Authors’ response to Dr. Z. A. Kanji

We thank Dr. Z. A. Kanji for raising this topic, which we will address in this reply.

The major reason for testing Clay Minerals Society kaolinite (KGa-1b) and China loess soil dust in the present paper were twofold; 1) to compare the ice nucleation properties of a typical clay mineral particle and a typical natural soil dust particle to those of the agricultural soil dust particles, and 2) to show the treatments to remove/deactivate organic matter had a very small impact on the ice nucleating ability of clay mineral and natural soil dust particles. As we note, the ice nucleating ability of the kaolinite, as measured by the Colorado State University CFDC, is in reasonable agreement with what we measure for the agricultural soil dusts when organic matter is removed/deactivated. Through comparing to previous literature data, we show that the ice nucleating abilities of the natural and agricultural soil dusts without any treatments, as measured by the CFDC, are quite similar to those of natural soil dusts measured using a cloud chamber (Niemand et al., 2012) and those of agricultural soil dusts measured using a droplet freezing technique on a cold plate (O’Sullivan et al., 2014). Nevertheless, it should be noted here that the use of the CFDC for measurements is the constant factor in our studies, while the other literature data are shown for reference only.

While the intention of our paper was not to focus on how we measure ice nucleation from any particle types in comparison to other methods, we will now include a comparison for Clay Minerals Society kaolinite (KGa-1b) results for the interest of others. In Fig. A1b of the revised manuscript (see the attached figure), we compare our kaolinite fit for ice nucleation active site density (n_s) with other results in the literature (Murray et al., 2011; Kanji et al., 2013; Wex et al., 2014). Murray et al. (2011) applied a droplet freezing technique on a cold stage. Kanji et al. (2013) reported immersion freezing for polydisperse kaolinite particles using the IMCA-ZINC device combination. Wex et al. (2014) studied immersion freezing for 300 nm kaolinite particles using both the LACIS and CFDC. Also, Murray et al. (2011) estimated the n_s values based on surface area determined using the N_2 gas adsorption method, whereas all other studies report the n_s values based on geometric surface area. Murray et al. (2012) noted that the gas adsorption method would produce smaller n_s values than the geometric surface area (e.g., four times smaller when using 800 nm particles). We may note that the n_s values from our study, Murray et al. (2011) and Wex et al. (2014) are relatively similar to each other. On the other hand, the n_s values from Kanji et al. (2013) are almost comparable to those of all other studies at around –36°C, but show much higher values at warmer temperatures. Differences in the n_s values of the kaolinite between Kanji et al. (2013) and the other studies may reflect some combination of instrumental- and/or dust aerosol generation-related factors, although the prime source is not yet known. How the method for immersion freezing used by Kanji et al. (2013) would see differences between agricultural soil dusts and other types examined here, we cannot say. Extended discussion of these points within our report would not seem to be productive for focusing on the key point of the research.

To briefly describe these points, we have added the following sentences in the main text:

Page 9710, Line 12: "*The n_s values for the kaolinite presented here are relatively similar to those reported by Murray et al. (2011) and Wex et al. (2014), but are more than one order of magnitude lower than those reported by Kanji et al. (2013) at temperatures warmer than about –30°C despite the use of the same Clay Minerals Society kaolinite (Fig. A1b). The difference may be related to different experimental technique; however, further speculation concerning this issue is beyond the remit of this study and is not pertinent to the major conclusions drawn from the comparison of our experimental results obtained using the CFDC.*"

Then, we have removed some statements regarding the comparison with the ice nucleating ability of clay minerals:

Page 9706, Lines 12-13: "*to almost the same level as that of clay minerals (e.g., kaolinite)*"

Page 9710, Lines 11-12: "*and other clay minerals (e.g., montmorillonite, Atkinson et al., 2013)*"

