Response to Referee #1 (Gabor Vali):

The work described in this paper is based on a simple and powerful idea: a direct way to determine the potential for ice formation in a cloud is to collect cloud water and determine the content of ice-forming nuclei in it. Furthermore, whether those ice nucleating particles (INPs) are of biological origin can be determined via some direct and some indirect tests. The authors' practical approach to this idea was to collect cloud water from a mountain peak when a cloud envelops it.
Not surprisingly, it is difficult to realize the idea in its pure form. Complications arise from a number of directions. The main ones can be put in question form: 1. How complete is the transfer of all potential INPs from the air in the which the cloud forms? 2. How many ice particles have already formed in the cloud and have fallen out before sampling? 3. Is the exclusion of other modes of ice nucleation, other than immersion freezing, justified? 4. Is there any evidence for aging of the sample after collection?
In spite of the fact that answers to the questions raised are missing in the paper, or are minimal, it is a valuable contribution. The paper demonstrates that detection of INPs in cloud water is a promising approach to shedding light on long-standing questions.

1) **The main shortcoming of the paper is that little information is provided about the clouds that were sampled. Was there precipitation occurring at the same time? Were the clouds forming in the uplift forced by the mountain slope or were they part of extensive cloud layers? How deep were the clouds? What can be said about the age and history of the cloud parcels? Clearly, it would take a project of much greater complexity to gain information on these aspects, the lack of even some broad descriptions and possible sorting of the data according to these variables weaken the results obtained.**

We agree that any information concerning cloud's history, IN partitioning and process by which freezing occur is important for data interpretation. In the original paper, in addition of ice nucleation data, we considered in our analysis sampling temperature, liquid water content, pH, ion composition and backtrajectories. For the revised version of the manuscript, we gathered and included the following additional data or information:

- Sampling times and the periods of time during which clouds were present at the sampling site based on continuous measurements of relative humidity. From these, we obtained information such as cloud duration at the sampling site and the time spent in cloud before and after sampling (did we sample the "edge" or the inside of the clouds?).
- The amount of precipitation cumulated downwind the puy de Dôme Station during the sampling period.
- Satellite visible images (Eumetsat) during the sampling period, showing an overview of the meteorological situation over Europe. These are available for academic purposes on the Wokingham Weather's website (http://www.woksat.info/wwp.html).

All these are now presented in Table 1 and supplementary material (Figure S1). Text sections have also been inserted accordingly in "2.1 Materials and Methods – Cloud water sampling and meteorological measurements" (Lines 132-148).

2) **At what temperatures were the collections made? It is mentioned that some samples froze onto the plate, but it is not clear if that made any difference.**

The information about whether or not samples were collected frozen was already present in the original paper (indicated in italic in Table 1): sampling temperature ranged from -1.5°C to

13.3°C, and 5 of the 12 samples were collected as ice formed by supercooled droplets on the impaction plate (#80- #84).
As suggested by the reviewer, we examined whether the fact that samples were collected frozen or liquid impacted ice nucleation data (concentration of total and biological IN and proportion of biological IN at each temperature, and onset temperature of freezing, *i.e.* the highest temperature at which at least one droplet froze during IN assays). The number of samples analyzed was < 30 and data were not normally distributed, so the non-parametric test of Mann-Whitney was utilized for comparing the two groups (frozen vs liquid). We found the following significant differences (95% confidence):

- the concentration of total IN at -8°C, -9°C and -10°C was significantly higher in samples collected frozen (n = 5) than in liquid samples (n = 7): at -8°C, z = -2.621; p = 0.009; at -9°C, z = -2.298; p = 0.022; at -10°C, z = -2.2; p = 0.028. (Medians: 10.9, 17.6 and 19.9 vs 3.2, 8.5 and 14.4 mL^{-1}, respectively).
- Logically, the concentration of biological IN at -8°C and -9°C was also significantly higher in samples collected frozen (n = 5) than in liquid samples (n = 7): at -8°C: z = -2.621; p = 0.009; at -9°C: z = -2.212; p = 0.027. (Medians: 10.9 and 14.3 vs 3.2 and 8.5 mL^{-1}, respectively).
- The proportion of biological IN at -9°C was lower in samples collected frozen (n = 5) than in liquid samples (n = 7): z = -2.276; p = 0.035. (Medians: 95% vs 100%, respectively).
- The onset temperature of freezing was warmer in samples collected frozen (n = 5) than in liquid samples (n = 7): z = -2.5618; p = 0.028. (Medians: -6 vs -8°C, respectively).

