clouds influence the functioning of airborne microorganisms

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Abstract. Airborne micro-organisms can remain at altitude for several days exposed to multiple environmental constraints that prevent or limit microbial activity, the most important of which is probably the lack of available liquid water. Clouds, *i.e.* air masses containing liquid water, could offer more favorable conditions. In order to investigate the influence of clouds on the functioning of airborne microorganisms, we captured aerosols into a nucleic acid preservation buffer from a high-altitude mountain meteorological station during cloudy and clear atmospheric conditions, and examined metatranscriptomes. The specificities of aeromicrobiome's functioning in clouds compared to the clear atmosphere were then decrypted from differential functional expression analysis (DEA). The data reveal higher RNA-to-DNA content in clouds than in the clear atmosphere suggesting higher metabolic activity, and an overrepresentation of microbial transcripts related to energy metabolism, the processing of carbon and nitrogen compounds, intracellular signalling, metabolic regulations, transmembrane transports, and others. Stress response orients towards responses to osmotic shocks and starvation, rather than toward oxidants in clear atmosphere. Autophagy processes in eukaryotes, (macropexophagy, *i.e.* the recycling of peroxisomes) could help to alleviate the limited amounts of nutrients in the restricted microenvironments provided by cloud droplets. The whole phenomenon resembles the rapid resumption of microbial activity in dry soils after rewetting by rain, known as the "Birch effect", described here for the first time in the atmosphere. This work provides unprecedented information on the modulations of aeromicrobiome's functioning in relation to atmospheric conditions. In addition of contributing to the processing and fate of chemical compounds in the atmosphere, cloud-induced modulations of biological processes could have ecological repercussions by shaping airborne microbial diversity and their capacity to invade surface environments.

1 Introduction

It is well established that biological material circulates in the atmosphere. This includes taxonomically and functionally diverse microorganisms, with frequent saprotrophs (Amato et al., 2007b; Tignat-Perrier et al., 2020), methylotrophs (Amato et al., 2007a), phototrophs (Dillon et al., 2020) and others including obligatory or opportunist plant, animal or human pathogens (Brown and Hovmøller, 2002). Once aerosolized as individual cells or fragments of biofilms, they can remain airborne for up to several days (Burrows et al., 2009), at concentrations typically ranging from ~10^3 to ~10^6 cell $m_{(air)}^{-3}$ (Amato et al., 2023; Šantl-Temkiv et al., 2022). Close to the ground, in the planetary boundary layer, the airborne microbial diversity reflects that of the emitting surfaces and follows its spatial and temporal variations in relation to meteorological and (micro)climatic conditions (Bowers et al., 2011; Fierer et al., 2008; Gusareva et al., 2019; Prass et al., 2021; Tignat-Perrier et al., 2020). At high altitudes in the free troposphere, the plumes from multiple sources mix which results in more evenly distributed assemblages (Péguilhan et al., 2021) at extremely low biomass (Smith et al., 2018).

Viable microbial cells suspended in the air are exposed to conditions at the limits of life's capacities including low water and nutrient availability, low temperatures, and high levels of UV radiation and oxidants (Šantl-Temkiv et al., 2022). Water availability in particular is among the most limiting factors of biological processes in nature (Stevenson et al., 2015).

Clouds are air volumes where relative humidity exceeds 100%, resulting in the condensation of water vapor on the surface of aerosol particles, including microbial cells. This leads to the formation of droplets of a few micrometers in diameter (*i.e.* individual volumes of ~10^{-6} µL), with a typical liquid water content of ~0.1-1 $g.m_{(air)}^{-3}$. Chemical compounds from the gas and particle phases dissolve into the aqueous phase, and complex chemical processes take place with notable influence the composition of air masses (Ervens et al., 2018; Herrmann et al., 2015; Lelieveld and Crutzen, 1990; Li et al., 2023). Microbiological processes as well can, to some extent, participate to process organic compounds and oxidants (Bianco et al., 2019; Khaled et al., 2021; Vaïtilingom et al., 2013). From the perspective of the microbiologist, cloud droplets can thus be considered short-lived aquatic microhabitats providing microorganisms with liquid water and a range of dissolved nutrients at nano- to micro-molar concentration (carboxylic acids, amino acids, ammonium, nitrate, metals, etc.) (Deguillaume et al., 2014; Šantl-Temkiv et al., 2013). Bulk cloud water was indeed evidenced during laboratory incubations to offer nutritional conditions compatible with microbial development, with impacts on the chemical composition (Amato et al., 2007a; Bianco et al., 2019; Sattler et al., 2001; Vaïtilingom et al., 2013). In addition, clouds, through precipitation, provide efficient access routes to the ground for micro-organisms airborne at high altitude and thus contribute to aerial dissemination (Péguilhan et al., 2021; Woo and Yamamoto, 2020).

The highly diluted microbial biomass in the atmosphere, along with short residence time, make any in-situ assessment challenging, so how the functioning of living cells may be modulated during atmospheric transport remains largely unexplored. If conditions allow, airborne microorganisms can maintain or activate metabolic processes in response to environmental conditions (Amato et al., 2017; Hill et al., 2007; Klein et al., 2016; Šantl-Temkiv et al., 2018). For instance, bacteria (*Sphingomonas aerolata*) aerosolized in a simulation chamber increases ribosome numbers when exposed to volatile organic

compounds (ethanol, acetic acid) and so, potentially, metabolic activity (Krumins et al., 2014). Data also suggest modulations of the energy metabolism of living bacteria in natural clouds in relation with oxidants (Wirgot et al., 2017).

So far, current knowledge of microbial functioning in the atmosphere and clouds is thus based almost exclusively on laboratory incubations of samples and isolated strains (Amato et al., 2007a; Bianco et al., 2019; Jousse et al., 2018; Vaïtilingom et al., 2013; Wirgot et al., 2019) or, at best, on experiments in atmospheric simulation chambers (Amato et al., 2015; Krumins et al., 2014), *i.e.*, in conditions that do not fully reflect the *in-situ* natural atmospheric conditions in which the cells are actually exposed. Metagenomics and, in particular, metatranscriptomics can provide instant snapshots of the biological processes taking place in a system. Over the last decade, the advent of high-throughput sequencing techniques stimulated such approaches. These led to unprecedented insights into the functioning of microbiota in humans (Franzosa et al., 2014; Jorth et al., 2014), oceans (Salazar et al., 2019), rivers (Satinsky et al., 2014), soils (Rosado-Porto et al., 2022), and highly polluted environments (Chen et al., 2015). Clouds were explored once, revealing multiple biological processes including responses to stresses, transport and central catabolic and anabolic processes (Amato et al., 2019). By comparing cloud transcriptomes with other data available in the literature, this work highlighted functional peculiarities compared with surface biomes. Still, there is no information regarding possible specificities of microbial functioning in clouds compared to the clear, cloud-free atmosphere, which occupies most of the atmospheric volume.

Here, we postulate that clouds could act as atmospheric "oases", *i.e.*, specific volumes providing water and nutrients to living organisms and allowing them to thrive within an otherwise vast and hostile atmospheric environment. By using an innovative combined non-targeted metagenomics/metatranscriptomics approach, we examine the functioning of airborne microbial cells in clouds as compared with clear atmosphere, and specify if and which biological processes are indeed affected. Given that airborne particles, including bacteria, spend on average 10 – 15% of their atmospheric residence time in clouds (Ervens and Amato, 2020; Lelieveld and Crutzen, 1990), such oases would provide conditions of (temporary) habitats or 'airborne ecosystems' and therefore could lead to enhanced survival, persistence and dispersal of bacteria similar to features of other dynamic environments. This study, based on unique and unprecedented data sets, provides valuable information regarding the active aeromicrobiome and its environmental drivers.

2 Materials and methods

2.1 Sample collection

Samples were collected from the summit of Puy de Dôme Mountain (PUY; 1 465 m a.s.l., 45.772° N, 2.9655° E, France, ~400 km East from the Atlantic Ocean and ~300 km North of the Mediterranean Sea), located in an area composed of deciduous forests and pastoral landscapes and exposed most of the time to air masses from North and West (Deguillaume et al., 2014; Renard et al., 2020). This mountain station is part of the Cézeaux-Aulnat-Opme-Puy-de-Dôme (CO-PDD) instrumented platform network for atmospheric research (Baray et al., 2020). Meteorological variables are monitored and the station is fully equipped for on-site sample processing and conditioning, including for microbiological and molecular analyses.

The main information pertaining to sample acquisition is summarized in Table 1. A total of nine cloud and six clear air events were sampled in 2019 and 2020, for periods of about two to six consecutive hours during daytime. In both conditions, two to four high-flow-rate impingers (HFRi; model DS6, Kärcher SAS, Bonneuil-sur-Marne, France) sampling with an air-flow rate of 2 m^3 min^{-1} were deployed in parallel. More details about these samplers and their applicability to collect biological material are provided in (Šantl-Temkiv et al., 2017). Nucleic acid analyses were carried out from samplers filled with filtered and autoclaved Nucleic Acid Preservation (NAP) buffer solution (Camacho-Sanchez et al., 2013; Menke et al., 2017) as the collection liquid (1.7 L of 0.5X NAP for clear atmosphere, or 850 mL of 1X NAP for clouds, in order to account for expected liquid evaporation or accumulation), following the procedures detailed in (Péguilhan et al., 2023a, b), including decontamination and controls. Negative controls consisted of unexposed collection liquid, and of collection liquid exposed to the sampling tank for 10 minutes, sampler off. These were taken immediately before sampling, and processed in parallel of samples. For atmospheric samples, the volume of the collection liquid was checked by weighting every sampling hour, and compensated if necessary with autoclaved ultrapure water. Samples and controls were processed immediately after sampling using the PUY station's microbiology facility, within a laminar flow hood previously exposed to UV light for 15 min. The collection liquid from each individual sampler was filtered through 0.22 µm porosity mixed cellulose esters (MCE) filters (47 mm diameter; ref. 0421A00023; ClearLine®, Bernolsheim, France) using sterile Nalgene filtration units. Filters were rolled using sterile forceps and placed into 5 mL Type A Bead-tubes (ref. 740799.50; Macherey-Nagel, Hoerdt, France). A volume of 1,200 µL of MR1 lysis buffer (ref. 744351.125; Macherey-Nagel) was then added to each tube, and a bead-beating step of 10 min was performed using a Genie2 vortex set at maximum speed. Filters and lysates were finally stored at -80°C in the bead tubes until further processing as detailed in the next section. Meteorological variables during sampling were monitored by the PUY meteorological station, including temperature, relative humidity, liquid water content, wind speed and direction (https://www.opgc.fr/data-center/public/data/copdd/pdd). The planetary boundary layer height (BLH) was extracted from ECMWF ERA5 global reanalysis (https://www.ecmwf.int/en/forecasts/datasets/reanalysis-datasets/era5) (Hoffmann et al., 2019). The geographical origin of the sampled air masses was derived from 72-hour backward trajectories computed using the CAT trajectory model (Baray et al., 2020), which uses dynamical fields extracted from the ERA-5 meteorological data archive with a spatial resolution of 0.125° for the present work. This tool was used for estimating percentages of air mass trajectory points in each of the eight direction sectors (Renard et al., 2020).

Table 1. Conditions of sample acquisition.

