

Opponent's report and evaluation

on the dissertation thesis entitled

"Effect of distinct soil horizon and vegetation on soil microbiome abundance, composition, and activity of Arctic permafrost"

by Milankumar VARSADIYA

The thesis deals with the interesting and currently important topic of soil microbiome involved in the transformation of soil organic matter in Arctic cryosols. This topic has attracted the interest of microbiologists and soil eco-biologists in recent years, particularly in view of increasing concentrations of greenhouse gases in the atmosphere and the concomitant rise in average temperature, raising concerns about the consequences of melting Arctic permafrost. Higher permafrost thawing rates bring with them higher activities of microorganisms involved mainly in the carbon and nitrogen cycles, leading to higher production of greenhouse gases such as CO₂, CH₄ and N₂O, thus amplifying positive feedbacks on microorganisms and their activities in the carbon and nitrogen cycles. The thesis is based on four publications that focus on the composition and distribution of microorganisms in different parts of Arctic cryosols, their activities in the carbon and nitrogen cycle and their role in the decomposition of organic matter buried in cryosols. The dissertation is written in English and consists of nine main chapters: introduction, aims and objectives, description of the methods used in the experiments, summary of results, conclusions and future implication, a list of references used in the dissertation and, finally attached publications. The attached publications consist of two articles already published in respected microbiological journals (FEMS Microbiol Ecol, Microorganisms) and two manuscript, one of which has already been submitted to the journal Biogeochemistry. Milankumar Varsadyia is first author on three papers.

Chapter 1 *Introduction* is divided into four main sections dealing with general characteristics of arctic cryosols, description of the microbiome of Arctic soils, the influence of vegetation on microbial communities and microbial activity in cryosols. This chapter represents solid introduction to the major themes that are addressed later in the thesis. It is very well written, and provides deep and comprehensive information for the readers which is supported with comprehensive literature review. I have only one question to this chapter:

1) p. 36: in a sentence „...growth of Thaumarchaeota, because this phylum has lower affinity for ammonium and mostly thrive under low or moderate availability of ammonium“ I'd expect the competitive advantage of this phylum to be that it has a higher affinity..., is not it?

Chapter 2 *Aims and objectives* is rather short but clearly describes well all objectives and refer properly to conducted experiments, while **Chapter 3-4** *Sampling localities and methods* provides a brief overview of both the localities and methods, which are described in more details in the corresponding supplemented papers in the last part of the Thesis.

Chapter 5 *Results and discussion* introduces the reader to the main research results, which are simultaneously discussed and compared with the results of other, already published papers in the field. Since this is in fact the most important part of the dissertation, I will allow myself few comments:

1) How old can a buried cryoOM be? Can the composition of the microbial community then differ between cryoOMs of different ages?

2) p. 37 and later in Conclusion (p. 45) you mention that ...“higher proportion of methanogens and methylotrophs but not obligate methanotrophs puts cryoOM in the position of a potential

hotspot for CH₄ emissions in Arctic cryosols". This conclusion undoubtedly has merit, but I would like to point out the results of our own study of the distribution and abundance of methanogens at two different sites in the sediments of a small watercourse: site: This conclusion undoubtedly has merit, but I would like to point out the results of our own study of the distribution and abundance of methanogens at two different sites in the sediments of a small watercourse: site: „If we compare both locations, we see that the overall *mcrA* copy numbers (as well as the group specific copy numbers) are relatively stable along the depth profiles. Astonishingly, the lower methane-emitting site (Location I) has on average higher cell counts for all tested methanogenic groups when compared to the higher methane-emitting site (Location IV (Chaudhary et al. 2017 - *MicrobiologyOpen*. 2017;6:e454, <https://doi.org/10.1002/mbo3.454>). Our results show that the previously observed 10-fold difference in methane emission of the two sites could not be explained by molecular methods alone, in fact, the activity of the methanogenic community is rather controlled by other factors (e.g., substrate supply) than by size of the community.

3) pp. 38 and 45– Because of the high proportion of methylotrophic families in cryoOM, but which do not use CH₄ as a C source, you infer that cryoOM may be a hotspot for CH₄ release. Were any methanotrophic bacteria (MOB or AOM) found in cryosols or cryoOM during your research? If not, do you have any explanation for this?

Chapter 6 *Conclusions* summarise main results and try to explain the research's significance as a new addition to already existing knowledge, while in **chapter 7** *Future Perspectives* based on the results obtained, the author suggests challenges and directions for further research on Arctic soils.