So overall, samples collected frozen had higher IN activity. This information has been added in the manuscript and discussed. (From line 264)

3) **How long were the sampling periods?**

This information is now included in Table 1.

4) **Some information on the sampling intake and the general setup of the apparatus would be helpful.**

The reference describing it has been included (Kruisz et al., 1992) (Line 133).

5) **The absence of data on cloud liquid water content is handled in the paper by using historical data with three different values assigned according to the collection rate of the sample. One wonders why the sample collections rates were not considered reliable enough to be used as a measure of cloud liquid water content. Changing droplet size distributions and variable collection efficiencies due to different wind conditions clearly weaken the reliability of such an evaluation. To what degree? The authors' reasoning for not using that approach should perhaps be in the paper.**

We rephrased the section "2.1 Materials and Methods – Cloud water sampling and meteorological measurements" for trying to make it clearer and add information about the additional parameters taken into account.

6) **The presentation of the results of the measurements is not always clear. Do expressions such as " . . . samples froze at -8◦C . . .. " (3715/6), ". . . none remained supercooled .." refer to one drop (sample tube) from the sample or some other measure?**

We agree that these sentences were confusing, so we rephrased it as for example "In 11 of the 12 cloud samples (92%), the onset temperature of freezing (i.e. temperature at which the first

droplet froze) was -8°C or warmer. Only sample #87 started to freeze at colder temperature (-11°C).” (Line 228).

7) **Comparisons based on “maximum freezing temperature” and “highest temperature” are subject to large errors and should be viewed as rough indications. More extensive use of the concentration functions and comparisons of concentrations at fixed temperatures, as in Table 2, would improve the paper. What is the reason for stating -11◦C as the lowest observed freezing temperature (3715/7) when Table 2 and the figures show data to lower temperatures?**

We think that the sentence “Eleven of the 12 cloud samples (92 %) froze at −8°C or warmer, and none remained supercooled at temperatures below −11°C” was confusing. In fact, we meant that in eleven of the samples, freezing occurred at -8°C or warmer in at least one of the droplets testifying of the IN presence. For the last sample, the first freezing event occurred at -11°C. This does not mean that all of the droplets were frozen at this temperature, but it is the lowest temperature at which freezing was initiated in a sample.
This sentence was modified (lines 228).

8) **How can the data in Fig. 4 extend to -14◦C when the last points on Fig. 3 are at -13◦C? The impression is that the low number of samples that provided data at -13◦C and -14◦C lead the authors to some hesitation about the data presentation. It would improve the paper if the results were presented in a more consistent way. In Fig. 4, the substitution of lower bound values for those not detected introduces an upward bias in the data. How would the analysis look if only samples with measured values were included at all temperatures?**

Both Fig. 3 and Fig. 4 were constructed using data presented in Table 1. However, in Fig. 3 the values above the detection limit were omitted. On the contrary, in Fig. 4 we included the lowest possible bounds of these values, as indicated in the legend. We fully agree that this can be confusing and decided to consistently show data only down to -13°C in Figure 4, i.e. when at least 3 absolute values were available. We still included the lowest possible bounds for concentrations above the quantification limit in order to avoid to artificially decreasing the values by ignoring them, but still showing conservative estimates.

9) **The data in Table 2 gives the impression that the most heat-labile samples had relatively low total concentrations of IN. Could the authors comment on this?**

Non-parametric Spearman’s rank correlations were calculated between the different variables. The corresponding matrices (p-values, ρ, and n) are shown in Table S2. This impression would mean, if we understand it correctly, that the concentration of total IN at given temperature would be inversely correlated with the proportion of biological (heat-labile) IN.
In reality there is a trend in that direction (see the correlation matrix in Table S2), but no significant relationship between these 2 parameters at any temperature was detected. So we do not think that it is relevant to comment more on this. A sentence stating that “The proportion of biological IN in samples did not depend on the absolute total IN concentration (Table S2; $p > 0.05$).” was inserted in the text (line 252).

10) **The higher values of INPs detected in cloud water compared to precipitation (3715/17) is a significant result and, if confirmed by more data, calls for an intensive search for explanations. Even as an early indication, it is a strong motivation for more work with cloud samples even if they are considerably more difficult to obtain. It would be good to know whether the correlation stated on**

3717/10 would also hold between bacterial concentration and total INP concentration.

