The authors would like to thank the Anonymous Reviewer 1 for his/her valuable comments and suggestions to strengthen the analysis presented in our manuscript. The comments and suggestions have been addressed in the revised manuscript (our responses in blue), as follows (reviewer's comments in bold):

The manuscript 'Hysteretic temperature sensitivity of wetland CH4 fluxes explained by substrate availability and microbial activity' by Chang and co-workers describes a modelling study in which the authors investigate the reasons for the differences in temperature sensitivity of methane emissions at the beginning and the end of the thawing season in two permafrost affected landscapes. They present observational data on this 'hysteretic temperature sensitivity' from one of the investigated sites (Stordalen Mire). However, to investigate the reasons for the observed temperature response they use data generated by their model. Based on the modeling results, the different temperature responses of methane emission during the thawing season is due to higher methanogen biomass and substrate production for methanogenesis in the later thaw season. This results in higher methane production and emissions at the same temperature in the later season compared to the early seasons.

The manuscript is concerned with a very important topic and there is no doubt that we need a better understanding of the factors regulating the different processes in the wetland CH4 cycle. This improved understanding has to inform models simulating methane emissions and their response to changes in environmental conditions. In this respect, the objective of the current study is highly relevant. On the other side, this study almost exclusively presents model-generated data on the regulation of methane emission in Stordalen Mire. The authors should make this clear and furthermore more critically evaluate the outcome of their model.

The authors thank the reviewer for the valuable comments. We have carefully revised our manuscript based on the suggestions provided by the reviewer. For example, we have added text in the abstract (Lines 30-36) and introduction (Lines 96-99) indicating that we are using model simulations to interpret the relative role of the complex set of interacting processes responsible for emergent CH_4 emissions observed at the Stordalen Mire. We also discuss further observations that would be required to evaluate our proposed mechanisms (Lines 334-342).

We have added the following in the revisions:

Lines 30-36

Here, we show that apparent CH_4 emission temperature dependencies inferred from year-round chamber measurements exhibit substantial intra-seasonal variability, suggesting that using static temperature relations to predict CH_4 emissions is mechanistically flawed. Our model results indicate that such intra-seasonal variability is driven by substrate-mediated microbial and abiotic interactions: seasonal cycles in substrate availability favors CH_4 production later in the season, leading to hysteretic temperature sensitivity of CH_4 production and emission.

Lines 96-99

We focus most of the detailed analysis at Stordalen Mire, where we recently validated the modeled CH_4 production pathways using acetoclastic and hydrogenotrophic methanogen relative abundance inferred from 16S rRNA gene amplicon sequencing data (Chang et al., 2019).

Lines 334-342

Although the CH_4 emission rates and CH_4 production pathways modeled in the Stordalen Mire fen have been examined (Chang et al., 2019), continuous substrate concentration measurements are lacking for validating the substrate-mediated hysteretic temperature responses proposed here. Wide ranges of acetate and hydrogen concentrations have been reported from incubation experiments studying methanogenesis (e.g., Hines et al., 2008; Tøsdal et al., 2015; Zhang et al., 2020); however, those values may not be used to validate the time and space specific substrate concentrations modeled at our study sites. Therefore, further studies and additional field measurements are needed to test our proposed hypothesis of the causes of observed CH_4 emission hysteresis.

First of all, the model simulates a very low contribution of aerobic methane oxidation, which seems to be constant, irrespective of methane production (Fig. 3). The absence of methane oxidation makes the whole story much easier, since in this case, methane emission almost exclusively depend on methane production. However, several studies demonstrated the importance of methane oxidation in Stordalen myre (e.g. Perryman et al., (2020) or Singleton et al., (2018)) and numerous studies on other bogs and fens have shown the utmost importance of methane oxidation for methane emissions. The authors should comment on this, in particular since the unequal importance of methane production and methane oxidation during one thaw season may contribute to the 'hysteretic temperature sensitivity' of methane emissions observed. The bottom soil, where methane production takes place experiences in the early thaw season deeper temperatures than the surface soil,

where aerobic processes like methane oxidation take place. In the late season, this pattern is reversed, since the soil starts freezing from the surface, which means that aerobic processes are earlier affected by freezing than anaerobic processes. Therefore, methane oxidizers and methane producers are exposed to different temperatures at the start and the end of the thaw season despite similar mean soil or air temperature. The very low contribution of methane oxidation in their model should be critically discussed on the background of the whole relevant literature and not only by considering the study supporting their findings. Furthermore, it would be interesting if the model is simulating a substantial contribution of methane oxidation at other sites, e.g. in Barrow.

