



Direct quantification
of total and biological
ice nuclei in cloud
water

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Direct quantification of total and biological ice nuclei in cloud water

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Abstract

The distribution, abundance and nature of ice nucleation active particles in the atmosphere are major sources of uncertainty in the prediction of cloud coverage, precipitation patterns and climate. Some biological ice nuclei (IN) induce freezing at temperatures at which most other atmospheric particles exhibit no detectable activity (> -10 °C). Their actual contribution to the pool of IN in clouds remains poorly known, but numerical studies suggested their likely significance in atmospheric processes. In this study, cloud water was collected aseptically from the summit of puy de Dôme (1465 m a.s.l., France) within contrasted meteorological and physico-chemical conditions. Total and biological (i.e. heat sensitive) IN were quantified by droplet-freezing assay between -5 °C and -14 °C. Freezing was systematically induced by the presence of biological material, between -6 °C and -8 °C in 92 % of the samples. Its removal by heat treatment led to a decrease of the temperature of freezing by 3 °C to 4 °C. At -10 °C, there were 0 to ~220 biological IN mL⁻¹ of cloud water (i.e. 0 to ~22 m⁻³ of cloudy air based on cloud liquid water content estimates) and these represented 65 % to 100 % of the total IN. Based on back-trajectory plots and on physico-chemical analyses, the high variability observed resulted probably from a source effect, with IN originating mostly from continental sources. Assuming that biological IN were all bacteria, at maximum 0.6 % of the bacterial cells present in cloud water samples could have acted as IN at -8 °C, 1.5 % at -10 °C, and 3.1 % at -12 °C. The dataset generated here will help elucidating the role of biological and bacterial IN on cloud's microphysics by numeric modelling, and their impact on precipitation at local scale.

1 Introduction

The formation of clouds and their evolution have global impacts on Earth's climate. Within the last decade, considerable efforts have been made in order to identify and quantify the particles acting as ice nuclei (IN) in the atmosphere. Those particles are

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responsible for the heterogeneous nucleation of ice in supercooled clouds, leading to modifications of their radiative properties and initiating precipitation. At temperatures colder than about -15°C , feldspar particles were recently demonstrated to account for a great part to the pool of IN in mixed-phase clouds at a global scale (Atkinson et al., 2013). However, at warmer temperatures, most of the mineral aerosols as well as metallic and soot particles exhibit only very low or undetectable IN activity (INA), and the best candidate ice nuclei are biological (bacteria, fungi), or biogenic (macromolecules derived from living organisms, such as proteins) (Conen et al., 2011; DeMott and Prenni, 2010). Hence, biological IN are thought to largely influence clouds' evolution within the upper range of temperatures around freezing (e.g. Möhler et al., 2007). Among those, the most efficient natural IN described so far are bacteria, with representatives active at temperatures as warm as -2°C (Maki et al., 1974); other very active biological IN different from bacteria were also detected in the air, but their exact nature remains unknown (Garcia et al., 2012). Specimens of INA bacteria have been recovered from all the compartments of the water cycle: freshwaters (Maki and Willoughby, 1978; Morris et al., 2008), clouds (Joly et al., 2013) and precipitation at high altitude (Sands et al., 1982) or closer to the ground (Constantinidou et al., 1990; Maki and Willoughby, 1978; Šantl-Temkiv et al., 2009; Stephanie and Waturangi, 2011). This supports the hypothetical concept termed “bioprecipitation” that such bacteria could participate to hydrological cycles by triggering precipitation (Morris et al., 2004).

Figure 1 summarizes our current quantitative knowledge about high-temperature ($> -15^{\circ}\text{C}$) IN in the atmosphere and in the environmental compartments of the water cycle. The main results of the present study are also indicated. Most plants harbor relatively large populations of epiphytic ice nucleation active (INA) bacteria (Constantinidou et al., 1990; Lindemann et al., 1982; Lindow et al., 1978; Maki and Willoughby, 1978; Morris et al., 2008). So, despite low emission rates disconnecting the concentration of INA bacteria existing at the surface of plants from their concentration in the air above (Garcia et al., 2012), the main source of atmospheric biological IN is probably

vegetation (Pöschl et al., 2010). Recently, oceans were also cited as possible emitters of biogenic IN into the atmosphere (Burrows et al., 2013).

In the air at low altitude, Garcia et al. (2012) observed concentrations of 90 to 460 IN m^{-3} active at -10°C over vegetated agricultural areas, most of which were classified as biological based on their sensitivity to heat. In the latter study, INA bacterial cells were estimated to represent only a small fraction of the total airborne bacteria ($\sim 0.002\%$). Nevertheless, some specimens of INA bacterial strains have been recovered by culture from atmospheric samples (e.g. Stephanie and Waturangi, 2011). At high altitude, notwithstanding their suspected importance in atmospheric processes, much less quantitative data of high-temperature IN are available. Their concentration there is in general much below 25 m^{-3} , but it can vary drastically between < 1 and $\sim 100\text{ m}^{-3}$ within very short timeframes (Bowers et al., 2009; Conen et al., 2012; Xia et al., 2013). Interestingly, the highest concentrations were observed at high relative humidity.

