Referee2

Guitián et al. built two new records of ε_p from the Oligocene to early Miocene based on IODP Site 1406 and ODP Site 1168, along with the new ε_p record from Site 925 to supplement previous published low-resolution record. As ε_p , the carbon isotopic fractionation during the photosynthesis of phytoplankton, is determined by both aqueous CO₂ levels and physiological parameters of phytoplankton. To extract variations in atmospheric CO₂ levels based on changes in ε_p from the Oligocene to the early Miocene, the authors evaluated the influence of varying physiological parameters, including cell size and growth rate, on ε_p evolution. The influence of changing cell size was assessed using measured coccolith sizes and a statistical multilinear regression model developed by Stoll et al. (2019), which shows that ε_p is a function of aqueous CO₂ concentration, light, growth rate, and cell radius. For growth rate, they assumed it is controlled by temperature and used the sensitivity of ε_p to temperature, as derived from the culture experiments by Torres Romero et al. (2024), to represent the sensitivity of ε_p to growth rate. They used changes in biogenic silica (bioSi) concentrations in sediments to indicate variations in nutrient concentrations; higher bioSi concentrations suggest increased nutrients and growth rates. They conclude that size and temperature effects have a negligible impact on the long-term declining trend of ε_p , and that a global CO₂ decline is the most likely cause of the decrease in ε_p .

Overall, the manuscript reads well, but a lot of phrases/sentences are confusing and difficult to understand. Please look at the specific comments below. In addition, I see several major shortcomings of the analysis and presentation of the results.

We would like to thank the detailed revision of the manuscript provided by the reviewer that we believe substantially improves the discussion of the dataset. We provide a response to the comments below.

First, I do not think it is appropriate to use a single value for temperature- ε_p sensitivity (0.48‰ decrease in ε_p per 1°C warming) to represent the sensitivity during the Oligocene to early Miocene. The 0.48‰ decrease in ε_p per 1°C warming is based on the linear regression of temperature and ε_p data (22 samples) from culture studies by Torres Romero et al. (2024), conducted at 22 different combinations of temperature, CO₂(aq), and light. Torres Romero et al. (2024) demonstrates that the sensitivity of ε_p to temperature varies significantly across different CO₂(aq) ranges: 0.37‰ decrease in ε_p per 1°C warming when CO₂(aq) ranges from 4 to 22.5µmol/kg, and 0.95‰ decrease in ε_p per 1°C warming when CO₂(aq) ranges from 22.5 to 41 µmol/kg. Given that CO₂(aq) likely fluctuated across these ranges during the Oligocene to early Miocene, the temperature- ε_p sensitivity may also have varied. Therefore, relying on a single value derived from the culture experiment is not reliable for representing the entire period.

Second, the sensitivity of ε_p to temperature derived from culture experiments does not necessarily represent the sensitivity of ε_p to growth rate in real geological environments. Growth rate is influenced by a combination of factors, including light, temperature, nutrient availability, CO₂ levels, cell size, and other variables, all of which varied significantly over the geologic past. Although Torres Romero et al. (2024) demonstrates that the temperature-sensitive ε_p variation can be fully explained by the temperature sensitivity of growth rate, this does not imply that the relationship is directly applicable to real-world conditions of the geologic past.

We appreciate these suggestions from the reviewer. In this study we provided the temperature- ϵ_p growth rate correction as a sensitivity analysis and chose the 0.48‰ dependence because this was also shown to be consistent with the growth rate effect on ϵ_p in culture studies compiled in Stoll et al., (2019) given the

temperature-growth dependence observed for phytoplankton in the modern ocean (Fielding, 2013). This comparison discussed in Torres-Romero et al., (2024) suggests consistency between field and culture experiments. We acknowledge the diverse slopes found in the culture study and that it is also possible that the modern temperature growth dependence observed by Fielding, (2013) may be different in an ocean with higher CO_{2laql} .

In this direction and to clarify the interpretation of the dataset in the revised version of the manuscript, we propose to adjust the Figures 6, and 7 in the main text as described in our response to Reviewer 1 (providing a set of panels with measured Ep and a separate set with Ep corrected for temperature). The set with Ep corrected for temperature, we propose to add an error bar illustrating the range in Ep when the slope of the correction ranged from 0.37 to 0.95 ‰ per 1°C warming.

Third, more evidence is needed to justify using changes in biogenic silica (bioSi) content in sediments to represent variations in surface ocean nutrient concentrations, particularly nitrate and phosphate, which are critical for coccolithophore growth. Sedimentary bioSi is primarily linked to the Si biogeochemical cycle, which likely differs from N and P cycles. Additionally, sedimentary bioSi is influenced by factors such as dissolution and preservation, limiting its reliability as a proxy for ocean nutrient concentrations, especially for nitrate and phosphate. Furthermore, the interpretation of bioSi results is inconsistent, with the authors at times referring to bioSi content as the delivery rate (Line 305) and at other times as the burial rate (Line 316).