Chapter 9 *Attached publications* - Paper IV

p. 161 - Figure 3f: Can you explain within Metazoa SSU rRNA the occurrence of the phylum Cnidaria in Arctic soil?

Conclusion (Final evaluation):

Milan Varsadiya proved his ability to set up experiments and soundly evaluate and interpret the results obtained from these experiments. I recommend to accept his dissertation for the defence and after successful defence to award the title of Ph.D.

Olomouc, August 4th, 2022

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Review of Doctoral Dissertation

Author	MSc. Milan Varsadiya
Topic of Doctoral Dissertation	Effect of distinct soil horizon and vegetation on soil microbiome abundance, composition, and activity of Arctic permafrost
Reviewer	prof. Ing. Ondřej Uhlík, Ph.D.
Reviewer's Workplace	Vysoká škola chemicko-technologická v Praze, Ústav biochemie a mikrobiologie

Milan Varsadiya has submitted a doctoral dissertation that deals with the abundance, composition, and activity of soil microbial communities involved in the decomposition of soil organic matter in different horizons and under distinct tundra vegetation of Arctic permafrost soils, in particular buried topsoil caused by cryoturbation processes. The thesis significantly contributes to our understanding of the Arctic cryosol microbiome and its potential to decompose soil organic matter. Milan's major findings include, but are not limited to, the following:

- abundance and activity of functional guilds in cryoturbated soils significantly differ from other soils;
- bacteria play a more important role in extracellular enzyme activity than fungi in cryoturbated soils;
- some fungal taxa change their role in the community in the cryoturbated soil, which can be associated with changed fluxes of C and N;
- cryoturbated soils are potential hotspots of methane emissions in Arctic cryosols;
- diazotrophs are quite abundant in cryoturbated soils, and N in cryoturbated soil organic matter is recycled mostly in prokaryotic biomass.

The thesis is based on four publications, two of which were published in 2021 while the other two have been submitted or resubmitted after revisions. The choice of presentation of the thesis as a summary and commentary on the selected papers is, in my opinion, suitable, as it provides an additional independent review of both experimental design and results and their interpretation.

From a scientific point of view, it is difficult to raise any criticism of the submitted thesis. Already the introduction shows that Milan studied a lot of literary sources, and their synthesis into a very clear and readable text points out the author's excellent orientation in the given issue. The results that Milan obtained are very valuable and advance our understanding of the roles that microbes and their

interactions play in cryoturbated soils. The discussion of the results is also at a very high professional level and points to the scientific erudition of the author. Finally, it should be mentioned that the thesis is written in very good English and contains only a minimum of typos or other shortcomings.

I have the following questions/comments for the author:

1. When this review has been completed, the two papers (III and IV) have not been published yet. Can the author provide this reviewer and the committee with the current status of the manuscripts?
2. In the annotation, the three domains of life are written as follows: bacteria, archaea, Eukaryota. Why does not the author use capitals for bacteria and archaea when they are spoken about as the domains of life?
3. The author repeatedly mentions hydrogenotrophic and acetoclastic methanogens, while completely omitting the third group of methanogens. Is there a reason for this in relation to the research of the author?
4. The author claims that *iron minerals have also been reported to stimulate methanotrophy in methanogens*. This does not seem right; can the author provide a correction to this statement?
5. The author denotes generalists as keystone taxa. Specialists cannot be denoted as keystone taxa?
6. The author claims that the *bacterial communities in the top soil can be similar in different ecosystems*. Given the many factors that influence the structure and activity of the microbial community in soils, I doubt that such a conclusion can be made. Can the author explain better what he meant?

In conclusion, I state that the dissertation submitted is prepared at a very good level and has a high scientific outreach and informational potential. The author has carried out an enormous number of experiments and analyses, and his expertise and scientific erudition is clearly demonstrated by the quality of the entire submitted dissertation. I consider the results obtained to be beneficial for further development of knowledge in the field of microbial ecology.

Milan Varsadiya has demonstrated the ability to independently work in science and to use logic and critical thinking. In my opinion, the submitted work meets all the criteria and requirements for a doctoral dissertation in the given field. On the basis of this presented work and after its successful defense, I recommend that the committee awarded the degree of Ph.D. to Milan Varsadiya.

In Prague on July 30, 2022

