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Bacterial contribution to nitrogen processing in the atmosphere

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Abstract. This study investigates potential microbial interactions with nitrogen compounds in the atmosphere, with a focus on inorganic forms (mainly NH_4^+, NO_3^-, and N_2). The reanalysis of metagenomes and metatranscriptomes from cloud-free and cloudy collected at the mountain site of puy de Dôme (1465 m asl, France) indicate equivalent representation of genes involved in organic and inorganic nitrogen utilization processes. Glutamate metabolism and denitrification (in particular nitrite reduction) contributed most (70%) of the microbial sequences of genes and transcripts linked to nitrogen utilization pathways. Other prevalent processes included assimilatory and dissimilatory nitrate reduction, and nitrogen fixation, with the latter being overexpressed in particular during clear atmospheric conditions. The screening of bacteria isolates revealed that 15% of them carry the biomarker gene for biological N_2 fixation (*nifH*). In addition, laboratory incubations of rainwater points towards the processing of NH_4^+. The decay rate of NH_4^+ concentration correlated positively with the relative abundance of *Sphingomonadales*, and negatively with that of *Burkholderiales*. The latter may rather obtain nitrogen from N_2 and organic forms. Overall, these results demonstrate multiple potential microbiological roles in the processing of inorganic nitrogen in the atmosphere, in relation with atmospheric conditions and microbial diversity. This opens up new perspectives in our understanding of biogeochemical cycles and chemical processing in the atmosphere, as well as microbial functioning in this major part of the Earth system.

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

1.Introduction

The atmosphere carries diverse living microorganisms and plays an important role in the dispersal of microorganisms and genetic material across ecosystems (Fröhlich-Nowoisky et al., 2016). Biological indicators of viability and metabolic activity like ATP and RNA could be identified in aerosol particles and cloud water, and regularly associated with bacteria taxa such as *Alphaproteobacteria* (*Rhodospirillales*, *Sphingomonadales*, and *Rhizobiales*) and *Gammaproteobacteria* (*Pseudomonadales*) (Amato et al., 2017; Klein et al., 2016; Šantl-Temkiv et al., 2018). As in other environments, their nutritive requirements include the uptake of carbon (C), nitrogen (N), phosphorous (P) and other elements from dissolved or gaseous inorganic and organic compounds, with potential impacts on biogeochemical cycles. However, the microbial processing of chemical compounds in the atmosphere is still very poorly studied.

A large variety of bioavailable organic and inorganic chemicals, such as carboxylic acids, aldehydes, sugars etc, are present in the atmospheric system as gas, particles, or dissolved in water (clouds, precipitation), and these are potential nutrients for microbial cells (Jaber et al., 2021; Renard et al., 2021). So far, the few experimental and model investigations of carbon utilization by microorganisms in clouds (Nuñez López et al., 2024; Vaïtilingom et al., 2013) led to the estimation that microbes may biodegrade up to 8-11 Tg yr^{-1} water-soluble organic carbon (WSOC) on a global scale (Ervens and Amato, 2020). Although some studies suggest interactions between airborne bacteria and nitrogen (Hill et al., 2007; Jaber et al., 2021; Péguilhan et al., 2025), they have not been quantified so far.

Nitrogen is an essential element for the biosphere. It is abundant in the atmosphere, which represents the planet's largest reservoir of inorganic nitrogen, primarily in the form of dinitrogen (N_2), with 3.7×10^9 Tg (Sorai et al., 2007). Many other inorganic nitrogen compounds (e.g., NH_4^+ / NO_3^- / NO_2^- / NOx), potentially bioavailable, are also present in the gas and aqueous phases (**Table 1**). They originate from natural sources (ocean, soils, biomass burning, etc.), anthropogenic activities (agriculture, industry), or are formed in the atmosphere from the processing of more complex molecules (Almaraz et al., 2018; Fowler et al., 2013).

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

Table 1. Most common nitrogen compounds reported in the atmosphere and their concentration (WSON = Water Soluble Organic Nitrogen).

Nitrogen compound	Gas phase mixing ratio	References
Dinitrogen - N_2	≈ 780 000 ppmv	(Lide, 2004)
Nitric oxide - NO	≤1 - ≈ 5 ppbv	(Hargreaves et al., 1992)
Nitrogen dioxide - NO_2	≤1 - ≈ 10 ppbv	(Cape et al., 1992)
Nitrous oxide - N_2O	≈300 ppbv	(Machida et al., 1995; Syakila and Kroeze, 2011)
Dinitrogen pentoxide - N_2O_5	≤15 ppbv	(Atkinson et al., 1986)
Nitrous acid - HNO_2	≤3 ppbv	(Bari et al., 2003)
Nitric acid - HNO_3	≤2 ppbv	(Bari et al., 2003)
Ammonia - NH_3	≤1 - ≈ 30 ppbv	(Nair and Yu, 2020)
	Concentration in cloud water	
Ammonium - NH_4^+	≤1 - ≈ 500 µM	(Hill et al., 2007; Renard et al., 2020; Vaïtilingom et al., 2010)
Nitrite - NO_2^-	≈ 1 µM	(Cape et al., 1992)
Nitrate - NO_3^-	≤1 - ≈ 500 µM	(Hill et al., 2007; Renard et al., 2020; Vaïtilingom et al., 2010)
Organic nitrogen (Amino acids, Amines, Urea etc) (WSON)	≤1 - ≈ 1000 µM	(Cape et al., 2011; Zhang and Anastasio, 2001)

By their composition, microbial cells directly contribute to the nitrogen pool, with molecules such as proteins, DNA, pigments, etc, and they can release N-containing organic compounds like glycoproteins, peptides and amino-acids (Decho and Lopez, 1993; Leck and Bigg, 2005). On the other hand, extracellular organic (amino-acids, peptides, etc) and inorganic (NH_4^+, NO_3^- etc) compounds can be utilized as sources of nitrogen by living cells, detoxified, such as dinitrogen trioxide (N_2O_3) and

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Preprint. Discussion started: 30 July 2025

peroxynitrite ($ONOO^-$) that causes cellular damages (Espey et al., 2000; Maes et al., 2011), and also in some cases (for instance nitrates under low-oxygen conditions), used as electron acceptors for the generation of biochemical energy (Bernhard, 2010; Galimand et al., 1991). Regarding inorganic nitrogen, microbially-mediated transformation includes N_2 fixation, nitrification, denitrification, anaerobic oxidation of ammonium, ammonification and assimilation/dissimilation of nitrate. These processes have been well characterized in environments such as soil and freshwater and specific enzymes and genes could be identified (Bernhard, 2010) (**Table 2**). In the atmosphere, the reported microbial nitrogen-related processes include nitrosative stress, through atmospheric metagenomes and metatranscriptomes, and in cloud water, the processing of dissolved amino-acids (Amato et al., 2019; Jaber et al., 2021; Péguilhan et al., 2025).

In the present study, a range of approaches, from the targeted reanalysis of sequence data to the observation of natural samples, were used in order to provide a large picture of the possible interactions between bacteria and inorganic nitrogen in the atmosphere. This work provides new insights into the functioning of microorganisms in the atmospheric system and their potential contribution to the nitrogen cycle.

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

Table 2. Main known biological transformations of inorganic nitrogen mediated by microorganisms. AOB = Ammonia-Oxidizing Bacteria; AOA = Ammonia-Oxidizing Archaea; NOB = Nitrite-Oxidizing Bacteria; Comammox defines the process including all the steps of nitrification (1+2+3).

Biological process	Chemical reactions	Enzyme(s)	Main Biomarker genes	Organisms	References
a - Biological nitrogen fixation (BNF)	$N_2 + 8H^+ + 8e^- \rightarrow 2\ NH_3 + H_2$	Nitrogenase	*nifDKHW; anfG; vnfDKGH*	Bacteria and Archaea; functional groups: diazotrophs; *Pseudomonadota* such as *Rhizobiales*, *Burkolderiales*, *Pseudomonadales* and *Sphingomonadales*	(Fani et al., 2000; Joerger et al., 1989, 1990; Koirala and Brözel, 2021)
b - Nitrification	1) $NH_3 + O_2 + 2e^- \rightarrow NH_2OH + H_2O$	Ammonia monooxygenase	*amoABC*	Bacteria and Archaea; functional groups: AOB, AOA and NOB; *Pseudomonadota* such as *Chloroflexi* and *Nitrospirae* (*e.g.*, *Nitrospira*, *Nitrobacter*, *Nitrococcus*, *Nitrospina*, *Nitrotoga* and *Nitrolancetus*)	(Dworkin et al., 2006; Hollocher et al., 1981; Norton et al., 2002; Rotthauwe et al., 1997)
	2) $NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$	Hydroxylamine oxidoreductase	*hao*		(Hommes et al., 2001)
	3) $NO_2^- + ½\ O_2 \rightarrow NO_3^-$	Nitrite oxidoreductase	*nxrAB*		(Dworkin et al., 2006; Pester et al., 2014)
c - Denitrification	1) $NO_3^- + 2\ H^+ + 2\ e^- \rightarrow NO_2^- + H_2O$	Nitrate reductase	*narGHIVJWYZ; napABGC*	Bacteria (*e.g.*, *Bacillus*, *Paracoccus*, *Pseudomonas*), certain unicellular eukaryotes and Archaea	(Berks et al., 1995; Blasco et al., 1990; Gregory et al., 2000; Sohaskey and Wayne, 2003; Stolz and Basu, 2002)
	2) $NO_2^- + 2\ H^+ + e^- \rightarrow NO + H_2O$	Nitrite reductase	*nirKS*		(Braker and Tiedje, 2003; Suzuki et al., 2006; Zumft, 1997)
	3) $2\ NO + 2\ H^+ + 2\ e^- \rightarrow N_2O + H_2O$	Nitric oxide reductase	*norBC*		(Braker and Tiedje, 2003; Heiss et al., 1989; Suzuki et al., 2006)
	4) $N_2O + 2\ H^+ + 2\ e^- \rightarrow N_2 + 2H_2O$	Nitrous oxide reductase	*nosZ*		(Coyle et al., 1985; Scala and Kerkhof, 1999; Zumft and Kroneck, 2006)
d - Anaerobic oxidation of ammonium (Anammox)	$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$	Hydrazine hydrolase	*hdh*	Bacteria (*e.g.*, *Candidatus Brocadia*, *Kuenenia*, *Anammoxoglobus*, *Jettenia* and *Scalindua*)	(Kartal et al., 2011)
		Hydrazine oxidoreductase	*hszABC*		(Kartal et al., 2011)
		Nitrite reductase	*nirKS*		(Braker et al., 2000; Cantera and Stein, 2007; Zumft, 1997)

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

2. Materials and Methods

To study the possible interactions occurring between inorganic nitrogen and atmospheric microorganisms, complementary approaches were combined: the prospection of sequences affiliated with biomarkers of the microbial nitrogen cycle in atmospheric metagenomes and metatranscriptomes (**section 2.1**); the screening of bacteria isolates from clouds for biomarkers of nitrogen fixation (**section 2.2**); the analysis of bacterial diversity and dissolved nitrogen ions during rainwater incubations (**section 2.3**).

