Answer to reviewer 1

Below we provide a point-by-point response to all individual comments of the review. We indicate the reviewer's comments by black text, our answers are given in blue text, and new text that will appear in the revised manuscript in green.

Eickhoff et al. present valuable results with a potential significant contribution to a better understanding of the microphysical processes in the ocean and the (polar) atmosphere.
However, in my opinion, some chapters could benefit from some shortening/rewriting, while the major findings should be rather put in a nutshell. Furthermore, I think, this manuscript is still lacking a look on the bigger picture and a critical discussion of its (atmospheric) significance and implications. See below for more specific comments.

We thank the reviewer for highlighting the importance of our contribution for the polar ocean and atmosphere and for the very detailed comments that have improved the revised version of the manuscript.

1. Introduction

L34-39 and L61-71.: Could you be more specific, which of the findings hold true for the Arctic, the Antarctic or both polar regions? This appears very important to me to not mix them up, since certain features of the Arctic and Antarctic are quite different.

The comments on the *Fragilariopsis cylindrus* hold for both the Artic and the Antarctic and we have updated that sentence accordingly.

The diatom *Fragilariopsis cylindrus* (see Fig. 1) is widespread in polar environments and is one of the predominant species within Arctic and Antarctic microbial assemblages (Kang and Fryxell, 1992; Poulin et al., 2011; van Leeuwe et al., 2018).

Regarding atmospheric INP, the we have added the following new paragraph at the end of the introduction to discuss the differences in more detail:

There are some differences regarding the relevance of INPs in the Arctic and Antarctic polar regions. While in both polar latitudes the absolute concentrations of INPs are low, the influence of anthropogenic aerosols and INPs is much larger in the Arctic due to long-range transport during the Arctic winter (Šantl-Temkiv et al., 2019; Šantl-Temkiv et al., 2020; Ekman and Schmale, 2022). During the Arctic summer, aerosol lifetimes are much shorter due to increased wet removal preventing long range transport and thus increasing the importance of locally emitted INPs. In the Antarctic, the influence of anthropogenic aerosols and INPs in generally much smaller (Stohl and Sodemann, 2010; Ekman and Schmale, 2022). During winter, blowing snow from the sea ice is the main aerosol source in the southern polar region, while DMS and other organic compounds from algae bloom are the main source during summer, while the anthropogenic influence is negligible (Ekman and Schmale, 2022).

L34-35 Please add a reference.

The references were added as requested:

The diatom *Fragilariopsis cylindrus* (see Fig. 1) is widespread in polar environments and is one of the predominant species within Arctic and Antarctic microbial assemblages (Kang and Fryxell, 1992; Poulin et al., 2011; van Leeuwe et al., 2018).

L40-42 Please recheck, if Wilson et al. (2015) or Aslam et al. (2018) are suitable references for supporting the statement of "the production of so-called ice-binding proteins (IBPs)"

The two references referred to only the second part of the sentence "and of other EPS also found in other diatom species". We have now added a reference for the first part of the sentence "the production of so-called ice-binding proteins (IBPs)" and separated the two parts with a comma:

One prominent example is the production of so-called ice-binding proteins (IBPs) (Bayer-Giraldi et al., 2011), and of other EPS that are also found in other diatom species (Wilson et al., 2015; Aslam et al., 2018).

L52-54 Which reference states that EPS do have good ice-binding properties? Is there any knowledge under which conditions (chemical composition) EPS do have these properties?

We have added the appropriate references and added the info that these EPS are mainly polysaccharides and proteins:

The very good ice-binding properties of *fc*IBP and EPS (mainly polysaccharides and proteins) under sea ice brine conditions have been reported in previous studies (Krembs et al., 2002; Bayer-Giraldi et al., 2011; Krembs et al., 2011).

L46-60 It might be important to clarify the relationship between the terms "ice-binding protein", "antifreeze protein" and "ice-nucleating protein" here. Do I get it right that less efficient ice-nucleating substances (in the sense that they are active at lower temperatures) can be at the same time antifreeze-substances at higher temperatures?

