Response to reviewers

"Sedimentary organic matter signature hints at the phytoplankton-driven Biological Carbon Pump in the Central Arabian Sea"

Response: We gratefully acknowledge the time and effort the reviewers took to evaluate this manuscript, and specifically, the supportive comments and positive criticism from two reviewers. We replied to all points raised by the reviewers and took most of their suggestions, or rebutted.

Reviewer 2 (Katrin Schmidt)

1. The manuscript 'Sedimentary organic matter signature hints at the phytoplankton-driven biological carbon pump in the Central Arabian Sea' by Pandey et al. looks at the composition of top sediment cores along a productivity gradient in the central Arabian Sea with the aim to evaluate the relative contribution of diatoms, flagellates, coccolithophores and zooplankton to carbon sequestration. This is an interesting study and Figure 5 nicely illustrates how the regional differences in the physical and biogeochemical environment lead to a different community structure of primary producers, different grazer abundances and different export. The manuscript builds on a previous study by the same lead author ('Interlinking diatom frustule diversity from the abyss of the central Arabian Sea to surface processes: physical forcing and oxygen minimum zone, Environmental Monitoring and Assessment, 195(1), 161, 823 https://doi.org/10.1007/s10661-022-10749-7, 2023), but introduces lipid biomarker results as a novelty. The authors' main conclusion is that dinoflagellates rather than diatoms or coccolithophores contribute most to the sedimentary flux. This conclusion derives from higher amounts of dinosterol (used for dinoflagellates) compared to brassicasterol (used for diatoms) and alkenone (used for coccolithophores) per unit organic matter in the sediment.

Response: We highly appreciate Katrin Schmidt for providing valuable suggestions and comments. We agree with most of the comments and our specific responses are provided below. We have tried to revise the manuscript keeping the comments in mind and hopefully, the modified version will be satisfying for the reviewers.

2. However, brassicasterol is not a reliable marker for diatoms. Rampen et al. (2010, 'A comprehensive study of sterols in marine diatoms…') analysed the sterol composition of > 100 marine diatoms species and in regard to brassicasterol they wrote: 'As this sterol is only the fifth most common sterol and absent in all radial centric diatoms and some important groups of bi(multi)polar centrics, our data support the statement by Barrett et al. (1995) that 24- methylcholesta-5,22E-dien-3b-ol should not be considered a general biomarker for diatoms. Furthermore, this sterol has also been found in many other groups of algae like Haptophyceae, Cryptophyceae, Chrysophyceae, Bangio[1]phyceae, and in a number of dinoflagellates (Volkman 2003)'. In line with Rampen (2010), I found more brassicasterol in Emiliania huxleyi than in 30 polar diatom species. The occurrence of brassicasterol in coccolithophores has also been described by others (e.g. Ding et al. 2019, 'Lipid biomarker production …'). Looking at the 8 diatom species that the authors mentioned in Fig. 4 (Coscinodiscus,

Thalassiosira etc..), none of them has been found producing brassicasterol in Rampen et al. (2010).

Response: We understand the concern raised by the reviewer about using Brassicasterol as a reliable biomarker for diatoms, however, we have three points to make.

We agree that sterols in general, could be derived from multiple sources (also including other microalgae). The same is true for Brassicasterol, which is known as a reliable and dominant sterol of most Pennate diatoms, as well as in varying percentages in some centric diatoms like *Probocia*. The same is valid for pigments like Fucoxanthin and the degradation products Lolliolides, which are popularly used as marker pigments for diatoms but can also be produced by coccolithophores. However, the ratio between Fucoxanthin and Chlorophyll-a can be used to assign the source of the algal group. Likewise, brassicasterols can be produced by other phytoplankton sources like dinoflagellates and coccolithophores, too, but the major is still diatoms. Since brassicasterol is the most prominent C28 sterol in the samples, it can be assumed that diatoms are the most likely producers. Because diatoms the one of the dominant phytoplankton in this region. The 24-methylcholesta-5,24(28)-dien-3β-ol, which is most abundant in cultured *Thalassiosira* and *Coscinodiscus* (Rampen et al., 2010), was not detected in our samples.

