

**Journal:** *bg-2017-89*

**Author:** *Janina Stapel et al.*

**Title:** *Substrate potential of Eemian to Holocene permafrost organic matter for future microbial greenhouse gas production*

*Revised parts in the manuscript are highlighted by a yellow background!*

**To the comments of reviewer 2**

Based on the comments of 3 reviewers, Stapel et al have revised their manuscript on substrate potential of permafrost OM for greenhouse gas production. The current version the manuscript has improved compared to the first version, but still needs further improvement in order to be acceptable for publication in Biogeosciences. Next to the interpretation (see detailed comments below), also the grammar will need a more thorough check. The long sentences, grammatical mistakes, and needless repetitions distract from bringing the main message across.

*We separated and simplified long sentences, removed needless repetitions and streamlined the overall paper. Due to the streamlining process parts of the manuscript were substantially revised or removed.*

**Based on reviewers comments (in random order):**

**- OM quality**

There is no clear definition of OM 'quality' in the introduction, and to me it is not clear what is exactly meant by 'quality', and what makes the quality of OM of good or bad. To me it would make more sense if the terms OM composition and/or OM properties are being used. Or even OM biodegradability. Since OM 'quality' is what the paper is about, this needs to be clarified.

A better definition may also prevent strange constructions like on P3L6-9: To access (better: obtain?) information on quality in terms of biodegradability of freeze locked OM, we examined characteristic OM parameters (amount and quality) and LMWOAs. In other words: you assess quality in order to examine quality....

*We used the "OM quality" term since it was used in the literature before. However, following the suggestions of the reviewers we removed the term throughout the whole manuscript and directly defined in the text in which context the OM is assessed.*

**- Since biomarker concentrations and TOC follow the same trends, both reviewer 1 and 3 suggest to normalize biomarker data on TOC. Doing this would help make the case that downcore trends in biomarker concentrations are real, and not related to changes in TOC. In their rebuttal, the authors indicate that biomarker trends remain after normalizing. To prevent future readers asking the same question, I strongly suggest to present TOC normalized data in the next version.**

*We now added S-Fig. 2 with biomarker data related to gTOC to the supplements. The data show comparable trends with exception of two samples with very low TOC contents (core L14-05 and*

coreL14-04). Additionally, a second table with the GDGT data related to gTOC is added to the supplements.

**- source of OM (terrestrial vs lacustrine)**

As already pointed out by reviewers 1 and 3 there are still contradictions in the description of the source of OM in the permafrost deposits. The origin is claimed to be terrestrial, but site descriptions clearly mention that the depositional environment was lacustrine. Also, all aliphatic-rich sediments are attributed an algal source. This needs to be discussed or stated more carefully.

*The Holocene and Eemian samples were deposited in a thermokarst lake environment. However, for this OM the pyrolysis data indicate a clear terrestrial (Typ III) OM. It is the Yedoma OM which shows a tendency towards Typ II OM. Thus, overall the samples contain mainly terrestrial organic matter but most of the Yedoma samples show an increased proportion of aliphatic structural moieties. This causes most likely this shift into the direction to a more Typ II OM. We now stated this more carefully in the manuscript. Lines 314-324*

**- past vs present biomass (reviewer 1 comments 8 and 11)**

I get that you choose to use PLFAs as a marker for living biomass, but I still don't see how that automatically makes GDGTs markers for past biomass. If you want to use GDGTs as such, you will have to provide a reference, or present this as a finding of this study rather than introducing them this way. Be careful with the ether-ester bond interpretation for the qualification of fossil/living-derived lipids, as this concerns headgroups, and not bonds within membrane lipids.

The extraction technique used here only extracts core lipid GDGTs. The vast majority of the GDGT pool in soils (also modern ones) is present as core lipid, so their presence as such does not tell you anything about the vitality of the microbial community, in past nor present. Instead you would have to look at IPL-GDGTs, i.e. with a phospho- or glyco-headgroup attached, and then I do agree that the phospho-GDGTs (attached with an ester bond) would indeed be a better marker for living organisms than glyco-GDGTs (attached with an ether bond).