Airborne IN can be transported to regions very distant from the source of emission and affect rain patterns after being incorporated into clouds (Creamean et al., 2013). In the single orographic cirrus cloud event studied by Pratt et al. (2009), about half of the 46 ice crystals residues (140–700 nm in diameter; -31°C ambient temperature) had a mass spectrometry signature typical of mineral dust, while about 33% were biological particles. More recent and more extensive in situ observations of cirrus clouds at temperatures $< -30^\circ\text{C}$ showed that biological particles are probably much more scarce among the solid residues of ice crystals (i.e. less than 1%), but that rather mineral dust and metallic particles dominate (Cziczko et al., 2013). However, observing crystal residues does not guaranty the identification of the actual IN, nor does give information about its activity. A quantitative study of high-temperature atmospheric IN was led at the Jungfrauoch summit in the Alps (3450 m a.s.l.); concentrations of 0 to 3.8 m^{-3} were measured when clouds were present on the site (Xia et al., 2013). Albeit, as emphasized by authors, their “precision was low” due to a limited air sample volume of less than 3 m^3 .

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Fresh snow and rain collected at different locations over the planet, from poles to sub-equatorial regions, carried ~ 1 to ~ 100 IN active at -7°C per liter of water. The large majority were altered by heat treatment and were thus categorized as biological; about half of these were probably bacteria (Christner et al., 2008a, b). INA bacteria were reported to be relatively more abundant in rainfall than in the air at the same site (Stephanie and Waturangi, 2011), and this may indicate that INA bacteria are preferentially incorporated into rainfall than other bacteria.

Based on these studies, biological IN are undoubtedly present all throughout the water cycle. They represent an important fraction of the pool of high-temperature IN where they were unambiguously quantified: in the air at low altitude, and in precipitation. However, our knowledge about their relative abundance in clouds is still scarce, which limits the evaluation of their impact on hydrological cycles through modeling approaches (Hoose et al., 2010; Phillips et al., 2008). As stressed by DeMott and Prenni (2010), it is technically not possible to provide any realistic concentration of airborne IN particles at the altitude of a cloud from measurements in precipitation, due to possible dilution/concentration effects and to non-nucleation particle scavenging. With the objective to provide quantitative data of IN concentration in clouds that could be utilized for modeling purposes, cloud water samples were collected throughout the year and under various meteorological situations from the summit of the puy de Dôme mountain in France (1465 m a.s.l). The concentrations of total and biological IN were then directly measured by the droplet-freezing method between -5°C and -14°C in 12 independent cloud water samples. From these, the maximum possible values of concentration of INA bacteria were then inferred.

2 Material and methods

2.1 Cloud water sampling

Twelve random cloud events were sampled from the puy de Dôme station (45°46'20'' N, 2°57'57'' E, 1465 m.a.s.l.) between June 2011 and October 2012. These were numbered from #76 to #87 following numbering of the cloud events sampled at puy de Dôme since 2001 and for which chemical and microbiological datasets are publically available at <http://wwwobs.univ-bpclermont.fr/SO/beam/data.php>. Cloud droplets were collected with single-stage aluminum droplet impactors (cut-off diameter: $\sim 7 \mu\text{m}$) sterilized by autoclave, as in Vaïtilingom et al. (2012).

2.2 Physico-chemical characterization and total cell counts

Cloud water samples were recovered either liquid or frozen onto the impaction plate depending on ambient temperature during sampling. For each sample, pH and conductivity were measured (Consort multiparameters C830) and major inorganic and organic ions were examined by ion chromatography (Dionex DX320 for anions and Dionex ICS1500 for cations). As the liquid water content (LWC) was not available directly during our sampling, we assigned a theoretical value based on a 10 yr monitoring of cloud at the puy de Dôme (Deguillaume et al., 2013). The minimum, average or maximum value (0.1, 0.3 or 0.6 gm^{-3}) was attributed according to the volume of water collected over the duration of sampling. Finally, 72 h back-trajectories of the sampled air masses were computed using HYSPLIT model (HYbrid Single-Particle Lagrangian Integrated Trajectory) (Draxler and Rolph, 2010).

Total bacteria were counted by epifluorescence microscopy on DAPI stained samples as in Vaïtilingom et al. (2012). Directly after collection, samples were fixed by the addition of 2% formaldehyde (final concentration; from 20% stock solution prepared in phosphate buffer 0.1 M, pH 7.0), and incubated in the presence of $2.5 \mu\text{g mL}^{-1}$ of DAPI (4',6-diamino-2-phenylindol) in the dark for at least 20 min before filtration

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on GTBP black filters (0.22 μm porosity; Millipore). Filters were then mounted on microscope slides and observed under UV-epifluorescence microscopy ($\lambda_{\text{exc}} = 365 \text{ nm}$; $\lambda_{\text{em}} = 420 \text{ nm}$) (Leica DM-IRB).

