Point-by-point reply to the comments made on cp-2021-76:

1. Comment by Referee #1

I have two concerns / suggestions I would like the authors to consider and one request.

First, the framing of the paper, from the title and abstract, is about "developing a new paleo- CO2 probe" (…). Although they do have some good proxy records to compare against their data, it leaves open a question of whether "mismatches" (assuming there should be a relationship) between [CO2]aq and large-small vital effects are due to physiological or oceanographic confounding effects, or both. This is a problem if the paper is setting out to make a solid contribution towards a new pCO2 proxy (no major step forward in quantifying a robust pCO2 - coccolith isotope relationship), but is less so if just trying to gather good data and understand how these size-specific coccolith isotope records actually behave in practice and especially across a range of temporal scales and magnitudes of pCO2 change (…). So, I would strongly recommend reorienting the paper towards the best interpretation of the data you have rather than trying to reach for a CO2 proxy which isn't there (yet).

We understand this remark and fully acknowledge that taken alone, this manuscript does not offer a ready-to-go novel pCO_2 proxy. We have probably not made sufficiently clear in our introduction that this manuscript is part of a series of papers which collectively support that the coccolith vital effects convey a pCO_2 signal. As such, we (and other teams) have tried to develop this exciting research avenue for several years now. This is something that we could better introduce in a revised manuscript, should we be invited to submit one by the Handling Editor.

Concerning the paper's orientation, as we highlighted in the discussion, the direct comparison of fossil coccolith $\delta^{13}C_{small}$- $\delta^{13}C_{large}$ and a theoretical CO_2 concentration would greatly benefit from better constraints on both the productivity changes and on the evolution of the chemical disequilibrium at the air-sea interface in the mid-latitude North Atlantic during Termination II. Although our results indicate that $[CO_2]$ might exert a first order control on coccolith differential vital effects, we agree that better knowledge is needed before we can (collectively) come up with a more robust transfer function between these two parameters.

In this regard:

- Although we've left the current title as is, as we feel it is rather objective, we would be also happy to change it to "Parallel between the isotopic composition of coccolith calcite and carbon levels across Termination II: *Is there a CO_2 signal in the magnitude of the vital effects?*" – this is dependent on Reviewer 1 and the Editor's advice.

- In the revised manuscript, we modified parts of the abstract **[l. 28]** and of the conclusion **[l. 446-449]** that probably oversold the transfer function and will focus instead on how this study fits in with previously obtained data on the tight link between the coccolith vital effect and CO_2 levels.

Second point, and related to the first, is that I'd like a more detailed consideration of the underlying

driver of your change in the large-small coccolith isotope offsets, especially for carbon. The raw data - Figure 2 - shows a ~1‰ negative shift in small coccolith $\delta^{13}C$ across Termination II whereas the large fraction hardly changes. The *G. bulloides* record trends slightly positive. In this instance, I can't see how this can be explained other than that the vital effects in the small cell sizes are increasing across TII - i.e. going further from equilibrium calcite - as CO_2 rises. Whereas the large cell sizes change less. OK, this gives you a reduction in large-to-small vital effects, as you'd expect with increasing CO_2, BUT it's the small coccolithophores that are driving this change, not the large cell sizes that should be most limited and sensitive to changing $[CO_2]_{aq.}$. With no major change in local $\delta^{13}C_{DIC}$ (based on G. bulloides) how do you account for this big shift in the small coccolith $\delta^{13}C$? This seems to me to be at the heart of understanding this record but is never really addressed.

The main difficulty in dealing with an offset is indeed to determine which of the large $\delta^{13}C$ or the small $\delta^{13}C$ (or both) changed throughout the interval. We agree that a clear understanding of what drives the isotopic evolution of the size fractions taken separately would massively contribute to our understanding of the evolution of the small-large coccolith offset across the interval of study. In culture experiments, increased photosynthetic activity under increasing $[CO_2]$ concentrations tends to build a ^{13}C-enriched internal carbon pool of large cells, which leads to higher $\delta^{13}C$ in large coccoliths. This biogeochemical phenomenon reduces the isotopic offset of $\delta^{13}C_{large}$ *both* with inorganic calcite (the absolute vital effect) *and* with smaller and less carbon limited cells (the differential vital effect).

