Revised alkenone $\delta^{13}C$ based CO$_2$ estimates during the Plio-Pleistocene

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Abstract. Here we revisit reported alkenone $\delta^{13}C$ ($\delta^{13}C_{C37}$) based CO$_2$ records during the Plio-Pleistocene and apply a refined approach to better constrain the dynamic range of CO$_2$ during the time. Specifically, we consider ways to correct for regional differences in physical oceanographic factors. As a result of our relatively simple approach we find that offsets of ~150 ppm between reported $\delta^{13}C_{C37}$ CO$_2$ records from different sites can be significantly reduced. This confirms that better constraints on environmental variables, including physical oceanographic controls on depth and season of production are key aspects for improving $\delta^{13}C_{C37}$ based CO$_2$ estimates. The revised $\delta^{13}C_{C37}$ CO$_2$ datasets suggest that Plio-Pleistocene CO$_2$ levels are 180-350 ppm, which is consistent with the most of reported CO$_2$ reconstructions, though their upper end of Pliocene CO$_2$ levels are lower than that of some CO$_2$ estimates.

1 Introduction

The reconstruction of CO$_2$ levels in the past is a crucial objective in palaeoclimate research. Continuous, reliable CO$_2$ records in the past have come from direct measurements of CO$_2$ in ice-cores (Siegenthaler et al., 2005; Lüthi et al., 2008). However, the currently available (continuous) ice-core based CO$_2$ record is limited to the past 800 krys (Lüthi et al., 2008). To reconstruct the continuous CO$_2$ record in the past, through warmer climatic base-lines (which predate the ice core record), indirect methodologies such as proxies and models must be applied. Several proxies have been proposed and applied to reconstruct CO$_2$ in the geologically deep past (Beerling and Royer, 2011). Reconstructed CO$_2$ data significantly contribute to constraining climate evolution and linkages to the carbon cycle. For instance, recent CO$_2$ reconstructions in the Cenozoic era indicate that a long-term decline in CO$_2$ levels was a key driver of global cooling and major glaciations in the Cenozoic (Kürschner et al., 2008; Hönisch et al., 2009; Pearson et al., 2009; Pagani et al., 2011; Beerling and Royer, 2011; Zhang et al., 2013; Greenop et al., 2014; Anagnostou et al., 2016; Cui et al., 2020; Anagnostou et al., 2020; Raitzsch et al., 2021).

The climate evolution from the Pliocene to the Pleistocene, characterized by a transition from warmer than the present climate to the Pleistocene cold-house, is one of the most dramatic climatic changes of the late Neogene. In order to better understand the role of CO$_2$ in Plio-Pleistocene climate evolution, CO$_2$ reconstruction has been conducted based on geochemical and botanical approaches including phytoplankton $\delta^{13}C$ (Pagani et al., 2010; Seki et al., 2010; Badger et al., 2013; Stoll et al., 2019;
Badger et al., 2019), carbonate δ11B (Bartoli et al., 2011; Martínez-Botí et al., 2015; Chalk et al., 2017; Dyez et al., 2018; de la Vega et al., 2020), paleosol (Da et al., 2019), terrestrial plant δ13C (Cui et al., 2020) and leaf stomata (Kürschner et al., 1996). The most widely applied in recent decades derive from analyses of δ13C C37 (Pagani et al., 2010; Seki et al., 2010; Badger et al., 2013; Badger et al., 2019) and planktic foraminiferal δ11B (Bartoli et al., 2011; Martínez-Botí et al., 2015; Dyez et al., 2018; de la Vega et al., 2020) in marine sediment cores recovered from the different parts of the global ocean (Fig. 1).

The accumulated data from this research effort suggests that CO2 played a substantial role in Plio-Pleistocene climate evolution (Fig. 2b). However, although all the proxy CO2 records show the long-term decreasing trend throughout the Plio-Pleistocene, absolute estimates of CO2 level markedly differ among the data, ranging from 200 to 600 ppm (Fig. 2b). The wider range of CO2 estimates may be attributed to variable δ13C C37 CO2 estimates depending on distinct oceanographic settings (Seki et al., 2010; Pagani et al., 2005) and large offset of δ11B estimates between the different foraminifera species (Martínez-Botí et al., 2015; Dyez et al., 2018). Of course, fundamental advances and refinements in our understanding of the δ13C C37 proxy, beyond the model conceptualized by Pagani et al. (2002) are possible, and progress is being made in this regard. For example in a) constraining the influence of growth rate (μ), cell size (r), and membrane permeability (P) on the empirical ‘b’ factor (Bolton et al., 2015; Stoll et al., 2019; Zhang et al., 2020) and b) on emerging culture-based models, which will attempt to quantitatively consider the influence of cell size and irradiance (Phelps et al., 2020). However, here we use the original “Pagani” et al. (2002) diffusive transport model which built on earlier work by Bidigare et al. (1997, 1999). Our aim is not to provide newly definitive CO2 estimates but to demonstrate the importance of physical oceanography, as all δ13C C37 CO2 reconstructions (even using refined models) rely on some assumptions that are sensitive to regional oceanographic settings (Pagani et al., 2011). Not correcting for these differences can yield errors up to 150 ppm for individual sites and regions (Pagani et al., 2010). This is demonstrated by the relatively higher alkenone based δ13C C37 CO2 values (>350 ppm) in the Pleistocene (Pagani et al., 2010), which are inconsistent with blue ice derived CO2 data which show that the late Pliocene CO2 levels do not exceed 280 ppm (Voosen, 2017; Yan et al., 2019).

