

We thank Anonymous Referee #2 for his/her thoughtful comments and useful discussion. Below are our point-by-point responses.

**Reviewer's comment [1]:**

page 9706, line 2-3: Is the importance of desert soil dusts for ice nucleation really relatively well understood? “Relatively” in that context could be misunderstood, and I’d recommend weakening the statement a bit more.

**Authors' response [1]:**

We have replaced “*relatively well understood*” with “*widely acknowledged*” [Page 9706, Line 4].

**Reviewer's comment [2]:**

page 9706, line 22: It is textbook-knowledge that IN are required to trigger ice nucleation at temperatures warmer than about  $-36^{\circ}\text{C}$ , and instead of citing these three newer publications, I recommend citing the Pruppacher and Klett book (e.g. already the version from 1978 or any of the newer prints, and the knowledge as such has been around even longer).

**Authors' response [2]:**

We have added Pruppacher and Klett (1997). However, the three publications (Szyrmer and Zawadzki, 1997; Hoose and Möhler, 2012; Murray et al., 2012) are review papers and cover more recent knowledge about the role of specific particle types (e.g., dust, soot, volcanic ash and biological materials) as IN active at temperatures warmer than about  $-36^{\circ}\text{C}$ . For this reason, we believe that these three publications are also appropriate.

**Reviewer's comment [3]:**

page 9706, line 26: The work by Hoose et al. (2010) you cite here and a second paper by Hoose et al. (2010b) both came to the conclusion (based on modeling), that there is only a marginal / negligible contribution of biological material to global ice nucleation and hence to not support your statement here. The Hoose et al. (2010) cite should be removed, or you have to discuss that it comes to a controversial conclusion. However, there is literature around

which explicitly dealt with the importance of soil dust for ice nucleation (Conen et al. (2011) and O’Sullivan et al. (2014)) – you cite this later, but please cite these two here, too.

**Authors’ response [3]:**

In this statement, we intended to explain about “desert soil dusts” and not agricultural soil dusts. Thus, we believe that we cite the work by Hoose et al. (2010) correctly. We feel that this reviewer’s comment (misunderstanding?) results from the fact that we used the wording “soil dusts” in this sentence, and then explained that “all soil dust IN are regarded as minerals from desert sources” in the following sentence. To avoid misunderstanding, we have rewritten the sentences as follows:

*Page 9706, Line 23 to Page 9707, Line 3: “In particular, laboratory and modeling studies suggest that desert soil dusts composed predominantly of minerals are the most important IN sources at temperatures between about  $-36^{\circ}\text{C}$  and  $-15^{\circ}\text{C}$  because of their ice nucleation properties and abundances in the global atmosphere (Hoose et al., 2010; Murray et al., 2012). In the estimates from these studies, the contribution of other dusts (e.g., agricultural soil dusts) to the global atmospheric IN population has not been taken into account.”*

**Reviewer’s comment [4]:**

page 9707, line 2-3: “has not been taken into account” – this contradicts the previous sentence, where you stated that “suggest that soil dusts are the most important IN sources at temperatures between about  $-36^{\circ}\text{C}$  and  $-15^{\circ}\text{C}$ ”. This sentence needs to be rewritten, also based on the fact that I suggest changing the citations in the previous sentence.

**Authors’ response [4]:**

See Authors’ response [3].

**Reviewer’s comment [5]:**

page 9707, line 13: “The chemical composition of individual particles capable of nucleating ice has remained uncertain.” More work than you suggest here has been done in this respect. E.g. Szyrmer and Zawadski (1997) list a variety of biogenic IN (fungi, bacteria, pollen, etc.). A recent publication by Hartmann et al. (2013) summarizes exemplary work of the past 4 decades, describing that it is a protein complex which causes the ice nucleation activity for

bacteria. There, it is also argued that these complexes can occur separated from the original bacteria, attached only to some membrane fragment, and that those were found to be preserved and maybe accumulated when being connected to mineral surfaces (Kleber et al., 2007). Furthermore, Pummer et al. (2012) and Augustin et al. (2013) both examine pollen washing water, showing that there is a small macromolecule (likely a polysaccharide) which is the ice active entity in case of pollen. All of this should be included / discussed in your text.

**Authors' response [5]:**

In this sentence, we intended to describe the chemical composition of individual agricultural soil dust IN. For clarity, we replaced the statement “*individual particles*” with “*individual agricultural soil dust particles*” [Page 9707, Lines 12-13]. To our knowledge, our study is the first example investigating the chemical composition of individual agricultural soil dust IN.

Kleber et al. (2007) studied organo-mineral complexes, but not their role as IN. Here, we simply added the following sentence:

Page 9707, Line 12: “*Agricultural soils are known to be complex mixtures of minerals, organic matter and so on.*”

Since the reviewer suggests the need to include the description on biogenic IN as discussed in previous studies (Szyrmer and Zawadzki, 1997; Hartmann et al., 2013; Pummer et al., 2012; Augustin et al., 2013), we have added some descriptions in the Results and discussion section (see Authors' response [17]).

