We would like to thank reviewers for re-reviewing our manuscript and providing detailed feedback. Below, we have addressed each comment individually, with the original comments in the back text and our responses in blue.

They have provided minimal to moderate updates from the initial submission, and do not adequately address the major points raised in the first round of review. As such, my review remains largely unchanged. The paper provides an irresponsible representation of the GDGT proxy literature, which is presented in a way to facilitate a climatic interpretation but not grounded in data. The interpretation additionally leans heavily on a lake model but the study provides no demonstration of how well that model performs for lakes similar to these small, high-arctic lakes, particularly for stratification dynamics. As such, it is difficult to conclude that the data fully support their interpretation of climate dynamics in southern and south western Greenland during the Holocene. The data they have prepared will be a meaningful contribution to the literature and an interesting comparison to other lake data points in the region, but like all proxy inferences is subject to the limitations of the proxy, and needs to be more thoughtfully/carefully evaluated before publication.

I will highlight the key points made in the first round of review and how the revisions failed to address them.

1) “At the very least, the model needs to be validated by observational data on temperature and mixing status from these or several similar Arctic lakes that have documented summer temperatures and mixing regimes”

The authors did not try to do this:

They make the case that the model captures warming accurately, in terms of LSWT sensitivity to air temperature anomalies, and is therefore robust. However, the literature they point to (i.e. Piccolroaz et al, 2020) records observations on lakes that are many orders of magnitude larger than the lakes modelled here (~50-82,000 km2 area, median ~270km2, vs <1 km2 surface area), containing data from just 19 polar lakes with a median (mean) area of 30km2 (500km2). Other datasets show lots of site-to-site variability with tons of heterogeneity in the trends dependent on lake size, elevation, morphometry, etc. (e.g., Dokulil et al, 2021; Zhang 2024 and references therein). But, even if this model is capturing LSWT correctly, this still doesn’t provide validation of the stratification dynamics expressed by the model.

Dokulil: https://doi.org/10.1007/s10584-021-03085-1

Zhang: https://doi.org/10.33265/polar.v43.9580

The authors need to validate this model against real data from a few lakes of comparable size in comparable regions. It is the most basic test of model validation to ask that the authors show that the model can accurately replicate real data in real lakes, similar in size and location to those here, before accepting the model output for these lakes (and the interpretations that rely on it). It is otherwise an assertion that this model is adequate for these lakes, and one major assumption used in the interpretation of their brGDGT data (i.e., that these lakes do not summer-stratify) hinges on this assertion. Yes, Carrera et al documents larger lakes than those in this study, but testing the model against these lakes is better than nothing. I agree with them that smaller lakes more similar to their sites would be even better.

Reply: We appreciate the feedback on this point and agree that model validation is important before relying on model outputs.

One key limitation is the scarcity of observational data for small, high-latitude lakes against which to calibrate the model. We have now calibrated and validated the lake model using observation data from Lake 578 in southwestern Greenland (Zhao et al., 2021). The lake has six-hourly temperature data for three years, from July 2016 to August 2019, collected from multiple depths, and is similar in geographic location and size to the lakes in our study, for which we have little to no observational data.

To calibrate the model and identify the best-performing parameter for the lake's thermodynamic balance (snow albedo, slush albedo, shortwave extinction coefficient, neutral drag coefficient, sediment albedo, sediment conductivity, and sediment specific heat), we follow established methods (Cluett et al., 2023; Longo et al., 2020; Morrill et al., 2019). An ensemble of 1,000 parameter sets was generated by simultaneously sampling each parameter from a uniform distribution using Latin Hypercube Sampling. Initial parameter distributions were wide but physically plausible ranges for natural lakes. The model was then run for each parameter set using ERA5 forcing data, and the resulting simulations were compared against observed lake temperature profiles for 2016 and 2017 (Figure 1). The 30 parameter sets that yielded the lowest root mean squared error (RMSE) were selected as the best-performing configurations.

We then conducted a validation step: we ran the lake model using the identified best-performing parameters and the ERA5 forcing data. The simulated lake temperatures were compared with modern lake-temperature observations from 2018 and 2019. While the model estimates a slight delay in spring warming relative to observations, it accurately reproduces observed ice-free season water temperatures at different depths (2.5, 5, 7.5, 10, and 14.5 m) (Figure 2). Further, the lake model successfully simulates the timing of ice-free season stratification in the lake from 2018 to 2019, albeit with an overestimated stratification duration (Figures 2 and 3). The model successfully simulates the fact that the lake is isothermal at the beginning and end of the ice-free season. These results demonstrate that the model effectively simulates the thermodynamic processes, including temperature and stratification in Lake 578, despite a minor cold bias in spring. These results provide confidence that the subsequent sensitivity experiments examining lake surface temperature and ice-free-season stratification duration responses to prescribed changes in air temperature are reliable, although it should be noted that the ice-free-season isothermal duration might be underestimated in the model. In other words, the model is biased towards conditions favoring ice-free season stratification and thus bottom water anoxic conditions, a key consideration for interpreting model results.