δ11B CO2 (Foster et al, 2008; Chalk et al., 2017) seems to represent better the late Pleistocene glacial-interglacial CO2 fluctuations observed in ice-core CO2 records (Siegenthaler et al., 2005), and thus likely provide more reliable estimates than alkenone δ13C C37 CO2 (Pagani et al., 2010; Seki et al., 2010; Badger et al., 2013). However, a recent study has exhibited Pliocene CO2 estimates markedly differ among planktic foraminiferal species (Globigerinoides ruber (G. ruber) vs. Torilobatus sacculifer (T. sacculifer)) (Dyez et al., 2018). Subsequently, an attempt was made to reconstruct CO2 based on the δ13C C37 method with a statistical model approach that constrained the physiological factors and the active carbon transport mechanism, which affects CO2 reconstruction (Stoll et al., 2019). The attempt resulted in a much wider range of CO2 in the Pliocene (200-620 ppm). Thus, there is still room for verification in terms of an absolute values and dynamic ranges of CO2 (Fig. 2b). Here, we revisit globally distributed δ13C C37 CO2 estimates from the Pliocene to today, refine the methods (within the framework of the original Pagani model), and then attempt to reconcile the discrepancies in the published δ13C C37 CO2 records that span the last 5 Myrs.
2 Material and Methods

2.1 Sample information and chronology

Locations of ODP sediment cores used in this study are shown in Fig. 1. We utilize and reinterpret previously published Plio-Pleistocene CO$_2$ datasets (Sites 806, 925, 982, 999, 1208, and 1241) from Pagani et al. (2010) and Seki et al. (2010). Table 1 provides sample information for the data compiled in this manuscript. Information used in revising CO$_2$ estimates are shown, including the surface mixed layer (SML) depth and phosphate concentrations ([PO$_4^{3-}$]) derived from the WOA09 (Locarnini et al., 2009), the air-sea CO$_2$ disequilibrium from Takahashi et al. (2009), the regressions applied to calculate the physiologically dependent b-term, and sea surface temperature (SST).

2.2 $\delta^{13}C_{C37}$ CO$_2$ proxy based on the diffusive transport model

The $\delta^{13}C$ values of alkenones produced by haptophyte algae, combined with foraminiferal records of surface ocean dissolved inorganic carbon $\delta^{13}C$, can be used to calculate haptophyte carbon isotopic fractionation of ($\epsilon_p$) during photosynthesis. This, in turn, is related to the concentration of aqueous CO$_2$ in ocean surface waters (Pagani et al., 2002; Bidigare et al., 1997, 1999). In areas of air-sea equilibrium and where paleo-SST can be determined from $U_{37}^R$ paleothermometry, [CO$_2$(aq)] can be used to determine CO$_2$.

2.3 Calculation of mixed layer temperatures

For all previously published data, temperatures in the surface mixed layer were estimated from $U_{37}^R$ paleothermometry. In this study, $U_{37}^R$ indices in sediments were converted into growth temperatures using the equation of Sonzogni et al. (1997) that is optimized to high SSTs $>24\,^\circ C$ for Sites 806, 925, 999, and 1241, whereas the global core top derived calibration of Conte et al. (2006) was applied to the extratropical sites (Sites 982 and 1208). The analytical precision of $U_{37}^R$-derived temperature from replicate analyses is $\pm0.3\,^\circ C$.

2.4 Calculation of $\epsilon_{p37:2}$ values

The carbon isotope fractionation by haptophyte algae during photosynthesis ($\epsilon_{p37:2}$), is calculated from the $\delta^{13}C$ values of di-unsaturated alkenone and the calcite shells of planktic foraminifera using the following equation:

$$\epsilon_{p37:2} = \left[\frac{\delta CO_2(aq) + 1000}{\delta org + 1000}\right] - 1 \times 1000$$

where $\delta CO_2(aq)$ and $\delta org$ are the $\delta^{13}C$ values of CO$_2$(aq) and haptophyte biomass, respectively. The former is determined from the carbon isotopic composition of $T.\,sacculifer$ and $G.\,ruber$, as well as the temperature-dependent relationship between $\delta CO_2(aq)$ and $\delta$CaCO$_3$ (Romanek et al., 1992; Mook et al., 1974). SSTs were estimated from the $U_{37}^R$ paleotemperature proxies.
as discussed above. $\delta_{\text{org}}$ is derived from the $\delta^{13}$C values of di-unsaturated alkenones and corrected for the 4.2‰ depletion of alkenones relative to biomass (Bidigare et al., 1997).

