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

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We thank Reviewer 1 for their constructive and critical review of our manuscript. There are some valuable observations in the review that will lead to improvements in our revised manuscript. We take this opportunity to respond to comments and highlight what changes we intend to make in the revised manuscript. Our replies to each comment begin with "»>".

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 ex-

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perimental question on the following grounds: 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 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 repositry of genetic potential for later stages of soil development? Very differ-

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ent 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.

»>The reviewer is correct - we used the term “viable”, which is rather old-fashioned and inaccurate terminology, and therefore misleading. We will replace the term with “cultivable”, in the revised manuscript. The reviewer also states that the cultivation technique is inadequate to answer our experimental question. However, the rationale for carrying out cultivable, in addition to total, counts was given in the methods as “In order to analyse if the community inhabiting the basal ice supported alive and viable. . .”. Environmental microbial cultivation, using classical dilution plating on diluted TSA, obviously does not provide an absolute quantification of the viable microbial load in the glacier and we do not claim this to be the case in our study (excepting the error of terminology noted above). In any case, we distinguish the difference between cultivable and viable cells in the text and Figure 2, in presenting the cultivable counts as “conservative estimates of the delivery of viable cells to the ice margin”. We stand by this – what we are presenting is a lower limit of viable cells and there is plenty of scope for future work to refine our estimates to further highlight the significance of basal ice derived microbial cell discharge. There is no guarantee that cultivation with 10 % TSA is “optimal” but there is also no requirement for it to be optimal and no reason to expect it not to be “representative” – at least for the purpose of comparisons between our samples. A look at several studies where microorganisms have been recovered from cold/icy environments reveals that there have been a range of different isolation media used (e.g. R2A e.g. Montross et al., 2014 , TSA e.g. Miteva 2004, LB e.g. Shivaji et al., 2011 and even other media, Foght et al., 2004), and with different media strengths (2%, 10%, 50%, 33%). Our purpose was simply to provide support for the microscopic counts, plus indicating the presence of viable cells. The reviewer seems to think we had a greater purpose than this, which is probably because we used in-

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accurate terminology (now removed). As the reviewer points out, to properly quantify the viable fraction of cells in the glacier is no trivial task, and as such it is way outside the scope of this work. Assessing microbial community composition and community assembly was also outside the scope of this work, in which we chose to focus on microbial cell transfer rates as a discrete subject that is clearly identifiable in the title of the paper. The underlying motivation of the paper is, we believe, clearly stated and not "limited in grasp" as suggested by the reviewer. The focus is a first assessment of transfer of microbial cells in the basal glacial environment and not their taxonomy. We will perform detailed geo-microbial community analyses of basal ice using culture-dependent and culture-independent methodology that will be reported separately. The results presented in the current study will inform interpretation of functional basal ice geo-microbiology data.

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.

»>Yes, the heart of this work is total counts and CFU counts – coupled with glaciological data about the nature of ice facies, included sediment, and sediment discharge. As mentioned above, this provides a discrete result and this was our aim. The findings are relevant to both the glaciology community and the microbiology community. As stressed above, to describe exhaustively microbial taxonomic composition in basal ice is outside the scope of the current study, and warrants reporting separately in a follow up microbial diversity focused paper. We also wanted to make the point in our paper that there are key differences between ice types, which might affect the microbiology – this is usually overlooked in glacial microbiology studies, and in our opinion worthy of discussion. So we feel our results and message are somewhat broader than suggested

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above by Reviewer 1.

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?

»>As the reviewer states, a key advantage of working at this site is that the sediment transfer system is well known to the authors – in particular, Cook has worked here for over a decade, and published a number of studies on sediment transfer at Svínafellsjökull. But the reviewer raises an important point about the extent to which this site is representative of other glacial systems more generally. We suggest that this site is representative of many of the glaciers in this region, and could be considered representative of other temperate glaciers elsewhere. Of course, we acknowledge that there can be significant variability between glaciers, even of the same thermal regime, size, prevailing climate, etc. Svínafellsjökull is a temperate valley glacier that has experienced periods of advance and recession contemporaneously with other glaciers in southern Iceland (Hannesdottir et al., 2015). During recession, there has been opportunity for soil and vegetation development in the area currently occupied by glacier ice. This is true of many of the glaciers in this region – for example, Ives (2007) highlights documentary reports from neighbouring Skaftafellsjökull where the glacier had once been mined for birch wood overridden by the glacier. Other glaciers globally have experienced similar patterns of recession and advance during the Holocene. Given that glaciers commonly experience phases of recession and advance, we suggest that Svínafellsjökull is a representative site at least regionally, and potentially globally for other valley glacier systems. In our revised manuscript, we will highlight how our results are applicable more generally.

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 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?

