We thank the Referees for their constructive suggestions, which were of great help to improve the quality of the work and to present the results in a clearer way. Here is a point-by-point reply to their proposed corrections. We merged the replies into a unique file to improve its clarity and the level of detail of the answers.

Referees’ comments are in italic font, authors’ responses in blue normal font, changes in the manuscript are in blue underlined and refer to the version of the document published on BGD. To facilitate the discussion, we have numbered the comments.

Referee #1

This manuscript presents the analysis of 56 fresh fallen snow sampled at Jungfraujoch for which concentrations of Ice Nucleating Particles (INPs) and bacteria have been determined and the fraction of water vapour lost by the air mass prior the sampling has been assessed by oxygen isotopic ratio. Based on the difference of dynamic ranges between INPs and bacteria concentrations, authors propose that INPs are more rapidly deposited from the atmosphere than bacteria. They confirmed it by a faster decrease of INPs than bacterial cells when air masses are submitted to larger losses of water vapour. A second part of the manuscript emphasizes the wind as a determining factor in the concentration of INPs. Finally, the authors isolated Pseudomonas syringae from the snow and compared them to strains isolated from a large panel of environments.

General comments: The manuscript is clear, well-written, and in perfect adequacy with the scope of the journal. It brings arguments to elucidate what happens in clouds, and more generally in the atmosphere, where a lot of processes concerning microorganisms are supposed but not clearly demonstrated. Still, I have some concerns/questions about different parts of the manuscript:

(1) I regret the structure of this paper in two parts: the first concerning INPs and bacteria persistence in the atmosphere and the second part concerning P. syringae and its belonging to specific phylogenetic groups. These two parts although comprehensible in atmospheric microbiology form a disconnected content.

To improve the level of connection of the contents of the manuscript, we made some adjustments in the abstract at page 1 line 20, which now reads as: “A prominent ice nucleating bacterium, *Pseudomonas syringae*, has been previously supposed to benefit from this behaviour as a means to spread via the atmosphere and to colonise new host plants. Therefore, we targeted this bacterium with a selective cultivation approach. *P. syringae* was successfully isolated for the first time at such an altitude in 3 of 13 samples analysed”. Paragraph 3.3 at page 5 line 22 was renamed in “Abundance and diversity of the prominent ice nucleating bacterium *P. syringae*”. Finally, according to the comment 4 Referee #1 and the Editor’s comment, the title of the manuscript has been changed into: “Ice nucleators, bacterial cells and *Pseudomonas syringae* in precipitation at Jungfraujoch”.

(2) In the first Results section, authors determine “dynamic ranges” to evaluate atmospheric residence times and support this citing “Fig 2 in Griffiths et al., 2014”. First, I do not believe this citation is relevant in this context.

We removed this sentence and the reference.

Second, I wonder how efficient this index would be for example in the case of a particle with a short residence time and important emission sources all around the sampling site?

In this case, the number concentrations of this type of particle would highly vary with the changing strength of the drivers of its emission (wind speed, radiation, surface wetness, etc.). At the same time, its background from upwind the source would be relatively small (short residence time, large local source). Consequently, we would also expect to see a large dynamic range in the concentrations of this particle type.

Nevertheless, at Jungfraujoch, we are about 1000 m above the nearest alpine meadows which could provide INPs. Furthermore, we do not expect the difference between alpine meadows and other
sources of bacteria and INPs, like lower lying meadows, forests and crops to be so large that this difference would be an alternative explanation for our observations.

In case glaciers are addressed as local sources of INPs, we have indications to exclude their influence in our measurements. We collected precipitation horizontally on the terrace of the observatory to maximize the recovery of falling snowflakes instead of particles floating. Lloyd et al., 2015 indicate that snow blown from the local sources around the observatory can increase the numbers of airborne ice particles (IPs) specifically under low wind speed. In our case, though, we measure INPs by immersion freezing and not IPs or secondary IPs, moreover we observe the opposite behavior with more INPs in association with high wind speed. Furthermore, if INPs are picked up from the surface of the glacier, these should have been freshly deposited on the snow surfaces around the station by the same air masses originating precipitation above the station, which makes them not much different from the one we collect on the terrace of the observatory.

(3) **Authors measured total bacteria concentration and INPs**. Is there a statistical correlation between these two parameters?

The two parameters are correlated, with Spearman’s correlation coefficient $r_S = 0.45$ and a statistical significance $< 0.001$. This justifies their similar trend, therefore at page 4 line 10 the sentence has been changed into: “Concentrations of INPs active at -8 °C or warmer (INPs$_{-8}$) (Fig. 1, data taken from Stopelli et al., 2016) presented a similar trend (Spearman’s correlation coefficient $r_S = 0.45$, $p < 0.001$) but with an order of magnitude larger dynamic range...”

At a closer look, though, this correlation is principally due to just a small set of samples where the total number of bacteria is larger than 1000 mL$^{-1}$. Excluding those data, no correlation is present anymore. Therefore, we prefer to consider such correlation with care and to focus in this manuscript on the reasons why the numbers of bacteria do not vary as largely as INPs$_{-8}$ do.