*– I am not sure whether I really understand what you are claiming here. Intact phospholipid esters (with fatty acid side chains) are used as life markers, since they rapidly degrade after cell death (White et al., 1979 and Logemann et al., 2011). Especially due to hydrolysis of the ester linked fatty acid side chains. In contrast intact phospholipid or glycolipid ethers (diethers or tetraethers) are more stable and degrade not along the ether bond side chain but finally at the head group. Logemann et al., 2011 indicated that it seems to make no difference, whether we have a phosphoester bound head group or a glycosidic bound head group. Both seem to have the same degradation rates (Logemann et al., 2011). Finally, the head group cleavage leads then to the accumulation of diether (archaeol) or tetraether (GDGTs) core lipids in the sediments after degradation. Thus, that bacterial phospholipids with ester linked side chains are less stable cannot be a matter of head group stability but of side chain stability. Since intact ether lipids are more stable, they only have restricted potential to act as life markers (Logemann et al., 2011) and thus, we did not use or measure these components. Instead we used the PLFAs as a general indicator for present microbial life here. At least this is my state of*

knowledge. If there are new publications stating the opposite, I would be grateful if you could provide me these references.

*We interpret br-GDGTs and iso-GDGTs as well as archaeol as degradation products of intact membrane lipids (loss of head groups). These biomarkers are widely used as past biomarkers for paleo-climatic and -environmental interpretation also in permafrost environments (Peterse et al., 2011; Schouten et al. 2013; Weijers et al., 2006). However, it is clear that the loss of the head group makes them not automatically to past markers. They could also be the remnants of an actual living microbial community in the deeper parts of the permafrost successions. That is the reason why we compare the GDGT and archaeol data sets with the PLFA depth profiles (see lines 367-369). The fact that abundant microbial life in permafrost regions is restricted to the surface active layer and that the depth profiles are different makes us confident, that these ether biomarkers represent the past microbial community. Line 370-374 and 385-396*

**- Comment 10 of reviewer 1: Space wise I can see why you choose to plot isoGDGT-0 and archaeol in the same panel. However, since they do not necessarily share the same source, please plot them as separate lines, so it is clear when they follow the same trend and when they do not.**

*– We separated iso-GDGT-0 and archaeol now in different plots in Fig .2.*

**- Introduction:**

The transition to the paragraph with the study area (P2L30) is still very sudden. From the previous section it is not clear what the open question actually is that will be addressed in this study. This only starts to make sense after reading the actual aim in the very last paragraph on P3L26. I suggest to move this paragraph up, prior to the site introduction. The permafrost feedback that is the whole driver of this study can also be better introduced, as it is now only mentioned on the side (P2L13).

*– We revised and restructured the introduction chapter. The aim is pointed out in the abstract. Then we start a general introduction into the background of the current study leading again to the aim of the study at the end of the introduction. I guess this is the usual structure for the introduction part in a paper. We shifted the part on Bol'shoi Lyakhovsky Island to the "Study area and materials chapter" and we added a sentence on the carbon climate feedback cycle. Lines 46-47*

**- Results:**

The results are presented as values, rather than what they really are (e.g. concentrations). For example, P7L8: TOC values instead of TOC concentration. Check this in the whole section.

*– Changed to "concentrations or contents" in most cases. The Hydrogen index are usually used with the term "values".*

- Statistics: I am happy to see that the authors have included statistical support for their statements.