The reviewer's observations on the conflicting behavior of the small fraction's absolute vital effect and differential vital effect are correct. Although our work originally attempted to use the foraminiferal record (*G. bulloides*) to constrain the absolute vital effect, we concluded that *G. bulloides* cannot be used to derive a reliable inorganic reference against which it would be possible to compare the isotopic changes of the individual size fractions. Indeed, we detail in **paragraph 3.2.** the uncertainties surrounding the calcification depth and the biogeochemistry of this particular foraminifera (between 70-100m depth according to Rebotim et al., Biogeosciences, 14, 827–859, 2017), which might record changes in seawater chemistry at a different depth from where the coccoliths are biomineralised (*i.e.* predominantly in the uppermost meters of the water column, O'Brien et al. Earth Syst. Sci. Data 5, 259–276, 2013). Therefore, without a reliable inorganic reference for the surface ocean, it is difficult to say which of the 2-3 µm or 5-8 µm fraction is responsible for the observed changes in the $\Delta^{13}C_{small-large}$ and $\Delta^{18}O_{small-large}$, a caveat that also captured our frustration. But we hope that our data are sufficiently convincing in that we can work with an offset that does not require an inorganic reference.

Finally, a request. Although there are lovely SEMs of the size splits in the SI, and I'm sure that all the splits look equally lovely, it would help enormously if you could provide some assemblage composition data for some selected representative samples through your record - both species composition and coccolith size distribution. I know this is some extra work, but at the moment it's impossible to properly compare data from coccolith separates like these from different studies (using different methods) unless there's reporting of what is actually being measured. This kind of quantitative assemblage data would also allow better comparisons between studies and across timescales - e.g. knowing that we're comparing small (of defined size range) retics to small retics. Your samples look quite confined in their taxonomic composition - and I'd want to document that (and get others to do the same) - so that we can spot issues if a (90%) Calcidiscus-rich assemblage is compared to a (50%) Calcidiscus-rich assemblage.

We fully acknowledge the point made by the Reviewer, as it would indeed be ideal to compare the isotopic signals from different sites/studies with strictly comparable coccolith assemblages. We also agree that the assemblage itself, and changes thereof, is a valuable source of information. Unfortunately, we do not have these micropalaeontological data. The rationale of comparing our data with other published datasets relies on the fact that a number of studies (from culture, sediment and numerical experiments) have linked the magnitude of individual coccolithophore vital effects to the degree of carbon limitation experienced by the cell, which to first order depends on the cell's size (and ultimately coccolith size). Thus, it was important that we carefully check our coccolith fractions for signs of (foram/coccolith) fragments that could eventually pollute (as they convey a distinct isotopic signal) the size-restricted fractions that we obtained.

Line 29 – would recommend rephrasing, especially the use of "overtakes"

This sentence has been removed and replaced by another which makes explicit mention of the factors complicating the use of differential vital effects for the reconstruction of surface ocean CO_2 (see first comment).

Line 63 – lower case "a" after the colon.

Corrected.

Line 64 – late Miocene not Late Miocene – informal division.

Corrected.

2. *Comment by Referee #2*

In the following, I develop several points that require more detailed explanation and several minor points

The first point concerns the discussion about the coccolith Δd13Csmall-large over the studied interval (Termination II) (mainly Section 3.3.2):

The relatively acceptable statistical correlation between [CO2aq] and $\Delta d^{13}C_{small-large}$ (Fig. 4) relies on 2 points with low $\Delta d^{13}C_{small-large}$ and relatively high [CO2aq] values. When looking at the downcore records (Fig. 3), these 2 points correspond to the H11 interval. This interval indicates noisy isotope values (Fig. 3). How robust is it? (if these 2 points were removed, the statistical correlation would probably be less significant): can you comment on this?

