

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 addresses 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₋₈. 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 $\delta^{18}\text{O}$. 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 $^{18}\text{O}:$ ^{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_g 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

IAEA International Atomic Energy Agency: Environmental isotopes in the hydrological cycle. Principles and applications. Vol. 2. Atmospheric Water, available at: http://www-naweb.iaea.org/napc/ih/IHS_resources_publication_hydroCycle_en.html (last access on 30.01.2017), 2001.

Lloyd, G., Choularton, T. W., Bower, K. N., Gallagher, M. W., Connolly, P. J., Flynn, M., Farrington, R., Crosier, J., Schlenczek, O., Fugal, J., and Henneberger, J.: The origins of ice crystals measured in mixed-phase clouds at the high-alpine site Jungfraujoch, *Atmos. Chem. Phys.*, 15, 12953-12969, doi:10.5194/acp-15-12953-2015, 2015.

Stopelli, E., Conen, F., Zimmermann, L., Alewell, C., and Morris, C. E.: Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation, *Atmos. Meas. Tech.*, 7, 129-134, doi:10.5194/amt-7-129-2014, 2014.

Stopelli, E., Conen, F., Morris, C. E., Herrmann, E., Bukowiecki, N., and Alewell, C.: Ice nucleation active particles are efficiently removed by precipitating clouds, *Sci. Rep.*, 5, 16433, doi:10.1038/srep16433, 2015.

Stopelli, E., Conen, F., Morris, C. E., Herrmann, E., Henne, S., Steinbacher, M., and Alewell, C.: Predicting abundance and variability of ice nucleating particles in precipitation at the high-altitude observatory Jungfraujoch, *Atmos. Chem. Phys.*, 16, 8341-8351, doi:10.5194/acp-16-8341-2016, 2016.

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₋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 (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 sampling, in order to reduce the influence of scavenging of particles on our measurements. 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 Stopelli 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

Conen, F., Morris, C. E., Leifeld, J., Yakutin, M. V., and Alewell, C.: Biological residues define the ice nucleation properties of soil dust, *Atmos. Chem. Phys.*, 11, 9643–9648, doi:10.5194/acp-11-9643-2011, 2011.

Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Yordanova, P., Franc, G. D., and Pöschl, U.: Ice nucleation activity in the widespread soil fungus *Mortierella alpina*, *Biogeosciences*, 12, 1057–1071, doi:10.5194/bg-12-1057-2015, 2015.

Hill, T. C. J., DeMott, P. J., Tobo, Y., Fröhlich-Nowoisky, J., Moffett, B. F., Franc, G. D., and Kreidenweis, S. M.: Sources of organic ice nucleating particles in soils, *Atmos. Chem. Phys.*, 16, 7195–7211, doi:10.5194/acp-16-7195-2016, 2016.

Morris, C. E., Sands, D. C., Glaux, C., Samsatly, J., Asaad, S., Moukahel, A. R., Goncalves, F. I. T., and Bigg, K. E.: Urediospores of rust fungi are ice nucleation active at > -10 °C and harbor ice nucleation active bacteria, *Atmos. Chem. Phys.* 13, 4223–4233, doi:10.5194/acp-13-4223-2013, 2013.

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”
8
- 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) 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. Furthermore, concentrations of INPs₋₈ 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 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 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

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1 they can proliferate (Morris et al., 2013a). In fact, favouring the growth of ice crystals could be a powerful and
2 effective means for airborne microorganisms to reduce their residence time aloft. Previous research has
3 illustrated that bacterial strains capable of nucleating ice can deposit rapidly under laboratory simulated cloud
4 conditions, corresponding to a cold temperature regime and supersaturation (Amato et al., 2015). Modelling
5 studies suggest that the atmospheric residence time of singularly airborne bacterial cells is highly reduced if such
6 bacteria act as condensation and ice nuclei in clouds (Burrows et al., 2009). Furthermore, snowfall has been
7 shown to enrich for the presence of ice nucleation active strains of *P. syringae* compared to a range of other
8 environmental contexts including within clouds (Morris et al., 2008; Morris et al., 2013a). Therefore, more direct
9 evidence from field observations on the selective deposition of ice nucleation active microorganisms with
10 precipitation is wanting.