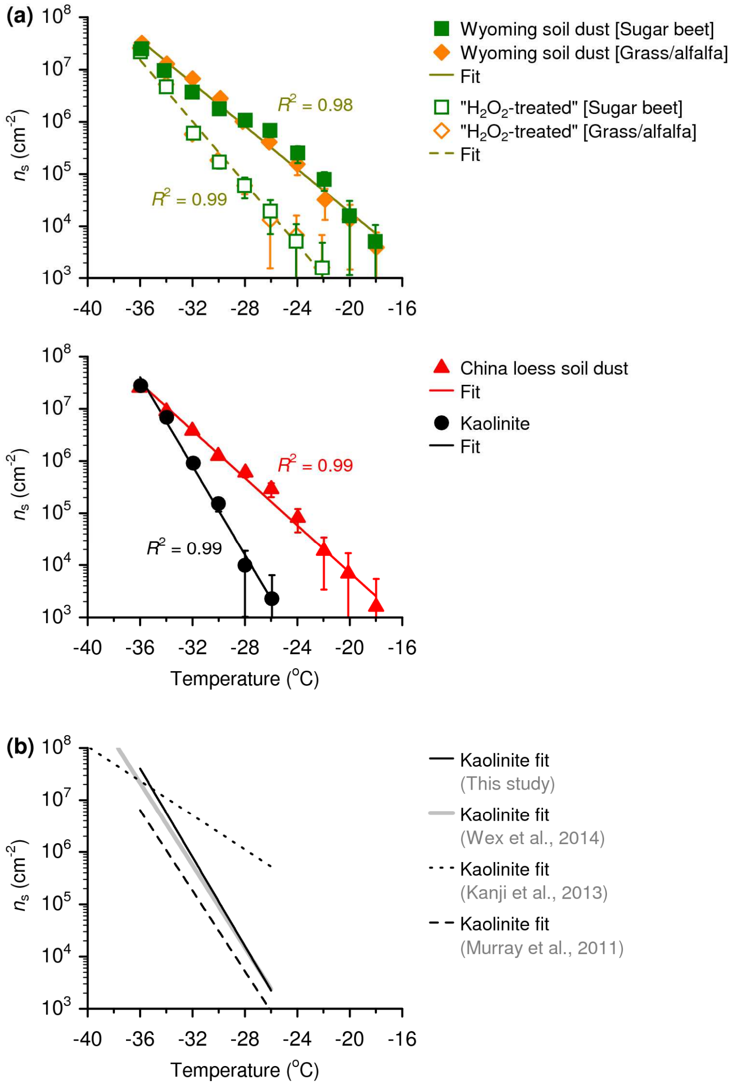

Fig. A1. Fit to ice nucleation active site densities for various dusts. **(a)** Parameterizations of n_s for untreated agricultural soil dusts ($\ln(n_s) = -0.4736T + 0.3644$; validity range: $-36°C < T < -18°C$) and for H_2O_2-treated agricultural soil dusts ($\ln(n_s) = -0.6773T - 7.8436$; validity range: $-36°C < T < -22°C$). Parameterizations of n_s for untreated China loess soil dust ($\ln(n_s) = -0.5230T - 1.5767$; validity range: $-36°C < T < -18°C$) and for untreated kaolinite ($\ln(n_s) = -0.9803T - 17.7764$; validity range: $-36°C < T < -26°C$) are also shown. Error bars represent standard deviations. **(b)** Comparison of the n_s parameterizations for kaolinite from this study, Murray et al. (2011), Kanji et al. (2013) and Wex et al. (2014).

Authors’ response to Dr. F. Conen

We thank Dr. F. Conen for his positive comments and for his suggestions for extending the work to include potential number concentrations of agricultural soil dust IN active at warmer temperatures. As you point out, because of very small IN number concentrations, it is difficult for the CFDC to obtain the number fraction of agricultural soil dust particles capable of nucleating ice at temperatures warmer than about –18°C. To investigate the ice nucleation properties of the soil dusts at warmer temperatures, your suggestion to collect particles into water followed by analyses using immersion freezing is a good one, and we have indeed tested this approach. This is still an ongoing study, although some preliminary results have been reported in the 19th International Conference on Nucleation and Atmospheric Aerosols (Hill et al., 2013). We will report the comprehensive results at warmer temperatures in a forthcoming paper (Hill et al., in preparation). In the revised manscript, we have added the following description:

<u>Page 9713, Line 17</u>: “*Given the results of immersion freezing experiments showing that soils having higher contents of organic matter can serve as efficient IN even at temperatures warmer than about –15°C (Schnell and Vali, 1972; Conen et al., 2011; Hill et al., 2013; O’Sullivan et al., 2014), the contribution of agricultural soil dusts to the IN population at these temperatures may exceed that of natural soil dusts (not shown here).*”

Authors’ response to Anonymous Referee #1

We thank Anonymous Referee #1 for his/her thoughtful comments and useful discussion. Below are our point-by-point responses.

Reviewer’s comment [1]:

How long were samples boiled in H_2O_2? How was it determined that all the organic material was removed? Why were these two treatments (heat and H_2O_2) chosen?