**Reviewer's comment [6]:**

page 9708, line 6: When reading the text the first time, I thought China loess soils were thought to represent an agricultural soil dust sample, too, as arid regions can be irrigated. Therefore, please clarify here if the China loess soil is or has been used for agriculture or not.

**Authors' response [6]:**

For clarity, we have rewritten the following sentence:

Page 9708, Lines 5-7: “*As a reference for natural desert soil dusts, we used China loess soils (CJ-1) (Nishikawa et al., 2000), which were collected in an arid area in Gansu Province, China.*”

**Reviewer's comment [7]:**

page 9709, lines 1-5: From what I remember from measurements made with your CFDC as shown in literature, immersion freezing is the most active heterogeneous freezing process of those mentioned here and hence should overwhelm all others? If you agree with me on that point, please mention this here or change the sentence accordingly.

**Authors' response [7]:**

We have added the following sentence:

*Page 9709, Line 5: "In particular, we expect the predominance of immersion freezing in this regime, since the relative humidity is high enough to activate cloud droplet formation."*

**Reviewer's comment [8]:**

page 9709, line 8: Some more information on the sampling by the impactors is needed (e.g. impactor type, how they were operated, lower size cut, . . . .) Also mention explicitly that you sampled behind the CFDC and in parallel to it. Also, when sampling behind the CFDC: How were IN distinguished from droplets and non-frozen dry particles, and how did you make sure that ice crystals in the CFDC did not evaporate prior to sampling?

**Authors' response [8]:**

We have added more information on the sampling by the impactors as follows:

*Page 9709, Line 8: "The dust aerosol particles were collected using a two-stage jet impactor (Matsuki et al., 2010a, b; Tobo et al., 2010). We used only the second stage of the impactor, because the 50% cutoff aerodynamic diameters of the first and second stages at a sample flow of  $1 \text{ L min}^{-1}$  are estimated to be 1.6 and  $0.2 \mu\text{m}$ , respectively, and the mobility diameter of the particles is  $600 \text{ nm}$  ( $= 0.6 \mu\text{m}$ ). For the IN sampling, the particles that nucleated ice and were grown to ice crystal sizes were collected using a single jet impactor (Prenni et al., 2013; Tobo et al., 2013) installed at the outlet of the CFDC. The 50% cutoff aerodynamic diameter at a sample flow of  $1.5 \text{ L min}^{-1}$  is estimated to be  $2.9 \mu\text{m}$ ."*

Furthermore, we have added more descriptions on the CFDC experiments as follows:

*Page 9709, Line 5* (just after the sentence indicated in Authors' response [7]): “*Following such a particle nucleation/growth section, the CFDC has a droplet evaporation section where only ice saturation exists. Since cloud droplets cannot survive through the evaporation section unless the relative humidity with respect to liquid water in the particle nucleation/growth section exceeds ~108% (Sullivan et al., 2010a, b), only particles that form ice crystals are counted as IN with an optical particle counter at the outlet.*”

Testing of methods to isolate and capture ice crystals exiting the CFDC instrument dates back to literature in the late 1990's (Chen et al., 1998; Kreidenweis et al., 1998) and the methods have been applied in numerous studies since that time.

**Reviewer's comment [9]:**

page 9709, line 21: Motivate why you think that H<sub>2</sub>O<sub>2</sub> treatment most likely destroys organic matter, or else remove the statement.

**Authors' response [9]:**

H<sub>2</sub>O<sub>2</sub> treatment is a common procedure to oxidize organic matter from soil samples for subsequent analysis of the remaining minerals (e.g., Conen et al., 2011; O'Sullivan et al., 2014). We have added the following sentence:

*Page 9709, Line 18: “Treatment with H<sub>2</sub>O<sub>2</sub> is a commonly used technique to oxidize organic matter from soils and to obtain the remaining minerals (Conen et al., 2011; O'Sullivan et al., 2014).”*

**Reviewer's comment [10]:**

page 9709, line 25ff: In some of your own work (Wex et al., 2013) it was shown that kaolinite from Fluka changed its ice nucleation activity when coated with H<sub>2</sub>SO<sub>4</sub>, while the kaolinite you chose for your study (KGa-1b) did not. This difference is argued to occur due to the presence of K-feldspar, which is not present in the KGa-1b kaolinite. Hence it is possible that this mineral is also not present in the China loess soil dust but in both agricultural soil dust samples you examined, and that it is not biological material but K-feldspar which you destroy by H<sub>2</sub>O<sub>2</sub> treatment. Can you argue against that? However, it helps that you also did a heat treatment, because with this you can argue that the K-feldspar is heat resistant (work from

your lab (Sullivan et al., 2010) showed in the past that Arizona Test Dust did not decrease in ice nucleation activity when heated up to 250°C, so you could use that as an argument) while the organic matter likely is not heat resistant. Rewrite this part of your text according to the points I raise in this comment.