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

17 2 Materials and methods

18 2.1 Sampling and counting of INPs and bacteria

19 Fifty six snow samples were collected at the High Altitude Research Station Jungfraujoch (7°59'06'' E,
20 46°32'51'' N, 3580 m a.s.l., Switzerland) during 11 short sampling campaigns from March to September 2013
21 and May to October 2014. The Station was always inside precipitating clouds while collecting snow. Samples
22 were collected with a 0.1 m² Teflon-coated tin for periods of 1.5 to 8 hours depending on precipitation intensity.
23 The tin was rinsed several times with 70 % ethanol and sterile Milli-Q water between samples to avoid cross-
24 contamination. Snow samples were melted at room temperature (about 16 °C) and adjusted to physiological
25 conditions (9 ‰ NaCl) to prevent osmotic stress on cells and improve the detection of freezing events. They
26 were analysed immediately on site for concentration of INPs active between -2 and -12 °C in a drop freeze assay
27 with the LINDA device loaded with 52 Eppendorf Safelock tubes containing 100 µL of sample each, as
28 described in Stopelli et al. (2014). For each sample, two filters were prepared for later analysis of bacterial
29 number concentration. Twenty mL of sample were passed through the active area (glass vacuum filter funnels
30 were equipped with an inlay to reduce the whole area of the filter into an active area of 6 mm diameter, to
31 improve the possibility of counting enough cells per unit area of filter) of two 0.22 µm black polycarbonate
32 membranes (Whatman). The filters were rinsed with 3 mL of sterile phosphate-buffered saline (PBS) and the
33 staining agents were added: 10 µL SYBR green (100x) for total cell count, and 10 µL SYBR green and 10 µL
34 propidium iodide (in Milli-Q water, 1% wt/wt) to facilitate counting cells that are alive (i.e with intact
35 membranes) and dead or dying (with permeable membranes). After incubation in the dark for 10 min, the stains
36 were filtered away and the filter columns rinsed with 3 mL of sterile water. Once dry, the filters were put on
37 glass slides, 15 µL of antifading agent (5 mL PBS, 5 mL glycerol, 10 mg p-phenylenediamine) was added for
38 preservation in the dark at -20 °C until analysis. Blanks for the determination of INPs and bacterial cells were

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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.

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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, cephalexin 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 10⁸ 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.

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Neighbour-joining phylogenetic trees of the strains of *P. syringae* isolated from Jungfrauoch 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'-CCCGTCGAGCTGCCAAT(AT)TTGCTGA-3' for sequencing (Morris et al., 2010; Sarkar and Guttman, 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; Vaitilingom 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

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1 al., 2011). Reported sequences are deposited in the GenBank Archive under the accession numbers GenBank
2 KY379248-KY379271.

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3 2.3 Environmental parameters and statistics

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

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14 Univariate and non-parametric statistics were carried out with PAST software version 2.17 (Hammer et al.,
15 2001).

16 3 Results and Discussion

17 3.1 Variability of the concentrations of bacterial cells and INPs_g

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18 Concentrations of total bacterial cells in the 56 samples collected over the course of 18 months (excluding the
19 winter period between 2013 and 2014) were mostly between $3.0 \cdot 10^3$ (10th percentile) and $3.9 \cdot 10^4$ mL⁻¹ (90th
20 percentile), a range which is coherent with previous observations carried out on precipitation and cloud water
21 samples collected at several places around the world (Bauer et al., 2002; Joly et al., 2014; Šantl-Temkiv et al.,
22 2013; Sattler et al., 2001; Vaitilingom et al., 2012). Concentrations of INPs active at -8 °C or warmer (INPs_g)
23 (Fig. 1, data taken from Stopelli et al., 2016) presented a similar trend (Spearman's correlation coefficient $r_s =$
24 0.45, $p < 0.001$) but with an order of magnitude larger dynamic range (DR, i.e. the ratio between the largest and
25 the smallest values) (10th percentile: 0.2 mL⁻¹; 90th percentile: 68.1 mL⁻¹).

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26 The DR of atmospheric particles or molecules is largely determined by their atmospheric residence time. In fact,
27 species with longer residence time have a higher chance to mix and integrate more sources and over wider areas.
28 Furthermore, a longer residence time leads to a higher background concentration of a species in the atmosphere.
29 Changing inputs due to changing source strength, or changing losses due to changing sink strength, only make
30 smaller differences to this larger pool. Hence, the relatively small DR of bacterial cells compared to that of INPs.
31 suggests that the majority of bacterial cells has a longer residence time in the atmosphere than INPs_g. The
32 shorter residence time of INPs_g is likely to be due to their capacity to catalyse ice formation and growth at -8 °C,
33 leading to their rapid deposition with the growing crystal. Such INPs_g include not only bacterial cells, but also
34 fungal spores, pollen, parts thereof, and soil particles associated with biological ice nucleation active fragments

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(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).

