A 40-million-year history of atmospheric CO$_2$

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The alkenone–pCO$_2$ methodology has been used to reconstruct the partial pressure of ancient atmospheric carbon dioxide (pCO$_2$) for the past 45 million years of Earth’s history (Middle Eocene to Pleistocene epochs). The present long-term CO$_2$ record is a composite of data from multiple ocean localities that express a wide range of oceanographic and algal growth conditions that potentially bias CO$_2$ results. In this study, we present a pCO$_2$ record spanning the past 40 million years from a single marine locality, Ocean Drilling Program Site 925 located in the western equatorial Atlantic Ocean. The trends and absolute values of our new CO$_2$ record site are broadly consistent with previously published multi-site alkenone–CO$_2$ results. However, new pCO$_2$ estimates for the Middle Miocene are notably higher than published records, with average pCO$_2$ concentrations in the range of 400–500 ppm. Our results are generally consistent with recent pCO$_2$ estimates based on boron isotope-pH data and stomatal index records, and suggest that CO$_2$ levels were highest during a period of global warmth associated with the Middle Miocene Climatic Optimum (17–14 million years ago, Ma), followed by a decline in CO$_2$ during the Middle Miocene Climate Transition (approx. 14 Ma). Several relationships remain contrary to expectations. For example, benthic foraminiferal $\delta^{18}$O records suggest a period of deglaciation and/or high-latitude warming during the latest Oligocene (27–23 Ma) that, based on our results, occurred concurrently with a long-term...
1. Introduction

Knowledge of the partial pressure of atmospheric CO2 (pCO2), beyond direct measurements of atmospheric [1] and ice-core gas compositions over the last 800,000 years [2–4], derives from a range of techniques including stomatal indices of fossil leaves (e.g. [5–9]), the carbon isotopic compositions of palaeosol carbonate nodules (e.g. [10–12]), the boron isotopic compositions of shallow-dwelling foraminifera (e.g. [13–17]) and the stable carbon isotope compositions of algal biomarkers, such as alkenones (e.g. [18–24]). Most Cenozoic CO2 reconstructions indicate substantially higher CO2 values in the Early Eocene with a broad decrease during the Middle Eocene into the Neogene. However, a twofold variation in absolute pCO2 concentrations persists among the available proxies during key climatic intervals [25].

Following the initial development of alkenone-based CO2 reconstructions for the Pleistocene and Holocene [26,27], a revised methodology was applied for long-term CO2 reconstructions for the Middle Eocene to the Pleistocene [18–20,22,23,28]. More than 20 marine localities have been used for Cenozoic reconstructions. However, the published Cenozoic alkenone–CO2 record represents a stacked record from various ocean localities (e.g. [28]). This potentially introduces bias into CO2 trends and magnitudes because temperature, nutrient conditions, seasonality and thermocline depth can impact algal growth conditions and contribute to variability in reconstructed CO2 estimates. Thus, temporal CO2 patterns from composite records could have trends specific to individual sites and not representative of changes in long-term global atmospheric conditions [21].

In this study, we present a continuous alkenone-based CO2 record from one marine site for the past 40 million years to limit variability introduced by composite results. With this new record, we attempt to reconcile the discrepancy of CO2-level reconstructions between alkenone and other proxies and explore CO2 forcing in the context of global climate change since the late Middle Eocene. Also discussed is a preliminary assessment of the potential bias related to algal carbon concentrating mechanisms (CCMs) and spontaneous bicarbonate–CO2 conversion, when using the alkenone–pCO2 method to estimate the CO2 levels in the Cenozoic.

2. Background

(a) The alkenone–pCO2 method

(i) Diffusion versus active carbon uptake models of carbon transport

Alkenones occur as a suite of long-chained (C37–C39) unsaturated ethyl and methyl ketones produced by a few haptophyte algae in the modern ocean [29,30]. Today, the predominant sources of alkenones derive from *Emiliania huxleyi* (originating in the Late Pleistocene [31]) and *Gephyrocapsa oceanica* (originating in the Pliocene [32]). Sedimentary alkenones that pre-date the Pliocene were probably produced by haptophytes related to the family Noelaerhabdaceae and genera *Reticulofenestra* and *Dictyococcites* [33–37].
Alkenone-based pCO2 reconstructions require paired measurements on stable carbon isotope composition of the di-unsaturated C37 methyl ketone (δ13C37:2) and carbonates. Differences between the δ13C of algal carbon and carbonate represent the total carbon isotope fractionation (εp) that occurs during algal growth. εp derived from δ13C37:2 values (εp37:2) is calculated by the following equation:

\[
ε_{p37:2} = \left[ \frac{δ_{CO2(aq)} + 1000}{δ_{org} + 1000} - 1 \right] \times 1000,
\]

