

Reply to referee 1

"I believe there could be a more explicit discussion of the implications of the investigation's results – moving beyond generalized statements of importance/warranting future attention toward isolating how specific simulated dynamics (e.g., cyclic convection induced by microbes) could influence broader Earth systems (e.g., nutrient/salt flux into the ocean and the impact on ocean circulation or biological production in the water column). I feel that such targeted discussion could amplify the impact of the paper by providing readers with tangible examples of how this type of modeling work can be helpful for the broader scientific community."

Although we are a little bit cautious about claiming too much (for example, modeled induced fluxes are on a relatively slow, biological time scale), it is interesting to note

"In the Introduction – While the addition of the biological antifreeze component to a sea ice model is novel, there is a general lack of discussion of previous modeling work that has been done exploring other biogeochemical processes (which is akin to and relevant to this publication, as these processes go hand in hand – e.g., nitrogen, carbon, iron, silica cycling, growth/death rates of resident in-ice biological populations). This is a new and dynamic field, and much work has been done in the past 12 years since the Vancoppenolle et al., 2013 paper cited at the end of the introduction. I think including some additional background on the current state of biogeochemical processes modelling in sea ice is likely warranted."

We thank the referee for this nudge. We've added some more reference to recent literature. We suspect though that the referee has something particular in mind, and welcome additional comment.

"The experimental component of the paper attributes the additional  $\sim 1^\circ\text{C}$  of freezing point depression (below that of the seawater samples) to the presence of osmolytes in the system. However, there are no experimental results to

show that this additional freezing point depression (e.g., that seen in Figure 2) could not be attributed to the f/2 media contained in the biological and supernatant samples. f/2 media contains a range of sodium salts that would be present in both samples, and as such could be the cause of the additional freezing point depression if the concentrations are non-negligible. I think it would be valuable to demonstrate that the seawater + f/2 media in the absence of cells/osmolytes cannot produce the observed signal, either experimentally or theoretically (by quantifying the level of sodium salts in the samples and ensuring they cannot impact the freezing point by 1°C)."

In fact, we did test the "pure" media to see that it didn't depress the freezing point by itself, see Figure 2, left panel. The figure caption was not well-worded though. We've changed its wording so as to, hopefully, clarify. Note: the concentration of f/2 is comparatively quite low (approximately 26.5 mg/L). The base sodium level in artificial seawater at 36 ppt is approximately 11.1 g/L whereas we estimate that, with f/2 medium added, 11.1 g/L would be increased to approximately 11.13 g/L. So the impact of f/2 is quite small here.

"The authors state "In the second case, there is the possibility of transient occupation in which microbes from an ocean reservoir only pass through the ice without any significant residence time, so we also consider growth and decay processes. In the sessile case, though, transience is not an issue so for simplicity we do not include growth/decay in the microbial model." I do not understand why growth and decay would not be relevant to the sessile case. I would imagine growth and decay would be more important, given no new organisms can be advected into the system like in the planktonic case. The impacts of salinity and nutrient concentration would likely be critical to these sessile organisms that rely on the upwelling of seawater to replenish nutrient stores and/or maintain a non-toxic level of salinity. Further explanation for why this choice was made in the model is warranted. I think it could be incredibly interesting to see the link between nutrient availability, osmolyte production, and the convective regimes."

We make an assumption that is frequently reasonable for environmental biofilms, namely that biofilm communities are approximately at equilibrium with respect to growth/decay processes, that is, growth and decay roughly balance. (This is not to say that growth and decay

are not occurring and not interesting individually, although in our experience microbes in biofilms are not as active, growth/decay wise, as planktonic ones.) So the presumption is that, if it is able to establish a convective flow, then adequate nutrient will be available to keep the biofilm roughly at balance.

As the reader suggests, though, biofilm (and planktonic) activity in the ice should be limited by something. Here we use the combined osmolyte and salinity (which we call effective salinity), see equation (1). A paragraph has been added (at approximately line 140). Motivation is discussed in a bit more detail down below.

Note: while editing, we noticed a notational inconsistency in equations (1) and (3), as osmolyte production (first term on the left on eqn (1)) depends on B but osmolyte decay (second term on the left in eqn (1)) does not. We've changed the definition of P, with accompanying minor change in eqn (3), to correct this.

"I think a plot of porosity evolution for the simulations should definitely be included in the manuscript. This is a critical factor governing the fluid flow throughout the system and is a property that is tied directly to the microbial dynamics explored in the investigation. An accompanying discussion of the feedback between salinity/osmolyte concentration, porosity, temperature, and biological activity (and associated observable features in the model results) could also help describe the system to non-experts who don't necessarily think about the interdependent nature of these systems."

