



Review of the Doctoral Thesis

Mgr. Petra Klímová

“Ecological processes associated with the glacial–proglacial ecosystem transition”

The submitted doctoral thesis is based on a collection of four publications (three already published papers and one manuscript currently in preparation for publication). Thesis deals with ecological and biogeochemical processes associated with the transition between glacial and proglacial environments in Arctic regions. The contribution of the candidate to each publication is clearly indicated in the thesis, and it is evident that she significantly contributed to most of the studies, particularly as the first author of one paper and as the leading author of the submitted manuscript.

The topic of the thesis is highly relevant as the rapid retreat of Arctic glaciers mobilizes organic matter and microbial communities that have been isolated beneath the glaciers for long periods of time. Understanding these processes is essential for interpreting global biogeochemical cycles of carbon and nitrogen and for predicting future ecosystem changes associated with ongoing climate change.

The introductory chapter provides a very good overview of the current knowledge about supraglacial and subglacial ecosystems and their importance in global biogeochemical cycles. The author clearly explains the major processes occurring in these environments and outlines their ecological significance. The structure of the introduction is logical and the individual sections are well connected. I particularly appreciate the emphasis on linking microbial processes with glacier hydrology and the development of proglacial ecosystems.

However, some terms could be explained more clearly in the introductory section. For example, the abbreviation CPI (Carbon Preference Index) appears later in the interpretation of the results (e.g., in the Figure 6 summarizing results of Chapter I), but it is not sufficiently explained in the introduction. Similarly, alkenes, alkanic acids are not mentioned in the introductory section, although they are subsequently used in the interpretation of organic matter degradation. In this context, it would be useful to provide some introduction to the concept of mineral-associated organic matter together with particulate and dissolved organic matter and its role in carbon stabilization in sediments.

The individual chapters based on publications are thematically well connected. The first study focuses on the characteristics of organic matter in subglacial sediments and its influence on microbial communities. The second paper analyses microbial assemblages exported from glaciers and their potential as indicators of subglacial processes. The third study uses an experimental approach to investigate methane production during simulated incubation experiment with cryoconite and sediment microbiome. The final article examines methylotrophic communities associated with methane emissions at the margin of the Greenland Ice Sheet.

Taken together, the thesis provides a comprehensive view of the links between microbial processes, organic matter, and biogeochemical fluxes in glacial systems.

Methodologically, the thesis is extensive and combines geochemical analyses, molecular biology approaches, and experimental incubations. The author uses modern sequencing methods for the analysis of microbial communities and applies bioinformatic approaches for their interpretation.



A very interesting and positive methodological aspect is the use of a mock microbiome with a known microbial community composition that was transported together with the samples as a control for PCR and sequencing processes. I consider this approach very valuable for any expedition into these extreme environments and all microbial ecologists should use it.

Overall, I consider the submitted thesis to be a very high-quality and valuable piece of scientific work. The author demonstrates the ability to combine multiple methodological approaches and interpret the results within a broader ecological framework.

Questions

1. The Figure 9 indicates that sequencing read counts were used, but it remains unclear to what extent these values can be interpreted as a quantitative estimate of the abundance of particular taxa. Can you discuss other methods which can be used to confirm the abundance of methanotrophic/methylotrophic microorganisms in the analysed samples?
2. You used three bioinformatic pipelines (SEED, USEARCH, and mothur) for the processing of sequencing data. Each of these tools has its advantages and limitations, and it would be interesting to explain why this combined approach was chosen and whether the analysis could have been performed using only a single pipeline. Why did you use three different bioinformatic pipelines (SEED, USEARCH, and mothur) for the processing of sequencing data? What were the main reasons for this choice?
3. In Chapter I the procedure used for constructing consensus sequences of OTUS was based on MAFFT alignments and using BLAST and NCBI nt/nr (protein?) database which is interesting but it is not entirely clear to me, particularly because the analysis is based on 16S rRNA amplicon data, if I understood it right. How did you identify the potential metabolism/functional genes of individual taxa using BLAST NCBI nt/nr databases based on 16S rRNA amplicon data?
4. Another question concerns the definition of “*core taxa*”. In the thesis summary, genera such as *Delftia*, *Rhodoferrax*, and *Polaromonas* are described as “*core taxa*,” whereas in the associated publication they appear to be described mainly as the most abundant taxa. It would therefore be useful to clarify what exactly is meant by the term “*core microbiome*” in this context. How do you define the *core microbiome* in the context of your study, and how does it differ from simply identifying the most abundant taxa? How do *core taxa* influence the microbiome assemblages in glaciers forefields?
5. In Chapter III you are often mentioning “*priming effect*” introduced by Kuzyakov in 2000. Do you plan to use stable isotope-labelled substrates ($\Delta^{13}\text{C}$) in future experiments to study the priming effect or methane production in glacial sediments? If so, how would you design such experiments?
6. Which microorganisms were included in the control DNA sample used during sample transport, and did their composition change during transport or sequencing?
7. How do you expect microbial community assembly in newly exposed proglacial sediments to be driven by deterministic environmental filtering versus stochastic colonization processes?



8. Do you expect functional succession of microbial metabolism during proglacial ecosystem development (e.g., from chemolithotrophy to heterotrophy)?
9. To what extent do microbial communities in proglacial soils originate from glacier-derived inoculum compared to atmospheric or surrounding terrestrial sources?
10. In the thesis you refer to the glacier catchment. Based on your field experience, how would you design an optimal sampling strategy for such a catchment in future expeditions?

Conclusion

The doctoral thesis of Mgr. Petra Klímová represents a high-quality scientific work based on a set of publications in international journals. The thesis brings new insights into the ecology of microorganisms in glacial systems and their role in biogeochemical cycles.

Therefore, I **recommend the thesis for defence and, upon successful completion of the defence, for the awarding of the Ph.D. degree.**

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