We have added some text to clarify the relationships of the different terms as requested:

The very good ice-binding properties of *fc*IBP and EPS (mainly polysaccharides and proteins) under sea ice brine conditions have been reported in previous studies (Krembs et al., 2002; Bayer-Giraldi et al., 2011; Krembs et al., 2011). Ice-binding proteins (IBPs) bind to ice crystal surfaces and by doing so can control crystal growth rate, inhibit ice recrystallization or help to adhere their host to ice (Davies, 2014; Bar Dolev et al., 2016; Guo et al., 2017). Originally, IBPs were known as antifreeze (glyco)proteins, which protect fish and insects by thermal hysteresis, i.e. by depressing the temperature where active crystal growth occurs to below the equilibrium melting point temperature (Bar Dolev et al., 2016). However, not all IBPs have such thermal hysteresis antifreeze properties. For example, a recently discovered IBP from the Antarctic bacterium *Marinomonas primoryensis* serves to bind its bacterial host to diatoms and Antarctic sea ice layer (Guo et al., 2017). Furthermore, even ice-nucleating proteins are sometimes considered to be a subgroup of IBPs, because their active sites appear to be structurally quite similar, just much larger, than those of regular IBPs with antifreeze properties (Davies, 2014; Bar Dolev et al., 2016; Eickhoff et al., 2019; Hudait et al., 2019). These considerations may imply that the much smaller ice-binding sites of 'antifreeze' IBPs could also stabilize the formation of small ice embryos and thereby promote the nucleation of ice from liquid water, however, only at very low temperatures (Davies, 2014; Bar Dolev et al., 2016; Eickhoff et al., 2019; Hudait et al., 2019). Indeed, it has been shown both experimentally as well as in molecular dynamics simulations that the ice-binding antifreeze proteins of the mealworm beetle *Tenebrio molitor* (*tm*AFP) can also trigger the nucleation

of new ice crystals just a few degree Celsius above the homogenous freezing temperature of water or an aqueous solution (Eickhoff et al., 2019; Hudait et al., 2019). Here, we explore whether a similar ice-nucleating effect also occurs for IBPs from *F. cylindrus*.

L59-60 "Here we explore whether a similar ice-nucleating effect does occur also for IBPs from *F. cylindrus*" -> What is the outcome? This might be an important result that deserves to be mentioned in chapter "5. Summary and Conclusions"

The results of the ice nucleation experiments are now mentioned in the Summary:

For the ice-binding (antifreeze) protein fcIBP11, we did not observe any evidence for promoting ice nucleation at low temperatures.

2. Material and methods

L76 Please add the months, when ANT XVI/3 took place.

The information has been added:

The investigated *F. cylindrus* cells belong to the strain TM99 isolated in 1999 from the sea ice of the Weddel Sea, Antarctica, by Thomas Mock (*Polarstern* ANT XVI/3 expedition, which took place in the early spring from March to May 1999).

L76-81 When did the laboratory steps happen after the isolation in 1999? Back in 1999 or just recently, just before the ice nucleation experiments started? I just wonder how many cells (of those 108 cells) were still alive after a storage of approximately 20 years.

The cells were kept in culture since the expedition, the laboratory steps took place just recently, immediately before the experiments started. This information was added to the manuscript.

Since then, stock cultures were kept in f/2 medium (Guillard and Ryther, 1962) set up with Antarctic water and cultivated at 0°C and under continuous illumination of approximately 25 µE m-2 s-1. Before starting the experiment, cell numbers of the F. cylindrus cultures were monitored using a Coulter Counter, and cells were harvested during the exponential growth phase.

L84-94 This section could be shortened, since it contains much redundant information. It might be enough to refer to Tabel S1 in regard of the exact composition of the artificial sea salt.

Section has been shortened as suggested by removing the detailed information on the salts and referring to table S1.

The composition of the salts and their concentrations are given in Supplemental Information Table S1.

L 94, 100, 104, 110, 118, … If you only used one type of filter throughout this study, it might be enough to mention the filter type just once in the beginning.

