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 major revisions. We took in to account all suggestions and comments provided by reviewers, and we feel that manuscript was significantly improved upon the revision.

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

Sincerely yours,

Elizaveta Rivkina

Answers to comments of anonymous reviewers

Report #1

Suggestions for revision or reasons for rejection (will be published if the paper is accepted for final publication)

The revised version of the manuscript takes several of the critical points into account, which were raised in the first review; however some fundamental issues were not met sufficiently. First of all, the authors should clearly describe the rationale of the study in the introduction. It is still unclear why a metagenomic analysis is required when the authors aim to elucidate the reason for low methane concentrations and low methanogenic activity in permafrost samples of the late Pleistocene. The question is not if a metagenomic analysis gives valuable information (as the authors response implies) but why it is necessary to conduct a metagenomic analysis if only the methane cycle is of interest. The results on microorganisms of the sulfur and nitrogen cycle are prominently presented in the manuscript including two figures (none for the methane cycle), hence it seems that not only the methane cycle was of interest in this study and this should be considered when presenting the objectives.

A. We revised wording to more clearly state aim and rationale of the study.

Secondly, the authors cited further literature showing elevated methane concentrations, viable methanogens and methanogenic activity in syngenetic permafrost of the late Pleistocene Ice Complex, but they do not clearly consider these results in the introduction and discussion (see specific comments). The generalization that syngenetic Yedoma deposits do not contain

methane (see l. 406) and viable or active methanogens is not correct considering current literature.

A. We added more description of Yedoma sediments, which were formed at the East Siberian coastal plains, where these sediments subdivided by locations to Yedoma present on Lena-Anabar, Yana-Indigirka, and Kolyma lowlands.

If the samples should be used for paleo-reconstructions the authors should comment in more detail on the climate 30.000 years ago in the area where the samples were taken. Currently the authors describe the climate in the late Pleistocene during Yedoma formation as "cold-arid" but this is a too general description. There were substantial changes in temperature and precipitation in northern Siberia over the ten-thousands of years during Yedoma formation. In particular 30.000 years ago, the age of the samples, the climate was more moderate and humid.

A. We reviewed two studies (Schirrmeister et al, 2011, 2013- ref L685, L689) which indicate that climate during period of 32,000 to 16,000 years ago was cold and dry/arid. In paper we discuss samples with age of 30,000 and 32,000 and do not attempt discussing climate during the whole late Pleistocene Period.

All these issues were raised in the review of the original submission but not considered sufficiently. Furthermore, several of the specific comments were not considered in the author's response or the revised version. There is no comment on the concentration of methane in the pore water and no comment on the higher functional diversity of Eukaryotes in the anaerobic lake sediments, which is quite surprising.

A. In our previously revised manuscript we gave references to paper where method of the methane detection in permafrost has been described. In this revised version we along with reference gave brief description how gas was collected from permafrost (L121-127), we did not analyze pore water.

L188-215 and L341 – We added discussion on the higher functional diversity of Eukaryotes

Specific comments:
L48ff: Please clarify what you mean by "low concentrations". Brouchkov & Fukuda 2002 call their methane concentrations in Yedoma (up to 6.000 ppm in air) "high" and methane concentrations in the Yedoma reported by Bischoff et al. 2013 are up to twice as high as in the sample IC4 of the current study. Hence the two cited publications do not support "low" methane concentrations in Yedoma. Furthermore Brouchkov & Fukuda 2002 did not study Yedoma in the Lena Delta.

A. It is impossible to compare results obtained in ppm by Brouchkov & Fukuda 2002 with our results, because Brouchkov & Fukuda 2002 did not give weight of the sample they used for methane measurement. If we attempt of using online convertor ppm to mmol/kg, 6,000 ppm comes to <0.26 mmol/kg and this number is 3-times lower than methane concentration in sediments of lake origin. We added more background about Yedoma formation, and Yedoma sediments found in different locations, see L43-82. We reviewed paper by Bischoff et al. 2013 ref L560, and this study showed that Yedoma samples from 32,000 to 16,000 years ago were characterized by low methane, low methanogens, and low methanogenic activity. This actually support our findings that Yedoma sediments of 32,000 years old contained methane at detection level and low presence of methanogenic archaea. High concentration of methane in Bischoff et al. 2013 study was detected in young Holocene sediments or in sediments older than 40,000 years, however we do not analyze or describe any such sediments in our paper, so there is no point in comparison of our samples with any younger or older sediments. However we added brief description of methane versus age (L52-70).

L54ff: Please consider that Bischoff et al. 2013 reports elevated methane concentrations, viable methanogens and methanogenic activity in syngenetic permafrost of the late Pleistocene Ice Complex in the Lena River Delta. A general absence of methane and methanogenic archaea in late Pleistocene Yedoma deposits cannot be concluded from the current literature.

A. Bulk of samples collected from 32,000-16,000 sediments and analyzed by Bischoff et al. 2013 do not contain or contain low methane and methanogenic activity. Bischoff et al. 2013 analyzed Yedoma from the Lena River Delta, our Yedoma samples came from Kolyma lowland, we extended Introduction.

L86ff: This sentence is still unclear. Which response is referred to? Please explain how the description of a microbial community composition will help to elucidate the response (of this community?) to permafrost thawing.

A. Text was revised

L188: must read Table 2

A. Text is revised (L353-433) and Table 2 has been cited.

L291ff: It is essential to discuss the implications of these potential changes in the permafrost community over a period of 30.000 years on paleo-reconstructions, which is based on this (potentially altered) community.

A. Discussion of the implications of the potential changes in the permafrost community over a period of 30.000 years is beyond of scope of this paper. We used obtained findings about microbial community from 2 permafrost sediments to understand how these sediments were formed. We added some discussion on climate relevant to our study, L 449-486.

L394ff: Please clearly explain which role late Pleistocene Ice Complex deposits in the Kolyma lowland may play in climate warming, permafrost degradation and greenhouse gas emission.

A. Text was revised

L422ff: This sentence is unclear. Please clearly explain how a metagenomic analysis may characterize the contribution of a frozen community to climate warming and permafrost thaw.

A. Text was revised

L432f: the sampled Yedoma…

A. Changed as suggested

Report #2

Suggestions for revision or reasons for rejection (will be published if the paper is accepted for final publication)

The 3rd reviewer have not found time to perform the review, therefore I reviewed the paper myself as the editor. In general, the revised manuscript has been improved, as the authors took most of reviewer comments into account. However, few points require further improvement. Firstly, the comment of reviewer #3 on the lack of replications and variability for the samples needs deeper attention – right now it is not accounted at all. I understand that it is not possible to provide additional replicated measurements to increase statistical significance of results, but this limitation of the study needs to be acknowledged. Secondly, the link between the results of analysis and implications for paleo-environment remains to be vague, especially if the microbial community was active over many thousand years in permafrost. I strongly suggest adding a new section “Potential limitation of analysis” where limitations of the author’s approach including the upper two points are discussed in details.

A. The manuscript was extensively revised. We added suggested section "Potential limitation of analysis", L487-508.