### 2.5 Calculation of CO$_2$

The $\epsilon_p$ values of marine algae are a function of not only CO$_2$(aq) but also growth rate (Bidgare et al., 1997) and cell geometry (Popp et al., 1998). Therefore, correction of those effects on $\epsilon_p$ values is required. Field and experimental work both indicate that the relationship between $\epsilon_p$ and related factors is expressed as the following:

$$\epsilon_p = \epsilon_f - \frac{b}{[\text{CO}_2(\text{aq})]}$$

(2)

where $\epsilon_f$ is the isotope fractionation associated with carbon fixation by the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme (25‰; Bidigare et al., 1997). The b-term is an expression of physiological factors— including growth rate, membrane permeability, and cell geometry— that affect carbon discrimination by regulating the flux of CO$_2$ into and out of the cell. For $\delta^{13}$C$_{\text{C37}}$-derived $\epsilon_p$ values, and thus for the limited range of organisms synthesizing such compounds and for which cell geometry effects are thought to be minimal, field data reveal a significant correlation between the b-term and $[\text{PO}_4^{3-}]$ (Bidigare et al., 1997; Pagani et al., 2005) (Fig. 3):

$$b = 118.52x[\text{PO}_4^{3-}] + 84.07 (r^2 = 0.78)$$

(3)

The modern range of $[\text{PO}_4^{3-}]$ in the surface mixed layer is used to calculate b-term. Because a reliable $[\text{PO}_4^{3-}]$ proxy is not available so far, we assume that $[\text{PO}_4^{3-}]$ did not differ for the open marine settings in Fig.1 over the last 5 Myr. Finally, atmospheric CO$_2$ levels are calculated using these b-term values and assuming air-sea equilibrium (using salinity, SSTs, and Henry’s Law to convert CO$_2$(aq) to gas CO$_2$; Weiss, 1974).

### 3 Result and discussion

#### 3.1 $\delta^{13}$C$_{\text{C37}}$ derived CO$_2$ records based on the standard diffusive model approach

Figure 4a shows CO$_2$ records derived from the standard approach used in most previous publications: We have calculated the b-term using the global regression (Pagani et al., 2005), which assumes alkenone production depths at the surface of the mixed layer, and which assumes air-sea equilibrium at all sites. Although the trends are generally similar, indicating decreasing CO$_2$ levels over the past 5 Myr, the ranges are large (>150 ppm) in both recent and older sediments. In particular, CO$_2$ estimates are relatively low in the extra-tropics (Sites 982 and 1208) and higher in the tropics (Sites 806, 925, and 1241) except for the Caribbean Sea (Site 999).

The first application of the alkenone $\delta^{13}$C$_{\text{C37}}$ CO$_2$ proxy suggested it could be used to accurately reconstruct $[\text{CO}_2(\text{aq})]$ when SSTs and the b-term are well constrained (Pagani et al., 2002). If constraints on those parameters are the key to reconstruct
CO₂ from the proxy, the observed large gaps among the sites could be attributed to insufficient constraints on such key parameters. It should be noted that the $\delta_{p}$ value of marine algae is a function of not only $[\text{CO}_2(aq)]$, but also the b-term. Modern ranges of $[\text{PO}_4^{3-}]$ in the euphotic zone are typically used to determine the b-term in paleo-investigations (Seki et al., 2010; Pagani et al., 2010). However, the relationship between the b-term and $[\text{PO}_4^{3-}]$ depends on the oceanographic setting, resulting in significant variability in the global regression equation (Bidigare et al., 1997; Pagani et al., 2005) (Fig. 2). Therefore, we applied regional rather than global regressions to better constrain the b-term at specific sites (Site 806 and 1241; Fig. 4c; see Section 3.2. for the detail). Another factor in CO₂ determination is regional variation in air-sea disequilibrium ($\Delta$CO₂). Previous studies have reconstructed CO₂ levels based on the assumption of equilibrium between CO₂ in seawater and the atmosphere. However, air-sea ∆CO₂ spatially varies, generally with excess $[\text{CO}_2(aq)]$ in the tropics and deficiencies in the extratropics (Takahashi et al., 2009). Hence, we have reconsidered these data by first correcting for disequilibrium between ocean and atmosphere (Fig. 4b; See Section 3.3. for details). Also, we discuss other factors that potentially influence CO₂ reconstruction in Sections 3.4. and 3.5.

3.2 Geographical and temporal variations in the b-term

As suggested by the empirical relationship between the b-term and $[\text{PO}_4^{3-}]$ in modern surface ocean waters (Bidigare et al., 1997), the growth rate is a primary factor controlling the b-term in diverse phytoplankton, including haptophytes (Bidigare et al., 1997, 1999; Popp et al., 1999). Although that is factored into our calibration at each site, significant past changes in growth rate (which we infer to be broadly correlated to a regime’s primary productivity history) will bias CO₂ trends and estimates (Mix et al., 2003). For these reasons, we have precluded Sites 882 and 1012, for which there is evidence for strong past oceanographic and productivity variations which are hard to constrain (Pagani et al., 2010). All of the remaining CO₂ data used in this study exhibit similar long-term decreasing trends (Fig. 4a), suggesting that growth rate variations are subordinate controls on long-term trends. However, there are likely local variations in the controls on the b-term. Although there is a strong positive relationship ($r^2 = 0.78$) between phosphate concentration and the b-term in the global dataset (Bidigare et al., 1997, 1999; Pagani et al., 2005), there are systematic variations in the relationship among different oceanographic regimes. For example, b-term values are lower than the global regression trend in the Equatorial Pacific, Southern Ocean, and sub-Antarctic and higher in Santa Monica, the Northeast Pacific, and the Peru upwelling margin (Fig. 3). Such regional differences are not surprising because phosphate is unlikely to limit directly haptophyte growth, which is instead is probably governed by micronutrients (such as iron, zinc, and cobalt) (Bidigare et al., 1997). The relationships between concentrations of phosphate and the actual growth-limiting micronutrients might differ among regions. Thus, we explore here the application of regional regressions to better constrain the b-term. Currently, regional regressions are available for Sites 806 and 1241 (Equatorial Pacific regression) (Bidigare et al., 1997).