(4) **Title should clearly expose authors were interested in INPs active for temperatures warmer than -8°C (and as much as possible an insight of the work on P. syringae).**

The title was changed into: “Ice nucleators, bacterial cells and *Pseudomonas syringae* in precipitation at Jungfraujoch”. Considering the Editor’s comment, we decided not to provide too much level of detail in the title, also because the reference to INPs active at temperatures warmer than -8 °C is immediately visible in the first lines of the abstract. The title has been changed accordingly also in the Supplement.

**Other comments:**

(5) **P1 Line 22: It sounds like it is the first time P. syringae is found in snow: it is the first time at such an altitude, please precise it.**

This part of the abstract now reads as: “*P. syringae* was successfully isolated for the first time at such an altitude in 3 of 13 samples analysed”.

(6) **P1 Line 27: Hemsfield (missing “i”)**

Corrected

(7) **P1 Line 30: Modify this sentence: Sands et al. (1982) did not demonstrate that P. syringae actually act as an INP in clouds**

In that study, *P. syringae* was isolated from clouds and the ice nucleation activity of the strains tested under laboratory conditions. Therefore, the sentence at page 1 line 30 has been changed and now reads
as: “*Pseudomonas syringae* was the first organism found to produce an ice nucleation active molecule (Maki et al., 1974) and to occur in clouds as potential biological INP (Sands et al., 1982)”.

(8) P2 Line 23: why did you adjust samples to physiological conditions? What was the range of temperatures tested?

The addition of small amounts of salts improves the detection of freezing events using our device LINDA. Furthermore, the final concentration 9 ‰ of NaCl should avoid osmotic stress to living cells, preventing breaking of cells and potentially multiplication of INPs of biological origin in samples. As discussed in Stopelli et al., 2014, the addition of this amount of salt does not depress the temperature of freezing of a sample. We analysed the temperatures between -2 and -12 °C. Considering these observations, and comment 5 Referee #2, the title of the section 2.1 now reads as: “2.1 Sampling and counting of INPs and bacteria”; while the paragraph has been modified into: “Snow samples were melted at room temperature (about 16 °C) and adjusted to physiological conditions (9 ‰ NaCl) to prevent osmotic stress on cells and improve the detection of freezing events. They were analysed immediately on site for concentration of INPs active between -2 and -12 °C in a drop freeze assay with the LINDA device loaded with 52 Eppendorf Safelock tubes containing 100 µL of sample each, as described in Stopelli et al. (2014). For each sample, two filters were prepared for later analysis of bacterial number concentration.”

(9) P2 Line 25: Are you sure the filter active area is 6 mm diameter?

Yes, we mounted an inlay of 6 mm diameter on the glass funnels used to filter the sample, in order to concentrate cells on a smaller active area, thus improving the quality of cell counts per unit area of filter. This detail has been inserted in the sentence, which now reads as: “Twenty mL of sample were passed through the active area (glass vacuum filter funnels were equipped with an inlay to reduce the whole area of the filter into an active area of 6 mm diameter, to improve the possibility of counting enough cells per unit area of filter) of two 0.22 µm black polycarbonate membranes (Whatman)”.

(10) P3 Line 10: How long were the plates incubated?

We changed the sentence into: “Plates were incubated at 20–25 °C for 3 days.”

(11) P3 Line 37: I would appreciate a brief description of this method.

Page 3 line 37 has been enriched by a brief description of this method and now reads as: “Particles tend to be removed from the atmosphere by precipitation, specifically INPs (Stopelli et al., 2015). According to this, it is relevant to know the fraction of water vapour lost as precipitation from an air mass prior to sampling, which is calculated from the relative abundance of $^{18}$O and $^{16}$O in precipitation, expressed as $δ^{18}$O. Water molecules containing the stable isotope $^{18}$O have a greater propensity to condense, hence to precipitate, than those containing the more abundant stable isotope $^{16}$O. Therefore, the relative abundance $^{18}$O/$^{16}$O in an air mass decreases during precipitation. The fraction of water vapour lost can be easily calculated comparing the isotopic composition of the water vapour in an air mass at the moment of its formation, assuming marine origin, with the composition at the moment of sampling, according to Rayleigh’s fractionation model (IAEA, 2001). More details on this calculation are provided in Stopelli et al., 2015”. Coherently, the reference to IAEA, 2001 has been added to the reference list.

(12) P4 Line 5: Is there a scientific reason you excluded the winter period for sampling?

Winter months have been sampled for the abundance of INPs between the years 2012 and 2013 and the results are fully reported in Stopelli et al., 2016. Measurements of bacterial cells have been introduced from March 2013 on and in this paper we report only samples on which both measurements have been carried out. For the second year of observations (2014), we preferred to focus on the period
stretching from Spring to Autumn since we were expecting to sufficiently cover both small and large numbers of INPs and bacterial cells, whereas in winter numbers were suspected to be only very small as Jungfraujoch is surrounded by free tropospheric air for most of the time. Sampling revealed large variability of INPs not only across a year but also within sampling campaigns and in summer months. For a detailed discussion on the role of environmental parameters on the abundance of INPs please refer to Stopelli et al., 2016. Concerning bacterial cells, such large variability in the abundance does not occur also for Summer months and we examine possible causes of this different behaviour in this manuscript.