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_g. This finding does not exclude that bacteria can constitute an important fraction of INPs_g. 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_g in precipitation with that of particles in air. In this work, by comparing number concentrations of both INPs_g 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_g 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_g. 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_g and bacteria

Wind speed is an important factor associated with enhanced number concentrations of INPs_g in air masses at Jungfraujoch (Stopelli et al., 2016). At high wind speeds (> 50 km h⁻¹) numbers of INPs_g were 10⁻² to 10⁻³ times the number of bacterial cells in precipitation, independent of prior precipitation (Fig. 3, red symbols). At lower wind speeds, the number of INPs_g 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_g and bacterial cells (Fig. 2; Lindemann and Upper, 1985). The atmospheric residence time of total bacterial cells is longer than that of INPs_g. 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_g means their background number concentration tends to be smaller, relative to the emission strength of these particles. Consequently, numbers of INPs_g increase more substantially above background values at high wind speed, than in the case of bacterial cells. Hence, the ratio of INPs_g 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_g are not restored from regional sources and we see more clearly the effect of a preferential washout of INPs_g relative to bacterial cells (blue symbols in Fig. 3). The median ratio of INPs_g to bacterial cells we observed at Jungfraujoch was 6.6·10⁻⁴,

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very close to what Joly et al. (2014) had found over the course of a year in cloud water at Puy de Dôme ($5.5 \cdot 10^4$).

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_g 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 $2 \cdot 10^7$ 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_g 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 10^{-4} times the number of INPs_g 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

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1 Based on observations of INPs_g and bacterial cells in 56 precipitation samples collected at Jungfraujoch we
2 have shown that ice nucleation activity at -8 °C and warmer temperatures contributes to shorter atmospheric
3 residence times due to a greater probability for wet deposition. For bacterial cells that are disseminated via the
4 atmosphere, this property is advantageous because, by enhancing deposition, the bacteria reduce the time of
5 exposure of their cells to hostile conditions such as UV radiation, extreme cold temperatures and to drying. The
6 [decrease of INPs relative to bacterial cells](#), with precipitation can be delayed by high wind speed, which promotes
7 the continuous uptake, mixing and transport of INPs over longer distances. Over half of the bacterial cells in
8 precipitation that fell on Jungfraujoch were viable and among those *P. syringae* could be cultured from 3 of 13
9 samples. But their concentration in precipitation was less than 50 culturable cells per litre of snow meltwater,
10 about 10⁴ times less than the concentration of INPs_g. Therefore, culturable *P. syringae* appeared as [a minor](#)
11 component of the ensemble of INPs_g collected in precipitation at Jungfraujoch. Nevertheless, the presence in
12 snowfall on Jungfraujoch of a bacterium such as *P. syringae* sheds new light on the possibilities for this
13 bacterium to survive journeys through the atmosphere and colonise new plants [and new habitats](#). At the same
14 time, it opens exciting research perspectives. For *P. syringae* there are a wide array of techniques for the
15 [characterisation](#) of its phenotypic and genotypic variability and banks of strains and genomic data related to
16 habitats and geographic origin. This makes *P. syringae* a powerful model for attempting to identify specific
17 sources of INPs in precipitating air masses and for better defining the extent of their trajectories.

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18 5 Data availability

19 The data set for this paper is publicly available as Table in the Supplement.

20 6 Author contributions

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

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27 The authors declare that they have no conflict of interest.

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30 for making it possible for us to conduct our measurements at Jungfraujoch; Urs and Maria Otz, [as well as](#) Joan
31 and Martin Fisher [are acknowledged](#) for their support on site. Corinne Baudinot measured the abundance of INPs
32 in snow samples during the second year of observations. Dr Thomas Kuhn and Mark Rollog analysed the stable
33 isotope ratio in our snow water samples. We thank MeteoSwiss for providing data on meteorology at
34 Jungfraujoch. The work described here was funded by the Swiss National Science Foundation (SNF) through
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References

- Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., and Delort, A. M.: Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: major groups and growth abilities at low temperatures, *FEMS Microbiol. Ecol.*, 59, 242-254, doi:10.1111/j.1574-6941.2006.00199.x, 2007.
- Amato, P., Joly, M., Schaupp, C., Attard, E., Möhler, O., Morris, C. E., Brunet, Y., and Delort, A.-M.: Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber, *Atmos. Chem. Phys.*, 15, 6455-6465, doi:10.5194/acp-15-6455-2015, 2015.
- Bauer, H., Kasper, Giebl, A., and Lo, M.: The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols, *Atmospheric Research*, 64, 109-119, doi:10.1016/S0169-8095(02)00084-4, 2002.
- Berge, O., Monteil, C. L., Bartoli, C., Chandeysson, C., Guilbaud, C., Sands, D. C., and Morris, C. E.: A user's guide to a data base of the diversity of *Pseudomonas syringae* and its application to classifying strains in this phylogenetic complex, *PLoS ONE*, 9, 105547, doi:10.1371/journal.pone.0105547, 2014.
- Burrows, S. M., Butler, T., Jöckel, P., Tost, H., Kerkweg, A., Pöschl, U., and Lawrence, M. G.: Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems, *Atmos. Chem. Phys.*, 9, 9281-9297, doi:10.5194/acp-9-9281-2009, 2009.
- Cantrell, W. and Heymsfield, A.: Production of Ice in Tropospheric Clouds: A Review, *B. Am. Meteorol. Soc.*, 86, 795-807, doi:10.1175/BAMS-86-6-795, 2005.
- Conen, F., Morris, C. E., Leifeld, J., Yakutin, M. V., and Alewell, C.: Biological residues define the ice nucleation properties of soil dust, *Atmos. Chem. Phys.*, 11, 9643-9648, doi:10.5194/acp-11-9643-2011, 2011.
- Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Yordanova, P., Franc, G. D., and Pöschl, U.: Ice nucleation activity in the widespread soil fungus *Mortierella alpina*, *Biogeosciences*, 12, 1057-1071, doi:10.5194/bg-12-1057-2015, 2015.
- Guilbaud, C., Morris, C. E., Barakat, M., Ortet, P., Berge, O.: Isolation and identification of *Pseudomonas syringae* facilitated by a PCR targeting the whole *P. syringae* group, *FEMS Microbiol. Ecol.*, 92, doi:10.1093/femsec/fiv146, 2016.
- Hammer, Ø., Harper, D. A., and Ryan, P. D.: PAST: PAleontologi- cal STatistics software package for education and data analysis, *Palaeontol. Electron.*, 4, available at: http://palaeo-electronica.org/2001_1/past/issue1_01.htm (last access: 14.11.2016), 2001.
- Hill, T. C. J., DeMott, P. J., Tobo, Y., Fröhlich-Nowoisky, J., Moffett, B. F., Franc, G. D., and Kreidenweis, S. M.: Sources of organic ice nucleating particles in soils, *Atmos. Chem. Phys.*, 16, 7195-7211, doi:10.5194/acp-16-7195-2016, 2016.
- IAEA International Atomic Energy Agency: Environmental isotopes in the hydrological cycle. Principles and applications. Vol. 2. Atmospheric Water, available at: http://www-naweb.iaea.org/naweb/ih/IHS_resources_publication_hydroCycle_en.html (last access on 30.01.2017), 2001.
- Joly, M., Attard, E., Sancelme, M., Deguillaume, L., Guilbaud, C., Morris, C. E., Amato, P., and Delort, A. M.: Ice nucleation activity of bacteria isolated from cloud water, *Atmos. Environ.*, 70, 392-400, doi:10.1016/j.atmosenv.2013.01.027, 2013.
- Joly, M., Amato, P., Deguillaume, L., Monier, M., Hoose, C., and Delort, A. M.: Quantification of ice nuclei active at near 0 °C temperatures in low-altitude clouds at the Puy de Dôme atmospheric station, *Atmos. Chem. Phys.*, 14, 8185-8195, doi:10.5194/acp-14-8185-2014, 2014.
- Lamichhane, J. R., Varvaro, L., Audergon, J. M., Parisi, L., and Morris, C. E.: Disease and frost damage of woody plants caused by *Pseudomonas syringae*: seeing the forest for the trees, in: *Advances in Agronomy*, Spark, D. L., ed., Academic Press, pp. 235-295, 2014.
- Lamichhane, J. R., Messean, A., and Morris, C. E.: Insights into epidemiology and control of diseases of annual plants caused by the *Pseudomonas syringae* species complex, *J. Gen. Plant Pathol.*, 81, 331-350, doi:10.1007/s10327-015-0605-z, 2015.
- Lindemann, J. and Upper, C. D.: Aerial dispersal of epiphytic bacteria over bean plants, *Appl. Environ. Microbiol.*, 50, 1229-1232, 1985.
- Maki, L. R., Galyan, E. L., and Caldwell, D. R.: Ice Nucleation Induced by *Pseudomonas syringae*, *Appl. Environ. Microb.*, 28, 456-459, 1974.