**Textual (a selection, the text needs editing):**

P1L16:...acetate are used. – *Abstract was revised.*

P1L16: replace highest by largest (pool). – *Changed. Line 13*

P1L17: ...pools in permafrost are present in the layers that cover MIS 3 and MIS 4. – *Changed. Line 14*

P1L18: ...deposits from MIS5e contain only a small pool of substrate. – *Changed as suggested. Line 15*

P2L8: replace formerly preserved OM by accumulated OM – *Changed to “accumulated and freeze-locked OM”. Line 36*

P2L14: replace former by Previous – *Changed. Line 38*

P2L15: remove comma after in – *Revised. Line 39*

P2L31: ‘The last interglacial deposits have been interpreted as Eemian’ -> of course they are, otherwise they would not be from the last interglacial. – *Sentence part was removed due to restructuring.*

P4L24: replace which are distinct between by which occur between – *Changed. Line 121*

P4L28: how can a site drain and then freeze over? – *After lake drainage the sediments left behind froze over again/ becoming permafrost again. We added a reference. Line 125*

P5L16: indicate how samples were decalcified (with HCl?) – *Yes, information added. Line 144*

P6L4: indicate that free acetate was measured in the pore water – *Added. Line 162*

P8L7: curves do not correlate but have R<sup>2</sup> of 0.8, p=0.02? Seems like a nice correlation to me though... – *Text revised. Lines 233-235*

P8L16: How can you have an average concentration in a range? – *Revised. Line 244*

P11L30: if you use significant provide p-value – *This part was rewritten. Lines 347-360*

P12L7: how can OM stimulate a microbial community? I think you need to rephrase. – *We write now: “stimulated a diverse bacterial and archaeal community”. Line 363*

P12L12: archaeol without a headgroup is not an IPL! See earlier comment on IPL vs CLs and present vs past biomarkers. – *This was related to “ether bond moieties”. We rephrased this part. Line 372*

**To the comments of reviewer 1:**

I find the manuscript improved, but also that some of my concerns have been only partly addressed (previous comments 1, 2, 4, 8):

**(1) I agree with the authors that chemical composition of organic matter can influence its degradability. However, we know that chemical composition is not the only factor involved. Since the data on chemical composition of organic matter presented here are used to make conclusions about its degradability, I think a comment on other parameters that can have an impact is necessary, and that the data need to be discussed more carefully. Apart from temperature, I am for instance thinking of oxic versus anoxic conditions and association with soil minerals.**

*Of course OM degradation is the result of different factors such as OM composition, environmental*

*conditions and microbial controls. However, the focus of this paper is clearly placed on the evaluation of specific compositional characteristics of the OM of different ages. Nevertheless, we discussed already the accumulation and deposition of permafrost OM within the context of the environmental conditions such as soil moisture including aerobic and anaerobic soil conditions and temperature in the paper (see discussion). Thus, this criticism is only partly justified. In the revised version we now broaden this discussion starting the Discussion chapter with a general introducing paragraph on the factors influencing OM accumulation and degradation (Lines 298-305). Furthermore, environmental and microbial controls are considered at several positions in the discussion.*

**(2) I still find the motivation for looking at past and present microbial biomarkers poorly justified. For instance, on page 12, lines 7-8, the authors write: “In order to investigate whether the freeze-locked OM already stimulated a microbial community during deposition in the past, biomarkers for past microbial communities were examined.” Why would anybody assume otherwise? Microorganisms exist in almost every environment. There is no reason to assume that there were no microbial communities in the Pleistocene.**

*Yes, this was maybe a bit misleading. Our intention was to show whether specific kind of OM stimulated already an abundant microbial community for the production of greenhouse gases in the past. In addition to methanogenic archaea this would include also bacterial microorganisms needed to degrade the OM by fermentative processes to provide the substrate pool for methanogens. However, in the current version we now strengthened the focus on the biomarkers representing methanogenic archaea. We made this point clearer in the Abstract (Lines 18-20) and in Chapter 5.2 (Lines 363-367).*

**(4) Please add a list with the specific compounds considered for total PLFAs. There is a lot of variation between different studies.**

*We added a list of PLFAs found in the active layer and mention that the PLFA diversity decreases with depth (Lines 200-205).*

**(8) While my technical comments have been addressed, there are plenty of new grammatical errors and unclear sentences (see below). Please check your language and consult a professional service if necessary.**

*We significantly revised the manuscript concerning unclear sentence structures.*

#### **Further general comments:**

**(10) I find the discussion of soil moisture conditions during deposition very speculative (e.g., page 13, lines 13-28).**