This is an interesting point. Rather than porosity, we've included a plot of permeability (Figure 6), which is more directly connected to transport. Permeability is a function of brine volume fraction (we don't really ever estimate porosity per se, although brine volume fraction is similar), and is a natural way to discuss the connection of our model results to ice structure. See the new text at the end of Section 4, and also in Section 5.

"There is an initial spike of osmolytes in the upper reaches of the ice in both simulations due to the initiation of the model – what conditions cause this spike (particularly near around 20cm below the ice-atmosphere surface)? Additionally, these high concentrations seem to then diffuse away – what causes this reduction in osmolyte concentration after the initial spike if the

permeability of these upper reaches is quite low so as to not allow fluid flow or osmolyte diffusion?"

Yes, the spikes are a result of initialization – the initial conditions are set as the final state of the model run for long time without any microbial contribution (an initialization run). Abrupt introduction of the biology results in some transient behavior. In this case, the final state of the initialization run is still a bit unsettled in the upper regions because it doesn't drain properly due to the very cold temperature there (we're not sure why 20cm is particularly noticeable) – note that even in the initialization stage, we maintain a 1m thick ice sheet and hence the upper region has not drained as it would with a growing ice sheet. For these reasons there are some more noticeable transients there.

The reason that the osmolyte concentration drops is that there is an osmolyte decay term see equation (1). We actually do not include osmolyte diffusion in the computations presented, with a decay time scale of order 100 hours. We've played with osmolyte diffusion a bit, and it doesn't seem to effect results all that much.

"The authors say that the decrease in temperature above the convective layer in the sessile simulations are related to a reduction in thermal conductivity associated with increased brine volume “temperature above the convective layer actually decreases with introduction of biofilm activity. This seems to be a consequence of an increase in brine volume fraction there due to osmolyte production, which results in a drop in thermal conductivity “. Is this not just purely a consequence of the defined liquidus relationship – i.e., osmolyte concentration increases, local thermodynamic equilibrium is assumed, and therefore the temperature of the system is required to decrease?"

Yes, the liquidus equation couples effective salinity and temperature, but there is also an additional constraint, namely the transport equation for enthalpy, see equation (A1). This extra constraint also couples the two, strongly in regions where ice flow is non-zero, and more weakly where it isn't (through the thermal conductivity, which is dependent on brine volume fraction). At the same time, the enthalpy transport equation is global in nature, and also introduces temperature boundary conditions from the top and bottom of the ice sheet. So it's a little difficult to say what causes what!

Regardless of causality, though, in the region where ice flow is zero, at steady state heat is transported diffusively (see again equation

A1) and the heat transport term  $kVT$  is constant. Since  $k$  is nearly unchanged in regions near the top of the ice sheet, we can infer that less bulk transport of heat is occurring. A clarifying remark to his effect has been added to the manuscript.

"The authors state "Note also the slow build up of osmolyte in a layer near the top of the convective region, Fig. 3 middle left, where convection is slow due to low ice permeability; this layer eventually discharges as well (not shown)." But do not expound on this further. I think this is an interesting result that could have some substantial implications – deep convection that exceeds the more continually convecting layer near the ice-ocean interface could provide substantial (and apparently punctuated) fluxes of material (salts, organics, etc.) to the underlying ocean that could impact ocean dynamics and biological productivity. I think it could be worth discussing this further, if only hypothetically."

We haven't pressed this point in the manuscript because of the possibility that it is an artifact of the model construction. To explain: the model needs to include a mechanism to inhibit microbial activity (otherwise activity might occur throughout the ice sheet). There are a number of factors that might influence inhibition, e.g., salinity, nutrient or byproduct concentrations, temperature, etc. For the purposes of our objective (i.e., testing *in silico* the hypothesis of microbially-induced convection), it doesn't matter so much how inhibition arises. Thus, to reduce model uncertainty, we choose only one, namely generalized salinity (actual salinity plus osmolyte effective contribution). To make sure that model inhibition does not directly interact with the main focus of induced convection, we tune down inhibition so that it does not have a significant impact within the convective region. As a result, there is a layer above the convective region which still shows activity in the form of osmolyte production, but we don't want to claim that this may not be model dependent.

Note: this is somewhat related to a point discussed above, and some text has been added to the manuscript around line 140.

"Digging into the numerical methods section of Appendix B here. Steps 1–3 state that equations A12, A11, and A10 are solved using the bulk fields. Are these equations solved iteratively? They appear interdependent, such that solving for temperature, will impact the porosity, which will impact salinity and osmolyte

concentration – BUT this will in turn impact temperature again via the liquidus (equation A7). I believe these will ultimately converge/stabilize to any desired tolerance if iterated (and perhaps this is already being done, in that case this comment can be ignored and I would just mention this is being done in the text), but if it isn't being iterated the choice of timestep could impact the results – it may not if the timestep is sufficiently small – but given this is the thermodynamic equilibrium assumption that governs the phase, temperature, and salinity of the system the authors may want to check if this is impacting the results."