Authors’ response [1]:

We repeated the H_2O_2 treatment until no visible reaction could be detected by addition of more H_2O_2, and used the lack of visible bubbling as an indicator that almost all soil organic matter had been removed. For clarity, we have rewritten the following sentence:

Page 9708, Lines 10-12: “*The former samples were prepared by boiling gently in a 30%* H_2O_2 *solution until almost all organic matter was expected to be digested (i.e., until no visible reaction could be detected by addition of more* H_2O_2*), followed by rinsing with deionized water and drying.*”

H_2O_2 digestion was used to remove organic matter almost completely, and dry heating was initially pursued as an alternative way of removing/deactivating organic matter without liquid immersion. As a result, we could confirm that dry heating to 300°C has a similar impact to H_2O_2 digestion [*Page 9710, Line 1-2*], although wet heating to 90-100°C is less effective [*Page 9712, Line 12-14*]. We have written the last part of the first paragraph in Section 3.1 as follows:

Page 9710, Line 1-2: “*Dry heating is also known as a technique to remove and/or deactivate organic matter in soils. For example, Fernández et al. (1997) reported that soils heated at 150°C exhibit no significant loss of organic matter, whereas those heated at 490°C lose almost all the organic matter. As for the ice nucleation properties, we confirmed that dry heating to 300°C has a similar impact to* H_2O_2 *digestion (Fig. 3).*”

Reviewer’s comment [2]:

Please point out early in the paper that China Loess is used for comparison purposes as a desert dust rather than an agricultural soil.

Authors' response [2]:

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 [3]:

Are the images in Fig. 4 examples of 600 nm particles even though the scale bar is 300 nm?

Authors' response [3]:

Yes. As described in the Materials and methods section, all data are the results from the experiments with particles having a mobility diameter of 600 nm.

Reviewer's comment [4]:

Do you expect all organic compounds of interest to contain both N and S? Aren't there lots of organic compounds that just contain C, H, O? Were all the CHO particles just soot? Is there a reason why organic compounds in agricultural soils would contain N and S rather than N or S or just CHO? You may be obtaining an underestimate of the number of organic compounds.

Authors' response [4]:

It is well known that SOM serves as a reservoir of nutrients, such as N, P and S (Paul, 2007). We did not necessarily expect all organic particles to contain both N and S, but our results showed that all the particles categorized as "organics" contained both N and S, but not P. We have added the following sentences in Section 3.2:

Page 9710, Line 20: "*It has long been known that SOM serves as a reservoir of nutrients, such as N, P and S (Paul, 2007). As for the samples analyzed here, all the particles categorized as organics contained both N and S, but P was not found.*"

We classify carbonaceous particles lacking N and S as "others" and not "organics", because most of them have a morphology like soot (see Fig. 4e). As seen in Fig. 5a, such particles categorized as “others” are very minor components and their exclusion does not result in a serious underestimate of the number of organic compounds.

Reviewer’s comment [5]:

Pg 9710 line 25: My understanding is that a sample of the soil was aerosolized and imaged. Other samples of soil were run through the CFDC or treated and run through the CFDC. The particles that nucleated ice were then collected after the CFDC for imaging. This should be stated in the Materials and Methods Section.

Authors’ response [5]:

We have added more detailed explanations in the Materials and methods section 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 stage at a sample flow of 1 L min^{-1} are estimated to be 1.6 and 0.2 µm, respectively, and the mobility diameter of the particles is 600 nm (= 0.6 µm). 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 L min^{-1} is estimated to be 2.9 µm.”*

Reviewer’s comment [6]:

Pg. 9710 line 27: The DMA size selection only produces monodisperse particles if the particles are spherical. It is unlikely that you have imaged enough particles to plot a size distribution, though it would be helpful if it could be provided. The distribution of particles studied can be referred to by their 600 nm mobility diameter, but cannot be called monodisperse.

Authors’ response [6]:

According to your suggestion, we have avoided using the expression “monodisperse” in the revised manuscript [*Page 9710, Line 27; Legends in Figs. 2 and 5*].

Reviewer’s comment [7]:

Pg 9711 line 7: A few more sentences could be added to this paragraph to clarify Fig. 5b.

