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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Sources of organic ice nucleating particles in soils" *by* T. C. J. Hill et al.

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Biological ice nucleating particles (INP) in the atmosphere have raised the attention in the last decades for their probable implication in cloud freezing and precipitation. Among the different sources of primary aerosols, soils could contribute to the release of biological INPs.

The aim of this study is clearly presented: decipher the nature of INPs in soils. To address this, several soil samples were subjected to a range of treatments targeting different types of biological material: heating at 60°C and 105°C (heat-labile proteins), H2O2 (total organic matter), acid (humics), chloroforme (sterols and aliphatics), enzymes (lysozyme against bacterial IN activity, papain protease and proteinase K against proteins), the rest, resistant to all treatments, was considered mineral. The abundance of INP in treated and in untreated samples was measured by immersion freezing assays. The results show that ice nucleation is largely dominated by bio-

logical material at temperatures > -9°C, with different types of structures contributing: bacteria, proteins and other macromolecules, and others unidentified compounds recalcitrant to most treatments.

In parallel, the authors looked for the presence of known IN bacteria in the soils by targeted PCR, but failed to detect any copy of the Ina gene partly due to PCR inhibition by the soil matrices. In addition, serial dilutions of samples that froze during IN assays were done for attempting to isolate and observe by microscopy the particle from which ice was likely originally formed.

This is an interesting study bringing new information about INPs in soils. It gathers many extraction techniques for selectively remove various types of organic compounds and try to determine the relative contribution of each to the global ice nucleation capacity of soil samples. It is well written, well constructed, potential weaknesses are discussed in each subsection, and it is well referenced.

I have a few more or less major/minor comments and criticisms that I hope the authors can answer for finalizing the manuscript.

First, I found the term "source" quite misleading at some places in the manuscript (including the title) as it generally refers to a process, a geographical area or something related to it (category of landscape or else). Here it refers to different families of molecules of unidentified origin, for most, so the word "nature" rather than "source" would be more appropriate, to my opinion. Then, one of my concerns is about sample storage. It is mentioned that these were kept at 4°C, but for how long? Could the biological content of the samples have been modified during storage? The range of treatments used for targeting different classes of organic material is interesting, but probably a bit too affirmative concerning the actual efficiency and specificities. My main point here is the different treatments are presented as quite specific, i.e. targeting very narrow families of molecules, but they probably also alter untargeted (organic or mineral) molecules or incompletely remove those targeted, and this is not always really

discussed.

Concerning H2O2 treatment, it is said in the abstract “Removal of SOM with H2O2 effectively removed all INPs active >-18 °C”, which is obviously not right, or I am missing something, looking at Figure 2 (some of the treated samples were frozen by this temperature). Furthermore, the method involves 15% H2O2 and boiling for 1 day. Since there is no mention of it, I assume this was done in the dark in the absence of UV light. How was it determined that “all SOM and then all residual H2O2 was decomposed”? (page 4, line 9).

Hydrogen peroxide treatment is used as a procedure for degrading OM in soils. However, a treatment at 15% H2O2 followed by 30% H2O2 at 70°C for 1-3 days only removes ∼80-90% of it (Leifeld and Kögel-Knabner, 2001). Even worst: there is sometimes still more than 50% of the original OM left in soils after 20-40 days treatment at 30% H2O2 (Eusterhues et al., 2005). The action of H2O2 on OM oxidation is based on the production of OH radicals, which requires the presence of catalysts like UV, O3 or iron for example (Kitis and Kaplan, 2007; Matilainen and Sillanpää, 2010). So, without addition of such catalysts in your samples along with H2O2, the efficiency of removal is totally dependent on the intrinsic chemical properties of your soils. Hence, I am wondering how much OM is left in your treated samples. In any case, you cannot affirm that OM is removed completely from the soils, and should acknowledge on the fact that a fraction of your “mineral INP” in figure 12 is actually probably still organic. I think that this is consistent with the fact that “mineral INPs”, as named in fig 12, start inducing freezing at as warm as -6°C while this is generally not observed with minerals tested pure. If you still have some samples of your soils, it would be interesting to determine how much OM is left after such H2O2 treatment, and what is the iron content in these samples.