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- Möhler, O., DeMott, P. J., Vali, G., and Levin, Z.: Microbiology and atmospheric processes: the role of biological particles in cloud physics, *Biogeosciences*, 4, 1059–1071, doi:10.5194/bg-4-1059-2007, 2007.
- Monteil, C. L., Guilbaud, C., Glaux, C., Lafolie, F., Soubeyrand, S., and Morris, C. E.: Emigration of the plant pathogen *Pseudomonas syringae* from leaf litter contributes to its population dynamics in alpine snowpack, *Environ. Microbiol.*, 14, 2099–2112, doi:10.1111/j.1462-2920.2011.02680.x, 2012.
- Monteil, C. L., Bardin, M., and Morris, C. E.: Features of air masses associated with the deposition of *Pseudomonas syringae* and *Botrytis cinerea* by rain and snowfall, *The ISME Journal*, 8, 2290–2304, doi:10.1038/ismej.2014.55, 2014.
- Morris, C. E., D. C. Sands, J. L. Vanneste, J. Montarry, B. Oakley, Guilbaud, C., and Glaux, C.: Inferring the evolutionary history of the plant pathogen *Pseudomonas syringae* from its biogeography in headwaters of rivers in North America, Europe, and New Zealand, *mBio*, 1, e00107-10, doi:10.1128/mBio.00107-10, 2010.
- Morris, C. E., Sands, D. C., Vinatzer, B. A., Glaux, C., Guilbaud, C., Buffière, A., S. Yan, H. Dominguez, and Thompson, B. M.: The life history of the plant pathogen *Pseudomonas syringae* is linked to the water cycle, *The ISME Journal*, 2, 321–334, doi:10.1038/ismej.2007.113, 2008.
- Morris, C. E., Monteil, C. L., and Berge, O.: The life history of *Pseudomonas syringae*: linking agriculture to earth system processes, *Annu. Rev. Phytopathol.*, 51, 85–104, doi:10.1146/annurev-phyto-082712-102402, 2013a.
- [Morris, C. E., Sands, D. C., Glaux, C., Samsatly, J., Asaad, S., Moukahel, A. R., Goncalves, F. I. T., and Bigg, K. E.: Urediospores of rust fungi are ice nucleation active at > -10 °C and harbor ice nucleation active bacteria, *Atmos. Chem. Phys.* 13, 4223–4233, doi:10.5194/acp-13-4223-2013, 2013b.](#)
- Morris, C. E., Conen, F., Huffman, J. A., Phillips, V., Pöschl, U., and Sands, D. C.: Bioprecipitation: a feedback cycle linking earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere, *Glob. Change Biol.*, 20, 341–351, doi:10.1111/gcb.12447, 2014.
- Mülmenstädt, J., Sourdeval, O., Delanoë, J., and Quaas, J.: Frequency of occurrence of rain from liquid-, mixed-, and ice-phase clouds derived from A-Train satellite retrievals, *Geophys. Res. Lett.*, 42, 6502–6509, doi:10.1002/2015GL064604, 2015.
- Murray, B. J., O’Sullivan, D., Atkinson, J. D., and Webb, M. E.: Ice nucleation by particles immersed in supercooled cloud droplets, *Chem. Soc. Rev.*, 41, 6519–6554, doi:10.1039/c2cs35200a, 2012.
- O’Sullivan, D., Murray, B. J., Ross, J. F., Whale, T. F., Price, H. C., Atkinson, J. D., Umo, N. S., and Webb, M. E.: The relevance of nanoscale biological fragments for ice nucleation in clouds, *Sci. Rep.*, 5, 8082, doi:10.1038/srep08082, 2015.
- O’Sullivan, D., Murray, B. J., Ross, J. F., and Webb, M. E.: The adsorption of fungal ice-nucleating proteins on mineral dusts: a terrestrial reservoir of atmospheric ice-nucleating particles, *Atmos. Chem. Phys.*, 16, 7879–7887, doi:10.5194/acp-16-7879-2016, 2016.
- Pummer, B. G., Budke, C., Augustin-Bauditz, S., Niedermeier, D., Felgitsch, L., Kampf, C. J., Huber, R. G., Liedl, K. R., Loerting, T., Moschen, T., Schauperl, M., Tollinger, M., Morris, C. E., Wex, H., Grothe, H., Pöschl, U., Koop, T., and Fröhlich-Nowoisky, J.: Ice nucleation by water-soluble macromolecules, *Atmos. Chem. Phys.*, 15, 4077–4091, doi:10.5194/acp-15-4077-2015, 2015.
- Sands, D. C., Langhans, V. E., Scharen, A. L., and de Smet, G.: The association between bacteria and rain and possible resultant meteorological implications, *J. Hungarian Meteorol. Serv.*, 86, 148–152, 1982.
- Šantl-Temkiv, T., Finster, K., Dittmar, T., Hansen, B. M., Thyrhaug, R., Nielsen, W. N., and Karlson, U. G.: Hailstones: A Window into the Microbial and Chemical Inventory of a Storm Cloud, *PLoS ONE*, 8, e53550, doi:10.1371/journal.pone.0053550, 2013.
- Sarkar, S. F. and Guttman, D. S.: Evolution of the core genome of *Pseudomonas syringae*, a highly clonal, endemic plant pathogen, *Appl. Environ. Microbiol.*, 70, 1999–2012, doi:10.1128/AEM.70.4.1999-2012.2004, 2004.
- Sattler, B., Puxbaum, H., and Psenner, R.: Bacterial growth in supercooled cloud droplets, *Geophys. Res. Lett.*, 28, 239–242, 2001.
- Stopelli, E., Conen, F., Zimmermann, L., Alewell, C., and Morris, C. E.: Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation, *Atmos. Meas. Tech.*, 7, 129–134, doi:10.5194/amt-7-129-2014, 2014.