2.1 Reanalysis of metagenomes and metatranscriptomes from clouds and clear atmosphere

A set of atmospheric metagenomes (MG) and metatranscriptomes (MT) reported previously (ENA project PRJEB54740; Péguilhan et al., 2025) was reprocessed and reanalyzed specifically for genes and transcripts related to the processing of nitrogen compounds, at the sequence level. Briefly, aerosol and cloud samples were originally collected from the instrumented atmospheric station of puy de Dôme Mountain summit (1,465m asl, France) (Baray et al., 2020), into a nucleic acid preservation buffer using high-flow rate impingers. The DNA and RNA extracts obtained from each sample were sequenced by Illumina HiSeq (paired end reads of 150 bp). In our study, the reads of MG and MT were trimmed (quality>30, length>145bp), and 20 million randomly selected of them were aligned against the protein sequences of the NCycDB database, using MMseqs2 (e-value threshold of 10^{-7}). NCycDB was designed for metagenomic profiling of N-cycling genes (Tu et al., 2019). This contains 68 gene (sub)families, grouped into 8 main functions: nitrification, denitrification, assimilatory nitrate reduction, dissimilatory nitrate reduction, nitrogen fixation, annamox, organic degradation and synthesis, and "other". For each sample, the number of reads similar to one of the 68 gene families (Tu et al., 2019) was normalized by the total number of reads in the dataset and by the average size (base-pairs) of orthologs inside each family. Values are presented in parts per million base pairs (ppmbp). In order to obtain taxonomic affiliations, the matching sequences (best hits) were compared with the generalist UniRef100 protein database (January 2025) (Suzek et al., 2015).

The proportion of each gene involved in each N-cycling functions (i.e., nitrification, denitrification etc) in each MG and MT were summed, and the corresponding "RNA:DNA ratio", i.e., the proportion reads in MT with respect to that in MG, was calculated for each sample.

2.2 Screening for *nifH* in bacteria strains isolated from clouds

The presence of the biomarker gene for nitrogen fixation, *nifH*, was tested in 34 bacterial strains previously isolated from clouds (Amato et al., 2007; Renard et al., 2016; Vaïtilingom et al., 2012; Vinatier et al., 2016) (**Supplementary Table S1**). These were selected to include known nitrogen-fixing taxa such as *Pseudomonadales*, *Sphingomonadales*, *Burkholderiales*, *Rhizobiales*, *Mycobacteriales*, *Rhodospirillales*, *Hyphomicrobiales*, *Rhodobacterales*. Bacteria cells from cultures stored at -80°C in 10% glycerol were re-cultured on R2A medium at 17°C. DNA was then extracted from colonies (QIAamp DNA Mini

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

Kit; QIAGEN; ref 51304) following the manufacturer's protocol. A polymerase chain reaction targeting the gene *nifH* was performed from DNA extracts (~20 ng), using the primers polF: 5'-TGCGAYCCSAARGCBGACTC-3' and polR: 5'-ATSGCCATCATYTCRCCGGA-3' from (Poly et al., 2001). Amplification was carried out at final concentrations of, 200 µM of each dNTP (Qiagen), 0.2 µM of each primer and 0.04 U of Platinum II Taq Hot-Start DNA Polymerase (Invitrogen; ref 14966005), in Platinum II PCR buffer (1X) and Platinum GC enhancer (1X) provided with the Taq, in a total final volume of 25 µL. The PCR was performed with the following thermocycling parameters: 7 min of initial DNA polymerase activation and DNA denaturation at 95°C, followed by 30 cycles of denaturation (95°C, 1 min), hybridization (55°C, 1 min), elongation (72°C, 2 min), and a final elongation step of 7 min at 72°C. The length of the amplicons (~450 bp) was controlled by electrophoresis on 2% agarose gel.

2.3 Analysis of rain water samples

2.3.1. Sample collection, processing and storage

Rain samples were collected in 2023 at the Theix INRAe meteorological station, France (GPS point: 45°43'22"N 3°01'09"E), a meadow at an altitude of 881 m asl located nearby (~10 km) puy de Dôme observatory, where the samples for MG and MT were obtained (**section 2.1**). The samples were collected over 24-h periods using an automated refrigerated (4°C) precipitation collector (Eigenbrodt NSA 181/KHS; Königsmoor, Germany), with a collection diameter of 47.4 cm (area of 1764 cm^2), into autoclaved one-liter glass bottles (Pouzet et al., 2017). The samples maintained at 4°C in the sampler were processed within 2 days. Due to practical limitations, only samples with volumes >30mL were analyzed for chemical and biological variables (see below). In addition, samples >100 mL were distributed into sterile Erlenmeyer flasks as triplicates and incubated for 5 days under laboratory conditions (17°C, 130 rpm shaking, dark conditions), in order to evaluate temporal changes in the microbial community and inorganic nitrogen concentrations.

2.3.2 Meteorological data and air mass back-trajectory analyses

Meteorological variables (temperature, wind, humidity) (**Supplementary data**) were monitored by the meteorological station at the Theix INRAe. Boundary layer height (BLH) and the geographical origin of the air masses (cardinal points, terrestrial or oceanic, altitude) were obtained from 72-h backward trajectory plots computed using the CAT trajectory model (Péguilhan et al., 2021).

2.3.3 Chemical analyses

The pH was measured immediately after sampling from a subsample of ~1.5 mL. For quantifying major inorganic ions, a volume of 5 mL was transferred into a 15 mL sterile polypropylene vial and stored at -25°C until analysis. The main dissolved inorganic cations and anions (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^- and SO_4^{2-}) were examined by ion chromatography using

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

a Dionex ICS1500 (column CS16) for cations as in (Deguillaume et al., 2014), and a Dionex DX320 (column AS11) for anions as in (Péguilhan et al., 2021).

In incubation experiments, ammonium and nitrate concentration decay rates (Δ, µmol h^{-1} $cell^{-1}$) were calculated as:

$$\Delta = \frac{d[N]/dt}{x_{bact}} \quad (1)$$

Where *d[N]/dt* (µmol L^{-1} h^{-1}) corresponds to the change in ammonium or nitrate concentration during the incubation time of120 h and x_{bact} corresponds to the initial number concentration of microorganisms in the sample in cells L^{-1}.

2.3.4 Total cell counts

For the total microbial cell quantification by flow cytometry (FCM), rain samples were prepared as in (Amato et al., 2017). Briefly, for each sample, triplicate volumes of 450µL were mixed with 50µL of 5% glutaraldehyde (0.5% final concentration; Sigma-Aldrich G7651, St-Louis, MO, USA), gently vortexed and stored at 4°C until analysis, within 3 weeks. Total cell concentration (or number? or counts?) was quantified using a LSR Fortessa flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Just before analysis, samples were mixed with 500 µL of 0.22 µm porosity filtered (47mm diameter; ClearLine 0421A00023) Tris-EDTA pH 8.0 (40 mM Tris-Base, 1 mM EDTA, acetic acid to pH 8.0) and stained with SYBRGreen I (Molecular Probes Inc., Eugene, OR, USA) from a 100X solution, and incubated for 15 min in the dark. Cell counts were performed at excitation and emission wavelengths of λexc = 488 nm and λem = 530 nm, respectively, at a flow rate of ~90 µL min^{-1} further determined by weighting.

For each rainwater incubation experiment, a microbial growth rate (µ) in gen h^{-1} was calculated as:

$$\mu = \frac{\log_2 N - \log_2 N_0}{dt} \quad (2)$$

Where *N* and N_0 are the number concentrations of cells at the end and at the beginning of the incubation period, respectively, in cells mL^{-1}; *dt* corresponds to the incubation time (120 h).

The generation time (Θ), in h gen^{-1}, is then:

$$\Theta = \frac{1}{\mu} \quad (3)$$

2.3.5 DNA extraction

For DNA extraction, the remaining volume of rain water (30 to 200 mL depending on the sample) was extracted from mixed cellulose esters (MCE)-filtered samples (0.22 µm porosity, 47mm diameter; ClearLine 0421A00023), using Macherey-Nagel NucleoMag® DNA/RNA Water Kit (744220.1) with. Each filter was stored in 5 mL Type A NucleoSpin Bead Tubes (Macherey-Nagel, 740799.50), added with 1.2 mL of lysis buffer MWA1, as in (Rossi et al., 2023). For DNA extraction, bead-beating lysates (10 min at maximum vortex speed on a Genie2 vortex) were processed following a protocol adapted for 47 mm

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

filter membranes (manufacturer's protocol: https://www.mn-net.com/media/pdf/ce/b5/38/Instruction-NucleoMag-DNA-RNA-Water.pdf). DNA was finally eluted into 50 µL RNase-free H_2O. The quality (260/280 and 260/230 ratios) and concentration (ng μL^{-1}) of the extracts were assessed using a BioSpec-nano (SHIMADZU BIOTECH corporation). The extraction products were stored at -25°C until DNA gene quantification (q-PCR) and 16S rRNA gene amplification and sequencing.

