Response to RC1

(comments in black, response in blue, *changes made to manuscript in italic [page and line numbers refer to revised version]*)

In this measurement report, the authors present ice nucleation data of aerosols sampled on filters between January 2019 and March 2021 at the high-altitude research station Jungfraujoch in Switzerland. The authors present concentrations of ice nucleating particles active above a supercooling temperature of -10°C and -15°C in the lower free troposphere (FT). Radon isotope data was used to distinguish between the FT and planetary boundary layer (PBL). The authors chose to perform a heat treatment experiment to study the composition of the aerosol samples and they discuss the influence of occasional PBL events (Sahara dust) on the INP concentration. The work presented merits publication in Atmospheric Chemistry and Physics after minor revisions.

In general, the study provides further evidence that the role of biological INPs to ice formation in the lower troposphere should not be underestimated. However, one major concern to me is that the authors only use heat treatment tests to evaluate the composition of aerosols. Heat treatment experiments have gained acceptance in the scientific community to broadly categorize INPs. However, heat tests alone do not identify certain microorganism. Have you considered additional investigations of the studied samples to get more thorough information (e.g. cultivating microorganisms)? If not, alternative methods should be mentioned in the paper and the limitation of the results needs to be discussed in more detail.

We are grateful to the reviewer for having read our manuscript and for the overall judgement.

Indeed, the use of heat tests provides only for a coarse categorisation of INP, whereas cultivation methods are more precise but also require much larger sample volumes. In an earlier study at Jungfraujoch, Stopelli et al. (2017) have targeted by selective cultivation *Pseudomonas syringae* in freshly fallen snow. In only 3 of 13 samples P. syringae was found in form of 2, 4 and 45 colony-forming units per litre of melted snow. The maximum of these three values corresponds to 0.02 colony forming units in 1 m^{-3} of air (45 colony forming units / 1000 g water * 0.4 g water m^{-3} air), assuming a condensed water content of 0.4 g m^{-3} (Petters and Wright, 2015). Overall, cultivable cells of *P. syringae* constituted a tiny fraction (10^{-4}) of all INP_{-8} in the investigated snow samples.

Apart from the limitations imposed by sample volume, INP in the atmosphere probably include a substantial fraction of cell-free INP that are not cultivable, such as macromolecules shed by hyphae of *Mortierella alpina* (Fröhlich-Nowoisky et al., 2015).

Introduction:

The introduction is written very clearly. However, the section would be improved if the authors would elaborate more on aerosol transportation. The authors suggest epiphytic microorganisms (MO) to be the major INP. How do you think MO are transported from the PBL to the FT? In last years, many studies have suggested that rain events over vegetation lead to an increase in bio-INP concentration (e.g. Huffman et al.2013, Prenni et al., 2013, Iwata et al., 2019). Do you think these events are important for the transportation of MO to the free troposphere?

Yes, we definitively think the aerosolisation of MO is intensified by rain events. When rain comes with a frontal system or a thunderstorm part of the MO are probably mixed with or injected to the

FT. Another possibility is injection to the FT from PBL air masses advected to mountains. We have added the following text near the end of the introduction:

Aerosolisation of biological particles from vegetated land surface to the PBL is intensified during rainfall (Huffman et al. 2013; Prenni et al., 2013; Iwata et al., 2019). Subsequent transport from the PBL to the FT may happen through mixing in frontal systems, cloud convection, or by mountain venting (Henne et al., 2005). [page 2, lines 51 to 53]

In line 36 the authors write: "[…] at more moderate supercooling (here ≥ -15°C) biological particles constitute the main part of the INP population, at least in the PBL." Doesn't that strongly depend on the location? There are also mineral particles which nucleate ice at temperatures >-15°C (see e.g. Harrison et al., 2016). Please elaborate more clearly.