**Authors' response [10]:**

As the reviewer points out, Wex et al. (2014) (published in Atmos. Chem. Phys.) indicated that Fluka kaolinite loses its ice nucleating ability after H<sub>2</sub>SO<sub>4</sub> coatings, suggesting the possibility that H<sub>2</sub>SO<sub>4</sub> treatment has the potential to alter the ice nucleating ability of the K-feldspar component of the Fluka kaolinite. On the other hand, H<sub>2</sub>O<sub>2</sub> treatment is a common procedure to oxidize organic matter from soils for subsequent analysis of the remaining minerals (see Authors' response [9]). In fact, it has already been confirmed that the ice nucleating ability of K-feldspar is resistant to H<sub>2</sub>O<sub>2</sub> treatment (O'Sullivan et al., 2014). We admit that since we have not evaluated the feldspar content in dust samples used here, further investigations are required to verify the contribution of the feldspar component to their ice nucleating abilities. However, a high-resolution mineralogical database of dust-productive soils have indicated that soils in China are expected to have much higher contents of feldspar than those in North America (Nickovic et al., 2012). To clarify these points, we have rewritten a sentence in Section 3.2 as follows:

*Page 9712, Line 27 to Page 9713, Line 1: "It is likely that the ice nucleating ability of the feldspar component is resistant to H<sub>2</sub>O<sub>2</sub> treatment (O'Sullivan et al., 2014). It may also be important to note that dust-productive soils in China tend to have much higher contents of feldspar than those in North America (Nickovic et al., 2012). Since we have not evaluated the feldspar content in dust samples used here, further investigations are required to verify the hypothesis concerning the contribution of the feldspar component to their ice nucleating abilities."*

As the reviewer notes, Sullivan et al. (2010b) indicated that dry heating to 250°C has a small impact on the ice nucleating ability of Arizona test dust. On the other hand, we are not sure if we should conclude that the K-feldspar is resistant to dry heating to 300°C, based on this result. At least, we hesitate to do it in our paper.

More importantly, the main focus of this study is on the evaluation of the role of organic matter as ice nuclei of agricultural soil origin. In this regard, we confirmed that agricultural soil dusts experienced a significant reduction in their ice nucleating ability at temperatures

warmer than about  $-36^{\circ}\text{C}$  after  $\text{H}_2\text{O}_2$  treatment (Fig. 2a). We further confirmed the presence of organic-rich particles based on SEM/EDX analysis (Fig. 5a) and then examined that the reduction of the ice nucleating ability of the agricultural soil dusts after  $\text{H}_2\text{O}_2$  treatment can be explained mainly by the removal of such organic-rich particles (Fig. 5b). Thus, it is evident that materials other than organic matter (e.g., minerals including K-feldspar) do not play a significant role in reducing the ice nucleating ability of the agricultural soil dusts after  $\text{H}_2\text{O}_2$  treatment.

**Reviewer's comment [11]:**

page 9710, lines 19/20 and lines 22/23: It is not entirely clear what you mean by “examples of the other elements”. Do you mean “some of the elements which were present only in smaller amounts”? Please rephrase!

**Authors' response [11]:**

We have replaced “*examples of the other elements*” with “*some of the elements found in smaller amounts*” [Page 9710, Lines 19-20; Page 9710, Line 22].

**Reviewer's comment [12]:**

page 9710, line 25: The caption of Fig. 5 does give the number of particles you examined, but I suggest you also give this information in the main text.

**Authors' response [12]:**

We have added the information as follows:

Page 9710, Lines 25-27: “*In Fig. 5a, we summarize the results of SEM/EDX analysis for the total dust aerosol particles ( $n = 95$ ) and IN active at temperatures of  $-36^{\circ}\text{C}$ ,  $-30^{\circ}\text{C}$  and  $-24^{\circ}\text{C}$  ( $n = 58$ ,  $52$  and  $68$ ) in the untreated sugar beet soil dust particles.*”

**Reviewer's comment [13]:**

page 9710, line 26: This refers to a point I already made earlier (page 9709, line 8, concerning the impactor sampling): How exactly did you isolate those particles which are IN active at the

different temperatures for separate examination? Please describe the method used in more detail somewhere in your text.

**Authors' response [13]:**

See Author's response [8].