Next, we validated these parameters for Lake N3, the lake in our study for which we have an ice-free-season temperature profile. We used the 30 best parameter sets identified in the calibration for Lake 578, and ERA5 data to simulate Lake N3 temperature for 30 years, including 2010, when we have temperature profile observations (Fig. 4). There are minimal differences between parameter sets, and all agree well with observed temperature both at the surface and at depth in the lake.

Given the similar size, morphometry, and climate conditions of Lake 578, Lake N3, and our other study lakes, we used the average of the 30 best-performing parameter values identified for Lake 578 to simulate all our study lakes. We used the average parameter values, rather than 30 individual parameter sets, as conducting sensitivity tests on all lakes using all 30 sets would be prohibitively computationally intensive. Furthermore, the changes forced by large climatological shifts dwarf the inter-parameter differences. Thus, we have used the best available data, to our knowledge, to both calibrate and validate the model for lakes that are similar in size and geographic location to the study sites. We believe that the parameter values we use for sensitivity tests in our study lakes provide accurate estimates of their thermodynamic responses, including lake temperature and stratification, albeit with a slight bias towards more summer stratification than in observations.

Further, we would like to highlight that the lake model used in this study has been extensively calibrated and validated in prior studies for simulating lake thermodynamics and stratification across a broad range of lake sizes, including lakes comparable to those investigated here (Almeida et al., 2022; Hostetler, 1987, 2009; Hostetler and Bartlein, 1990; Longo et al., 2020; Perroud et al., 2009). One of these is Lake E5 on the north slope of Alaska, with a surface area of 0.019 km^2 and a maximum depth of 12 m, where the lake model was first calibrated using modern datasets (the mean lake temperature, maximum summer lake temperature, ice-break-up date, freeze-up date, and mixing depth) and then simulated for Middle Holocene conditions (Longo et al., 2020). These studies demonstrate that the model reliably captures thermodynamic and mixing regimes in lakes like those studied here, across a range of climatic conditions. We believe this evidence provides strong support for the lake model's reliability in simulating temperature and stratification under modern and Holocene conditions in our study lakes in Greenland.

Moreover, we would like to highlight that additional calibration and validation of the lake model using the datasets from Carrea et al. (2025), Dokulil et al. (2021), and Zhang et al. (2024) require more extensive work, beyond the scope of this study, especially considering that the studied lakes by Carrea et al., (2025) are an order of magnitude large than our study lakes and by Dokulil et al. (2021) and Zhang et al. (2024) have different climate regimes.

We primarily used the lake model to assess the sensitivity of lake surface water temperature and mixing duration in the studied lakes to changes in air temperature, to explain the reconstructed Holocene temperature trend, rather than to simulate absolute temperatures; thus, we assessed the sensitivity of lake surface water temperature to air temperature anomalies.

We will incorporate Figures 1-4 into the main text and/or supplemental material, and we revised the text in Section 2.4 of our manuscript as:

“We calibrated and validated the lake model using observation data from Lake 578 in southwestern Greenland (Zhao et al., 2021). The lake has six-hourly temperature data for three years, from July 2016 to August 2019, collected from multiple depths, and is similar in geographic location and size to the lakes in our study, for which we have little to no observational data. To calibrate the model and identify the best-performing parameter for the lake's thermodynamic balance (snow albedo, slush albedo, shortwave extinction coefficient, neutral drag coefficient, sediment albedo, sediment conductivity, and sediment specific heat), we follow established methods (Cluett et al., 2023; Longo et al., 2020; Morrill et al., 2019). An ensemble of 1,000 parameter sets was generated by simultaneously sampling each parameter from a uniform distribution using Latin Hypercube Sampling. Initial parameter distributions were wide but physically plausible ranges for natural lakes. The model was then run for each parameter set with ERA5 forcing data, and the resulting simulations were compared with observed lake temperature profiles from 2016 and 2017. The 30 parameter sets that yielded the lowest mean squared error (MSE) were selected as the best-performing configurations. We then conducted a validation step: we ran the lake model using the identified best-performing parameters and the ERA5 forcing data. The simulated lake temperatures were compared with modern lake-temperature observations from 2018 and 2019. While the model estimates a slight delay in spring warming relative to observations, it accurately reproduces observed ice-free season water temperatures at different depths (2.5, 5, 7.5, 10, and 14.5 m) (Figure 2). Furthermore, the lake model successfully simulates the timing of ice-free season stratification in the lake from 2018 to 2019, albeit with an overestimated stratification duration (Figures 2 and 3). The model successfully simulates the fact that the lake is isothermal at the beginning and end of the ice-free season. These results demonstrate that the model effectively simulates the thermodynamic processes, including temperature and stratification in Lake 578, despite a minor cold bias in spring. These results provide confidence that the subsequent sensitivity experiments examining lake surface temperature and ice-free-season stratification duration responses to prescribed changes in air temperature are reliable, although it should be noted that the ice-free-season isothermal duration might be underestimated in the model.