2.3 Droplet-freezing assays

The ice nucleation activity (INA) of the cloud water samples was determined within 2 h after collection following the well-tried droplet-freezing method (Vali, 1971). Thirty-two to 160 drops of 20 μL were distributed in 0.2 mL microtubes designed for high thermal conductivity and preventing aerial contamination and evaporation (Stopelli et al., 2013). These were placed in a cooling bath (Julabo F34-ED) at decreasing temperatures from -5°C to -14°C , with 1°C intervals for 8 min. The tubes were visually inspected at the end of each temperature step and those still liquid were counted. The concentration (mL^{-1}) of ice nuclei CIN at the temperature T in the suspensions was calculated using the equation in Vali (1971):

$$C_{\text{IN}} = [\ln(N_{\text{total}}) - \ln(N_{\text{liquid}})]_T / V \cdot (1/D_f)$$

where N_{total} is the total number of droplets, N_{liquid} the number of droplets still liquid after 8 min at the temperature T , V the volume of the droplets assayed (mL) and D_f the dilution factor of the suspension. Under our experimental conditions, the limits of quantification ranged from 1.59 to 173.3 IN mL^{-1} in the case where 32 droplets were assayed, and from 0.31 to 253.8 IN mL^{-1} in the case where 160 droplets were assayed. Negative controls consisted of ultrapure sterile water droplets and these remained liquid over all the range of temperatures investigated.

2.4 Biological IN quantification

For each sample, the concentration of biological IN (INA_{bio}) was calculated as the difference between the concentration of IN measured in untreated sample ($\text{INA}_{\text{total}}$) and the concentration of IN measured after heating for 10 min at 95°C ($\text{INA}_{\text{heated}}$), as in

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Christner et al. (2008a) and in Garcia et al. (2012). Heat denatures protein structures, so it eliminates at least a certain fraction of biological IN without altering non-biological material. When $[(\text{INA}_{\text{heated}})_{T-1} - (\text{INA}_{\text{heated}})_T]$ exceeded $[(\text{INA}_{\text{total}})_{T-1} - (\text{INA}_{\text{total}})_T]$, this calculation artificially led to a decrease in the concentration of INA_{bio} at $T - 1$ compared to T and values of $\text{INA}_{\text{heated}}$ were corrected for being consistent with the values of $\text{INA}_{\text{total}}$. Following this rule, three values of $\text{INA}_{\text{heated}}$ were corrected: -12°C in sample #79, -10°C in sample #82 and -11°C in sample #86.

2.5 Statistical analyses

All statistical analyses were made using the R software version 2.12.2 (R Core Team, 2011).

3 Results and discussion

3.1 Main characteristics of the cloud water samples

The main biological and physico-chemical characteristics of the 12 cloud samples studied are presented in Table 1. Ion composition is given in more details in Table S1 (data also available online at <http://www.obs.univ-bpclermont.fr/SO/beam/data.php>). Ambient temperature during collection ranged from -1.5°C to 13.3°C , so, as indicated in Table 1, some samples consisted of ice formed upon impaction on the collectors (samples #80 through #84); others samples were collected as liquid. Most of the air masses sampled originated from west (Atlantic Ocean) and travelled over different continental areas in Europe before hitting the puy de Dôme, following different trajectories (Fig. S1). Consistently, the chemical composition varied greatly from one sample to another. The pH ranged from 4.5 to 6.2, which are typical values for cloud water (e.g. Deguillaume et al., 2013). Ammonium (16.8 to $531.1\ \mu\text{M}$), sodium (0.6 to $145.7\ \mu\text{M}$), nitrate (1.0 to $126.0\ \mu\text{M}$) and sulfate (0.5 to $52.2\ \mu\text{M}$) dominated among inorganic ions, and formate was the most abundant dissolved carboxylic acid (3.2 to $109.6\ \mu\text{M}$) (Table S1).

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The chemical signature of the samples attested of mixed influences from oceanic and continental sources, the respective contributions to the global chemical composition of which were more or less marked depending on the origin of the air mass.

3.2 Quantification of total and biological ice nuclei

5 The total concentration of IN active between -5°C and -14°C was determined by direct droplet freezing assays. Eleven of the 12 cloud samples (92 %) froze at -8°C or warmer, and none remained supercooled at temperatures below -11°C (Table 2; Fig. 2). Ice initially formed due to the presence of 0.6 to 8.5 IN mL^{-1} (Table 2; Fig. 3a). Two samples (#81 and #83) were clearly outlying with much higher IN concentrations ($\sim 70\text{ mL}^{-1}$ at -8°C). After correction for LWC (Fig. 3b), the concentration of IN per volume of cloudy air ranged from 0.06 to more than 71.1 m^{-3} between -6°C and -14°C . This is in the range of concentrations typically observed in the air at high altitude (Fig. 1) (Bowers et al., 2009; Xia et al., 2013), and one order of magnitude lower than the concentrations measured at low altitude (Garcia et al., 2012).