Authors’ response [7]:

We have added/rewritten the following sentences:

Page 9711, Lines 11-12: “*~ that all organic compounds can be digested and removed via H_2O_2 treatment, but inorganic components are not altered by H_2O_2 treatment*”

Page 9711, Lines 13-17: “*The temperature-dependent changes in the fractions of organic and inorganic IN estimated from this calculation (Fig. 5b) are roughly consistent with the results from the SEM/EDX analysis (Fig. 5a). If H_2O_2 treatment can cause a certain reduction in the ice nucleating ability of inorganic components, the possibility remains that the results in Fig. 5b may somewhat overestimate the fractions of organic IN. We have not exhaustively analyzed the influence of H_2O_2 treatment on all known minerals that may be present in the soil dusts. Nevertheless, the results presented here indicate that the reduction of the ice nucleating ability of the agricultural soil dusts after H_2O_2 treatment (Fig. 2) can be explained mainly by the removal of organic matter.*”

Reviewer’s comment [8]:

Kaolinite is unlikely to be affected by 300°C temperatures or H_2O_2. What are the other common minerals in the soil? How do these differ from the common minerals in China Loess? Could any of these minerals be altered by heat or H_2O_2? If minerals are also deactivated, Fig. 5b may overestimate the amount of organic.

Authors’ response [8]:

In the calculation, we assume that inorganic components are not altered by H_2O_2 treatment. As you point out, Fig. 5b may somewhat overestimate the amount of contributions of organics to ice nucleation activity if minerals are deactivated, and we have not exhaustively analyzed the influence of H_2O_2 treatment on all known minerals that may be present in the soil dusts (see Authors’ response [7]). Nevertheless, our results demonstrate that the differences in the IN fraction of kaolinite and China loess soil dust between before and after treatments (i.e., H_2O_2 digestion or dry heating to 300°C) are relatively small (at least, within the range of uncertainty of the CFDC measurements; see Figs. 2 and 3).

Reviewer's comment [9]:

Pg 9712 line 5: The structure of this paragraph is confusing. Please start with the main point (the last sentence) and then compare to literature. Is soil from England and Wyoming very different in composition?

Authors' response [9]:

As suggested by the reviewer, we have largely changed the structure of this paragraph as follows:

Page 9712, Lines 5-19: "*It is noteworthy that although agricultural soil dusts collected in Wyoming have similar ice nucleating abilities to those collected in England (Fig. 2b), the major component responsible for ice nucleation at temperatures between about –36°C and –15°C has been interpreted in different ways. Our results demonstrate that the presence of organic compounds (i.e., SOM particles) has a significant influence on the ice nucleating ability of the Wyoming soil dusts throughout the entire temperature range down to –36°C. In contrast, O'Sullivan et al. (2014) suggested that mineral components are more important than biogenic components for the ice nucleating ability of the England soil dusts at temperatures colder than about –15°C. The suggestion by O'Sullivan et al. (2014) was based on the results from freezing experiments with the soil dusts before and after wet heating to 90°C. It is expected that wet heating to 90-100°C deactivates only certain organic matter (e.g., heat-sensitive proteins or proteinaceous compounds, Christner et al., 2008). In fact, it has been reported that wet heating to 90-100°C is less effective than* H_2O_2 *digestion in reducing the ice nucleating ability of fertile soil dusts (Conen et al., 2011; O'Sullivan et al., 2014). In this study, we applied treatments designed to remove and/or deactivate almost all organic matter (i.e.,* H_2O_2 *digestion or dry heating to 300°C). Thus, although the possibility remains that the soil dusts from Wyoming and England are very different in composition, we speculate that the different interpretations of the major component responsible for ice nucleation are in part attributable to the different experimental approaches.*"

In the revised version, we have described the main point in the first sentence. Unfortunately, it is not clear whether the compositions of the soil dusts from Wyoming and England are similar or not. Although the possibility remains that these soil dusts are very different in composition, we speculate that the different interpretations of the major component responsible for ice nucleation are in part attributable to the different experimental approaches. In the revised version, we have described this point in the last sentence of this paragraph.

Reviewer's comment [10]:

Pg 9712 line 23: It is hard to tell whether the impact on the ice nucleating ability of China Loess is small because of the log scale. Please give numbers rather than referring to changes as "small" or "large".

Authors' response [10]:

A problem is that it is difficult for the CFDC to obtain the IN fraction data accurately, especially at warmer temperatures. The differences in the IN fraction of China loess soil dust between before and after treatments are "within the range of uncertainty" of the CFDC measurements. For this reason, we consider that although it is hard to discuss the quantitative differences, the impact on the ice nucleating ability of China Loess must be relatively small as compared to the case of the Wyoming soil dusts. Thus, instead of giving numbers, we have added the statement "*~ is relatively small (within the range of error).*" to a sentence in Section 3.1 [*Page 9710, Line 1*].