We reanalyzed data, and stats have been redone. It appears that a mistake was done in particular here, and the concentration of bacteria is actually not correlated with IN data. This is now indicated in the text (line 319) and shown in Table S2.

11) Minor points (page/line):

3711/8 "all" and "throughout" are redundant.

We removed the word "all" in this sentence.

3711/9 "high-temperature IN" is difficult to replace with better wording, yet is awkward to call sub-zero temperatures 'high'

We agree but did not find better expression, so we used terms such as "high negative temperature IN" or "high subzero temperature IN" to be more precise.

3713/12 CIN instead of CIN

We modified this.

3713/18-19 Fewer significant figures would be sufficient (1.6 rather than 1.59 etc.)

We agree and corrected it in the manuscript.

3716/2 As shown in Table 2 "at least 77%" should be "as low as 77%"

This sentence was modified.

3722/27 Initials for first author missing.

There is no initial for the first author of this reference (Stephanie and Waturangi, 2011).

Response to Referee #2:

General comments:
This paper adds very valuable new information to the current debate about bio-precipitation.
In particular, the authors found that
(1) Biogenic ice nuclei were active at <= -6°C and were the dominant active ice nuclei between -6 and -10°C. At -11°C and below the non-biological IN accounted for more than 1/2 of the ice nucleation activity, at -13°C reaching more than 90 % of total activity. (Fig. 2).
(2) The number of total biogenic IN (Table 2) that are active at high T (-6 to -8°C) was larger than expected based on earlier findings, e.g. approx. 100 x of Christner et al. The authors also speculate about the total biogenic IN being made up largely or even totally by intact bacteria. While that part of the paper is not convincing, it is still an interesting discussion – nevertheless the paper could stand alone without that part.

1) **Critical to the quality of the paper is in my view the severe absence of statistical analysis.**

We do not agree that no statistics was done in the original manuscript. Among many examples, we presented a PCA analysis of the data in Figure S2. In the revised manuscript, we extended this analysis with new parameters that we were able to collect afterwards (precipitation, cloud event duration, …), and we performed new stats. Correlation matrices are shown in Table S2, and text has been added accordingly (Lines 232-234, 251-253, 264-281, notably).

2) **In addition, as nice and new as the data are, a principle limitation of this work is that the mountain peak can produce orographic clouds, and thus the clouds can be contaminated by bacteria derived from the side of the mountain. Without addressing this issue, the authors cannot remove my suspicion that not all the clouds investigated were true high altitude clouds. It would be better to discuss this matter (instead of not mentioning the problem): Maybe the authors could have a meteorologist make an educated estimate how large the contribution from the surface of mountain side possibly could be?**

It is right that we cannot exclude the possible influence of local sources, so a sentence has been modified in the text to indicate it (line 289-290). However, concerning the fact that clouds could have been orographic, great care was taken for avoiding such situation. Note that new variables have been added in the analysis (see section 2.1 and answer 1) to referee 1).

Specific comments:

3) **Page 3709, line 26 - : It appears that the authors have not considered the papers by Santl-Temkiv on hailstones' bacterial content and bacteria's origin (FEMS Microbiol Ecol 81: 684–695; PLoS ONE 8(1): e53550).**

Those references are not about IN, so we think that citing these would be out of purpose here. The only reference by this group that deals with IN that we found (Šantl-Temkiv et al., 2009) originated from a conference:
Šantl-Temkiv, T., Gosewinkel-Karlson, U., Finster, K., and Munk Hansen, B.: The diversity and proportion of ice nucleation active bacteria in rain and their ability to produce extracellular ice nucleation active particles, 18th International Conference on Nucleation and Atmospheric Aerosols (ICNAA), 1460–1466, Prague, Czech Republic, 10–14 August 2009.

4) **Page 3716, line 23: The statement on the bacteria targeted by lysozyme is not correct. See also the interactive comment by C. Morris on this matter.**

See answer 2) to C. Morris's comments.

5) **Page 3719, line 1-2: OVERREPRESENTATION does not seem to be what the authors mean here. Either the use of this term should be explained, or the expression deleted.**

We agree that the term "overrepresentation" was not appropriate as this referred to an inferred maximum possible value, rather to an actual value. The corresponding sentence has been removed.

Pages 3718-3719: Otherwise I agree with the authors' conclusions. See my general comments, above.