We fully agree with the reviewer that biogenic silica is an imperfect indicator for surface ocean nutrient concentrations in the sites in the Oligocene. We had initiated the discussion in line 305 with cautious tone "*as one possible nutrient indicator*". Yet, in the absence of any superior indicators of surface ocean nutrient content, we suggest it is worth including biogenic Si in the comparison because nutrient content is one factor which affects the production and export of biogenic Si. We do not feel it is the place to go into discussion about opal export vs burial because opal accumulation rate is widely used in many settings for the Pleistocene as an indicator of opal export because the sediment dissolution is buffered when accumulation rates are high, and this is not the key relevant caveat for the current discussion. Line 305 refers to the delivery as part of the explanation of the proxy process, whereas line 316 refers to the observed burial.

To address this limitation we propose to expand the paragraph beginning in line 305 (new sentences in bold):

As one possible nutrient indicator, a higher concentration of biogenic silica (bioSi) in sediments may reflect a higher rate of bioSi delivery to the seafloor due to higher export production produced by siliceous organisms (mainly diatoms) in the ocean (Ragueneau et al., 2000). In the modern ocean, regions with abundant dissolved Si in the photic zone are regions also characterized by higher concentrations of macronutrients such as P and N. However, bioSi is an imperfect indicator of past surface nutrient content because coccolithophores have a minimal Si requirement, and Si remineralization in the ocean does not occur at the same rate as soft-tissue nutrients such as N and P. At IODP 1406, bioSi concentrations generally increase from the Oligocene to earliest Miocene, potentially indicating a gradual increase in the concentration of dissolved Si in surface waters at the site (Fig. 5). If the increase in dissolved silica observed at the North Atlantic is correlated to an increase in dissolved P or N, it could contribute to increase in growth rate, and therefore likely increase in biomass and chlorophyll, which would reduce light in the water column both being part of the observed long term decrease of εp . However, the actual correlation between bioSi and εp is not that strong (Fig. S2), suggesting that while increased nutrient

concentrations could contribute to the long-term evolution of εp , the specific steps of εp decline are less likely to be driven by increased nutrients and growth rate.

Fourth, the linear relationship between ε_p and SST (or benthic δ^{18} O) shown for several time slices in Figure 6 is not statistically meaningful, as the sample sizes for most of these time slices are fewer than 10. Therefore, the conclusions drawn from Figure 6 are unreliable.

We fully agree with the reviewer, that many of the time slices do not have significant correlations. Our conclusion drawn from Figure 6 is that across the overall time interval there are weak positive correlations with SST in Site 1168 and weak negative ones with SST in Site 1406.

To further clarify our conclusion, as suggested in response to Reviewer 1, in new Figure 6 we propose to leave the symbols color coded by time interval but illustrate only the single overall correlation in each site (and to provide a separate panel for measured Ep and for growth-rate corrected Ep, each with their single correlation). The correlations by time period are given in Supplementary S3 and we propose to annotate S3 to indicate which relationships have statistical significance. We propose to eliminate the paragraph beginning in line 372 since it will no longer be part of the main text presentation.

Lastly, the relationship between ε_p and benthic δ^{18} O at orbital scales (Figures 7b and 7c) does not yield a clear conclusion, as variations in benthic δ^{18} O are influenced by both deep-water temperature and ice volume. A more meaningful comparison would be between ε_p and estimated global mean SST (Gaskell et al., 2022; https://doi.org/10.1073/pnas.2111332119) or surface temperature (Evans et al., 2024; https://doi.org/10.1029/2023PA004788).

We thank the reviewer for bringing this discussion to our attention. We would like to mention that Figure 7 presents results on orbitally resolved Ep and δ^{18} O benthic as well as bulk sediment δ^{18} O, extracted from the same samples. These are provided to assess the trend in the relationship between Ep and benthic (or surface ocean carbonate) δ^{18} O, and no absolute comparison of temperature or sensitivity is derived from them in our paper. Consequently, a linear transformation of the benthic δ^{18} O to global temperature, such as described in previous studies such as Evans et al (2024), would not change the conclusion we make from this figure: data suggest that there is no direct relationship between lower CO₂ and colder temperatures. The δ^{18} O bulk, dominated by surface dwelling coccolithophorids, is not discussed by the references provided. Therefore we propose to leave Figure 7b in the original unit of measured benthic δ^{18} O.