»>The reviewer is correct in stating that we are not the first researchers to investigate microbial prevalence in basal ice, nor are we the first to document the potential for basal ice microbes to promote proglacial soil and vegetation development. We will re-word the abstract and the Introduction in the revised manuscript to reflect the fact that there has been literature to suggesting that microbial inocula are important for proglacial ecosystems. We now recognise that the way in which we had written this appeared to be disingenuous. The reviewer is also correct in stating that it is the combination of our microbiological data with the glaciological/geographical data about basal ice thickness, sediment content and extent that adds novelty to our study. We see no basis for the reviewer's first criticism here about us not being the first researchers to examine microbial prevalence in basal ice – we never make such a claim. The point of our paper is that we are the first researchers to quantify the discharge of microbes from basal ice – not to be the first to quantify microbial prevalence in basal ice. We feel that we have already been clear about this throughout, including in the statements cited by the reviewer above.

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,

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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.

»>Yes, the heterogeneity in basal ice is a key point that we want to get across to those interested in sampling basal ice for microbes. It was for this reason that we developed a targeted basal-ice sampling methodology described in detail in Toubes-Rodrigo et al. (2016), which is cited in the text. Ultimately, we think the reviewer has slightly misunderstood the aims of our study, but this has prompted us to make some minor clarifications to the text. Briefly, previous studies of basal ice microbiology, with the exception of Yde et al. (2010) and Montross et al. (2014), have not accounted for the fact that basal ice typically comprises different ice types/facies of different origins and characteristics. Our point really is that it is too crude to state that one sampled "basal ice" that is likely to be comprised of different ice facies, that could potentially explain differential glacier-specific microbial content. Our study acknowledges that the two basal ice types, stratified facies and dispersed facies, exhibited different physical characteristics. Whilst there might be slight differences between, for example, a piece of dispersed facies sampled from the north of the glacier versus a piece that is extracted from the south, the samples are from descriptively the same ice type with the same origins – as demonstrated by Cook et al. (2007, 2010, 2011a). We will provide photos of the sampled sites to try to clarify this in the revised manuscript, and will clarify the lack of intra-site variation in the text.

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?

»>Our cell discharge values are based on glacier velocities ranging from 4 to 8 cm/day (or 14.6 to 29.2 m/year), as reported in Cook et al. (2010). These data were acquired over a 4-week survey period in Summer 2007 using a total station. We have undertaken subsequent satellite remote sensing work (feature tracking), which confirms velocities

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in the terminus to be <40m/year, averaged over a year from 2002 to 2003. It is perhaps unfortunate not to have convergence on a closer range in velocity, and hence, cell discharge values. We could instead have reported the mean velocity rather than the range, but decided it best to be transparent about the range of values observed. In reality, it is likely that velocity changes rather a lot as subglacial hydrological networks evolve seasonally, for example. We think the range in values is a better way to reflect this natural variability, which is important because this is the first time anyone has attempted to quantify cell discharge from basal ice. We will clarify this in the revised manuscript. We also argue that we are making an important separate point about cell release from different basal ice facies at this glacier, which is a function of sediment content and basal ice extent and thickness. As we have discussed, known basal ice heterogeneity is an important justification for the rationale of our work and it is the first time anyone has examined variations in cell discharge between different basal ice facies.

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

»>Frankly, we don't know! This is something that needs to be addressed by the glacial microbiological community. This could be relatively straightforward to assess for subglacial meltwater – ideally, one would want a glacier where the majority of the water is discharged at the glacier front through a single portal. Irvine-Fynn and Edwards (2014) estimated that 3.2 x 1021 cells a-1 were discharged from glacial meltwater systems worldwide. Studies on cell discharge from till would be more challenging. There is still much disagreement on the pervasiveness and depth of till deformation, and how to model its flow (e.g. Clarke, 2005), and instrumenting the till in a representative way to gain insights into rates of movement would be difficult. Certainly, we can consider adding some context to our results, but it is difficult to address the reviewer's comment more fully due to the lack of available data from other studies.

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?

»>This paper does not directly lead to ecological insight. Rather, it provides data that can support such insight in future work - for example, our own microbial community analysis mentioned earlier. We believe that the addition of ecological interpretation is out of the scope of this publication. The aim of this paper is to present a calculation of microbial delivery from basal ice to the glacier margin.

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.

»>Thanks. Yes, we will add more detail in the revised manuscript.

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.

»>Yes, that's a good point.

L30: Formamide? or formaldehyde? The reader needs to be reassured the microbial population is adequately fixed for enumeration. & L32:

»>Formaldehyde in both cases.

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?

»> The use of DAPI is a well-established technique, and it has been used previously to investigate basal ice microbiology (Yde et al., 2010). For valid comparisons the same concentration has been applied in this study.

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