We now mention the filter type only once with a more generalized statement:

The artificial seawater was filtered through a syringe-filter (0.22 µm, Polyethersulfone, SimplePure) in order to exclude any effect of suspended dust particles on ice nucleation. This type of filter has been used for all filtrations in this study, unless not mentioned otherwise.

L103 I guess replacing "*F. cylindrus* cells" with "*F. cylindrus* samples" might make it more accurate.

Changed as suggested.

L106 Any reference that states that fcIBP11 is a soluble macromolecule detached from the cell? Or could it be connected to the cell surface of the diatoms as well?

According to Bayer-Giraldi et al., 2011, all analysed fcIBP11 isoforms are neither intracellular nor transmembrane, but secreted into the extracellular space. We have added the reference to the sentence.

L109-110 Is it possible to give an approximate estimate of the extend of cell loss?

We have added an approximate estimate as requested:

From comparison of the frozen fraction curves obtained with the sample with those of unfiltered samples (see below) our best estimate of the concentration is about of 2×10^6 cells per mL (estimated uncertainty range $1\times10^6 – 1\times10^7$).

L136 "…belongs to the DUF3494 IBP family,….".. was already mentioned in the introduction (L 43). Why is it relevant to mention it here again?

We have removed the sentence in question as requested.

L145-184 Is it possible to shorten theses sections onto the relevant information since all these methods have been published before? Of course, the main principle and deviations from the original protocol should be mentioned. If you want to keep all details, maybe it is possible to shift part of these descriptions to the SI? Instead I would appreciate a short explanation, why you chose two different setups (2.3.1, 2.3.2) for the ice nucleation experiments.

We have shortened these paragraphs by shifting parts of the text to the SI. We have also added a short explanation, why we have used two different methods for our ice nucleation experiments:

The DSC experiment has been used as a simple and direct method to check whether F. cylindrus diatoms are potential ice nucleators or not. The method does not allow for the observation of single droplets, and we can only study cell fragments but not intact cells because the latter are disrupted during the emulsion preparation process. Therefore, we have used the WISDOM microfluidic device, which is described below, as the main experimental method in this study.

L185. You should check the appropriate use of “INP” throughout the manuscript. Maybe “ice nucleating molecules” or “ice nucleating entities” could be more correct here? I recommend to check the terminology proposed by (Vali et al., 2015).

We have used “INP”, because we think “particle” is the most accurate term for the diatom cells and their fragments. This usage is also in line with many papers in the literature (including those of authors of the Vali et al. (2015) paper), in which diatoms were termed INPs, e.g.: (McCluskey et al., 2018; Creamean et al., 2021).

L186-188 Is this true? I think the methodology in the literature is just different to yours, where bigger droplets with higher volume per droplet were used. E.g. Budke and Koop (2015) used in the BINARY 1 µL droplets. Why did you choose for these small volumes of 380 pL (l 190) then?

We think there is a misunderstanding here. What we mean is that ~microliter droplet experiments usually employ a larger number of INPs per droplet (not INP concentration per volume). Moreover, these large droplet experiments are often subject to heterogeneous ice nucleation by impurities and the supporting surfaces at low temperatures, thus making it nearly impossible to study INPs with very low activity that nucleate only at temperatures just above homogeneous nucleation temperature. (The median freezing temperature of ‘pure’ water in the BINARY device is about -30°C, while the freezing temperature of the diatom experiments presented in Fig. 2 is between -32°C and -37°C.) For this purpose, small volume experiments such as the ~nanoliter microfluidic device used here are much more suitable, as they allow to reach homogeneous ice nucleation because the smaller droplets usually contain less impurities per droplet (The median freezing temperature of artificial seawater in the WISDOM device is about -40°C, see Fig.2). We have rewritten the text to make this clearer.