2.3.6 Gene quantification, reaction mixture and amplification conditions for q-PCR

In order to quantify the ammonium-to-nitrate transformation gene (*amoA*), homemade standard assays were performed using plasmids (pEX-A128; Eurofins Genomics), in which sequences of *amoA* (*Nitrosomonas europaea* N accession L08050) (McTavish et al., 1993) and 16S rRNA gene (*Pseudomonas syringae* isolate PDD-32b-74 accession HQ256872.1) were ligated. Plasmids containing the genes of interest were linearized with the restriction enzyme EcoRI FastDigest (ThermoFischer; ref FD0274) during 15 min at 37°C and the enzyme was inactivated during 5 min at 80°C. The concentration of the plasmid DNA solutions was measured spectrophotometrically, and converted into gene copies μL^{-1}, using the following formula:

$$Gene\ abundance = \frac{[DNA] \times N_A \times 10^{-9}}{(n \times mw)} \quad (4)$$

Where *[DNA]* is the concentration of recombinant plasmids in ng μL^{-1}, N_A is Avogadro's constant (6.023×10^{23} molecules mol^{-1}), *n* is the length of the gene sequence in base pairs and *mw* is the average molecular mass of a base pair (660 g $mole^{-1}$).

Standards for quantification were obtained by decimal dilutions of a stock solution containing 10^9 copies of the constructed plasmid, considering concentrations between 2 and 10^8 copies μL^{-1}. Limits of quantification were determined using the linearity of the standard curve for Ct between 10-30/ 5-20 [*amoA*/ *16S*].

The q-PCR analyses were performed as technical triplicates, in 96-well reaction plates along with a minimum of two no-template controls and standard samples consisting of 2–10^6 *amoA* copies or 10^2–10^8 16S rRNA gene copies per reaction. Each 20 µL qPCR reaction volume contained 10 µL of IQ™ SYBR® Green Supermix assays (BIO-RAD; ref 1708882), 300 nM of each primer [amoA-1F: 5'- GGGGTTTCTACTGGTGGT -3' and amoA-2R: 5'- CCCCTCKGSAAAGCCTTCTTC -3' for *amoA* (Rotthauwe et al., 1997); EUBf : 5'-GGTAGTCYAYGCMSTAAACG-3' and EUBr : 5'-GACARCCATGCASCACCTG-3' for the 16S rRNA gene (Bach et al., 2002)], 8 µL or 5 µL (at a concentration of 15-50 ng μL^{-1}) of DNA extract as the template for *amoA* and 16S rRNA genes, respectively, and PCR grade water (20 µL final). The q-PCR was performed with the following thermocycling parameters: 3 min of initial DNA polymerase activation and DNA denaturation at 95°C followed by 35 cycles of denaturation (95°C, 15 s), hybridization and elongation (60°C for amoA or 62°C for 16S), 1 min). The specificity was finally assessed by following up melting curves at temperatures from 60°C to 95°C, with a heating rate of 0.1°C/s). The standard curves had a linearity $r^2 > 0.99$ and an efficiency (E) between 80% and 120%. The

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

maximum standard deviation for each triplicate of q-PCR was set at 0.3 cycle threshold (C_T). The q-PCR results were converted to gene copy numbers per mL of rainwater using the standard curves as a reference.

2..3.7 Ribosomal gene amplification and sequencing, bioinformatics and statistics

Amplification of the V4 region of the 16S sub-unit of bacterial ribosomal genes was performed from genomic DNA extracts by PCR, using the universal primers 515f (5'-GTGYCAGCMGCCGCGGTAA-3') and 806r (3'-GGACTACNVGGGTWTCTAAT-5') (Apprill et al., 2015; Parada et al., 2016) and the Platinum II Taq Hot-Start DNA Polymerase (Invitrogen ; ref 14966005), following the conditions specified in (Bulgarelli et al., 2012). Amplicons were purified using the QIAquick PCR Purification kit (QIAGEN; Hilden, Germany) and sequenced on Illumina Miseq 2×250 bp (GenoScreen; Lille, France).

The sequence data have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under the accession number PRJEB91356. A total of 9,292,119 sequences were obtained following demultiplexing for the 57 samples investigated. Each sample contributed between 8,350 and 50,157 16S rRNA gene reads. For taxa annotation, the FROGS pipeline [Find Rapidly OTUs with Galaxy Solution; (Escudié et al., 2018)] was used through the Metabarcoding.useGalaxy v4.1.0 (**Supplement Materials and Methods**).

All statistical tests were carried out using Past software (v.4.03) (Hammer et al., 2001).

3. Results

3.1 Atmospheric metagenomes reanalysis

In atmospheric MG, organic and inorganic nitrogen processing genes are equivalently represented ($p>0.05$, Student's test), and contributing a total of 4.08 ± 2.33 parts per million base pairs (ppmbp). Organic nitrogen processes are dominated by glutamate and urea metabolisms (2.01 ± 0.53 parts per million) (**Fig. 1A; Supplement Fig. S1** and **Supplement Table S2)**. Regarding inorganic processes, together, denitrification, assimilatory and dissimilatory nitrate reduction, and biological nitrogen fixation contribute ~99% of the related reads, with respective contributions of 46% ± 15% (0.96 ± 0.36 ppmbp), 25% ± 3% (0.51 ± 0.09 ppmbp), 19% ± 10% (0.40 ± 0.24 ppmbp) and 9% ± 4% (0.18 ± 0.11 ppmbp). Nitrification and anammox contribute <1% (**Fig. 1A; Supplement Fig. S1** and **Supplement Table S2**). Within denitrification, nitrite reduction prevails (0.71 ± 0.25 ppmbp), with 2 genes (*nirK* and *nirS*) contributing 75% ± 26% of the denitrification genes, and 35% ± 12% of the genes involved in inorganic nitrogen transformations. Nitrate reduction genes (*napABC*, *narGHIJVWYZ*) evenly contribute between 0 and 40% of the denitrification genes (**Supplement Fig. S2**). Within biological nitrogen fixation, the two most represented biomarker genes are *nifH* and *nifW*, whereas *nifD* and *nifK* are underrepresented (**Supplement Fig. S2**).

Overall, nitrogen processing genes are more represented in MG during clear atmospheric conditions (4.79 ± 1.66 ppmbp) than in clouds (3.36 ± 0.65 ppmbp) ($p<0.05$, Student's test). Inorganic nitrogen processing genes contribute 2.50 ± 0.90 ppmpb and

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

1.63 ± 0.47 ppmbp in clear atmosphere and in clouds, respectively, of which biological nitrogen fixation genes 0.26 ± 0.11 ppmbp (i.e., 10 ± 4% of the inorganic N-related reads) and 0.10 ± 0.05 ppmbp (i.e., 6 ± 3%) ($p<0.05$, Student's test), respectively (**Fig. 1A; Supplement Fig. S1** and **Supplement Table S2**).

3.2 Active microbial processes in the atmosphere and difference between clouds and clear atmosphere

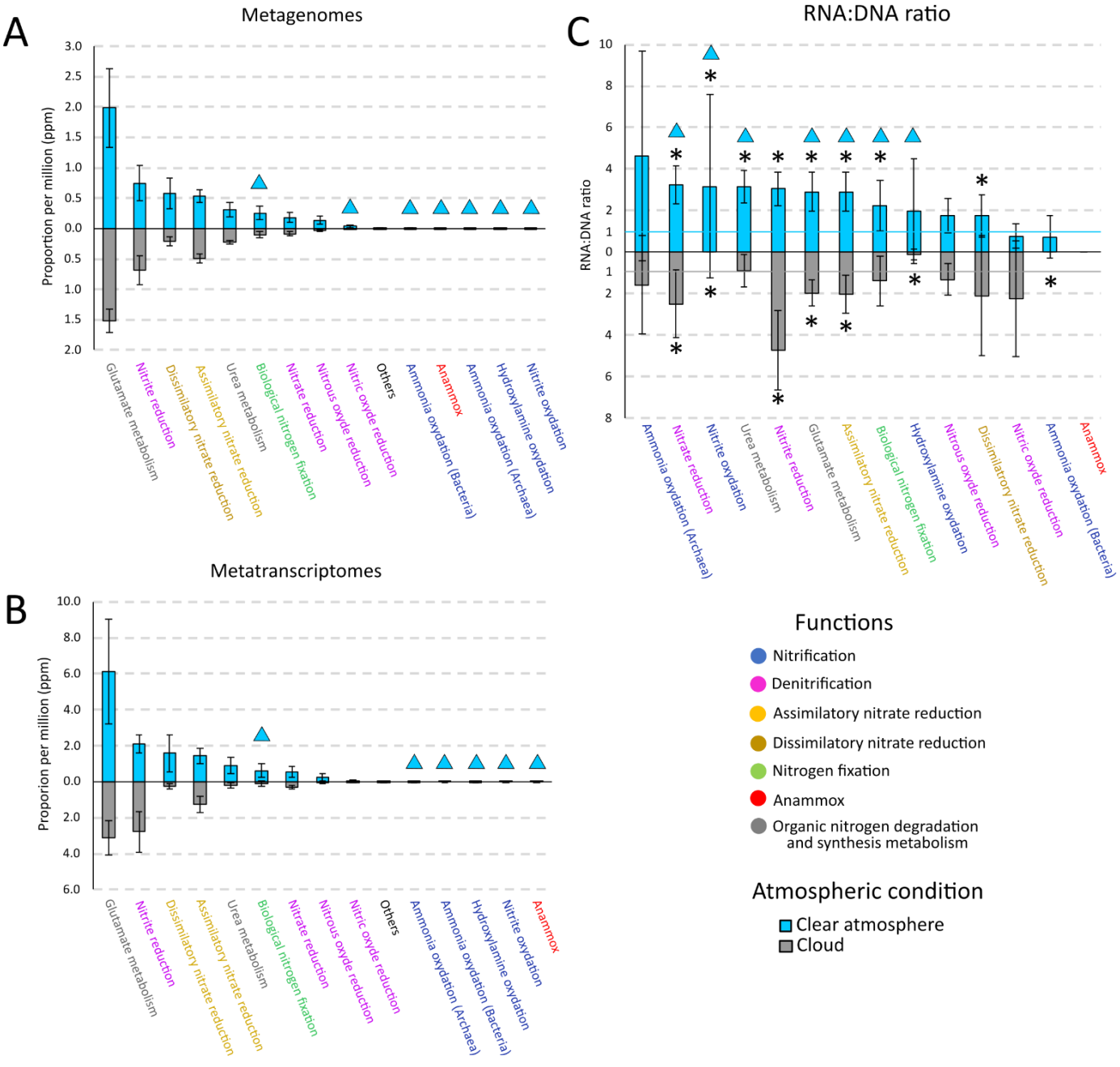
Functional gene expression was evaluated considering their relative representation in MT respect to their corresponding MG (RNA:DNA ratio, with higher values indicating higher expression levels). On average, nitrogen processing genes are more represented in MT than they are in the corresponding MG (RNA:DNA ratio > 1) ($p<0.05$, One-sample Wilcoxon test). Nitrate reduction, nitrate assimilation, biological nitrogen fixation, as well as glutamate and urea metabolisms all have an RNA:DNA ratio > 1 ($p<0.05$, One-sample Wilcoxon test), and this is higher in clear atmospheric conditions than in clouds ($p<0.05$, Mann-Whitney test) (**Fig. 1C**).