$$b = 109.8 \times [\text{PO}_4^{3-}] + 65.6 \quad (r^2 = 0.74)$$

We apply the regional regression (4) to these sites (Table 1).
3.3 Geographical and temporal variations in air-sea disequilibrium

As described earlier, the conversion from \([\text{CO}_2\text{(aq)}]\) to atmospheric \([\text{CO}_2]\) is based on Henry’s Law (Weiss, 1974) and using SSS (Table 1) and SST (described above), while assuming air-sea equilibrium. However, this assumption is likely invalid in some of the settings investigated. There is excess \([\text{CO}_2\text{(aq)}]\) in the equatorial ocean due to active upwelling and deficiency of \([\text{CO}_2\text{(aq)}]\) in extratropical regions (Takahashi et al., 2009). Therefore, we also show \([\text{CO}_2]\) reconstructions, in which we have corrected for air-sea \(\Delta\text{CO}_2\) at each site (Fig. 4b; correction shown in Table 1).

3.4 Temporal variations in cell geometry

Previous studies showed that variations in haptophyte cell geometry, if significant enough, can potentially affect \(\varepsilon_p\) values (Henderiks et al., 2007; Bolton et al., 2015; Stoll et al., 2019; Zhang et al., 2020). In a previous study (Seki et al., 2010), we explored the potential effects of such changes based on the approach of Henderiks et al. (2007), who proposed a geometry-corrected b-term using the coccolith size record of \textit{Reticulofenestra}, the postulated alkenone-producing haptophytes in the Caribbean Sea prior to the Pliocene (Kameo and Bralower, 2000). However, applying this approach to Site 999 results in significantly lower \([\text{CO}_2]\) levels (<150 ppm) for the Pliocene and Pleistocene compared to the other \([\text{CO}_2]\) estimates (Kürschner et al., 1996; Siegenthaler et al., 2005; Badger et al., 2013; Pagani et al., 2010; Martínez-Botí et al., 2015; Cui et al., 2020; de la Vega et al., 2020). This underestimation potentially illustrates the limitations for applying the relationship between \textit{Reticulofenestra} coccolith and cell sizes obtained from early Miocene species to the smaller species of the Pliocene.

Moreover, it has been suggested that \textit{Cyclicargolithus} was the most important alkenone producer during the Miocene (Plancq et al., 2012). Similarly, the alkenone concentration record in Caribbean Sea sediments (Seki et al., 2010) does not resemble that of \textit{Reticulofenestra} abundances (Sites 998, 999, and 1000) (Kameo and Sato, 2000). This decoupling suggests that \textit{Reticulofenestra} may not be a major alkenone producer. Thus, we suggest that a cell geometry correction based on \textit{Reticulofenestra} is not appropriate for the Caribbean Sea. Therefore, the effect of changes in growth rate and cell geometry of alkenone producers remains uncertain for \([\text{CO}_2]\) reconstruction. However, the effect of geometry change on b-term is probably not significant on Plio-Pleistocene timescales, since cell size does not significantly change in the tropical ocean during the Plio-Pleistocene (Bolton et al., 2015; Badger et al., 2019).

3.5 Other factors affecting \(\varepsilon_{p37:2}\)

As outlined above, \([\text{CO}_2\text{(aq)}]\) estimates are highly sensitive to the b-term, which represents an integration of various physiological variables but is especially dependent on haptophyte growth rate (Bidigare et al., 1997). Equation (2) implicitly assumes that \([\text{CO}_2]\) diffuses into the haptophyte cell, even though there is much evidence that haptophytes, including those that produce alkenones, employ carbon concentrating mechanisms (CCMs) (Raven et al., 2011; Reinfelder, 2011). Nor does the model account for the competing requirements for dissolved inorganic carbon for both cell growth and coccolith production and the resultant internal partitioning of the DIC pool. These factors are implicitly considered because Equation (2), as well as
the relationships between the b-term and nutrient concentrations, are empirical (Bidigare et al., 1997). However, the recently developed ACTI-CO model of Bolton and Stoll (2013) reveals the complexity of the controls on haptophyte \( \varepsilon_p \) values; in particular, it illustrates how isotopic partitioning between organic and inorganic components will be governed by a complex range of environmental and physiological factors. As such, we consider our CO\(_2\) estimates to be semi-quantitative and include them primarily to allow comparison to previous investigations. Nonetheless, the ACTI-CO model still indicates that higher [CO\(_2\)\(_{oa}\)] will yield higher \( \varepsilon_p \) values; as such, we consider the trends in atmospheric CO\(_2\) levels generated from \( \delta^{13}C_{C37} \)-derived \( \varepsilon_p \) values to be robust.

