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Interactive comment on "Quantification of basal ice microbial cell delivery to the glacier margin" *by* Mario Toubes-Rodrigo et al.

Anonymous Referee #1

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Toubes-Rodrigo et al present evidence in support of their assertions regarding the delivery of cells from basal ice to the glacier forefield of an Icelandic glacier. The manuscript is clearly structured, and is to be commended for integrating key glaciological constraints on subglacial microbiology. Unfortunately I have a number of major reservations about the manuscript in its present form. Principally these are:

1. Claims of cell discharge relate to total cells derived from DAPI counts and viable cells from CFU counts obtained from the inoculation of a specific growth medium under one set of incubation conditions for five weeks. While total counts from microscopy are acceptable, I firmly disagree with the notion that the authors have determined viable counts, and whether the procedures employed are adequate to answer their experimental question on the following grounds:

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A: Viable does not mean culturable. Consider the very paradox of the acronym "Viable But Not Culturable" which has been explored for >30 years by many investigators. As the issue of VBNC sets out, one of the challenges of contemporary microbial ecology is understanding the gap between who appears on your agar plate (culturable), who is present (total, including dead cells) and who might live in situ (viable) and those who are able to live in situ but not on your agar plate (VBNC).

The paper needs to take into account that viability is non synonymous with culturability. Here culturability under one set of conditions is presented. This means very little for quantitative estimation of viability. If one is minded to determine the abundance of viable cells within an environmental habitat, very different tools are required, typically in the vein of Live/Dead stains and microscopy. These are not without their problems of course.

B: One set of conditions are tested: 10% TSA, 4 deg C for five weeks. No data is presented setting out whether this set of conditions is representative or optimal. What assurance does the reader have that this protocol provides consistent counts?

C: So if viability itself is not quantified what about culturability itself - is it meaningful? What does growth in vitro really tell us about those cells' ability to colonize proglacial environs? I believe it was the eminent microbiologist John Postgate who stated that "every colony is an artefact". It is difficult to convincingly aruge that culture of cells in vitro under one set of fixed conditions necessarily provides quantitative insights to the in situ actuality.

D: No information is provided on the community composition of the inhabitants of the basal ice or the proglacial habitats they may be discharged into. Clearly, not all microbes have the same potential to colonize an environment. The ecological impact of inoculating a trillion cells which cannot persist and grow in the forefield will be very different to just one cell immured in basal ice which can also thrive in the forefield. As such, the implications of mass transfer of cells are poorly developed, and assume

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equivalency of outcome across what are very different scenarios: are these cells likely to pioneer the forefield community's development, or are they simply a source of nutrients as necromass? Or will dormant cells provide a long term repository of genetic potential for later stages of soil development? Very different ecological scenarios arising from the physiological state and colonization potential of the source microbiota which are beyond the scope of the analyses performed. As such I feel the development of the rationale of this underlying motivation of the paper is limited in its grasp, and the paper would really benefit from careful consideration of the processes underlying the assembly of microbial communities.

2. At the heart of this paper are total counts and CFU counts. While their use coupled with expert interpretation of the basal ice facies is important, this seems a little preliminary and the conclusions drawn risk superficiality as a result. The paper would be greatly strengthened as an offering to the literature if it described the taxonomic composition of the cultured and total community. As noted above, simply dumping cells into an environment has radically different outcomes dependent on the identity of the cells.

3. How representative is this site of other locations? I appreciate that its history of circumspect glaciological investigation lends itself for this study, but considering its history of advance over soils, what lessons can be learned from this site that would be applicable to sites with very different histories?

4. L8: "We present the first assessment of microbial cell discharge from sediment-laden glacier basal ice." L28: "We report the first quantification of microbial discharge to a glacier margin, and demonstrate that there is viable microbial inoculum released to the proglacial environment"

Respectfully, I disagree with the assertion of priority made for this claim, and the emphasis provided by placing it at the start of the abstract. Starting from the seminal paper of Sharp et al (1999) microbial prevalence in basal ice has been widely documented as has its potential for inoculating forefields as well as demonstratable culturable bacteria

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using a range of methodologies, as well as culture-independent strategies (e.g. Kaštovská et al 2007; Yde et al 2010 Ann Glaciol; Montross et al 2015 Geomic J; Rime et al 2016 ISMEJ). I would highly recommend a more circumspect statement regarding the motivation of this study which clearly and fairly asserts the scientific novelty of the work. Perhaps the emphasis of integration with basal ice extent is required?

5. L16: The authors emphasize the heterogeneity inherent to these ice facies. The methods section does not set out how the samples collected were distributed across the ice facies to describe this hetereogeneity and minimise potential biases. In short, what was the specific survey design, and the extent of replication. Fig1a goes some way to explain the number of sites sampled, but more clarity is needed here, especially on potential intra-site variation.

6. Uncertainties in sediment transfer rates. These seem pretty broad, and incur a two-fold variation in the potential discharge of cells. Can the authors justify the insights afforded by this calculation considering this considerable uncertainty?

7. How does basal ice microbial discharge scale up relative to fluxes from meltwater or till? Context could be provided here.

8. Discussion needs to draw out the insights into the ecological processes affected by cell discharge from basal ice. What does it all mean for the downstream habitat?

In summary, many of the assumptions made in this paper merit careful contemplation and the datasets presented could be supplemented by orthogonal information regarding community composition. In critiquing the work offered I really do not wish to deflate the very commendable initiative shown by the early career reesearcher in the process of learning how to correspond his work. I would hope all authors to work carefully with him to strengthen a future embodiment of this paper.

Minor comments.

L27: More detail is needed here on sampling protocol and precautions to allow readers

without access to the cited source to evaluate the protcol applied. L29: Ballpark figure provided by Shivaji et al (2011). Microbial abundance changes considerably as soil develops over a chronosequence. Perhaps your basal ice abundances matter more when meeting the depauperate bare till of the immediate glacier margin. L30: Formamide? or formaldehyde? The reader needs to be reassured the microbial population is adequately fixed for enumeration. L32: Formaldehyde L36: Using DAPI on small and dormant cell populations. What assurances does the reader have about the sensitivity of DAPI in this context given its lower quantum yield of fluorescence relative to SYBR stains?

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