Reviewer 2

Permafrost soils store more than 30% of the global surface organic carbon. The thaw-induced carbon release in the form of greenhouse gases would create a positive feedback to amplify climate warming. In this paper, Jongejans et al., present the valuable records of TOC, TN and multiple lipid biomarkers to assess organic matter quality in a 650-ka ancient permafrost of east Siberia. Such old records cover glacial-interglacial climate variations, which provides a valuable opportunity to explore potential influence of climate changes on permafrost carbon cycling. Their biomarker evidences show higher organic matter decomposition during interglacial periods. This will be very useful for understanding of carbon cycles in permafrost regions as global climate warms. I have no major comments on this valuable paper and hence recommend to accept it after the following minor/moderate comments have been covered.

Thank you for this overall positive assessment of our work! We responded to all comments below. Note: the line numbers refer to the preprint version

L22: Add what lipid biomarkers did you analyze in this study, such as alkanes, fatty acids.
We added "(alkanes, fatty acids and alcohols)" to the abstract as suggested.

L29: Delete "or bacterial". Microbial origin contains bacterial origin.
Changed accordingly.

L36: the world's surface soil carbon?
The global soil estimate (3350 Gt) is based on soils to 3 m (2800 Gt) as well as other pools in deep permafrost (500 Gt) and tropical peatlands (50 Gt; Jackson et al., 2017). Therefore, it is not just the surface soil C, but also includes C of deeply buried deposits. We changed the wording to make this clearer: "The global permafrost region contains roughly half of the world's soil carbon (3350 Gt) and in addition, a large deep permafrost carbon pool (>3 m) which is often not accounted for and its amount is uncertain (~500 Gt) (Strauss et al., 2021)."

L57-58: Biomarker tools as tracing permafrost thaw and carbon cycling are very important in this study. I suggest more previous publications are needed to introduce here. Multiple lipid biomarkers have been applied to sediment records for reconstructing carbon perturbations of permafrost (e.g., Evert et al. 2016, https://doi.org/10.1177/0959683616645942; Yao et al., 2021, https://doi.org/10.1130/G48891.1).
Thank you for your recommendation. We rephrased the sentence and added a few relevant references: "The study of lipid biomarkers has been proven useful in previous work to characterise permafrost OM and carbon cycling as well as tracing permafrost thaw has proven useful in previous work (Zech et al., 2009; Strauss et al., 2015; Elvert et al., 2016; Stapel et al., 2016; Jongejans et al., 2018, 2020; Martens et al., 2020; Bröder et al., 2021; Yao et al., 2021)."

L115: What solvents (including solvent volume) do you use for separation the aliphatic, aromatic and polar NSO?

The MPLC is an instrument for the chromatographic separation of the usually complex extract into fractions of different polarity namely an aliphatic, aromatic and NSO fraction. Due to the instrumental design only n-hexane is used as the main transport medium. During this process, the NSO compounds are separated from the aliphatic and aromatic components by a pre-column. Then the aliphatics are passing through the main column to form the aliphatic fraction, while the aromatics will stick on top of the main column. With the help of a backflush vent the aromatics are eluted from the main column with n-hexane. Subsequently, the NSO compounds are eluted from the pre-column using dichloromethane with 1% methanol. We added now the reference describing the MPLC methods in detail to avoid extending the method description too much.

L127: The first mention of abbreviation "FA" is fatty acids?

Yes. We introduced the abbreviation in line 21 and used the abbreviation throughout the manuscript.

L127: ACL can be also affected by climate changes and resulting alkane degradation, such as temperature and relative humidity. Terrestrial plants tend to produce longer n-alkanes to protect their water loss under higher temperature and drier conditions. Moreover, higher temperature and wetter conditions may facilitate higher microbial activities, may resulting in the faster degradation of organic matter.

Thank you for clarifying this. Indeed, Sachse et al. (2006, https://doi.org/10.1016/j.orggeochem.2005.12.003) found that the ACL index of all analysed *Betula* species increased along a transect from Northern Finland to Southern Italy. It is possible that trees (and with them also other plants) in areas with a longer vegetation period and inhabiting regions with more potential incoming radiation protect their leaves from water loss with longer chain *n*-alkanes. Alternatively, Sachse et al. stated that the observed phenomenon could be caused by evaporative loss of the shorter *n*-alkanes due to increased evaporation southward along their transect. Besides a faster degradation rate, also the annual air temperature could affect *n*-alkane chain length in leaf waxes. Moreover, it has been shown that environmental factors such as moisture and temperature affect the hydrocarbon composition in *Rosmarinus officinalis* leaves on a seasonal scale (Maffei et al., 1993).