Reviewer's comment [11]:

Fig. 4 legend: Please specify that the particles are also Au-coated.

Authors' response [11]:

We have added an explanation that "*The SEM/EDX images were acquired after Au-coating.*" in the legend of Fig. 4.

Reviewer's comment [12]:

Figs. 4&5: EDX is not generally used as a quantitative technique, and it is challenging to make it quantitative. When imaging the particles, the background is also imaged, and the peaks due to the background should be reduced in intensity. The amount these peaks come through the particles of the sizes given in Fig. 4 could be estimated using a SEM simulator like Casino. How far into the particle does the EDX beam penetrate? Why is there so little variation in the intensities of the carbon peaks? What is being normalized? The minerals have additional carbon; I suspect they are coated with CHO compounds. Why are so many particles pure organic or pure mineral and so few internally mixed? Is this typical of soils? Why does this separation between organic particles and mineral particles occur in soils?

Authors' response [12]:

Thank you for pointing out a need for clarification in the manuscript. The X-ray spectra from the particles were acquired for 20 sec of live time at an acceleration voltage of 20 kV. At this voltage, it is expected that the electron beam penetrates the entire particle (Laskin and Cowin, 2001). Here, we have added the following explanations:

<u>Page 9709, Line 12</u>: *"After the SEM images of individual particles were taken, the X-ray spectra from the particles were acquired for 20 sec of live time at an acceleration voltage of 20 kV."*

<u>Page 9710, Line 17</u>: *"A major difficulty in the EDX analysis of submicron particles is their high transparency for the primary electron beam (Laskin and Cowin, 2001). Since the electron beam penetrates the entire particle, the EDX spectrum from the particle projection area contains the background signal from the butvar film supported by Ni-grids as well as the signal of the particle coated with Au. For this reason, as illustrated in Fig. 4, we compare the EDX spectrum of the particle projection area with that from the particle-free area."*

As the reviewer points out, the particles categorized as "minerals" have additional carbon. However, since the particles categorized as minerals contained Ca and Mg, we consider that the detection of the C peak in particles categorized as "minerals" is mostly attributable to the presence of carbonates (e.g., $CaCO_3$, $CaMg(CO_3)_2$). It is also important to note that the major elements of particles categorized as organics are C, N and S. For these reasons, 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". It is difficult to explain the reason why so many particles are pure organic or pure mineral and so few internally mixed and why this separation between organic particles and mineral particles occur in soils. It is also difficult to know if this is typical of soils. This is because few studies have reported the composition and mixing state of agricultural soil dusts in the submicron size range (i.e., agricultural soil dusts emitted at this atmospherically-relevant size) using single particle analysis. However, we have to admit that 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”. Therefore, we have added/rewritten the following descriptions:

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.,* $CaCO_3$*,* $CaMg(CO_3)_2$*). 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.*”

Authors' response to Anonymous Referee #2

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°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°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°C and –15°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°C and -15°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's 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 L* min^{-1} *are estimated to be 1.6 and 0.2 μm, respectively, and the mobility diameter of the particles is 600 nm (= 0.6 μm). 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 L* min^{-1} *is estimated to be 2.9 μm.”*

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 though 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_2O_2 treatment most likely destroys organic matter, or else remove the statement.

Authors' response [9]:

H_2O_2 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_2O_2 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_2SO_4, 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_2O_2 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_2SO_4 coatings, suggesting the possibility that H_2SO_4 treatment has the potential to alter the ice nucleating ability of the K-feldspar component of the Fluka kaolinite. On the other hand, H_2O_2 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_2O_2 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_2O_2 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°C after H_2O_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 H_2O_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 H_2O_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°C, –30°C and –24°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., $CaCO_3$, $CaMg(CO_3)_2$). 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_2O_2 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_2O_2 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.

Additional authors’ note to reviewers and editor

1. We have added the present addresses of two co-authors as follows:

   Y. Tobo[1,*]

   [*]{now at: National Institute of Polar Research, Tachikawa, Tokyo 190-8518, Japan}

   A. J. Prenni[1,**]

   [**]{now at: Air Resources Division, National Park Service, Lakewood, CO 80225, USA}

2. We updated the corresponding author’s e-mail address:

   ytobo@atmos.clostate.edu => yutaka.tobo@gmail.com

3. We have added a sentence in Acknowledgement:

   We also thank Naruki Hiranuma for helpful discussions.