Specific comments

Comments to the abstract:

Line 12-13: The statement "most based on the phytoplankton carbon isotopic fractionation (ϵ_p) proxy" is not accurate. Between 25 and 16 Ma, most of CO2 estimates are based on boron isotopes, not alkenone carbon isotopic fractionation.

Line 17: Full name of "Ma" is needed here Line 17-18: "a higher resolution sampling" — higher than what?

Line 20: Please specify "the two sites". Line 20: climate dynamics is a broad concept. Please clarify it.

Line 21-22: This sentence is confusing, especially the phrase 'average earth surface temperature evolution.' Are the authors referring to the global mean surface temperature?

Line 22-23: what does the inverse relationship between ϵ_p and benthic $\delta 180$ indicate? Line 25: what do "specific time intervals" represent?.

Line 26: this sentence is incomprehensible.

Line 25-27: Confusing. How does the changing cell size and growth rate explain the divergence between $\epsilon_{\rm p}$ and benthic δ^{18} O?

Line 29: "While CO_2 changes likely caused significant changes in radiative forcing" is not connected to the following sentence "SST variation at the examined sites may have been conditioned by regional heat transport".

Line 31: How does "the relationship between benthic δ 18O and ϵ_p " reflect the phasing between ice growth and global temperature?

We thank the reviewer for carefully revising the abstract details. To address all the specific comments we propose the following text in the new version of the abstract:

Atmospheric carbon dioxide decline is hypothesized to drive the progressive cooling over the Cenozoic. However, the long term CO_2 record from the early Oligocene to Miocene time interval, derived from the phytoplankton carbon isotopic fractionation (*cp*) proxy, differs from what is expected to drive the climate observations. Here, we produce two new long-term records of sp over the Oligocene to early Miocene time interval from widely separated locations at IODP Site 1406 and ODP 1168 and increase the resolution of determinations at the equatorial Atlantic ODP 925. These new results confirm a global footprint of *εp* shift occurring during this interval. Rapid 3 ‰ declines are found from 27 to 24.5 million years ago (Ma) and 24 to 22.5 Ma, and minimum p is attained at 19 Ma. Between 28.7 and 29.7 Ma at IODP 1406, a 20-30 ky sampling resolution at Site 1406 reveals orbital scale 100 kyr cyclicity in ɛp. Making use of alkenone-based sea surface temperature (SST) estimates and benthic $\delta^{18}O$ estimates extracted from the same samples, we perform a direct comparison with sp to evaluate the relationship with climate. We observe that across the long Oligocene to early Miocene interval, sp is positively correlated to SST only at the southern ocean Site 1168, but not with SST at the North Atlantic Site 1406. Accounting for the temperature-driven growth rate or cell size effects on sp does not lead to stronger correlations between ϵp and benthic δ^{18} Onor stronger correlations between ϵp and SST at Site 1406. Moreover, at orbital timescale, the relationship between εp and benthic $\delta^{18}O$, albeit weak, implies greater ice volume or colder deep ocean at higher CO₂. Despite the persistence of climate paradox, the reproducible albeit trends in three widely separated sites, which experienced contrasting temperature evolution and likely experienced different variations in nutrient availability, suggest that a common CO₂ forcing is likely the dominant control on the long term trends in *cp*. Changing ocean heat transport to the North Atlantic may contribute to the observed decoupling of long term Ep and SST in this location.

Line 34: Please specify "long-term trends". What trend?

Sentence in the short summary will be clarified: "*Records confirm long-term* CO_2 *record* but show contrasting relationships with the sea surface temperatures evolution"

Line 44: please specify what time interval shows "multimillion year warming" and what time intervals shows "cooling trends"

We propose to revise to:

"However, the long term decline in CO2 estimated by existing proxy records contrasts with the rather stable climatic state with multimillion year warming (e.g. Late Oligocene Warming) and cooling (e.g. Mi1 glaciation) trends interpreted from deep ocean (Cramer et al., 2011; Lear et al., 2000), and surface ocean records (Guitián et al., 2019; Liu et al., 2009; O'Brien et al., 2020) and with estimated Antarctic Ice sheet volume and sea level (Lear et al., 2004;Liebrand et al., 2017; Miller et al., 2020)."

Line 54: delete "globally"?

We will revise to: "at any given site"

Line 56: References are needed.

We will add here Rau *et al.*, (1996), as well as Stoll *et al.*, (2019), which discusses both growth rate and light.

Line 60: Full name of "m. y." is needed

Will be adjusted in the new version of the text

Line 60: Please specify "two sites on the south American margin".