We would like to clarify that the modeled CH_4 oxidation rate is not constant, although it appears to be constant in Fig. 3 due to its relatively low values compared with the modeled CH_4 production and emission rates. At the Stordalen site, our simulation suggests that CH_4 oxidation is unlikely to be a dominant factor controlling thawed-season CH_4 emissions (e.g., less than 5% of the modeled CH_4 production is oxidized to CO_2 from July to August), which is consistent with isotopic evidence from this site (McCalley et al., 2014). Although Perryman et al. (2020) and Singleton et al. (2018) discussed the importance of CH_4 oxidation on CH_4 cycling, the extent to which CH_4 emissions are regulated by CH_4 oxidation remains uncertain since their results did not estimate the relative strengths of CH_4 production and oxidation. For example, Singleton et al. (2018) reported a disconnection between methanotroph abundance and activity in the Stordalen Mire fen, suggesting that the metabolic potential may not necessarily represent microbial activity. In the revised manuscript, we show that seasonal cycles in CH_4 production, oxidation, and emission rates modeled at the Stordalen Mire fen are consistent from 2011 to 2013 (Supplementary Fig. 6) while the apparent temperature dependencies of CH_4 emissions exhibit consistent intra-seasonal variation during the corresponding thaw season (Fig. 2d, e, f). Therefore, the hysteretic temperature sensitivity discussed in our study is not caused by the unequal importance of CH_4 production and CH_4 oxidation modeled in one thaw season. We have included these discussions in the revised manuscript (Lines 298-302; 305-313).

We have added the following in the revisions:

Lines 298-302

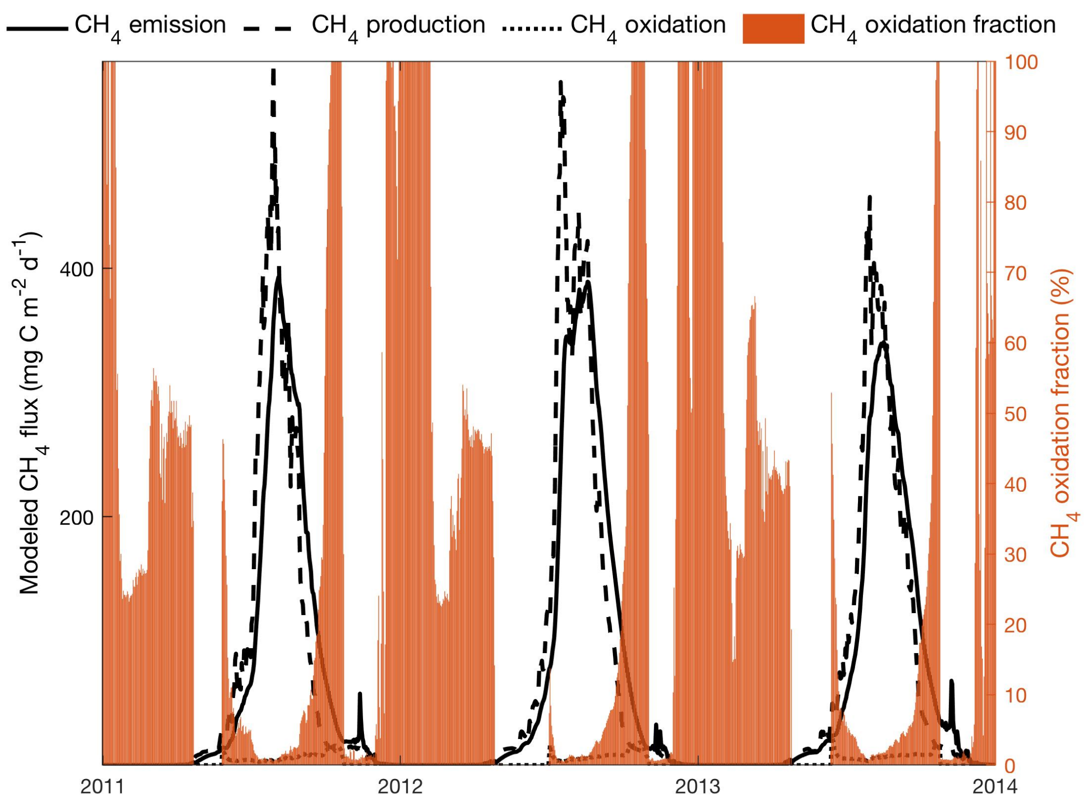
Further, the consistent seasonal cycles in CH_4 production, oxidation, and emission rates modeled from 2011 to 2013 (Supplementary Fig. 6) indicate that the CH_4 emission hysteresis modeled in that period (Fig. 2d, e, f) is not caused by relatively low CH_4