Next, we validated these parameters for Lake N3, the lake in our study for which we have an ice-free-season temperature profile. We used the 30 best parameter sets identified in the calibration for Lake 578, and ERA5 data to simulate Lake N3 temperature for 30 years, including 2010, when we have temperature profile observations (Fig. 4). There are minimal differences between parameter sets, and all agree well with observed temperature both at the surface and at depth in the lake.

Given the similar size, morphometry, and climate conditions of Lake 578, Lake N3, and our other study lakes, we used the average of the 30 best-performing parameter values identified for Lake 578 to simulate all our study lakes. We used the average parameter values, rather than 30 individual parameter sets, as conducting sensitivity tests on all lakes using all 30 sets would be prohibitively computationally intensive. Furthermore, the changes forced by large climatological shifts dwarf the inter-parameter differences. Thus, we have used the best available data, to our knowledge, to both calibrate and validate the model for lakes that are similar in size and geographic location to the study sites. We believe that the parameter values we use for sensitivity tests in our study lakes provide accurate estimates of their thermodynamic responses, including lake temperature and stratification. We acknowledge that using the same lake model parameters might lead to slight mismatches between the absolute and modelled temperature values;

however, for sensitivity tests at orbital scales, such changes can only have a minor impact (Fig. S17–S19)."

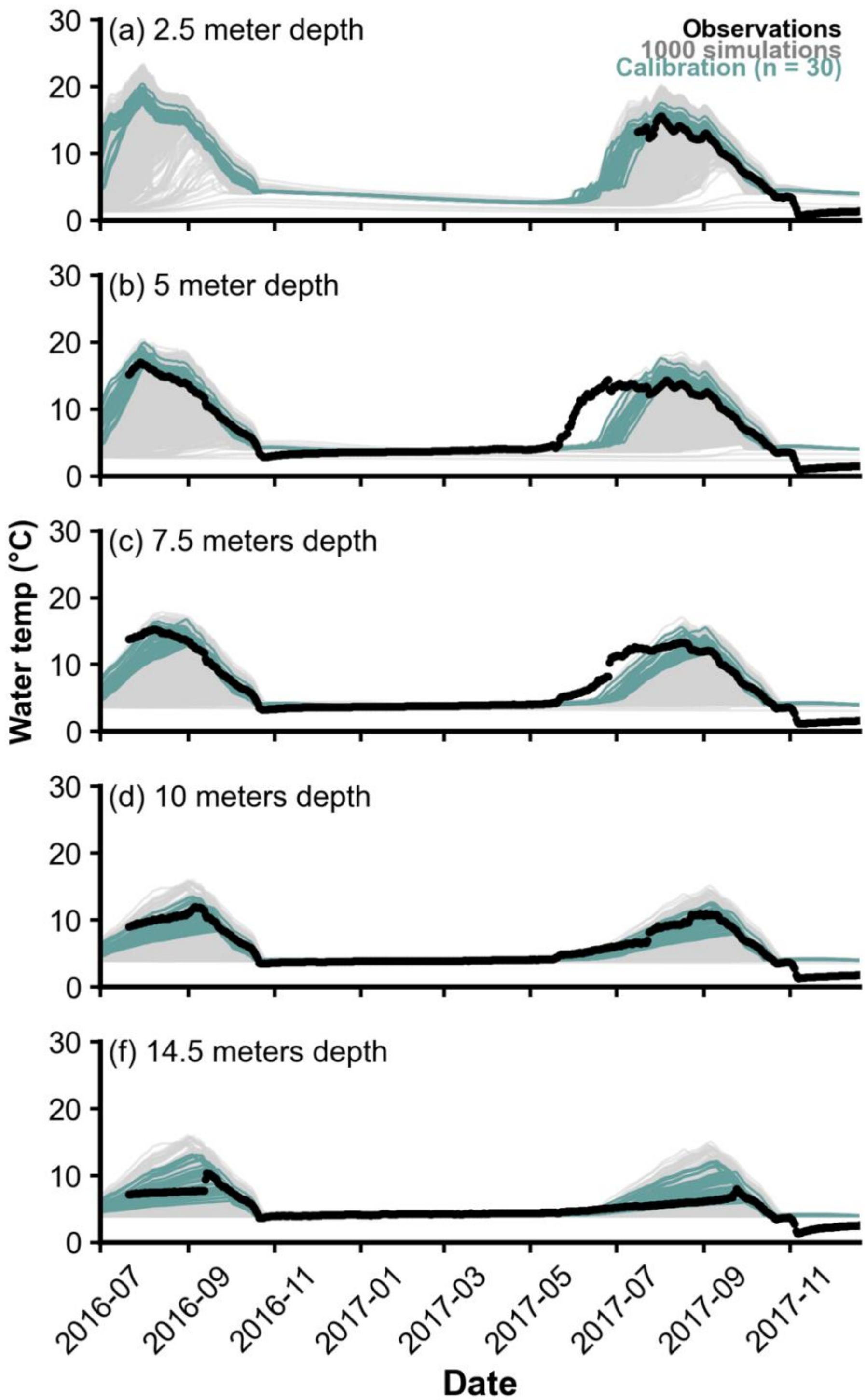

Figure 1: Lake model parameter calibration. Water temperatures at depths 2.5, 5, 7.5, 10, and 14 meters in Lake 578 with black dots indicating observations, the grey lines indicate simulations