(13) P4 Line 20: This sentence is ambiguous, please reformulate.
We merged the answer to this comment with the one to comment 2 Referee #2.

(14) P4 Line 10: I would remove “seasonal” as you sampled only 7 months of the year. Furthermore, bacterial concentration in May 2014 is higher or equivalent to those in July/August.
“Seasonal” was removed from the sentence.

(15) P5 Line 16: Replace “is” with “in”
This sentence now reads as: “…increase more substantially above background values at high wind speed, than in the case of bacterial cells”.

References mentioned in the reply


Referee #2

General comments

This study represents the results of INP measurements and bacteria cell counting of 56 snow samples collected at Jungfraujoch over an 18-month sampling period. They found a larger dynamic range of INP active at -8°C compared to the number of bacterial cells and a high fraction of living bacteria cells. Correlations with water vapor loss prior sampling show that INP have shorter atmospheric residence times than bacterial cells, in particular at low wind speeds. Furthermore, 24 strains of Pseudomonas syringae were isolated from selected samples and phylogenetically characterized. The manuscript is well written and the results are clear and well presented.

Specific comments:

(1) I suggest renaming the section 3 from “results” to “results and discussion” as there is no separate discussion section.

Done

(2) When discussing the higher concentration dynamics of the INP compared to bacteria the authors mention changing source and sink strength of INP and bring a one short statement about “other particles carrying a biological INP” on P4 Line20. This discussion seems very general and should be extended. Other possible biological INP sources (at least fungi) should be specifically mentioned and more references should be cited. This would also help to explain the INP-8 concentrations at JFJ as P. syringae was found only in three out of 13 tested samples. Given the high diversity of P. syringae strains as found in those samples and an average of 60% of total living bacteria cells in the samples one could actually expect to find P. syringae in more samples. Preferential removal of P. syringae with precipitation and loss of culturability during atmospheric transport as stated in the MS seems possible, but possible sources of non-P. syringae INP active at -8°C should be discussed in more detail.

Also in the light of comment 13 Referee #1, we changed the paragraph involving page 4 line 20, which now read as: “Hence, the relatively small DR of bacterial cells compared to that of INPs at -8°C suggests that the majority of bacterial cells has a longer residence time in the atmosphere than INPs at -8°C. The shorter residence time of INPs at -8°C is likely to be due to their capacity to catalyse ice formation and growth at -8°C, leading to their rapid deposition with the growing crystal. Such INPs at -8°C include not only bacterial cells, but also fungal spores, pollen, parts thereof, and soil particles associated with biological ice nucleation active fragments (Conen et al., 2011; Fröhlich-Nowoisky et al., 2015; Hill et al., 2016; Joly et al., 2013; Morris et al., 2013b; O'Sullivan et al., 2015, Pummer et al., 2015).”

Coherently, newly cited articles were added to the reference list, specifically: Conen et al., 2011; Fröhlich-Nowoisky et al., 2015; Hill et al., 2016 and Morris et al., 2013b.

(3) Some more information about the sampling conditions would be helpful as the station at JFJ is not always within clouds. Did the authors only collect precipitation/snow when the station was within clouds? And if not, did they found differences in the number of INP and bacterial cells in samples collected in a cloud compared to samples collected below a cloud? Why was there no sampling in winter?

The Station was always inside precipitating clouds while collecting snow. At page 2 line 19 this sentence has been added: “The Station was always inside precipitating clouds while collecting snow”. Concerning sampling in winter, please refer to our reply to comment 12 Referee #1.

Other comments/typos:

(4) P2 Line 23: Although the math is correct here, I think it is better to consistently use either 0.9 % (as on P3 Line 18) or 9 ‰ NaCl.

Changed into 9 ‰ for consistency, also in the Supplement
(5) P2 Line 23: Please provide some basic information here. How many droplets did you measure? What was the volume of the droplets? What kind of controls were measured?

Also according to comment 8 Referee #1, this paragraph has been changed into: “Snow samples were melted at room temperature (about 16 °C) and adjusted to physiological conditions (9 ‰ NaCl) to prevent osmotic stress on cells and improve the detection of freezing events. They were analysed immediately on site for concentration of INPs active between -2 and -12 °C in a drop freeze assay with the LINDA device loaded with 52 Eppendorf Safelock tubes containing 100 µL of sample each, as described in Stoplelli et al. (2014). For each sample, two filters were prepared for later analysis of bacterial number concentration”. The reference to controls has been introduced at page 2 line 32: “Blanks for the determination of INPs and bacterial cells were periodically prepared using the Milli-Q water used to rinse the tin as control sample.”

