Anonymous referee # 1 comments and reply.

Dear Referee # 1,

Thank you for your contribution in reviewing this manuscript. We have outlined considerable changes that could be made to the manuscript in order to address your concerns that oxygenation of biogenic methane could produce the $\delta^{13}C$-CH_4 signatures observed in this study.

This manuscript aims to determine the contribution of biogenic and thermogenic methane (CH_4) to CH_4 fluxes from the Mackenzie River Delta (MRD) into the atmosphere. Therefore, the authors collected surface air samples from several sampling sites in the MRD and analysed their CH_4 concentration and the $\delta^{13}C$ value of CH_4. To differentiate between thermogenic and biogenic CH_4 they used two thresholds, assuming that CH_4 with a carbon stable isotope value of > -50‰ is of thermogenic origin and CH_4 with a value < -70‰ of biogenic origin. Values between -50‰ and -70‰ would indicate a mixture of both sources. The main conclusions of the manuscript are based on this assumption, which is, however, a substantial oversimplification. There are numerous studies demonstrating carbon stable isotope signatures of biogenic CH_4 of > -70‰, in particular CH_4 from acetoclastic methanogenesis (see e.g. Bréas et al. (2001), Chanton & Smith (1993), Conrad (2005)), including from permafrost affected wetlands (Nakagawa et al., 2002). Furthermore, CH_4 emitted from highly heterogeneous wetlands as the one studied here are affected by microbial CH_4 oxidation, which causes the carbon stable isotope signature of released CH_4 to increase, also to values above -50‰. There are many studies about the impact of CH_4 oxidation in northern wetlands on CH_4 fluxes and the carbon stable isotope signatures of released CH_4 (e.g. Happell et al. (1994), Vaughn et al. (2016)), but the effect of CH_4 oxidation on carbon stable isotope values of CH_4 is mentioned only very briefly. Since the carbon stable isotope values of released methane may vary strongly, e.g. due to different CH_4 production pathways, CH_4 transport and CH_4 oxidation, carbon stable isotope values between -42 ‰ and -88 ‰, as presented in this manuscript, may be explained by biogenic sources alone and are also reported for northern wetlands not affected by fluxes of thermogenic methane. Hence, I do not see that carbon stable isotope values of released methane alone provide robust information to answering the central research question of this manuscript, the contribution of biogenic and thermogenic methane to methane release in the MRD. To give substantial information on this question, further data are needed, e.g. the δD signatures of CH_4, its ^{14}C age, or the concentration of further hydrocarbons.

Furthermore, methods of gas sampling and analysis and calculation of the source $\delta^{13}C$ value should be described in more detail. What was the gas flow while flushing the Synflex tube, how often was it flushed with the air sample to ensure that no contaminations remained? How was gas collected with the LI-7810, how often and at which positions? Why were gas samples collected in the Synflex tube, if they were not analysed for $d^{13}C$ of CH_4? How far is ‘as close as possible’? Please clearly describe which samples were collected for which analysis. Particularly for the Keeling-plots and the calculation of the $\delta^{13}C$ source values it should be clearly explained from which collected sample the CH_4 concentrations and $\delta^{13}C$ values were analysed.

Finally I suggest restructuring the Results and Discussion section. In the current version of the manuscript a substantial part of the results are presented (or repeated) in the Discussion. Specific comments:

We agree that measuring deuterium as well as $^{13}CH_4$ would significantly strengthen the conclusions made in this study. We also agree that our interpretation of signatures may be an oversimplification. Sites with stable carbon isotope signatures observed in this study which we have described as "mixed" sources could have potentially been produced by oxidized biogenic methane. Specifically, sites Pingo 1 (-53.0 ‰) and Pingo 2 (-63.6 ‰). We could make changes to the first paragraph of the discussion around our determination of the source of CH_4 at these sites. The potential effect of oxidation is somewhat understated in the manuscript. In response, we can add existing literature values for biogenic CH_4 with the highest stable carbon isotope signatures to the discussion. We find that that biogenically derived, stable carbon isotope signatures as high as - 50‰ are exceedingly rare for arctic permafrost environments, but they do exist. Values as high as -42 ‰ are not present in the literature. The highest signatures reported in literature for arctic lakes or wetlands are -58.2 in Nakagawa et al. (2002), -52.3 in Vaughn et al. (2016), -49.2 ‰, (Thompson et al., 2016) and -44.9 ‰ (Preuss et al., 2013). The isotopic values from Siberian alasses reported in Nakagawa (2002) were misrepresented in Conrad (2005) as -43 ‰ to -27 ‰ when they are actually -63.9 ‰ to -58.2 ‰. This is the only Arctic site referenced by Conrad (2005). Happell et al. (1994), Chanton & Smith, (1993) and Bréas et al. (2001) observed $^{13}CH_4$ values greater than -50‰ (as high as -37 ‰, -41 ‰, and -31 ‰). All of these studies were south of 30°N where $\delta^{13}C$ signatures are higher than those found in the Arctic due to the prevalence of C4 plants (Chanton & Smith, 1993; Fisher et al., 2017; Nakagawa et al., 2002; Oh et al., 2022).