under the full range of uncertain thermodynamic balance parameters across 1000 ensembles, and the green line indicates the simulations under the parameters that match observations closely.

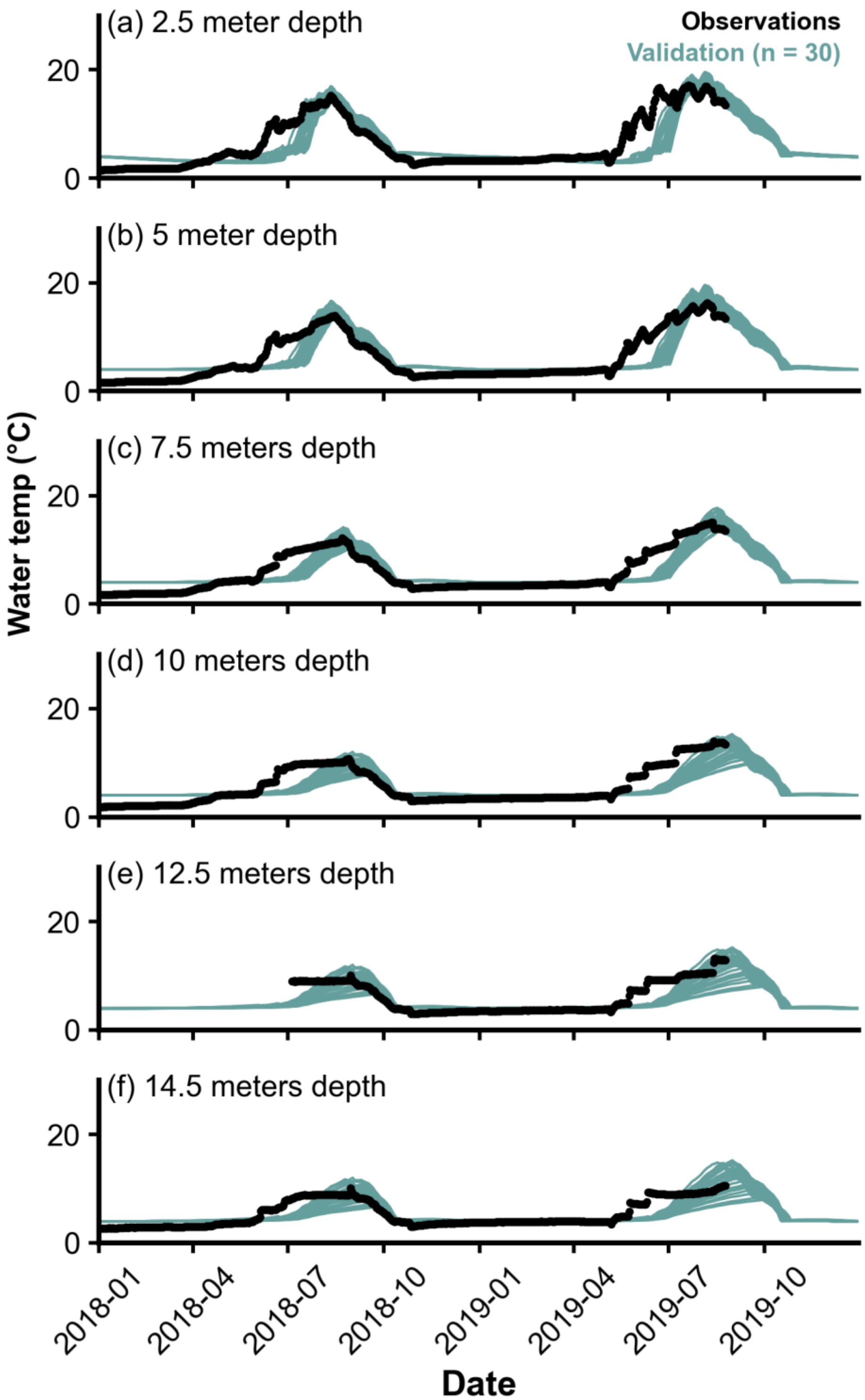

Figure 2. Lake model parameter validation. Comparison of water temperature at depths 2.5, 5, 7.5, 10, 12.5, and 14 m in Lake 578 between model outputs and observations, with black dots indicating observations and the green line indicating the simulations using ERA5 data and identified best-performing parameters (Zhao et al., 2021).