Functions with RNA:DNA ratios that are significantly greater than unity ($p<0.05$, One-sample Wilcoxon test) in clear atmosphere include nitrate reduction (3.23 ± 0.92), nitrite reduction (3.04 ± 0.81), nitrate assimilation (2.90 ± 0.94), dissimilatory nitrate reduction (1.73 ± 1.01), biological nitrogen fixation (2.22 ± 1.21), and genes associated with organic nitrogen metabolism, i.e., glutamate (2.90 ± 0.92) and urea metabolisms (3.15 ± 0.80). In clouds, functions with a ratio significantly > 1 include nitrate reduction (2.49 ± 1.62), nitrite reduction (4.71 ± 1.92), nitrate assimilation (2.01 ± 0.91), and glutamate metabolism genes (1.98 ± 0.64).

Functions with similar representation in MT as in MG (RNA:DNA ratio ~ 1) ($p>0.05$, One-sample Wilcoxon test) include, in clear atmosphere conditions, nitric oxide reduction (0.76 ± 0.58) and nitrous oxide reduction (1.77 ± 0.83). In cloud conditions, the same applies to nitric oxide reduction (2.25 ± 2.77), nitrous oxide reduction (1.33 ± 0.76), dissimilatory nitrate reduction (2.10 ± 2.89), biological nitrogen fixation (1.40 ± 1.19), and urea metabolism (0.91 ± 0.78) (**Fig. 1C**).

In turn, other functions exhibit RNA:DNA ratio < 1 ($p<0.05$, One-sample Wilcoxon test), specifically the steps involved in nitrification and anammox in both clear atmosphere and cloud conditions (**Fig. 1C**).

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025



245 **Figure 1. Proportions (per million base pairs) of nitrogen processing genes in (A) atmospheric metagenomes, (B) metatranscriptomes, and (C) corresponding RNA:DNA ratios, in clouds (grey) or clear atmosphere (blue) at the puy de Dôme mountain station. For (C), the blue line corresponding to the value 1 for clear atmosphere condition and the grey line corresponding to the value 1 for cloud condition. Triangles represent significant differences between clear atmosphere and cloud condition (Mann-Whitney test). Asterisks (*) indicate that the RNA:DNA ratio is different from 1 (Student or Wilcoxon test).**

250 **3.3 Bacteria associated with functional genes and transcripts**

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

The bacteria associated with atmospheric nitrogen processing genes in MG and MT are affiliated with 61 distinct phyla and 331 orders (**Fig. 2**). Within each nitrogen function, the contributing taxa are evenly distributed at the phylum level, with no difference between clouds and clear atmospheric conditions, except for the rare taxa related to nitrification. The most represented phyla for most functions are *Pseudomonadota* (contributing from 5% to 32% of the N-associated functional sequences), *Acidobacteriota*, *Actinomycetota*, *Bacteriodota*, *Bacillota* and *Planctomycetota* (**Fig. 2A**). Some functions involve specific phyla, such as *Nitrospirota* and *Nitrososphaerota* for nitrification, and *Cyanobacteriota* for N_2 fixation.

At the order level, the dominant orders include *Acetobacterales*, *Burkholderiales*, *Hyphomicrobiales*, *Sphingomonadales*, and *Rhodospirillales* (**Fig. 2B**). Nitrification processes are contributed by orders such as *Nitrososphaerales*, *Nitrosomonadales*, *Nitrospirales*, *Isosphaerales*, and *Cytophagales*, which account for 100% of the orders contributing transcripts in clouds. Certain bacterial orders are associated only with certain functions, such as *Methylococcales* for N_2 fixation and *Pseudomonadales* for urea metabolism.

In terms of diversity, similar taxa contribute to MG and MT for a given process, with differences in the relative abundance of taxa's representation. The major phyla in MG are also those dominating in MT, notably *Planctomycetota* and *Acidobacteriota*, which, for the denitrification process, account for <5% and 7% of the reads in MG and 36% and 31% in MT, respectively, under cloud conditions. Regarding nitrification, *Acidobacteriota* is the only phylum represented in MT, while this contributes only 10% of N-related sequences in MG.

3.4 Screening for potential diazotrophic microorganisms among isolates

Among the 34 strains of *Alphaproteobacteria* and *Gammaproteobacteria* isolated from clouds in earlier work, and tested here by PCR for the presence of the N_2 fixation biomarker gene (*nifH* gene), 5 (~15%) were positive, of which 4 *Sphingomonadales* and 1 *Rhizobiales* (**Supplement Table S1**).

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

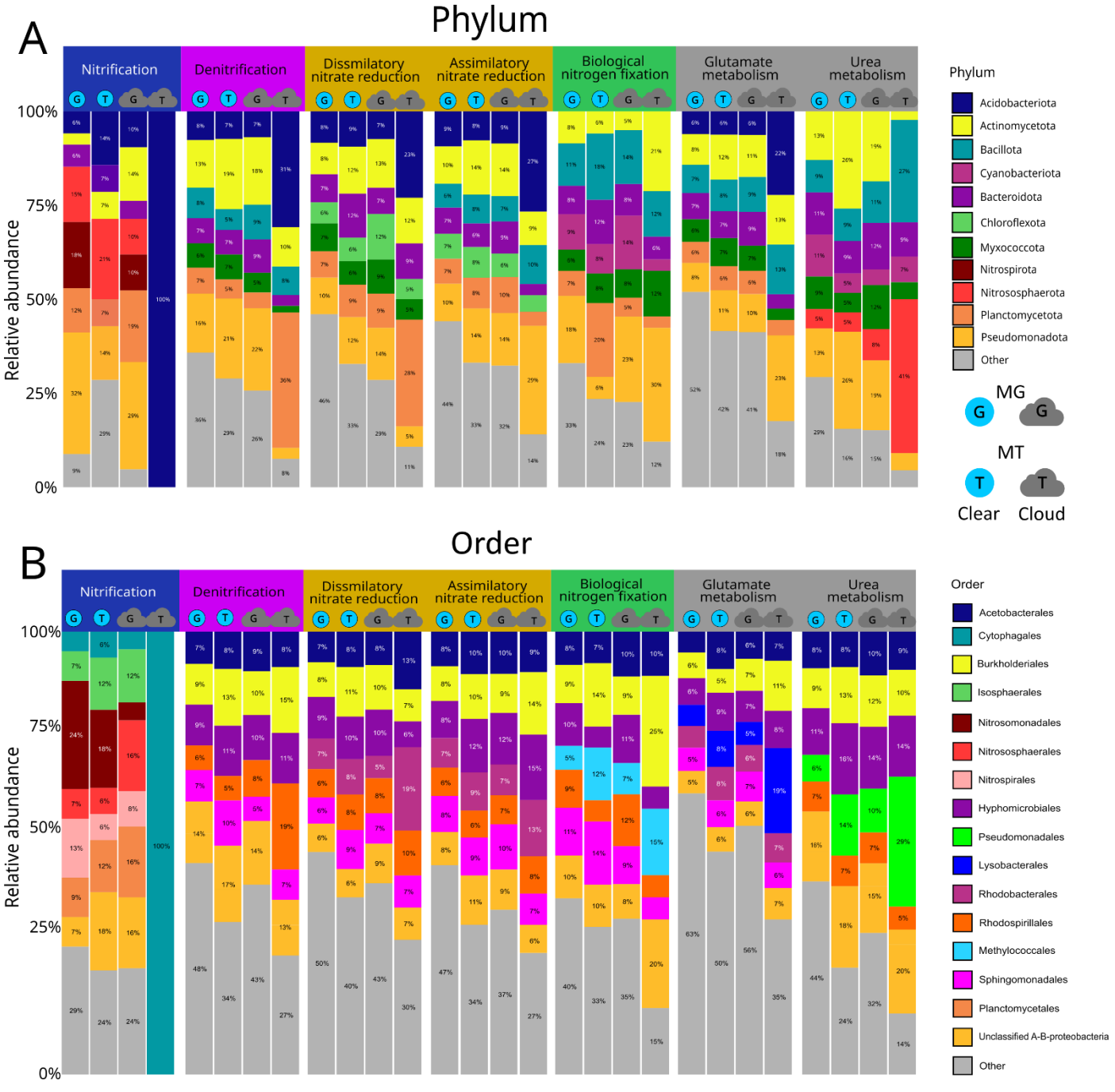


Figure 2. Proportion of bacterial phyla (A) and orders (B) in clear atmosphere and cloud metagenomes (MG) and metatranscriptomes (MT). For each active biological function linked to the nitrogen cycle, the 8 major phyla and orders are represented by different colors. Proportions greater than or equal to 5% are displayed directly on the figure.