Ice nucleation studies using larger-volume droplet arrays usually employ relatively high concentrations of INPs per droplet, e.g. mineral dust particles or bacterial cells (Budke and Koop, 2015; Hiranuma et al., 2015; Wex et al., 2015; DeMott et al., 2018; Hiranuma et al., 2019; Kunert et al., 2019; Ickes et al., 2020), to ensure that freezing is induced at a temperature that is higher than that triggered by the supporting surface or minute amounts of impurities contained in the water In the present study, the total amounts of INPs was small due to the limited availability of *F. cylindrus* cells, suggesting the use of small droplet methods which require less total INP material. We investigated droplets with a diameter of 90 µm, corresponding to a volume of about 380 pL. Another, probably more important advantage of using these small droplet volumes is that we are able to measure ice nucleation down to the homogenous freezing temperature of water (Riechers et al., 2013; Reicher et al., 2018; Tarn et al., 2021), enabling also the investigation of rather poor ice nucleators.

L189-192: I find this statement quite surprising, since you started your experiments with a solution of a highly concentrated culture (see 2.1). Then you performed several dilutions. And now you are stating that the number of INP was not enough for your experimental setup. Why did you perform dilutions then?

This question probably arises from the same misunderstanding discussed in the previous comment. We did these dilution experiments to obtain more accurate cumulative numbers of ice active sites per mass n_{m_total} and over a broader temperature range such that we can compare the data better to other literature data (see Figure 7).

L199-308 Is it possible to shift this section (or parts of it) into the SI?

As suggested, we have shifted the entire section (former lines L199-308) into the SI.

3. Results and Discussion
General:

Is it possible to bring this part more on a point? It feels like reading and rereading the same or similar aspects.

We have shortened this part by shifting the detailed description of DSC experiments including the former Fig.4 into the SI. We have also rewritten some sentences or parts and now term this section "Results" (see your comment on section names further below).

L 326 How can freezing at -44°C be still relevant, when water droplets usually freeze at -38°C homogeneously? Is it because of the small volume of the droplets in your setup?

The droplets in the DSC experiments (and also in the microfluidic experiments) are all large enough not to be affected by the Gibbs-Thomson effect. The homogeneous freezing temperature of water droplets in the DSC experiments is indeed -38°C, as defined by the onset of the exothermic signal. The corresponding onset temperatures for the two samples, sea-water and sea-water containing *F. cylindrus*, are shifted to lower temperatures (-39°C to-40°C) due to the colligative freezing point depression effect of the salts contained in the seawater. We added this information to this text (now in the SI):

Because of the colligative freezing point depression of the seawater, the freezing temperatures of the reference and the sample are shifted to lower temperatures, compared to pure water.

Figure 5: Just as another example, where sentences can be shortened. Instead of: "b): Freezing temperatures of the same samples shown in panel (a), but after filtration with a pore size of 0.22 µm." you could write: "b): Freezing temperatures of the filtered (0.22 µm) samples."

Changed as suggested.

Line 369 "all diatoms" (?) or all cells?

We agree and changed the term to 'all *F. cylindrus* cells' to make this point more clear. We also made corresponding changes at several other places throughout the text where appropriate.

L378 Within the whole manuscript, proteins are proposed as likely ice nucleating molecules. What about polysaccharides? Any tests performed into this direction?

Yes, it is known from the literature that polysaccharides can also act as ice-nucleating molecules (e.g. Dreischmeier et al. (2017)), so we added the term polysaccharides to the sentence in question:

We suggest that their freezing is induced by cell fragments or by INPs released by the *F. cylindrus* diatoms, e.g. soluble species from the EPS such as proteins or polysaccharides.

However, as the experiments on soluble components described further below in the paper did not reveal any ice nucleating effect, we did not analyse our samples for polysaccharide content.

L379 “macromolecules” or maybe “polysaccharides”, to be more specific. Why is the comparison of diatoms with birch pollen relevant here?

We used the comparison to birch pollen here just to mention that by filtering the *F. cylindrus* samples, it is entirely possible that ice nucleation activity can remain, when either smaller fragments or soluble ice-nucleating molecules passes through the filter, thus resulting in n_N values larger than 1. This is exactly what happens when pollen suspended in water are filtered and the remaining washing water contains ice nucleating molecules. Also in the case of pollen, the n_N values reach values far above 1 (about 10^4 per pollen grain to be precise).