15 Rain and surface snow samples analyzed using similar methods by Christner et al. (2008a, b) had total IN concentrations of about ~ 1 to ~ 300 per liter of water at -8°C , i.e. 2 orders of magnitude fewer than in our cloud water samples. This probably resulted from the relative dilution of insoluble particles in precipitation compared to cloud water (Flossmann and Wobrock, 2010), and from differences in sample handling: Christner et al. filtered samples for concentrating particles larger than $0.22\text{ }\mu\text{m}$, so smaller IN particles were missed, among which some could have originated from bacteria (Phelps et al., 1986). In addition, it is possible that a fraction of IN particles was not recovered from the filters.

25 Heating samples for 10 min at 95°C invariably decreased the highest temperature of freezing (Fig. 2), in general by 3°C to 4°C , and by 1°C to more than 5°C in samples #81 and #77, respectively (Table 2). This indicated that heat sensitive IN (thereafter termed biological IN) were systematically responsible for freezing at the warmest temperature. As other IN were activated at lower temperature, the relative contribution of biological

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IN decreased with decreasing temperature, from 97 % to 100 % of the total number of IN active at -8°C to at least 77 % at -12°C (Table 2). These are in accordance with observations of IN in the air (Garcia et al., 2012) and in precipitation (Christner et al., 2008a, b).

The average absolute concentrations of biological and non-biological IN are represented on Fig. 4. Since heat treatment does probably not inactivate every IN site of biological material such as fungi or pollen, the concentrations of biological IN reported here should be seen as conservative (i.e. lowest possible) values. Clearly, non-biological (i.e. heat resistant) particles contribution became significant only around -12°C and colder. Principal component analysis (PCA) revealed 2 different groups of IN depending on their temperature of activity, with a net separation between -10°C and -11°C (Fig. S2). This demonstrated differences in the origin of the two sets of IN and so probably in their nature as well. The clear positive correlation existing between $\text{IN}_{T \leq -11^{\circ}\text{C}}$ and soluble inorganic ions concentrations supports their inorganic composition (Fig. S2). $\text{IN}_{T > -11^{\circ}\text{C}}$, i.e. biological IN, were not related to any of the chemical parameters measured, except a negative correlation with the concentration of chloride, which mostly originates from marine environment (Warneck, 1999). This tends to situate the sources of biological IN on the continent, for the puy de Dôme site.

3.3 Estimation of the contribution of bacteria to biological IN

Joly et al. (2013) proposed an estimation of the concentration of INA bacteria in clouds based on laboratory results. It was proposed that between 0 and ~ 500 bacterial cells mL^{-1} could act as IN in cloud water at -10°C . This very wide range needed clarification. In order to discriminate bacterial IN from others biological IN, Christner et al. (2008a, b) suggested treating samples with lysozyme. This was intended to alter bacterial cell wall and selectively eliminate bacterial IN. Lysozyme is indeed responsible for the lysis of peptidoglycans (hydrolysis of the 1,4- β -linkages between N-acetylmuramic acid and N-acetylglucosamine) and thus specifically targets Gram-positive bacteria. So far, all INA bacteria described in literature including those

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encountered in clouds were Gram-negative species (Cochet and Widehem, 2000; Joly et al., 2013) and they are thus expected to be insensitive to lysozyme. This was verified on 2 of our cloud samples and on laboratory cultures of INA Gamma-Proteobacteria isolated from cloud water (those reported in Joly et al., 2013): lysozyme had no effect on the freezing profiles (not shown). So, this treatment was finally judged not relevant here and it was not further applied.

In our samples, bacteria concentration ranged from 1.6×10^3 to 3.4×10^4 mL⁻¹, which is within the range of concentrations typically observed in cloud water at the puy de Dôme site (Vaïtilingom et al., 2012) (Table 1). This was significantly correlated with the proportion of biological IN at -9 °C (Spearman correlation test; $p = 0.043$, $n = 8$), suggesting that either bacteria or some particles associated with the presence of bacteria contributed for a great part to heat-sensitive IN. Hence, in order to provide an estimation of the proportion of INA bacteria in our samples, biological IN concentration was normalized to bacteria concentration (Table 3 and Fig. 5). This has to be considered as an upper estimate as it obviously assumes only one IN site per cell, which is the most likely (Hartmann et al., 2013), and it ignores the fact that a certain but unknown fraction of biological materials other than bacteria could also have been inactivated by heat and contributed to the population of biological IN, such as cell fragments for example (Hartmann et al., 2013). At the temperature of -6 °C, a maximum of 0.1 % of the bacteria could have been responsible for freezing (sample #82). This proportion reached maxima of 1.24 % at -9 °C and 3.06 % at -12 °C (in samples #83 and #85, respectively), or about 200 INA cells mL⁻¹. In the air over vegetated areas, INA bacteria were estimated to contribute only ~ 0.002 % of the total cells (Garcia et al., 2012), and this proportion falls to less than 0.001 % at high altitude (Xia et al., 2013). In snowfall, comparable estimations gave a very similar fraction of 0.4 % of bacterial cells acting as IN between -4 °C and -7 °C (Christner et al., 2008a) (Fig. 1). In laboratory cultures of INA bacteria, the proportion of individual cells actually acting as IN largely depends on the strain. Except in some exceptionally efficient microorganisms for which this can reach up to more than 4 %, this is often around 1 % at -9 °C, and in general well below

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and precipitation, respectively (Christner et al., 2008b). If confirmed, such an over-representation of high-temperature INA cells in cloud water compared to other places in nature would raise the question of the existence of a particular link of ice nucleation active microorganisms with these environments.