If we remove the two points mentioned for a moment, the statistical correlation between CO_{2aq} and both $\Delta^{13}C_{small-large}$ and $\Delta^{18}O_{small-large}$ remains significant (**Figure 1 below**). We chose to keep these points in the study because we found no evidence that these samples were more contaminated than other levels by fragments from either the adjacent fractions or foraminifera.
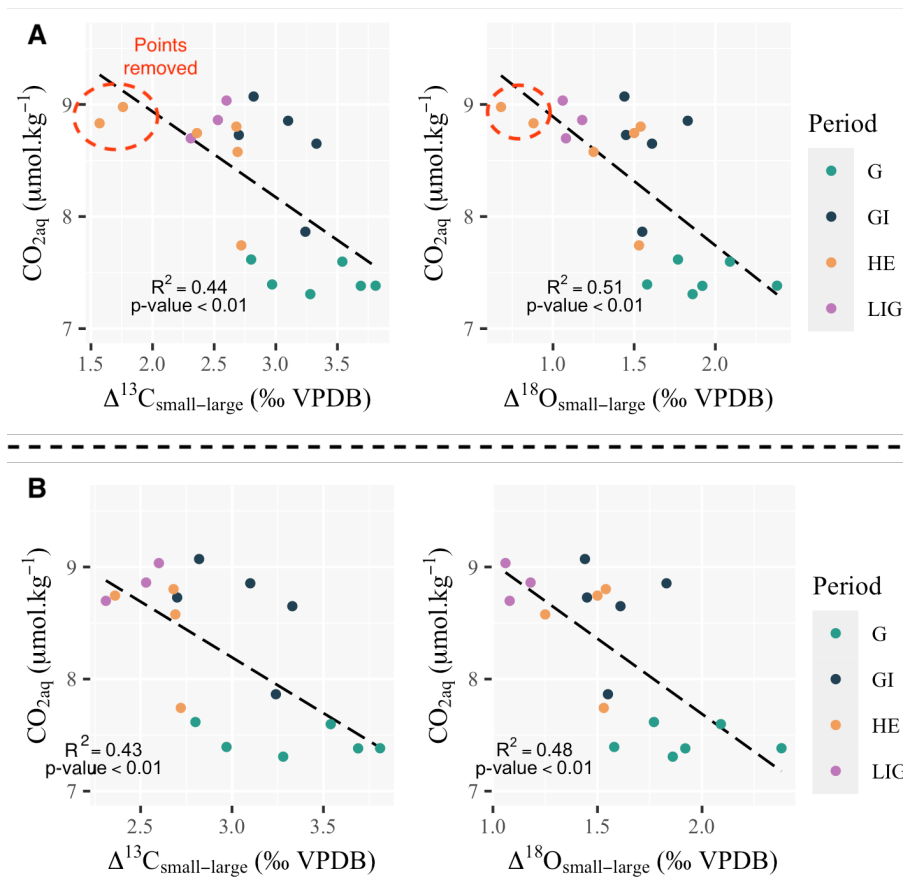
Figure 1: Impact of the removal of two points with High CO₂ – Low Differential Vital Effect on the correlation. In panel A, we've represented the correlation as it appears in the manuscript. The point colors correspond to the time periods identified in the original manuscript. In panel B, we've represented the results for the same dataset excluding the two points with low differential vital effects.

Some information is needed about isotope measurements on the different size fraction : are they based on replicates ? (triplicates ?)