### 3.6 Revised \( \delta^{13}C_{C37} \) CO\(_2\) over the past 5 Myrs

With taking the factors described above into account, we revised published CO\(_2\) data from the Pacific Ocean and the Atlantic Ocean. Figure 4d shows revised CO\(_2\) records based on the refined approach (e.g. correcting offsets estimated for both \( \Delta CO_2 \) and regional [PO\(_4^{3-}\)]). The large difference (>150 ppm) in CO\(_2\) estimates among sites based on the standard approach (Fig. 4a) has been markedly reduced by ~100 ppm when we apply this refined method (Fig. 4d). Indeed, a combination of corrections, using both regional b-term regressions and accounting for air-sea \( \Delta CO_2 \), yields similar Pliocene to Pleistocene CO\(_2\) records (Fig. 4d). Importantly, the late Pleistocene CO\(_2\) estimates fall within the range of ice core CO\(_2\) records (Siegenthaler et al., 2005; Lüthi et al., 2008), blue ice CO\(_2\) (Higgins et al., 2015; Voosen, 2017; Yan et al., 2019) and T-inverse model derived CO\(_2\) (van de Wal et al., 2011) except for the glacial periods (for detail in Section 3.7) and ODP 982 (Figs. 4d). Overestimation of CO\(_2\) in ODP 982 suggests that the global regression for calculation of b-term is not suitable to the site. Despite the importance of oceanographic conditions – export production depth of alkenones, [PO\(_4^{3-}\)] depth profiles and their relationship to b-term, air-sea \( \Delta CO_2 \) – we have assumed that they did not differ markedly in the past. If these parameters have changed significantly over time, this would impact absolute reconstructions of CO\(_2\) levels as well as the temporal trends. As records from several sites all exhibit similar long-term trends, despite representing diverse oceanographic settings, we suggest that these parameters have been relatively stable, or at least a relatively minor factor, over the past 5 Myrs for the open ocean environment. Indeed, a relatively stable b-term on Plio-Pleistocene timescales has been suggested in some regions (Bolton et al., 2015; Mejia et al., 2017; Stoll et al., 2019).

### 3.7 Comparison of \( \delta^{13}C_{C37} \) CO\(_2\) records with those derived from the ice cores and other CO\(_2\) proxies

Comparison of ice core and \( \delta^{13}C_{C37} \) CO\(_2\) shows that some \( \delta^{13}C_{C37} \) CO\(_2\) records based on the global b-term regression are higher than ice core CO\(_2\) data (Fig. 4a). However, the offset is significantly reduced if regional b-term regressions and air-sea \( \Delta CO_2 \) are applied (Fig. 4d) although \( \delta^{13}C_{C37} \) CO\(_2\) estimates are still significantly higher than that of ice core records during the glacial periods. Downward revision of the \( \delta^{13}C_{C37} \) based CO\(_2\) estimates in the Pleistocene are consistent well with recent CO\(_2\) estimates based on paleosol, which show low CO\(_2\) values (below 300 ppm) throughout the Pleistocene (Da et al., 2019) (Fig. 2b). The large offsets during the glacial periods are possibly attributed to dramatic changes in growth rates of haptophytes (Benthi
al., 2005; Zhang et al., 2019), air-sea ΔCO2 (Jasper et al., 1994) during the pronounced glacial-interglacial cycles in the late Pleistocene or upregulation of algal CCM in the low CO2 glacial period (Stoll et al., 2019; Badger et al., 2019). Further consideration of reported δ13C C37 CO2 records in the late Pleistocene reveals that the relationship between δ13C C37 and ice core CO2 breaks down when dissolved CO2 concentration falls below 7 µmol/L (Badger, 2021).

It remains unclear how these factors will affect CO2 estimates in the Pliocene (5.4-2.4 Ma) (see below), and therefore I compare our CO2 estimates to those recently obtained using the G. ruber and T. sacculifer δ11B values of planktonic foraminifera (Seki et al., 2010; Martínez-Botí et al., 2015; Bartoli et al., 2011; Dyez et al., 2018; de la Vega et al., 2020). The revised δ13C C37 CO2 data suggest relatively low CO2 levels in the warm Pliocene (250-340 ppm from 3-4.5 Ma). This suggests that CO2 decreased from the Pliocene to the Pleistocene. Our results agree well with T. sacculifer δ11B CO2 (230-330 ppm) (Bartoli et al., 2011; Dyez et al., 2018) rather than the G. ruber δ11B CO2 reconstructions (Seki et al., 2010; Martínez-Botí et al., 2015; de la Vega et al., 2020), which exhibit relatively higher CO2 levels (250-470 ppm) (Fig. 2b).

The relatively higher G. ruber δ11B CO2 estimates in the Pliocene could largely reflect an inherently greater CO2 variability than that recorded by alkenones (Badger et al., 2013). This might be due to the aforementioned impact of important factors not explicitly considered in the Bidigare et al. (1997) model, i.e. CCMs (Stoll et al., 2019) and G-IG variabilities of growth rate (Zhang et al., 2019), cell size, and membrane permeability (Zhang et al., 2020). Zhang et al. (2019) and (2020) argued that term b value substantially varies associated with G-IG cycles in some regions. Discounting this results in an underestimation of δ13C C37 CO2 variability over the glacial cycles while Badger et al. (2019) suggest the importance of CCMs effect for overestimation of δ13C C37 CO2 in the glacial low CO2 conditions. However, CCM unlikely explains relatively low CO2 estimates in the Pliocene since upregulation of CCM results in overestimation of pCO2 when dissolved CO2 level is lower than 7 µM (Badger, 2021). Also, it should be noted that the CCM effect is unlikely upregulated in mid to high latitude oceans where the surface water temperature is enough cold and thus dissolved CO2 concentration in the surface mixed layer is enough high to allow algae to adopt diffusive carbon transport.