Such estimates of in-cloud biological IN concentrations will allow the community of atmospheric scientists to explore, e.g. using cloud-resolving models, the extent to which these particles can contribute to cloud glaciation, to modification of cloud radiative properties and to regional precipitation patterns.

Supplementary material related to this article is available online at

<http://www.atmos-chem-phys-discuss.net/14/3707/2014/acpd-14-3707-2014-supplement.pdf>

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Table 1. Meteorological, physico-chemical and biological parameters of the cloud water samples investigated in this study. The samples that were recovered as ice are indicated in italic. See detailed ion composition in Table SM1.

Cloud sample event #	Date	Temperature (°C)	pH	LWC (gm ⁻³)	Total bacteria (mL ⁻¹)
# 76	29 Jun 2011	11.5	5.88	0.6	n.d.*
# 77	7 Jul 2011	12.0	5.95	0.1	n.d.*
# 78	20 Jul 2011	8.3	5.50	0.3	12 355
# 79	7 Nov 011	7.0	4.57	0.6	10 825
# 80	<i>20 Jan 2012</i>	<i>-0.4</i>	<i>4.90</i>	<i>0.3</i>	<i>9980</i>
# 81	<i>23 Jan 2012</i>	<i>-1.2</i>	<i>5.82</i>	<i>0.1</i>	<i>33 724</i>
# 82	<i>19 Mar 2012</i>	<i>-1.5</i>	<i>5.25</i>	<i>0.1</i>	<i>1648</i>
# 83	<i>4 Apr 2012</i>	<i>-0.4</i>	<i>5.60</i>	<i>0.1</i>	<i>14 914</i>
# 84	<i>18 Apr 2012</i>	<i>0.2</i>	<i>5.47</i>	<i>0.1</i>	<i>3902</i>
# 85	25 Jun 2012	13.3	5.52	0.3	4474
# 86	13 Jun 2012	6.0	5.89	0.6	5199
# 87	10 Oct 2012	9.4	6.22	0.6	19 658

* n.d.: not determined.

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Table 2. Concentration of total IN and proportion of heat-sensitive IN in the cloud water samples between -5°C and -14°C . Values below the detection limit are presented as “0” for visual clarity, and a “>” indicates values higher than our detection limit.

Sample	IN mL ⁻¹ [total (% heat sensitive)]									
	Temperature (°C)									
	-5°C	-6°C	-7°C	-8°C	-9°C	-10°C	-11°C	-12°C	-13°C	-14°C
# 76	0 (- %)	0 (- %)	0 (- %)	4.92 (100 %)	18.73 (100 %)	31.63 (100 %)	45.04 (100 %)	118.36 (99 %)	n.d.*	n.d.*
# 77	0 (- %)	0 (- %)	0 (- %)	4.92 (100 %)	12.34 (100 %)	14.38 (100 %)	92.81 (100 %)	138.63 (100 %)	n.d.*	n.d.*
# 78	0 (- %)	0 (- %)	0 (- %)	1.59 (100 %)	8.49 (100 %)	16.51 (100 %)	26.06 (94 %)	69.31 (88 %)	n.d.*	n.d.*
# 79	0 (- %)	0 (- %)	0 (- %)	3.23 (100 %)	8.49 (100 %)	12.34 (100 %)	16.51 (100 %)	16.51 (100 %)	n.d.*	n.d.*
# 80	0 (- %)	0 (- %)	0 (- %)	4.92 (100 %)	12.34 (100 %)	16.51 (100 %)	18.73 (92 %)	53.39 (91 %)	n.d.*	n.d.*
# 81	0 (- %)	0 (- %)	8.49 (100 %)	63.43 (97 %)	> 173.3 (< 99 %)	> 173.3 (< 97 %)	> 173.3 (< 95 %)	> 173.3 (< 92 %)	n.d.*	n.d.*
# 82	0 (- %)	1.59 (100 %)	6.68 (100 %)	10.38 (100 %)	18.73 (74 %)	21.06 (66 %)	28.77 (70 %)	53.39 (77 %)	n.d.*	n.d.*
# 83	0 (- %)	0.63 (100 %)	7.40 (100 %)	73.21 (100 %)	184.44 (100 %)	217.51 (99.3 %)	> 253.8 (< 97 %)	> 253.8 (< 93 %)	n.d.*	n.d.*
# 84	0 (- %)	1.59 (100 %)	2.40 (100 %)	11.35 (100 %)	16.51 (90 %)	18.73 (92 %)	30.18 (95 %)	41.33 (88 %)	110.65 (66 %)	> 207.9 (< 55 %)
# 85	0 (- %)	0 (- %)	0 (- %)	2.24 (100 %)	3.23 (100 %)	5.27 (100 %)	34.66 (100 %)	138.63 (99 %)	> 253.8 (< 91 %)	> 253.8 (< 45 %)
# 86	0 (- %)	0 (- %)	1.59 (100 %)	3.23 (100 %)	8.49 (100 %)	14.38 (89 %)	14.38 (89 %)	18.73 (83 %)	49.04 (93 %)	> 173.3 (< 56 %)
# 87	0 (- %)	0 (- %)	0 (- %)	0 (- %)	0 (- %)	0 (- %)	3.23 (100 %)	28.77 (100 %)	83.70 (72 %)	> 173.3 (< 46 %)
Min	0 (- %)	0 (- %)	0 (- %)	0 (- %)	0 (- %)	0 (- %)	3.23 (100 %)	18.10 (91 %)	49.04 (93 %)	> 173.3 (< 46 %)
Max	0 (- %)	1.59 (100 %)	8.49 (100 %)	73.21 (100 %)	184.44 (100 %)	217.51 (99.3 %)	> 253.8 (< 97 %)	> 253.8 (< 93 %)	> 253.8 (< 91 %)	> 253.8 (< 45 %)

