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Interactive comment on "Substrate potential of Eemian to Holocene permafrost organic matter for future microbial greenhouse gas production" *by* Janina G. Stapel et al.

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Responses to the reviewer's comments: "Substrate potential of Eemian to Holocene permafrost organic matter for future microbial greenhouse gas production"

By Janina G. Stapel et al.

We thank the reviewer for his/her thoughtful and very constructive comments and suggestions on our manuscript which will improve the clarity and the quality of the paper. Below, we will address all listed issues, which are relevant for discussion.

Reviewer #2

General comments

1) I recommend to be careful with conclusions on organic matter degradability based on organic matter chemistry (see e.g. Schmidt et al., 2011, Nature).

In our understanding Schmidt et al. 2011 claimed that the persistence of soil OM primarily not depend on the molecular properties of the OM itself but on the physico-chemical and biological properties of the surrounding environment. In permafrost regions the main factor controlling the ecosystem is the cold temperature, which causes reduced OM decomposition and therefore increased OM accumulation. Thus, permafrost can contain OM which is highly vulnerable to microbial degradation as it was not fully degraded due to the low environmental temperature conditions (short periods of microbial activity during summer season). Although microbial degradation might be impeded by the conditions in permafrost areas, microorganisms will not degrade OM randomly upon thaw. Specific structural moieties are better degradable (less energy demanding) than others for the microbial community involved in the degradation processes (aromatics vs. aliphatics). These structural differences in the OM depend on the OM source and the level of microbial degradation before freeze-locking. In the current paper we addressed whether we can (i) trace these structural differences in the deposited OM by pyrolysis and (ii) characterize a stored potential substrate pool for microbial turnover. Thus, we apply these two parameters as quality indicators for the stored OM to act as a substrate provider for microbial degradation upon permafrost thaw. The detected microbial abundance and activity in the active layers on top of the permafrost (Fig. 2) as well as incubation experiments of old permafrost material indicate that old OM indeed can act as a substrate provider. Thus, our approach is more to discuss the OM quality in terms of potential substrate provision as outlined in the final conclusions.

2) I do not understand the importance of past and present microbial biomass for this research question. There is no doubt about the presence of a living, active microbial decomposer community in the active layer, and the data are not interpreted to more

detail. I would also like to point out that I am not aware of studies testing how fast PLFAs are degraded in continuously frozen soils, and that we therefore do not know for sure if PLFAs in permafrost really represent the living microbial community.

In order to investigate whether the freeze-locked OM stimulated already a microbial community during its deposition in the past, biomarkers for past microbial communities were examined. Since past microbial biomarkers could also be a product of microbial degradation by the presently living microbial community, the Bol′shoy Lyakhovsky samples were also screened with regard to microbial life markers to compare both biomarker records. PLFA life marker profiles indicate abundant microbial life in the active layers compared to the permafrost deposits and do not correlate with the past markers profiles. Thus, the data suggest that in the permafrost sequence the past marker represent a paleo-signal. The significant difference in PLFA concentration between the active layer and the permafrost deposits suggest that PLFAs also in permafrost environments can be used as a life marker. Other studies have shown that microorganisms can survive in deep permafrost sediments (e.g. Gilichinsky and Wagener, 1995; Rivkina et al., 2004; Bischoff et al., 2013) and incubation experiments have measured microbial produced CO2 after thawing permafrost sediments (e.g. Knoblauch et al., 2013; Walz et al., 2017), indicating living microbial cells in frozen permafrost sediments, which can be “re-activated” with permafrost thaw and then consume the stored OM (We will add this to the text). Thus, the low numbers of PLFA in the permafrost seems indeed represent living microorganisms in the permafrost sequence. For comparison also see Stapel et al., 2016. Overall, PLFA life marker profiles only indicate abundant microbial life for the active layers and do not correlate to the past markers in the permafrost sequence. Thus, the data suggest that in the permafrost sequence the past marker represent a paleo-signal (see chapter 5.2).

3) As the authors themselves acknowledge (page 11, lines 12-14), acetate concentrations say little about acetate availability as this depends on the production rates of acetate from organic matter. I am therefore not sure about the value of this parameter

in this context.

This was misunderstood; free acetate is an easily consumed substrate for microbial metabolism. Thus, free acetate concentration is a good tool to assess the potential of the OM to provide substrates for microbial turnover. The same is valid for the bound acetate concentrations indicating the future potential of the OM for substrates release upon degradation. In the respective sentence we wanted to explain why the free acetate concentration might be more different from the TOC values than the bound acetate concentration. We will re-phrase this part.