(6) Table 1 caption: I suggest to change the beginning to “Diversity of Pseudomonas syringae strains”

Done

References mentioned in the reply


Additional insertions from the authors of the manuscript

- *Cts* gene sequences have been in the meanwhile deposited in the online repository GenBank. Therefore we added at the end of section 2.2 the sentence: “Reported sequences are deposited in the GenBank Archive under the accession numbers GenBank KY379248-KY379271.” As a consequence, we remove the provisory Excel table reporting such sequences which was previously sent a supplementary material to the article.
- “Colony forming units” changed into “colony-forming units” through the text
- Page 1 line 13 sentence in brackets changed into: “(of which 60 % was on average alive)”
- Page 1 line 17 changed “perhaps” into “likely”
- Page 1 line 23 changed “plants” into “habitats”, now reads as: “and its propensity to spread to colonise new habitats”
- Page 1 line 32 we removed “the spread of microbial”
- Page 2 line 2, added “a”, now reads as: “corresponding to a cold temperature regime and supersaturation”
- Page 2 line 28 substituted “live” with “alive”
- Page 2 line 31 added “the” to “in the dark”
Page 3 line 2 substituted “to study” with “to assess”
Page 3 line 7 substituted “successive” with “subsequent”
Page 3 line 11 added “of KBC”, now reads as: “without the antibiotics and boric acid of KBC”
Page 3 line 14 added “Research”, now reads as: “Plant Pathology Research Unit”, and removed “the” from “for molecular identification”
Page 3 line 22 modified “tobacum” into “tabacum”
Page 3 line 30 modified the beginning of the sentence into “Analysis of partial…”
Page 3 line 38 modified into “and data were stored”
Title of Paragraph 3.1 now reads as: “Variability of the concentrations of bacterial cells and INPs”
Page 4 line 23 added “a” to the sentence “with a small standard deviation”
Page 5 line 3 sentence modified into: “This opens the question whether we can find evidence also for the replenishment of their atmospheric concentrations at shorter time scales.”
Page 5 lines 12/13 modified “is” into “would be” and removed “it is”, sentence now reads as: “Therefore, their background number concentration would be relatively large and stable and not changed much by the additional cells aerosolised at high wind speeds in the region.”
Page 6 line 23/24 beginning of the sentence modified into: “The decrease of INPs relative to bacterial cells with precipitation…”
Page 6 line 28 added “a” to “P. syringae appeared as a minor component”
Page 6 line 31 added “… and new habitats.”
Figure 1 and 2: units in the y-axes have been inserted in brackets
Page 7 lines 5 and 6 now read as: “Caroline Guilbaud provided fundamental support in the isolation of P. syringae and did the phylogenetic analysis on the isolated strains.”
Page 7 line 13 modified into: “Urs and Maria Otz, as well as Joan and Martin Fisher are acknowledged for their support on site.”
Page 13 lines 4/5: substituted “where” with “with” and “for” with “with”, this sentence now reads as: “Red symbols correspond to values with average wind speeds > 50 km h⁻¹ during samplings and blue symbols correspond to data with lower wind speeds.”
In the Supplement changed “Supplement of” into “Supplement to”
In Supplemental Table 1, column Pseudomonas syringae, substituted “under 2” with “≤ 2”
Ice nucleators, bacterial cells and Pseudomonas syringae in precipitation at Jungfraujoch.

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Abstract Ice nucleation is a means by which the deposition of an airborne microorganism can be accelerated under favourable meteorological conditions. Analysis of 56 snow samples collected at the high altitude observatory Jungfraujoch (3580 m a.s.l.) revealed an order of magnitude larger dynamic range of ice nucleating particles active at -8 °C (INPs -8°C) compared to the total number of bacterial cells (of which 60 % was on average alive). This indicates a shorter atmospheric residence time for INPs -8°C. Furthermore, concentrations of INPs -8°C decreased much faster, with an increasing fraction of water precipitated from the air mass prior to sampling, than the number of total bacterial cells. Nevertheless, at high wind speeds (> 50 km h⁻¹) the ratio of INPs -8°C to total bacterial cells largely remained in a range between 10⁻² to 10⁻³, independent of prior precipitation, likely because of recent injections of particles in regions upwind. Based on our field observations, we conclude that ice nucleators travel shorter legs of distance with the atmospheric water cycle than the majority of bacterial cells. A prominent ice nucleating bacterium, Pseudomonas syringae, has been previously supposed to benefit from this behaviour as a means to spread via the atmosphere and to colonise new host plants. Therefore, we targeted this bacterium with a selective cultivation approach. P. syringae was successfully isolated for the first time at such an altitude in 3 of 13 samples analysed. Colony-forming units of this species constituted a minor fraction (10⁻⁴) of the numbers of INPs -8°C in these samples. Overall, our findings expand the geographic range of habitats where this bacterium has been found and corroborates theories on its robustness in the atmosphere and its propensity to spread to colonise new habitats.

1 Introduction

The nucleation of ice in clouds is a process of primary relevance both for the radiative budget of clouds and for the development of precipitation (Cantrell and Heymsfield, 2005; Mülmenstädt et al., 2015; Murray et al., 2012). Most ice nucleating particles (INPs) active at moderate supercooling in the atmosphere are of biological origin (Murray et al., 2012). Pseudomonas syringae was the first organism found to produce an ice nucleation active molecule (Maki et al., 1974) and to occur in clouds as potential biological INP (Sands et al., 1982). As it is also a plant pathogen it received and continues to receive attention from biologists in the perspective of improving the protection of crops from diseases (Lamichhane et al., 2014; Lamichhane et al., 2015). The combination of both roles, of an ice nucleator and of a plant epiphyte and pathogen, sparked the bioprecipitation hypothesis (Morris et al., 2014; Sands et al., 1982). Part of the hypothesis is the idea that ice nucleation activity contributes preferentially to the deposition of the organisms with this property helping them to return to plant surfaces where
they can proliferate (Morris et al., 2013a). In fact, favouring the growth of ice crystals could be a powerful and
effective means for airborne microorganisms to reduce their residence time aloft. Previous research has
illustrated that bacterial strains capable of nucleating ice can deposit rapidly under laboratory simulated cloud
conditions, corresponding to a cold temperature regime and supersaturation (Amato et al., 2015). Modelling
studies suggest that the atmospheric residence time of singularly airborne bacterial cells is highly reduced if such
bacteria act as condensation and ice nuclei in clouds (Burrows et al., 2009). Furthermore, snowfall has been
shown to enrich for the presence of ice nucleation active strains of *P. syringae* compared to a range of other
environmental contexts including within clouds (Morris et al., 2008; Morris et al., 2013a). Therefore, more direct
evidence from field observations on the selective deposition of ice nucleation active microorganisms with
precipitation is wanting.