Sample ID	Sampling date (dd/mm/yyyy)	Sampling time period (local)	Sampling duration (h)	Geographical origin of the air mass†	Boundary layer height (min-max [average]) (m above sea level)‡	Position of the sampling site relative to the boundary layer	Temperature (°C)§	Relative humidity (%)§	Wind speed ($m.s^{-1}$) §	Liquid water content (LWC) ($g.m^{-3}$) §
CLEAR ATMOSPHERE										
20200707AIR	07/07/2020	9:00 AM - 3:30 PM	6.5	NW	1268-1834 [1626]	In	11.1	61	3.6	< 0.01
20200708AIR	08/07/2020	8:35 AM - 2:40 PM	6.1	NW	623-1675 [1253]	In	14.2	53	3.1	< 0.01
20200709AIR	09/07/2020	8:35 AM – 2:35 PM	6.0	N	651-2377 [1487]	In	20.3	48	3.4	< 0.01
20200922AIR	22/09/2020	8:51 AM – 2:46 PM	5.9	W	665-1334 [972]	Out	12.4	78	1.0	< 0.01
20201118AIR	18/11/2020	9:00 AM – 2:50 PM	5.8	W	680-1142 [870]	Out	14.1	41	6.4	< 0.01
20201124AIR	24/11/2020	10:00 AM – 3:55 PM	6.0	W	644-740 [699]	Out	8.6	50	3.4	< 0.01
Minimum	-	-	**5.8**	-	-	-	**8.6**	**41**	**1.0**	**< 0.01**
Maximum	-	-	**6.5**	-	-	-	**20.3**	**78**	**6.4**	**< 0.01**
Median	-	-	**6.0**	-	-	-	**13.3**	**52**	**3.4**	**< 0.01**
Mean	-	-	**6.1**	-	-	-	**13.5**	**55**	**3.5**	**< 0.01**
Standard error	-	-	**0.2**	-	-	-	**4.0**	**13**	**1.7**	-
CLOUDS										
20191002CLOUD	02/10/2019	2:47 PM – 5:10 PM	2.4	NW	1422-1505 [1465]	In	6.5	100	3.0	NA
20191022CLOUD	22/10/2019	10:06 AM – 4.30 PM	6.4	S	698-957 [813]	Out	5.7	100	8.7	NA
20200311CLOUD	11/03/2020	8:55 AM – 1:00 PM	4.1	W	964-1145 [1060]	Out	5.0	100	7.4	NA
20200717CLOUD	17/07/2020	11:45 AM – 3:00 PM	3.3	NW	1271-1437 [1343]	Out	10.1	100	1.6	0.08
20201016CLOUD	16/10/2020	8:50 AM – 1:30 PM	4.7	NE	917-1034 [958]	Out	1.1	100	1.8	0.35
20201028CLOUD	28/10/2020	9:13 AM – 3:15 PM	6.0	W	1026-1529 [1269]	Out	5.2	100	11.0	0.23
20201103CLOUD	03/11/2020	11:00 AM – 2:30 PM	3.5	W	1126-1593 [1390]	In	2.2	100	8.7	0.06
20201110CLOUD	10/11/2020	11:02 AM – 2:10 PM	3.1	SW	691-1276 [1016]	Out	5.9	100	2.5	0.07
20201119CLOUD	19/11/2020	8:54 AM – 11:47 AM	2.8	W	1207-1234 [1215]	Out	0.3	100	7.7	0.11
Minimum	-	-	**2.4**	-	-	-	**0.3**	**100**	**1.6**	**0.06**
Maximum	-	-	**6.4**	-	-	-	**10.1**	**100**	**11.0**	**0.35**
Median	-	-	**3.5**	-	-	-	**5.2**	**100**	**7.4**	**0.10**
Mean	-	-	**4.0**	-	-	-	**4.7**	**100**	**5.8**	**0.15**
Standard error	-	-	**1.4**	-	-	-	**3.0**	**0**	**3.6**	**0.11**
P-value (Mann-Whitney test; clouds *vs* clear conditions)			0.04*	-	-	-	0.003**	0.001**	0.44	0.003**

2.2 Nucleic acid extraction and shotgun sequencing

For each sample, DNA and RNA were extracted in parallel from single MCE filters using NucleoMag® DNA/RNA Water kit (Macherey-Nagel, Hoerdt, France), following the protocols recommended by the manufacturer for filter membranes. All facilities were previously treated with RNase-away spray solution (Thermo Scientific; Waltham, USA). For DNA extraction, half of the lysate was processed (600 µL), and a final step consisted of the removal of RNA by adding 1:50 volume of RNase A (12 mg.mL^{-1}, stock solution from Macherey-Nagel). DNA was finally eluted into 50 µL of DNase-free H2O after 5 min of incubation at 56°C, then quantified by fluorescence using the Quant-iT™ PicoGreen® dsDNA kit (Invitrogen; Thermo Fisher Scientific, Waltham, MA USA). For RNA extraction, the remaining 600 µL of lysate was processed, and the final step consisted of removal of DNA by the addition of 1:7 volumes of reconstructed rDNase (as provided with kits, Macherey-Nagel). RNA was finally eluted into 30 µL of RNase-free H_2O after 10 min of incubation at room temperature. Only trace amounts of DNA could be obtained from negative controls (7.3 ng of DNA on average, 11.4 ng at maximum), and these were, thus, not processed for sequencing. In contrast, the total amounts of DNA and RNA recovered from environmental samples ranged from 42.6 to 838.7 ng and 22.5 to 244.8 ng, respectively. The corresponding total DNA and RNA concentrations in the air volumes sampled as inferred from concentrations in the extracts, ranged from 0.03 to 0.73ng DNA.m^{-3} and from 0.02 to 0.42 ng RNA.m^{-3}, respectively (Table S1).

Individual DNA or RNA extracts from individual samplers from the same sampling event were pooled, and 30 µL were transferred to GenoScreen (Lille, France) for further processing of RNAs (quantification, reverse-transcription to cDNAs), and shotgun sequencing of the metagenomes (MGs) from DNAs, and metatranscriptomes (MTs) from cDNAs, on Illumina HiSeq (paired end reads of 150 bp). A first sample (20191022CLOUD) was deeply sequenced (~200 M reads) and used to check the feasibility of the approach, adjust the sequencing depth, and elaborate bioinformatics workflows. The other samples were sequenced at a lower sequencing depth (40-60 M reads per sample). Raw sequencing MGs and MTs data are available as fastq.gz files through the European Nucleotide Archive at EBI, under the project accession PRJEB54740, samples ERR9966616 to ERR9966643.

2.3 Bioinformatics and differential expression analyses

Raw MGs contained approximately between 30 and 260 million (M) reads (68.7 M in average), and raw MTs from 65 M to 195 M reads (Tables S2-S3). The bioinformatics workflow is detailed in Supplementary Material and summarized in Fig. S1. Briefly, this consisted of (i) sequence preprocessing (quality control, trimming, etc.), (ii) taxonomic annotations of MGs and MTs [Kraken2 v2.1.1 (Wood and Salzberg, 2014) and "PlusPF" database], (iii) construction of a gene catalog to serve as a unique reference for the study, as inspired from (Salazar et al., 2019) (Fig. S2). This was elaborated by (I) merging all the contigs from each individual MG, (II) predicting genes [MetaGeneAnnotator v1.0.0 (Noguchi et al., 2008)], (III) clustering in order to remove redundancy [CD-Hit v4.8.1 (Li and Godzik, 2006)], and (IV) annotating functions and taxonomy [using DIAMOND v2.0.8.0 (Buchfink et al., 2015) and the UniProtKB Swiss-Prot database (The UniProt Consortium, 2019)]. Finally, (iv) non-rRNA reads in each MG and MT were mapped toward the annotated gene catalog. The log ratios of the number of reads associated with a gene, taxon, E.C. or GO in a MT dataset to that in the corresponding MG (abbreviated as RNA:DNA log ratios) were calculated using data normalized to total counts. RNA:DNA ratios are commonly used as an

appraisal of the relative level of metabolic activity, with higher ratios indicating potentially higher metabolic activity (Baldrian et al., 2012; Zhang et al., 2014). In addition, statistical differential expression analysis (DEA) was performed on the MT to MG mapping coverages ratio towards the gene catalog in order to detect overrepresented genes and functions, and those significantly overrepresented in clouds compared to clear conditions, or conversely [MTX model v1.5.1 (Zhang et al., 2021); see Supplementary Material for details]. Metabolic pathways were reconstructed from the E.C. numbers obtained from UniprotKB identifiers using KEGG database and resources (Kanehisa et al., 2023).

3 Results

3.1 Microbial taxonomy in metagenomes and metatranscriptomes

The datasets include sequences from eukaryotes, bacteria, archaea and viruses. Bacteria dominate in clear atmosphere (~88% and 71%, on average, of the total number of reads in MGs and MTs, respectively), while eukaryotes (mainly fungi) prevail in clouds (~51% and 87% on average, respectively) (Fig. S3), but both Prokaryotic and Eukaryotic diversity indices are statistically similar between cloud and clear atmosphere samples (Kruskal-Wallis tests, $p > 0.05$) (Fig.S4 1). Archaea (Euryarchaeota, Thaumarchaeota and Crenarchaeota) and viruses (mainly bacteriophages) both contribute very low proportions of sequences (< 0.1%).

In Bacteria, a total of 32 distinct phyla, 159 orders and 1 249 genera are identified in MGs (Data S1). The dominant are Micrococcales, Corynebacteriales, Propionibacteriales (Actinobacteria), Pseudomonadales, Sphingomonadales, Burkholderiales and Hyphomicrobiales (Proteobacteria) (Fig 1A). The observed bacteria richness in samples varies between 532 and 826 genera, the vast majority of which (963 out of 1,249 genera; ~77%) are common to clouds and clear air (Fig 1C). Shannon's diversity index ranges from ~2.6 to ~4.3 depending on samples (Fig. S4).

In Eukarya, identified richness distributes among 8 phyla, 21 orders and 54 genera (Data S2); all are shared between cloudy and clear atmospheric conditions (Fig. 1D). Fungal taxa largely predominate, with the most abundant affiliated to Helotiales, Hypocreales and Mycosphaerellales in Ascomycota, and Ustilaginales in Basidiomycota (Fig 1B). Other unicellular eukaryotic phyla detected include Apicomplexa (parasites of Metazoa), Bacillariophyta (microalgaes), Euglenozoa, Evosea, and Cercozoa (amoeboids and flagellates). The observed eukaryotic richness in samples varies between 41 and 43 genera depending on samples, with Shannon's index ranging from ~1.1 to ~2.4 (Fig. S4).

The taxa significantly overrepresented in MTs compared with MGs include 77 families of bacteria (198 genera) and 10 families of eukaryotes (27 genera) (Data S3). These are subsets of the total biodiversity seen in MGs, and they are not distinguishable between clouds and clear conditions (PCA, Fig. S5). Bacterial taxa tend to exhibit higher relative representation in MTs compared to MGs (termed as RNA-to-DNA ratio) in clouds than in clear atmosphere, contrary to eukaryotes (Fig. S6-S7).

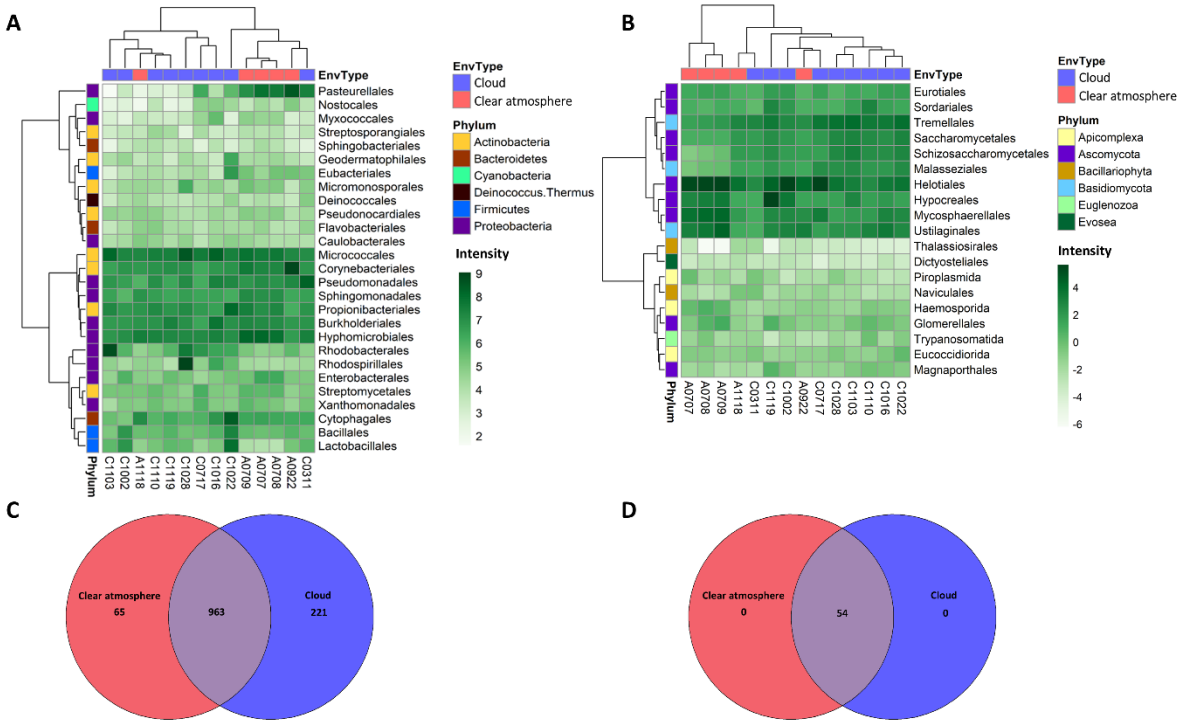

Figure 1. Bacterial and eukaryotic diversity from metagenomes. (A, B) Distribution of the most abundant bacterial and eukaryotic orders in the metagenomes, and corresponding hierarchical clustering (Ward's method, "ward.D2"). The intensity scale depicts centered-log ratio (clr) abundance. EnvType: environment type (the samples are identified as follows: "A" for clear atmosphere (air) or "C" for clouds, followed by the sampling date in the format "mmdd"); (C, D) Venn diagrams depicting the distribution of bacteria and eukaryotic genera between clouds and clear atmosphere.