4. Discussion and Implications

General:

What is the difference between the chapters “3. Results and Discussion” and “4. Discussion and Implications”? It appears to me that the text (or at least parts) of chapter 4 in the current version of the manuscript might still represent a subchapter of “3. Results and Discussion”.

As suggested we have renamed chapters 3-5 and also shifted some of their contents. Chapter 3 is now simply termed “Results”. Chapter 4 remains “Discussion and Implications” because we compare our results to those of other diatoms and combine these datasets into a single parameterization. Moreover, we added two new paragraphs to discuss implications of our results for the atmosphere and compare them to (the very sparse) Southern Ocean diatom and INP measurements (see your next comments).

This study was mainly motivated with the atmospheric relevance of INPs (e.g lines 64-72, lines 534-536). However, a critical discussion of these (new) findings for atmospheric implications are still missing and could fit here. Some of the following aspects should be discussed in this section:

Which atmospheric residence time would you expect for diatoms, their fragments and exudates? Whole marine diatoms are rather big and might precipitate within few seconds or minutes. Can it be expected that complete cells/fragments will make it into the atmospheric layers relevant for mixed-phase clouds or even cirrus clouds? (as implied in lines 66-70)

We have added some of this information to the text.

Which are the atmospheric concentrations of diatoms/*Fragilariopsis cyclindus* or fragments in the ambient air?

As far as we now there are no such measurements.

In Figure 10, you nicely compare the ice nucleating activity of *Fragilariopsis cyclindus* with several diatoms from other studies. However, a rating of the importance of diatoms as INP in the polar regions/Southern Ocean in comparison to other types of INP (such as marine bacteria, mineral dust, …) in regard of abundance and/or ice nucleating efficiency is missing. The Antarctic is known for a low number of efficient atmospheric INPs in comparison to the Arctic (e.g. (McCluskey et al., 2018; Wex et

al., 2019; Hartmann et al., 2021; Zeppenfeld et al., 2021)). Considering this fact, how would you evaluate the importance of your findings for a better understanding of the Antarctic environment/atmosphere?

We have added two new paragraphs relating our data on *F. cyclindus* and sea ice diatoms to marine INP data and also try to compare the potential importance of our experimental data to Southern Ocean INP abundances. As there are only very sparse data on diatoms and INP in the Southern Ocean and the Antarctic marine environment these can only be regarded as order of magnitude estimates. Clearly, more field experiments in these regions are highly desirable. The new text reads:

In the following, we will try to put the ice nucleation data of *F. cylindrus* and the other sea ice diatoms into context by comparing to field studies. Wilson et al. (2015) provided experimental evidence for a marine biogenic source of ice nucleating particles, and suggested that exudates and fragments of diatoms as a source of the ice nucleating material located in the sea surface microlayer. Their low-temperature freezing data reveals a cumulative number of ice nucleating active sites per total organic carbon mass n_{m_TOC} of ~1.3x10^{10} g^{-1} at -27 °C, which is the low temperature end of their data, and the most relevant to the present study (calculated from the equation given in the caption of their Fig. 2). To compare this value to the n_{m_total} values given in Fig.7, we estimated that the organic carbon content of the samples varies between 39.32% (representing the organic carbon content of *F. cylindrus* cells, see above) or 100% (representing a purely organic carbon composition), resulting in a range of n_{m_total} of ~5.0x10^{9}-1.3x10^{10} g^{-1} for their Arctic sea surface microlayer samples. These are compared to n_{m_total} values of 8.2x10^{7} g^{-1} (2σ prediction bands: 2.8x10^{7}-2.4x10^{8} g^{-1}) for *F. cylindrus* and of 5.8x10^{8} g^{-1} (2σ prediction bands: 7.0x10^{7}-4.8x10^{9} g^{-1}) for sea ice diatoms, respectively, at -27°C, indicating that *F. cylindrus* and other sea ice diatoms may contribute to the marine INP in Southern Oceans and Antarctic seawater, assuming the Wilson et al. parameterization is applicable also to these areas.