(2.1)

where δorg is the carbon isotopic composition of the algal cell estimated from the δ13C of alkenone (δ37:2). The isotopic difference between δorg and δ37:2 is 4.2‰, following earlier studies [26,27,38]:

\[
δ_{org} = \left[ (δ_{37:2} + 1000) \times \left( \frac{4.2}{1000} + 1 \right) \right] - 1000,
\]

(2.2)

where δCO2(aq) is the δ13C value of dissolved CO2(aq) approximated from the δ13C values of shallow-dwelling foraminifera assuming isotopic and chemical equilibria among all inorganic carbon species, atmospheric CO2 and foraminifera calcite tests (e.g. [26]).

The alkenone–CO2 method is based on the assumption that the transport of inorganic carbon (dissolved CO2) across the cell membrane to the site of carbon fixation within the algal cell predominantly occurs by diffusion. For diffusion transport, the total stable carbon isotope fractionation, which occurs during marine photosynthesis, is described by the following equation [39,40]:

\[
ε_p = ε_f - b \left( \frac{C_i}{C_e} \right),
\]

(2.3)

where C_e represents the ambient concentration of CO2(aq) ([CO2(aq)]), C_i is the intracellular [CO2(aq)], ε_f is the total carbon isotope fractionation that occurs during photosynthesis and ε_t is the carbon isotope fractionation associated with carbon transport. Most alkenone-based pCO2 reconstructions have adopted a range of ε_f values from 25 to 28‰ (e.g. [18–20,22–24]), consistent with the majority of algal growth experiments [41,42].

Chemostat incubations for two strains of alkenone-producing haptophyte algae and two diatoms, under continuous light- and nitrate-limited conditions show that εp linearly varies with [CO2(aq)], specific growth rate (μ) and cell geometry [42–44], consistent with a predominantly diffusion carbon transport model. By contrast, dilute batch cultures of E. huxleyi grown under nutrient-replete conditions and variable irradiance yield lower εp37:2 values, minor response to [CO2(aq)] changes [45] and an irradiance effect on the magnitude of εp37:2 [46]. Such results suggest that assumptions of a strictly diffusion carbon uptake model may not be valid under some nutrient and light conditions. For example, low [CO2(aq)] chemostat experiments for diatom Phaeodactylum tricornutum resulted in a nonlinear response of εp to μ/[CO2(aq)], indicating that a CCM was triggered when the supply of CO2(aq) became limiting to growth (in this case, approx. less than 7 µmol kg\(^{-1}\) [44,47]). CCMs are known to be available to a broad range of algal species [48–51]. However, whether or not ancient alkenone-producing algae were characterized by CCMs is presently unknown.

(ii) Simplified model of diffusion carbon uptake and palaeoclimate reconstructions

Various physiological factors have been shown to impact C_i (equation (2.3)) [52]. However, because C_i is difficult to constrain, Jasper & Hayes [26] recast equation (2.3) as

\[
ε_p = ε_f - \frac{b}{[CO2]}.
\]

(2.4)

where the term b represents a range of physiological variables that encompass the combined effects of growth rate, cell geometry and other potential factors.

Surface water analysis of δ13C37:2 and seawater chemistry show a strong relationship between b in equation (2.4) and the concentration of soluble phosphate [21,53]. Dissolved reactive phosphate ([PO_4^{3-}]) is a key macronutrient that often covaries with other biolimiting trace-metal
Figure 1. Global plate reconstruction shows the location of ODP Site 925 at 40, 20 Ma and the present, as well as a detail of the northward movement of Site 925 since 40 Ma [54]. Continents are shown as tectonic terrains rather than shorelines.

micro-nutrients, such as iron, zinc and cobalt [53], providing a link between $[\text{PO}_4^{3-}]$, growth rate and the value of $\varepsilon_p$.