oxidation modeled in a particular site-year.

Lines 305-313

Although CH_4 oxidation has been proposed to be an important control regulating wetland CH_4 emissions, e.g., Perryman et al. (2020) and Singleton et al. (2018), the competitive dynamics between methanogens and methanotrophs throughout the year has not been included in such studies. The modeled CH_4 oxidation rate is relatively low during the thawed season when CH_4 production is strongest, and relatively high during the shoulder season when CH_4 production is weakest (Supplementary Fig. 6). These strong seasonal variations suggest that the relative importance of CH_4 production and oxidation on regulating CH_4 emissions may fluctuate throughout the year, highlighting the need to properly represent the underlying dynamics controlling CH_4 biogeochemistry.

Supplementary Fig. 6



Supplementary Figure 6. Daily CH_4 emissions, CH_4 production, CH_4 oxidation, and CH_4 oxidation fraction modeled in the Stordalen Mire fen from 2011 to 2013. CH_4 oxidation fraction is defined as the ratio of daily CH_4 oxidation to daily CH_4 production.

A second critical point is the simulated extremely high concentration of substrates for methanogens. The simulated maximum acetate concentration is above the substrate concentration that is used to cultivate methanogens in the laboratory. Both simulated acetate and hydrogen concentrations are at least an order of magnitude above those concentrations measured in the presence of active methanogens and also much higher than concentrations that might enable fermenting organisms to gain energy by the production of these end-products. Previous investigations have shown an accumulation of substrates (but not to such high concentrations) if the consumers, in this case the methanogens, are inactive. In case of methanogenic activity, much lower concentrations are present to enable an energy gain for all organisms involved in the anaerobic food chain. Also in this case, the findings should be discussed on the background of available observations.

We appreciate the reviewer's comment on the modeled acetate and hydrogen concentrations. We have examined our simulations and found that there was an error in our post processing script converting hourly acetate and hydrogen concentrations modeled at individual soil layers into daily means at the given soil column. We have corrected our post processing script and the corresponding figures (Fig. 4 and 5), and included discussions of available observations and uncertainty of modeled substrate dynamics (Lines 334-342). The acetate concentrations were not within expected ranges, as pointed out by the reviewer.

We have added the following in the revisions:

Lines 334-342

Although the CH_4 emission rates and CH_4 production pathways modeled in the Stordalen Mire fen have been examined (Chang et al., 2019), continuous substrate concentration measurements are lacking for validating the substrate-mediated hysteretic temperature responses proposed here. Wide ranges of acetate and hydrogen concentrations have been reported from incubation experiments studying methanogenesis (e.g., Hines et al., 2008; Tøsdal et al., 2015; Zhang et al., 2020); however, those values may not be used to validate the time and space specific substrate concentrations modeled at our study sites. Therefore, further studies and additional field measurements are needed to test our proposed hypothesis of the causes of observed CH_4 emission hysteresis.