Another potential reason for the overestimation of glacial CO2 is an underestimation of G-IG variability of dissolved CO2 δ13C.

δ13C of dissolved CO2 in the past is estimated from δ13C of foraminifera carbonate shells, which is used to calculate aC. A previous study shows that carbon isotope fractionates into foraminiferal calcite as a function of seawater pH/CO2 (Spero et al., 1999’97) which must vary associated with the late Pleistocene G-IG cycle given that ~100 ppm variation of CO2 as suggested by the ice core CO2 record. Not considering this effect results in an overestimation of glacial CO2 levels. However, according to the relationship between foraminiferal δ13C and CO2 (Spero et al., 1997), this effect only explains approximately 10 ppm overestimation during the glacial. Thus, this effect is not sufficient to explain the mute signal of G-IG δ13C C37 CO2 variability.

Stoll et al. (2019) revisited δ13C C37 CO2 records in the two tropical sites (ODP 925 and 999) and revised estimates based on a statistical multilinear regression model, which accounts for light, growth rate, and [CO2(aq)] (Fig. 2b). The statistical model δ13C CO2 data broadly agrees with G. ruber δ11B CO2 estimates in the Pliocene. However, it should be noted that our revised δ13C C37 CO2 agrees well with not only T. sacculifer δ11B CO2 (Dyez et al., 2018) but also T-inverse model derived CO2 (van...
245 de Wal et al., 2011; Berends et al., 2019) during the Pliocene in terms of both long-term trend and orbital-scale fluctuation. Pliocene CO₂ estimates based on other independent proxies such as leaf stomata are below 360 ppm (Kürschner et al., 1996). Also, the dynamic range of G. ruber δ¹¹B CO₂ (300-480 ppm) in the Pliocene is approximately twice as much as that of the deep glacial-interglacial cycles (180-280 ppm) in the late Pleistocene (Siegenthaler et al., 2005; Lüthi et al., 2008), despite the latter exhibiting smaller orbital-scale climate variations compared to that of the late Pleistocene. Furthermore, the fluctuation range of the statistical model CO₂ in the Pliocene (200-620 ppm) is much larger than that of G. ruber δ¹¹B CO₂ (Stoll et al., 2019) (Fig. 2b). Such a large fluctuation is difficult to explain given that G-IG climate fluctuation in Pliocene is much smaller than that of the late Pleistocene.

3.7 Consideration of the discrepancy among the CO₂ proxies

A large offset of CO₂ estimates also exists between G. ruber and T. sacculifer δ¹¹B proxies throughout the Plio-Pleistocene (Dyez et al., 2018). T. sacculifer δ¹¹B CO₂ records show relatively low values (well below 350 ppm) during the Pliocene (Bartolli et al., 2011; Dyez et al., 2018), while many G. ruber δ¹¹B CO₂ values are above 350 ppm (Seki et al., 2010; Martinez-Botí et al., 2015; de la Vega et al., 2020) (Fig. 5b). Potential causes for the discrepancy have been discussed in Dyez et al. (2018) and de la Vega et al. (2020). They argued that biological effects such as long-term evolution of either foraminifera species might alter the relationship between δ¹¹B and carbonate chemistry. On the other hand, de la Vega et al. (2020) argue that the offset may be attributed to an analytical issue with T. sacculifer δ¹¹B. However, the revised alkenone CO₂ records agree well with T. sacculifer δ¹¹B CO₂ (Fig. 5b), suggesting alternation of G. ruber δ¹¹B CO₂ due to the biological effect. Alternatively, another possible explanation for the high estimates of G. ruber δ¹¹B CO₂ is that those CO₂ records overestimate variability ranges possibly due calibration for the conversion of G. ruber δ¹¹B into CO₂ concentration not being well-optimized. It is worth noting that the T. sacculifer δ¹¹B CO₂ of Dyez et al. (2018) is re-estimated based on updated calibrations, with a significant downward revision of Pliocene CO₂ compared to the original values (Bartolli et al., 2011) (Fig. 6). For conversion of G. ruber δ¹¹B into CO₂, the culture and global core top calibration of Henehan et al. (2013) have been applied. However, it should be noted that the number of the culture data, which is used to establish the calibration, is limited. Although G. ruber δ¹¹B CO₂ in ODP 999 seems to well represent the CO₂ variability during the glacial-interglacial cycles documented in the Antarctic ice core record (Foster et al., 2008; Chalk et al., 2017), a close look reveals that the variability range of G. ruber δ¹¹B CO₂ (150 to 340 ppm) is significantly larger than that of the ice-cores (180 to 280 ppm) (Fig. 2c). This suggests that G. ruber δ¹¹B CO₂ overestimates the variability of CO₂ in the G-IG cycles. On the other hand, the dynamic range of T. sacculifer δ¹¹B CO₂ (175-300 ppm) (Dyez et al., 2018), which shows relatively low CO₂ in the Pliocene, agrees well with ice core values (180-280 ppm) (Fig. 2c) during the past 800 kyrs, supporting the reliability of CO₂ estimates. The variation range (150-350 ppm) of the statistical model δ¹³C_{C37} CO₂ in the late Pleistocene also significantly exceeds that of ice core (180-280 ppm) (Fig. 2c), suggesting overcorrection of environmental factors. These considerations depict that the CO₂ records, which overestimate CO₂ variation range during the Late Pleistocene G-IG cycles, show high CO₂ estimates in the Pliocene.
Further studies for developing the calibrations and constraints of environmental and physiological factors will be needed to generate robust CO₂ estimates. As for the alkenone δ¹³C CO₂ proxy, there are ongoing efforts within the community (Zhang et al., 2020; Phelps et al., 2020; Badger, 2021) and include refinements to the alkenone CO₂ model (Zhang et al., 2020), culture based constrains (Phelps et al., 2020) and consideration of the lower limits of the proxy due to CCMs (Badger, 2021). Here we highlight how relatively simple refinements for export production depth of alkenones (based on U³⁷C temperature) and using regional [PO₄³⁻] depth profiles can lead to major reconciliation of CO₂ estimates with other lines of evidence.