3.2 Functional aspects and differential expression analyses

The RNA-to-DNA concentration ratio is significantly higher in clouds than in a clear atmosphere (range 1.49-3.62 and 0.21-1.64, Mann-Whitney test, p-value=0.004) (Table S1; Fig. 2A). No relation is observed with relative humidity in clear atmosphere.

Independently from atmospheric conditions, abundant transcripts in the datasets are related to central biological functions and their regulation, including carbon, amino-acid and protein processing, energy production, signaling, response to stresses, transports and others (Fig. 3; panels A, C, E in Fig. 4 and Fig. S8-S9). From a total of 21,046 unique genes (Uniprot IDs) in MGs constituting the reference catalog, 488 are found significantly more represented in MTs than in MGs (Data S4). These correspond to, at the GO term level, 419 Biological Processes, 284 Molecular Functions, and 140 Cellular Components overrepresented in transcriptomes (Data S5). Most

of these (~80%) are affiliated with Eukaryotes, in particular Fungi, or with Gamma-Proteobacteria and Actinobacteria in Bacteria (~48% and ~25% of the bacteria transcripts, respectively) (Fig. S10).

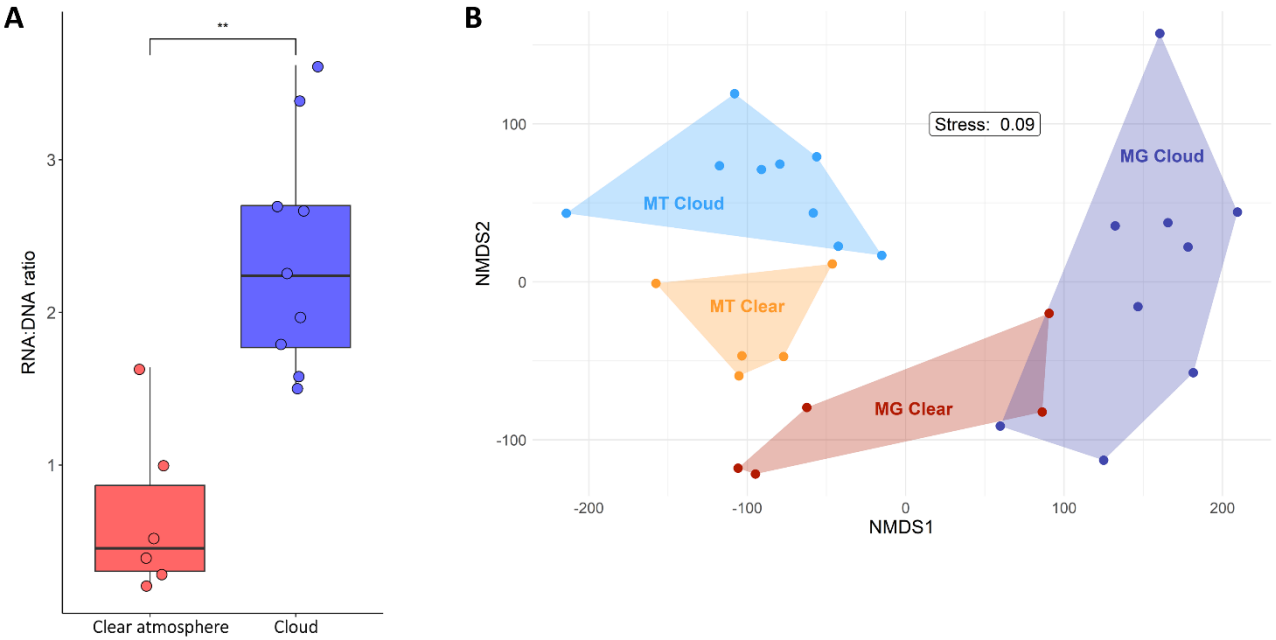

Figure 2. (A) RNA-to-DNA concentration ratio in clouds and clear atmosphere; dots indicate individual samples, boxplots display medians, 25th and 75th percentiles, and whiskers are 1.5 interquartile ranges; ** indicates a significantly higher ration in clouds (Mann-Whitney test, p-value=0.004); (B) Non-Metric Multidimensional Scaling (NMDS) analysis based on the 21 046 functional gene entries detected in total, depicting clear distinctions between MGs and MTs, and between MTs of cloudy and clear conditions.

These genes and functions occur and distribute differentially depending on the presence of condensed water (panels B, D, F in Fig. 4 and Fig. S8-S9). Multivariate analysis (NMDS) indeed indicates distinct transcriptional patterns depending on atmospheric conditions (Fig. 2B), and differential expression analysis (DEA) specifies it (Fig. 5; Data S6-S7): among the 488 genes significantly more represented in MTs than in MGs, 320 (~66%) are also significantly differentially represented between clouds and clear conditions, about two thirds of which in clouds, contributed by Eukaryotes (Fig. S11). In total, differentially represented transcripts belong to 394 Biological Processes, 147 Cellular Components, 279 Molecular Functions, and correspond to 200 unique E.C. numbers (see distribution of subclasses in Table S4). Overall, the most diverse enzyme transcripts are NADH:ubiquinone oxidoreductase (E.C. 7.1.1.2, 11 distinct entries), RNA polymerase (E.C. 2.7.7.6), RNA helicase (E.C. 3.6.4.13) and cytochrome-c oxidase (E.C. 7.1.1.9; 6 distinct entries each), and non-specific serine/threonine protein kinase (E.C. 2.7.11.1; 5 distinct entries). We examined below the

similarities and specificities of microbial transcriptomes in and outside of clouds for different categories of functions and metabolisms based on the distribution of GOs and E.C. numbers (Fig. 5-6; Fig. S12-S15; Table S4; Data S6-S7).

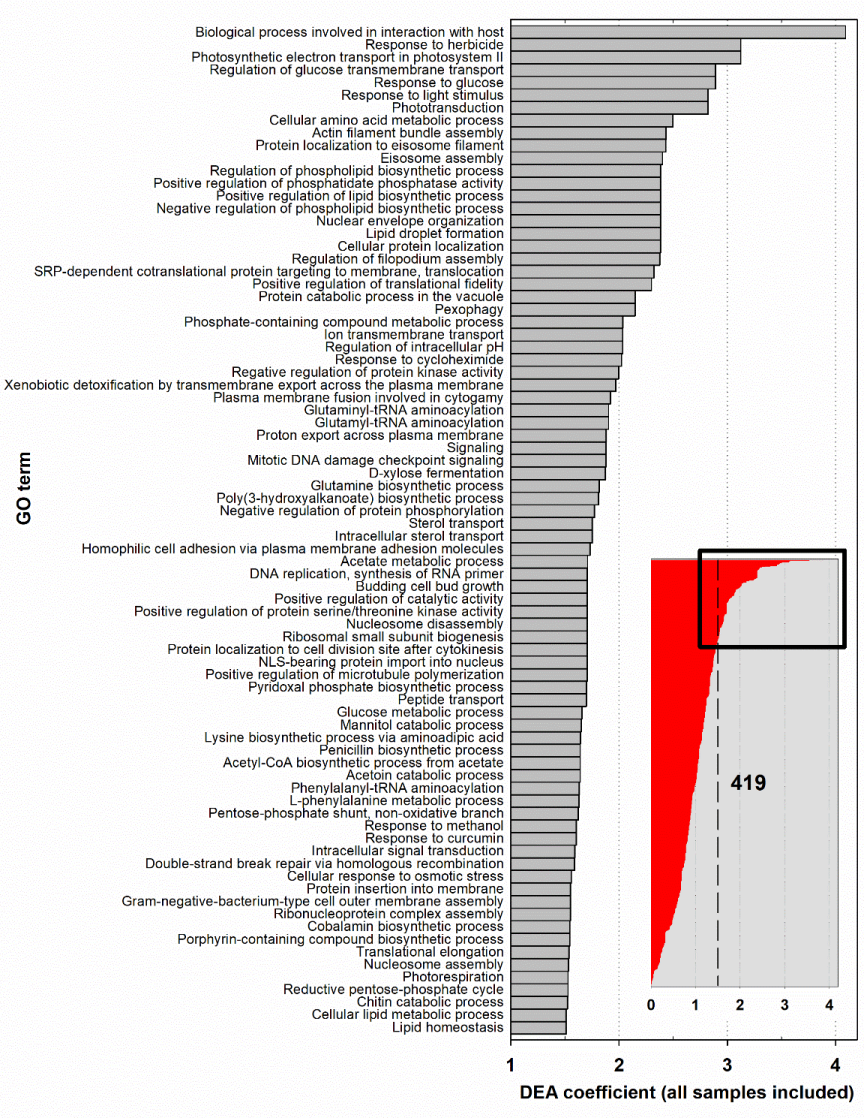

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Figure 3. Biological Processes GO terms associated with overrepresented transcripts in samples, as compared with their representation in metagenomes, from Differential Expression Analysis (DEA). Only the 80 GO terms with DEA coefficients > 1.5, out of 419 in total, are shown.

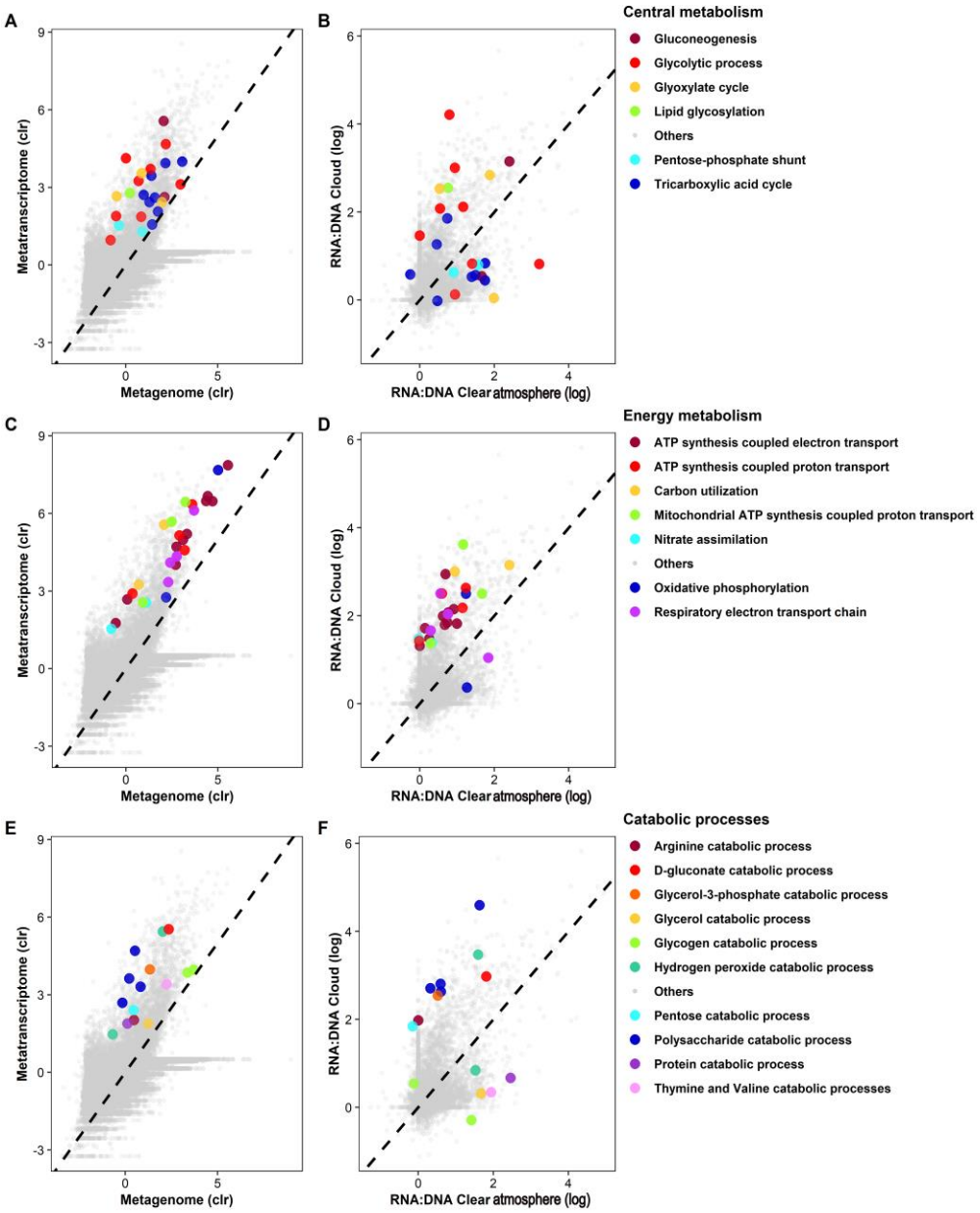
A
B
C
D
E
F
Metatranscriptome (clr)
Metagenome (clr)
RNA:DNA Cloud (log)
RNA:DNA Clear atmosphere (log)
Central metabolism
Gluconeogenesis
Glycolytic process
Glyoxylate cycle
Lipid glycosylation
Others
Pentose-phosphate shunt
Tricarboxylic acid cycle
Energy metabolism
ATP synthesis coupled electron transport
ATP synthesis coupled proton transport
Carbon utilization
Mitochondrial ATP synthesis coupled proton transport
Nitrate assimilation
Others
Oxidative phosphorylation
Respiratory electron transport chain
Catabolic processes
Arginine catabolic process
D-gluconate catabolic process
Glycerol-3-phosphate catabolic process
Glycerol catabolic process
Glycogen catabolic process
Hydrogen peroxide catabolic process
Others
Pentose catabolic process
Polysaccharide catabolic process
Protein catabolic process
Thymine and Valine catabolic processes

Figure 4. GO terms representation in metagenomes and metatranscriptomes (A; C; E), and relative representation (termed as RNA:DNA) in metatranscriptomes in clouds *versus* clear atmosphere (B; D; F), for Biological Processes related to: central metabolism (A, B), energy metabolism (C, D), and catabolic processes (E, F); clr: centered log-ratio transformation. Other GO terms of interest are presented as Fig. S9-S10.