Calibration of $b$ with respect to $[\text{PO}_4^{3-}]$ using all available data (see [21]) results in the following relationships assuming $\varepsilon_f = 25^\circ/\text{permil}$:

$$ b = (118.52 \times [\text{PO}_4^{3-}]) + 84.07. \tag{2.5} $$

(iii) Uncertainties in $p\text{CO}_2$ calculations

If carbon assimilation is primarily determined by diffusion transport, $\varepsilon_p$ is well described by equation (2.3). However, various other parameters, in addition to $\text{CO}_2(\text{aq})$, are known to play a role in the expression of $\varepsilon_p$. Some of these parameters influence $\varepsilon_p$ through a direct impact on $C_i$, including growth rate, irradiance, cell membrane permeability and cell size. $\text{CO}_2$ estimates are further compromised by uncertainties in phosphate concentrations used to estimate the physiological-dependent term $b$, as well as the potential of regionally different $b$ versus $[\text{PO}_4^{3-}]$ relationships relative to the global calibration. Assumptions regarding the value of enzymatic isotope fractionation ($\varepsilon_f$), as well as knowledge of sea surface temperatures (SSTs)—generally obtained from various geochemical proxies—further contribute to $\text{CO}_2$ uncertainties [21]. Finally, other factors, such as CCMs, probably arise when the intracellular concentration of $\text{CO}_2$ becomes limiting. CCMs can impact the value of $\varepsilon_p$ by transporting different carbon species with specific carbon isotopic compositions, and altering $C_i$ relative to diffusive flux. Also, isotope fractionations associated with active transport mechanisms are likely distinct.

(b) Site location

Ocean Drilling Program (ODP) Site 925 (4°12.25′N, 43°29.33′W, 3042 m water depth) is located on Ceara Rise in the western equatorial Atlantic Ocean (figure 1) [55]. Modern surface waters at this site are characterized by high mean annual SST (approx. 27.5°C) and low nutrient levels (surface $[\text{PO}_4^{3-}]$ approx. 0.14 μmol l$^{-1}$ [56]). The Middle Eocene to Pleistocene sedimentary succession recovered at Site 925 consists of pelagic carbonate oozes and chalks, primarily foraminifer-bearing nannofossil oozes with minor amounts of clay. All samples used in this study were taken from ODP Hole 925A. Previously reported Plio-Pleistocene $p\text{CO}_2$ data are based on Hole 925C and 925D [22].

Palaeogeographic reconstructions indicate that this site has moved northward over the past 40 Ma, from just south of the equator during the Late Eocene, to approximately 4°N today.
Site 925 was right on the equator around 30 Ma. Available mass accumulation rates of biogenic barium and reactive phosphorus suggest stable productivity and nutrient conditions from 36.9 to 32.7 Ma [57]. Calcareous nannofossils indicate weak to moderate tropical upwelling history [55] consistent with a minor air–sea CO2 disequilibrium expressed today, with modern surface waters enriched by approximately 22 ppm [58].

3. Material and methods

(a) Lipid extraction and analysis

Approximately 40–100 g of sediment (dry weight) were used for biomarker analysis. Samples were freeze-dried and extracted with dichloromethane/methanol (2 : 1, v/v) using an Accelerated Solvent Extractor (ASE 300, Dionex) at 120°C and 10.3 MPa. Total lipid extracts (TLEs) were concentrated under a stream of purified N2 using a Zymark Turbovap II, and then separated into three fractions using silica gel chromatography. TLEs were separated into compound classes using ashed Pasteur pipettes loaded with approximately 4 g deactivated silica gel (70–230 mesh), and sequentially eluted with 2 ml hexane, 4 ml dichloromethane and 4 ml methanol to obtain aliphatic, aromatic and polar fractions, respectively.

The dichloromethane fraction containing ketones was subject to basic hydrolysis and silver nitrate column chromatography to purify long-chain alkenones. Relative abundances of di- and tri-unsaturated methyl ketones were analysed on a Thermo Trace 2000GC equipped with an Rxi-1 ms column (60 m × 0.25 mm × 0.25 μm), a programmed temperature vaporization (PTV) injector and a flame ionization detector with He as the carrier gas. The oven was set to 90°C upon sample injection, held for 1 min, ramped up at 20°C min⁻¹ to 280°C, then 2°C min⁻¹ to 320°C. Temperature was held constant at 320°C for 30 min. The relative abundance of C37 : 2 and C37 : 3 alkenones was used to compute U’k37, a proxy developed to reconstruct ancient SSTs [59,60]. Analytical precision, determined through multiple analyses of an in-house alkenone standard, represents an uncertainty of ±0.005 U’k37 units (see electronic supplementary material).

