

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.

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?

In line 36 the authors write: *"[...] at more moderate supercooling (here $\geq -15^{\circ}\text{C}$) 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 $>-15^{\circ}\text{C}$ (see e.g. Harrison et al., 2016). Please elaborate more clearly.

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?

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.

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

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

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.

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?

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

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?

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.

Technical comments:

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

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

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

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

Line 46: Superscript number 3: “[...] air was sampled at a rate of 720 m³” day⁻¹

Line 60: It should be “Sample values [...]” not Sample“s”

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

Line 102: “*Fusarium graminearum*” and “*Puccinia sp.*” should be written in italic

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

Line 106: “*Mortierella alpine*” should be written in italic

Line 123: change phrase to “[...] but excluded *one sample* from further analysis [...]”

Line 373: Superscript -3: “[...] 0.095 INP m⁻³”

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

Line 444: It should be Figure “3”

References:

Harrison, Alexander D., et al. "Not all feldspars are equal: a survey of ice nucleating properties across the feldspar group of minerals." *Atmospheric Chemistry and Physics* 16.17 (2016): 10927-10940.

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Iwata, A.; Imura, M.; Hama, M.; Maki, T.; Tsuchiya, N.; Kunihiya, R.; Matsuki, A. Release of Highly Active Ice Nucleating Biological Particles Associated with Rain. *Atmosphere* 2019, 10, 605.

Vali, G. Revisiting the differential freezing nucleus spectra derived from drop-freezing experiments: Methods of calculation, applications, and confidence limits. *Atmos. Meas. Tech.* 2019, 12, 1219–1231.

Polen, M., Lawlis, E., & Sullivan, R. C. The unstable ice nucleation properties of Snomax® bacterial particles. *Journal of Geophysical Research: Atmospheres*, 2016, 121(19), 11-666.