4 Conclusion

In this study, previously reported δ¹³C₃₇ based CO₂ records from the Plio-Pleistocene were reexamined to better constrain CO₂ evolution. We refined the conventional approach, which assumes constant physiological factors and a carbon diffusive model over the time by including considerations of regional differences in physical oceanographic factors, specifically the mixed layer depth, production season and air-ocean disequilibrium of CO₂ for each site (Fig. 4). This simple approach refines estimates of CO₂ significantly, reducing large offsets previously observed in the reported δ¹³C₃₇ CO₂ records from different oceanographic settings. However, some of our data still do not obtain sufficiently low glacial CO₂ values during the Pleistocene (Fig. 4d). This suggests varying physiological factors associated with G-IG cycles or upregulation of carbon concentration mechanisms in glacial low CO₂ conditions at some sites. Nevertheless, this study shows that better constraints on past environmental variables can be obtained using literature data and this this approach can improve δ¹³C₃₇ CO₂ estimates. Our revised δ¹³C₃₇ CO₂ datasets are consistent well with the previously reported CO₂ estimates in the early to mid Pleistocene (Da et al., 2019; Dyez et al., 2018; Cui et al., 2020) with CO₂ levels below 300 ppm. On the other hand, the revised δ¹³C₃₇ CO₂ CO₂ levels in the Pliocene are lower than 350 ppm. Our low CO₂ values in the Pliocene are consistent with other CO₂ estimates such as a T-inverse model (van de Wal et al., 2011; Berends et al., 2019), T. sacculifer δ¹¹B (Bartoli et al., 2011; Dyez et al., 2018) and leaf stomata (Kürschner et al., 1996) based CO₂ reconstructions (Fig. 5b), albeit the upper end of the Pliocene CO₂ are lower than that of G. ruber δ¹¹B (Seki et al., 2010; Martínez-Boti et al., 2015; de la Vega et al., 2020) and statistical model δ¹³C₃₇ (Stoll et al., 2019) CO₂ estimates. Further efforts are needed to constrain the dynamic range of CO₂ for time periods pre-dating the ice core records. It is particularly important to generate continuous, high temporal resolution CO₂ records which overlap and match the Antarctic ice-core CO₂ records.

Author contributions

O.S. and J.B. analysed the paleoclimatic data and wrote the manuscript.
Competing interests

The authors declare that they have no conflicts of interest.

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Table 1. Location of ODP samples, surface mixed layer depth (SML), sea-air $\Delta$CO$_2$ in surface water, sea surface salinity (SSS) and regression applied to calculate b-term.

| Site      | Modern location | SML (m) | $[PO_4^{3-}]$ at SML (uM/L) | $\Delta$CO$_2$ (µatm) | SSS (psu) | b-term regression | SST calibration |
|-----------|-----------------|---------|------------------------------|------------------------|-----------|--------------------|-----------------|
| ODP 806   | 0.5N, 159.5E    | 0-50    | 0.14-0.18                    | 30                     | 35        | EP                 | Sonzogni et al. (1997) |
| ODP 925   | 4.5N, 43.5W     | 0-30    | 0.16-0.08                    | 22                     | 35        | Global             | Sonzogni et al. (1997) |
| ODP 982   | 57.5N, 16W      | 0-30    | 0.52-0.55                    | -32                    | 35        | Global             | Conte et al. (2006)   |
| ODP 999   | 12.5N, 78.4W    | 0-30    | 0.05-0.08                    | 17                     | 36        | BA                 | Sonzogni et al. (1997) |
| ODP 1208  | 36.5N, 158.5E   | 0-20    | 0.22-0.28                    | -38                    | 35        | Global             | Conte et al. (2006)   |
| ODP 1241  | 5.5N, 86.5W     | 0-10    | 0.32-0.38                    | 14                     | 32        | EP                 | Sonzogni et al. (1997) |