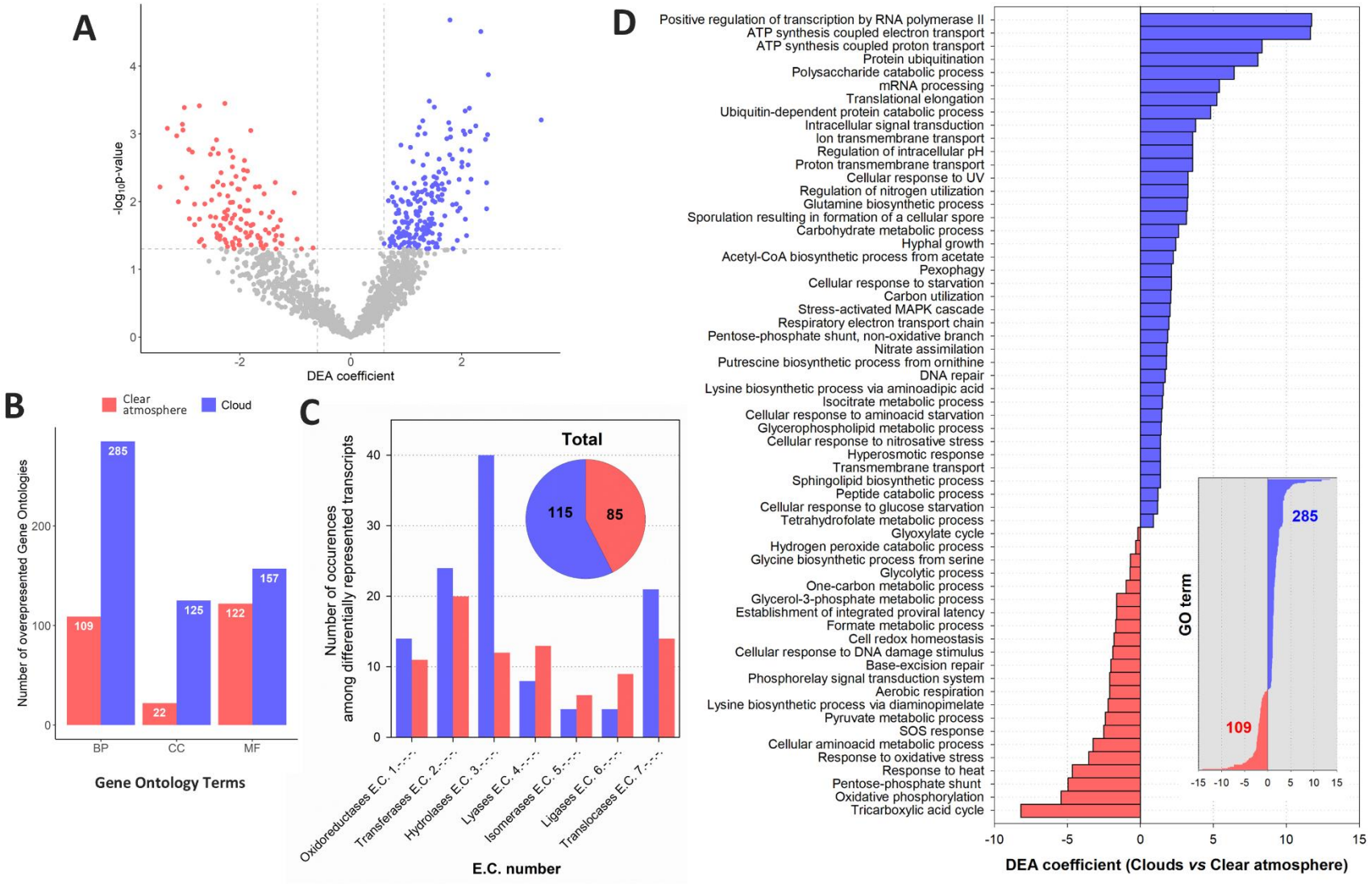

Figure 5. Results of Differential Expression Analysis (DEA) of transcripts overrepresentation in clouds (blue) compared with clear atmosphere (red), at the gene (A), GO term (BP: Biological Processes; CC: Cellular Component; MF: Molecular Function) (B) and E.C. number (C) levels. In (D) the DEA coefficient associated with selected Biological Processes GO terms are shown (39 GO terms out of 285 in total for clouds and 22 out of 109 for clear atmosphere).

3.2.1 Central, carbon and energy metabolisms

Numerous transcripts related to translation and elongation factors in Eukaryotes are overrepresented in clouds (Data S6) suggesting metabolic regulations and the production of new biomass. Large numbers of transcripts relate to central functions of carbon and energy metabolisms in both clouds and clear air samples (Fig. 4, Fig. S8-S9): glycolysis and glucose-related metabolic processes (GO:0006096; GO:0006006), glyoxylate and TCA cycles (GO:0006097; GO:0006099), and carbohydrate metabolisms (GO:0005975; GO:0006083; GO:0019427). Consistently, transcripts coding for key enzymes of these pathways are abundant in both prokaryotes and eukaryotes, such as isocitrate dehydrogenase (IDH) (EC 1.1.1.42 and EC 1.1.1.41), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12) and aconitase (EC 4.2.1.3) (Fig. 6, Fig. S12-S14).

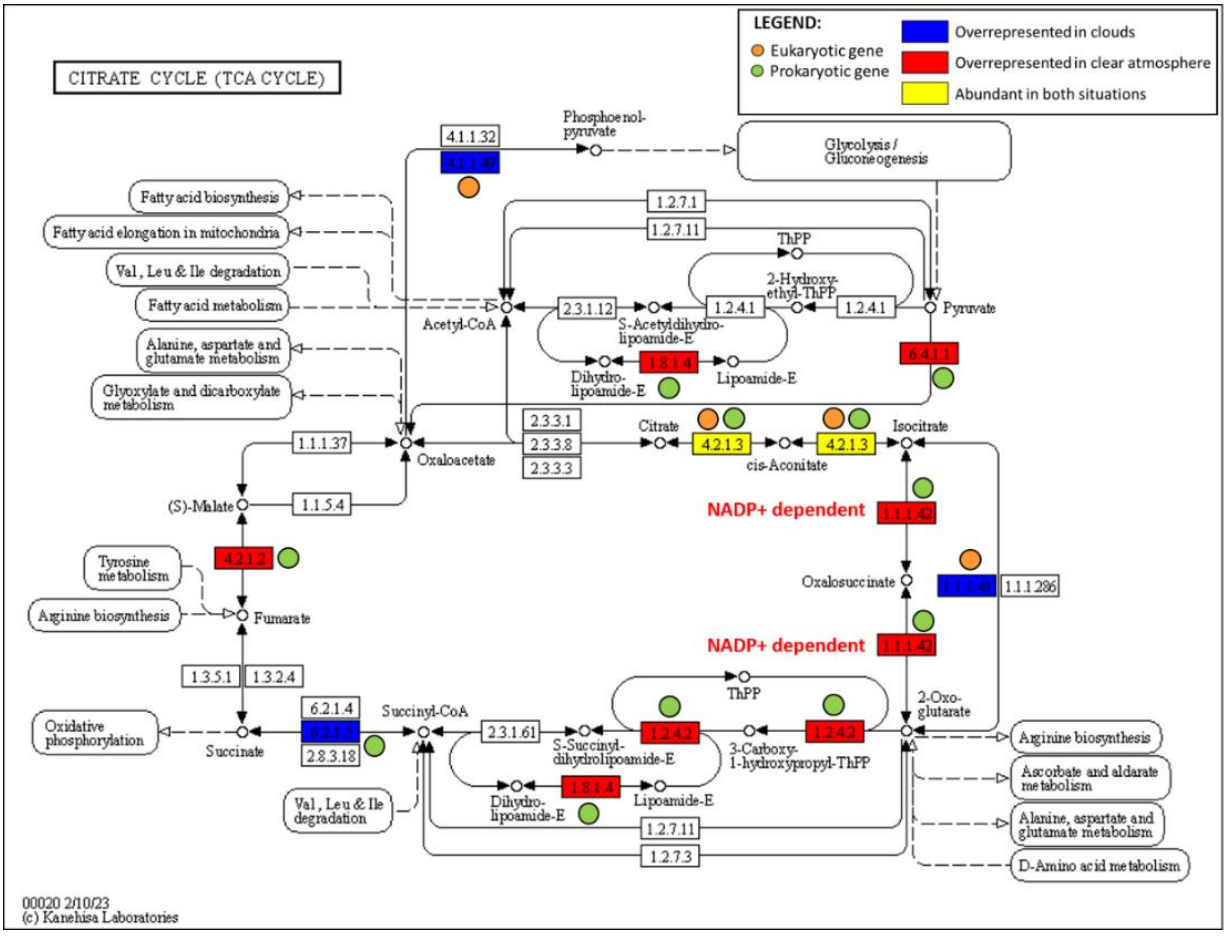

Figure 6. The TCA cycle metabolic pathway, depicting overrepresented enzyme transcripts in clouds and/or in clear atmosphere by eukaryotes and/or by prokaryotes (from UniprotKB identifiers and KEGG database).

DEA analysis indicates a strong overrepresentation in clouds of functional transcripts coding for hydrolases (E.C. 3.-.-.-) and, to a lesser extent, translocases (E.C. 7.-.-.-), particularly those involved in the translocation of protons (E.C. 7.1.-.-). These relate to GOs including carbon utilization (GO:0015976), polysaccharide catabolism (GO:0000272), and ATP production-

coupled electron and proton transport (GO:0042773; GO:0015986) (Fig. 5). The pentose phosphate pathway (non-oxidative phase) (GO:0009051) also tends to be more represented in clouds than clear atmosphere, with an over-representation in particular of fructose-bisphosphate aldolase transcripts (*fba*; EC 4.1.2.13) (Fig. S13). These observations concur with increased biochemical energy needs in clouds.

In turn, transcripts of lyases (E.C. 4.-.-.-) and ligases (E.C. 6.-.-.-) are overall more prevalent in clear atmosphere than in clouds (Fig. 5; Table S4). In particular the TCA cycle pathway (GO:0006099) appears upregulated by prokaryotes, with transcripts coding for enzymes including pyruvate carboxylase (*pyc*, *pycA*, *ylaP*; E.C. 6.4.1.1), isocitrate dehydrogenase [NADP-dependent] (IDH) (*icd*, *Cgl0664*, *cg0766*; EC 1.1.1.42), fumarate hydratase (*fumC*; E.C. 4.2.1.2), and alpha-ketoglutarate dehydrogenase (*sucA*, *odhA*, *Oant*; EC 1.2.4.2) (Fig. 6). Glycolytic processes and the glyoxylate cycle are overall barely affected by clouds based on DEA. Nevertheless, transcripts of enzymes involved in specific steps of these pathways are detected in higher proportions in clouds, including enolase (2-phospho-D-glycerate hydro-lyase; EC 4.2.1.11), phosphoenolpyruvate carboxykinase (EC 4.1.1.49), and acetyl-coenzyme A synthetase (E.C. 6.2.1.1) (Fig. S12-S13).