Will be adjusted in the new version of the text

Line 61: I would add the name of the Site for the additional North Atlantic record

Will be adjusted in the new version of the text

Line 56-63: please reorganize these sentences. The current sentences are not in logic order. I would put the sentence "In this study, we produce a new long-term record of ε_p over the Oligocene to Miocene time interval at two new, widely separated locations" right after "One approach to evaluate the relative contribution of physiological factors vs CO2 is to produce ε_p records from sites of widely contrasting oceanographic setting...". The difference of environmental factors (important for physiological factors of coccolithophores) between Site 1406 and Site 1168 should also be clarified in order to make it connected to the previous sentence.

We appreciate the suggestion and propose to revise as a start of a new paragraph and to read:

"One approach to evaluate the relative contribution of physiological factors vs CO_2 is to produce εp records from sites of widely contrasting oceanographic setting, where the CO_2 signal may be expected to be common to both locations but the environmental factors such as nutrient availability might not be expected to change in unison. In this study, we produce a new long-term record of εp over the Oligocene to Miocene time interval at two new, widely separated locations: IODP Site1406 in the subtropical North Atlantic off the Newfoundland coast, and ODP 1168 in the Southern Ocean off of Tasmania. We also increase the resolution of determinations at the equatorial Atlantic ODP 925. The existing εp -based CO_2 estimations for the Oligocene are derived from ~1 million year resolution measurements from two sites (Site 925 and 516) on the South American margin of the equatorial and South Atlantic; in the early Miocene an additional North Atlantic record (Site 608) provides data (CenCO2PIP Consortium, 2023)."

Line 68-69: "an indicator of high-latitude temperature and Antarctic ice sheet extent and/or volume" is not accurate. Variations in Benthic δ 18O are controlled by changes in both deep-water temperature and ice volume.

We propose to revise to the strict proxy interpretation: "*Variations in benthic* $\delta^{18}O$ are controlled by changes in both deep-water temperature and deep ocean $\delta^{18}O_{sw}$ which reflects ice volume."

Line 69: what do "These long-term relationships" indicate? "higher resolution" - higher than what?

For clarification we propose to rewrite to:

"We further measure Ep and benthic $\delta^{18}O$ *at approximately 20-30 ky resolution over a series of eccentricity cycles in the early Oligocene at IODP 1406."*

Line 70: climate dynamics is a broad concept. Please clarify it.

We clarify by proposing to rewrite to "*climate*" to refer the broad climate indicators ($\delta^{18}O$, SST) used.

Line 74: Please add the full name of CO2[aq].

Full name will be added in the new version of the text.

Line 75: what do "These" refer to?

We will replace it with: "Physiological factors were initially ... "

Line 80-81: Please add the equation $\varepsilon_{p} = \varepsilon_{f} - b/CO2[aq]$, which makes it easy to read.

Following the reviewer suggestion we will add the equation in the new version of the manuscript.

Line 83-87: Some statements are incorrect. Zhang et al. (2013) also applied modern relationships between b and phosphate. Bolton et al. (2016) and Henderiks and Pagani (2007) do not estimate the difference between the modern b value at the site and the paleo-setting b value. Bolton et al. (2016) uses previous formulations of the relationship between cell size and b, which is derived from Henderiks and Pagani (2007).

In this overview of the introduction we stand by the accuracy of the descriptions included. Zhang *et al.*, (2013) applied the range of phosphorus concentrations in the modern surface water above the site to estimate the modern *b* value (from regressions between *b* and phosphate) and applied this modern *b* value to the past calculation of CO_2 . This is equivalent to our concise statement that their study assumed the modern *b*-value for that oceanographic setting remained constant in the past.

The reviewer's assertion that Bolton *et al.*, (2016) use previous formulations of the relationships between cell size and *b* is not a more accurate characterization of the correction applied. Bolton *et al.*, (2016) have generated a curve of variation in *b* using additional productivity indicators, the alkenone accumulation rate and the coccolith Sr/Ca rate (which have not been discussed in Henderiks and Pagani, (2007)) and computed these as variations between the paleo- and modern *b* value. Thus, we believe that our original statement that this work "*estimated the difference between the modern b value at the site and the paleo-setting b value from productivity proxies or proxies for coccolithophore size*" is both concise and accurate for the scope of the introduction section.

In the new version of the text, following the reviewer suggestion we will segregate the reference to Henderiks and Pagani, (2007) to indicate that it exclusively evaluated the relationship between *b* and size

variations (and not other growth rate proxies) to avoid confusion by juxtaposition with the description of Bolton *et al.*, (2016).