6) **Page 3722, lines 23-25: I should think that this conference presentation can be replaced by citable publications originating from the same authors.**

See answer 3) above. No other publication concerning IN from these authors seems to exist.

7) **Table 1: I strongly suggest that the sample volumes should be added as a separate column. This will enable the readers to fully appreciate the work done, and to use the data for their own considerations and estimates in the future. Another information that I feel is missing is the type of cloud(s) encountered in each event, e.g. convective, stratus, orographic.**

Samples volumes are now presented in Table 1. Concerning the type of clouds sampled, we now show satellite images which show overviews of the meteorological situation over Europe and France at the moments of sampling.

8) **Table 2, and corresponding discussion in the text: I miss a calculation of the number of IN per cloud droplet. Based on their LWC, the authors should be able to provide such an estimate. For the readers, such numbers would aid in appreciating the significance of the authors' findings for cloud physics.**

We agree that the number of IN per droplet is an interesting feature so we added a simple estimation based on the droplet diameter to the manuscript (lines 291-295). We considered cloud droplets as spherical objects, with a volume (mL) $= \frac{4}{3}\,\pi\, r^3$, where r is the droplet radius (cm). From this value, we calculated the concentration of IN per droplet ($[IN]_d$) as $[IN]_d = V \times [IN]$ with [IN] the concentration of ice nuclei per mL of cloud water.

9) **Table 3, legend: (1) DETECTION limit is the wrong term, as the authors are referring to an upper limit of quantification (accidentally) caused by how much the droplets were diluted.**

We replaced "our detection limit" by "experimental quantification limit"

(2) Please change first sentence to "Inferred maximum possible fraction of INA bacteria among total bacteria . . ."

This has been changed.

10) **Fig. 2: (1) Should the y-axis label not read ". . .frozen droplets", instead of ". . . samples"?**

The Y-axis was referring to the proportion of cloud samples for which at least one droplet froze during droplet freezing assays.

2) Please change first sentence to "Cumulative proportion of frozen samples (droplets?) at specific freezing temperatures . . .".

It is now changed by "Cumulative proportion of cloud samples for which at least one freezing event was observed during IN assays, in the absence of treatment (shaded bars) or after heating at 95°C for 10 minutes (black bars)."

(1)Please include error bars.

It is not possible to include error bars here as this figure shows absolute frequencies.

11) **Fig. 3: What is the reason for showing every single data point, i.e. individual samples? The whole paper lacks statistics. Here is one obvious place to offer to the reader a regression, with corresponding statistics, over all samples.**

We showed, already in the original manuscript, both every single data point, in Figure 3, to allow the reader to figure out the variability of profiles, and consensus, i.e. averages, in Figure 4.

12) **Fig. 4: Also here the use of statistics would improve the paper. Here the reader would appreciate seeing error bars, confidence intervals, or similar measures of statistical significance.**

Error bars have been inserted in Figure 4.

13) **Fig. 5: (1) Again, what is the reason for showing every single data point, i.e. individual samples? Also here is one obvious place to offer to the reader a regression, with corresponding statistics, over all samples.**
(2) The data points are the same as in Table 3. There is no need to show the data twice – unless . . . see remark no. 1). (3) The y-axis scale is correct, but confusing. Please eliminate the "%" and change the numbers on the scale accordingly (e.g. 10% becomes 10e1). (4) Please change the sentence to "Inferred maximum possible fraction of INA bacteria among total bacteria . . ."

Figure 5 is not presented anymore in the manuscript.

14) **Technical corrections:**

Please re-work the whole manuscript for using grammatically and syntactically correct English. Here is a non-exhaustive list of spelling errors and wrong use of words:
CloudY air, several places in the paper, including abstract and legends
Back trajectory PLOTS (these are called back trajectories, not plots)
Cloud'S microphysics
At –10°C there WERE . . ., seen in the abstract (correct to say "were observed, were measured, but not just "there were")
guarantY, page 3710
wrong use of "nor", page 3710
MaterialS, Page 3712, line 1
CIN, page 3713, line 12, (please put IN in subscript)
HITTING the puy de . . ., page 3714 (better: "reaching" or "arriving at")
otherS, page 3716, line 23

All these have been corrected.

