

The Manuscript entitled “The adsorption of fungal ice-nucleating proteins on mineral dusts: a terrestrial reservoir of atmospheric ice-nucleating particles” deals with the potential of proteins to serve as cell free and available IN in soils. In detail, clay and cell free ice nuclei washed from the mycelial cell wall from *Fusarium avenaceum* are used in laboratory experiments as demonstration. Basically, the manuscript is very well written, and the background is precisely elaborated. Therefore I can easily give the manuscript the excellent for the form. The topic of organic and biological residues is of major interest in this field of science. As stated in the manuscript, most mineral particles are covered with carbonaceous matter originating from organic and/or biological sources. I agree here with the authors these might be of significant impact and also might have relevance in water cycling.

Fusarium has spread to the whole World. Especially *Fusarium avenaceum* as generalist in plant pathogenicity commonly found to be IN active contribute a huge amount of biological particles on our Earth. However, there would be a gain for the data. It is obvious, that there is a chemical or biochemical modification of the clay upon incubation with *F.avenaceum* washing water. But there is no experiment showing it to be proteins. A protein extraction to recover proteins from the clay or incubated clay used in a protein assay (e.g. BCA or Bradford) could show proteins or proteinaceous compounds bound to the clay. Although it is known that proteins adsorb to minerals, this would fortify the interpretations, and could also be related to the impact of the different cations in either protein or proteinaceous IN adsorption to the clay. Because of the complex composition of biological samples, an experiment showing protein being enriched in the treated clay would be a gain. Anyhow, the results are related to the conclusion as well as I generally agree with the interpretation. The protein assay is not mandatory for the manuscript and the conclusions are concise. Therefore I recommend publication of the manuscript in ACP after the open discussion.

We thank referee #1 for both taking the time to review our manuscript and for his/her insightful comments. Our replies are given in blue, while changes to the text are listed in *blue italic*.

With regards to there being no experiment showing that the modification of the clay upon incubation with *Fusarium* washing waters is caused by a protein, on the basis of evidence in the literature, it is very unlikely that proteinaceous compounds are not involved. The proteinaceous characteristics of INPs from *F. avenaceum* include the thermal lability of INPs, and susceptibility to treatment with proteinase K (*Hasegawa et al (1994)*). Agents such as phenylboric acid and chloroform did not affect activity, suggesting that lipids and saccharides are not central to its nucleating ability (*Hasegawa et al (1994)*). Also of note, for the ice nucleating fungus *Fusarium acuminatum*, genes coding for the ice-nucleating protein have reportedly been expressed in *E. coli* (*Lagzian et al., 2014*).

On the basis of this, within the manuscript we referred to the INPs as “proteinaceous-INPs”, consistent with the literature. We agree that further purification, isolation and characterisation of the macromolecule(s) responsible for activity in *F. avenaceum* is certainly of interest, but we suggest that this is an area of future research, rather than being critical for the conclusions drawn in this article.

We have highlighted the previous literature which supports INPs in the washing waters as being proteinaceous in the introduction for the interested reader to gain an understanding of the current state of knowledge:

“Similarly, a single gram of *Fusarium avenaceum* harbours 10⁸ nano-sized INPs active at temperatures above -7 °C (O’Sullivan et al., 2015). These INP appear to be proteinaceous as inferred from their thermal lability, combined with their susceptibility to treatment with Proteinase K (*Hasegawa et al (1994)*)”

References

Hasegawa, Y., Ishihara, Y., and Tokuyama, T.: Characteristics of ice-nucleation activity in *Fusarium avenaceum* IFO 7158, *Biosci., Biotechnol., Biochem.*, 58, 2273-2274, 10.1271/bbb.58.2273, 1994.

Lagzian, M., Latifi, A. M., Bassami, M. R., and Mirzaei, M.: An ice nucleation protein from *Fusarium acuminatum*: cloning, expression, biochemical characterization and computational modeling, *Biotechnol. Lett.*, 36, 2043-2051, 2014.