3.5 Fresh rain sample analysis

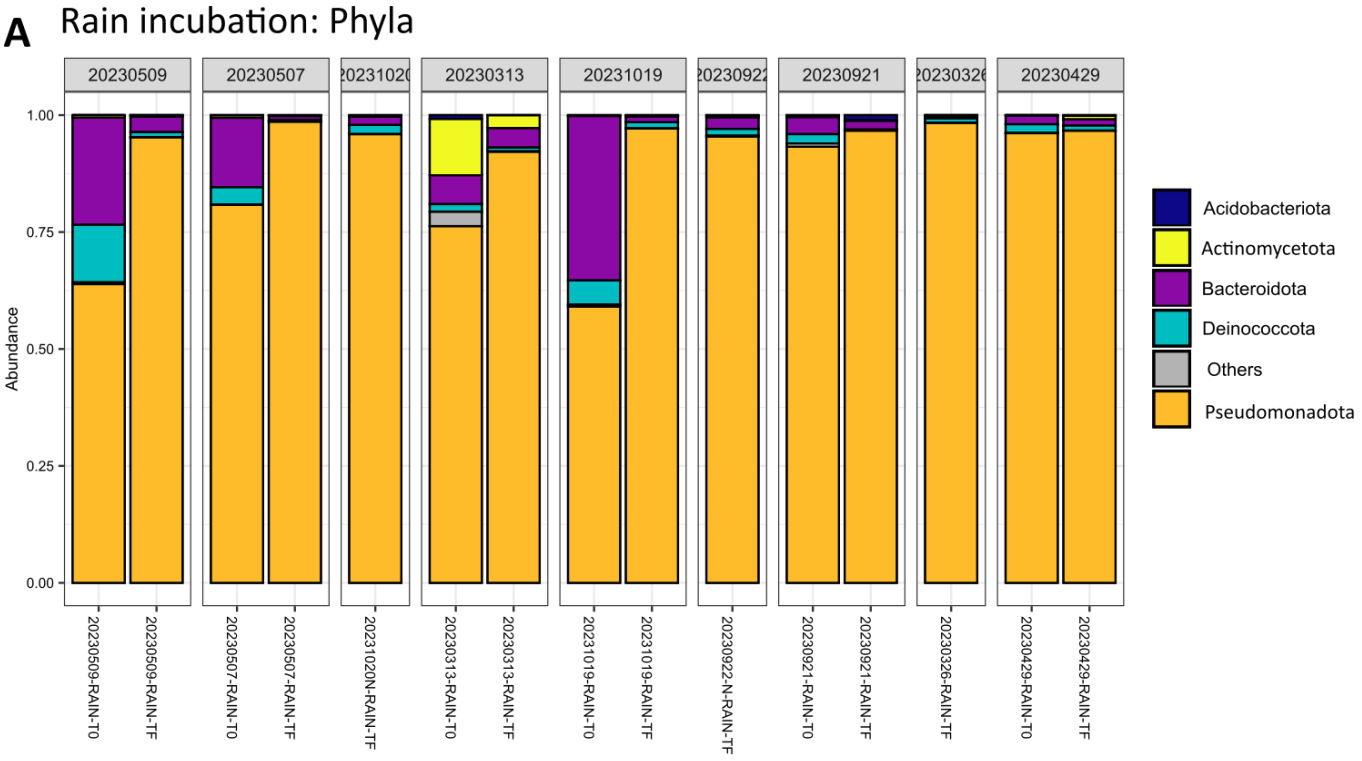
https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

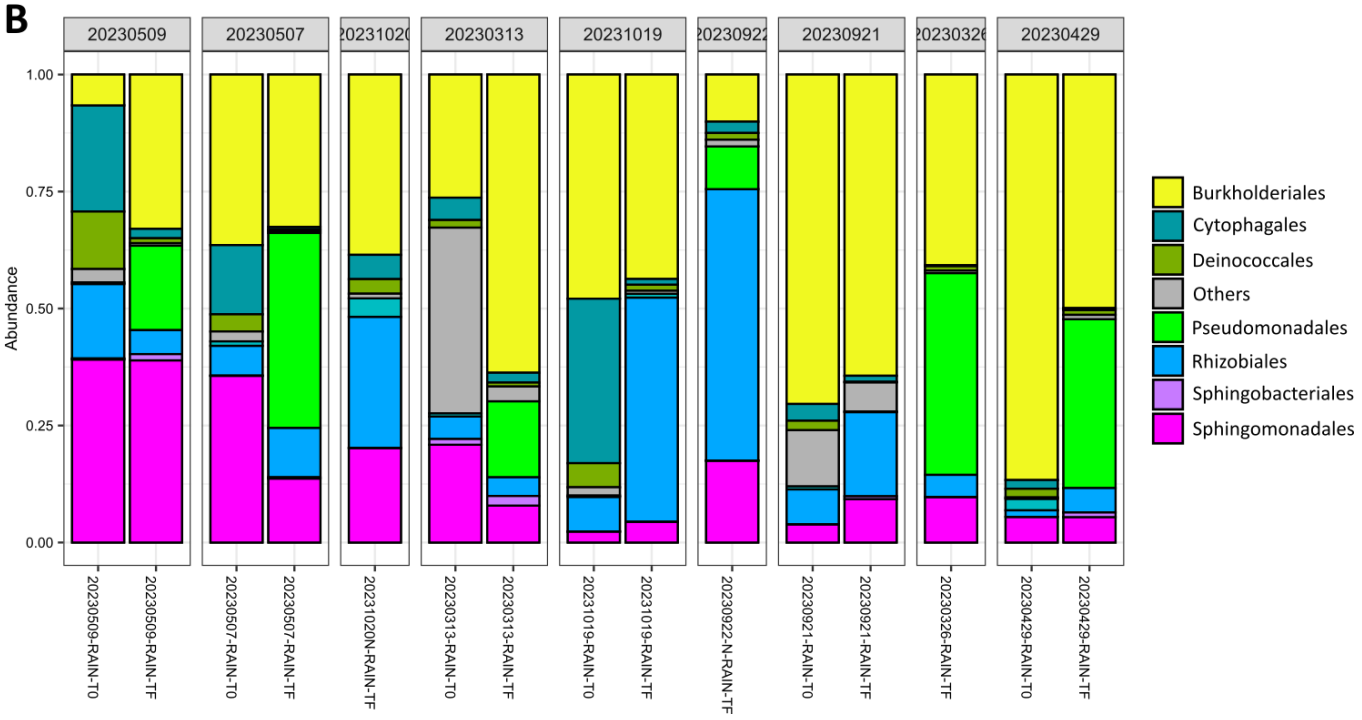
Among the 30 rain samples assessed in this study, ammonium and nitrate concentrations ranged from 3.3 ± 0.1 to 79.8 ± 0.1 µmol L^{-1}, and from 1.0 ± 0.1 to 34.8 ± 0.1 µmol L^{-1}, respectively (**Supplement Table S3**). This was linked with the geographical origin of the air masses, as attested by backward trajectories, with continental sources as major contributors (**Supplement Fig. S3**) ($p<0.05$, Pearson test, on the correlation between nitrate + ammonium concentration and the origin of air masses).

Bacterial concentration in the samples ranged from 1.94 ± 0.88 x10^2 cells mL^{-1} to 6.73 ± 1.31 x10^4 cells mL^{-1} (**Supplement Table S3**). This was not correlated with the origin of the air masses ($p>0.05$, Pearson test, **Supplement Table S4**).

The majority of bacteria in rain samples are affiliated with *Pseudomonadota* (ranged between 80 and 90% on average, **Fig. 3**). The major orders are *Burkholderiales*, *Cytophagales*, *Pseudomonadales*, *Sphingomonadales*, *Sphingobacteriales* and *Rhizobiales* (**Fig. 3**). There are clear differences in the distribution of these orders, in particular *Sphingomonadales* and *Burkholderiales*, depending on the sample (**Fig. 3**). The *amoA* gene, related to ammonium utilization in bacteria (ammonia monoxygenase), was below the detection limit of qPCR (less than $10^{0.25}$ copies mL^{-1}) in all rainwater samples, except 20230506_RAIN where 14 copies mL^{-1} could be quantified.

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025





290

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

Figure 3. Proportion of sequences affiliated with (A) bacterial phyla and (B) orders of *Pseudomonadota* in fresh rain samples and after incubation for 5 days at 17°C and 130 rpm.