Heat-treatment is currently widely used as a method for suppressing proteinaceous IN activity. It is mentioned in the conclusion that it may also modify the IN ability of crystals of organic material by dissolving them. Concerning mineral crystals, the reference cited

(Zolles et al. 2015) indeed reports a little effect of heating at 250°C on the IN activity of feldspar due to surface modification. To me this is a different phenomenon which does not attest of the absence of mineral dissolution in your samples. Also, I do not get why the lack of effect of heating at 60°C demonstrates that this was not the case at 105°C (page 14, line 14). Can you clarify this?

Acid treatment for removing humic and fulvic acids: a method employed by the International Humic Substance Society was used. It involves concentrated HCl and further neutralization with NaOH. Even if the soils were decanted upon treatment, concentrated HCl and NaOH have probably modified the ionic strength of the samples (these were apparently not rinsed), and I am wondering to which extent this affected the results observed. Could you give information on that? Acid treatment also denaturate many other organic molecules than humic and fulvic acids (as mentioned page 9 line 24), and it also probably solubilizes metals (Snape et al., 2004) which are known IN (Chen et al., 1998; Phillips et al., 2008). Maybe metals are comprised into the definition of “fulvics” given page 9 line 6? This should at least be mentioned and discussed. This is also relevant since, independently of their IN activity, metals like iron are suspected to be complexed with HULIS in the atmosphere (e.g., Parazols et al., 2006).

Similarly, chloroform extracts lipids and so it is likely to inactivate IN due to bacteria. Results shows that chloroform treatment had no significant effect so suggesting to me that bacterial IN was not significant in the samples, also confirmed by PCR approach. However, lysozyme had little effect, leading to the conclusion that bacterial INPs were present but below the detection limit of PCR. Do you have evidence that bacterial INP could resist chloroform treatment? If not, how can you explain that chloroform did not affect bacteria (discrepancy between chloroform and lysozyme treatments)? About the PCR products showed in figure 7, it seems to me that a band corresponding to the gene targeted was actually present in the pasture soil (the gel is not completely horizontal in the image). How can you affirm this was not the right band? Have you any other information not mentioned in the text that helped you decide?

The exploratory method attempted for isolating particles by dichotomy and observe them, is new, to my knowledge, and I found the idea quite interesting for further investigations. Just for this reason it deserves to be presented here. However, at this stage of development this did not bring much information about soil INPs, except these are indeed particles and that they are aggregates of multiple unidentified compounds. It was to me mainly "recreational" in the paper, and probably a bit too affirmative, with no evidence for it, that the particle observed was indeed the INP (notably the first sentence page 12 line 28).

Fig 12 is an interesting summary of the results for the pasture soil, but it needs to be completed and probably modified. First, this sample was not subjected to chloroform, so the conclusion that the refractory biological IN are not removed by chloroform in this sample (page 15, line 4) is obviously erroneous. Also, considering my comments about H2O2 treatment, the mineral fraction should be even smaller, or uncertainties somehow indicated. Finally, legend is incomplete: why are there dotted and straight lines? What do represent the green line? And it is not clear to me (although I can guess) where is the "notable segment of organic INPs active below -10 to -12 °C that was unaffected by any challenge short of oxidation with H2O2" mentioned in the text. Perhaps I would be a good idea to indicate it on the Figure.

Typing and references errors: - Page 6, line 2: some words are missing in this sentence. - Bigg et al. 2015, Wright et al. 2015 (page 2 line 16), O'Sullivan et al. 2011 (page 2 line 22) and Popovitz et al. 1994 (page 10 line 2) - are missing in the list of references; - Check Tobo et al. (2104!) (page 4 line 15) and Zolles et al (2105!) (Page 6, line 17); - Pouleur et al. 92 should be 1992 (page 5 line 19); - Gavish et al. 1980 should be 1990 (page 18); - Balch et al. 2013 (page 16), DeMott and Prenni 2010 (page 17), Hayes et al. 2001 (page 18), Rigg et al. 2013 (page 22) and Wagenbrenner et al. 2013 (page 23) are not cited in the text.

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