Dear Dr. Brovkin,

Please accept a resubmission of our revised manuscript entitled “Metagenomic analyses of the late Pleistocene permafrost – additional tools for reconstruction of environmental conditions” after minor revisions. We took in to account all suggestions and comments provided by reviewer #1 and editor.

We would like to thank reviewers and you for useful comments and constructive suggestions.

Sincerely yours,

Elizaveta Rivkina

Answers to comments of reviewer #1

1. Line 47: Better change unit to µmol kg^{-1}.

A1. We did not change mmol to µmol since the concentration of methane in the permafrost is given in mmol kg^{-1}, and it seems logical to give the trace concentrations of CH_4 in the same units.

2. Line 96: Please quote the respective study.

A2. We quoted papers: Mackelprang et al 2011, Jansson and Tas, 2014 (New Lines 98-99).

3. Line 125 and 127: Please give the company additionally to the country of the supplier of the equipment.

A3. That was done (New Lines 129-131).

4. Line 135: Better change unit to µmol kg^{-1}

A4. See answer A1. (New Lines 139).

5. L273: Several microbial processes are involved in the N cycle, please rephrase.

A5. We rephrased this paragraph as: Bacteria of nitrogen cycle. The nitrogen cycle includes several microbial processes such as N2 fixation, ammonification, nitrification, and denitrification. Nitrogen-fixing bacteria more abundant in the IC4 were represented by the genera…….(New Lines 280-283).

6. L309ff: The description of Desulfitobacterium is a bit misleading. The use of very low H_2 concentrations would not result in facilitating sulfate reduction and methanogenesis but in outcompeting these two processes under natural conditions.

A6. We rephrased this sentence as: However, another stricly anaerobic bacterium Desulfitobacterium, which is capable of using a wide variety of electron acceptors, such as nitrate, sulfite, metals, humic acids, and halogenated organic compounds can use H_2 as an electron donor to facilitate sulfate reduction and methanogenesis (Villemur et al 2006) was twice as abundant in the IC4 (0.2%) than in the IC8 sample (0.1%). (New Lines 320-324).

7. L523-525: The final conclusion is not completely substantiated by the data. The study shows that metagenomics analyses may add information to the environmental conditions during permafrost sediment formation, but it may not be used as an "instrument for paleo-reconstruction" alone. Due to the lack of replicates in the study, it remains unclear if the data are representative for the investigated permafrost deposits. I suggest rephrasing the final sentence.

A7. We rephrased final sentence as "The obtained results demonstrate that the metagenomic analysis of permafrost may give additional information on the environmental conditions during permafrost sediment formation." (New Lines 539-541).

Answer to comments of Associate Editor

Associate Editor Decision: Publish subject to minor revisions (Editor review) (25 Mar 2016)
by Dr. Victor Brovkin
Comments to the Author:
Dear Dr. Rivkina,

the referee #1 have read your manuscript, found that it has been substantially improved, and suggested only minor comments for revision. Please revise manuscript accordingly and submit revised paper and point-to-point response to the comments.

My other concern as the editor is that in the new section "Potential limitation of analysis" you discuss technical limitations of your methods, and not limitations of interpreting the results of metagenomic analysis in terms of paleo-environment. Assuming that you get correct sampling and processing, representativeness of samples, etc., in my understanding there is still a big gap in going from reconstructed genomes to environment. I would appreciate if you add into this section a paragraph on limitations of INTERPRETATION of the metagenomic analysis.

Yours sincerely,
Victor Brovkin

A: We added phrase to clarify "limitations of INTERPRETATION» – Line 520-524:

"Keeping in mind that interpretation of metagenomic data is highly dependent upon the depth of sequencing, accurate annotation, and comprehension of database (Hazen et al, 2013), bioinformatic analyses were performed in the same fashion for both samples that allowed accurate comparison of these metagenomes".