3.6 Incubation of rainwater samples

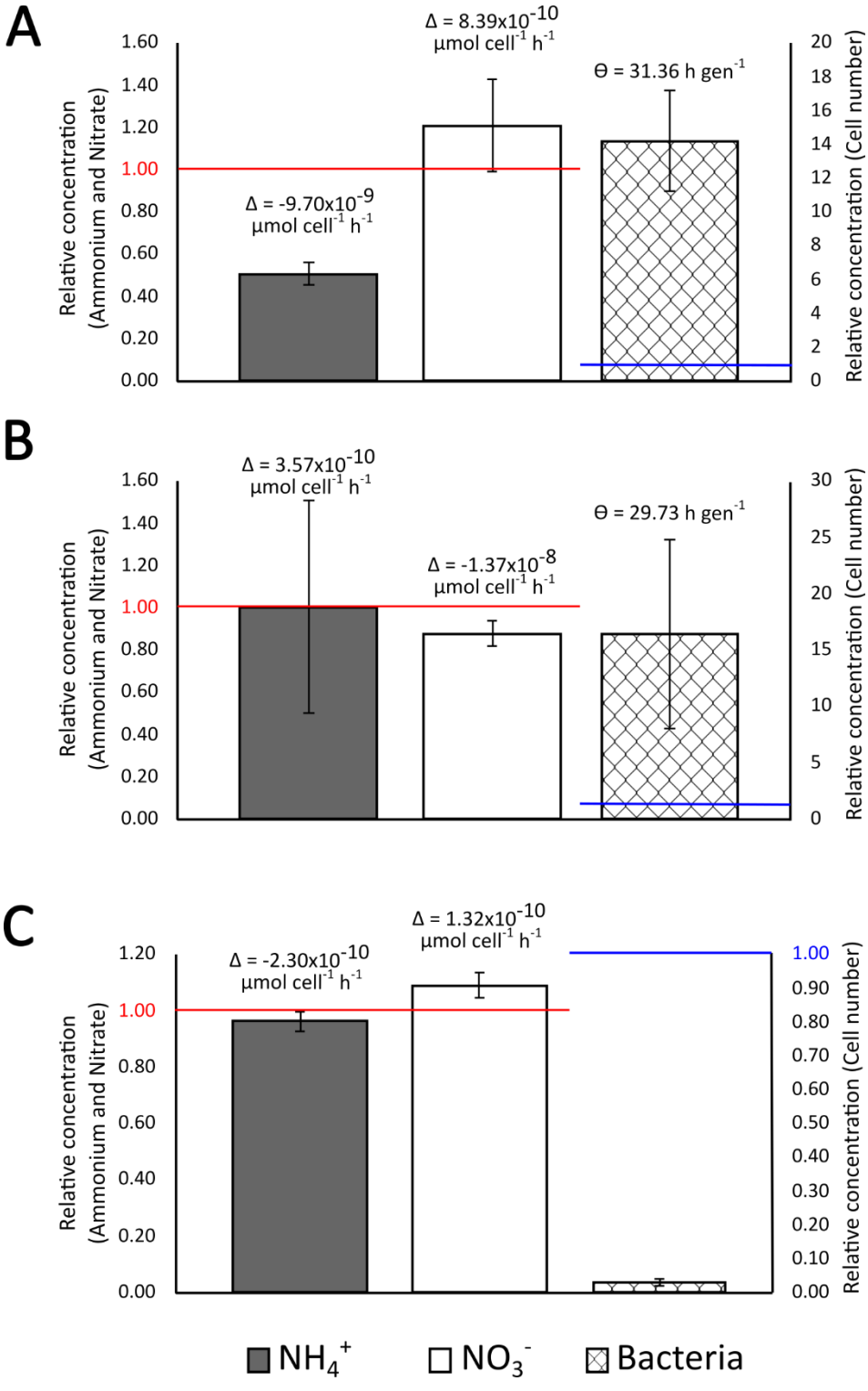
The bacteria biomass and diversity in the 9 rain water samples investigated changed during the 5 days of incubation (**Fig. 4** and **Table 3**). Depending on samples, at the end of incubation, the cell number concentration ranged from 1/35 to 400 times that initially present. When increasing, this corresponded to generation times of 13.45 to 56.26 h gen^{-1}.

The dominant orders initially were *Burkholderiales*, *Cytophagales*, *Pseudomonadales*, *Sphingomonadales*, *Sphingobacteriales* and *Rhizobiales* (**Fig. 3**). In some of the samples, their relative abundance was modified after incubation. For example, *Burkholderiales*' representation increased from 7% to 30% in the 20230509_RAIN sample, and from 26% to 65% in the 20230313_RAIN sample. Similar trends were observed for *Rhizobiales* in 20231019_RAIN, and for *Pseudomonadales* in 20230507_RAIN, 20230313_RAIN, and 20230429_RAIN, in these cases at the expense of the initially dominant order *Burkholderiales*.

Similar to the correlation observed between the origin of air masses and ion patterns (**Fig. 5** and **Supplement Fig. S3**), a source effect could be related with the proportion of *Burkholderiales* initially present in the rain samples: this significantly decreased with the level of marine influence ($p<0.05$, Spearman rs test) (**Fig. 5**).

The *amoA* gene could be quantified in the samples only upon incubation for 5 days (**Table 3**), with values ranging from 20 to 40 gene copies mL^{-1} of rainwater.

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025



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Preprint. Discussion started: 30 July 2025

Figure 4. Typical evolution of the concentration of ammonium (grey histogram) nitrate (white histogram) and microbial cell number concentration (hatched histogram) in rainwater samples after incubation for 5 days at 17°C and 130 rpm shaking, respect to initial values, and corresponding inferred average ammonium and nitrate bioassimilation rate (Λ) and bacteria generation time (Θ), when cell number concentration increased. (A) 20230922_RAIN, (B) 20230326_RAIN and (C) 20230429_RAIN. The results for the other incubations are shown in Supplement Fig. S4.

On average, NH_4^+ concentration decreased over the 5 days of incubation (Wilcoxon test; $p<0.05$) from 28.43 ± 18.09 µmol L^{-1} to 21.38 ± 18.02 µmol L^{-1}, i.e., between 0.26 ± 0.18 and 1.00 ± 0.50 of its initial concentration, depending on samples (Wilcoxon test; $P<0.05$) (**Table 3**). The inferred corresponding NH_4^+ bioassimilation rates ranged between $2.34x10^{-7}$ and $2.30x10^{-10}$ µmol h^{-1} $cell^{-1}$ ($4.61x10^{-8}$ µmol h^{-1} $cell^{-1}$ on average) i.e., this ranged over 3 order of magnitude. On the contrary, NO_3^- concentration did not significantly change, with concentration of 7.99 ± 4.66 µmol L^{-1} in fresh samples and 7.87 ± 4.83 µmol L^{-1} after incubation, i.e., 0.64 ± 0.41 to 1.25 ± 0.23 its initial values (**Fig. 4** and **Table 3**).

The rate of ammonium bioassimilation was independent from the initial concentrations of ammonium, bacteria cell or *amoA* gene copies ($p>0.05$, Spearman rs test). In turn, it was positively correlated with the relative abundance of *Sphingomonadales* in the samples, and negatively with that of *Burkholderiales* ($p<0.05$, Spearman rs test) (**Fig. 5** and **Supplement Table S5)**.

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

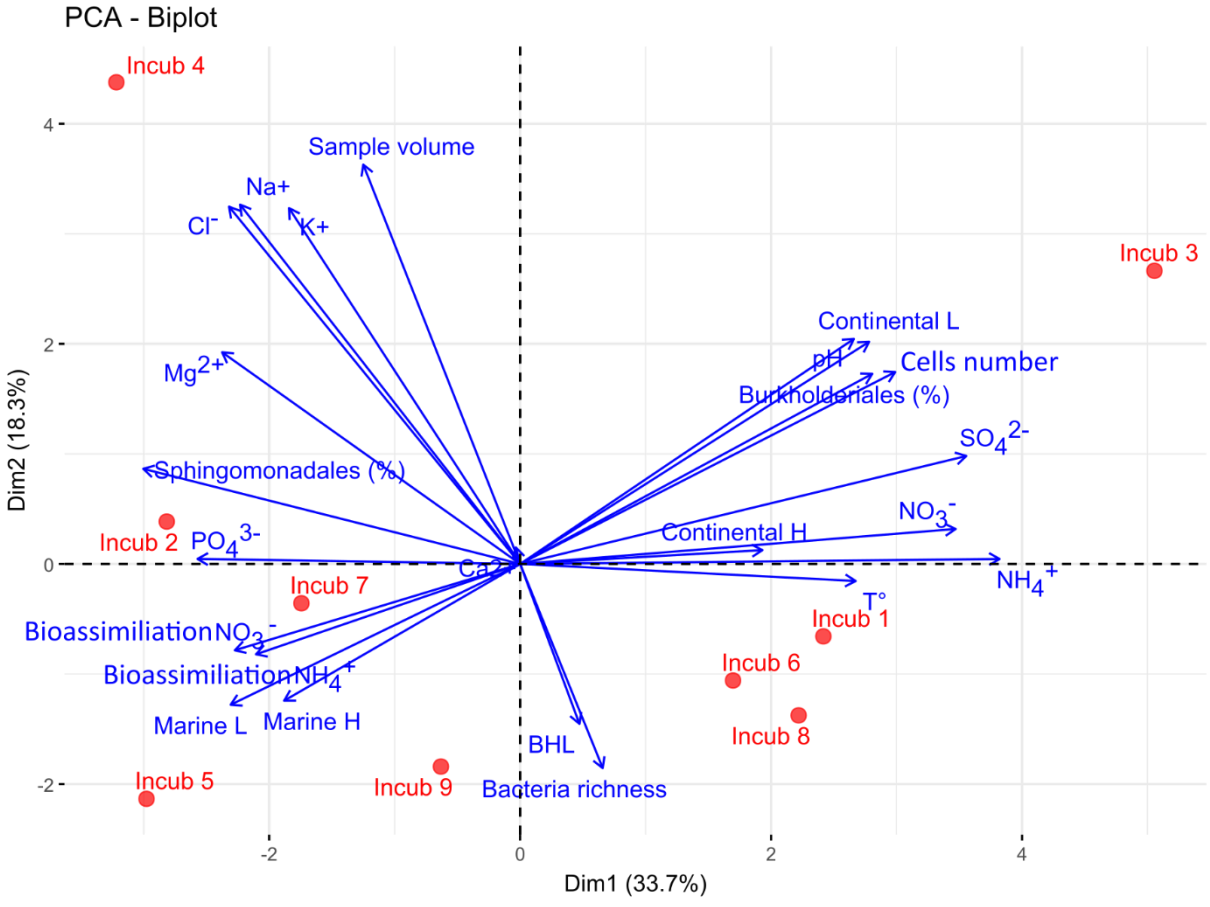

Figure 5. Principal component analysis (PCA) between the initial chemical, biological and meteorological variables of rainwater samples and inorganic nitrogen bioassimilation rates during incubations (blue arrows). The red dots represent the different incubations numbered in chronological order. BLH: Boundary Layer Height; L and H for "Marine" and "Continental" air mass origins stand for Low and High altitude, respectively.