Response to Referee #3:

The study under review quantified the amount of biological particles or better bacterial cells /INA proteins in cloud water samples. As there is still a controversial discussion about the importance of biological particles for atmospheric processes, this study provides important information about the possible number of bacteria in clouds.
The freezing method used in the study is comparably insensitive, due to the use of large droplets containing a lot of material, but it is still a powerful method to investigate whether a sample freezes or not and how a sample changes if it is treated for example by heat, as it was done here.
The authors focused on the determination of heat sensitive INA proteins in the samples which are the most active biological particles known so far. It would have been interesting to see if the samples changed further when they were treated with other procedures, which can also destroy heat insensitive biological IN. So, as already stated in the paper, this study gives only the lowest possible value for concentrations of biological IN, which nevertheless still is important information.
However, the title of the study promises that information about biological particles in general would be given. As this is not done, the authors should change their title as to not give promises that cannot be fulfilled.

We do not agree on the fact that the tittle promises such result. Yet, we modify it by "Quantification of ice nuclei active at near 0°C temperatures in low altitude clouds at the puy de Dôme atmospheric station (1465 m a.s.l.)" for giving more precise information about the content of the manuscript.

> **1) In the presentation of the procedure and the data I found some missing and contradictory information. p3712, 11: Some samples already froze during the sampling procedure. Is it possible that this makes any differences?**

See answer 2) to referee 1.

> **2) p3714, 6: Why do you use sometimes 32 and sometimes 160 droplets? The information how many droplets were used for each experiment could be added to table 2. What is the uncertainty for the two types of experiments? Is it possible to get some error bars to figure 3 and 5?**

We actually replicated 5 times assays of 32 droplets in some samples. Since the standard error was very low at all temperatures (< 4 IN mL^{-1}) and that these replicates in reality corresponded to a larger amount of droplets, basically, we decided to present it as 160 droplets rather than replicates of 32 (5*32 = 160). We have included the number of droplets used for each sample in Table 2. The number of droplet assayed had no influence on the data ($p > 0.05$; Spearman's correlation test).

> **3) - p3714, 16: It would be interesting to know the value of the dilution factor Df and how it is determined.**

In our case, the dilution factor is 1: we used cloud water directly without any dilution.

> **4) - p3716, 7: I think it is better to say:"none of the samples remained completely supercooled at temperatures below -11°C", because if I understand it correctly,**

you want to say that at -11°C every sample showed at least one frozen droplet. In your statement it sounds that all droplets of all samples were frozen at -11°C.

We agree that these sentences were confusing, so we rephrased it as for example "In 11 of the 12 cloud samples (92%), the onset temperature of freezing (i.e. temperature at which the first droplet froze) was -8°C or warmer. Only sample #87 started to freeze at colder temperature (-11°C)." (Line 228).

5) - p3716, 7: a reference would be nice at that point (e.g. Pummer et al., 2013)

We added the following reference: Pummer, B. G., Bauer, H., Bernardi, J., Bleicher, S. and Grothe, H.: Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen, Atmos. Chem. Phys., 12(5), 2541–2550, doi:10.5194/acp-12-2541-2012, 2012.

6) - p3717, 10: Why at -9°C?

We reanalyzed data, and stats have been redone. It appears that a mistake was done, and the concentration of bacteria is actually not correlated with IN data. This is now indicated in the text and shown in Table S2.

7) - Fig1: This is potentially a very interesting sketch, but the font is quite small. If you want that your readers can get anything from this plot, you either have to make sure that it will cover one complete page in a possible final publication, or better still increase the size of the fond!

We were not able to increase the size of the font without altering the figure. So this will be presented as a complete page if possible in the manuscript.

Main changes in our manuscript:

In this new version, we took in consideration the reviewers critics and advices, as well as the comments posted during the interactive discussion step. We modified the manuscript title as recommended by one of the referees. Our manuscript is now entitled "Quantification of ice nuclei active at near 0°C temperatures in low altitude clouds at the puy de Dôme atmospheric station (1465 m a.s.l.)".

We included:

- new information about meteorological conditions before, during and after cloud sampling and we notably added a new figure in supplement (Fig S1) showing relative humidity and liquid water content measured at the sampling site, precipitation rates around the collection site and satellite images ;
- new statistics, in particular a new supplementary table (Table S2) gathering correlations between meteorological, physico-chemical parameters and IN measurements.

Modifications were also performed according to particular comments. In particular, we modified:

- Table 1 to add new information about volumes sampled and the duration of the collection
- Figure 4 according to Reviewer 1's comments (x-range was reduced to fit data presented on Figure 3).
- Figure 5 was removed as suggested as it was redundant with Table 2.

Finally, we took in consideration the reviewers corrections concerning English mistakes.