Dear Reviewer #2 (Dr. Hagemann):

Reply: We thank the reviewer for their thorough evaluation and positive assessment of our work. Below we indicate with each comment how we intend to adjust the manuscript. Below, we list each of the comments provided by the reviewer in bold black text and our response to them in plain blue text.

This manuscript presents a new index and calibration for GDGTs based on surface sediments in the Southern Ocean. The authors used PCA biplots to determine the main factors that influence the GDGT distribution. Furthermore, they used the new index to correlate it with water temperatures at different depths and found the best correlation at 400m. In a last chapter, they compare their new calibration with previous calibrations on five sites in the Indian and Atlantic Ocean.

Overall, the article is good, especially the PCA analysis, and I appreciate the publication, even though I think the article needs a bit more work and reorganization.

1. **The introduction lacks some important information about GDGTs. For example, that the number of rings decreases with decreasing temperatures, which becomes important later in the article. I also think it would be useful to include the current debate about whether the signal is a depth signal or a surface signal, as the type of calibration (SST or subST) has an enormous influence on the results. It should be noted here that this discussion has not been adequately addressed in the SO so far. Since the authors discuss depth in detail in the later part, I would even include this in the scientific question here.**

   Reply: We appreciate this helpful feedback on the introduction. We agree that additional background information would strengthen the manuscript. We will expand the introduction to include:

   (1) a more detailed explanation of the number of rings/structures in GDGTs and their significance by adding the following sentences.

   "The degree of cyclisation of isoGDGT is strongly correlated with ocean temperature in global core-top datasets (Schouten et al., 2002), attributed to adaptive changes in archaeal membrane ring structures that maintain optimal fluidity under varying ambient temperatures (Fietz et al., 2020; Gabriel & Chong, 2000; Schouten et al., 2002). In low temperature environments, archaea reduce the number of cyclopentane rings in their membrane structures, to prevent membrane rigidity. Based on this relationship, Schouten et al. (2002) proposed the first isoGDGT index (TEX_{86})."

   (2) Adding a discussion of the current debate regarding the most appropriate water mass depth for calibration (SST vs. subST), especially in the SO. We will add the following paragraph into introduction.

   "There is an ongoing debate about the depth of production and export of sedimentary GDGTs. In the Antarctic Zone, GDGTs are thought to predominantly reflect subsurface rather than surface ocean temperatures (Fietz et al., 2016; Hagemann et al., 2023; Ho and Laepple, 2016; Jaeshke et al., 2017; Kim et al., 2012; Lamping et al., 2021; Park et al., 2019). This is supported by observations of elevated archaeal abundances in Circumpolar Deep Water (Alonso-Sáez et al., 2011; Church et al., 2003; Kalanetra et al., 2009; Sow et al., 2022; Spencer-Jones, 2021). These genomic studies have also revealed differences in archaeal communities and archaeal diversity across water masses, reflecting the influence of oceanographic features (Kolody et al., 2025; Raes et al., 2018). Additionally, the seasonality of isoGDGT production (Chandler

& Langebroek, 2021; Church et al., 2003; Park et al., 2019) and polar-related biases such as seasonal change in sea ice (Xu et al., 2020) have been thought to contribute to GDGT variability.

2. **I also believe that the authors should compare the indices (Figure S2) with subsurface temperatures. It is not surprising that satellite data, which only reflect the top few centimeters of the ocean (and thus the cold freshwater cover), show little correlation with the GDGT indices in the SO, which occur throughout the water column. If possible, I would add a second graph here showing that the already existing indices do also not correlate with subsurface data. In this context, you could refer to the data from Kim et al. (2012), which present a TEX86L subsurface calibration based on WOA09 data. Kim et al. (2012) https://doi.org/10.1029/2012GL051157**
   Reply: We thank the reviewer for this comment. We will analyse the relationship between traditional indices and subsurface temperatures and incorporate this into Figure S2.

3. **In the last chapter - when comparing all cores - the SSTs derived from satellite data are compared with the subSTs based on the new calibration. Based on the results that GDGTs reflect a subsurface signal there, the comparison is biased from the start. I recommend two articles (Kim et al., 2012 and Hagemann et al., 2023; https://doi.org/10.5194/cp-19-1825-2023), which also present a TEX86L-based subsurface calibration for the SO for a TEX86L based comparison instead.**
   Reply: We thank the reviewer for this suggestion. We agree that comparing SST-calibrated indices with subsurface temperatures may not provide a fair assessment. We recalculated TEX_{86}^{L} temperatures using the Kim et al. (2012) subsurface calibration (0-200 m) for MD11-3357 (north of SAF), and the Hagemann et al., 2023 calibration for the four sites south of SAF. The recalculated temperature values are compared with WOA18 temperatures at subsurface depths (e.g., 0-200 m). As a result, these recalculated values, especially using Hagemann et al. (2023) calibration, improved the reconstructed temperature range. However, our conclusions regarding the AIZ index and its calibration remain unchanged, as it shows the best performance in terms of temperature range and representation of glacial-interglacial cycles. This provides a more equitable comparison between traditional indices and our new index, with each now applied at their intended depth range.

Minor Comments:

Line 34: Add Reference Brochier-Armanet et al., 2008, who named the phylum of the *Thaumarchaeota* https://www.nature.com/articles/nrmicro1852v
Reply: We will add the reference to the revised manuscript.

Line 38: Write isomere instead of regioisomere.
Reply: We will replace te term “regioisomer” with “stereoisomer.”

Line 81: Change last part of the sentence and add unit e.g., "In this zone, SSTs drop below 4°C with a salinity around ~34.0 PSU."
Reply: We will change this accordingly.