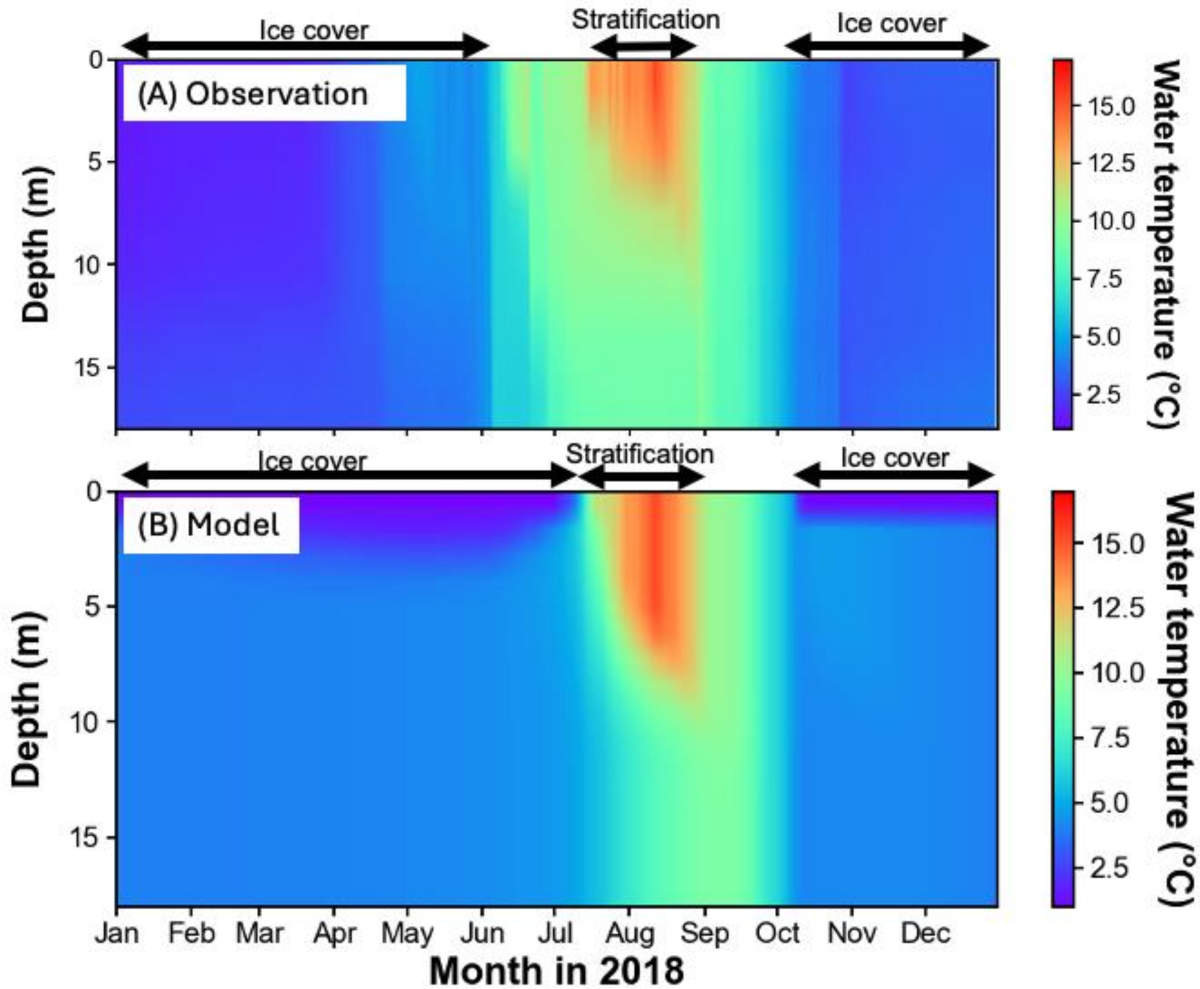

Figure 3: Comparison of thermal structure of Lake 578 based on (a) observations and (b) model simulations (Zhao et al., 2021) for 2018. The ice cover and stratification duration in the model simulations and observations are indicated by the arrows at the top of each plot. It should be noted that observation temperaure data for are only available at 2.5, 5, 7.5, 10, 12.5, and 14 m water depths, and are interpolated for other depths. The lake is isothermal during the periods between stratification and ice cover.