BA = Bermuda Atlantic. EP = Equatorial Pacific.
Figure 1: Locations of ODP sediment core sites used for CO$_2$ reconstructions.
Figure 2: (a) Benthic $\delta^{18}$O stack (Lisiecki and Raymo, 2005), $\delta^{13}$C$_{C37}$, $\delta^{11}$B and paelosol CO$_2$ records over the past (b) 5 Myrs and (c) 2 Myrs together with Antarctic ice core and blue ice CO$_2$. Open purple triangle: *G. ruber* $\delta^{11}$B CO$_2$ records in ODP 999 (Foster et al., 2008; Seki et al., 2010; Martinez-Botí et al., 2015; Chalk et al., 2017; de la Vega et al., 2020). Solid purple triangle: *T. sacculifer* $\delta^{13}$B CO$_2$ records in ODP 668 and 999 (Hönisch et al., 2009; Bartoli et al., 2011; Dyez et al., 2018). Light blue line: ice core CO$_2$ (Siegenthaler et al., 2005; Lüthi et al., 2008). Open light blue circle: blue ice CO$_2$ (Higgins et al., 2015; Yan et al., 2019). Bar: $\delta^{13}$C$_{C37}$
CO₂ records in ODP 806 (orange), ODP 925 (light blue), ODP 982 (light green), ODP 999 (red), ODP 1208 (dark green) and ODP 1241 (blue) (Pagani et al., 2010; Seki et al., 2010). Error bars represent a range calculated assuming production depths (surface mixed layer). Open red circle: δ¹³C₃° CO₂ records in ODP 999 (Badger et al., 2013, 2019). Open and solid brown circles: statistical model δ¹³C₃° CO₂ records in ODP 925 and 999, respectively (Stoll et al., 2019). Open blue square: Stomata CO₂ records (Kürschner et al., 1996). Solid blue diamond: paleosol CO₂ (Da et al., 2019). Grey and black lines: T-inverse model CO₂ (van de Wal et al., 2011; Berends et al., 2019).

Figure 3: Scatter plot of phosphate concentration and b-term (Bidigare et al., 1997, 1999; Eek et al., 1999; Laws et al., 2000). Open red circles represent Equatorial Pacific data including Equatorial Pacific (fall and spring) and Iron Ex (Bidigare et al., 1997) while open black circles are data from other regions. Red line: Equatorial Pacific (EP) regression. Black dashed line: the global regression (Pagani et al., 2005).
Figure 4: $\delta^{13}$C$_{C37}$ CO$_2$ records over the past 5 Myrs together with ice core CO$_2$ (light blue line) (Siegenthaler et al., 2005; Lüthi et al., 2008), blue ice CO$_2$ (open light blue circle) (Higgins et al., 2015; Yan et al., 2019) and T-inverse model derived CO$_2$ (grey and black lines) (van de Wal et al., 2011; Berends et al., 2019). (a) CO$_2$ records based on the global regression (original data are from Pagani et al. (2005) and Seki et al. (2010)). (b) CO$_2$ records based on the global regression with accounting for air-sea $\Delta$CO$_2$. (c) CO$_2$ records based on the regional regressions. (d) CO$_2$ records based on the regional regressions records of (b) but also after correction of air-sea $\Delta$CO$_2$. Bar: $\delta^{13}$C$_{C37}$ CO$_2$ records in ODP 806 (orange), ODP 925 (light blue), ODP 982 (light green), ODP 999 (red), ODP 1208 (dark green) and ODP 1241 (blue) (Pagani et al., 2010; Seki et al., 2010).
Figure 5: (a) Benthic δ¹⁸O stack (black line) (Lisiecki and Raymo, 2005) and (b) Corrected δ¹³C₃₇, statistical model δ¹³C₃₇, δ¹¹B and paleosol CO₂ records over the past 5 Myrs together with ice core CO₂ (light blue line) (Siegenthaler et al., 2005; Lüthi et al., 2008), blue ice CO₂ (open light blue circle) (Higgins et al., 2015; Yan et al., 2019) and T-inverse model derived CO₂ (grey and black lines) (van de Wal et al., 2011; Berends et al., 2019). Open blue triangle: G. ruber δ¹¹B CO₂ records in ODP 999 (Foster et al., 2008; Seki et al., 2010; Martínez-Botí et al., 2015; Chalk et al., 2017; de la Vega et al., 2020). Solid purple triangle: T. sacculifer δ¹¹B CO₂ records in ODP 668 and 999 (Hönisch et al., 2009; Bartoli et al., 2011; Dyez et al., 2018). Open light blue circle: blue ice CO₂ (Higgins et al., 2015; Yan et al., 2019). Bar: δ¹³C₃₇ CO₂ records in ODP 806 (orange), ODP 925 (light blue), ODP 982 (light green), ODP 999 (red), ODP 1208 (dark green) and ODP 1241 (blue) (Pagani et al., 2010; Seki et al., 2010). Open red circle: δ¹³C₃₇ CO₂ records in ODP 999 (Badger et al., 2013, 2019). Open and solid brown circles: statistical model δ¹³C₃₇ CO₂ records in ODP 925 and 999, respectively (Stoll et al., 2019). Open blue square: Stomata CO₂ records (Kürschner et al., 1996). Solid blue diamond: paleosol CO₂ (Da et al., 2019).
Figure 6: Original (red triangle; Bartoli et al., 2011) and revised (purple triangle; Dyez et al., 2018) T. sacculifer $\delta^{13}$B CO$_2$ records from the Pliocene to early Pleistocene. Yellow and grey areas represent ranges of reported (Pagani et al., 2010; Seki et al., 2010; Badger et al., 2013, 2019) and corrected (this study) $\delta^{13}$C$_{\text{C37}}$ CO$_2$, respectively.