* n.d.: not determined.

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Table 3. Inferred upper proportion of INA bacteria in the cloud water samples based on the concentration of heat-sensitive IN and on total bacteria counts. A “>” indicate values higher than our detection limit for heat-sensitive IN.

Sample	IN/bacterial cell Temperature (°C)									
	-6°C	-7°C	-8°C	-9°C	-10°C	-11°C	-12°C	-13°C	-14°C	
# 78	0.00 %	0.00 %	0.01 %	0.07 %	0.13 %	0.20 %	0.49 %	> 0.49 %	> 0.49 %	
# 79	0.00 %	0.00 %	0.03 %	0.08 %	0.11 %	0.15 %	0.15 %	> 0.15 %	> 0.15 %	
# 80	0.00 %	0.00 %	0.05 %	0.12 %	0.17 %	0.17 %	0.49 %	> 0.49 %	> 0.49 %	
# 81	0.00 %	0.03 %	0.18 %	> 0.51 %	> 0.51 %	> 0.51 %	> 0.51 %	> 0.51 %	> 0.51 %	
# 82	0.10 %	0.41 %	0.63 %	0.84 %	0.84 %	1.23 %	2.49 %	> 2.49 %	> 2.49 %	
# 83	0.00 %	0.05 %	0.49 %	1.24 %	1.46 %	> 1.66 %	> 1.66 %	> 1.66 %	> 1.66 %	
# 84	0.04 %	0.06 %	0.29 %	0.38 %	0.44 %	0.73 %	0.93 %	1.86 %	> 2.95 %	
# 85	0.00 %	0.00 %	0.05 %	0.07 %	0.12 %	0.77 %	3.06 %	> 5.15 %	> 5.15 %	
# 86	0.00 %	0.03 %	0.06 %	0.16 %	0.25 %	0.25 %	0.30 %	0.88 %	> 1.87 %	
# 87	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.02 %	0.15 %	0.31 %	> 0.41 %	
Mean	0.01 %	0.06 %	0.18 %	> 0.35 %	> 0.40 %	> 0.57 %	> 1.02 %	> 1.40 %	> 1.62 %	

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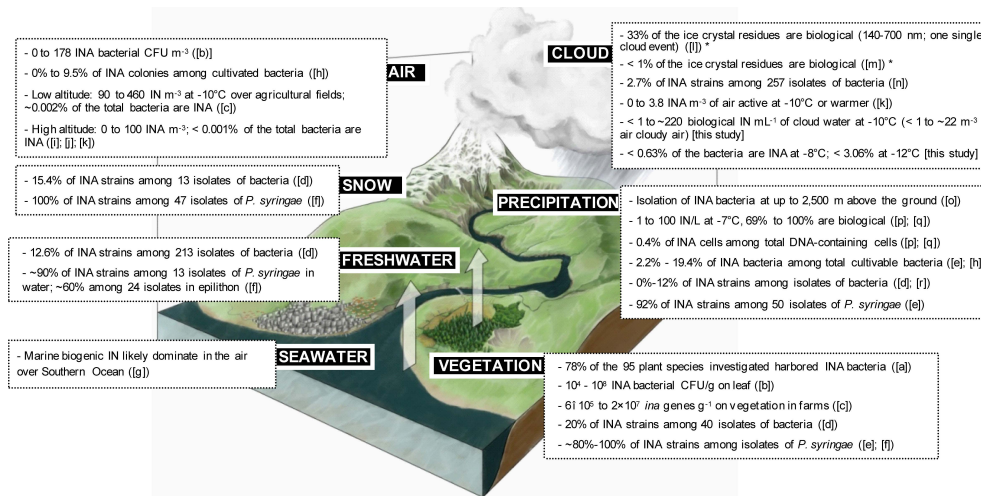