We are aware that running small aliquots of foraminiferal assemblages (<15-20 specimens) may lead to biases. Unlike foraminifera, the ≈ 80 μg of coccolith fractions we run for isotopic analyses integrate an appreciable number of coccoliths and thus we do not routinely run them for duplicate analysis. For the sample we did run for duplicates, however, the standard deviation fell well within the standard deviation determined from the different NBS19 values ($\delta^{13}C = 1.95 \pm 0.05$, $\delta^{18}O = 2.20 \pm 0.1$), which is known to be a homogenous standard material. In any case, we are confident that the magnitude of the observed changes between our fractions and adjacent levels is much higher that the reproducibility of our measurements. In the revised manuscript, we specified how standard errors on the isotopic analyses had been calculated **line 142**: *"Standard errors (1σ), which are calculated by running a minimum of six samples of NBS-19 per series of analyses, are ±0.05‰ for $\delta^{13}C$ and ±0.1‰ for $\delta^{18}O$ values."*

The second point concerns the downcore isotope records: -some information is missing why d13C of large is more stable than d13C of small coccoliths; -another particular feature is the stability of the d13C bulloides record; even if it is not the main topic of the study, reasons why this former record is stable

over Termination II needs a comment (since it is not observed in other d13C records from other planctonic species).

This remark, pertaining to the *G. bulloides* δ^{13}C reference was somewhat made by Reviewer 1, which we hope we satisfactorily addressed. We would specifically like to add the following:

Two observations can be made on the variations in δ^{13}C$_{G.\ bulloides}$:

- The amplitude of the δ^{13}C$_{G.\ bulloides}$ change across the interval is 1.16‰ for the samples considered (**Figure 2 below**). It appears stable because this change is smaller than for the total δ^{13}C change of the 2-3 μm coccolith fraction (a 1.84 ‰ change).

- Neither the δ^{13}C$_{2-3\mu m}$ nor the δ^{13}C$_{5-8\mu m}$ parallel the change in δ^{13}C$_{G.\ bulloides}$ (**Figure 2**) nor the δ^{13}C$_{DIC}$ we derive from it:

These two observations show that the foram and the coccolith record are disconnected from each other over the interval. We interpret these results to indicate that the coccoliths and *G. bulloides* record variations in δ^{13}C$_{DIC}$ of different water masses (see **section 3.2.** of the manuscript and response to RW1's query). The discrepancy between the foram and coccolith record can be accounted for by distinct documented living depths, as *G. bulloides* is found to live between 70-100m depth, below the preferred living depths of coccolithophores (Rebotim *et al.*, Biogeosciences, 14, 827–859, 2017). Thus, we are *a posteriori* of the opinion that the uncertainties pertaining to the inorganic reference make it difficult to conclude as to which of the 2-3 μm or 5-8 μm fraction is responsible for the observed changes in the Δ^{13}C$_{small-large}$ and Δ^{18}O$_{small-large}$. We would like to stress that, as a result, we do not base our interpretations on the isotopic composition of the forams.
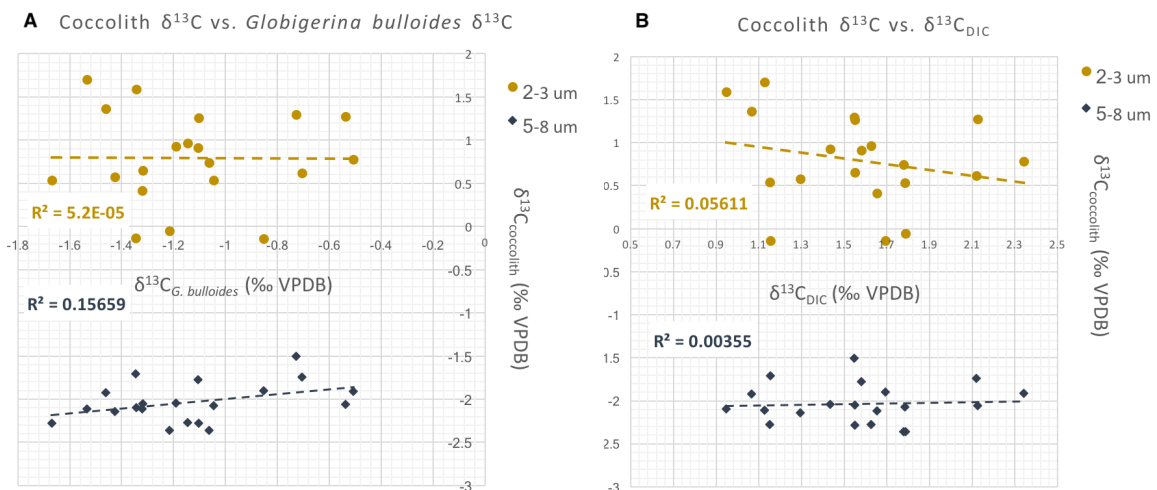
Figure 2: Comparison of the δ^{13}C composition of the two coccolith size fractions considered with the foraminifera reference (A) and the inorganic reference (B).