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1 Stopelli, E., Conen, F., Morris, C. E., Herrmann, E., Bukowiecki, N., and Alewell, C.: Ice nucleation active
2 particles are efficiently removed by precipitating clouds, *Sci. Rep.*, 5, 16433, doi:10.1038/srep16433,
3 2015.

4 Stopelli, E., Conen, F., Morris, C. E., Herrmann, E., Henne, S., Steinbacher, M., and Alewell, C.: Predicting
5 abundance and variability of ice nucleating particles in precipitation at the high-altitude observatory
6 Jungfraujoch, *Atmos. Chem. Phys.*, 16, 8341-8351, doi:10.5194/acp-16-8341-2016, 2016.

7 Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S.: MEGA5: molecular evolutionary
8 genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods,
9 *Mol. Biol. Evol.*, 28, 2731-2739, doi:10.1093/molbev/msr121, 2011.

10 Vaitilingom, M., Attard, E., Gaiani, N., Sancelme, M., Deguillaume, L., Flossmann, A. I., Amato, P., and Delort,
11 A. M.: Long-term features of cloud microbiology at the Puy de Dôme (France), *Atmos. Environ.*, 58, 88-
12 100, doi:10.1016/j.atmosenv.2012.03.072, 2012.

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

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Strain	Date	Phylogroup ¹	INA ²	HR ³
JFJ-0007	22 May 2014	2	-4.2	+
JFJ-0008	22-23 May 2014	3	-	-
JFJ-0011	22-23 May 2014	5	-3.7	+
JFJ-0039	21-22 Oct 2014	1	-	+
JFJ-0045	21-22 Oct 2014	1	-	+
JFJ-0048	21-22 Oct 2014	1	-	+
JFJ-0061	21-22 Oct 2014	1	-	+
JFJ-0043	21-22 Oct 2014	2	-2.6	+
JFJ-0047	21-22 Oct 2014	2	-	-
JFJ-0050	21-22 Oct 2014	2	-3.6	+
JFJ-0052	21-22 Oct 2014	2	-2.7	+
JFJ-0054	21-22 Oct 2014	2	-2.2	+
JFJ-0055	21-22 Oct 2014	2	-2.2	+
JFJ-0058	21-22 Oct 2014	4	-	+
JFJ-0056	21-22 Oct 2014	7	-2.9	+
JFJ-0059	21-22 Oct 2014	7	-4.9	+
JFJ-0040	21-22 Oct 2014	10	-4.2	+
JFJ-0044	21-22 Oct 2014	10	-2.1	+
JFJ-0049	21-22 Oct 2014	10	-2.8	+
JFJ-0051	21-22 Oct 2014	10	-2.9	+
JFJ-0053	21-22 Oct 2014	10	-4.0	+
JFJ-0060	21-22 Oct 2014	10	-2.8	+
JFJ-0046	21-22 Oct 2014	13	-	-
JFJ-0057	21-22 Oct 2014	13	-	+

¹Full details of the phylogenetic situation of these strains compared to a range of reference strains is presented in the Supplemental Figure 1.