We do not exclude that also mineral particles contribute to the INP population in the PBL. Yet, heat tests suggest they constitute a minor fraction even in dust events. The debatable part of the introduction is changed accordingly, also in response to a comment made by Reviewer #2:

Heat deactivates biological INP but leaves mineral INP largely unaffected (Hill et al., 2016). Chen et al. (2021) found during dust events sampled in Beijing that 70% of INP active at ≥ -15 °C (INP_{-15}) were heat-sensitive. Observed heat-sensitive fractions of INP_{-15} in the PBL of agricultural areas in the USA (Suski et al., 2018) and in Argentina (Testa et al., 2021) were even larger (> 90%). These findings contrast with a small biological fraction detected among ice particle residuals collected at Jungfraujoch from mixed-phase clouds and classified by physico-chemical analyses (Mertes et al., 2007) or by laser ablation mass-spectrometry (Schmidt et al., 2017; Lacher et al., 2021). Not every ice particle residual has initiated as INP the formation of the ice particle it was recovered from, in particular not when it was recovered from a secondary ice particle. An ice particle residual recovered from a primary ice particle and classified by mass spectrometry as mineral dust may have been activated at moderate supercooling by a minor, ice-nucleation active biological component sticking to its surface (Augustin-Bauditz et al., 2016). A heat test, which specifically targets the ice-nucleation active component, would have classified the same assembly as biological INP. [page 1, line 38 to page 2, line 49]

Materials and methods:

2.1.

In total 72 punches were analysed for their ice nucleation activity. For me it is unclear, how many punches per filter were used for the ice nucleation measurements? Could you please clarify this in the manuscript?

The issue was clarified by changing the sentence:

From each selected filter we took 72 punches with 2 mm diameter each; all 72 punches together contained aerosol particles from a total of 10.6 m^{-3}. [page 2, lines 66 and 67]

Additionally, I am wondering whether such large filter pieces influence the measurement. The paper would greatly benefit if results of a background measurement are provided in the appendix.

We have added results of the background (blank) measurements to the appendix (Table A3). They show that the filter material has little influence on the measurement. To the first paragraph of the Results and discussion section we have added:

A majority of INP_{-10} (83%) and IN_{P-15} (57%) in the FT were heat sensitive and lost their activity after exposure to 60 °C (Table 1; for INP active at other temperatures between -8 °C and -15 °C see Table A3). [page 4, lines 125 and 126; added Table on page 18]

The authors state that the ice nucleation data was adjusted with a background correction. Was this done in accordance to Vali, 2019?

The background correction according to Vali (2019) is a powerful tool when analysing freezing spectra. In our manuscript we focus on INP active at two temperatures only, -10 °C and -15 °C. Therefore, we have simply subtracted the cumulative blank value from the cumulative sample value at either temperature.

Could you please clarify what filter-fringes are? (line 59)

We have expanded the description accordingly:

Two blank (background) assays were done with punches from 5 mm wide fringes of sample filters. This part of a filter is covered by the clamp rings holding it in place during sampling. That way, sampled air does not pass through it and it remains clean. However, during handling and transport some particles may get smeared from the active filter area onto this narrow fringe. Consequently, these blank values are a conservative (upper) estimate of a field blank. Each of these blanks was composed of punches from four filters. Sample values were corrected for blank values by subtracting the average of both blanks, which on average was 7% of a sample value. [page 3, lines 82 to 89]

Results and discussion

My main criticism on the paper is the identification of microorganisms, based only on heat treatment experiments. How can you exclude that repeated freeze-thaw cycles be partially responsible for the loss of ice nucleation activity (see e.g. Polen et al., 2016)? Please discuss more clearly.

This is an important issue. We think it is best discussed in the materials and methods section. We have added several lines to the end of its first paragraph:

Loss of INP active < -10 °C due to the repeated freezing is unlikely in this study. Although Polen et al. (2016) have observed throughout five repeated freezing assays some loss of INP active > -5 °C, little had changed in INP active < -5 °C (see Fig. 6 in Polen et al., 2016). A more extensive set of tests was conducted by Vali (2008) on a soil sample. After 55 refreezing cycles an increasingly larger fraction of INP had been lost above -10 °C toward the warmer end of the freezing spectrum, but the concentration of INP active < -10 °C had remained practically the same (Fig. 1c in Vali, 2008). [page 2, lines 72 to 77]

Table 1: The table would be improved if the authors could write the amount of analysed samples to each category. In addition, I was wondering why the sum of the categories heat sensitive, moderately heat tolerant and heat tolerant do not reach the value of the category all? The table caption starts with an explanation of the standard deviations; wouldn't it be better to first mention the INP concentration?