Here we explore, through analysis of snow samples collected at a high altitude station, whether the capability to
induce the formation and growth of ice crystals makes a discernible difference to the atmospheric residence time
of INPs compared to the majority of bacterial cells, which are not INPs. We have sought to isolate the prominent
ice nucleation active bacterium *P. syringae* at high altitude and compare its abundance with numbers of INPs.
We have also carried out phylogenetic analysis in an attempt to identify potential sources of this bacterium in the
atmosphere.

2 Materials and methods

2.1 Sampling and counting of INPs and bacteria

Fifty six snow samples were collected at the High Altitude Research Station Jungfraujoch (7°59′06″ E,
46°32′51″ N, 3580 m a.s.l., Switzerland) during 11 short sampling campaigns from March to September 2013
and May to October 2014. The Station was always inside precipitating clouds while collecting snow. Samples
were collected with a 0.1 m² Teflon-coated tin for periods of 1.5 to 8 hours depending on precipitation intensity.
The tin was rinsed several times with 70 % ethanol and sterile Milli-Q water between samples to avoid cross-
contamination. Snow samples were melted at room temperature (about 16 °C) and adjusted to physiological
conditions (9 ‰ NaCl) to prevent osmotic stress on cells and improve the detection of freezing events. They
were analysed immediately on site for concentration of INPs active between -2 and -12 °C in a drop freeze assay
with the LINDA device loaded with 52 Eppendorf Safelock tubes containing 100 µL of sample each, as
described in Stopelli et al. (2014). For each sample, two filters were prepared for later analysis of bacterial
number concentration. Twenty mL of sample were passed through the active area (glass vacuum filter funnels
were equipped with an inlay to reduce the whole area of the filter into an active area of 6 mm diameter, to
improve the possibility of counting enough cells per unit area of filter) of two 0.22 µm black polycarbonate
membranes (Whatman). The filters were rinsed with 3 mL of sterile phosphate-buffered saline (PBS) and the
staining agents were added: 10 µL SYBR green (100x) for total cell count, and 10 µL SYBR green and 10 µL
propidium iodide (in Milli-Q water, 1% wt/wt) to facilitate counting cells that are alive (i.e with intact
membranes) and dead or dying (with permeable membranes). After incubation in the dark for 10 min, the stains
were filtered away and the filter columns were rinsed with 3 mL of sterile water. Once dry, the filters were put on
glass slides, 15 µL of antifading agent (5 mL PBS, 5 mL glycerol, 10 mg p-phenyldiamine) was added for
preservation in the dark at -20 °C until analysis. Blanks for the determination of INPs and bacterial cells were
periodically prepared using the Milli-Q water used to rinse the tin as control sample. Blank counts were generally less than 10% of sample counts. Filters were analysed with a fluorescence microscope (Leica DM2500) with a 100x ocular lens and an objective with 10x10 10 µm grids. Bacterial cells were recognised by shape and size. Each time, at least 10 fields and 300 cells were counted. In filters stained solely with SYBR green all cells were visible under blue light, while in filters treated with SYBR green and propidium iodide only living cells were counted.

2.2 Selective isolation and phylogenetic characterization of Pseudomonas syringae

Enough precipitation volume was available in 13 samples in a total of 15 samples collected in 2014 to assess the presence of culturable P. syringae. The method employed for the selective isolation of P. syringae and its metabolic characterisation is described in Monteil et al. (2014) and in Morris et al. (2008). In addition to the above described procedure, the snow collecting tin was sterilised by dry heat (150 °C) for 20 min. Samples were concentrated 500 times by filtration of about 1 litre of melted snow across sterile 0.22 µm pore size polycarbonate filters and subsequent resuspension of the particles by stirring the surface of the filter into 2 mL of filtered precipitation water. The concentrated samples were dilution-plated on KBC, King’s medium B supplemented with boric acid, cephalixin and cycloheximide, to isolate and calculate the abundance of P. syringae. Plates were incubated at 20–25 °C for 3 days. Putative strains of P. syringae were purified on KB media (without the antibiotics and boric acid of KBC) where their production of fluorescent pigment could be observed under UV light (312 nm) and were then tested for the absence of arginine dihydrolase and for the absence of cytochrome C oxidase. Those that were negative for both the oxidase and arginine tests were suspended in sterile phosphate buffer and sent to the Plant Pathology Research Unit, INRA, Montfavet, France for molecular identification using PCR primers specific for P. syringae: Psy-F: 5’- ATGATCGGAGCGGACAAG-3’; Psy-R: 5’-GCTCTTGAGGCAAGCACT-3’ (Guilbaud et al., 2016). The strains confirmed to be P. syringae were also tested for their ice nucleating activity. After 3 days of growth on KB, suspensions of pure colonies corresponding to 10⁸ cells mL⁻¹ in 9‰ NaCl were incubated 1 h in melting ice and subsequently tested for ice nucleation activity between 0 and -8 °C, with the freezing apparatus LINDA at 200 µL per tube (about 2-10⁷ cells in each tube). The capacity of strains of P. syringae to induce a hypersensitive response (HR), indicative of the presence of a functional type III secretion system used in pathogenicity by the strains, was determined on tobacco plants (Nicotiana tabacum) by infiltrating bacterial suspensions of 48 h cultures at approximately 10⁶ cells mL⁻¹ into the leaves of the plant. After 24-48 h of incubation HR was revealed by the appearance of necrotic lesions at the site of infiltration.