In answer, no, there is no intra-step iteration, and, yes, this could potentially have some impact on accuracy. However, our time step is actually very small. This is by necessity as we are using an explicit-in-time method for integrating the enthalpy equation (A1) which is subject to numerical instability. We ended up using a time step of  $5 \times 10^{-6}$  hr (i.e., each time step is approximately 0.001 seconds), which is very short in comparison to physical time scales in the problem. Additionally, we conducted convergence tests by varying the time step, so we are confident in the accuracy of our computations. With apologies, we didn't provide the time step in the original manuscript, but have now included it, see the end of the first paragraph of Appendix B.

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"I like the current modeling approach as it clearly is geared towards isolating the effect that is examined here. However, it would be helpful for the reader to maybe see results from an additional model setup that might be more relevant for the real world: In reality, previous studies have found an opening of the pore space in spring related to the warming of the ice from above. This can lead to full-depth convective overturning and a substantial nutrient supply to the interior of the ice around the time of spring bloom. I was not sure (but maybe I just missed it) which surface temperature was chosen for the simulations presented here, but it might be worthwhile to add model simulations with a relatively warm surface temperature that allows for substantial convection even without microbes added. It would be interesting to see how the addition of microbes affects convective efficiency in such warm ice, maybe in percentage of convective volume of the case without microbes."

The question about springtime, and spring bloom, is really an

interesting one. it is, however, not at all simple especially mathematically but also physically. Mathematically, the system turns into a moving boundary problem which is a significant complication. Adding to the difficulty are ambiguities in the interface conditions with regards to heat transfer at the ice-ocean interface (how much heat goes into melting and how much is exchanged with the ocean, which depends on local fluid dynamics). This is really an entire project by itself, and one we are thinking about in fact. We agree that microbial influence could be of interest!

Yes, the boundary temperatures were unfortunately omitted in the original manuscript. We will add them to the revision ( $T=-20$  at the air-ice interface and  $T=-2$  at the ice-ocean interface). Also, we did in fact look at changing the surface temperature to an extent - we didn't notice much effect other than changing the size of the convective region.

"l.49: I feel the description of initial brine convection to be a bit misleading: Brine inclusions do not "begin to be interconnected", but instead the growing brine network is initially interconnected and then the ice matrix causes the interconnection to eventually become too small for effective convection. The lack of convection in very thin ice can be attributed to too low a driving potential energy despite a fully connected pore space. The current description is correct for existing ice that is being warmed (as described above), but this was not the context of this paragraph in the manuscript as far as I could tell. (On re-reading it, one could also understand the "begin" to refer to a spatial position as one moves downward in the cold ice, but also this I do not find to be too clear a description). Generally, I felt it might be helpful for the reader to have the discussion framed around a critical Rayleigh number already here, as this captures the underlying physics of the convection."

In fact, the second (re-reading) interpretation is correct, i.e., "begin" does not refer to time dependence here. We will delete "begin to" to remove this ambiguity.

The suggestion about a discussion of underlying physics of a Rayleigh instability is good - we will add something along these lines in a revised version.

"l.52: You might want to add "and thermal dissipation", as in sea ice the driving force of brine convection becomes weaker as the brine warms (and thus gets diluted) while it moves downward through the ice, as captured by the heat diffusivity within the calculation of the Rayleigh number"

We're not sure precisely what is intended here. Changes due to warming might indeed change dynamics (we're not actually aware if such an effect has been considered in the Rayleigh–Taylor instability literature, which is very large but mostly in contexts other than ice) because of dilution and also because of reduction in effective viscosity (large brine channels result in less viscous drag). The parameterized approximation we use here is too crude to capture either of these effects in any case. This is a quite interesting point though.

"l.57 I find it misleading to state that the drainage relies on freezing of new seawater. Drainage can also occur as the ice gets warmer and the porosity increases, for example. This description could be clarified by framing this part around the Rayleigh number, I think."

We're a bit puzzled by this remark. Drainage occurs when colder temperatures result in brine concentration which drive density inversions, at least drainage via Rayleigh–Taylor instability, so we're not sure how warming plays in. Perhaps the trade-off in increased porosity vs decreased density can still allow instability? If the referee could expand and/or provide a reference?

"l.89 ff: Again, I felt that framing this discussion around the potential energy needed for convection versus the impact of porosity in determining the energy required for convection could be helpful in framing this discussion. If I understand correctly, you are saying that  $Y_{sal}$  does barely influence the Rayleigh number, is that correct?"

Yes, while  $Y_{sal}$  does not directly impact Rayleigh number, we have not seen significant impact indirectly either. There might very well be biological reasons to favor higher or lower values though.