Fig. 1. Schematic summarizing our current knowledge about the abundance of biological IN active at temperatures $\geq -10^\circ\text{C}$ in the different environmental links of the water cycle. A “*” indicates data relative to ice crystal residues in clouds at much colder temperatures. [a] Lindow et al. (1978); [b] Lindemann et al. (1982); [c] Garcia et al. (2012); [d] Maki and Willoughby (1978); [e] Constantinidou et al. (1990); [f] Morris et al. (2008); [g] Burrows et al. (2013); [h] Stephanie and Waturangi (2011); [i] Bowers et al. (2009); [j] Conen et al. (2012); [k] Xia et al. (2013); [l] Pratt et al. (2009); [m] Cziczo et al. (2013); [n] Joly et al. (2013); [o] Sands et al. (1982); [p] Christner et al. (2008a); [q] Christner et al. (2008b); [r] Šantl-Temkiv et al. (2009).

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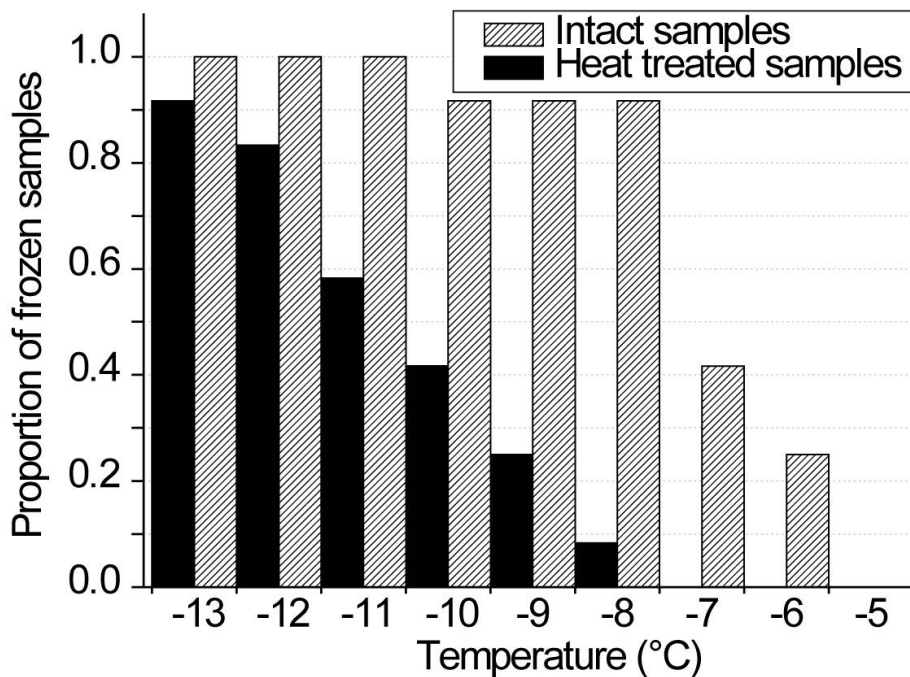


Fig. 2. Cumulative proportion of maximum freezing temperature of the 12 cloud water samples, in the absence of treatment (shaded bars) or after heating at 95 °C for 10 min (black bars).

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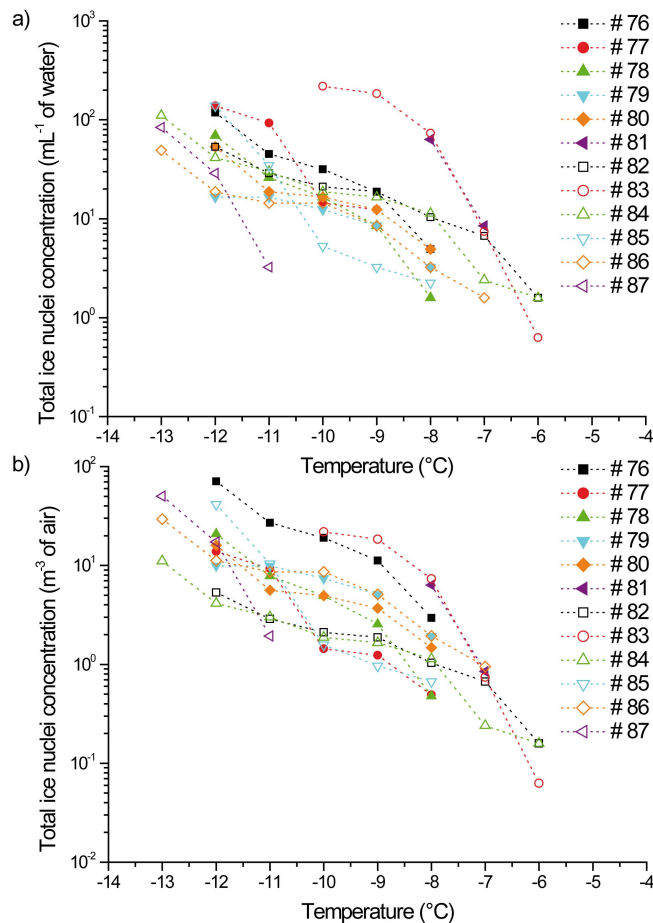


Fig. 3. Cumulative concentration of total IN in the cloud samples, **(a)** per volume of water sample (mL⁻¹) and **(b)** per corresponding volume of cloudy air (m⁻³).