*Generally, literature data show that the Yedoma successions have been deposited during slightly warmer and wetter conditions leading to higher soil moisture with anaerobic soil conditions. These conditions were favorable for the accumulation of presumably less degraded OM, which we tried to link to the OM characteristics on Bol'shoy Lyakhovsky Island (higher TOC, higher HI, higher aliphatic character, higher acetate/substrate concentrations). Using these relations we speculate a bit on the conditions for OM accumulation during the Holocene and especially during the Eemian. Nevertheless, we strongly revised and restructured the discussion and reduced the speculative parts.*

#### **Technical corrections**

Page 2, line 7: “resulted in”. – Changed. Line 35

Page 2, line 9: Delete “again”. – Removed and sentence revise. Line 37

Page 2, line 12: “enhanced”. I’m also not sure what you mean with “microbial production”. Activity?

Growth? – We removed “the” to change sentence to: “microbial production and release of greenhouse gases”

Page 2, lines 13-14: I suggest a separate sentence for the global change feedback. - Changed to “This enhanced release is expected to have strong feedback on global warming and further permafrost degradation.” Line 44

Page 2, line 15: Delete “in”. – Removed. Line 39

Page 2, line 18: “at today’s coasts and islands”. You could also delete the insert. – Removed. Line 48

Page 2, line 19: Split the sentence. E.g., “These deposits provide a unique paleo-environmental archive ...”. – Changed as suggested. Line 49

Page 2, line 21: What do you mean with “here”? – Removed. Line 51

Page 2, lines 30-31: This sentence is incomprehensible. I suggest: “We selected Bol’shoi Lyakhovsky Island in the Laptev Sea (NE Siberia) as our study area, since it provides an excellent opportunity to investigate permafrost OM deposited from the last interglacial to the Holocene.” – Revised and shifted to Study area. Lines 93-119

Page 2, lines 33-34: I suggest: “These Eemian deposits form a paleo-equivalent to the Holocene and are otherwise rather difficult to assess.” – Changed as suggested. Lines 103-104

Page 3, line 2: “Oyogos Yar”. – Corrected. Line 104

Page 4, lines 26-28: Please add a reference. – Reference added. Line 125

Page 5, line 28: I think the reference should be in brackets. – Changed. Line 156

Page 5, line 29: With C1-C5 gases, do you mean alkanes? – This is the usual assignment, but we added now “alkane gases”. Line 157

Page 6, line 4: What other anions? This is the only time this is mentioned. If other compounds were quantified originally but are not presented in this paper, I would not mention them. – Removed! Line 161

Page 6, line 23: Change “were” to “was”. – Changed. Line 180

Page 6, line 25: What do you mean with “optical correlations”? – Changed. Line 184

Page 6, line 29: Add “communities” after “bacterial”. – Changed to “past bacterial and archaeal communities”. Lines 188-189

Page 6, line 30: The bracket after “archaeal communities” is incomprehensible. – Changed to (isoGDGT-0 and archaeol). Line 189

Page 7, line 22: Add a “the” to the “TOC curve”. – Added. Line 216

Page 8, line 7: How is  $R^2=0.8$ ,  $p=0.021$  not a significant correlation? – Changed. Line 234

Page 9, line 16: Remove “of”. – Removed. Line 278

Page 10, line 6: Remove “again”. – We cannot see what is wrong with “again”, because it was already bioavailable in the past when it was part of the active layer. Line 298

Page 10, line 9: Remove “rich” in both cases. – This part was removed due to the streamlining process.

Page 10, lines 8-14: See my general comments on organic matter chemistry. I do not question that chemical composition is important, but I think a more balanced view is necessary. Further, I don’t find it justified to claim that pyrolysis is established here as a new tool. It has been frequently used before and the ending of the sentence (“... as introduced in Stapel et al....”) is in fact a contradiction to that claim. The sentence in lines 10-12 is also not clear.