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

Table 3. Characteristics of rain sample incubations. T0 and TF indicate initial and final (5 days) incubation times, respectively; bdl: below detection limit; N.A.: not available.

Sample identifier (yyyymmdd)	Cell number			Ammonium (NH_4^+)			Nitrate (NO_3^-)			Copies *amoA* mL^{-1}	Bacteria richness (number of distinct ASVs)
	Concentration (mL^{-1})	Relative change during incubation	Inferred cell generation time (h gen^{-1})	Concentration (µmol L^{-1})	Relative change during incubation	Inferred bioassimilation rate (µmol $cell^{-1}$ h^{-1})	Concentration (µmol L^{-1})	Relative change during incubation	Inferred bioassimilation rate (µmol $cell^{-1}$ h^{-1})		
20230313_RAIN-T0	2.08×10^3	0.70	0	48.7	0.88	2.25×10^{-8}	13.1	1.06	-3.17×10^{-9}	bdl	383
20230313_RAIN-TF	1.45×10^3			43.0			13.9			4.99×10^1	184
20230326_RAIN-T0	2.49×10^2	16.41	29.73	13.1	1.00	-3.57×10^{-10}	3.3	0.88	1.37×10^{-8}	bdl	N.A.
20230326_RAIN-TF	4.09×10^3			13.1			2.9			4.31×10^1	119
20230429_RAIN-T0	6.73×10^4	0.03	0	48.4	0.96	2.30×10^{-10}	12.0	1.09	-1.32×10^{-10}	bdl	60
20230429_RAIN-TF	1.89×10^3			46.6			13.1			2.08×10^1	137
20230507_RAIN-T0	9.29×10^2	28.38	24.86	16.5	0.43	8.37×10^{-8}	7.1	0.90	6.61×10^{-9}	bdl	100
20230507_RAIN-TF	2.64×10^4			7.1			6.4			2.09×10^1	145
20230509_RAIN-T0	3.63×10^2	485.77	13.45	13.8	0.26	2.34×10^{-7}	4.7	0.64	3.90×10^{-8}	bdl	141
20230509_RAIN-TF	1.76×10^5			3.6			3.0			2.41×10^1	161
20230921_RAIN-T0	1.35×10^4	4.39	56.26	45.6	0.72	7.78×10^{-9}	12.2	1.01	-6.55×10^{-11}	bdl	178
20230921_RAIN-TF	5.91×10^4			33.0			12.3			bdl	87
20230922-RAIN-T0	3.96×10^3	14.19	31.36	9.3	0.51	9.70×10^{-9}	1.9	1.21	-8.39×10^{-10}	bdl	141
20230922-RAIN-TF	5.62×10^4			4.7			2.3			bdl	125
20231019_RAIN-T0	3.89×10^3	8.66	38.53	46.8	0.78	2.24×10^{-8}	13.3	0.88	3.46×10^{-9}	bdl	N.A.
20231019_RAIN-TF	3.37×10^4			36.4			11.7			bdl	117
20231020_RAIN-T0	2.08×10^3	20.53	27.53	13.7	0.36	3.53×10^{-8}	4.2	1.25	-4.22×10^{-9}	bdl	177
20231020_RAIN-TF	4.26×10^4			4.9			5.3			bdl	100
Average, all samples T0	1.05×10^4 ±2.17×10^4	4.26 ±158.32	57.39 ±87.59	28.43 ±18.09	0.75 ±0.27	4.61×10^{-8} ±7.49×10^{-8}	7.99 ±4.66	0.99 ±0.19	6.04×10^{-9} ±1.35×10^{-8}	-	169 ±103
Average, all sample TF	4.46×10^4 ±5.42×10^4			21.38 ±18.02			7.8 ±4.83			-	131 ±30

335

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

4. Discussion

Nitrogen compounds (NH_3, NH_4^+, NO_3^-, NO_2^-, NOx, HNO_3, N_2O, *e.g.*, urea, amines, proteins, etc.) are emitted to the atmosphere by a variety of natural and anthropogenic sources (Fowler et al., 2013; Sutton et al., 2013). In terrestrial ecosystems, excess reactive nitrogen disrupts the nutrient balance, reducing carbon storage (Galloway et al., 2002; Matson et al., 2002), acidifies soils and reduces biodiversity (Aber et al., 1995). The intensive use of nitrogen fertilizers in agriculture is therefore a major cause of soil pollution and degradation worldwide (Lal, 2015; Müller et al., 2018). This nitrogen is then washed into aquatic environments, causing eutrophication of fresh and coastal waters (Diaz and Rosenberg, 2008; Howarth, 2008). The nitrogen concentrations have been rising over the last few decades amplifying the nitrogen cycle, producing more NOx, causing health issues and accelerating climate change (Cowling et al., 1998; Krey, 2014; Myhre et al., 2013), so a better understanding of the interactions between atmospheric nitrogen and microorganisms is essential.

Although some studies suggest interactions between airborne bacteria and nitrogen, including the biological degradation of amino acids in clouds and nitrosative stress in airborne microorganisms (Amato et al., 2019; Hill et al., 2007; Jaber et al., 2021; Péguilhan et al., 2025), information remains very limited. This study provides new insights into the interactions that may exist between nitrogen and microorganisms in the atmosphere.

4.1 The atmosphere expands surface ecosystems

The biological and chemical composition of the atmosphere mirrors to some extent that of emitting surfaces. In atmospheric metagenomes and metatranscriptomes, we find approximately equal proportions (~50:50%) of genes associated with inorganic and organic nitrogen transformation. This is similar to what is found in other environments such as oceans, rivers, sediments and plants (Deng et al., 2024; Nie et al., 2021; Song et al., 2022; Tu et al., 2017; Wang et al., 2021). The predominant metabolic functions identified are glutamate metabolism, denitrification and nitrate assimilation/dissimilation, as in plants, rivers and oceans (Deng et al., 2024; Nie et al., 2021; Song et al., 2022). In these environments these processes can account for up to 80% of the genes associated with inorganic nitrogen utilization, versus ~90% in the atmosphere in our study.

Active nitrogen-related functions are maintained by bacteria in the atmosphere, which might be critical for their physiology despite much shorter residence times (a few days) than in any other environment (Burrows et al., 2009). Bacterial diversity associated with nitrogen-related processes is composed of a core of taxa, which are recurrently present and active regardless of atmospheric conditions (clear atmosphere or cloud), and also predominant in surface ecosystems (freshwater, ocean, phyllosphere) (Deng et al., 2024; Nie et al., 2021; Song et al., 2022). This is made up of six phyla: *Pseudomonadota*, *Acidobacteriota*, *Actinomycetota*, *Bacteroidota*, *Bacillota*, and *Planctomycetota*, which contrasts with other ecosystems (plants, river etc.) where nitrogen-associated processes are typically dominated by a single bacterial phylum or order (>50–75% relative abundance) (Deng et al., 2024; Song et al., 2022; Tu et al., 2017). Functions carried out by more diverse

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

assemblages of microorganisms are less sensitive to environmental changes and therefore more stable over time (Maron et al., 2018).

In contrast, certain functions and their associated taxa have very low representation in the atmosphere, such as nitrification and anammox, usually carried out by *Nitrososphaerales*, *Nitrosomonadales* and/or *Nitrospirales*. These occur mostly in sediments from the ocean's mesopelagic zones (Deng et al., 2024; Song et al., 2022), i.e. areas that are not in contact with the atmosphere.

4.2 Focus on the main nitrogen elements and processes

4.2.1 Organic nitrogen content in the biomass of airborne bacteria

In total, 5×10^{19} bacteria (Whitman et al., 1998) are estimated aloft in the Earth's atmosphere, roughly contributing ~500 kg of N, considering an average N content of 10^{-14} g per cell (Paul and Clark, 1996). According to the study by (Neff et al., 2002), the annual flux of atmospheric organic nitrogen emitted as bacterial biomass would be 9.5×10^{-6} kg of N ha^{-2}. Considering only the continents ($1{,}489 \times 10^{10}$ ha), we infer that this represents ~10^5 kg N yr^{-1}. In another study, the total number of bacteria emitted annually into the atmosphere from surface ecosystems is estimated at ~10^{24} cells (Burrows et al., 2009), corresponding to approximately 10^7 kg N yr^{-1} carried as bacterial biomass. In clouds, organic nitrogen compounds represent 18% of the total molar content of nitrogen present (Hill et al., 2007). It was estimated that bacterial biomass represents less than 1% of it (Hill et al., 2007).