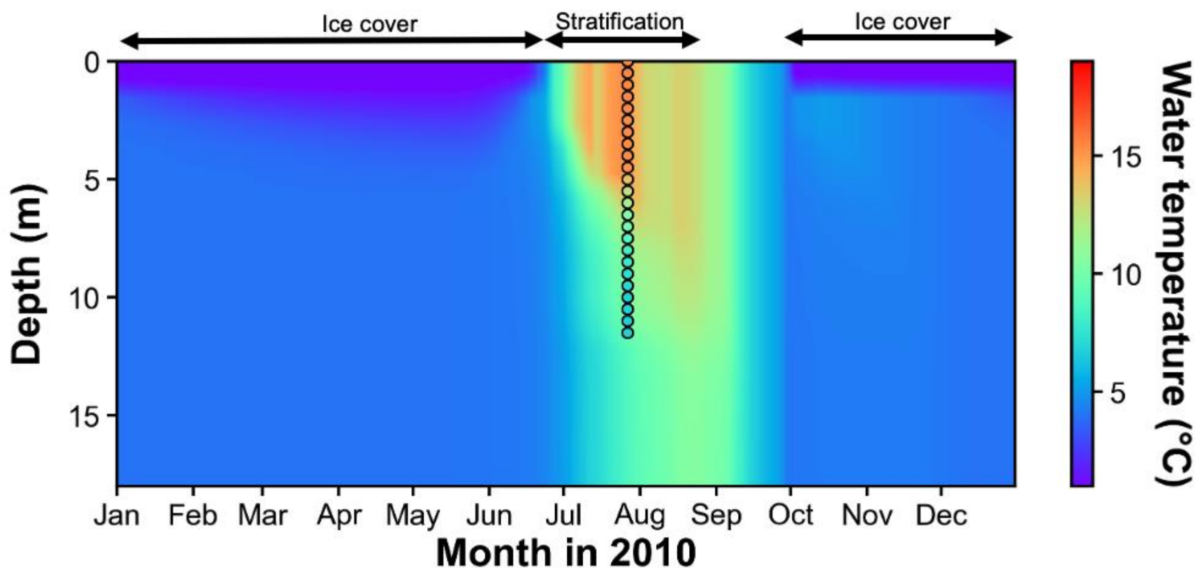

Figure 4: Comparison of thermal structure of Lake N3 based on model simulations and observations (indicated by circles) for 2010 (Corcoran et al., 2021). The ice cover and stratification duration in the model simulations are indicated by the arrows at the top of the plot. The lake is isothermal during the periods between stratification and ice cover.

2) "It is unclear what data exists to support that the production seasonality of archaeal vs. bacterial GDGT production would be so substantially disconnected"

This point was raised in the first round of review and the authors continue to present this in the manuscript as though there is an established and well-documented literature to support it. E.g., "Based on the evidence for seasonal production and the contrasting trends in these lake time series, we interpret the br-and isoGDGTs to be predominantly produced during different seasons: brGDGTs in ice-free season and isoGDGTs in ice covered season (Baxter et al., 2021; Blaga et al., 2011; van Bree et al., 2020; Loomis et al., 2014b; Shanahan et al., 2013)"

This point is important because it provides the rationale to rule out biogeochemical impacts on brGDGT distributions based on what is a hypothesis that one group is synthesized in the summer and the other the winter. In their rebuttal, the authors state, "The activity of these archaeal communities is higher during winter in ice-covered lakes" and point to Blaga et al (2011), Masse et al (2019) and Sinnghe-Damste (2022) as evidence for that activity to support winter-biased production of isoGDGTs. Blaga et al (2011) present on a very large, stratified (oligomictic) lake with no ice cover and show highest flux of isoGDGTs overall in late summer and fall (Figs 2, 5); Masse et al (2019) is a study on ammonia oxidation rates with no clear link to isoGDGT production, nevermind that it implicates ammonia-oxidizing bacteria more than archaea in the winter and that ammonia oxidation requires the presence of oxygen under ice; and Sinnghe-Damste et al (2022) present data also on a very large, permanently stratified lake but nevertheless show the highest flux rates of isoGDGTs in the spring-summer months (Fig. 8).

I restate that this is an interesting hypothesis that I hope is tested in future work but there is not independent data that shows isoGDGT production seasonality is clearly winter and brGDGT is clearly summer. There is evidence across the literature, however, to support substantial site-to-site variation in timing/depth/conditions of production dependent on the physical properties of the lake and surrounding watershed.

I note that their brGDGT-related tests for assessing competing gradients (e.g., HP5, %IIIa'') are interesting and provide some support for differential seasonality, albeit not strongly. There is some moderate correlation or anti-correlation in three out of six lakes (N3, Pluto, Rosaea) and the authors did not also include concentration data in these tests [see prior review, "is it equally or more plausible that higher seasonality and warmer summers in the early Holocene led to mixing regime changes at some of these sites (reflected currently in isoGDGT0 concentration and 0/cren ratios)…"]. Further, the HP5/%IIIa and IIIa'' trends themselves also suggest there is influence from oxygen in some of the lakes at different points in time (e.g., Bullet Lake). Correlation tests were conducted against MBT'5Me, which is not the temperature index used here. (I would also like to see how strongly their explanatory variables for the climate interpretation correlate to their reconstructions, e.g., SST reconstructions tested against brGDGT-temperatures.)

