**Author response for "Alkenone isotopes show evidence of active carbon concentrating mechanisms in coccolithophores as aqueous carbon dioxide concentrations fall below 7 µmol $L^{-1}$" by Marcus P. S. Badger**

Anonymous Referee #1

Many thanks to the reviewer for the kind words on the manuscript and the constructive comments and suggestions.

> The fact that these many studies have now all been calculated using the exact same methods should be included in the Abstract and Introduction (re: Line 100-101), as this alone is important for the community. Consider moving (or repeating) Line 173-175 in the Abstract and/or Introduction, as this is also an important point of this study.

I have included a line to this effect in the Abstract (lines 7-8; line numbers throughout refer to the latexdiff file for easy reference)

> The Introduction needs to establish some key topics. Most importantly, there is almost no introduction of carbon concentrating mechanisms, which is the entire crux of the study. In particular, it is important to establish how they work, the different kinds of mechanisms (e.g. external or intracellular carbonic anhydrase, HCO3� transport), and why CCMs are concerning for the proxy (e.g. isotopic difference between bicarbonate and CO2, different fractionation pathways). It might also be useful to further discuss alkenone-producers, including which have CCMs and possible differences among alkenone-producers. In the studies compiled here, is there an idea of which producers are present (i.e. coccoliths preserved in the sediment)? Any size-corrections?

I now include a new section in the Introduction outlining what is understood about CCMs in coccolithophores (new section 1.1, lines 45 -79) and further lines on alkenone producers in the introduction (lines 30-33). As noted within the methods I use a common methodology for all records which does strip away some secondary corrections (lines 99-104), for size corrections in particular, there is not a consensus about how to perform these (see discussion in Badger et al 2019), and very few records had lith size records available.

> Throughout the manuscript, there is almost no mention of the other possible causes for the breakdown relationship between epsilon p and dissolved CO2. Although CCMs are certainly a strong possibility, the author should also consider changes to the "b" factor or environmental conditions. These need to be addressed in the Introduction and later on in the Discussion. Has the author considered looking for indicators of e.g. upwelling (which would increase the availability of aqueous CO2) or the BIT index (indicating increased terrestrial input, effecting nutrient availability or species composition change)?

As noted in the discussion phase, and similar to lith size, not all records have suitable BIT or upwelling records to compare to, and the strength of the analysis presented is the ability to treat all records equally. I have expanded my discussion of 'b' corrections in the discussion (lines 260-265) however as noted there the recent attempts to correct the 'b' term has relied on the assumption that the proxy system is working in the Pleistocene, but with reduced sensitivity or secondary corrections. My work here suggest that for some of the Pleistocene at some sites the proxy system breaks down, and so this sort of correction is inappropriate. I have clarified this point (line 262-3).

> If possible, include site information such as estimated depth and distance from coast, as this is important to interpreting the results.
> Following up on this point, Lines 187-188, the author states that areas of warm water (i.e. tropical or shallow shelf regions) should be avoided. However, essentially all of the sites used in this study are tropical low latitude (30_ N to 30_ S) and all look quite close

> to the continents, likely to be shallow. Is there a possibility that the sites are the issue (e.g. warm waters, upwelling, growth factors), not necessarily the proxy mechanisms? This Lines 187-188 statement also seems at odds with Lines 50-51, where the author states that low latitude, gyre sites are likely more oceanographically stable.

This information now included in Table 1, from which it can be seen that although many of the sites are low latitude (this is noted lines 87-9) they are otherwise quite diverse and range in water depth and distance from the coast.

> In the Methods, the author discusses the use of phosphate to determine b. Could the author briefly include why they are not considering the findings of Zhang et al. (2019; 2020)? Any possibility of comparing with _15N values? (see Andersen et al. 1999 in Use of Proxies in Paleoceanography, Ch. 19, 469–488).

15N data is not available for most sites but could be of interest in future work, the reasons for not considering Zhang (2019; 2020) is, as noted above, because this work potentially makes the analysis of Zhang et al (2019;2020) incorrect, as noted in new lines 260-265.

> In the Results, Line 102-107, the author suggests that CCMs only come actively start pumping under a certain low-CO2 threshold. This needs further support/references, given that the literature has shown that CCMs are quite complex in their function and varied among species (e.g. Reinfelder, 2011, Annual Review of Marine Science). Line 105-107: rephrase as it is currently misleading. If we assume that carbon concentrating mechanisms are prevalent, then the proxy would perform least well under low CO2 concentrations. However, as currently phrased, if the alkenone-based proxy "relies on the assumption of a purely diffusive uptake of carbon", then actually, we would expect the proxy to perform the same as it does at any level.

I now include a much fuller discussion of CCMs in the Introduction (new section 1.1. lines 45-79) and have expanded the discussion section on this point (new and revised material lines 229-249) and have revised the phrase in question (line 243)

> Table 1: I suggest adding a column with the approximate age ranges for each site. I would also suggest giving this table some kind of structured order (maybe by latitude?)

Done (new table 1)

> Fig. 2: NIOP 464 should be a star but is expressed as a square (there are two square symbols). It is difficult to distinguish these shapes, as they are very small (e.g. the circle and hexagon look identical unless I zoom in). Please add the site numbers next to the locations or at the very least, make the symbols more distinct with an accompanying legend on the figure.

Fixed and site labels added (new Figure 2)

> Fig. 3: Consider including the site symbols.