We hope that the new revised text address this comment :

...previous pCO_2 calculates have either (1) assumed the modern b-value for that oceanographic setting remained constant in the past (e.g. Zhang et al., 2013), (2) applied modern relationships between b and phosphate and a simulated paleo-surface ocean phosphate concentration at the site (Pagani et al., 2011), (3) estimated the difference between the modern b value at the site and the paleo-setting b value from productivity proxies (Bolton et al., 2016) or (4) applied variation in the b value at the site based on proxies for coccolithophore size (Henderiks and Pagani, 2007).

Line 87-88: Please add references.

Line 88: The sentence "b term is not well predicted by growth rate, light or cell size alone in a diffusive model" is confusing.

To address both reviewer comments we propose combining with the next sentence:

"Despite the appeal of this approach, a recent re-evaluation of cultures and field observations suggest the *b* term is not well predicted by growth rate, light or cell size alone in a diffusive model but that additional effects occur from carbon concentration mechanisms (CCM) on carbon uptake at lower CO_2 concentrations, which cause a deviation in the CO_2 dependence from the theoretical hyperbolic relationship (Hernández-Almeida et al., 2020; Stoll et al., 2019)."

Line 93-94: Could the author provide a brief implication of lower Rubisco fractionation?

We suggest rewriting: "The lower Rubisco fractionation has implies a lower sensitivity of εp to CO2 (e.g. as explored in González-Lanchas et al., (2021))".

Line 96: what does "This approach" refer to? The previous sentence does not mention any approach. Line 96-97: "the observed slope of ε_{0} dependence on CO2" is difficult to understand.

We suggest rewriting as: "A meta-analysis of experimental culture data (Stoll et al., 2019) suggests that εp features a logarithmic dependence on CO_2 , rather than the hyperbolic dependence implied by (Rau et al., 1997). **This analysis** does not resolve the mechanisms for the form of the observed **relationship between \varepsilon p and** CO_2 , but over the range of $CO_2_{(aq)}$ from 5 to 30 μ M, it provides an empirical relationship for interpreting the magnitude of $CO_2_{(aq)}$ change implied by a given εp change."

Line 103: Change "growth rate" to "growth rate μ_i "

Symbol will be added to the sentence as suggested.

Line 107: Please add references after "While cell size can be estimated from coccolith length".

We will add here the Henderiks and Pagani, (2007).

Line 116-117: Please clarify how 0.5 ‰ decrease in ε_p per 1°C warming is indistinguishable from the prediction of growth rate effect on ε_p ? Krumhardt et al. (2017) only demonstrates the increases in sea surface temperature lead to faster coccolithophore growth rates.

Krumhardt *et al.*, (2017) includes a quantification of the temperature effect on growth rates which Torres *et al.*, (2024) show is of the proper magnitude to explain the observed temperature effect on Ep.

To provide a more detailed description we will expand this paragraph including references:

"Recent culture studies document a 0.5 ‰ decrease in ɛp per 1°C warming (Torres Romero et al., 2024), and show that this magnitude is identical to the product of ɛp dependence on growth rate (Stoll et al., 2019) and the modeled temperature dependence of coccolithophore growth rates (Krumhardt et al., 2017) derived from diverse culture and field studies (Fielding et al., 2013; Behrenfeld et al., 2005; Sherman et al., 2016)."

Line 134: Please specify "higher resolution".

The new text will clarify: Additionally, 61 samples (at approximately 15 ky sampling) interval for bulk carbonate isotopes were obtained from IODP 1406 within the 29-30 Ma time window, of which 29 were processed for benthic foraminiferal isotopes and 22 yielded biomarkers sufficient for analysis.

Line 139-140: Replace "The ODP Site 1168 age model" to "The age model of ODP Site 1168". Similar issues occur throughout this manuscript. Please revise accordingly.

This will be revised.

Line 146: what ages do "the two ODP 1168 samples deeper than the Sr isotope measurements" correspond to?

We will detail this refers to two samples deeper than 562 mbsf

Figure 1: what is ODSN?

New caption of the figure will include the complete reference. ODSN refers to Plate Tectonic Reconstruction Service from the Ocean Drilling Stratigraphic Network (<u>https://www.odsn.de/</u>) using the data from Hay et al., (1999).

Line 159, 170, and 189: Change the bold text to normal formatting. Similar issues are present throughout the manuscript. Please revise them accordingly.

This will be adjusted in revision.

Line 171: please specify the age of "the young set of samples"

The new version of the text will detail that this refers to samples younger than 23.1 Ma.

Line 173-176: the sentence flow is not clear. Please reorganize these sentences.