Reply: These are great points that we clarified in the updated manuscript as follows. Note that the figure references are those in the main text:

"Aquatically produced brGDGTs in the study lakes may be influenced by anoxic or suboxic conditions, potentially biasing temperature reconstructions (Acharya et al., 2023; Harning et al., 2025; Raberg et al., 2025). To assess this, we used the caldarchaeol-to-crenarchaeal ratio (Cald/Cren), a proxy for suboxic conditions in the water column and/or lake sediments where caldarchaeol is produced (Baxter et al., 2021; Blaga et al., 2009; Sinninghe Damsté et al., 2022). The Cald/Cren ratio ranged from 1 to 857 in all study lakes, with generally low values in lakes Marshall and Rosaea and high values in lakes Bullet, N3 and Pluto (Fig. 5). The ratio was higher from 10 to 7 ka in lakes Rosaea, Bullet, Marshall, and Pluto, suggesting reduced oxygen availability, while afterwards, it was lower, indicating higher oxygen availability. Similarly, the Cald/Cren ratio was higher in Lake N3 from 8 to 5 ka, suggesting lower oxygen availability before decreasing to low levels.

Correlations between Cald/Cren and reconstructed temperatures were weak or non-significant in Lakes N3 and Marshall throughout the Holocene (Table S4), indicating that suboxic conditions had little influence on reconstructed temperatures. In contrast, significant negative correlations were present in Lakes Rosaea, Bullet, and Pluto (Table S4), suggesting potential redox-related effects on reconstructed temperatures during periods of inferred suboxia (10–7 ka). Similar to our study sites, Lake 578 contains high cald/cren values during the Early Holocene (Schneider et al., 2024). This was interpreted to indicate anoxia during the ice-covered period and/or early in the ice-free season, and likely did not influence the brGDGT-inferred temperatures due to mixing during the ice-free season (Schneider et al., 2024). Moreover, agreement between the Lake 578 brGDGT-inferred temperature with temperature from other proxy time series suggests that anoxia is unlikely to be the main factor controlling brGDGT distributions in Lake 578.

Furthermore, the lake model sensitivity tests under Holocene climate conditions (±5 °C) indicate that stratification occurs early in the ice-free season in all study lakes but does not persist throughout the entire ice-free period (Fig. 3 and Fig. S18). As ice-free season duration increases, the duration of isothermal mixing increases and the duration of stratification remains constant. This is due to the relatively shallow depths and low dissolved-solids concentrations in these lakes, which make them prone to mixing by solar heating and wind. Such mixing during summer is observed today in Arctic lakes with similar size and chemical composition (Antoniades et al., 2024; Klanten et al., 2023, 2024; Lindborg et al., 2016). Due to the possible influence of oxygenation on brGDGT-distributions, we cannot completely rule out the effects of anoxia/suboxia and possible cold-bias on MAF-T in lakes Pluto, Bullet, Rosaea, Marshall, and N3 during the periods high Cald/Cren from 10 to 7 ka. Yet, based on the lake model sensitivity tests and evidence from Lake 578, we suggest that the impact of anoxia/suboxia on brGDGTs-inferred MAF-T is minimal.

We next examine brGDGT compounds that have been interpreted in some settings to indicate suboxic conditions. Although the ratio of 5-methyl hexa-methylated and penta-methylated brGDGTs with no cyclopentane rings (HP5) index and the fractional abundance of brGDGT IIIa (%IIIa) are suggested as indicators of anoxia in large and permanently stratified lakes (Weber et al., 2018; Yao et al., 2020), both 5-methyl hexa-methylated and penta-methylated brGDGTs exhibit a strong temperature dependence in Arctic lakes (Martínez-Sosa et al., 2021; Otiniano et al., 2023, 2024; Raberg et al., 2021) and can also be influenced by soil-derived input (Acharya et al., 2023; Martin et al., 2019). In Bullet Lake, Cald/Cren showed a non-significant correlation with both HP5 and %IIIa (Table S4). In Lake Rosaea, significant positive correlations were observed between Cald/Cren and both HP5 and %IIIa (Table S4). In Marshall and Pluto lakes, Cald/Cren was significantly positively correlated with HP5 but not with %IIIa. In Lake N3, no significant correlation was found with HP5, whereas a significant negative correlation was observed with %IIIa. As isoGDGTs and brGDGTs are produced by different microbial communities: brGDGTs by bacteria and isoGDGTs by archaea, these variable relationships may reflect seasonal and/or spatial differences in the niche of these microbial communities, which are subject to future study. Notably, in all our study lakes, both HP5 and %IIIa show a significant negative relationship with reconstructed temperature, aligning with previous studies that these proxies have a strong temperature dependence in Arctic settings.

Similarly, brGDGT IIIa″ has been suggested to be influenced by anoxia in a permanently stratified lake (Weber et al., 2015, 2018), but it shows no significant correlation with oxygen in Arctic lakes (Raberg et al., 2025). Moreover, brGDGT IIIa″ is present only in lakes Bullet and Rosaea, and the Holocene trends between brGDGT IIIa″ and cald/cren in these lakes are decoupled (Fig. S12 and S14). This evidence suggests that brGDGT IIIa″ is also not a reliable indicator of anoxia in these lakes."