3.2.2 Protein, amino-acids and nitrogen metabolism

Numerous of the most abundant transcripts related to protein, amino acids and nitrogen metabolisms are more prevalent in clouds than they are in clear atmosphere (Fig. 4-5; Fig. S8-S9). These include post-translational protein modifications (ubiquination, phosphorylation and glycosylation, and related processes; GO:0016567; GO:0006511; etc.), known to participate to the regulation of enzymatic activities and multiple other cellular and metabolic processes (Yang et al., 2022). The overrepresentation in clouds of transcripts of the regulatory gene *areA* suggests that multiple nitrogen sources are targeted (Kudla et al., 1990), likely as a response to limited resources. Clouds are also associated with aminoacid starvation (GO:0034198).

Among aminoacid related transcripts and functions, only glutamine and lysine biosynthesis are overrepresented in clouds (Fig. 5). Catabolism is rather directed towards serine and arginine, in link with ornithine and putrescine production pathways within glutathione metabolism, a major pathway in the recycling of NADP+ and the regulation of oxidants (Fig. S15). During clear conditions, processes of aminoacid biosynthesis prevail (arginine, leucine, threonine, lysine, beta-alanine, glycine), potentially involving gaseous dinitrogen fixation (Dalton and Kramer, 2006).

Among inorganic nitrogen metabolism, nitrate assimilation (GO:0042128) and the utilization of ammonium NH_4^+ (GO:0042128) are both overrepresented in clouds. Both ions are abundant in atmospheric water (Péguilhan et al., 2021). The latter function concords with the overrepresentation of L-glutamate:ammonia ligase transcripts (E.C. 6.3.1.2; GO:0004356, gln), an enzyme involved in the synthesis of glutamine, one of the main regulators of cellular development and oxidative stress response in fungi (Wang et al., 2022).

3.2.3 Transport, signalling and response to stress

In clouds, transcripts in link with transmembrane transports of ions and protons (GO:0034220, GO:1902600, GO:0055085) are among the most represented (Fig. S9). These participate in ATP synthesis (GO:0042773, GO:0015986 etc), the regulation of substrate utilization (GO:0006808, GO:0015976; GO:0009267) and homeostasis (GO:0045454) (Fig. 5; Fig. 7).

In turn, oxidative stress (GO:0006979) and SOS response (GO:0009432) dominate in clear atmosphere, whereas in clouds responses to osmotic and nitrosative stress (GO:0071470; GO:0071500), stress-activated MAPK cascade (GO:0051403), the regulation of intracellular pH (GO:0051453), processes of starvation towards carbon and nitrogen (GO:0042149, GO:0034198), and processes of autophagy and pexophagy (*i.e.*, macropexophagy; GO:0051403) prevail (Fig. 5; Fig. 7; Fig. S9). Such functional patterns can be interpreted as microbial responses to wetting. They indicate a probable sheltering effect of condensed water against oxidative stress, along with limited nutrient resources requiring metabolic adjustments.

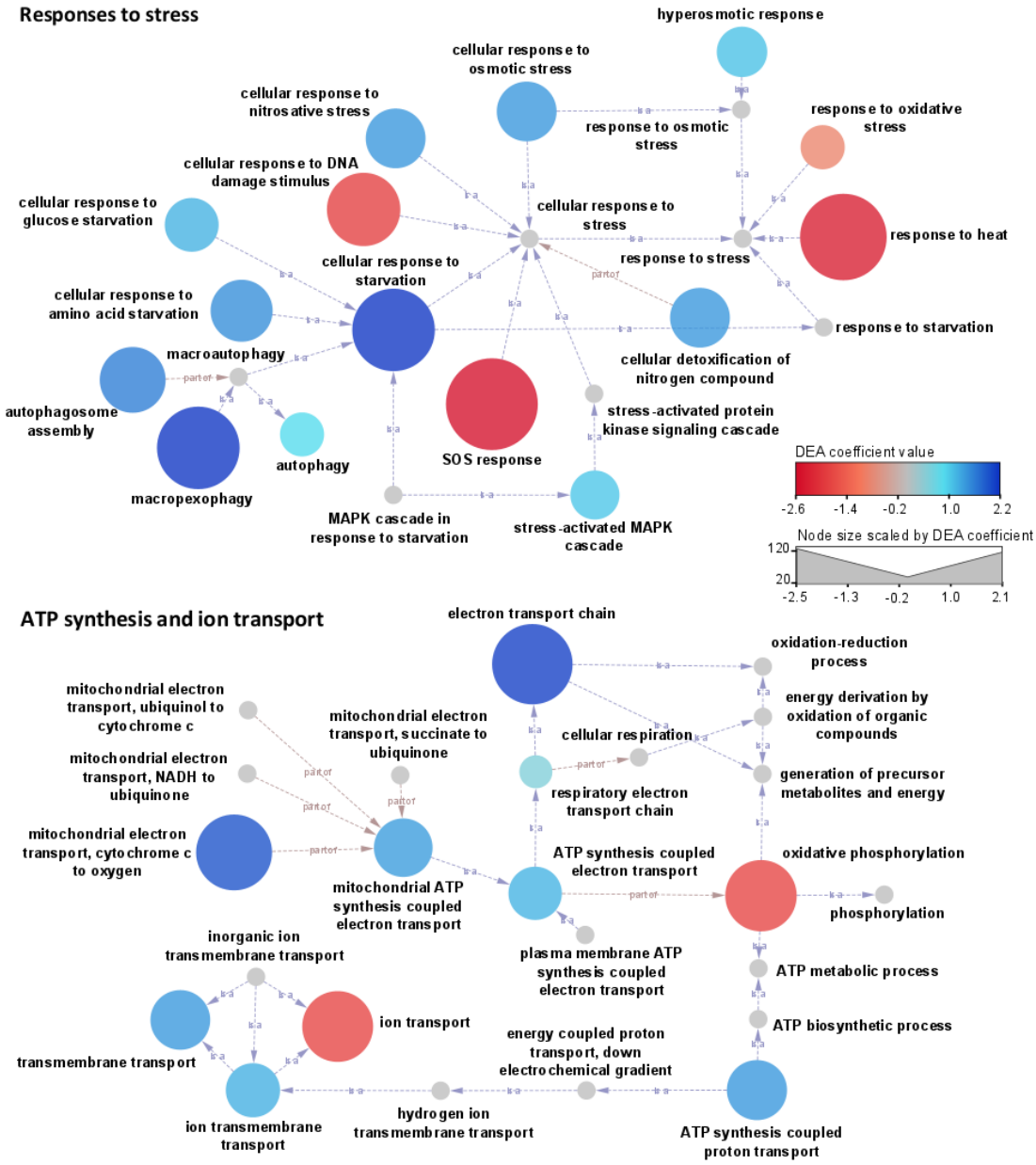

Figure 7. Networks linking Biological Processes (GO terms) related with stress responses, and ATP synthesis and ion transport. The colour scale represents associated DEA coefficients, with negative values (red shades) indicating a significant overrepresentation of related transcripts in clear conditions, and positive values (blue shades) in clouds. Node size is scaled to DEA coefficient absolute value. Arrows indicate relationships between GOs ("is a" or "part of" as specified).

4 Discussion

We report here the most comprehensive dataset to date regarding aeromicrobiome's functioning in the natural environment. For the first time, we demonstrate that multiple microbial functions are directly influenced by atmospheric conditions,

specifically by the presence of clouds. In previous work, using targeted and untargeted transcriptomics approaches, we identified metabolically active bacteria and fungi in clouds (Amato et al., 2017), then depicted their functioning through the analysis of transcriptomes (Amato et al., 2019). Here, we extend knowledge by integrating non-cloudy atmosphere in our analysis, so replacing clouds into the atmospheric context, *i.e.* as volumes embedded within clear atmosphere.

In terms of taxonomy, the airborne microbial assemblages in clouds are not distinguishable from those in clear atmosphere, as we recently reported from amplicons for bacteria (Péguilhan et al., 2023a). They consist of diverse Eukarya, Bacteria, Archaea and viruses, with Proteobacteria and Actinobacteria dominating in Bacteria, and Ascomycota and Basidiomycota in Eukaryotes, which is typical over continental vegetated areas [*e.g.*, (Bowers et al., 2013; Tignat-Perrier et al., 2020)]. Such a similarity was to be expected given the large size of microbial aerosols and so their ability to act as cloud condensation nuclei, and considering that cell multiplication in clouds is unlikely to significantly affect the structure of microbial assemblages due to limited residence time (Ervens and Amato, 2020).

4.2 A "Birch effect" up in the sky

Higher concentration of RNA, respect to DNA, in clouds than in cloud-free air suggests higher levels of microbial metabolic activity (Baldrian et al., 2012; Salazar et al., 2019). This remains to be assessed quantitatively through more direct activity measurements. Accordingly, transcripts related to multiple biological processes are overrepresented in clouds as compared with clear atmosphere, such as energy metabolism, carbohydrate and polysaccharide catabolism, transcription, translation, transmembrane transports, and metabolic regulation mechanisms. Homeostasis regulation, starvation and autophagy supplant the oxidative stress response and DNA repair functions (SOS response) that prevail during clear conditions. The switch of functional gene expression observed in microorganisms between clear atmosphere and clouds therefore suggests a phenomenon similar to the "Birch effect" that occurs in dry soils in response to rewetting by rain, where the sudden influx of water triggers a burst of microbial activity (Griffiths and Birch, 1961; Unger et al., 2010). The "Birch Effect" in soils typically lasts for a few days, hence, much longer than the lifetime of clouds and individual cloud droplets (Feingold et al., 1996). The metabolic modulations described here should therefore apply to any cloud regardless of the time elapsed since its formation. As with soils – but necessarily to a much smaller extent because of the low biomass – the resurgence of microbial activity in clouds may lead to the release of gaseous biogenic compounds such as N_2O through aerobic ammonium oxidation, *i.e.* nitrification (Jørgensen et al., 1998), which is also supported by the overrepresentation of ammonium utilization process.

Dry-wet alternance in ecosystems contributes to shaping microbial assemblages, activity and responsiveness to changing conditions. The lag-time after which microorganisms start recovering in soil upon rewetting shortens after repeated dry-wet cycles, due to selection processes towards the most responsive ones (Zhou et al., 2016). Transcriptionally active taxa in our observations represent ~20% of the richness, and this could result and attest from such selection processes during aerial transport. Typical atmospheric residence times of microbial cells are on the order of a few days (Burrows et al., 2009), during which they may undergo ~10 water evaporation-condensation cycles before precipitating (Pruppacher and Jaenicke, 1995).

Whereas clouds can last for several hours (Dagan et al., 2018), individual cloud droplets can form and evaporate within a few minutes (Feingold et al., 1996). Such rapid fluctuations of water availability could favour the most responsive microorganisms such as Proteobacteria and Actinobacteria, which include numerous generalists with high metabolic flexibility (Chen et al., 2021), and which in fact often dominate airborne microbial assemblages (Amato et al., 2017; Péguilhan et al., 2021; Šantl-Temkiv et al., 2022).

4.2 Responses to stress attest of multiple functional adjustments

Our data indicate that clear atmosphere is dominated by responses to oxidative stress and DNA damages, involving SOS response, while clouds are characterized by osmotic stress, starvation and autophagy. The functional patterns of aeromicrobiome' stress responses are therefore very consistent with environmental conditions, and help drawing a more complete picture of the multiple aspects of the microbial journey in the high atmosphere.

In clouds, liquid water shelters cells against oxidants and radiations, but the rapid condensation/evaporation processes along with the dissolution of solids and the solubilization of gases generate large fluctuations of water activity (*e.g.*, Koehler et al., 2006). Additionally, in the limited volumes provided by droplets, the nutrient requirements are likely not fully satisfied, and autophagy processes may contribute to alleviating the needs. Peroxisomes, organelles dedicated to the detoxification of oxidants in eukaryotes, are targeted in particular by autophagy (pexophagy), as during fungal spore germination. Such process could compromise survival if the cloud evaporates, but it may be a trade-off with increased chances in the race for surface colonization if the cloud precipitates.

Here, clear air was collected at relative humidity between 41%-78%, *i.e.*, at the limits of compatibility with biological processes, around ~0.6 a_w (water activity) for the most tolerant organisms (*i.e.*, 60% pure water rH) (Stevenson et al., 2015). At a_w below 0.55, DNA gets unstructured and metabolic regulations are no longer possible. Water limitation is a great challenge that many microorganisms have to face in their natural habitats. This affects cell turgor due to water efflux and slows down growth and metabolic activity (Chowdury et al., 2011).

In order to manage the numerous environmental factors related with variations of water activity, such as temperature or osmotic pressure, microorganisms have developed ranges of strategies: modifications of the saturation level of lipids in membranes to adjust fluidity, synthesis and accumulation of intracellular compatible solutes in order to prevent water efflux and maintain homeostasis (osmoprotectants and cryoprotectants such as K^+, sucrose, trehalose, amino-acids and others) (Poolman and Glaasker, 1998), chaperones to protect molecular structures, membrane canal proteins, such as aquaporins, to sustain water fluxes (Tong et al., 2019), etc.