4) I would appreciate more details on the applied methods that are also not contained in the cited previous publication (Stapel et al., 2016). In particular, what compounds were detected with pyrolysis-GC-MS and how were they evaluated to generate Figure 3?

As external standard we used n-butane and the pyrolysate products were identified with the aid of reference chromatograms. The peak areas were integrated and calculated using the AGILENT ChemStation software. For the Eglinton-diagram (Eglinton et al,. 1990) o-xylene, 2,3-dimethylthiophene and n-nonene and for the Horsfield-diagram Horsfield et al. (1989) C1-C5 gases, C6-C14-n-alkanes and n-alkenes as well as C15 and longer n-alkanes and n-alkenes were integrated. We will integrate this to the methods chapter.

5) What PLFAs were detected and used to quantify total PLFAs? Were only bacterial or also fungal markers considered?

Further information on the quantification of the PLFAs was added in the sub-chapter “microbial lipid biomarker analysis”. Bacterial PLFAs from 14:0 to 21:0 with corresponding iso- and anteiso-FAs as well as br- and unsaturated-FAs were considered. We will integrate this to the text.

6) The authors further present some interesting correlations between individual pa-

rameters, and the statistical approach should be described in the methods section. The underlying correlation matrix could also be presented in a separate table in the manuscript to give the reader a better overview. We will add an additional sub-chapter (3.4 Statistical approaches) to describe how the statistical parameters were calculated. All shown data will be available on https://www.pangaea.de for free download; therefore we decided not to include extra tables to present the underlying correlation matrix for every single calculation.

7) The authors use European terminology to describe the glacial cycles that is technically not correct for Siberia. I do not object in general since the European terms are well known and the authors also use Marine Isotope Stages to identify these periods, but I suggest to add at least a comment on the terminology to the text.

We totally agree to also add the Russian terminology into the text and into table 1.

8) If I understood correctly, the authors imply that old organic matter from deep, continuously frozen permafrost deposits might move upwards into the active layer and stimulate microbial activity there (e.g., page 10, lines 10-13; page 11, lines 20-24; Conclusions). While it is correct that an influx of additional organic carbon can stimulate microbial activity in soils ("priming effect"), I do not see by what mechanism organic compounds could move upwards from frozen into non-frozen parts of the soil.

This was also a misunderstanding. What is meant here is that old OM gets into the active layer with increasing active layer depth (deepening of thaw front) and therefore is incorporated into the active microbial carbon-cycle again during the thawing period. We will re-write the respective paragraphs making clear that the OM becomes available again due to permafrost thaw.

9) The manuscript contains many grammatical mistakes and would overall profit from some language polishing (some sentences are very long and difficult to understand).

We will revise the manuscript thoroughly.

10) As my last general comment, I want to mention that I think the authors do a good job in keeping overview of the different depositional ages across the different cores. This is not an easy task.

We thank the reviewer for the positive feedback.

Technical corrections:

Page 1, line 19: What do you mean with "representing at least a future substrate potential upon release during OM degradation"?

The investigated bound acetate is a not directly available substrate pool and can only be available upon liberation from the OM via geochemical or microbial alteration of the OM. We will add "(present substrate pool)" and "(future substrate pool upon degradation)" into the abstract. Additionally, to improve the understanding of the idea of this parameter sentences will be added into the introduction.

Page 2, lines 2-4: I suppose you mean that the freeze-locked OM might thaw and/or be converted into CO2 or CH4, potentially inducing a positive feedback to global warming. Since the consequences of permafrost thaw are described in more detail later in the paragraph anyway, I suggest deleting this sentence. If you want to keep the sentence, please be more concrete.

We will remove this sentence part, but explain the climate carbon feedback later.

Page 2, line 6: What do you mean with "drastic changes in the ecosystem"; - we will replace it by "changes in vegetation".

Page 2, lines 8-10: The sentence is not clear to me. The term "re-mobilization" usually refers to the export of previously frozen OM or nutrients into aquatic systems (e.g., in the cited Vonk et al. reference), but this is not the cause for increasing decomposition rates or accessibility of OM for microbial degradation. Rather, permafrost thaw leads to increased microbial activity and consequently increased decomposition rates, as well as to increased export into aquatic systems.

We were not aware that the term “re-mobilization” can only be used in the way the reviewer claimed. Here, we wanted to say that freeze-locked OM and nutrients are again taking part in the carbon cycling upon permafrost thaw. To avoid confusion we will replace this term by “Thawing of permafrost promotes the accessibility of the formally preserved OM and nutrients for microbial turnover again, which results in increased microbial activity and consequently in increased OM decomposition rates (Dutta et al., 2006; Schmidt et al., 2011).”