Neighbour-joining phylogenetic trees of the strains of P. syringae isolated from Jungfraujoch were constructed on the basis of partial sequences of the citrate synthase housekeeping gene (cts) as previously described (Berge et al., 2014). Primers Cts-FP (forward): 5’-AGTTGATCATCGAGGGCGC(AT)GCC-3’ and Cts-RP (reverse): 5’-TGATCGGTTTGATCTCGCACGG-3’ were used for amplification and primer Cts-FS (forward): 5’-CCGTCGAGCTGCAAT(CT)TTGCTGA-3’ for sequencing (Morris et al., 2010; Sarkar and Guttmman, 2004).

Analysis of partial cts gene sequences was performed as described previously using P. syringae reference strains (Berge et al., 2014), atmospheric strains (Amato et al., 2007; Joly et al., 2013; Vaïtilingom et al., 2012) and the 24 strains from this study that were positive with the PCR specific for P. syringae. Alignment of sequences was made with DAMBE (version 5.6.8) and a Neighbour Joining tree was built with MEGA (version 5.05; Tamura et
2.3 Environmental parameters and statistics

Particles tend to be removed from the atmosphere by precipitation, specifically INPs (Stopelli et al., 2015). According to this, it is relevant to know the fraction of water vapour lost as precipitation from an air mass prior to sampling, which is calculated from the relative abundance of $^{18}$O and $^{16}$O in precipitation, expressed as $\delta^{18}$O. Water molecules containing the stable isotope $^{18}$O have a greater propensity to condense, hence to precipitate, than those containing the more abundant stable isotope $^{16}$O. Therefore, the relative abundance $^{18}$O/$^{16}$O in an air mass decreases during precipitation. The fraction of water vapour lost can be easily calculated comparing the isotopic composition of the water vapour in an air mass at the moment of its formation, assuming marine origin, with the composition at the moment of sampling, according to Rayleigh’s fractionation model (IAEA, 2001). More details on this calculation are provided in Stopelli et al, 2015. Wind speed was measured at Jungfraujoch by MeteoSwiss and data were stored as 10-minute averages.

3 Results and Discussion

3.1 Variability of the concentrations of bacterial cells and INPs-8

Concentrations of total bacterial cells in the 56 samples collected over the course of 18 months (excluding the winter period between 2013 and 2014) were mostly between $3.0 \times 10^3$ (10th percentile) and $3.9 \times 10^4$ mL$^{-1}$ (90th percentile), a range which is coherent with previous observations carried out on precipitation and cloud water samples collected at several places around the world (Bauer et al., 2002; Joly et al., 2014; Šantl-Temkiv et al., 2013; Sattler et al., 2001; Vaïtilingom et al., 2012). Concentrations of INPs active at -8 °C or warmer (INPs-8) (Fig. 1, data taken from Stopelli et al., 2016) presented a similar trend (Spearman’s correlation coefficient $r_S = 0.45, p < 0.001$) but with an order of magnitude larger dynamic range (DR, i.e. the ratio between the largest and the smallest values) (10th percentile: 0.2 mL$^{-1}$; 90th percentile: 68.1 mL$^{-1}$).

The DR of atmospheric particles or molecules is largely determined by their atmospheric residence time. In fact, species with longer residence time have a higher chance to mix and integrate more sources and over wider areas. Furthermore, a longer residence time leads to a higher background concentration of a species in the atmosphere. Changing inputs due to changing source strength, or changing losses due to changing sink strength, only make smaller differences to this larger pool. Hence, the relatively small DR of bacterial cells compared to that of INPs-8 suggests that the majority of bacterial cells has a longer residence time in the atmosphere than INPs-8. The shorter residence time of INPs-8 is likely to be due to their capacity to catalyse ice formation and growth at -8 °C, leading to their rapid deposition with the growing crystal. Such INPs-8 include not only bacterial cells, but also fungal spores, pollen, parts thereof, and soil particles associated with biological ice nucleation active fragments.
Surprisingly, the fraction of living cells among total bacterial cells was on average 0.6, with a small standard deviation (0.1), despite the harsh environmental conditions such as low temperature (sometimes down to -25 °C) and intense solar radiation. The fraction of living cells was neither related to the fraction of water lost prior to sampling nor to wind speed, and had no relation with the number of INPs. This finding does not exclude that bacteria can constitute an important fraction of INPs. Rather, it suggests that i) the ice nucleating capability can be retained beyond cellular death under atmospheric conditions (Amato et al., 2015; Möhler et al., 2007) and/or ii) it can be linked not only to entire cells but also to cellular fragments or molecules released from cells (O’Sullivan et al., 2016; Pummer et al., 2015).