Line 83: Change: "with a salinity of" to "reaching near-freezing temperatures at a continuous salinity of 34.0 PSU"
Reply: We will change this accordingly.

Line 88: I would split the sentence, "but" is irritating → "...extends from ~1400 m to >3500 m depth. South of the PF, CDW upwells ..." OR "...extends from ~1400 m to >3500 m depth, with upwelling of the CDW south of the PF ..."
Reply: We will change this according to the first suggestion.

Line 90: Just a general comment: the upwelling event might be driven mainly by the Westerlies (haven't checked out the Carter paper yet), but the general driver of the entire ACC are the Westerlies in combination with buoyancy forcing (Rintoul, 2018 https://doi.org/10.1038/s41586-018-0182-3), maybe you want to mention it.
Reply: We agree and will add a description of ACC circulation drivers (westerly winds and buoyancy forcing) to the oceanographic settings section, following Rintoul (2018).

Line 96: The "are also indicated" is unnecessary. I would simply delete them. Anyway, if it is possible, I would add the long version like "Subtropical Front" to the legend, behind the shortcut STF and delete them fully of the description. And then I would write instead the Figure capture as: "Bathymetric map with sediment core location and oceanic fronts analyzed in this study."
Reply: We will change both the text and the figure (legend) accordingly.

Line 374: Why did you compared it with Kim 2010, which was a surface calibration and not with the subsurface calibration after you figured out that it is probably a subsurface signal?
Reply: As mentioned above, we recalculated TEX_{86}^{L} temperatures following Hagemann et al. (2023) recommendations: applying the Kim et al. (2012) subsurface calibration (0-200 m) for MD11-3357 north of the SAF, and the Hagemann et al. (2023) calibration for four sites south of the SAF.

Line 388: OPTiMAL derived SSTs of MD11-3351 look to me very noisy, especially during the glacial period, with temperatures compatible to LIG. This is not a typically G-IG pattern. I would add the wording "almost every site except MD11-3357, which shows temperatures during the glacial comparable to the Interglacials."
Reply: We will change this accordingly.

<u>**Table 4**</u>

- **Generally, if you do a sediment surface comparison, it is better to use WOA05 or WOA09 since global warming has less impact on the surface temperatures there.**
  Reply: We appreciate this comment and understand the concern. The reason we chose WOA18 is that it has higher resolution grid (0.25 x 0.25 degrees, compared to WOA09 (1 x1 degrees)). We have also checked the differences between WOA18 and WOA09 (using a 1.0x1.0 degrees grid for both), and found that the temperature differences at the study sites range from - 0.3 ~ +0.4 °C, confirming that these differences do not change our conclusion. Thus, we will continue to use WOA18 (0.25 x 0.25 degrees).

- **The placeholder characters are irritating.**
  Reply: We will delete the placeholder characters.

- **Satellite-derived temperatures at 400m? Is that possible? As far as I know it is only the surface. <u>https://podaac.jpl.nasa.gov/SeaSurfaceTemperature</u>**
  Reply: We appreciate this clarification. The temperature data used in this study are derived from in situ observations compiled in the World Ocean Atlas (WOA18), not satellite-derived sources. We will revise the terminology to 'WOA-derived temperatures' to ensure clarity.

Additional references (not found in our original submission) referred to by the reviewer and in our response – these will be incorporated into our final revised submission:

Alonso-Sáez, L., Andersson, A., Heinrich, F., and Bertilsson, S.: High archaeal diversity in Antarctic circumpolar deep waters: Biogeography of Archaea in Antarctic waters, Env. Microbiol. Rep., 3, 689–697, https://doi.org/10.1111/j.1758-2229.2011.00282.x, 2011.

Brochier-Armanet, C., Boussau, B., Gribaldo, S., and Forterre, P.: Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota, Nat. Rev. Microbiol., 6, 245–252, https://doi.org/10.1038/nrmicro1852, 2008.

Hagemann, J. R., Lembke-Jene, L., Lamy, F., Vorrath, M.-E., Kaiser, J., Müller, J., Arz, H. W., Hefter, J., Jaeschke, A., Ruggieri, N., and Tiedemann, R.: Upper-ocean temperature characteristics in the subantarctic southeastern Pacific based on biomarker reconstructions, Clim. Past, 19, 1825–1845, https://doi.org/10.5194/cp-19-1825-2023, 2023.

Kolody, B. C., Sachdeva, R., Zheng, H., Füssy, Z., Tsang, E., Sonnerup, R. E., Purkey, S. G., Allen, E. E., Banfield, J. F., and Allen, A. E.: Overturning circulation structures the microbial functional seascape of the South Pacific, Science, 2025.

Raes, E. J., Bodrossy, L., Van De Kamp, J., Bissett, A., Ostrowski, M., Brown, M. V., Sow, S. L. S., Sloyan, B., and Waite, A. M.: Oceanographic boundaries constrain microbial diversity gradients in the South Pacific Ocean, Proc. Natl. Acad. Sci. U.S.A., 115, https://doi.org/10.1073/pnas.1719335115, 2018.

Rintoul, S. R.: The global influence of localized dynamics in the Southern Ocean, Nature, 558, 209–218, https://doi.org/10.1038/s41586-018-0182-3, 2018.

Xu, Y., Wu, W., Xiao, W., Ge, H., Wei, Y., Yin, X., Yao, H., Lipp, J. S., Pan, B., and Hinrichs, K.: Intact Ether Lipids in Trench Sediments Related to Archaeal Community and Environmental Conditions in the Deepest Ocean, JGR Biogeosciences, 125, e2019JG005431, https://doi.org/10.1029/2019JG005431, 2020.