Page 3, lines 9-10: Reference missing.

We will add the references “Weijers et al., 2006; Schouten et al., 2013” and “Pancost et al., 2001; Koga and Morii”.

Page 3, lines 12-13: What do you mean with “feedback effects on permafrost deposits”?

We will rephrase the whole sentence: “. . . the contribution of thawing permafrost deposits of different ages to the carbon-climate cycle is still an open question”

Page 4, lines 30-31: Were samples acidified before TOC analysis?

Yes they were. We will add this information to the text.

Page 6, line 19: Do the PLFA and TOC concentrations also correlate?

There are some similarities between PLFA profile and TOC (especially MIS 1, MIS 5e) but they show no overall correlation. We will add this information to the text.

Page 7, line 2: What do you mean with a partial correlation between past bacterial and archaeal markers?

Past bacterial and archaeal biomarker show higher abundances in the same depth interval, but the curves do not directly correlate.

Page 7, line 23: Do p-value and R2 refer to correlations of both bacteria and archaea

with TOC?

The p-value and R^2 referred only to the correlation of the br-GDGTs with TOC. We will rephrase the sentence to make that more clear.

Page 8, line 30: What do you mean with “assigned”? How does OM degradability depend on the amount of OM? And how do you distinguish OM composition and OM quality?

We will add a paragraph at the beginning of each discussion chapter to introduce into the following discussion (chapter 5.1, 5.2, 5.3). Thus, the respective part will be rephrased becoming part of the starting paragraph: “When permafrost thaws, formerly freeze-locked OM becomes bioavailable again. In this context, it is of utmost interest for the assessment of the impact of this OM on future climate evolution not only to determine the abundance but also to learn more about the quality of the OM with regard to its potential degradability. For instance, terrestrial OM (more aromatic rich) is considered to be more recalcitrant than aquatic OM (more aliphatic rich) (Hedges et al., 2000). Thus, the quality of the OM in permafrost deposits is determined by its source and, therefore, structural composition as well as by its alteration due to early diagenetic degradation processes during its deposition in the past (White, 2013). In the following chapter we apply pyrolysis techniques (Rock-Eval pyrolysis and open-system pyrolysis GC-FID) of the OM to get a deeper insight into the structural composition and to establish a new tool for OM quality assessment as introduced in Stapel et al. (2016).”

Page 9, line 10: The main mechanism by which TOC/TN decreases during OM decomposition is the faster loss of C than N due to microbial respiration.

We agree with the reviewer and will add this to the sentence.

Page 9, line 19: Please add a reference here;- we will add “Andreev et al., 2009 “ as reference.

Page 9, lines 27-29: Are you referring to the last interstadial here? I also noticed that

Table 2 suggests both a dry climate and moist soils during that period, this seems rather strange. There is also something wrong with the grammar in the first part of the sentence.

In this sentence we are referring to the Late Pleistocene (LP) glacial period in general, which is characterized by cold-climate conditions with anaerobic soil conditions. These slowed down OM decomposition rates and increased the accumulation of OM during this period. In this study, we present permafrost deposits from a stadial and interstadial period within the LP glacial. Subordinated different climate conditions (e.g. dry and wet) during the overall cold-climate conditions during the LP glacial period characterize this interstadial and stadial period. We will rephrase the sentence.

Page 10, line 11: The Fontaine paper is not about permafrost; - we removed this reference and replaced it by Knoblauch et al., 2013.

Page 10, lines 11-12: I do not understand. + Page 10, line 12: Which is not surprising considering that the active layer is seasonally thawed and the deeper permafrost is continuously frozen.

We will delete this here and shift it to chapter 5.3, where it will be rephrased to: "However, the increased PLFA concentrations in all active layers indicate to a certain extent that the permafrost deposits at least from MIS 3, 4 and 1 can serve as good substrate providers when thawed. For MIS 5e this could not be evaluated due to the lack of MIS 5e deposits with an active layer on top."

Page 10, lines 13-14: PLFAs inform about microbial biomass, not activity. + Page 10, line 20: Please add a reference for GDGTs as indicators of microbial activity. Also, the data presented show TOC concentrations, not accumulation rates.