The third point is a general comment. The results of this study support findings that the isotopic composition of coccoliths (for different size ranges) is sensitive to CO2 concentrations at the glacial/interglacial scale. However, even if in Sections 3.3.3 and 3.4, different factors that could imprint

the coccolith vital effect are addressed, the conclusions about the use of this proxy as a paleo-CO2 indicator are slightly too optimistic. It should be mentioned that there are still a number of issues to be clarified (effect of productivity, stratification). (…)

As replied to Reviewer 1's comment, we understand that the phrasing of the conclusion might be a little optimistic in light of the issues with the transfer function that were discussed in the manuscript. Although our results indicate that $[CO_2]$ might exert a first order control on coccolith differential vital effects, we agree that better knowledge on variables such as productivity (which has an effect on cell physiology) and stratification (which has an effect on air-sea disequilibrium) is needed before we can define a robust transfer function between these two parameters.

We have toned down this proxy with an explicit mention to the issues that you identified as a complicating factor of the prospective proxy [**l. 31** of the abstract and **l. 436** of the conclusion].

It would be interesting to compare these data either for another Termination or another more distant site of events affecting oceanic conditions.

This is true and definitely belongs to a longer-term and cocco-community approach! We hope that the present study will stimulate such studies from6 other teams.

Some minor points :

-[CO2aq] calculation : mention the impact of salinity uncertainty on the estimation

Uncertainties on salinity estimates were already included in the calculation of $[CO_{2\ aq}]$ [at **l. 219** of the manuscript]. A ±1 psu conservative change in salinity across the interval leads to a maximum ±0.05 $\mu mol.kg^{-1}$ uncertainty on $[CO_{2\ aq}]$.

-What is the temporal resolution difference between atmospheric pCO2 records and SST reconstructions in core MD37?

The Antarctic pCO_2 records (with a mean temporal resolution of 760 yrs across the studied interval) were matched to SST records which have a mean temporal resolution of 1 kyr. We did our best to fit the two.

-in relation with section 3.3.3 : do you have you access to the coccolith counts/assemblages over the studied interval ?

Please refer to our response made to Reviewer 1 in his third comment. But a short answer is that we have not generated absolute or relative coccolith abundances for every size fraction because coccolith size in our approach matters more than the size of the coccolithophore taxon that mineralized it, although the two correlate. Therefore, we checked our coccolith fractions for signs of recrystallisation and contamination from larger coccolith fragments or foraminifera fragments rather than for assemblage changes.

3. *Additional changes made to the manuscript from comments of the community*

The article being freely available for download online, we received an email from a member of the paleoceanographic community with observations on the datasets used in **Figure 5**. As a result, we've chosen to use updated dataset for Figure 5 as follows:

Updated variable	Dataset used in the original submitted manuscript	Updated dataset in the revised manuscript	Updates
Miocene/Pliocene coccolith differential vital effects from Caribbean site 999	Bolton, C. T. & Stoll, H. M. Nature 500, 558–562 (2013)	Bolton, C. T., Stoll, H. M. & Mendez-Vicente, A., Paleoceanography 27, 1–16 (2012)	A larger number of values for site 999
Miocene/Pliocene pCO_2 data for the computation of $[CO_2]$	Zhang, Y. G., Pagani, M., Liu, Z., Bohaty, S. M. & DeConto, R. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 371, 20130096 (2013)	Rae, J. W. B. et al. Annu. Rev. Earth Planet. Sci. 49, 609–641 (2021)	An updated pCO_2 curve derived from boron isotopes rather than from alkenone ε_p values