Following the suggestion to improve the organization of this methods description we proposed the revised text:

"The RTX-200 column provided substantially improved resolution of C38 peaks, allowing quantification of C38:2 and C38:3 ME peaks. For samples between the ages of 23.1 and 29.1 Ma in ODP 1168 the RTX-200 column still did not sufficiently resolve coelutions on the C37:3 peaks. Therefore, for this interval we provide temperatures estimated from the *Uk'38ME* ratio applying the Novak et al. (2022) core top calibration."

Line 207-208: References are needed.

ODP 1168 evolving water depth is described in the Site and Sediments section referenced in Line 148. Following the suggestion, reference will be included here.

Line 209-210: Guitián et al. (2020) does not demonstrate that the bulk carbonate is dominated by Reticulofenestra coccoliths. Line 212-213: Here the authors assume that δ^{13} C of bulk carbonates is equivalent to the δ^{13} C of coccolith. However, they do not provide any evidence to support this assumption.

We thank the reviewer for arising that a clarification is needed here. Guitian *et al.*, (2020) describe that the coccolith fraction is dominated by *Reticulofenestra*. We will additionally clarify that the foraminfera content is very low. For Site 1406 sample content complete description can be found in Guitian *et al.*, (2019). We will revise lines 203-204:

"Although the foraminifera content in Site 1406 and 925 is very low, features sufficient well preserved benthic foraminifera, mainly epifaunal Cibicidoides spp. larger than..." and line 206 states: "...ODP Site 1168 benthic foraminifera were scarce for picking"

And lines 208-210

"Consequently, to follow the same approach for all studied records we calculate the $\delta^{13}C$ DIC from the $\delta^{13}C$ measured on the bulk carbonate, which is dominated by calcareous nannofossils, for which previous studies show Reticulofenestra to be the most abundant genera (Guitián et al., 2020)"

Line 213: The citation should be the original paper, McClelland et al. (2017), rather than Stoll et al. (2019).

The study by Stoll *et al.*, (2019) has aggregated results from cultures of multiple studies including McClelland *et al.*, (2017). Therefore in this case the meta analysis of Stoll *et al.*, (2019) would be the correct citation.

Line 215: Guitián et al. (2019) describes the method for measuring stable isotopes of benthic foraminifera, not bulk carbonate. Before the section "Estimation of aqueous carbon dioxide δ^{13} C", a section describing the method for measuring stable isotopes of bulk carbonate is needed, including details on sample preprocessing.

We thank the reviewer for noticing this gap in the method description. New text will be revised to: **Bulk** *carbonate and benthic foraminifera* were measured using analytical techniques described in in Guitián et al., (2019) with the guidelines from Breitenbach and Bernasconi, (2011) for small carbonate samples on a GAS BENCH II Delta V Plus irMS from Thermo Scientific with international (NBS-19 & 18) and in-house carbonate as standards achieving a precision of 0.07 ‰.

Line 237-238: Do the authors use the value in equation (1) or the linear relationship between ε_p and cell radius? The slope of the linear relationship between ε_p and cell radius, derived from a compilation of culture experiments, is certainly different from the value in equation (1). Please specify the sensitivity of ε_p to cell radius used here and provide justification for its selection.

For this exercise the sensitivity is the referred to the equation (1) following the empirical relationship from the culture dataset assuming only varying size. New text will clarify:

"We complete a similar exercise for cell radius, calculating the deviation in \varepsilon p only relative to the median cell size, for each point using the culture dependence of \varepsilon p on cell radius shown in equation (1)."

Line 247: Replace "26 ma" to "26 Ma".

This will be adjusted in revised text.

Line 249: Replace "from 28.8 to 29.6 Ma" to "from 29.6 to 28.8 Ma". Similar issues occur throughout this manuscript. Please revise accordingly.

These issues will be adjusted in the revised text.

Line 250: "Several ~ 100 ky orbital scale variations of 0.75 % benthic δ 18O and bulk δ 18O" is incomprehensible.

Will revise to: "Over several~ 100 ky orbital cycles, variations of 0.75 ‰ benthic $\delta^{18}O$ and bulk $\delta^{18}O$ are observed, consistent with previous findings of high 100 ky power in benthic $\delta^{18}O$ in other sites during this time period (Liebrand et al., 2017)."

Figure 2: Did the authors measure δ^{13} C of the bulk carbonate for all three sites? The methods section does not clarify which sites were analyzed for bulk carbonate carbon isotopes.

In this study bulk carbonate was measured for all three sites and values are reported in the data supplement. Method section will clarify in line 208:

"Consequently, to follow the same approach for all studied records we calculate the $\delta^{13}C$ DIC from the $\delta^{13}C$ measured on the bulk carbonate, which is dominated by calcareous nannofossils, for which previous studies show Reticulofenestra to be the most abundant genera (Guitián et al., 2020)"

Comments to figure 2:

Line 255: Instead of using solid and transparent lines, I recommend using different colors for the lines.