That is true, but high abundance of PLFAs in a surface near seasonally thawed deposit highly suggest that the living microbes are somehow active, especially if comparing with the PLFA data from the deeper permafrost deposits. We will rewrite the whole

paragraph: "According to Knoblauch et al. (2013), permafrost surface layers contain both fresh organic material and old OM (within the permafrost), which can stimulate microbial activity. The high concentrations of PLFAs in the active layer suggest that not only the abundance of microbial life is increased in this layer, but also the microbial activity." Also GDGTs provide no direct measure on the activity, but the high abundance of these past markers suggest that they have been active in the past during time of deposition. We will rephrase this sentence to: "...and indirectly their abundance might say something about their activity during time of deposition."

Page 10, lines 23-25: The observed coincidence of high GDGT and OM concentrations does not necessarily imply certain environmental conditions. Microbial biomass is often correlated with OM concentrations since most microorganisms use OM as substrates.

By normalizing the GDGT and archaeol concentrations to TOC, the same trends within the depth profiles of the cores are visible. Depths of increased TOC and OM quality (e.g. core L14-02 at about 1.5 m with a HI of 246 mg HC/ g TOC and TOC of 4.7 wt%) correlate with increased concentrations of br-GDGTs. On the opposite, depths of decreased TOC but increased HI (e.g. core L14-03 at about 1.4 m with a HI of 254 mg HC/ g TOC and TOC of 1.9 wt%) reveal increased br-GDGT and archaeol concentrations indicating that the concentrations of living microorganisms in the past not only depend on the amount of TOC but also on the quality of the OM (HI values). As the HI indicates a higher aliphatic character representing increased soil-moister conditions (Stapel et al., 2016), conclusions on changes in the past soil moisture can be derived. We will rewrite the paragraph about the past microbial markers.

Page 11, line 15: What do you mean with "soil biogeochemistry composition"?

We will rephrase this sentence to: "The reason for this might be that free acetate pool in permafrost pore waters is not only the result of acetate released from the OM, but also can be influenced by other factors e.g. lateral and vertical diffusion promoted by capillary pressure (Parlange, 1971), thawing and freezing processes as well as

microbial production and consumption."

Page 11, lines 16-17: Speculation.

The sentence will be rephrased to: "The very low concentrations of free acetate and elevated concentrations of PLFA life markers detected in the investigated active layer samples suggest a higher microbial consumption of free acetate by an active microbial community (Lee et al., 2012; Knoblauch et al., 2013; Stapel et al., 2016)."

Page 11, line 19: It is true that input of fresh OM by plants might additionally stimulate the microbial community in active layers, but I would expect that the main reason for the higher microbial biomass is the fact that the active layer is thawed in summer (i.e., provides liquid water).

Sure the reason for the activity in the surface layer is the fact that this part is thawing during summer, but we think also the kind of OM is important for microbial degradation (what is more easily degradable for a microorganisms). We will rephrase the paragraph and consider the reviewer′s comment.

Page 11, lines 20-21: What do you mean with the incorporation of frozen permafrost carbon into the active layer?

Due to increasing active layer thickness, more old freeze-locked permafrost carbon is integrated in the microbial degradation processes in the active layer during the thawing period. We will extend the sentence.

Page 11, line 26: "thermos"?

It has to be "thermoterrace" which is also known as "thermo-erosional valley" (Schirrmeister et al., 2011). We will replace it and add the reference to the text.

Page 12, line 1: Microbial consumption in the active layer is an ongoing process and not restricted to the "time of deposition".

This was a misunderstanding. It was meant when the Holocene deposits were part of

the active layer in the past. We will rephrase this to: "The minor free-acetate pool in the Holocene deposits may be the result of the OM composition or of intense microbial consumption during OM deposition in the past as has been proposed for the modern active layer."

Page 12, line 2: What do you mean with "stronger pronounced"? I'm afraid I cannot follow the entire sentence.

We will rephrase the sentence and replace "stronger pronounced" by "deeper and longer thawed"

Page 12, line 11: What do you mean with "microbial acetate consumption on a regional scale"?

Permafrost thaw enables lateral and vertical transportations of water, OM and sediment which can redistribute substrates. These redistributed substrates will probably be microbially consumed on/in another place (e.g. in a near located soil or lake). In other words, on a more regional scale (this study site only represents a small spot in the Siberian Arctic) lateral and vertical transportation of substrates probably will end up in microbial consumption. We will add further information into the text.

Page 29: How were the plus and minus signs assigned?

To visualize the OM quality and the substrate potential a relative scaling based on the results of this study was applied.

We thank the reviewer for his/her comments and will follow all his/her suggestions on the other (minor) technical corrections (not listed here).

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