In another comparison, we use measurements of insoluble aerosol particles made at Amsterdam Island in the Southern Indian Ocean (Gaudichet et al., 1989). These measurements show that marine biogenic particles make up between 8 and 28% of the number of detected particles and that these were predominantly assigned to Radiolaria and diatom fragments (identified as amorphous silicates), with about 27 % or 2.7x10^{4} m^{-3} particles observed in the southern winter (July) and fewer in fall (May, 8 %, 2.4x10^{4} m^{-3}) and spring (September, 7 %, 1.8x10^{3} m^{-3}). If we assume that all Radiolaria and diatom fragments can be attributed to *F. cylindrus* diatoms, we can calculate the mass concentration of *F. cylindrus* diatom cells per cubic meter of air from the mass per individual cell (m_{total} = 4.5x10^{-11} g, see above) yielding to values of 1.2x10^{-6} g m^{-3} air (July), 1.1x10^{-6} g m^{-3} air (May), and 8.1x10^{-8} g m^{-3} air (September). Using the parametrization of the cumulative number of ice nucleating active sites per mass *F. cylindrus* in Eq. (S10), we calculate a n_{m_total} value of 8.2x10^{7} g^{-1} (2σ prediction bands: 2.8x10^{7}-2.4x10^{8} g^{-1}) at -27 °C, see above, from which we can derive the number concentration of INPs per m^{3} air at -27 °C in fall (May) as a value of ~88 INP m^{-3} air (2σ: 3-250). This value can be compared to in situ total INP measurements in the Southern Ocean south of Australia in fall (March-April) yielding values between 34 and 207 INP m^{-3} air at -27 °C (McCluskey et al., 2018). Although the above calculations are an order of magnitude estimates at best, the comparison shows that it is not unreasonable that sea ice diatoms such as *F. cylindrus* and their fragments may constitute a significant fraction of the INP in the Southern Ocean and Antarctic waters.

Specific:

L494 You performed own elementary analysis for obtaining the carbon content in your samples? Could you please add the method to chapter “2. Materials and Methods”? Few lines might be sufficient.

As requested, we have added a few lines on the elemental analysis in the new section 2.4:

The total carbon content of the *F. cylindrus* samples has been determined using elemental analysis. For this purpose, an amount of 0.7 mg *F. cylindrus* diatoms was combusted at a high temperature ($T > 1000$ °C) in a Tin-crucible and the composition was analysed using a commercially available elemental analyser (EuroVector, Euro EA).

Figure 10: Is it anyhow possible to still extend this figure with the experimental freezing results on the diatom *Thalassiosira pseudonana* by (Wilson et al., 2015) or (Knopf et al., 2011)?

Knopf et al. (2011) were the first to show that marine diatoms can act as INPs. However, as they presented only freezing temperature data, but no n_m or n_N values, we could not include these data in our comparison in Figure 7 (former Fig.10)

The same is true for the *Thalassiosira pseudonana* data in Wilson et al., 2015. Nevertheless, we now make a numerical comparison in the text to the sea surface microlayer freezing data of Wilson et al., 2015 at -27°C, which is the low temperature end for the cumulative number of INPs of their study (their Fig.2b) and the high temperature end of our *F. cylindrus* data. Note, that the direct comparison is difficult, as the provide their data as cumulative number of INPs per gram of **total organic carbon** (TOC). As the source of the ice nucleator and the species distribution in the sea surface microlayer is not known to us, we had to estimate the mass fraction of the organic carbon. The related text now reads:

Wilson et al. (2015) provided experimental evidence for a marine biogenic source of ice nucleating particles, and suggested that exudates and fragments of diatoms as a source of the ice nucleating material located in the sea surface microlayer. Their low-temperature freezing data reveals a cumulative number of ice nucleating active sites per total organic carbon mass n_{m_TOC} of ~1.3x10^{10} g^{-1} at -27 °C, which is the low temperature end of their data, and the most relevant to the present study (calculated from the equation given in the caption of their Fig. 2). To compare this value to the n_{m_total} values given in Fig.10, we estimated that the organic carbon content of the samples varies between 39.32% (representing the organic carbon content of *F. cylindrus* cells, see above) or 100% (representing a purely organic carbon composition), resulting in a range of n_{m_total} of ~5.0x10^{9}-1.3x10^{10} g^{-1} for their Arctic sea surface microlayer samples. These are compared to n_{m_total} values of 8.2x10^{7} g^{-1} (2σ prediction bands: 2.8x10^{7}-2.4x10^{8} g^{-1}) for *F. cylindrus* and of 5.8x10^{8} g^{-1} (2σ prediction bands: 7.0x10^{7}-4.8x10^{9} g^{-1}) for sea ice diatoms, respectively, at -27°C, indicating that *F. cylindrus* and other sea ice diatoms may contribute to the marine INP in Southern Oceans and Antarctic seawater, assuming the Wilson et al. parameterization is applicable also to these areas.

Figure 10: Now you show values which are normalized on mass (total mass?) At which part did you include the carbon content (L 494-495) then?

Originally, we only know the number of diatom cells per mL of water, which is why we calculated the cumulative number n_N of ice nucleating sites per number of *F. cylindrus* diatoms. In the literature, many studies (including field studies) provide the cumulative number n_N of ice nucleating sites per mass. Hence, to compare our freezing data to other studies in the literature the mass concentration of the *F. cylindrus* diatoms was required and, thus, the mass per individual *F. cylindrus* diatom cell m_{total}. However, in the literature we only found a value for the mass of carbon per *F. cylindrus* diatom. Hence, we needed the mass fraction of carbon in the *F. cylindrus* diatoms (which we could determine experimentally, see new section 2.4). To make this procedure more clear, we added the calculation of m_{total} in the manuscript:

For the *F. cylindrus* samples investigated here, we used the total carbon mass per *F. cylindrus* cell from the literature (Kang and Fryxell, 1992) and performed elemental analysis to obtain the carbon content

of our samples, resulting in a value of 39.32 % to calculate the total mass per individual *F. cylindrus* diatom cell of m_{total} = 4.5x10^{-11} g. Using these values and our experimental data in Eq. (1), we have calculated the ice nucleating active sites n_{m_total} of the *F. cylindrus* diatoms

L505-507: "All temperatures were corrected for the freezing point depressions of different buffers…" How did you do it? Did you follow the approach by Koop and Zobrist (2009)?

For the data from Xi et al. (2021), we only received freezing temperature data that were already corrected for the freezing point depression by these authors. Hence, we have used their data for our comparison plot.
For the data from Ickes et al., we got their raw data with both the uncorrected freezing temperatures and those corrected for the freezing point depression. Hence, we have used their corrected ones for our comparison plot.
For our own data, we have obtained the freezing point depression by directly measuring the shift in ice nucleation temperature between pure distilled water and the artificial seawater that we used. We have corrected all measured nucleation temperatures of *F. cylindrus* diatoms in seawater by this temperature shift.

L508-510 Is it necessary to mention this in the main text? It could be sufficient to add this information as a footnote in Figure 10.

Changed as suggested.

L528 Is there any reason, why you convert °C to K at this late part of the manuscript? You have not done it before, so why here?

We removed the additional information on the Kelvin temperature range to avoid confusion.

5.Conclusions
General:

The current version of the text rather represents a summary of the manuscript. However, conclusions are still sparse in this section. I'd recommend adding some real conclusions and a renaming of this chapter "Summary" or "Summary and Conclusions".

We have renamed this Chapter to "Summary and Conclusions" as requested and also made some text changes to that part.

Minor comments:

L67 "can be transported"

We inserted missing word "be".

L538: Remove "s" from "seas-ice"

The extra letter has been removed.

L538 Check for a consistent writing of “sea-ice diatoms” versus “sea ice diatoms” throughout the manuscript

Checked and unified to „sea ice diatoms”.

References:

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