The paper by Jaramillo-Madrid, et al. (2019) (https://doi.org/10.1016/j.phytochem.2019.03.018) enlisted the diatoms producing brassicatserol and shows that diatoms like *Fragillaria* sp., *Amphiprora* sp., *Thalassionema* sp, *Thalassiosira* sp. produce brassicasterol. These frustules were also detected in our samples. On the other hand, there could be also some diatoms that were not detected in our sediment samples, but they were reported from the water column as their frustules might have been lost by dissolution. The most resilient and thickly silicified frustules (e.g. *Coscinodiscus* and *Thalassiosira*, etc) are preserved in the sediment, whereas, the thinly silicified frustules can be degraded on their way through the water column, yet their cellular organic matter could reach the sediment along with marine snow. Even during grazing when mesozooplankton bite diatom cells, the frustules may break and the organic matter can be released. The broken frustules can be dissolved faster, yet the cellular debris can reach the sediment if not degraded. So, in a nutshell, the community recorded from frustules may not necessarily be the producers of the brassicasterol stored in the sediment.

We would like to also stress on the point that in our study diatom frustule and brassicasterol (24-methyl cholest-5,22-dien-3β-ol), both used to show diatom abundance as independent proxies and eventually they revealed good correlation. However, we possibly were not clear enough to point out that the community seen from the frustules are not necessarily the producers of brassicasterol in the same samples. For exactly this reason, we have not assigned the observed sterols to any particular phylogenetic group of diatoms.

The last point we would like to emphasize that the cellular production of biomolecules could significantly differ with changing culture conditions, particularly temperature, light, and other nutrients and the reviewer also mentioned that in a comment. As rightly pointed out by the reviewer in a few studies brassicasterol was not noticed in the major taxa reported here (*Thalassiosira* and *Coscinodiscus*), as mentioned in Rampen et al. 2010. Unfortunately, the authors did not mention anything about the culture conditions and hence it is difficult to compare their culture results with those from other studies. To demonstrate this, we added a

Table where evidence for brassicasterol production was observed in *Thalassiosira* as published by Véron et al. (1998), particularly as steryl glycosides and acyl steryl glycosides. So, a change in the sterol inventory of individual diatom species is not unlikely and might depend on varying growth conditions, environmental conditions, etc., and may change sterol production in marine diatoms. We also provided the table from Jaramillo-Madrid, et al. 2019 to support the same.

TABLE 4. *Distribution pattern of different classes of sterols in* Thalassiosira pseudonana *(% of total sterols in each sterol class). SE: steryl esters (64.5%), FS: free sterols (32%), SG: steryl glycosides (2.5), ASG: acyl steryl glycosides (1%).*

Sterol	SE	FS	SG	ASG
Cholesta-5,22-dien-3β-ol	—[a]	2	—	—
Cholest-5-en-3β-ol	79	34.5	—	20
5α-Cholestan-3β-ol	—	1.5	—	—
24-Methylcholesta-5,22-dien-3β-ol	—	7.5	24	50.5
24-Methylcholesta-5,24(24[1])-dien-3β-ol	16	31	46	22.5
24-Methylcholest-5-en-3β-ol	—	6	—	—
24-Ethylcholesta-5,22-dien-3β-ol	5	—	18.5	38
24-Ethylcholest-5-en-3β-ol	—	8.5	12	—
24-Ethylcholesta-5,24(24[1])-dien-3β-ol	—	9	—	—
Total	100	100	100	100

[a] Not detected.

See details in Véron, B., Dauguet, J. C., & Billard, C. (1998). Sterolic biomarkers in marine phytoplankton. II. Free and conjugated sterols of seven species used in mariculture. Journal of phycology, 34(2), 273-279. https://doi.org/10.1046/j.1529-8817.1998.340273.x

A.C. Jaramillo-Madrid, et al.

Table 1
The occurrence of sterols in diatom species. Reported biological activities are noted.

