

Interactive comment on “Metagenomic analyses of the late Pleistocene permafrost – additional tools for reconstruction of environmental conditions” by E. Rivkina et al.

Anonymous Referee #1

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The manuscript “Metagenomic analyses of the late Pleistocene permafrost - additional tools for reconstruction of environmental conditions” by Rivkina and co-workers describes a metagenomic analysis of two permafrost samples of similar age retrieved from the Kolyma lowlands in northern Siberia. One sample originates from a lake sediment and contains elevated amounts of methane, the other from Ice Complex sediments which contains no methane. The authors present the taxonomic diversity of prokaryotes based on isolated DNA sequences followed by the abundance of functional genes in the two samples. The authors intend using the data from metagenomic analysis as an additional tool for environmental reconstructions, as indicated in the title and the conclusions. Furthermore, the data should help to evaluate the response of

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microbial communities to thawing permafrost due to climate change.

The main finding of the study is, that the microbial composition in the two samples are different. Furthermore, the sample with high methane content had a higher abundance of genes involved in methane production.

The authors present in the introduction some background information, previous findings and the rationale of their work. However, they mainly consider their own work and miss previous work from other groups. The absence of methane and methanogenic Archaea in late Pleistocene Ice Complex is not a general feature since studies of different groups in the Lena River Delta and the Kolyma lowlands have shown the opposite. Therefore, the basic question of the presented work should be revised. Furthermore, the advantage of a metagenomic analysis in the context of the work should be explained more clearly. E.g., if the focus of the work is on the reason for a lack of methane and methanogenic activity in one of the samples (L56ff), is it really necessary to analyze the whole metagenome or is it sufficient to look for functional genes of methanogens and methane oxidizers?

The Material and Methods parts lacks a detailed description of the settings where the samples were collected. If the data of the metagenomic analysis should be used for environmental reconstructions, all available information on the environmental conditions at the sampling sites during the deposition of the organic matter should be given.

The central part of the manuscript, the section Results and Discussion, mainly presents the relative abundance of different taxonomical units and functional genes among the millions of sequences retrieved from the samples. This section is very descriptive and there is almost no discussion on the reasons for the differences between the samples. E.g. the striking difference in the relative abundance of Proteobacteria and Actinobacteria, the most abundant groups of bacteria in the two samples, is ignored. If the analysis shall be used for pale-environmental reconstruction, the reader expects a discussion on the potential relation between obtained sequences and environmental

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conditions. Currently this section is more an inventory of isolated sequences. Only in the following conclusions the data are discussed on the background of previous work.

Some of the central conclusions are not backed by the data. E.g. the conclusion on metagenomic analysis as central key for paleo-reconstructions (I340ff) should be revised. Indeed the study has shown, that the abundance of genes related to methane production is higher in methane bearing sediments. No attempt was made to correlate the metagenomic data with the paleo-environment since no data on environmental conditions during deposition of the sampled sediment are presented. Was the climate 30,000 years ago at the sampling sites colder and dryer or similar as today? Furthermore, the description of the formation of the Ice Complex sediments is an oversimplification (see specific comments). The authors suggest, that the single sample from the Ice Complex they studied is representative for the whole Ice Complex sediments. This obviously cannot be the case.

Many abbreviations are not explained in the text

Specific comments:

line 31: should read "of the sampled late Pleistocene..."

I44ff: Please explain epigenetic and syncryogenic, since not all readers will be familiar with these two terms.

I43: Not only the findings of the authors should be considered, but also work from other groups (see general comments).

I57: This general research question is not supported by recent literature. Sediments of the late Pleistocene Ice Complex may contain methane and methanogenic activity (after thaw).

I58: It is unclear why metagenomic analysis should answer the question raised. Metagenomic analysis may be used to describe the microbial community present in the samples (as done in this study). If there is no methanogenic activity you would expect

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pect a low abundance or absence of methanogens (as shown in the current analysis). But the analysis will not tell you why this is the case.

I 67: Please quote the respective study.

I75f: Please explain in more detail, why the presented descriptive results will help evaluating the community response to permafrost thaw and global warming.

I91: Could you give the concentration in the pore water? 1.2 mmol kg⁻¹ seems above saturation concentrations of CH₄ in water (depending on the amount of water in 1 kg).

I95ff: Please describe how CH₄ was measured and give the detection limit of CH₄.

I145: It is surprising to me that the diversity of Eukaryotes is higher than of Bacteria. Has this been found before? How is the situation in active layer soils? And what could be the reason for high Eukaryotic diversity in anoxic soils, that should be almost free of Eukaryotes? Please discuss this finding. How can you be sure to detect all Bacterial species if you have only sequences with an average length of 150 base pairs?

I155: What means "significantly dominate"?

I156ff: dito

I158: Please do not use a statement as header

I240: Please explain SEED

I248: Please explain KEEG

I254: How did you test significant differences

I264: What means features in this context? Do you mean genes or sequences?

I351: This is an oversimplification. The authors of the cited study consider the Ice Complex development to MIS2 (last glacial maximum) and MIS3 (interstadial). Yedoma sediments from MIS3 are characterized by higher TOC and less decomposed organic matter than MIS2 deposits indicating anaerobic conditions during deposition.

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l364ff: This is not a conclusions from the data presented but well established knowledge that might be presented in the introduction with the respective citations.

l366 ff: The last sentence is unclear. Why will a method, which only describes the status quo help to understand how a community will respond to climate change?

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