²INA refers to ice nucleation activity of suspensions of $2 \cdot 10^7$ cells. The reported values are the freezing onset temperature.

³Capacity 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.

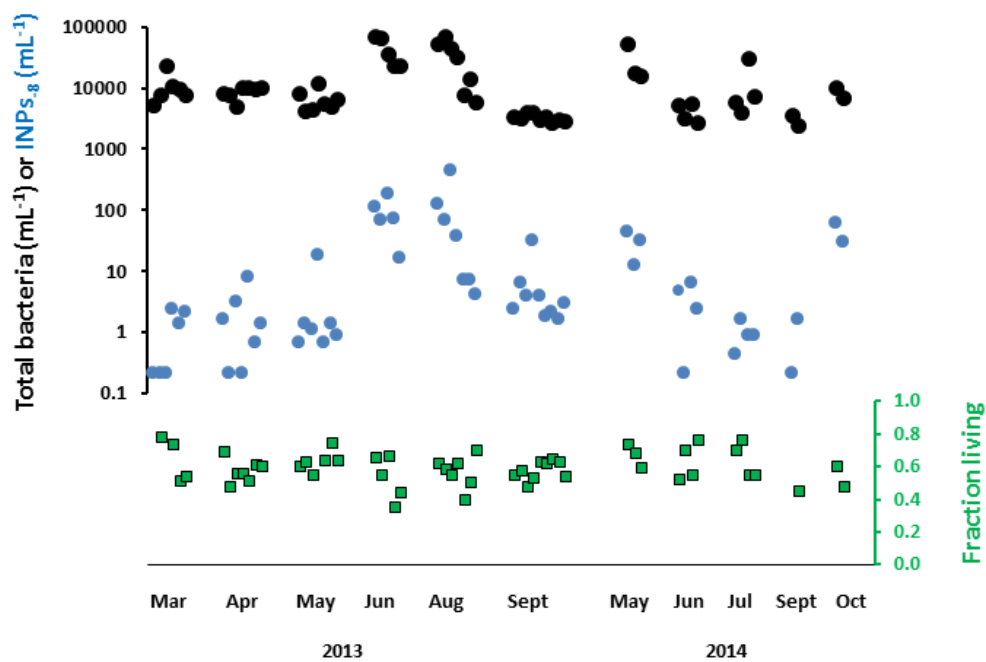
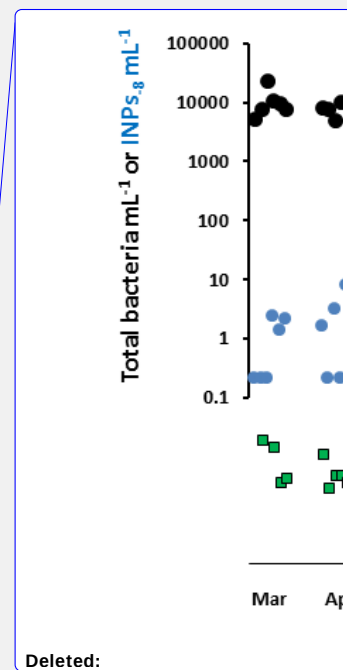


Figure 1. Number of total bacterial cells (black dots), fraction of living cells (green squares) and ice nucleating particles active at -8°C (INPs₈, blue, from Stopelli et al., 2016) in precipitation samples collected at the high altitude research station Jungfraujoch (3580 m a.s.l.) during 11 sampling campaigns between March 2013 and October 2014.



