Answer to reviewer 2

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.

This is a sound set of experiments showing an increase of 7.2 °C in the ice nucleation temperatures for seawater containing F. cylindrus diatoms when compared to pure seawater. The laboratory study seem carried out well and the literature review is state of the art. There are two important aspect to be considered before this paper can be accepted.

We thank the reviewer for this positive evaluation of our study and for the detailed comments that has improved the revised version of the manuscript.

The paper does not read well and it seems a bit too long. I suggest to merge the results discussion implication or to short by half all the text in the last two section. Once have a feeling there are many sentences saying the same and not really giving a clear simple message. Clean up and make a simple clear concise message.

We have significantly shortened the manuscript by deleting several parts of the Materials and Methods (subsections 2.3.1, 2.3.2, and nearly entirely 2.3.3 including Figs. 2 and 3) and also the entire subsection 3.1.1 of the Experimental Results (including Fig. 4) and moving them into the Supplementary Information. Furthermore, we have edited the sections 3. Results, 4. Discussions and Implications, and 5. Summary and Conclusions. Following suggestions by reviewer 1, we renamed these sections and also added some text on the atmospheric relevance of our experimental results on the ice nucleation of *F. cylindrus* diatom cells and fragments, as well as sea ice diatoms in general to section 4.

It seems to be that in the abstract and conclusion, and also in the introduction (well written) one of the main result is the results "that *F. cylindrus* diatom cells as well as cell fragments suspended in seawater can induce heterogeneous ice nucleation, while icebinding proteins produced by *F. cylindrus* such as *fc*IBP11 have negligible ice nucleation activity.". This is important and also compared with the literature, but what is the reason? Any literature support any speculation and possible reasons? This is in stark contrast with other literature supporting the idea of proteins being important in INP, but little is discuss in the text of this paper. I suggest to expand this extensively given it seems a major result. It is also important to give possible biogeochemical reasons of cell fragments being more important than proteins.

We have now stated this result also in the Summary and Conclusion section. The reason why some ice-binding 'antifreeze' proteins act as at least moderate ice nucleators (such as the *Tenebrio molitor tm*AFP) and others show only minute or now ice nucleation activity (such as the *fc*IBP11 studied here) is not entirely clear, but a recent modelling study suggest that this may have to do with the ice planes that they usually bind to (see statement at the end of section 3). However, typical ice-nucleating proteins are usually much larger than these ice-binding 'antifreeze' proteins and their large ice-active site may therefore be much better suited in supporting a newly forming ice embryo. We have enhanced the section explaining the differences and similarities between ice-binding proteins, antifreeze proteins and ice-nucleating proteins that may also help understanding these phenomena:

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*.

References:

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