Overall, the contribution of airborne bacteria to atmospheric N therefore ranging between 10^5 and 10^7 kg N yr^{-1} according to these rough estimates, remains minor compared to inorganic nitrogen emissions, whether anthropogenic or natural, reaching ~70 Tg NH_3 yr^{-1}, ~18 Tg N_2O yr^{-1}, and ~5 Tg NO yr^{-1} (Bouwman et al., 2013; Duce et al., 2008; Fowler et al., 2013; Pilegaard, 2013; Sutton et al., 2013; Voss et al., 2011).

4.2.2 NH_4^+ utilization and glutamate metabolism

In atmospheric MG and MT, the sequences related to glutamate metabolism prevail among N-related processes. Although the inorganic forms largely predominate, organic nitrogen therefore likely represents a major source of nitrogen and energy for airborne microorganisms (Jaber et al., 2020).

Ammonium/ammonia is one of the most abundant nitrogen elements in the atmosphere. This is present at concentration of up to several hundred micromolar in cloud water (Hill et al., 2007; Renard et al., 2020; Vaïtilingom et al., 2010). Emissions from surface ecosystems amount to ~70 Tg NH_3 $year^{-1}$, mainly from industry and agriculture (Bouwman et al., 2013; Duce et al., 2008; Fowler et al., 2013; Sutton et al., 2013).

From our data, we estimate a global ammonium processing by bacteria in clouds of ~5.5×10^7 kg $year^{-1}$, considering the average bioassimilation rate measured in rainwater incubations (4.61×10^{-8} µmol $cell^{-1}$ h^{-1}), a total airborne biomass of 5×10^{19} bacteria (Whitman et al., 1998), and a cloud fraction of 15% (Lelieveld and Crutzen, 1990). This represents approximately

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

~0.08% of NH_3 emissions from terrestrial ecosystems and oceans (Bouwman et al., 2013; Duce et al., 2008; Fowler et al., 2013; Sutton et al., 2013). The positive correlation between the proportion of *Sphingomonadales* and ammonium decay rate in the incubations suggests a direct impact of these bacteria.

The biosynthesis of glutamate is the primary pathway for the assimilation of nitrogen and this requires NH_4^+ (Merrick and Edwards, 1995). This process involves glutamine synthesis via the enzyme glutamine synthetase (GS), which catalyzes an ATP-dependent amidation of glutamate, utilizing ammonium as the nitrogen source (Walker and van der Donk, 2016). Glutamine then acts as a nitrogen donor for approximately half of the nitrogen atoms incorporated into purines and pyrimidines, whereas glutamate provides nitrogen atoms for these nucleic acid bases, as well as amino groups for all other amino acids. Therefore, the GS/Glutamate synthase biosynthesis pathway, also known as the GS-GOGAT pathway, constitutes a major route for nitrogen assimilation, from ammonium (Walker and van der Donk, 2016).

4.2.3 Nitrate utilization (NO_3^-)

After glutamate/ammonium metabolism, denitrification (mainly nitrate reduction) and nitrate assimilation and dissimilation are the most represented processes in atmospheric MG and MT. Like ammonium, nitrate is also usually present at concentrations of several hundred micromolar in cloud water (Hill et al., 2007; Renard et al., 2020; Vaïtilingom et al., 2010).This is a major target of microbial processes as well in ecosystems. Nitrate concentration decreased in ~40% of the rainwater incubation experiments. Extrapolating these observations to the global scale, this corresponds to the transformation of ~2×10^7 kg $year^{-1}$ of NO_3^- in the atmosphere on a planetary scale, which is 47% less than predicted for ammonium (**section 4.2.2**). In all experiments, ammonium concentration decreased simultaneously to that of nitrate. Ammonium is preferentially used by microorganisms to recover nitrogen, over nitrates (McCarthy et al., 1977). The ability to respire nitrate is widely spread among bacteria that can dissimilate the produced nitrite into gaseous compounds (denitrification) for energy production, or to ammonium (dissimilatory nitrate reduction) for nitrogen assimilation (Philippot and Højberg, 1999).

4.2.4 Biological nitrogen fixation (N_2)

The third most represented function in inorganic nitrogen processes in the atmosphere is biological nitrogen fixation (N_2 -> NH_3). The corresponding biomarker genes (*nifDHKW*) represent ~10% of all genes associated with the atmospheric inorganic nitrogen cycle in MG and MT. Moreover, the screening of isolates indicates that ~15% of *Pseudomonadota* strains isolated from the atmosphere carry this function. N_2 is the most abundant inorganic nitrogen form in the atmosphere. Consistently, biological nitrogen fixation transcripts were more expressed in clear atmosphere than in clouds.

In some rain incubation experiments, we observed bacteria multiplication but no decrease of ammonium or nitrate concentration, so other source(s) of nitrogen, such as N_2 and/or organic forms supported bacterial growth. In these cases, *Burkholderiales* dominated the bacteria community; this order is known to include nitrogen-fixing bacteria (Bahulikar et al., 2021; Jean et al., 2020), including anoxygenic phototrophs, *i.e.* photosynthetic bacteria using light to produce energy, with no

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

production of molecular oxygen (Imhoff et al., 2019; Vergne et al., 2021). In these bacteria, phototrophy supports atmospheric nitrogen fixation (through the enzyme nitrogenase), one of the most energy-intensive reactions in living organisms (Kim and Rees, 1994). In addition, a study conducted in an atmospheric simulation chamber using a cloud-isolated *Methylobacterium* sp. demonstrated that this auxiliary metabolism enhances bacterial fitness during atmospheric transport and may facilitate subsequent environmental colonization (Mathonat et al., 2025).

In our work, attempts to quantify N_2 fixation using acetylene reduction assay (ARA) (Hardy et al., 1968) failed, likely because of the limitations due to low microbial biomass. Considering that half of the airborne bacteria (5×10^{19} cells) are *Pseudomonadota*, and that 15% are diazotroph, biological N_2 fixation in the atmosphere would account for approximately 18 $\times 10^6$ kg N_2 year^{-1} (=0.018 Tg year^{-1}), assuming an average nitrogen fixation rate of 10 fmol N_2 cell^{-1} h^{-1} by a marine diazotroph (Foster et al., 2022). Although a more precise quantification would be needed, this likely represents an unsignificant fraction of global biological nitrogen fixation compared to estimates for terrestrial (58 Tg N_2 year^{-1} for natural ecosystems and 60 Tg N_2 year^{-1} for agricultural crops (Herridge et al., 2008)) or marine systems (140 Tg N_2 year^{-1} (Voss et al., 2013)). However, this may support microbial maintenance during atmospheric transport.

4.3 Perspectives

Atmospheric bacteria assimilate and transform more nitrogen than they contribute through their biomass, suggesting that they may act as sinks of atmospheric nitrogen. However, the accuracy of these transformation estimates at global scale could be improved. For instance, the measured bioassimilation rates were obtained from rainwater incubations at 17°C, which do not fully represent atmospheric aerosol conditions, as the liquid bulk phase does not account for the particulate nature of aerosols, and the temperature is relatively high compared to actual atmospheric conditions.

Water availability is another important factor to consider. While ammonium and nitrate assimilation was extrapolated to cloud environments based on rainwater data, it is important to note that even under non-cloud conditions, aerosol particles are not entirely devoid of liquid water (Ervens et al., 2025; Pandis and Seinfeld, 1989), making nitrogen assimilation potentially feasible in clear atmosphere conditions as well. This hypothesis is further supported by the high proportion of nitrogen-related transcripts detected in atmospheric MT from clear atmosphere samples.

Moreover, while these estimates are scaled to the global level, atmospheric nitrogen concentrations are subject to strong spatial and temporal variability. One could envision enhanced microbial nitrogen cycling activity following major nitrogen release events (e.g., fertilizer application in agriculture). Conversely, such nitrogen "overloads" might induce nitrosative stress, potentially reaching toxic levels for airborne microorganisms. This is supported by chamber simulation studies showing that high NO and N_2O concentrations can reduce bacterial culturability (Vernocchi et al., 2023).

5. Conclusion

https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

This study identified that atmospheric microorganisms are potential contributors to the atmospheric nitrogen cycle. Using a combination of metagenomic, metatranscriptomic, and experimental approaches, we showed that atmospheric microorganisms exhibit genes associated with nitrogen cycling, including both inorganic and organic nitrogen pathways, with near-equal representation (~50:50), similar to other environments such as oceans, rivers, and plant-associated microbiomes. Denitrification, nitrate assimilation/dissimilation, and glutamate metabolism emerge as the dominant functional pathways, both in terms of genetic presence and transcriptional activity, highlighting the metabolic versatility of airborne microbial communities.

Rainwater incubations showed bioassimilation of ammonium and, to a lesser extent, nitrate with an important link with the microbial diversity. The microbial community composition plays a significant role in nitrogen transformation potential. Atmospheric back-trajectories provide contextual information and help link air mass origin (marine vs. continental) to both concentrations of nitrogen-containing ions and microbial community composition in precipitation. For example, we show that air masses of continental origin carry higher levels of ammonium and nitrate and are associated with microbial communities enriched in taxa such as *Burkholderiales*, thereby influencing nitrogen transformation dynamics. Overall, our findings suggest that atmospheric microbial communities, though transient and exposed to harsh conditions, maintain stable and functionally resilient nitrogen-cycling capabilities.

6. Data availability

The sequence data of 16S rRNA gene amplicons have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under the accession number PRJEB91356.

7. Authors contributions:

FM, PA, MJ designed the experiments. FM performed the experiments. FM and PA collected samples and data. FM, RP and FE conducted the bioinformatics analysis. J-LB performed the backward trajectory analysis. FM and PA wrote the paper. All authors have contributed to the editing and formatting of figures and text. PA and BE supervised the research, coordination and strategy.

8. Competing interest

At least one of the (co-)authors is a member of the editorial board of Biogeosciences.

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https://doi.org/10.5194/egusphere-2025-3534
Preprint. Discussion started: 30 July 2025

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