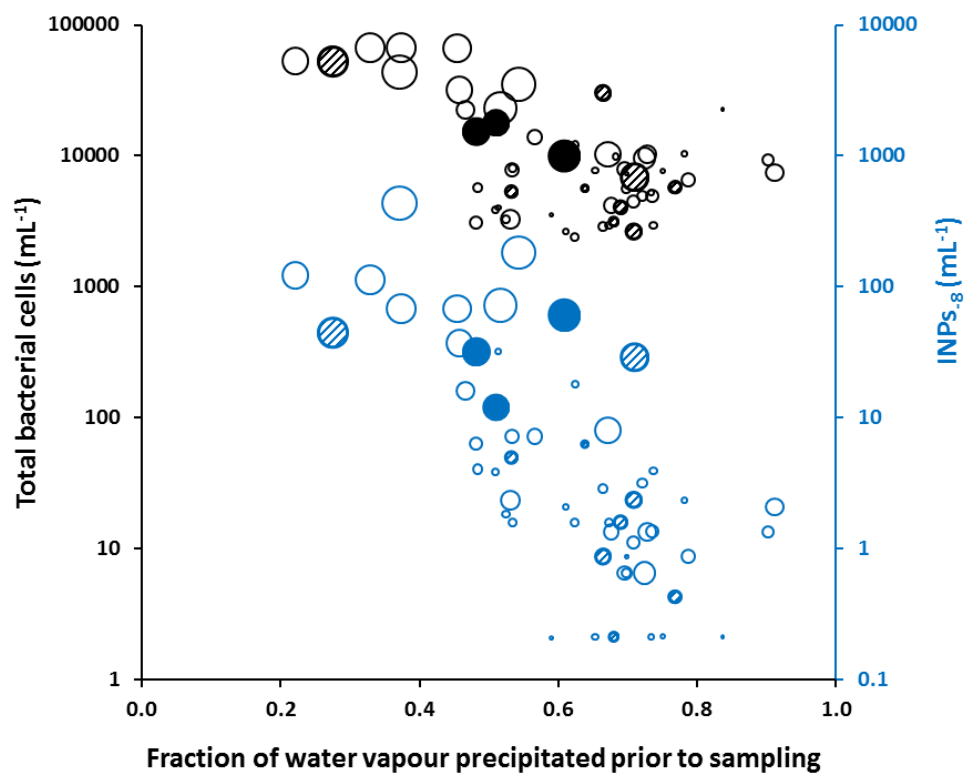


Figure 2. Number of bacterial cells (black) and INPs₈ (blue, from Stopelli et al., 2016) versus the fraction of precipitation lost from the air mass prior to arrival and sampling at the observatory. The width of the symbols is proportional to wind speed (minimum = 2 km h⁻¹, maximum = 89 km h⁻¹). Patterned 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.

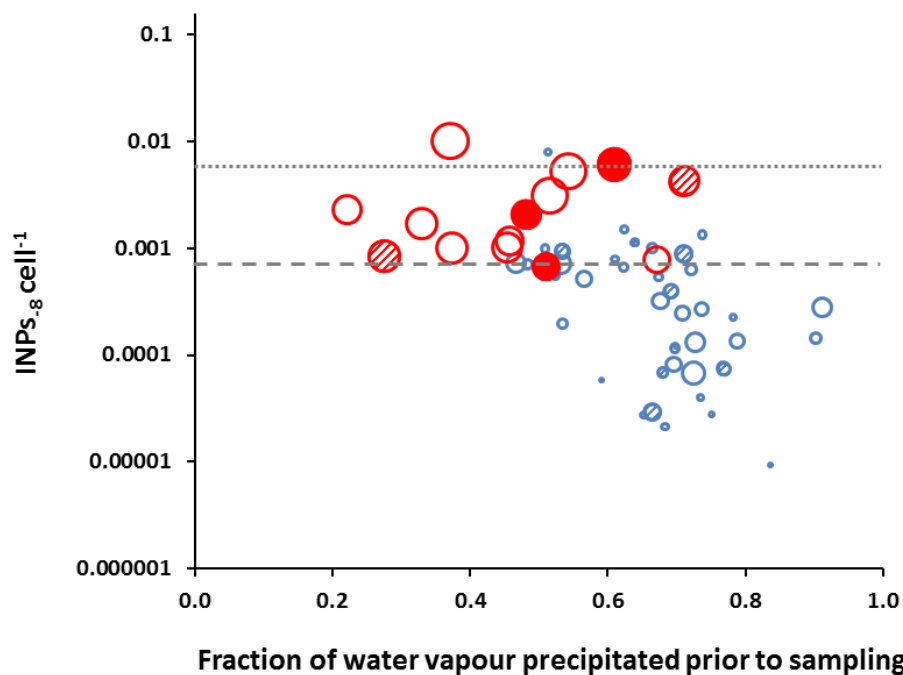


Figure 3. Ratio of INPs_8 to bacterial cells in precipitation samples collected at Jungfraujoch (3580 m a.s.l.). The width of symbols is proportional to wind speed (minimum = 2 km h^{-1} , maximum = 89 km h^{-1}). Red symbols correspond to values with average wind speeds $> 50 \text{ km h}^{-1}$ during 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).

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