4.3 Airborne fungal spores initiate germination in clouds

In agreement with the overrepresentation of transcripts, it is likely that fungal spores initiate germination in clouds. These include translation initiation and elongation factors affiliated with several taxa of fungi (elF4E, eEF3 and others) (van Leeuwen et al., 2013; Li et al., 2022; Osherov and May, 2001), chitin deacetylase (Leroch et al., 2013) and other regulatory protein genes such as *areA* (Kudla et al., 1990). Fungal spores are propagules designed for (aerial) dispersion (Brown and Hovmøller, 2002), which germinate (i.e., initiate growth) when they reach favourable conditions of water availability. During germination, functions of cell protection give way to anabolic processes within minutes (van Leeuwen et al., 2013; Leroch et al., 2013). Cellular growth and respiration are promoted by the sudden availability of water, associated with the release and solubilization of readily bioavailable organic compounds. While dormant, functions of cell protection prevail in spores, against osmotic stress, heat, and oxidants. In the presence of water, mitogen-activated protein kinase (MAPK) cascade signalling pathways mediate the activation of central metabolic functions of energy production and biosynthesis (van Leeuwen et al., 2013).

Autophagy processes in particular can participate to the early steps of appressorium synthesis in parasitic and symbiotic fungi (Veses et al., 2008), a structure designed to invade host cells. Although we did not consider time series, our observations therefore strongly concur with such a sequence, and it is reasonable to assert that airborne fungal spore germination occurs in clouds.

4.4 Utilization of nutrients and interactions with chemistry

Microbial activity is driven by the balance between water availability and accessibility to substrates (Skopp et al., 1990). Although not evaluable here, the amounts of water retained by efflorescent aerosols below water vapor saturation may be sufficient to sustain microbial activity, down very low values of relative humidity (Cruz and Pandis, 2000; Ervens et al., 2024). In clouds, *i.e.*, above saturation levels, the large amounts of available water make it even conceivable that bacterial multiplication occurs. Bulk cloud water indeed contains enough nutrients to sustain microbial growth including carboxylic acids, aldehydes, sugars, amino-acids, ammonium, nitrate, etc. (Amato et al., 2007a; Bianco et al., 2016, 2018, 2019; Deguillaume et al., 2014; Renard et al., 2022), and the level of microbial activity at 0°C was shown to be compatible with it (Sattler et al., 2001). Field observations indicate that fog carries higher biomass than clear atmosphere (Fuzzi et al., 1997; Saikh and Das, 2023), while estimations suggest that microbial mass may double during cloud's lifetime (Ervens and Amato, 2020). The fact that statistically only 1 out of ~10 000 droplets contains a microbial cell in aerially suspended water, as opposed to bulk water, potentially causes a very efficient and rapid depletion of nutrients in these small biotic volumes (Khaled et al., 2021) (~10^{-6} µl for 20 µm diameter droplets, so a cell concentration of at least ~10^{9} cells mL^{-1} in biotic droplets), which exposes cells to starvation and may limit metabolic processes (Gray et al., 2019).

The overrepresentation of transcripts related to carbon, ammonium and nitrate utilization in clouds supports that carbon and nitrogen biological processing occurs. Despite the low biomass, the impact of microbial activity on organic carbon chemistry

has been assessed as potentially significant, depending on the volatility and solubility of the compounds (Ervens and Amato, 2020; Khaled et al., 2021; Nuñez López et al., 2024). The impacts on nitrogen species have been barely examined (Hill et al., 2007; Jaber et al., 2021). Estimates indicate potential biodegradation of amino-acids, but there is as yet no information on inorganic compounds such as ammonium and nitrate, which are among the most abundant ions in cloud water (Deguillaume et al., 2014).

5 Concluding remarks and perspectives

The recovery of active metabolic processes in airborne microorganisms prior to their deposition could facilitate surface or host invasion if cloud precipitates. In turn, in the likely event where the cloud evaporates instead of precipitating, triggering germination and sacrificing essential cellular structures while conditions may soon become inhospitable could compromise future chances of survival, and so of further dispersion. It thus remains to be evaluated whether these metabolic regulations offer an ecological advantage to microorganisms in such transient environments as clouds where they have no chance to establish due to limited residence time, or whether they may influence survival in the atmosphere and invasion processes upon deposition.

Metatranscriptomics approaches are undoubtedly among the most powerful methods to examine microbial functioning. Still, they remain limited by multiple constraints inherently associated with them. For instance, they do not allow attributing biological processes to individual cells or to specific taxa with certainty. They are also limited by current functional genomic knowledge and existing databases, in particular in natural environments like the atmosphere where rare taxa often represent an important fraction of the richness (Péguilhan et al., 2023b). In the atmosphere, micro-organisms have a short residence time of a few days, and air volumes contain mixed populations of cells of different origins and atmospheric ages. Such analyses as metatranscriptomics, based on RNA, assume a high turnover of these molecules in cells, in particular mRNA, and are therefore considered to reflect quasi-instantaneous cell activity. Nevertheless, ribosomes (rRNA) can persist several days at low temperatures (Schostag et al., 2020) and may therefore be the result of recent past activity. These "residual" signatures of activity, along with the high level of mixing, can blur our vision of the actual situation in such highly dynamic environment as the atmosphere.

Transcriptomes attest of potential cellular activity, but they do not provide quantitative information of microbial activity in terms of fluxes of elements or energy. Quantitative measurements of microbial activity therefore remain necessary to confirm the "atmospheric Birch effect" caused by clouds. The transitions between clear and cloudy conditions in particular remain to be examined to evaluate the temporal responsiveness of airborne microbial assemblages to cloud formation and evaporation. While this is potnetially achievable in an atmospheric simulation chamber, assessing microbial activity in naturally aerially suspended biological microorganisms remains highly challenging, if not impossible (yet). The development of methods able to detect and quantify microbial metabolic activity in air-suspended cells and at high frequency appears therefore as a prerequisite.

Our study focused in particular on the potential impact of clouds on microbial functioning, and it relies on samples collected on a single site, using unique sampling methods in order to avoid introducing site effects and methodological bias. We could qualitatively show that there are differences in microbial gene expressions in samples collected in cloud-free *vs* cloudy air masses. We used 'water availability' as a proxy to distinguish the two air mass types. However, cloudy air masses also differ from those outside clouds in a multitude of other environmental factors which are expected to play roles on aeromicrobiome's functioning, and they still need to be evaluated (Amato et al., 2023). Such variables include temperature, solar radiation, chemical composition, etc, and they are linked not only to clouds but also to altitude, location, day/night cycles and season. The synergy, temporal dynamics and arrangement of these variables (shocks, cloud cycles, freezing events, combination of chemicals, etc…) could also participate in shaping the aeromicrobiome in even more complex ways.

6 Data availability

Raw sequencing MGs and MTs data are available as fastq.gz files through the European Nucleotide Archive at EBI, under the project accession PRJEB54740, samples ERR9966616 to ERR9966643.

7 Author contribution

Conceptualization: PA
Methodology: RP, FR, FE, BB, EN
Investigation: RP, PA
Visualization: RP, MJ, PA
Funding acquisition: PA, BE
Supervision: PA
Writing – original draft: RP, PA
Writing – review & editing: RP, PA, FR, MJ, FE, EN, BB, BE

8 Competing interests

The authors declare that they have no conflict of interest.

9 Acknowledgments and funding

We thank OPGC's facility for running the PUY atmospheric station and sharing meteorological data, and for logistical help during sampling. We are also grateful to Nadia Goué at the computational center of Clermont Auvergne University

(Mésocentre) and the network Auvergne Bioinformatique for providing computational power and excellent support with software deployment in the Galaxy environment. This research has been supported by the French National Research Agency (ANR) grant no. ANR-17-MPGA- 0013.

10 References

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

Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., and Delort, A.-M.: 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, 59, 242–254, https://doi.org/10.1111/j.1574-6941.2006.00199.x, 2007b.

Amato, P., Joly, M., Schaupp, C., Attard, E., Möhler, O., Morris, C. E., Brunet, Y., and Delort, A.-M.: Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber, Atmos Chem Phys, 15, 6455–6465, https://doi.org/10.5194/acp-15-6455-2015, 2015.

Amato, P., Joly, M., Besaury, L., Oudart, A., Taib, N., Moné, A. I., Deguillaume, L., Delort, A.-M., and Debroas, D.: Active microorganisms thrive among extremely diverse communities in cloud water, PLOS ONE, 12, e0182869, https://doi.org/10.1371/journal.pone.0182869, 2017.

Amato, P., Besaury, L., Joly, M., Penaud, B., Deguillaume, L., and Delort, A.-M.: Metatranscriptomic exploration of microbial functioning in clouds, Sci Rep, 9, 4383, https://doi.org/10.1038/s41598-019-41032-4, 2019.

Amato, P., Mathonat, F., Nuñez Lopez, L., Péguilhan, R., Bourhane, Z., Rossi, F., Vyskocil, J., Joly, M., and Ervens, B.: The aeromicrobiome: the selective and dynamic outer-layer of the Earth's microbiome, Front Microbiol, 14, 2023.

Baldrian, P., Kolařík, M., Stursová, M., Kopecký, J., Valášková, V., Větrovský, T., Zifčáková, L., Snajdr, J., Rídl, J., Vlček, C., and Voříšková, J.: Active and total microbial communities in forest soil are largely different and highly stratified during decomposition, ISME J, 6, 248–258, https://doi.org/10.1038/ismej.2011.95, 2012.

Baray, J.-L., Deguillaume, L., Colomb, A., Sellegri, K., Freney, E., Rose, C., Baelen, J. V., Pichon, J.-M., Picard, D., Fréville, P., Bouvier, L., Ribeiro, M., Amato, P., Banson, S., Bianco, A., Borbon, A., Bourcier, L., Bras, Y., Brigante, M., Cacault, P., Chauvigné, A., Charbouillot, T., Chaumerliac, N., Delort, A.-M., Delmotte, M., Dupuy, R., Farah, A., Febvre, G., Flossmann, A., Gourbeyre, C., Hervier, C., Hervo, M., Huret, N., Joly, M., Kazan, V., Lopez, M., Mailhot, G., Marinoni, A., Masson, O., Montoux, N., Parazols, M., Peyrin, F., Pointin, Y., Ramonet, M., Rocco, M., Sancelme, M., Sauvage, S., Schmidt, M., Tison, E., Vaïtilingom, M., Villani, P., Wang, M., Yver-Kwok, C., and Laj, P.: Cézeaux-Aulnat-Opme-Puy De Dôme: a multi-site for the long-term survey of the tropospheric composition and climate change, Atmos Meas Tech, 13, 3413–3445, https://doi.org/10.5194/amt-13-3413-2020, 2020.

Bianco, A., Voyard, G., Deguillaume, L., Mailhot, G., and Brigante, M.: Improving the characterization of dissolved organic carbon in cloud water: Amino acids and their impact on the oxidant capacity, Sci Rep, 6, https://doi.org/10.1038/srep37420, 2016.

Bianco, A., Deguillaume, L., Vaïtilingom, M., Nicol, E., Baray, J.-L., Chaumerliac, N., and Bridoux, M.: Molecular characterization of cloud water Samples collected at the Puy de Dôme (France) by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry, Environ Sci Technol, 52, 10275–10285, https://doi.org/10.1021/acs.est.8b01964, 2018.

Bianco, A., Deguillaume, L., Chaumerliac, N., Vaïtilingom, M., Wang, M., Delort, A.-M., and Bridoux, M. C.: Effect of endogenous microbiota on the molecular composition of cloud water: a study by Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), Sci Rep, 9, 7663, https://doi.org/10.1038/s41598-019-44149-8, 2019.

Bowers, R. M., McLetchie, S., Knight, R., and 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, https://doi.org/10.1038/ismej.2010.167, 2011.

Bowers, R. M., Clements, N., Emerson, J. B., Wiedinmyer, C., Hannigan, M. P., and Fierer, N.: Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere, Environ Sci Technol, 47, 12097–12106, https://doi.org/10.1021/es402970s, 2013.

Brown, J. K. M. and Hovmøller, M. S.: Aerial Dispersal of pathogens on the global and continental scales and its impact on plant disease, Science, 297, 537–541, https://doi.org/10.1126/science.1072678, 2002.

Buchfink, B., Xie, C., and Huson, D. H.: Fast and sensitive protein alignment using DIAMOND, Nat Methods, 12, 59–60, https://doi.org/10.1038/nmeth.3176, 2015.