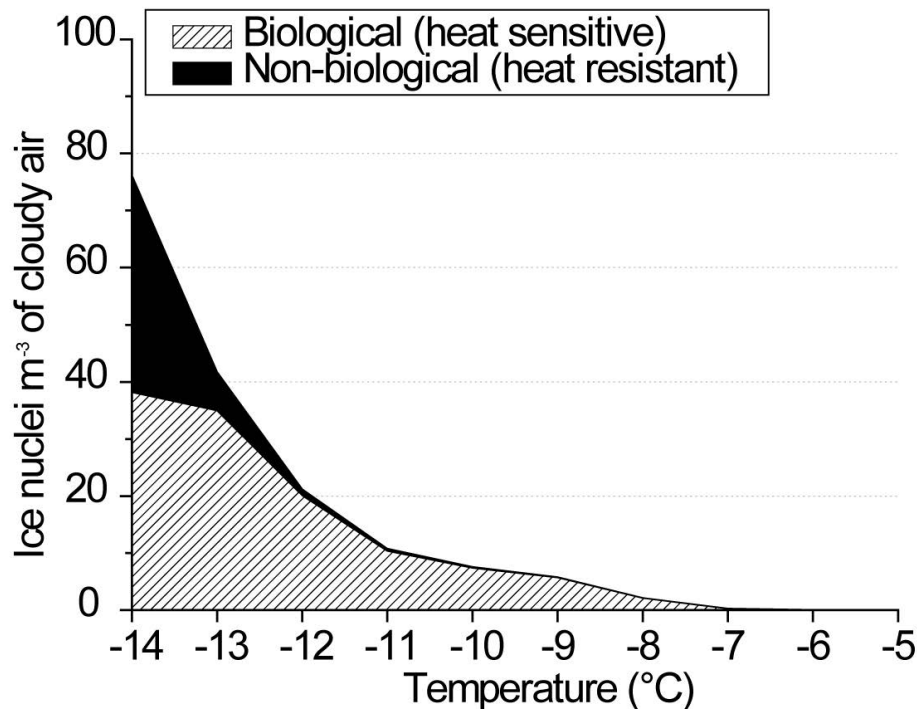


Fig. 4. Mean cumulative concentrations of biological (heat-sensitive, shaded area) and non-biological (heat-resistant, black area) IN in clouds ($n = 12$) per volume of air. The sum of the two categories corresponds to the mean concentration of total IN. The lower bound was considered for values below the detection limit.

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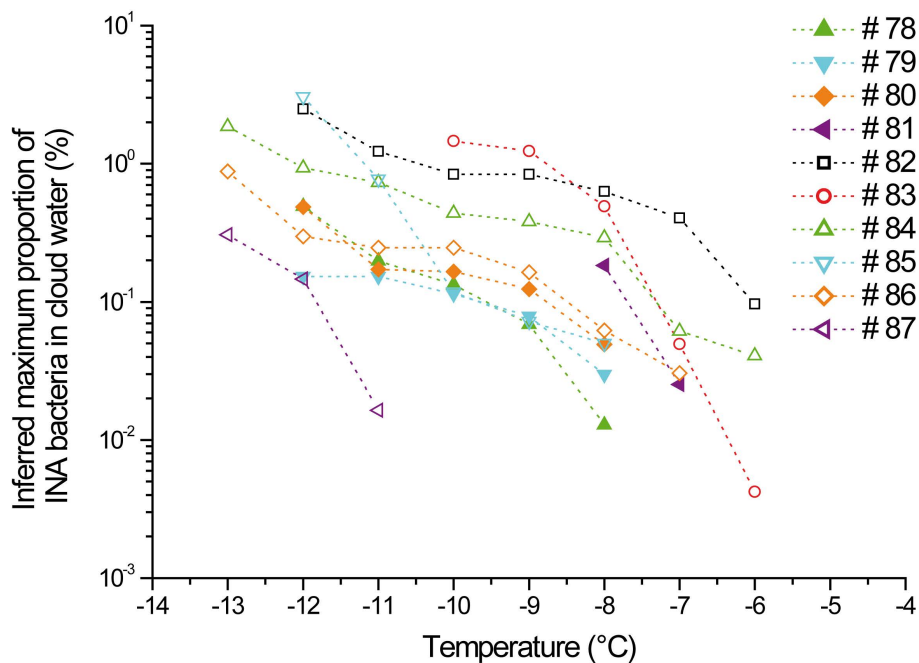


Fig. 5. Inferred upper proportion of INA bacteria in the cloud water samples based on the concentration of heat-sensitive IN and on microscopy counts.

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