Line 258: White symbols are not visible on this figure; consider using a more visible color.

Lines 259–260: To maintain consistency, I suggest using either 1σ or 2σ for all the error bars.

Figure will be adjusted following reviewer suggestions.

Line 267: Change "an overall low and stable early Miocene" to "overall low and stable values in the early Miocene"?

Text will be revised accordingly.

Line 272: Is Curry et al. (1995) the correct citation? Curry et al. (1995) is the Initial Report for Leg 154, covering ODP Site 925 alone. It does not include DSDP 516 or ODP 608.

We thank the reviewer for noticing the missing references for the age models datums as described in the supplementary material Table S2. Sites 925 and 516 events and source calibrations are detailed in Guitian *et al.*, (2020) and Site 608 are from CenCO2PIP Consortium, (2023). Citations will be included in the revised text.

Line 272: The phrase "As seen in sites 1168 and 1406" is confusing, as this paper does not present the Sr isotopic stratigraphy of Sites 1168 and 1406.

Following the reviewer comment, we revise the text to clarify: "As previous studies document for sites 1168 and 1406, Sr isotopic stratigraphy can adjust age determinations by 0.5 to 1 Myr. or even up to 2 Myr in a few cases (Stoll et al., 2024)."

Line 282 and 287: "within age uncertainty of the decrease" and "within the age model uncertainty of the minimum" are difficult to understand.

Following the reviewer comment, we revise the text to clarify:

"A steep cp decline between at 21 and 20 Ma in Site 516 may be within age uncertainty of the decrease observed between 20 and 19 Ma at ODP 1168 and ODP 925"

"The characteristic minimum in εp from 18 to 17 Ma is potentially within the age model uncertainty of the 19 Ma minimum in εp at in 1168 and the 18.5 Ma minimum identified at Site 1406."

Line 284: "5‰ peak" is confusing. Do the authors mean "5‰ increase"?

Text will clarify this with "a transient 5‰ positive excursion"

Line 295: the title is not accurate.

Following the reviewer suggestion, we propose to modify the section name to "*Potential for size and nutrient effects on ep*"

Line 296: The authors have not discussed the effect of CO2 on ε_p yet.

The background section (section 2) has discussed the influence of CO_2 and physiological factors on Ep. Here in the discussion we elect to first estimate the influence of physiological parameters on Ep before assessing the Ep variation which may be due to CO_2 . We hope that the new section name detailed in the previous comment emphasizes the aim of the section.

Line 296: replace "cell surface area to volume ratio" with "cell size"

We will revise the text accordingly.

Line 304: Confusing. How does the deeper mixing cause the lower mean light levels?

Deep mixing brings the cells more time into the lower photic zone where light levels are lower. We propose to cite Hernández-Almeida *et al.*, (2020) which discusses this correlation in detail:

Line 316-322: The main point of this paragraph is not clear.Line 318 and 323: Misra and Froelich (2012) do not suggest an increase in erosion and weathering rates from the Oligocene to the early Miocene. In fact, their $\delta^2 Li_{sw}$ data show little change from the middle Oligocene to the early Miocene.

We thank the reviewer for suggesting that a clarification is needed in this paragraph. This paragraph outlines the potential interpretations of bioSi burial, and presents the multiple caveats surrounding its interpretation as evidence for an increase in ocean nutrient concentrations. We agree that the data by Misra and Froelcih (2012) show little change, however there is an increase in $\delta^7 Li_{SW}$ in the early Miocene and have a data gap from much of the early Oligocene. The sentence refers to the evidence among all three isotopic systems (which have complementary data coverage across the Oligocene to early Miocene) and includes caveats about the interpretation of the isotopic systems (Rugenstein *et al.*, 2019).

To clarify the main point of the paragraph we suggest:

"The drivers for increasing bioSi burial rates at Site 1406 are not clear. They could reflect a global increase in nutrient delivery or local effects. Important changes in the rate of continental weathering within the Oligocene- early Miocene are often interpreted from the evolution of radiogenic isotopes of Sr, Li and Os (Misra and Froelich, 2012) including the steep rise in ⁸⁷Sr/⁸⁶Sr, although the precise origin of the late Eocene and Miocene increase in ⁸⁷Sr/⁸⁶Sr remains under discussion (Rugenstein et al., 2019). On a global scale, the nutrient delivery may be conditioned by the riverine supply of P from continental erosion and weathering of P containing minerals. Yet, on the time scales examined in our records, much longer than the residence time of P, the net effect on nutrient concentrations depends on the balance of the supply and the nutrient removal in sediments."