In an earlier study we had already found evidence for INPs being more efficiently deposited from precipitating clouds than the majority of particles larger than 0.5 µm (Stopelli et al., 2015). This evidence was based on the comparison of INPs in precipitation with that of particles in air. In this work, by comparing number concentrations of both INPs and bacteria in precipitation, we corroborate with field data the hypothesis that the ability to foster the formation and growth of ice crystals increases the chance of an airborne particle to be deposited. With an increasing fraction of water precipitated from air masses prior to arrival at the observatory, the number concentration of INPs decreased much faster than that of bacteria (Fig. 2). Therefore, a more rapid loss from the atmosphere with precipitation is one factor contributing to the greater dynamic range of INPs.

This opens the question whether we can find evidence also for the replenishment of their atmospheric concentrations at shorter time scales.

### 3.2 The influence of wind speed on INPs and bacteria

Wind speed is an important factor associated with enhanced number concentrations of INPs in air masses at Jungfraujoch (Stopelli et al., 2016). At high wind speeds (> 50 km h⁻¹) numbers of INPs were 10² to 10⁷ times the number of bacterial cells in precipitation, independent of prior precipitation (Fig. 3, red symbols). At lower wind speeds, the number of INPs decreased much more rapidly than the number of bacterial cells (blue symbols). We interpret this observation in the following way. High wind speeds at Jungfraujoch could be associated also with high wind speeds in source regions upwind (e.g.: Swiss Plateau, Po Valley) which promote the aerosolisation of INPs and bacterial cells (Fig. 2; Lindemann and Upper, 1985). The atmospheric residence time of total bacterial cells is longer than that of INPs. Therefore, their background number concentration would be relatively large and stable and not changed much by the additional cells aerosolised at high wind speeds in the region. The shorter atmospheric lifetime of INPs means their background number concentration tends to be smaller, relative to the emission strength of these particles. Consequently, numbers of INPs increase more substantially above background values at high wind speed, than in the case of bacterial cells. Hence, the ratio of INPs to bacterial cells remains large, even when a substantial fraction of water had already precipitated from the air mass prior to sampling. At lower wind speeds, concentrations of INPs are not restored from regional sources and we see more clearly the effect of a preferential washout of INPs relative to bacterial cells (blue symbols in Fig. 3). The median ratio of INPs to bacterial cells we observed at Jungfraujoch was 6.6 · 10⁻⁴,
very close to what Joly et al. (2014) had found over the course of a year in cloud water at Puy de Dôme (5.5×10−4).

### 3.3 Abundance and diversity of the prominent ice nucleating bacterium *P. syringae*

*Pseudomonas syringae* was successfully detected in three of the 13 samples analysed for the presence of this bacterium. These samples had over 1000 bacterial cells mL−1 and more than 10 INPs mL−1 and were from clouds that had precipitated less than 70% of their water vapor prior to sampling (Fig. 2). Two-thirds of the strains (16/24), after culture in the laboratory, produced at least 1 cell in a suspension of 2·107 cells that was ice nucleation active at temperatures warmer than −8 °C. The freezing onset temperature of all ice nucleation active strains was warmer than −5 °C and was −2.1 °C for the most active strain (Table 1). The precipitation samples from which these strains were isolated were characterised by a relatively warmer onset of freezing (median onset temperature of −5.0 °C for samples with *P. syringae* vs. −6.8 °C for those without *P. syringae*, p = 0.02, Mann-Whitney test). Although *P. syringae* was found in the samples with the largest numbers of INP−8 and with the warmest onset freezing temperature, only 2, 4, and 45 colony-forming units (i.e. culturable cells) of *P. syringae* were present per litre of sample. This corresponds to 2 orders of magnitude less than what has been found in snowfall at lower altitudes (Monteil et al., 2014). We suspect that this can be due both to the preferential removal of *P. syringae* with precipitation as soon as it develops at lower altitudes and to the larger distance of Jungfraujoch from the sources where *P. syringae* entered into air masses. This possibly leads to longer exposure of *P. syringae* to UV radiation and desiccation, reducing its culturability when collected at higher altitude. The largest number concentration of colony-forming units of *P. syringae* we found in snow water was 104 times the number of INPs−8 in the same sample.