– We revised this introductory paragraph of the discussion chapter presenting now a more balanced view on OM accumulation and degradation. However, the focus of this paper still are the different characteristics of the OM of different ages. Lines 298-305

Page 10, lines 20-21: This interpretation implies that there is strong algal growth now in the active layer. Is there any evidence for this from previous studies that use different approaches?

– Soil algae material can be one explanation. We revised this part to make this clearer and added a reference. Lines 322-324

Page 10, line 30: Nitrogen is not respired, please re-phrase. Page 10, lines 28-31: A common mechanism that can lead to low TOC/TN values in soils is the accumulation of inorganic N (binding of



ammonium to clay minerals).

– Due to streamlining the Discussion this part was completely removed.

Page 11, line 6: Dryer. – *Changed. Line 338*

Page 11, line 17: Change “into” to “in”. – *Changed. Line 327*

Page 11, line 27: Change “setting” to “settings”. – *Part was rewritten. Lines 347-360*

Page 11, line 28: “Reveals”. – *Changed.*

Page 12, line 3: What do you mean with “higher rates of decomposed OM”? – *The whole paragraph was removed in order to avoid repetitions and to streamline the manuscript.*

Page 12, line 14: “contain bacteria and archaea”. – *Changed to “consist of both”. Line*

Page 12, lines 18-19: Do you mean labile carbon in the permafrost? – *Changed to “newly produced and old”. Line 379*

Page 13, line 1: “varies between”. – *Changed. Line 393*

Page 13, line 3: “markers”. – *Changed. Line 395*

Page 13, lines 6-7: There is some mixup in the text. – *This paragraph was rewritten. Lines 401-402*

Page 13, line 9: I would change “greenhouse gas production” to “methane production”. – *Changed as suggested. Line 401*

Page 13, lines 9-11: What do you mean? I thought the whole paragraph is about correlations between TOC and past microbial markers. – *This sentence was introduced because the reviewers ask for relating biomarker data to gTOC. We added know a table and a figure into the supplements with biomarker data related to gTOC. Lines 403-404*

Page 13, lines 13-14: Please add a “might” or “is expected to” or similar. – *Changed as suggested. Line 405*

Page 13, line 30: Please clarify which periods the thermokarst lakes refer to. – *Holocene soil OM is suggested to be the largest source of brGDGTs supplied to the thermokarst lakes in the Kolyma region (Peterse et al., 2014). Also this paragraph was streamlined and revised. Line 351 and line 422.*

Page 14, line 3: Change to “... did not significantly affect the concentration ...” – *This part was removed.*

Page 14, lines 6-7: Please re-phrase. This sentence is incomprehensible. – *Sentence was completely removed.*

Page 14, line 26: I don’t find anything on the input of old and new carbon and a stimulation of microbial activity in the cited paper. – *I cannot reconstruct how this could happen. Maybe it is an artefact of a former revision of the text, where the text does not fit anymore to the reference. We removed this reference here.*

Page 14, line 27: “result in”. – *This part was rephrased. Lines 443-446*

Page 14, line 30 to page 15, line 6: Do you mean that when the active layer is deepened and reaches the now frozen MIS 3, 4, and 1 deposits, we expect a microbial biomass similar to the current active layer, and microbial consumption of the contained acetate? Can you write this more clearly? – *No, the active layer of the core containing MIS 3 deposits (core L14-02) contains MIS 3 OM which is thawed already. The fact that this material shows the highest PLFA concentrations indicate that the MIS 3 OM can act as a good substrate provider for microbial life. We revised this part. Lines 448-458*

Page 15, line 8: Article missing (“... that the free-acetate pool ...”) – *Changed. Line 460*

Page 15, line 17: I would replace “caused by the onset of the early Holocene” by “at the onset of the early Holocene”. I suppose this is what you mean. – *Changed to “starting with”. Line 469*

Page 16, lines 7-8: Please specific what you mean with “concerning” here. – *This paragraph was removed during the streamlining process.*

Page 3, lines 15-16: I don’t understand, please rephrase. – *Rephrased. Lines 72-74*