Burrows, S. M., Butler, T., Jöckel, P., Tost, H., Kerkweg, A., Pöschl, U., and Lawrence, M. G.: Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems, Atmos Chem Phys, 9, 9281–9297, https://doi.org/10.5194/acp-9-9281-2009, 2009.

Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I., and Leonard, J. A.: Preservation of RNA and DNA from mammal samples under field conditions, Mol Ecol Resour, 13, 663–673, https://doi.org/10.1111/1755-0998.12108, 2013.

Chen, L., Hu, M., Huang, L., Hua, Z., Kuang, J., Li, S., and Shu, W.: Comparative metagenomic and metatranscriptomic analyses of microbial communities in acid mine drainage, ISME J, 9, 1579–1592, https://doi.org/10.1038/ismej.2014.245, 2015.

Chen, Y.-J., Leung, P. M., Wood, J. L., Bay, S. K., Hugenholtz, P., Kessler, A. J., Shelley, G., Waite, D. W., Franks, A. E., Cook, P. L. M., and Greening, C.: Metabolic flexibility allows bacterial habitat generalists to become dominant in a frequently disturbed ecosystem, ISME J, 15, 2986–3004, https://doi.org/10.1038/s41396-021-00988-w, 2021.

Chowdhury, N., Marschner, P., and Burns, R.: Response of microbial activity and community structure to decreasing soil osmotic and matric potential, Plant Soil, 344, 241–254, https://doi.org/10.1007/s11104-011-0743-9, 2011.

Cruz, C. N. and Pandis, S. N.: Deliquescence and hygroscopic growth of mixed inorganic−organic atmospheric aerosol, Environmental Science & Technology, 34, 4313–4319, https://doi.org/10.1021/es9907109, 2000.

Dagan, G., Koren, I., Altaratz, O., and Lehahn, Y.: Shallow convective cloud field lifetime as a key factor for evaluating aerosol effects, iScience, 10, 192–202, https://doi.org/10.1016/j.isci.2018.11.032, 2018.

Dalton, D. A. and Kramer, S.: Nitrogen-fixing bacteria in non-legumes, in: Plant-Associated Bacteria, edited by: Gnanamanickam, S. S., Springer Netherlands, Dordrecht, 105–130, 2006.

Deguillaume, L., Charbouillot, T., Joly, M., Vaïtilingom, M., Parazols, M., Marinoni, A., Amato, P., Delort, A.-M., Vinatier, V., Flossmann, A., Chaumerliac, N., Pichon, J. M., Houdier, S., Laj, P., Sellegri, K., Colomb, A., Brigante, M., and Mailhot, G.: 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, https://doi.org/10.5194/acp-14-1485-2014, 2014.

Dillon, K. P., Correa, F., Judon, C., Sancelme, M., Fennell, D. E., Delort, A.-M., and Amato, P.: Cyanobacteria and algae in clouds and rain in the area of puy de Dôme, Central France, Appl Environ Microbiol, https://doi.org/10.1128/AEM.01850-20, 2020.

Ervens, B. and Amato, P.: The global impact of bacterial processes on carbon mass, Atmos Chem Phys, 20, 1777–1794, https://doi.org/10.5194/acp-20-1777-2020, 2020.

Ervens, B., Sorooshian, A., Aldhaif, A. M., Shingler, T., Crosbie, E., Ziemba, L., Campuzano-Jost, P., Jimenez, J. L., and Wisthaler, A.: Is there an aerosol signature of chemical cloud processing?, Atmos Chem Phys, 18, 16099–16119, https://doi.org/10.5194/acp-18-16099-2018, 2018.

Ervens, B., Amato, P., Aregahegn, K., Joly, M., Khaled, A., Labed-Veydert, T., Mathonat, F., Nuñez López, L., Péguilhan, R., and Zhang, M.: Ideas and perspectives: Microorganisms in the air through the lenses of atmospheric chemistry and microphysics, EGUsphere, 1–18, https://doi.org/10.5194/egusphere-2024-2377, 2024.

Feingold, G., Cotton, W. R., Stevens, B., and Frisch, A. S.: The Relationship between Drop In-Cloud Residence Time and Drizzle Production in Numerically Simulated Stratocumulus Clouds, Journal of the Atmospheric Sciences, 53, 1108–1122, https://doi.org/10.1175/1520-0469(1996)053<1108:TRBDIC>2.0.CO;2, 1996.

Fierer, N., Liu, Z., Rodríguez-Hernández, M., Knight, R., Henn, M., and Hernandez, M. T.: Short-term temporal variability in airborne bacterial and fungal populations, Appl Environ Microbiol, 74, 200–207, https://doi.org/10.1128/AEM.01467-07, 2008.

Franzosa, E. A., Morgan, X. C., Segata, N., Waldron, L., Reyes, J., Earl, A. M., Giannoukos, G., Boylan, M. R., Ciulla, D., Gevers, D., Izard, J., Garrett, W. S., Chan, A. T., and Huttenhower, C.: Relating the metatranscriptome and metagenome of the human gut, Proc Natl Acad Sci, 111, E2329–E2338, https://doi.org/10.1073/pnas.1319284111, 2014.

Fuzzi, S., Mandrioli, P., and Perfetto, A.: Fog droplets—an atmospheric source of secondary biological aerosol particles, Atmos Environ, 31, 287–290, https://doi.org/10.1016/1352-2310(96)00160-4, 1997.

Griffiths, E. and Birch, H. F.: Microbiological Changes in Freshly Moistened Soil, Nature, 189, 424–424, https://doi.org/10.1038/189424a0, 1961.

Gusareva, E. S., Acerbi, E., Lau, K. J. X., Luhung, I., Premkrishnan, B. N. V., Kolundžija, S., Purbojati, R. W., Wong, A., Houghton, J. N. I., Miller, D., Gaultier, N. E., Heinle, C. E., Clare, M. E., Vettath, V. K., Kee, C., Lim, S. B. Y., Chénard, C., Phung, W. J., Kushwaha, K. K., Nee, A. P., Putra, A., Panicker, D., Yanqing, K., Hwee, Y. Z., Lohar, S. R., Kuwata, M., Kim, H. L., Yang, L., Uchida, A., Drautz-Moses, D. I., Junqueira, A. C. M., and Schuster, S. C.: Microbial communities in the

tropical air ecosystem follow a precise diel cycle, Proc Natl Acad Sci, 116, 23299–23308, https://doi.org/10.1073/pnas.1908493116, 2019.

Herrmann, H., Schaefer, T., Tilgner, A., Styler, S. A., Weller, C., Teich, M., and Otto, T.: Tropospheric Aqueous-Phase Chemistry: Kinetics, mechanisms, and its coupling to a changing gas phase, Chem Rev, 115, 4259–4334, https://doi.org/10.1021/cr500447k, 2015.

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

Hoffmann, L., Günther, G., Li, D., Stein, O., Wu, X., Griessbach, S., Heng, Y., Konopka, P., Müller, R., Vogel, B., and Wright, J. S.: From ERA-Interim to ERA5: the considerable impact of ECMWF's next-generation reanalysis on Lagrangian transport simulations, Atmos Chem Phys, 19, 3097–3124, https://doi.org/10.5194/acp-19-3097-2019, 2019.

Jaber, S., Joly, M., Brissy, M., Leremboure, M., Khaled, A., Ervens, B., and Delort, A.-M.: Biotic and abiotic transformation of amino acids in cloud water: experimental studies and atmospheric implications, Biogeosciences, 18, 1067–1080, https://doi.org/10.5194/bg-18-1067-2021, 2021.

Jørgensen, R. N., JØrgensen, B. J., and Nielsen, N. E.: N_2O emission immediately after rainfall in a dry stubble field, Soil Biol Biochem, 30, 545–546, https://doi.org/10.1016/S0038-0717(97)00144-2, 1998.

Jorth, P., Turner, K. H., Gumus, P., Nizam, N., Buduneli, N., and Whiteley, M.: Metatranscriptomics of the human oral microbiome during health and disease, mBio, 5, e01012-14, https://doi.org/10.1128/mBio.01012-14, 2014.

Jousse, C., Dalle, C., Canet, I., Lagrée, M., Traïkia, M., Lyan, B., Mendes, C., Sancelme, M., Amato, P., and Delort, A.-M.: Metabolomic study of the response to cold shock in a strain of *Pseudomonas syringae* isolated from cloud water, Metabolomics, 14, 11, https://doi.org/10.1007/s11306-017-1295-7, 2018.

Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., and Ishiguro-Watanabe, M.: KEGG for taxonomy-based analysis of pathways and genomes, Nucleic Acids Res, 51, D587–D592, https://doi.org/10.1093/nar/gkac963, 2023.

Khaled, A., Zhang, M., Amato, P., Delort, A.-M., and Ervens, B.: Biodegradation by bacteria in clouds: an underestimated sink for some organics in the atmospheric multiphase system, Atmos Chem Phys, 21, 3123–3141, https://doi.org/10.5194/acp-21-3123-2021, 2021.

Klein, A. M., Bohannan, B. J. M., Jaffe, D. A., Levin, D. A., and Green, J. L.: Molecular evidence for metabolically active bacteria in the atmosphere, Front Microbiol, 772, https://doi.org/10.3389/fmicb.2016.00772, 2016.

Koehler, K. A., Kreidenweis, S. M., DeMott, P. J., Prenni, A. J., Carrico, C. M., Ervens, B., and Feingold, G.: Water activity and activation diameters from hygroscopicity data - Part II: Application to organic species, Atmos Chem Phys, 6, 795–809, https://doi.org/10.5194/acp-6-795-2006, 2006.Krumins, V., Mainelis, G., Kerkhof, L. J., and Fennell, D. E.: Substrate-dependent rRNA production in an airborne bacterium, Env Sci Technol Lett, 1, 376–381, https://doi.org/10.1021/ez500245y, 2014.

Kudla, B., Caddick, M. X., Langdon, T., Martinez-Rossi, N. M., Bennett, C. F., Sibley, S., Davies, R. W., and H N Arst, J.: The regulatory gene areA mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger, The EMBO Journal, 9, 1355, https://doi.org/10.1002/j.1460-2075.1990.tb08250.x, 1990.

van Leeuwen, M. R., Krijgsheld, P., Bleichrodt, R., Menke, H., Stam, H., Stark, J., Wösten, H. A. B., and Dijksterhuis, J.: Germination of conidia of *Aspergillus niger* is accompanied by major changes in RNA profiles, Studies in Mycology, 74, 59–70, https://doi.org/10.3114/sim0009, 2013.

Lelieveld, J. and Crutzen, P. J.: Influences of cloud photochemical processes on tropospheric ozone, Nature, 343, 227–233, https://doi.org/10.1038/343227a0, 1990.

Leroch, M., Kleber, A., Silva, E., Coenen, T., Koppenhöfer, D., Shmaryahu, A., Valenzuela, P. D. T., and Hahn, M.: Transcriptome profiling of *Botrytis cinerea* conidial germination reveals upregulation of infection-related genes during the prepenetration stage, Eukaryot Cell, 12, 614–626, https://doi.org/10.1128/ec.00295-12, 2013.

Li, C., Jia, S., Rajput, S. A., Qi, D., and Wang, S.: Transcriptional Stages of Conidia Germination and Associated Genes in *Aspergillus flavus*: An Essential Role for Redox Genes, Toxins, 14, 560, https://doi.org/10.3390/toxins14080560, 2022.

Li, K., Guo, Y., Nizkorodov, S. A., Rudich, Y., Angelaki, M., Wang, X., An, T., Perrier, S., and George, C.: Spontaneous dark formation of OH radicals at the interface of aqueous atmospheric droplets, Proc Natl Acad Sci, 120, e2220228120, https://doi.org/10.1073/pnas.2220228120, 2023.

Li, W. and Godzik, A.: Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences, Bioinformatics, 22, 1658–1659, https://doi.org/10.1093/bioinformatics/btl158, 2006.

Li, X., Gupta, D., Eom, H.-J., Kim, H., and Ro, C.-U.: Deliquescence and efflorescence behavior of individual NaCl and KCl mixture aerosol particles, Atmos Environ, 82, 36–43, https://doi.org/10.1016/j.atmosenv.2013.10.011, 2014.

Menke, S., Gillingham, M. A. F., Wilhelm, K., and Sommer, S.: Home-made cost effective preservation buffer is a better alternative to commercial preservation methods for microbiome research, Front Microbiol, 8, 102, https://doi.org/10.3389/fmicb.2017.00102, 2017.

Noguchi, H., Taniguchi, T., and Itoh, T.: MetaGeneAnnotator: Detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes, DNA Res, 15, 387–396, https://doi.org/10.1093/dnares/dsn027, 2008.

Nuñez López, L., Amato, P., and Ervens, B.: Bacteria in clouds biodegrade atmospheric formic and acetic acids, Atmos Chem Phys, 24, 5181–5198, https://doi.org/10.5194/acp-24-5181-2024, 2024.