Fig. 4

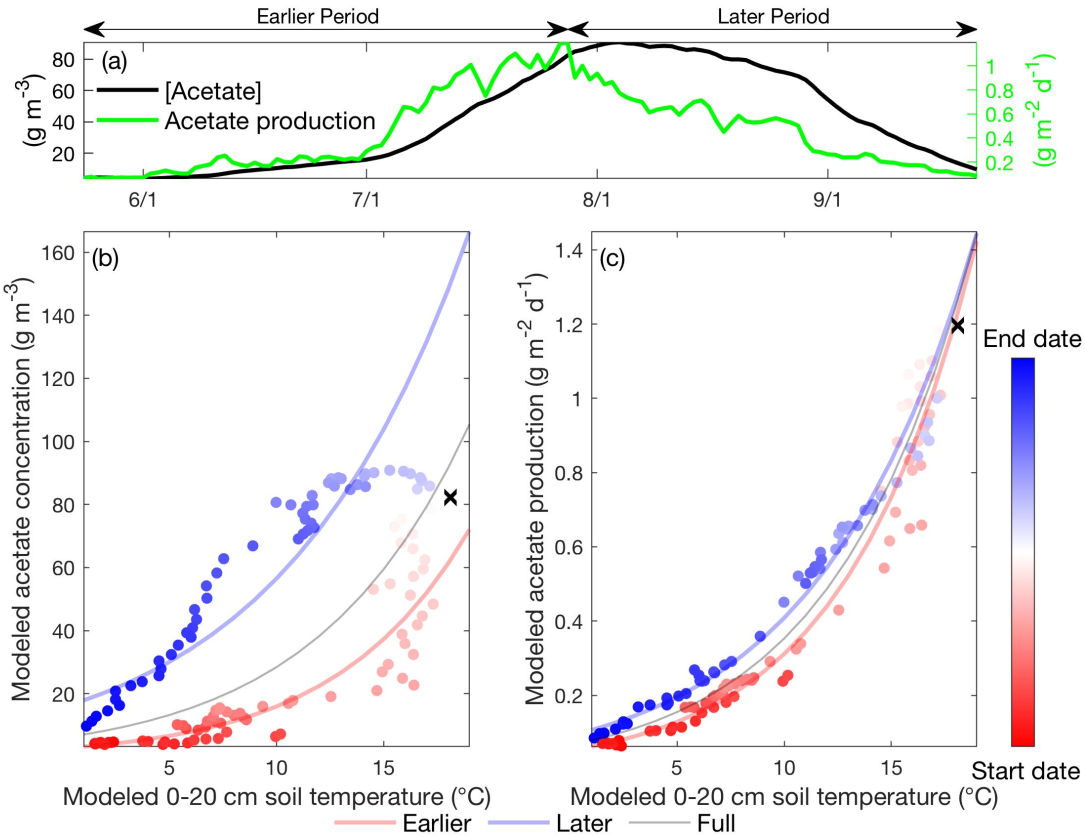


Figure 4. Daily acetate concentration and acetate production modeled in the Stordalen Mire fen during the 2011 thawed season (a). The corresponding apparent temperature dependence of the modeled acetate concentration (b) and acetate production (c) during the 2011 thawed season. Dots and lines represent the daily data points and the fitted apparent temperature dependence, respectively. The earlier, later, and full-season periods are colored in red, blue, and black, respectively. Earlier and later periods are defined as the time before and after the seasonal maximum 0-20 cm soil temperature denoted by black cross signs. Start date and end dates represent the beginning and ending of a thawed season defined as the period when modeled daily 0-20 cm soil temperature is above 1 ˚C, respectively.