We added this limitation in the methods: "Variations of the ACL can be caused by different plant type material and climatic-induced changes of the environmental conditions. For example, different temperature and wetness conditions as well as length of the vegetation periods can influence the long chain n-alkane distribution (e.g., Sachse et al., 2006)" (L129).

L148: Add "may" before "contain".

In line 148, there is no “contain”. Do you mean the word “contained”? For that we think that “may” is not the right word, so we added likely instead.

L145-146: Could you show a supplementary figure or table for these correlations?
Following your suggestion, we placed a table with correlation coefficients r in the revised supplements (Table S1). All p-values are below 0.01.

	alkylcyclopentanes	methylalkanes	diethylalkanes and ethyl-methylalkanes
alkylcyclohexanes	0.997	0.98	0.992
alkylcyclopentanes		0.974	0.986
methylalkanes	0.974		0.994

L227: Please specify what biomolecules or organic indices can give insight into different OM sources.
We added “such as the biomarker indices ACL and P_{aq}” to match our work

L240: Change to “higher ACL” and “lower P_{aq}”.
Changed accordingly

L260: Add a supplementary figure or table for these correlations. And elsewhere.
We placed accordingly a table with correlation coefficients r in the supplements (Table S2). All p-values are below 0.01.

	ACL	P_{aq}
CPI	0.74	-0.7
ACL		-0.94

L280: Higher ACL does not indicate higher terrestrial source. Higher temperature or drier climate can also lead to higher ACL values.
ACL values around 27 to 31 indicate usually higher plant material. In contrast, lower ACL values might be influenced by more C23/C25 n-alkanes indicating a higher contribution of aquatic OM and/or moss species. Thus, the ACL can provide general information on OM sources. In addition to this, you are right that the ACL is influenced also by climatic induced changes of the environmental conditions. Warmer temperature and more humid conditions seem to impact the ACL to higher ratios. This is sometimes also caused by shifts in the terrestrial plant community (e.g. C3 to C4 plants). However, although there are climate induced variations, the ACL can be used to generally assess the OM type. We explained the parameter in more detail in chapter 3.2 and we used the ACL in combination with the Paq to assess the higher plant and aquatic or moss like character of the OM.

L285: ACL can be affected multiple factors. Please see my comment “L127”.
Please see our answer to your comment above

L308: Are there aquatic plants grow around the study area? Higher P_{aq} ratios could also be due to input of mosses - they also produce lots of mid-chain n-alkanes.
That is right, *n*-C23 and *n*-C25 are common both in aquatic macrophytes as well as in (Sphagnum) mosses.. During all stages, ponding water or saturated soil conditions are likely, as the permafrost table prevents percolation below the seasonally thawed uppermost active layer. Ashastina et al. (2017) and Murton et al. (2017) suggested that the modern vegetation also includes mosses. However, there is no Holocene vegetation data from either of those studies (Ashastina et al., 2017; Murton et al., 2017) based on pollen and/or plant macrofossil data. We added in the text that the high Paq could also indicate moss rich OM and included this into the discussion (L130).

L318 and 330: Again, delete "or bacterial". Microbial origin contains bacterial origin.
Changed accordingly.

L346: Change to "rivers".
Changed accordingly.

L357: Add more references (e.g., Evert et al. 2016, https://doi.org/10.1177/0959683616645942; Yao et al., 2021, https://doi.org/10.1130/G48891.1).
We added the suggested manuscript of Yao et al. (2021) as well as a recent manuscript from Bröder et al. (2021) on permafrost C mobilisation from retrogressive thaw slumps.

L359: Please specify what past environments.
We refer to all environments which are not recent. We changed the wording to "palaeo environment".

L370: The impacts of findings should be described as well. E.g. why are these findings important and for whom?
We added the following sentences to the conclusion: "Our biomarker analyses of ancient permafrost sediments contribute to a better understanding of how OM is incorporated and preserved in permafrost deposits during glacial and interglacial periods. Furthermore, it helps to improve our comprehension of possible consequences resulting from future permafrost thaw and OM mobilisation." (L370)

Table 2: Add m/z data of molecular weight, base peak, and characteristic peak of each individual compounds.
We revised Table 2, now including the information on the M+ and characteristic fragments with marked base peak fragments.