Sterol common name and other nomenclatures	Diatom sources	Bio-f
Brassicasterol Brassicasterin (22E)-Ergosta-5,22-dien-3β-ol 24-Methylcholesta-5,22-dien-3β-ol ($\Delta^{5,22E}$) (24β = 24R) CAS # 474-67-9 $C_{28}H_{46}O$	*Amphiprora alata* (17), *Achnanthes brevipes* (72), *Achnanthes sp.* (76), *Achnathes cf. longipes* (85), *Amphiprora paludosa* (1), *Arcocellulus mammifer* (8), *Biddulphia sp.* (32), *Brockmanniella brockmannii* (11), *Cymatosira belgica* (3), *Delphineis sp.* (62), *Dickieia ulvacea* (97), *Ditylum brightwellii* (2), *Entomoneis cf. alata* (27), *Extubocellulus cribiger* (12), *Extubocellus spinifer* (13), *Fragilaria pinnata* (21), *Grammatophora oceanica* (61), *Helicotheca thamensis T* (32), *Hyalosira sp.* (67), *Leynella arenaria* (3), *Lithodesmium undulatum* (4), *Minutocellus cf. sp.* (2), *Minutocellus polymorphus* (5), *Nanofrustulum shiloi* (11), *Odontella aurita* (14), *Odontella longicruris* (42), *Papiliocellulus sp.* (5), *Pauliella taeniata* (86), *Plagiogrammopsis vanheurckii* (21), *Stauroneis constricta* (100), *Stauroneis simulans* (1), *Synedra fragilaroides* (85), *Synedra hyperborea* (21), *Synedropsis cf. recta* (10), *Talaroneis sp.* (88), *Thalassionema sp.* (1), *Thalassiosira stellaris* (tr)	Athe Hypo 2013
Campesterol Campesterin (24R)-Ergost-5-en-3β-ol Δ^5-24α-Methyl-cholesten-3β-ol (24R)-24-Methylcholest-5-en-3β-ol Campest-5-en-3β-ol (Δ^5) (24α = 24R) CAS # 474-62-4 $C_{28}H_{48}O$	*Achnanthes brevipes* (8), *Achnanthes sp.* (3), *Achnathes cf. longipes* (6), *Amphipora hyaline* (0.3), *Amphiprora alata* (8), *Amphora sp.* (47), *Arcocellulus mammifer* (1), *Attheya ussurensis* (1), *Attheya longicornis* (44), *Attheya septentrionalis* (17), *Attheya septentrionalis* (19), *Attheya septentrionalis* (36), *Aulacoseira granulate var. Angustissima* (61), *Bacteriastrum hyalinum* (29), *Biddulphia sp.* (7), *Brockmanniella brockmannii* (2), *Coscinodiscus granii* (17), (44.4), *Coscinodiscus sp.* (6), *Cyclotella cryptica* (7), *Delphineis sp.* (1), (1.6), *Dickieia ulvacea* (2), *Entomoneis cf. Alata* (14), *Extubocellulus cribiger* (1), *Extubocellulus cribiger* (2), *Fragilaria pinnata* (2.4), *Fragilaria striatula* (5), *Halassiosira punctigera* (18), *Hyalodiscus sp.* (13), *Hyalodiscus stelliger* (10), *Hyalosira sp.* (25), *Leynella arenaria* (3), *Melosira cf. Octogona* (9), *Minidiscus trioculatus* (41), *Minutocellus cf. Sp.* (tr), *Minutocellus polymorphus* (1), *Navicula phyllepta* (11), *Navicula sp.*(9), *Nitzschia thermalis* (46), *Odontella aurita* (9), *Odontella longicruris* (6).	Hypo 2013 Prote

3. The second point, I would like to bring across: Sterols and other lipid biomarker such as fatty acids have rarely a fixed ratio to carbon or biomass. The production of these

components can be highly sensitive to environmental conditions, e.g. light levels, nutrient supply, pH etc. Therefore, even though sediment cores contain more dinosterol than brassicasterol, this does not allow extrapolation to algae cell numbers or biomass. Ratios of two sterols have some potential for regional comparisons of relative abundance or presence vs absence, but not to quantify biomass.