Fig. 5

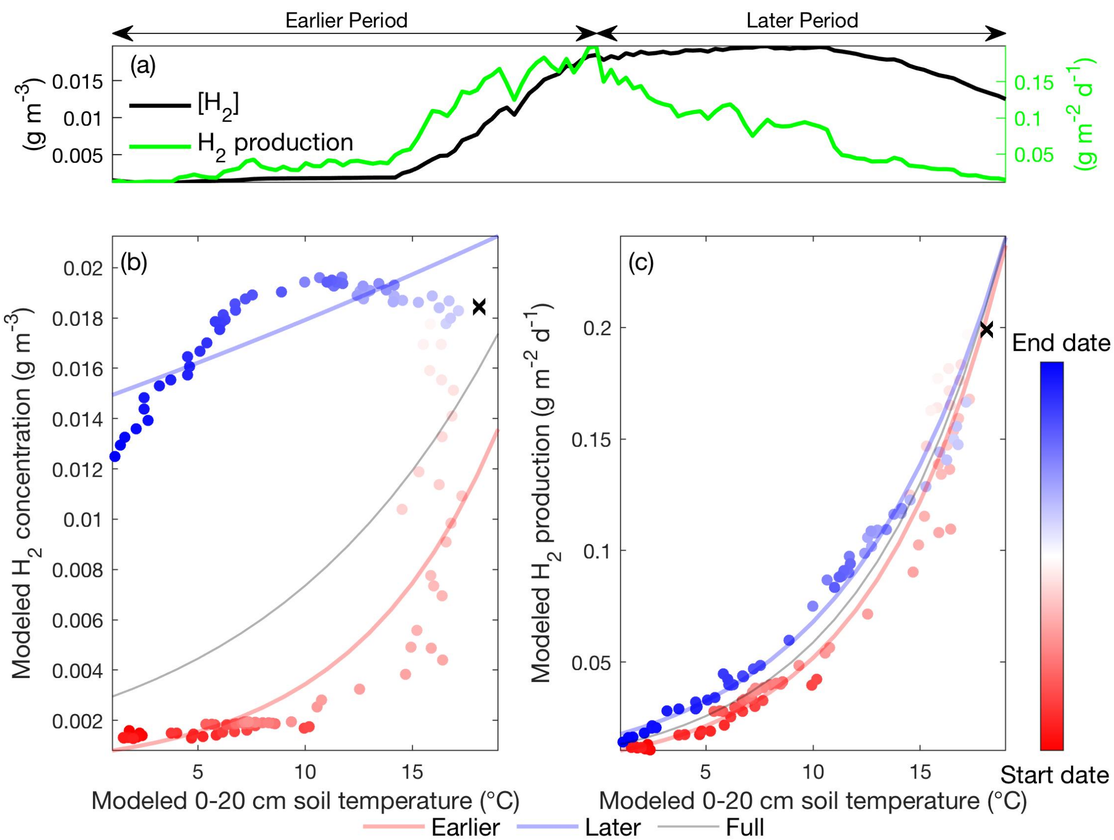


Figure 5. Daily hydrogen concentration and hydrogen production modeled in the Stordalen Mire fen during the 2011 thawed season (a). The corresponding apparent temperature dependence of the modeled hydrogen concentration (b) and hydrogen production (c) during the 2011 thawed season. Dots and lines represent the daily data points and the fitted apparent temperature dependence, respectively. The earlier, later, and full-season periods are colored in red, blue, and black, respectively. Earlier and later periods are defined as the time before and after the seasonal maximum 0-20 cm soil temperature denoted by black cross signs. Start date and end dates represent the beginning and ending of a thawed season defined as the period when modeled daily 0-20 cm soil temperature is above 1 ˚C, respectively.

Furthermore, it is not clearly described in the manuscript, which observations are part of the manuscript. After reading the abstract, I expected observational and mechanistic modelling data from two sites (Strodalen and Barows) but the manuscript indeed presents and discusses almost exclusively model generated data

on Stordalen. I suggest more clearly presenting, which kind of observational data are presented. As I understand, only Fig. 1 presents observational data to indicate that the 'hysteretic temperature sensitivity' is real and all the remaining data are generated by the model. I suggest either including more data and discussion on UtqiaÄavik, or omitting this site. In the current manuscript latter site is only represented in three panels in Fig. 2.

To sum this up: The manuscript lacks in large parts of the discussion a critical evaluation of the model output, which should be discussed on the background of the available observational data.

We appreciate the reviewer's comment that we need to better indicate which observations are used and how they were used, and to clarify where model simulations are used for analysis. We revised the manuscript according to these reviewer comments (e.g., Lines 30-36), and have included the term 'modeled' at dozens of locations in the manuscript to clarify when modeled results are being discussed. Also, the results collected at Utqiaġvik are now described as a case study to represent the robustness of the modeled CH_4 emission hysteresis, where similar hysteretic responses to temperature were found under very different model setup and microclimatic conditions. Although year-round chamber and eddy covariance measurements have indicated hysteretic apparent temperature dependence of CH_4 emissions from the beginning to the end of a thaw season, the underlying dynamic remains unclear. Here, we investigated the potential cause of such hysteresis and focused our discussion on modeled results from the Stordalen Mire because we previously validated the modeled CH_4 production pathway by the relative abundance of acetoclastic and hydrogenotrophic methanogens inferred from 16S rRNA gene amplicon sequencing data (Chang et al., 2019) (now mentioned on Lines 97-99). Our goal in the manuscript is to propose a CH_4 cycling mechanism that has the potential to (1) fill the knowledge gap of the observed CH_4 emission hysteresis, (2) help identify factors should be included in future measurements, and (3) shed light on future CH_4 model development.