Please see below for our responses to individual comments.

L11: To my understanding, CH_4 is released but not produced from thermogenic sources. Please clarify

Reply: "Production" can be changed to "emission"

L30: What means 'conductive for biogenic CH_4 production'? Please clarify
Reply: "Conditions conducive for biogenic CH_4 production" can be changed to "conditions where biogenic CH_4 production and potential atmospheric release is likely to occur."

L56f: This assumption is an oversimplification (see above)
Reply: "Intermediate values may indicate gases which are a mixture of biogenic and thermogenic CH_4. Complexities can result from geochemical processes such as CH_4 oxidation which can change the $\delta^{13}C$-CH_4 due to a preference for bacteria to oxidize CH_4 containing the lighter isotope (^{12}C) enriching the remaining CH_4 with ^{13}C."

Can be changed to:

"Intermediate values may result from the oxidation of biogenic CH_4 or from gas which contains a mixture of biogenic and thermogenic CH_4. Oxidation of CH_4 can occur if gas migrates through an oxidizing environment such as the aerobic zone of the soil or a wetland. This can result in a higher $\delta^{13}C$-CH_4 signature due to a preference for bacteria to oxidize CH_4 containing the lighter isotope (^{12}C), enriching the remaining CH_4 with ^{13}C (Chanton et al., 2005)."

L85: Do mixed sources contain other CH_4 than biogenic and thermogenic? Please clarify.
Reply: Mixed sources contain a mixture of thermogenic and biogenic methane. In order to more clearly identify the intent of the manuscript the final sentence of the introduction can be changed to read "We hypothesise that the largest hotspots in the MRD include contributions of biogenic CH_4 due to the abundance of environmental settings where modern methane is being produced (Cunada et al., 2021)."

L159f: The sampling of surface gas with the aluminium tubing is unclear to me. How was the tube filled and how it was possible to analyse discrete samples from this tube? Please explain in more detail.
Reply: The samples collected in aluminium tubing were intended to give a continuous transect of CH_4 and CO_2 concentrations over the hotspot and were not analyzed as discrete samples. The description of the methods can be rewritten as follows in order to clarify:

"Walking transects were carried out by filling a 30 m coil of 4 mm inside diameter aluminium Synflex tubing while walking at a steady pace across the ground. A constant flow rate of 20 standard cubic centimetres per minute (CCM) was maintained by attaching a small pump and a flow controller to the coil of tubing. Samples were analyzed using a Picarro G2210i analyser immediately on return from the field site. Five walking transects using Synflex tubing took approximately 20 minutes each to fill and covered a distance between 600-800 m. Methane and CO_2 concentrations were measured every 1-2 seconds on the air samples collected in aluminium tubing. This allowed for consideration of spatial variability in methane concentrations at each site. Mixing of the air sample inside the tube between collection and analysis is limited due to the small diameter of the tubing. A similar method was used during drone-based CH_4 measurements (Andersen et al., 2018)."

L 177: value not ratio
Reply: Ratio can be changed to value

L 275f: This might just indicate a higher contribution of CH_4 oxidation in summer than in winter, when the surface soil is frozen.
Reply: We have stated that the difference in ^{13}C source signatures could be caused by greater oxidation at this site. The existing text can be changed to the following to make that clearer:

"Discrete sampling at Wetland 1 yielded a $\delta^{13}C$-CH_4 Keeling plot source signature of -88.3 ‰ when sampled in October during freeze up and -53.4 ‰ during the summer. We conclude that

this demonstrates a biogenic source during the fall since biogenic production can persist late into the cold season (Zona et al., 2016). While the sampling was carried out at the same location, methane ebullition was seen while sampling during the fall, but not during the summer. The Wetland 1 site was dominated by sedge vegetation with areas of standing water. The lack of ebullition flux at the same site during the summer and the different Keeling plot estimate suggests methane flux in this wetland setting varies seasonally. The Keeling plot source signature of -53.4 ‰ during the summer could be caused from either oxidation of a biogenic source or contributions of both biogenic and thermogenic sources. Oxidation of CH_4 has been shown to be a significant source of fractionation in arctic lakes during the summertime (Thompson et al., 2016)."

L282f: Methane oxidation in permafrost-affected wetlands is most important in the ice- free summer. High CH_4 oxidation might even cause the lack of ebullition and explain the high $\delta^{13}C$ value of CH_4.
Reply: This paragraph can be rewritten to explain the potential for oxidation during the summer (see response to previous comment).

