INSTITUT MEDITERRANEEN D'OCEANOLOGIE
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**Reviewer report on the Doctoral thesis of Petra Klímová: "Ecological processes associated with the glacial-proglacial ecosystem transition"**

## Overall Assessment

This thesis investigates ecological and biogeochemical processes across the glacial–proglacial transition, with particular focus on processes occurring at the glacier bed, subglacial sediments, and ice margin, exported microbial communities, and methane cycling. The thesis is structured by a synthesis chapter and four research chapters (two published as first author, one published as a co-author, and one submitted as a co-author). The central contributions of the thesis are:

1. Demonstrating that subglacial sediments exposed by glacier retreat contain a chemically and microbially distinct "legacy" pool of organic matter and nutrients.
2. Showing that exported microbial assemblages retain signatures of subglacial microhabitats and hydrological processes.
3. Experimentally testing how mixing of supraglacial and subglacial substrates under oxic and anoxic conditions influences $CO_2$ and $CH_4$ dynamics.
4. Exploring the potential role of methylotrophs and methanotrophs at a methane hotspot at the Greenland Ice Sheet margin.

Overall, the thesis is comprehensive and of very high quality. The research spans an impressive amount of field sampling, microbial community analyses, geochemical analyses, incubation experiments, and ecological interpretations. The candidate's analyses integrates many datasets and their findings make meaningful contributions to the understanding of ecological and biogeochemical dynamics at glacial–proglacial margins. The thesis is also well written and contains multiple peer-reviewed publications.

## Comments on individual aspects

*1. Conceptual framing and synthesis*

The thesis is conceptually very strong. The glacial–proglacial transition is treated as an interacting biogeochemical continuum rather than a set of isolated environments. The notion of a subglacial "legacy package" comprising of organic matter quantity and quality, nutrients, and microbial inhabitants, is an important and conceptually robust framework. In some places, particularly when analysing taxonomic data and drawing functional inference, the interpretation could slightly exceed the strength of evidence, as detailed below.

*2. Chapter I: Subglacial sediment characterisation*

This chapter integrates an Arctic-wide sampling effort, organic matter degradation measurements, radiocarbon data, and microbial community composition data. A key conclusion is that subglacial organic matter is often substantially degraded prior to exposure following ice retreat. This is an important contribution as it challenges narratives of glaciers releasing labile organic carbon. For the defence, the candidate could reflect on the following:

- If subglacial organic matter is already substantially degraded prior to exposure following ice retreat, does the organic matter represent a preserved legacy pool, an end-product of subglacial processing, or a mixture? To what extent do the data allow for distinguishing between these

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processes? Building from this, to what extent might proglacial soil development and ecological succession be driven by (i) the characteristics of legacy material, (ii) the subglacial transformation processes prior to its exposure, and (iii) terrestrial conditions following exposure?

- What are the key determinants of microbial community structure in theses sediments? Underlying lithological characteristics vs subglacial hydrology vs glaciation history?
- To what extent is there collinearity among environmental variables and how does this affect the robustness of the inferred links between organic matter degradation state and microbial community composition?

*3. Chapter II: Exported microbial communities as indicators of subglacial habitats*

The concept of exported assemblages serving as biological tracers of subglacial micro-environments is compelling and well argued. The chapter relies largely on 16S rRNA gene amplicon sequencing data and geochemical/environmental data from sampled meltwaters. Taxonomic patterns are well described and linked to environmental characteristics. The interpretation occasionally makes functional claims (e.g., methanogenic or methanotrophic potential). For the defence, the candidate could reflect on:

- What level of functional inference is justified from 16S rRNA amplicon sequencing data? What cannot be supported by functional gene data alone and requires other data (what are these other data)?
- How might conviction in conclusions change if mcrA and pmoA genes were targeted, or if metagenomic data were incorporated?
- Even if mcrA and pmoA genes / metagenomic data analyses were incorporated, could one distinguish between active (or inactive) in situ populations, and exported (active/inactive) legacy populations in the proglacial melt outflow?

*4. Chapter III: Simulation of deglaciation*

In this chapter, mixing of subglacial sediments and supraglacial cryoconite under oxic and anoxic conditions provides a mechanistic test of how redox transitions influence greenhouse gas production in transitioning glacial/proglacial environments. The observation of strong methane production under anoxic mixed substrate conditions, despite a low relative abundance of methanogens, is interesting and raises some important discussion points.

- How can high $CH_4$ production be reconciled with less than 1% methanogen relative abundance?
- Methane production from cryoconite appeared to be site-specific, with strong production in some cases but not others. Is it possible to disentangle substrate quality, potential inhibitory compounds, the functional capacity of the microbial community, and/or other factors in explaining these differences? Which of these limiting mechanisms is most strongly supported by the data?
- How closely do the incubation redox regimes reflect realistic proglacial depositional environments? How do the microcosm results scale to landscape-scale ice marginal and glacier forefield environments?

*5. Chapter IV – Methane hotspot and methylotrophs*

Chapter IV addresses an ecologically and climatically important question: how microbial community structure relates to methane dynamics in a subglacial methane hotspot of the Greenland Ice Sheet. The integration of high-resolution microbial community data with methane measurements provides a valuable dataset that is analysed in depth. The chapter reports a high relative abundance of methylotroph- and methanotroph-affiliated ASVs. Several aspects require careful interpretation.

- Relative abundance is based on rarefied 16S rRNA gene read counts. This is not directly representative of absolute cell abundance.
- Functional inferences are made based on 16S rRNA gene amplification and sequencing.

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- In microbial relative abundance figures, it would be helpful to explicitly state in captions whether data are rarefied and whether axes represent relative abundance following rarefication or abundance based on raw reads.

For the defence, the candidate may be asked about the following:

- Although meltwaters were methane-saturated, methanotroph-affiliated taxa were not dominant. What are the potential ecological or geochemical constraints on methane oxidation in this system?
- What are the strongest lines of evidence in this dataset for a biological methane filter operating at the site?
- How can active methane oxidation and hydrological export of methanotroph DNA be distinguished?

**Summary**

This thesis clearly fulfils the requirements for a PhD. It contains multiple peer-reviewed publications (including first-author publications), demonstrates significant independent research, and makes a meaningful contribution to our understanding of ecological and biogeochemical processes in glacial–proglacial transitional zones. The work is scientifically sound, conceptually thoughtful, and clearly of publication quality. The questions raised above are intended to refine interpretation and probe depth of understanding during the defence, not to indicate weaknesses. Overall, I recommend that the thesis be accepted for defence and that the candidate be considered for the award of Doctor of Philosophy, subject to successful completion of the oral examination.

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