Also: yes, some discussion of the connection of porosity and Rayleigh–Taylor energetics is a good idea, and will be included in a revision.

"section 2/section 4.1: I did not fully understand how you differentiated between the impact of the microbes, the impact of their by-products/proteins/osmolytes, and the impact of the chemical composition of the supernatant which probably was salty. Can you add a few lines of discussion to help the reader understand what really caused the  $-1$  °C freezing-point depression identified in 4.1?"

Yes, we will add some discussion to the revised text. To answer the questions:

Diatoms were grown in artificial seawater plus a culture medium (no algae, no other component). The impact of the culture medium itself (~26.5 mg/L) was negligible compared to the salinity of the seawater (11.1g/L).

The entire culture was tested (Fig.2, middle) as well as the supernatant (Fig.2, right) which contains the seawater-media mixture plus whatever else is there after the diatoms themselves (and any other dense material) is centrifuged out. That is, Fig.2, right, includes by-products/proteins/osmolytes as well as the seawater and remaining media all as part of the supernatant.

Figure 2, left, tests the artificial seawater and "raw" media (no microbes ever added) as a control.

Because Figure 2, middle and right, are similar, and each show an approximately one degree C drop, we argue that most of the temperature drop is a consequence of what is in the supernatant and not of the microbes themselves.

"Figure 2: I was surprised to see the fairly large amount of supercooling in all experiments carried out here. Can you comment on this? Given that the water was stirred, I found it quite impressive that it would remain liquid in a supercooled state for several minutes, even with the diatoms added. Is the response time of the thermometer negligible?"

This is a great question. Our apparatus is not suitable for making conclusions about the degree of supercooling, as it is making some sort of averaged measurement of a spatially heterogeneous transient-in-time process, even with stirring. The reviewer is also correct in pointing to the thermometer response time; the data logging frequency (one measurement per minute) is not sufficient to accurately capture supercooling events. This may also contribute to the variability observed across replicates regarding the supercooling. Altogether, we don't know the answer to this question!

"section 4.2 ff: I suggest to have a short description of the model in the main paper. In order to understand, for example, l.164, one would need to turn to the appendix in any case. I suggest that only equations are left in the appendix that provide detailed information that can be skipped for understanding the main text. The formulation

of the model is a central part of your results and thus warrants some highlighting in the main part of the paper, I find.

Yes, this is a difficult point and one that we thought about quite a bit when writing the original manuscript. We haven't found a way to include equations into the main text without moving most of them, which is likely to negatively impact readability for many, and also break up the model presentation. Even accepting that, though, we take the referee's point that a description of the model in the main text is needed, so will add one to the revisions.

"Eq. 1 and 2: I would find it helpful if the choice of these functional forms were briefly motivated."

We will do so in the revised version.

"Figure 4: I couldn't fully follow the argument of the reason for the periodic cooling throughout the entire ice matrix above the convecting layer. It would be helpful to maybe show vertical profiles for the colder and the warmer periods, respectively. I was wondering if the "unusual" behavior is rather the warming of the ice column by the intrusion of warm sea water and the cooling is a recovery to the equilibrium state without convection. I think this could be differentiated more clearly if profiles were shown."

"Figure 4: It'd be helpful to use the same color scale on both figures, and maybe also the same x axis."

We think that there maybe a misunderstanding here. The periodicity in the upper regions in Figure 4, left, is present because of the periodicity in the lower regions. Both are a consequence of the periodic convection, as seen in Figure 3, top left, bringing in warm water indeed as the referee notes.

Rather, the discussion in Section 4.4 is about the reason that the upper regions in Figure 4, left, show ice sheet cooling relative to the microbe-free ice. That is, while periodic behavior is seen, the upper ice sheet is always cool relative to microbe-free ice (after initial transients). This was (to us anyway) unexpected because convection in the lower region draws in warm water and so might be expected to lead to relative warmth in the upper regions. We will look to clarify the discussion in the revision.

With regards to axis and color scales, with apologies, we'd prefer not to make changes for the following reasons. First, x-axis (which is

actually time): the biofilm case (left columns of Figures 3 and 4) are run out to  $t=2000$  to demonstrate clearly the approximate periodicity. This length of simulation is not needed for the planktonic case (right columns of Figures 3 and 4) so we only run to  $t=500$ . In principle, the planktonic case could be run out to  $t=2000$  as well, but this wouldn't add anything and would obscure some of the initial features a bit, which is in fact the reason we stop at  $t=500$ . Second, color scales: this is a close case, but because the temperature increments don't overlap very much between Figure 4 left and right, using the same temperature scale for both would compress the color range for both, lessening contrast in both. So we'd prefer not to make this change.

Figure 2: It'd be helpful to have the x axis labeled (with units)

We will do this in the revised version, thanks.