L306: It is unclear, which data indicate the multiple sources of CH_4. Please clarify.
Reply: "Flux chamber sampling over the terrestrial shrub tundra terrain immediately adjacent to Lake 1 indicated it was a source of CH_4 and CO_2 with flux rates of 2.25 mg CH_4-C $hr^{-1}m^{-2}$ and 52.73 mg CO_2-C $hr^{-1}m^{-2}$. The soil adjacent to Lake 1 was saturated with water, creating ideal conditions for biogenic production at the site. This shows that we sampled multiple sources of CH_4 at the site; biogenic methane production in and around the lake as well as a strong thermogenic seep. This could account for the low r^2 value (0.48) (Fig. 3) at Lake 1 despite highly elevated CH_4."

Can be changed to:

"The soil adjacent to Lake 1 was saturated with water, creating ideal conditions for biogenic production at the site. It is possible that we sampled multiple sources of CH_4 at the site; biogenic methane production in and around the lake as well as a strong thermogenic seep. This could account for the low r^2 value (0.48) (Fig. 3) at Lake 1 despite highly elevated CH_4.

Thermogenic seeps to the atmosphere have been documented in Arctic regions, including the Mackenzie River Delta, where thermogenic methane exists underneath the permafrost (Bowen et al., 2008; Osadetz & Chen, 2010; Walter Anthony et al., 2012). While biogenic CH_4 production typically results in $^{13}CH_4$ values less than -70 ‰, oxidation can result in a higher $\delta^{13}C$-CH_4 signatures which are closer to that of thermogenic CH_4. This is due to a preference for bacteria to oxidize CH_4 containing the lighter isotope (^{12}C), enriching the remaining CH_4 with ^{13}C (Chanton et al., 2005). Values of $\delta^{13}C$-CH_4 signatures as high as -44.7 ‰, which were observed at Lake 1, are not likely to be generated through oxidation of biogenic CH_4. Values almost as high are exceedingly rare at these latitudes, but have been observed before, in an Arctic lake (-49.2 ‰) (Thompson et al., 2016), and in a pond formed in polygonal tundra (-44.9 ‰, -52.3 ‰) (Preuss et al., 2013; Vaughn et al., 2016). In the case of Preuss et al.

(2013), almost complete oxidation of CH_4 to atmospheric levels was required to increase the $\delta^{13}C$-CH_4 signature from less than -50 ‰ up to -44.9 ‰. Additionally, these two sites are at a river channel and a lake, respectively, where oxidation would be minimal as compared to a wetland. The likelihood of the permafrost thawing completely through at these two locations is also higher than at the wetland locations, increasing the possibility of thermogenic migration."

L311 f: The second part of this sentence is unclear.
Reply: We stand by our assessment that at least 2 of these sites had $\delta^{13}C$-CH_4 signatures that indicate thermogenic origin. The values of -42.5 ‰ and -44.7 ‰ are higher than the proven range for biogenic methane in Arctic environments, even sites where there is fractionation due to excessive CH_4 oxidation.

We can still reworded this sentence to be clearer, as follows:

"Estimates of source isotope signatures from field sites varied substantially from biogenic to thermogenic, indicating that the largest sites of CH_4 production in the MRD are caused by a variety of sources." Can be changed to read: "Estimates of source isotope signatures from field sites ranged from -42 ‰ to -88 ‰, indicating that the largest sites of CH_4 production in the MRD are caused by both biogenic and thermogenic sources."

L318f: What are 'eddy covariance hotspot locations' and which data of this study verify these?
Reply: The airborne eddy covariance hotspot locations are described and cited (Kohnert et al., 2017) in the first paragraph of the Methods section and are further detailed in Figure 2.

Cited literature:

Bowen, R. G., Dallimore, S. R., Côté, M. M., Wright, J. F., & Lorenson, T. D. (2008). *Geomorphology and gas release from pockmark features in the Mackenzie Delta, Northwest Territories, Canada.* 6.

Bréas, O., Guillou, C., Reniero, F., & Wada, E. (2001). The Global Methane Cycle: Isotopes and Mixing Ratios, Sources and Sinks. *Isotopes in Environmental and Health Studies*, *37*(4), 257–379. https://doi.org/10.1080/10256010108033302

Chanton, J., Chaser, L., Glasser, P., & Siegel, D. (2005). Carbon and Hydrogen Isotopic Effects in Microbial, Methane from Terrestrial Environments. In *Stable Isotopes and Biosphere Atmosphere Interactions* (pp. 85–105). Elsevier. https://doi.org/10.1016/B978-012088447-6/50006-4

Chanton, J., & Smith, L. (1993). Seasonal variations in the isotopic composition of methane associated with aquatic macrophytes. In R. S. Oremland (Ed.), *Biogeochemistry of Global Change: Radiatively Active Trace Gases* (pp. 618–632). Springer US. https://doi.org/10.1007/978-1-4615-2812-8