Response: We also only partly agree with the reviewer that these sterols may not have a fixed cellular ratio and environmental conditions may modulate the production and concentrations of these markers in the cell. As mentioned above, we think that the culture experimental conditions are not representative of all oceanic environments, and due to changes in temperature up to 20 degrees (the average SST in our study locations is usually 28 °C, the sterol inventory may substantially vary. For example, the study by Piepho et al. (2012) reported an enhancement in sterol production in freshwater diatoms in response to a change in temperature from 10 °C to 25 °C.

Piepho, M., Martin-Creuzburg, D., & Wacker, A. (2012). Phytoplankton sterol contents vary with temperature, phosphorus and silicate supply: a study on three freshwater species. European Journal of Phycology, 47(2), 138–145. https://doi.org/10.1080/09670262.2012.665484

To conclude, we want to point out that diatom cells living in the water column experience several environmental variability throughout the year and specifically in the Arabian Sea, the seasonal change is distinct. Hence the diatoms may also show changes in sterol production and what we see on the surface sediment is the accumulated biomass over several years from different species.

4. If the authors would like to move forward with sterol biomarkers, I would suggest they analyze the sterol composition of their two main diatom species (Coscinodiscus and Thalassiosira) – either picking sufficient live cells from the sediment or water column, or culture them and grow sufficient biomass. Based on those findings, the samples from top sediment cores could be re-analysed for the 'right' sterols. Rampen et al. (2010) found chalinasterol (24- methylcholesta-5,24(28)-dien-3β–ol; m/z 470) in both Coscinodiscus and Thalassiosira (but likely different species). The same should be dome with their common platted dinoflagellates and coccolithophores. This will help to correctly interpret the sterol composition of the sediment cores. For a further read on sterols in microalgae (including the production of dinosterol) I would recommend Volkman (2017, 10.1007/978-3-319-24945-2_19) and for phyto-vs-zoosterol ratios (Kohlbach et al. 2021, https://doi.org/10.3389/fmars.2020.610248)

Response: We thank the reviewer for suggesting to improve our biomarker analysis and interpretation. However, we would also like to mention that 1) we have not attributed the Brassicasterol found in the sediment to the taxonomic group reported from the frustule analysis. We have also given justification for this point in the previous comments.

Coscinodiscus and *Thalassiosira* may not grow on the sediment, particularly since the station locations are on average 3.5 km deep, and after death when a frustule reaches the seafloor, there may not be any cellular materials in. So measuring biomarkers from live cells from sediment may not be feasible. Collecting living, planktonic *Coscinodiscus* and *Thalassiosira* for measuring biomarkers could be tried, but that needs thick blooms to have enough biomass in

quantifiable amounts for repeat measurements. This also could be challenging. Furthermore, to measure sterols in coccolithophore, needs really hard work as they are nanoplankton and even cannot be seen under a light microscope. I am not confident that picking live cells of coccolithophores under SEM could be a way to measure sterols.

Lastly we would also like to point out that many scientists have used brassicasterols as a potential biomarker for diatoms over the years as can be seen in many published papers and I show a few of them below:

- Schubert, C.J., Villanueva, J., Calvert, S.E., Cowie, G.L., Von Rad, U., Schulz, H., Berner, U. and Erlenkeuser, H.: (1998). Stable phytoplankton community structure in the Arabian Sea over the past 200,000 years, Nature, 394(6693), 563–566, https://doi.org/10.1038/29047

- Müller, J., & Stein, R. (2014). High-resolution record of late glacial and deglacial sea ice changes in Fram Strait corroborates ice–ocean interactions during abrupt climate shifts. Earth and Planetary Science Letters, 403, 446-455. https://doi.org/10.1016/j.epsl.2014.07.016
- Zimmermann, H. H., Stoof-Leichsenring, K. R., Kruse, S., Müller, J., Stein, R., Tiedemann, R., & Herzschuh, U. (2020). Changes in the composition of marine and sea-ice diatoms derived from sedimentary ancient DNA of the eastern Fram Strait over the past 30000 years. Ocean Science, 16(5), 1017-1032. https://doi.org/10.5194/os-16-1017-2020