This is the first time this bacterium has been isolated at such altitudes (3580 m a.s.l.), and therefore this result expands the established limits for *P. syringae*’s dissemination and survival under atmospheric conditions. Sequencing of the cts gene for phylogenetic analyses was conducted for all 24 strains of *P. syringae* from culture plates from the precipitation samples (Table 1) to obtain insight into the possible origin of these strains. For all dates where *P. syringae* was isolated, the strains in each precipitation event were genetically diverse and represented a broad range of known phylogenetic groups (Table 1, Supplemental Fig. 1). This high diversity suggests that the process of entry of *P. syringae* into air masses moving up to Jungfraujoch involves either multiple events from a wide range of sources along the trajectory or a few entry events from common sources that harbour a high diversity of *P. syringae* that can readily be wafted into the air. Leaf litter, for instance, is one substrate that could have been a source of a high diversity of *P. syringae*, since it contains a high density and high genetic diversity of *P. syringae*, many of which are ice nucleation active, no matter the geographic origin and trajectory of the air masses (Berge et al., 2014; Monteil et al., 2012).

All but 3 of the 24 strains had functional type III secretion systems (hypersensitivity test). This suggests that they had potential to cause plant disease on some crops and illustrates the potential extent of spread of diseases caused by this pathogen.

### 4 Conclusions
Based on observations of INPs and bacterial cells in 56 precipitation samples collected at Jungfraujoch we have shown that ice nucleation activity at -8 °C and warmer temperatures contributes to shorter atmospheric residence times due to a greater probability for wet deposition. For bacterial cells that are disseminated via the atmosphere, this property is advantageous because, by enhancing deposition, the bacteria reduce the time of exposure of their cells to hostile conditions such as UV radiation, extreme cold temperatures and to drying. The decrease of INPs relative to bacterial cells with precipitation can be delayed by high wind speed, which promotes the continuous uptake, mixing and transport of INPs over longer distances. Over half of the bacterial cells in precipitation that fell on Jungfraujoch were viable and among those *P. syringae* could be cultured from 3 of 13 samples. But their concentration in precipitation was less than 50 culturable cells per litre of snow meltwater, about $10^4$ times less than the concentration of INPs. Therefore, culturable *P. syringae* appeared as a minor component of the ensemble of INPs collected in precipitation at Jungfraujoch. Nevertheless, the presence in snowfall on Jungfraujoch of a bacterium such as *P. syringae* sheds new light on the possibilities for this bacterium to survive journeys through the atmosphere and colonise new plants and new habitats. At the same time, it opens exciting research perspectives. For *P. syringae* there are a wide array of techniques for the characterization of its phenotypic and genotypic variability and banks of strains and genomic data related to habitats and geographic origin. This makes *P. syringae* a powerful model for attempting to identify specific sources of INPs in precipitating air masses and for better defining the extent of their trajectories.

5 Data availability
The data set for this paper is publicly available as Table in the Supplement.

6 Author contributions
Emiliano Stopelli carried out the field measurements at Jungfraujoch on the abundance of INPs, bacterial cells and on the isolation of *P. syringae*, and together with Franz Conen analysed the data. Caroline Guilbaud provided fundamental support in the isolation of *P. syringae* and did the phylogenetic analysis on the isolated strains. Jakob Zopfi provided great help with the set up of epifluorescence microscopy technique. Christine Alewell and Cindy E. Morris provided strong conceptual frameworks. Emiliano Stopelli wrote the manuscript together with Franz Conen, with important contributions from all other co-authors.

7 Acknowledgements
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References


Table 1. Diversity of *Pseudomonas syringae* strains from fresh snow samples collected at Jungfraujoch.

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1 Full details of the phylogenetic situation of these strains compared to a range of reference strains is presented in the Supplemental Figure 1.
2 INA refers to ice nucleation activity of suspensions of 2·10^7 cells. The reported values are the freezing onset temperature.
3 Capacity to induce a hypersensitive reaction (HR) in tobacco indicative of the presence of a functional type III secretion system that is one of the fundamental traits usually required for pathogenicity of *P. syringae* to plants.
Figure 1. Number of total bacterial cells (black dots), fraction of living cells (green squares) and ice nucleating particles active at -8 °C (INPs₈, blue, from Stopelli et al., 2016) in precipitation samples collected at the high altitude research station Jungfraujoch (3580 m a.s.l.) during 11 sampling campaigns between March 2013 and October 2014.
Figure 2. Number of bacterial cells (black) and INPs$_{8}$ (blue, from Stopelli et al., 2016) versus the fraction of precipitation lost from the air mass prior to arrival and sampling at the observatory. The width of the symbols is proportional to wind speed (minimum = 2 km h$^{-1}$, maximum = 89 km h$^{-1}$). Patterned symbols represent the 10 fresh snow samples in which $P.\ syringae$ was searched for, but not found. The three full symbols represent the 3 samples where culturable $P.\ syringae$ was found.
Figure 3. Ratio of INPs$_8$ to bacterial cells in precipitation samples collected at Jungfraujoch (3580 m a.s.l.). The width of symbols is proportional to wind speed (minimum = 2 km h$^{-1}$, maximum = 89 km h$^{-1}$). Red symbols correspond to values with average wind speeds $> 50$ km h$^{-1}$ during samplings and blue symbols correspond to data with lower wind speeds. Patterned symbols represent the 10 samples in which $P$. syringae was searched for, but not found. The three full symbols represent samples where culturable $P$. syringae was found. Dashed and dotted lines indicate median and maximum ratios observed in cloud water collected over the course of a year at Puy de Dôme (1465 m a.s.l.), 350 km west of Jungfraujoch (data from Joly et al., 2014).