Done. (new Figure 3)

> Fig. 6: Consider indicating the breakdown point of proxy vs ice core (e.g. using a dashed line)

I try to avoid adding guides to figures – if the eye needs guiding often the point is not as strong as suggested, I hope that this point stands out sufficiently.

> Fig. 7: Between _400-500 [CO2(ep-alk)/uatm], why are there two separate estimates for [emulated uncertainty/uatm]?

This is just the result of the interaction of different parameters in the different datasets (I think the lower one is at a lower temperature).

> Fig. 8: Because Fig 6 and 8 are so similar and are constantly discussed together, it might be worth combining these into one figure with 4-quadrants (instead of two figures with 2-quadrants).

This is a good idea, which I have implemented in a new Figure 6.

> There are numerous spelling and grammatical issues throughout the manuscript, for example: Line 20 (isotopic), Line 26 (Noelaerhabdaceae), Line 106 (diffusive), Line 145 (calculated), Fig. 4 (estimate). Some of the references also have incorrect the incorrect doi or link. Please revise.

Done (see marked-up file).

> Line 20 (and throughout): Add “stable” before carbon isotopic composition
> Line 28 (and throughout): Add “concentrations” when regarding atmospheric CO2

Done (see additions marked throughout the marked-up file)

> Line 29: Consider including other CO2 proxies, e.g. paleosols, leaf gas exchange

As the two marine proxies are most comparable in terms of temporal usage (both range and resolution) I have kept at these two.

> Line 33: I would not consider less than 400 uatm “moderate to low”. The author may avoid the subjective term altogether by rephrasing to “: : : at atmospheric CO2 concentrations below 400 uatm of the Pleistocene”.

Done (line 39-40).

> Line 36: Although the Super et al. (2018) SST reconstruction is incredibly useful, I would not consider the Miocene a “resolved” issue. The Miocene CO2 is a highly debated topic at the moment.

Revised (lines 42-3).

> Line 46 (and throughout): Add “values” after all _13C

Done (throughout).

> Line 118-121: Remove “such as” throughout. Here, the author includes every single site, so there’s no need to express them as examples.

Done (lines 162-167 and throughout).

> Line 143-150: Please break down into several sentences, quite difficult to read.

Revised (lines 190-199).

> Line 175: Remove “even”

Done (line 233).

> Line 196: “Recent” what?

Revised (line 260)

Anonymous Reviewer #2

Thank you to the reviewer for their kind words about the manuscript and the constructive comments.

> As mentioned above, the main issue I have is the statement that the activation of CCMs is the sole explanation between the offset in CO2 reconstructions between the alkenone proxy and ice core record. There is no evidence that since the development of CCMs in haptophytes (which may have occurred during the late Miocene – early Pliocene) these CCMs could be turned on and off. On the contrary, a study from Van de Waal et al. 2019 (L&O Letters) suggests that haptophyte CCMs (measured in present day haptophytes) are not so adjustable even in high CO2 environments. Although this is from present day haptophytes, no mention of such findings is made, even though the data presented here is closer in age to the present-day haptophytes than those from late Miocene.
>
> The main conclusion of the paper also revolves around CCMs, but this topic is hardly introduced or properly explained in the introduction and explored in the discussion. This can certainly be improved. CCMs also comprise various mechanisms of acquiring C and it can be explored how alterations in these strategies may compromise alkenones being a reliable proxy for atmospheric CO2. It may have something to do with increased uptake of HCO3 relative to CO2, but no mention of this is made. Just stating CCMs are turned on or off is a bit oversimplified, especially since there is not a lot of evidence for this.
>
> A bit more emphasis on the comparison of the alkenone proxy to the ice core record may be made in the title and in the abstract, as it is very nice that data from multiple sites are combined and also demonstrates the pitfalls of this proxy.

I now include a new section in the Introduction outlining what is understood about CCMs in coccolithophores (new section 1.1, lines 45 -79) and have revised the Discussion (throughout the Discussion, lines 235-265), and now include more specifics of the comparison in the abstract (lines 7-8). As stated in the discussion phase, the work of Van de Waal is intriguing, although is based on a modern haptophyte evolved within the low $CO_2$ world of the Pleistocene. I have revised the language throughout to note that it is if CCMs dominate that the proxy seems to fail, and note the evidence (reviewed by Reinfelder 2011 and discussed in the new section 1.1) that coccolithophores likely supplement passive diffusion with CCMs but that the evidence is that coccolithophores on the whole do so much less effectively than some other algae.

> There are still a few mistakes and a few awkward sentences in the text.

These have now hopefully all been revised, along with all the minor revisions and corrections suggested (see marked up latexdiff version)

> Line 17-21 Quite a long and confusing sentence with conjugations that do not fit.

Revised and split (lines 19-24).

> Line 61, but also Line 84 here you state that additional corrections from the original records were removed, but you accounted for that in the fractionation with the “b” term,

right? How exactly is this term calculated for all the sites?

This is detailed in lines 128-133.

Line 175 what do you mean here? The study you did or the one from Laws? Not clear from sentence structure, although I assume you mean your study as you refer to alkenones. If so, I would not state it like this, as you only look at sedimentary records which is not clear behavior of activation of CCMs.

This passage has now been revised (lines 232-237).

Line 182 not sure if this is necessarily a CCM threshold or a switch maybe from one of mechanisms of the CCM (for instance a switch from CO2 to HCO3uptake)

Revised (line 244-7).

Line 187 maybe also state how SST influences aqueous CO2, as this is not yet mentioned.

Revised (line 250).