Conrad, R. (2005). Quantification of methanogenic pathways using stable carbon isotopic signatures: A review and a proposal. *Organic Geochemistry*, *36*(5), 739–752. https://doi.org/10.1016/j.orggeochem.2004.09.006

Fisher, R. E., France, J. L., Lowry, D., Lanoisellé, M., Brownlow, R., Pyle, J. A., Cain, M., Warwick, N., Skiba, U. M., Drewer, J., Dinsmore, K. J., Leeson, S. R., Bauguitte, S. J.-B., Wellpott, A., O'Shea, S. J., Allen, G., Gallagher, M. W., Pitt, J., Percival, C. J., … Nisbet, E. G. (2017). Measurement of the ^{13} C isotopic signature of methane emissions from northern European wetlands: Northern Wetland CH $_4$ Isotopic Signature. *Global Biogeochemical Cycles*, *31*(3), 605–623. https://doi.org/10.1002/2016GB005504

Happell, J. D., Chanton, J., & Showers, W. S. (1994). The influence of methane oxidation on the stable isotopic composition of methane emitted from Florida swamp forests. *Geochimica et Cosmochimica Acta*, *58*, 4377–4388.

Kohnert, K., Serafimovich, A., Metzger, S., Hartmann, J., & Sachs, T. (2017). Strong geologic methane emissions from discontinuous terrestrial permafrost in the Mackenzie Delta, Canada. *Scientific Reports*, *7*(1), 5828. https://doi.org/10.1038/s41598-017-05783-2

Nakagawa, F., Yoshida, N., Nojiri, Y., & Makarov, VladimirN. (2002). Production of methane from alasses in eastern Siberia: Implications from its ^{14} C and stable isotopic compositions: METHANE FROM ALASSES IN EASTERN SIBERIA. *Global Biogeochemical Cycles*, *16*(3), 14-1-14–15. https://doi.org/10.1029/2000GB001384

Oh, Y., Zhuang, Q., Welp, L. R., Liu, L., Lan, X., Basu, S., Dlugokencky, E. J., Bruhwiler, L., Miller, J. B., Michel, S. E., Schwietzke, S., Tans, P., Ciais, P., & Chanton, J. P. (2022). Improved global wetland carbon isotopic signatures support post-2006 microbial methane emission increase. *Communications Earth & Environment*, *3*(1), 159. https://doi.org/10.1038/s43247-022-00488-5

Osadetz, K. G., & Chen, Z. (2010). A re-evaluation of Beaufort Sea-Mackenzie Delta basin gas hydrate resource potential: Petroleum system approaches to non-conventional gas resource appraisal and geologically-sourced methane flux. *Bulletin of Canadian Petroleum Geology*, *58*(1), 56–71. https://doi.org/10.2113/gscpgbull.58.1.56

Preuss, I., Knoblauch, C., Gebert, J., & Pfeiffer, E.-M. (2013). Improved quantification of microbial CH_4 oxidation efficiency in arctic wetland soils using carbon isotope fractionation. *Biogeosciences*, *10*(4), Article 4. https://doi.org/10.5194/bg-10-2539-2013

Thompson, H. A., White, J. R., Pratt, L. M., & Sauer, P. E. (2016). Spatial variation in flux, $\delta^{13}C$ and $\delta^{2}H$ of methane in a small Arctic lake with fringing wetland in western Greenland. *Biogeochemistry*, *131*(1–2), 17–33. https://doi.org/10.1007/s10533-016-0261-1

Vaughn, L. J. S., Conrad, M. E., Bill, M., & Torn, M. S. (2016). Isotopic insights into methane production, oxidation, and emissions in Arctic polygon tundra. *Global Change Biology*, *22*(10), 3487–3502. https://doi.org/10.1111/gcb.13281

Walter Anthony, K. M., Anthony, P., Grosse, G., & Chanton, J. (2012). Geologic methane seeps along boundaries of Arctic permafrost thaw and melting glaciers. *Nature Geoscience*, *5*(6), Article 6. https://doi.org/10.1038/ngeo1480

Zona, D., Gioli, B., Commane, R., Lindaas, J., Wofsy, S. C., Miller, C. E., Dinardo, S. J., Dengel, S., Sweeney, C., Karion, A., Chang, R. Y.-W., Henderson, J. M., Murphy, P. C., Goodrich, J. P., Moreaux, V., Liljedahl, A., Watts, J. D., Kimball, J. S., Lipson, D. A., & Oechel, W. C. (2016). Cold season emissions dominate the Arctic tundra methane budget. *Proceedings of the National Academy of Sciences*, *113*(1), Article 1. https://doi.org/10.1073/pnas.1516017113