Line 399: Is "1 ‰ range" typo?

In the specified time interval there is a 1 % range in the δ^{18} O values as illustrated in Figure 7.

Line 403: what do "these variables" indicate?

We propose to rephrase for clarification: "... the impact of temperature-stimulated carbon fixation rates is not a significant impact on the relationship between εp and SST or $\delta^{18}O$ benthic – a temperature-corrected εp record for the 29 to 29.6 Ma interval would still not exhibit an inverse relationship between εp and $\delta^{18}O$ benthic as observed in the late Pleistocene glacial cycles (Hernández-Almeida et al., 2023)."

Figure 7: The numeric labels on the x- and y-axes (e.g., "2,2" and similar) are difficult to read. Please adjust them to a clearer format, such as "2.2." Similar issues occur in other figures. Please revise accordingly. Figure 7: please add a, b, and c to each panel of this figure.

Labels will be adjusted as suggested.

Line 409: r2=-0.34 is not possible. R-squared is always a positive value.

The typo will be corrected.

Figure 8: Please add the full name of MMCO, Mi-1, LOW, and MOGI.

These will be added to the figure caption.

Line 443: delete very. what do "a different set of feedbacks" mean? Different from what?

Propose to rephrase: If the interpretation of εp as a CO_2 decline is correct, it suggests that climate sensitivity was either significantly weaker so that no appreciable change in global mean surface temperature occurred, or that available paleotemperature records are significantly biased by regional heat transport effects or available paleotemperature estimates reflect a significant misinterpretation of measured biomarker signals.

Line 446: Please clarify "a substantially different relationship between ice expansion and CO2."

Sentence will be adjusted and word omitted

Line 451-452: Please delete "and decline in radiative forcing from the greenhouse effect."

Sentence will be adjusted as suggested

Line 453: there is no evidence to support the claim "the ODP Site 1168 temperature trend reflects global temperature". SST change of Site 1168 is likely a regional signal.

We propose rewording to state that we have raised it as a possibility, that among the two temperature time series, 1168 may potentially be more representative of a global trend. With two sites temperature trends, one from sediment drifts in the North Atlantic, there is no a priori reason to assume that the North Atlantic trend is more representative than the 1168 record:

"If the ODP Site 1168 temperature trend is more representative of global average temperature trends, whereas the long term alkenone temperature record at Newfoundland Ridge Site 1406 and Site 1404 (Liu et al. 2018) is dominated by variations in the heat transport from the Gulf Stream, then the 1168 temperature trend may reflect the signal of radiative greenhouse forcing."

Line 462: The term "late Oligocene divergence" is not easy to understand. Please consider replacing "divergence" with a clearer term throughout the manuscript to improve clarity.

This section has been reworded as described in the response to Referee #1 including this replacing suggestion.

Line 465: The conclusion section merely repeats the results presented in earlier sections. In addition to summarizing the findings, the conclusion should discuss the broader implications of the results.

We thank the reviewer for the suggestions to include in the conclusions section. However, we have introduced the broader implications of the results and suggestions for next steps in section 4.4. We follow the style suggestions from the EGU Journals Webinar of Ken Carslaw, that the Conclusion should not feature further discussion but summarize the findings.

Figures and supplementary figures: The current color scheme, particularly the use of red and green in the same figure, is not color-blind-friendly. Please adjust the colors to enhance accessibility and readability for all readers.

Following the reviewer some of the colors in the figure symbol will be revised. In Figure 4 the squares will be varied in size so that they are distinguishable by features other than color. Figure 8 will be revised with different dash patterns for the doubling pCO_2 lines. Additionally, figures will be tested through the color blindness simulator again to revise the color scheme accordingly.

References included in this reply not previously included in the manuscript:

Behrenfeld, M. J., Boss, E., Siegel, D. A., and Shea, D. M. (2005). Carbon-based ocean productivity and phytoplankton physiology from space. *Glob. Biogeochem. Cycles* 19. doi:10.1029/2004GB002299

Fielding, S. R. (2013). Emiliania huxleyi specific growth rate dependence on temperature. *Limnol. Oceanogr.* 58, 663–666. doi:10.4319/lo.2013.58.2.0663

Hay, W. W., DeConto, R. M., Wold, C. N., Wilson, K. M., Voigt, S., Schulz, M., ... & Söding, E. (1999). Alternative global Cretaceous paleogeography.

Sherman, E., Moore, J. K., Primeau, F., and Tanouye, D. (2016). Temperature influence on phytoplankton community growth rates. *Glob. Biogeochem. Cycles* 30, 550–559. doi:10.1002/2015GB005272