

Dear Prof. Silvio Pantoja,

Thank you very much for editorial efforts on our manuscript. My coauthors and I reviewed your comments carefully and found that they are very valuable to improve our manuscript. So, we made changes according to your suggestions. Also, for your convenience, I highlighted all those changes in the revised manuscript. Please see our detailed response as follows.

I am looking forward to hearing your decision or any comments.

Regards-Yunping

1) **Reviewer 2.** "The uniqueness of the study site is both a strength and a weakness of the presented work. "

**Editor:** The reviewer is asking to discuss this caveat in the context of your conclusions, not merely state that we "...we have to be cautious...". Please elaborate further on the new version.

**Response:** This is a good comment. In the revised manuscript, we added the content in the discussion and conclusion. From line 344-356 (sec. 4.4), we added a paragraph as "**The unique composition of brGDGTs in the Mariana Trench has significant implications on the brGDGTs-based proxies. As a remote setting from the landmass, the Mariana Trench provides an opportunity to distinguish marine in situ production from a terrestrial origin of brGDGTs that usually muddles the interpretation of shelf sediments. However, it is unclear what the similarity and difference are for brGDGTs-producing microbes and their response to environmental factors between the Mariana Trench and continental shelf. In addition, the weight contribution to the brGDGT pool from sediments and water column remains elusive. Since factors such as nutrients, particle loading, bacterial community, and oceanographic parameters (e.g., oxygenation, salinity, currents) vary significantly between the shelf and trench as well as among different hadal trenches, the brGDGT-producing microbes are likely different. Therefore, the investigation of brGDGTs in multiple hadal trenches and shallow marine regions are needed to decipher their source and environmental control, that are beneficial for accurate application of the brGDGTs-based proxies such as MBT, CBT and BIT.**"

We also added two sentences in the conclusion as "**4) The uniqueness of the Mariana Trench that is remote from any landmass allows distinguishing marine in situ production from a terrestrial origin of brGDGTs. However, it is unclear how comparable this unique site is to shallow marine settings and other hadal trenches. Therefore, the comparison studies of brGDGTs for different hadal trenches as well as between hadal and non-hadal sites are recommended.**" (line 377-381)

In the abstract, we added a sentence "**Given the uniqueness of the Marina Trench, we recommend more studies for different trenches and shallow regions in order to holistically understand the biosynthesis and environmental control of brGDGTs.**" (line 24-26)

2) **Reviewer 2.** Line 28-30: Please consider finding more appropriate citations. Sinninghe Damste 2000 and Weijers et al. are neither the first to report on iGDGTs/brGDGTs, nor are these the most comprehensive papers.

**Authors:** We have replaced previous citations with Schouten et al., 2013, which are the most comprehensive and cited papers about GDGTs.

**Editor:** Please revise. A 2013 paper is not older than a 2000 paper. Which one is the first to report on iGDGTs/brGDGTs?

**Response:** We searched the references and added two suitable references here. De Rosa and Gambacorta (1988) is a classic paper about lipids of archaeobacteria and introduced isoprenoid tetraethers (this paper has been cited by more than 700 times). Sinninghe Damsté et al. (2000) is the first report for non-isoprenoid dialkyl diglycerol tetraether lipids (BrGDGT) in sediments. The detailed information about these two references are:

- 1) De Rosa, M., Gambacorta, A., 1988. The lipids of archaeobacteria. *Prog. Lipid Res.* 27, 153-175. [https://doi.org/10.1016/0163-7827\(88\)90011-2](https://doi.org/10.1016/0163-7827(88)90011-2).
- 2) Sinninghe Damsté, J.S., Hopmans, E.C., Pancost, R.D., Schouten, S., Geenevasen, J.A.J., 2000. Newly discovered non-isoprenoid dialkyl diglycerol tetraether lipids in sediments. *Chem. Commun.* 23, 1683–1684, <https://doi.org/10.1039/B004517I>.

- 3) **Reviewer 2,** "5) Line 120-122: Without response factors of GDGTs relative to the C46 standard, concentrations cannot be determined. Please report concentrations as response units or peak areas normalized to OC.

**Authors:** "This is a good comment. In the revised manuscript, we added the sentences as Since all brGDGT isomers were assumed to have an identical response factors on the instrument, our analytical method is better regarded as semi-quantification." (Line 289-290). "However, it is common to report the concentration of GDGTs in ... without consideration of response factor in literatures. So, we still keep the current format for the concentration. "

**Editor:** "it is common to report the concentration of GDGTs ... without consideration of response factor"). This is not correct. Is there much variability in response factors for brGDGT isomers?

**Response:** This is a good comment. As the reviewer and the editor pointed out, we did not consider the response factors. In our study, the absolute concentration is actually not important, since we only discussed the composition of brGDGTs and derived proxies (such as BIT, CBT and MBT), all of which are based on relative abundance of brGDGTs. The reason we presented the absolute concentration is for the followers who may want to compare their GDGT concentrations with ours. In the revised manuscript, we accepted the reviewer's and editor's suggestion and removed the contents related to absolute concentrations. Since our original quantification is by an internal standard (C46 GDGT) method, and thus we did not measure the exact total volume of each sample loaded on HPLC which is not needed for the internal standard method. Given these facts, we rewrote the first paragraph of Sec. 3.2. We only reported the relative abundance of GDGTs based on peak area of each compound.

In sum, we changed the contents of Sec. "2.2 Lipid extraction and GDGT analyses" as well as "3.2 Concentration and composition of GDGTs". From line 111 to 114, we wrote the sentence as "**Since the response factors of GDGTs were not determined due to the lack of authentic standard, we did not calculate the absolute concentration of GDGTs. Instead, we reported the relative concentration based on peak areas (pa) of respective GDGTs normalized to total GDGTs.**" From line 173-179, we wrote as "**The fractional abundance of iGDGTs and brGDGTs were summarized in Table 2. iGDGTs were the dominant components, accounting for 96.8% to 98.6% of total GDGTs in Mariana Trench sediments. The proportion of brGDGTs was substantially lower than that of iGDGTs, ranging from 1.4%**

to 3.2%. For all sediment samples, the BIT index remained at a low level ( $0.03 \pm 0.01$ ).”.

- 4) Reviewer 1. "3. The application of soil pH index CBT' and mean annual air temperature index MAT<sub>mr</sub> in this marine setting is unconvincing. "

Authors: " As mentioned above, we accepted the reviewer's suggestion and removed all relevant figures and discussion."

Editor: Being that the case, the associated conclusion should be removed from the abstract as well (line 15).

**Response:** we accepted this suggestion and already deleted “This phenomenon may reflect an adaptation of brGDGTs-producing bacteria to weak alkaline and low temperature conditions” in the abstract.

Minor comment:

1. Line 30. Change IGDTs with iGDGTs

**Response:** we changed “IGDTs” into “iGDGTs” in the revised manuscript.

2. Line 15. Replace "Rota Evaporator" with "rotary evaporator "

**Response:** we changed “Roto Evaporator” into “rotary evaporator” in the revised manuscript.

3. Line 175.? Why "stated elsewhere"?

**Response:** In order to avoid confusing, we deleted “stated elsewhere”. In the revised manuscript, we wrote as “The significance level was set at  $p < 0.05$ .”

4. Line 190. Consider using gdw instead of "dry weight sediment (dws) " since it is more commonly used

**Response:** This is a good point and we accept it.

5. Figure 4. Remove comma in Luo et al., (2017)

**Response:** we already deleted comma here in the revised manuscript.