Osherov, N. and May, G. S.: The molecular mechanisms of conidial germination, FEMS Microbiol Lett, 199, 153–160, https://doi.org/10.1111/j.1574-6968.2001.tb10667.x, 2001.

Péguilhan, R., Besaury, L., Rossi, F., Enault, F., Baray, J.-L., Deguillaume, L., and Amato, P.: Rainfalls sprinkle cloud bacterial diversity while scavenging biomass, FEMS Microbiol Ecol, 97, https://doi.org/10.1093/femsec/fiab144, 2021.

Péguilhan, R., Rossi, F., Rué, O., Joly, M., and Amato, P.: Comparative analysis of bacterial diversity in clouds and aerosols, Atmos Environ, 119635, https://doi.org/10.1016/j.atmosenv.2023.119635, 2023a.

Péguilhan, R., Rossi, F., Rué, O., Joly, M., and Amato, P.: Experimental and methodological framework for the assessment of nucleic acids in airborne microorganisms, bioRxiv, 2023.10.10.561683, https://doi.org/10.1101/2023.10.10.561683, 2023b.

Poolman, B. and Glaasker, E.: Regulation of compatible solute accumulation in bacteria, Molecular Microbiology, 29, 397–407, https://doi.org/10.1046/j.1365-2958.1998.00875.x, 1998.

Prass, M., Andreae, M. O., de Araùjo, A. C., Artaxo, P., Ditas, F., Elbert, W., Förster, J.-D., Franco, M. A., Hrabe de Angelis, I., Kesselmeier, J., Klimach, T., Kremper, L. A., Thines, E., Walter, D., Weber, J., Weber, B., Fuchs, B. M., Pöschl, U., and Pöhlker, C.: Bioaerosols in the Amazon rain forest: temporal variations and vertical profiles of Eukarya, Bacteria, and Archaea, Biogeosciences, 18, 4873–4887, https://doi.org/10.5194/bg-18-4873-2021, 2021.

Pruppacher, H. R. and Jaenicke, R.: The processing of water vapor and aerosols by atmospheric clouds, a global estimate, Atmos Res, 38, 283–295, https://doi.org/10.1016/0169-8095(94)00098-X, 1995.

Renard, P., Bianco, A., Baray, J.-L., Bridoux, M., Delort, A.-M., and Deguillaume, L.: Classification of Clouds Sampled at the Puy de Dôme Station (France) Based on Chemical Measurements and Air Mass History Matrices, Atmosphere, 11, 732, https://doi.org/10.3390/atmos11070732, 2020.

Renard, P., Brissy, M., Rossi, F., Leremboure, M., Jaber, S., Baray, J.-L., Bianco, A., Delort, A.-M., and Deguillaume, L.: Free amino acid quantification in cloud water at the Puy de Dôme station (France), Atmos Chem Phys, 22, 2467–2486, https://doi.org/10.5194/acp-22-2467-2022, 2022.

Rosado-Porto, D., Ratering, S., Moser, G., Deppe, M., Müller, C., and Schnell, S.: Soil metatranscriptome demonstrates a shift in C, N, and S metabolisms of a grassland ecosystem in response to elevated atmospheric CO_2, Front Microbiol, 13, 2022.

Saikh, S. R. and Das, S. K.: Fog-Induced Alteration in Airborne Microbial Community: a Study over Central Indo-Gangetic Plain in India, Appl Environ Microbiol, 89, e01367-22, https://doi.org/10.1128/aem.01367-22, 2023.

Salazar, G., Paoli, L., Alberti, A., Huerta-Cepas, J., Ruscheweyh, H.-J., Cuenca, M., Field, C. M., Coelho, L. P., Cruaud, C., Engelen, S., Gregory, A. C., Labadie, K., Marec, C., Pelletier, E., Royo-Llonch, M., Roux, S., Sánchez, P., Uehara, H., Zayed, A. A., Zeller, G., Carmichael, M., Dimier, C., Ferland, J., Kandels, S., Picheral, M., Pisarev, S., Poulain, J., Acinas, S. G., Babin, M., Bork, P., Boss, E., Bowler, C., Cochrane, G., de Vargas, C., Follows, M., Gorsky, G., Grimsley, N., Guidi, L., Hingamp, P., Iudicone, D., Jaillon, O., Kandels-Lewis, S., Karp-Boss, L., Karsenti, E., Not, F., Ogata, H., Pesant, S., Poulton, N., Raes, J., Sardet, C., Speich, S., Stemmann, L., Sullivan, M. B., Sunagawa, S., Wincker, P., Acinas, S. G., Babin, M., Bork, P., Bowler, C., de Vargas, C., Guidi, L., Hingamp, P., Iudicone, D., Karp-Boss, L., Karsenti, E., Ogata, H., Pesant, S., Speich, S., Sullivan, M. B., Wincker, P., and Sunagawa, S.: Gene expression changes and community turnover differentially shape the global ocean metatranscriptome, Cell, 179, 1068-1083.e21, https://doi.org/10.1016/j.cell.2019.10.014, 2019.

Šantl-Temkiv, T., Finster, K., Dittmar, T., Hansen, B. M., Thyrhaug, R., Nielsen, N. W., and Karlson, U. G.: Hailstones: A window into the microbial and chemical inventory of a storm cloud, PloS one, 8, e53550, 2013.

Šantl-Temkiv, T., Amato, P., Gosewinkel, U., Thyrhaug, R., Charton, A., Chicot, B., Finster, K., Bratbak, G., and Löndahl, J.: High-flow-rate impinger for the study of concentration, viability, metabolic activity, and ice-nucleation activity of airborne bacteria, Environ Sci Technol, https://doi.org/10.1021/acs.est.7b01480, 2017.

Šantl-Temkiv, T., Gosewinkel, U., Starnawski, P., Lever, M., and Finster, K.: Aeolian dispersal of bacteria in southwest Greenland: their sources, abundance, diversity and physiological states, FEMS Microbiol Ecol, 94, https://doi.org/10.1093/femsec/fiy031, 2018.

Šantl-Temkiv, T., Amato, P., Casamayor, E. O., Lee, P. K. H., and Pointing, S. B.: Microbial ecology of the atmosphere, FEMS Microbiol Rev, fuac009, https://doi.org/10.1093/femsre/fuac009, 2022.

Satinsky, B. M., Zielinski, B. L., Doherty, M., Smith, C. B., Sharma, S., Paul, J. H., Crump, B. C., and Moran, M. A.: The Amazon continuum dataset: quantitative metagenomic and metatranscriptomic inventories of the Amazon River plume, June 2010, Microbiome, 2, 17, https://doi.org/10.1186/2049-2618-2-17, 2014.

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

Schostag, M. D., Albers, C. N., Jacobsen, C. S., and Priemé, A.: Low Turnover of Soil Bacterial rRNA at Low Temperatures, Front Microbiol, 11, https://doi.org/10.3389/fmicb.2020.00962, 2020.

Skopp, J., Jawson, M. D., and Doran, J. W.: Steady-State Aerobic Microbial Activity as a Function of Soil Water Content, Soil Science Society of America Journal, 54, 1619–1625, https://doi.org/10.2136/sssaj1990.03615995005400060018x, 1990.

Smith, D. J., Ravichandar, J. D., Jain, S., Griffin, D. W., Yu, H., Tan, Q., Thissen, J., Lusby, T., Nicoll, P., Shedler, S., Martinez, P., Osorio, A., Lechniak, J., Choi, S., Sabino, K., Iverson, K., Chan, L., Jaing, C., and McGrath, J.: Airborne bacteria in Earth's lower stratosphere resemble taxa detected in the troposphere: results from a new NASA aircraft bioaerosol collector (ABC), Front Microbiol, 9, https://doi.org/10.3389/fmicb.2018.01752, 2018.

Stevenson, A., Cray, J. A., Williams, J. P., Santos, R., Sahay, R., Neuenkirchen, N., McClure, C. D., Grant, I. R., Houghton, J. D., Quinn, J. P., Timson, D. J., Patil, S. V., Singhal, R. S., Antón, J., Dijksterhuis, J., Hocking, A. D., Lievens, B., Rangel, D. E. N., Voytek, M. A., Gunde-Cimerman, N., Oren, A., Timmis, K. N., McGenity, T. J., and Hallsworth, J. E.: Is there a common water-activity limit for the three domains of life?, ISME J, 9, 1333–1351, https://doi.org/10.1038/ismej.2014.219, 2015.

The UniProt Consortium: UniProt: a worldwide hub of protein knowledge, Nucleic Acids Res, 47, D506–D515, https://doi.org/10.1093/nar/gky1049, 2019.

Tignat-Perrier, R., Dommergue, A., Thollot, A., Magand, O., Amato, P., Joly, M., Sellegri, K., Vogel, T. M., and Larose, C.: Seasonal shift in airborne microbial communities, Sci Tot Environ, 716, 137129, https://doi.org/10.1016/j.scitotenv.2020.137129, 2020.

Tong, H., Hu, Q., Zhu, L., and Dong, X.: Prokaryotic Aquaporins, Cells, 8, 1316, https://doi.org/10.3390/cells8111316, 2019.

Unger, S., Máguas, C., Pereira, J. S., David, T. S., and Werner, C.: The influence of precipitation pulses on soil respiration – Assessing the "Birch effect" by stable carbon isotopes, Soil Biol Biochem, 42, 1800–1810, https://doi.org/10.1016/j.soilbio.2010.06.019, 2010.

Vaïtilingom, M., Amato, P., Sancelme, M., Laj, P., Leriche, M., and Delort, A.-M.: Contribution of microbial activity to carbon chemistry in clouds, Appl Environ Microbiol, 76, 23–29, https://doi.org/10.1128/AEM.01127-09, 2010.

Vaïtilingom, M., Deguillaume, L., Vinatier, V., Sancelme, M., Amato, P., Chaumerliac, N., and Delort, A.-M.: Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds, Proc Natl Acad Sci, 110, 559–564, https://doi.org/10.1073/pnas.1205743110, 2013.

Veses, V., Richards, A., and Gow, N. A.: Vacuoles and fungal biology, Curr Opin Microbiol, 11, 503–510, https://doi.org/10.1016/j.mib.2008.09.017, 2008.

Wang, S., Lin, R., Tumukunde, E., Zeng, W., Bao, Q., Wang, S., and Wang, Y.: Glutamine synthetase contributes to the regulation of growth, conidiation, sclerotia development, and resistance to oxidative stress in the fungus *Aspergillus flavus*, Toxins (Basel), 14, 822, https://doi.org/10.3390/toxins14120822, 2022.

Wirgot, N., Vinatier, V., Deguillaume, L., Sancelme, M., and Delort, A.-M.: H2O2 modulates the energetic metabolism of the cloud microbiome, Atmos Chem Phys, 17, 14841–14851, https://doi.org/10.5194/acp-17-14841-2017, 2017.

Wirgot, N., Lagrée, M., Traïkia, M., Besaury, L., Amato, P., Canet, I., Sancelme, M., Jousse, C., Diémé, B., Lyan, B., and Delort, A.-M.: Metabolic modulations of *Pseudomonas graminis* in response to H_2O_2 in cloud water, Sci rep, 9, 1–14, https://doi.org/10.1038/s41598-019-49319-2, 2019.

Woo, C. and Yamamoto, N.: Falling bacterial communities from the atmosphere, Environ Microbiome, 15, 22, https://doi.org/10.1186/s40793-020-00369-4, 2020.

Wood, D. E. and Salzberg, S. L.: Kraken: ultrafast metagenomic sequence classification using exact alignments, Genome Biol, 15, R46, https://doi.org/10.1186/gb-2014-15-3-r46, 2014.

Yang, K., Tian, J., and Keller, N. P.: Post-translational modifications drive secondary metabolite biosynthesis in *Aspergillus*: a review, Environ Microbiol, 24, 2857–2881, https://doi.org/10.1111/1462-2920.16034, 2022.

Zhang, Y., Zhao, Z., Dai, M., Jiao, N., and Herndl, G. J.: Drivers shaping the diversity and biogeography of total and active bacterial communities in the South China Sea, Mol Ecol, 23, 2260–2274, https://doi.org/10.1111/mec.12739, 2014.

Zhang, Y., Thompson, K. N., Huttenhower, C., and Franzosa, E. A.: Statistical approaches for differential expression analysis in metatranscriptomics, Bioinformatics, 37, i34–i41, https://doi.org/10.1093/bioinformatics/btab327, 2021.

Zhou, X., Fornara, D., Ikenaga, M., Akagi, I., Zhang, R., and Jia, Z.: The resilience of microbial community under drying and rewetting cycles of three forest soils, Front Microbiol, 7, 2016.