**Reviewer's comment [14]:**

page 9710, line 28ff: As mentioned before, in the literature (Hartmann et al. (2013) and many older references therein, Pummer et al. (2012), Augustin et al. (2013)) it has been shown that the ice nucleation by biological entities such as bacteria, pollen and fungi is caused by single very small INA-MM (size of a few 10nm) (for fungi this so far has only been shown in Fröhlich-Nowoisky et al., (2014)). Can you exclude that such a small macromolecule is present on those particles you define as "mineral particle"? Again, the heat treatment might help you, but you should discuss this explicitly in your work.

**Authors' response [14]:**

As the reviewer points out, we agree that we cannot rule out the possibility that such smaller amounts of organics are mixed with mineral particles. We have rewritten a sentence in the Materials and methods section as follows:

*Page 9710, Lines 23-24: "We consider that the detection of the C peak in particles categorized as minerals may be attributable to the presence of carbonates (e.g., CaCO<sub>3</sub>, CaMg(CO<sub>3</sub>)<sub>2</sub>). In this study, only particles containing the major elements of both organics and minerals (i.e., C, N, Si and S) are categorized as "organo-mineral mixtures". Therefore, the possibility remains that Si-rich particles containing N- and S-free organics or very small amounts of organics are categorized as minerals and not organo-mineral mixtures."*

**Reviewer's comment [15]:**

page 9711, line 8-10: Hint towards Fig. 5b, where you show the respective results.

**Authors' response [15]:**

As described in this paragraph [*Page 9711, Lines 12-13*], the calculation method is detailed in Appendix A1.



**Reviewer's comment [16]:**

page 9711, line 12: Again: Referring to H<sub>2</sub>O<sub>2</sub> treatment here might be too weak – you might want to mention that the heat treatment more certainly only affected the organic ice nuclei and resulted in a similar decrease of the ice nucleation activity.

**Authors' response [16]:**

Again, it is well known that H<sub>2</sub>O<sub>2</sub> treatment is a common procedure to oxidize organic matter from soil samples for subsequent analysis of the remaining minerals (e.g., Conen et al., 2011; O'Sullivan et al., 2014). Because we have explained it already (see Authors' response [9]), we do not repeat it in this part.

**Reviewer's comment [17]:**

page 9712, line 3-4: As mentioned before, more is known about this than you suggest here, and you should discuss this accordingly (see my comment to page 9707, line 13).

**Authors' response [17]:**

We have rewritten/added the last several sentences in this paragraph as follows:

*Page 9711, Line 26 to Page 9712, Line 4: "Organic matter in soils is composed of a variety of macromolecules, such as lignin, cellulose, hemicellulose, protein, lipids, humic-like substances (e.g., humic acid, fulvic acid) and so on (Paul, 2007). So far, freezing experiments with certain standard humic-like substances have indicated that while they can act as IN under mixed-phase cloud conditions (Fornea et al., 2009; Wang and Knopf, 2011; Knopf and Alpert, 2013; Rigg et al., 2013; O'Sullivan et al., 2014), they are much less effective as IN than fertile soil dusts (O'Sullivan et al., 2014). In addition, although the potential importance of ice nucleation by other macromolecules like protein (Hartmann et al., 2013), cellulose (Hiranuma et al., 2014) or fragments of pollen grains (Pummer et al., 2012; Augustin et al., 2013) has been suggested, it still remains unclear what materials are responsible for the major source of SOM particles having very high ice nucleating ability."*

**Reviewer's comment [18]:**

page 9713, line 18ff: You argue that the contribution from biological IN to the overall IN population is small. This might be misleading as SOM might consist of ice active macromolecules (see my respective comments above). It might be argued that the contribution from whole bacteria or pollen is small, but it might be wrong to extrapolate that to biological IN in general.

**Authors' response [18]:**

We have added the following sentence:

*Page 9713, Line 23: "However, further studies will be necessary to understand the contribution of various other microorganisms or their fragments."*

**Reviewer's comment [19]:**

page 9714, line 16: Typo: The author of the paper is Santl-Temkiv (i.e. an "l" instead of an "i")

**Authors' response [19]:**

We have revised it accordingly [*Page 9714, Line 16*].

**Reviewer's comment [20]:**

Appendix A, equation A5: Could it be that the nominator misses a "+ N\_IN[inorganic]"?

**Authors' response [20]:**

No. It is correct.

**Reviewer's comment [21]:**

Figure 4: Do you really mean "representative", or rather "exemplary". Just think about it and choose which term fits better.

**Authors' response [21]:**

We have replaced "*Representative*" with "*Exemplary*" [*Legend of Fig. 4*].

**Reviewer's comment [22]:**

Figure 4 and 5: The print in both figures is rather small, particularly for Fig. 5. If you can assure that the figures will be printed in the final version using two columns, you can leave it as is, but if they are thought to be printed in a single column, a copy of your paper when printed on paper will be indecipherable.

**Authors' response [22]:**

Thank you. We will confirm the size of these figures in the final typeset version.

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