To address the reviewer's comment regarding a critical evaluation of the model simulations, we acknowledge that additional measurements are needed to further evaluate the cause of the observed CH_4 emission hysteresis. However, we note that the substrate mediated CH_4 production hysteresis inferred from our model is consistent with the varying temperature responses to microbial thermal history reported in laboratory incubations (Updegraff et al., 1998). We have added text to the revised manuscript to clarify these points (Lines 334-342).

Specific comments:

L142 -144: The meaning of this sentence is unclear. Please clarify.

Unlike the peatland type specific CH_4 emissions measured at the Stordalen Mire, CH_4 emissions measured at the Utqiaġvik site come from different topographic features with distinct soil thermal and moisture conditions. To clarify this point, we describe this issue and modify the original Lines 142-144 sentence in the revised manuscript.

L 297: Hodgkins et al. (2014) gives no information on emissions, please revise

We have corrected the cited papers used to support this statement (Line 304).

This result is consistent with isotopic measurements which also indicated that changes in CH_4 production, not CH_4 oxidation, determine the CH_4 emissions observed in the Stordalen Mire sites (McCalley et al., 2014).

L305ff: The energy yield for methanogens indeed increases with rising substrate concentrations but the energy yield of fermenters decreases with rising end-product concentrations. Fermenters will most likely not be able to gain energy from fermentation at such high end-product concentrations. Please consider the whole anaerobic food web.

We greatly appreciate the reviewer's comment on the modeled acetate and hydrogen concentrations. As described above, we have corrected our post processing script and the modeled acetate and hydrogen concentrations.

L329f: In L107 a fluctuating water table between the surface and -35 cm is given. Please clarify.

The water table depth is fluctuating between the peat surface and -35 cm (negative values implying below the peat surface) in the Stordalen Mire bog site (in the original Line 107), and it remains around or above the peat surface in the Stordalen Mire fen site (in the original Lines 111-112 and Lines 329-330). Therefore, we do not expect seasonal variations in water table depth to be the dominant factor controlling CH_4 emission hysteresis observed in the Stordalen Mire fen site (Lines 353-356).

Reference:

Chang, K.-Y., Riley, W. J., Brodie, E. L., McCalley, C. K., Crill, P. M., & Grant, R. F. (2019). Methane Production Pathway Regulated Proximally by Substrate Availability and Distally by Temperature in a High-Latitude Mire Complex. *Journal of Geophysical Research: Biogeosciences*, 2019JG005355. https://doi.org/10.1029/2019JG005355

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McCalley, C. K., Woodcroft, B. J., Hodgkins, S. B., Wehr, R. A., Kim, E.-H., Mondav, R., et al. (2014). Methane dynamics regulated by microbial community response to permafrost thaw. *Nature*, *514*(7523), 478–481. https://doi.org/10.1038/nature13798

Perryman, C. R., McCalley, C. K., Malhotra, A., Fahnestock, M. F., Kashi, N. N., Bryce, J. G., et al. (2020). Thaw Transitions and Redox Conditions Drive Methane Oxidation in a Permafrost Peatland. *Journal of Geophysical Research: Biogeosciences*, *125*(3). https://doi.org/10.1029/2019JG005526

Singleton, C. M., McCalley, C. K., Woodcroft, B. J., Boyd, J. A., Evans, P. N., Hodgkins, S. B., et al. (2018). Methanotrophy across a natural permafrost thaw environment. *ISME Journal*, *12*(10), 2544–2558. https://doi.org/10.1038/s41396-018-0065-5

Tøsdal, A., Urich, T., Frenzel, P., & Marianne, M. (2015). Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming. *Proceedings of the National Academy of Sciences*, E2507–E2516. https://doi.org/10.1073/pnas.1420797112

Updegraff, K., Bridgham, S. D., Pastor, J., & Weishampel, P. (1998). Hysteresis in the temperature response of carbon dioxide and methane production in peat soils. *Biogeochemistry*, *43*(3), 253–272. https://doi.org/10.1023/A:1006097808262

Zhang, L., Liu, X., Duddleston, K., & Hines, M. E. (2020). The Effects of pH, Temperature, and Humic-Like Substances on Anaerobic Carbon Degradation and Methanogenesis in Ombrotrophic and Minerotrophic Alaskan Peatlands. *Aquatic Geochemistry*, (0123456789). https://doi.org/10.1007/s10498-020-09372-0