We have added the number of samples analysed in each category. The sum of the three categories does not to match the value of category 'all' because these values are not averages but medians of differently skewed distributions.

We changed the start of the caption to:

INP concentration found in the free troposphere (FT) and during Saharan dust intrusions (SD), categorised according to heat sensitivity. Median and multiplicative standard deviation are shown. [page 11, lines 429 and 439]

Table 2 gives a very good overview of the reported heat sensitivity of INPs in the literature.

Thank you for this comment.

Line 145: Do you think that impingers would be more efficient to sample and analyse INPs in general? Since INPs only occur in low concentrations in the FT, filter pose a challenge that an impinger may be able to handle. With an impinger, INPs are directly impacted in a solution which can be used in a freezing assay and the intermediate step of suspending INPs from a filter is avoided. Could you maybe discuss this issue in more detail?

That is correct. A high volume impinger, such as the one used by Mignani et al. (2021) with a flow rate of 300 L/min has the advantages you mention. In addition, it allows for a higher resolution in INP time series. But impingers also have an important disadvantage: They still require the continuous presence of an operator. Maybe, one day someone will develop an automated impinger similar to the automated continuous flow diffusion chamber developed by Brunner and Kanji (2021). Until this has happened, the use of impingers is limited to field campaigns. In contrast, the filters we have been using are the result of a continuous, ongoing monitoring effort and are available for almost every day of many years back. The sampling unit requires a little attention only once a fortnight. With the filter archive we can address questions arising after the events we eventually become interested in have happened. Impingers and filters are complementary approaches. We think the filters have well served the purpose of investigating INP composition throughout a longer time period in FT and during several SD events.

Figure 3. In contrast to the previous study, the authors do not see a seasonal trend in their data. However, in this study only one data point was considered during summer time. This seems too weak to clearly rule out seasonal dependency. I would encourage the authors to lower the strength of the argument.

We have changed the contentious beginning of the paragraph:

A surprising feature of INP_{-10} in FT samples is their narrow distribution (1.0 to 5.6 m^{-3}) throughout the year (Fig. 3), which is in contrast to what we had found earlier at Jungfraujoch in filters not selected for FT conditions that covered a range of three orders of magnitude (Conen et al., 2015). [page 5, lines 185 to 187]

Technical comments:

Line 27: A minus sign is missing [...] "-"5°C […]

Corrected.

Line 30: Another minus sign is missing [...] "-"31°C […]

Corrected.

Line 30: This sentence requires a citation. Which other regions? Please specify.

Completed the sentence:

In FT conditions at the high-altitude observatory Jungfraujoch (3580 m a.s.l.) in the Swiss Alps, Lacher et al. (2018) found similar concentrations of INP active at -31 °C (INP_{-31}) as had been reported for the FT in other regions of the world (summarised in Table 2 and Figure 6 in Lacher et al., 2018), and little seasonal variation. [page 1, lines 30 to 33]

Line 35: Another minus sign is missing [...] "-"15°C […]

Corrected.

Line 46: Superscript number 3: "[…] air was sampled at a rate of 720 m"³" day-1

Corrected.

Line 60: It should be "Sample values […]" not Sample"s"

Corrected.

Line 81: Saharan dust intrusion (SD) – the abbreviation was already introduced in the introduction (line 38).

Corrected.

Line 102: "Fusarium graminearum" and "Puccinia sp." should be written in italic

Done.

Line 105: Maybe reconsider the sentence position. We found …

Done.

Line 106: "Mortierella alpine" should be written in italic

Done.

Line 123: change phrase to"[…] but excluded one sample from further analysis […]"

Done.

Line 373: Superscript -3: "[…] 0.095 INP m-3"

Done.

Line 440: Remove space between the minus sign and the number "-10 °C"

Done.

Line 444: It should be Figure "3"

Corrected.

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