Overall, it is my position that the authors still do not consider that lake-to-lake variability likely plays a key role in the expression of brGDGTs in these lakes. Ultimately, I don't think this would be as much of a limitation for the paper if the authors had presented their interpretation as one plausible hypothesis, emphasizing that it is a hypothesis, for explaining the data along with the strong possibility that the spatial variance could also be explained by lake specific conditions, AND that their favored hypothesis is built on major assumptions (e.g., differential seasonality of iso vs. brGDGTs, an unvalidated model that suggests lakes don't stratify in the summer even under strong warming despite literature that suggests they can stratify at much lower temperature thresholds, clear evidence for anoxia developing in different lakes at different times that shows up in both types of GDGTs, signals that largely are not outside of brGDGT-temp calibration error). My suggestion to the authors would be to take a step back and instead look at what is consistent across the sites (e.g., approximately 1-2 deg of anomalous MAF warmth in the early-middle Holocene), which is more likely to be explained by broad climate than lake-to-lake variability. Reply: Thank you very much for these suggestions. Please refer to the reply above for lake model validation and the potential influence of suboxic conditions on reconstructed temperatures. We have also phrased the interpretations to clarify that this is one possible interpretation of the brGDGTs in these lakes as:

"While, due to the influence of multiple environmental factors on brGDGTs, we cannot entirely rule out the effects of anoxia on brGDGTs and cold-bias on MAF-T in lakes Pluto, Bullet, Rosaea, Marshall, and N3 during the periods of high Cald/Cren from 10 to 7 ka. Due to the possible influence of oxygenation on brGDGT-distributions, we cannot completely rule out the effects of anoxia/suboxia and possible cold-bias on MAF-T in lakes Pluto, Bullet, Rosaea, Marshall, and N3 during the periods high Cald/Cren from 10 to 7 ka. Yet, based on the lake model sensitivity tests and evidence from Lake 578, we suggest that the impact of anoxia/suboxia on brGDGTs-inferred MAF-T is minimal."

I note that the authors did address the IR ratio adequately by including all data and noting where it is below their flagged ratio, and that they have changed text to appropriately update e.g., Greenland ice sheet GISP2 records as impacted by elevation. I appreciate that they added new tests to address some of the prior review (e.g., HP5, %IIIa), that they updated the calibration approach, and that they recognized the major discrepancy with air temps if brGDGT IFSLWT followed their model output. I suggest more support in the next iteration for the averaging of values output from different calibrations, what the implications are for different calibrations showing varying trends in the same lake, as well as how they delt with compounded error from averaging.

Reply: We appreciate the feedback on these points. Given that Otiniano et al. (2024) provide the most recent calibration for Arctic lakes, using the extensive datasets synthesized from Otiniano et al. (2023), Raberg et al. (2021), and Zhao et al. (2021), and have the lowest prediction error (RMSE = 1.0 °C), we applied this calibration to estimate MAF-T in our study.

We clarified this point in section 3.1 as follows:

"While several lacustrine brGDGT–temperature calibrations are available globally (Martínez-Sosa et al., 2021; Raberg et al., 2021; Zhao et al., 2023), regionally (Bauersachs et al., 2023; Dang et al., 2018; Otiniano et al., 2023, 2024; Russell et al., 2018), and site-specifically (Bittner et al., 2022; Feng et al., 2019; Zhao et al., 2021), only Raberg et al. (2021), Zhao et al. (2021), and Otiniano et al. (2023; 2024) are based on the Hopmans et al., (2016) HPLC method separating GDGTs isomers and explicitly consider Arctic seasonal climate. The Raberg et al. (2021) and Otiniano et al. (2023; 2024) calibrations estimate mean air temperature for the months above freezing (MAF-T) while Zhao et al. (2021) estimate epilimnion lake water temperature for the ice-free season (Ice-free season lake water temperature, IFS LWT). Given that Otiniano et al. (2024) provide the most recent calibration for Arctic lakes, using the extensive datasets synthesized from Otiniano et al. (2023), Raberg et al. (2021) and Zhao et al. (2021) and have the lowest prediction error (RMSE = 1.0 °C), we applied this calibration to estimate MAF-T in this study. This calibration yields a Holocene temperature range from 3.8 to 8.6 °C for study lakes, which is comparable to the modern MAF-T range."

Other comments: I suggest the authors double-check they've processed all their indices correctly. It seems odd that the HP5 and %IIIa would not predict major changes in brGDGT-inferred temp, but I observe that there are places in different lake records where one or the other (abundances or temp) change substantially, but not both (e.g., ~4500 years at N3).

Reply: We reverified the processing and calculation of all brGDGT indices, including HP5 and %IIIa, making corrections where needed.

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