The methanol fraction containing glycerol dialkyl glycerol tetraethers (GDGTs) was further purified by passing through activated alumina dissolved in dichloromethane/methanol (1 : 1, v/v). The eluting fraction was dried under pure N2 stream, then dissolved in an azeotrope of hexane/isopropanol (99 : 1, v/v) and filtered through an ashed 0.7 μm glass microfibre filter. Analyses of GDGTs were conducted following a slightly modified method described by Hopmans et al. [61]. Compound identification and relative abundance analyses were determined using an Agilent 1200 high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometer (HPLC/APCI-MS). Separation of GDGTs was achieved on an Alltech Prevail cyano column (150 mm, 2.1 mm I.D., and 3 μm grain size) kept at 30°C. The following solvent polarity gradient was used, based on A: hexane/isopropanol (99 : 1, v/v), and B: hexane/isopropanol (90 : 10, v/v): 100% solvent A for 5 min, then solvent B was linearly increased from 0% at 5 min, to 7.4% at 40 min, and maintained from 40 to 50 min. After each sample analysis, the column was back flushed with 100% solvent B for 14 min, followed by 10 min equilibration with normal phase flow of 100% solvent A. Mass spectrometric identification and quantification were achieved using an Agilent 6130 ion-trap mass spectrometer coupled to the HPLC by an APCI interface. Ion scans were set to m/z 1200–1500. Quantification was based on peak intensities in the mass chromatogram of the [M + H]⁺ ions. Relative abundance of GDGTs was used to calculate TEX86, a proxy employed to estimate past SSTs [62]. Repeated measurements of an in-house laboratory standard showed the analytical precision represented ±0.01 TEX86 units (see electronic supplementary material).

(b) Carbon isotope measurements

Carbon isotopic compositions of C37:2 alkenones were analysed on a Thermo Finnigan MAT 253 mass spectrometer interfaced with a Trace GC Combustion III (GC-IRMS) equipped with
a PTV injector and a J&W Scientific DB-1 capillary column (60 m × 0.25 mm × 0.25 mm), using He as a carrier gas with a flow speed of 2.0 ml min⁻¹. GC temperature was held at 60°C for 1 min, increased to 320°C at 15°C min⁻¹ and held isothermally for 35 min. Carbon isotopes are reported relative to the VPDB standard based on an in-house reference gas calibrated to the OzTech standard (δ¹³C = −40.61‰). A C20 n-alkane standard was injected daily to determine the analytical accuracy of the carbon isotope measurements, yielding an uncertainty of ±0.2‰. Uk′37 and δ¹³C37:2 data reported for the Eocene–Oligocene climate transition [21] and the Pliocene–Pleistocene [22] have been previously published (see electronic supplementary material).

(c) Foraminifera stable isotope measurements

Splits of samples were wet sieved with deionized water at 63 μm. Surface-dwelling planktic foraminifera, including Globigerinoides ruber, ‘Globigerinoides ruber s.1.’ and Globigerinoides altiaperturus, were picked from the 250–300 μm sieve fraction in the Neogene section, and epifaunal benthic foraminifera (Cibicidoides spp.) were picked from greater than 250 μm sieve fraction throughout the entire Site 925 study section. Carbon stable isotope analysis of the picked foraminiferal samples was performed using a Europa GEO 20–20 mass spectrometer at the University of Southampton. Samples were reacted with phosphoric acid at 70°C using an automatic carbonate preparation system ‘CAPS’ in line with the mass spectrometer. Typically, five to eight benthic specimens from each sample were combined and analysed together, and 15–20 planktic specimens were analysed together. All values are reported relative to the VPDB scale, with an external analytical precision estimated at 0.08‰ for δ¹³C (see electronic supplementary material).

(d) Chronology

Age determinations for ODP Hole 925A are based on biostratigraphy planktic foraminifera [63,64] with 75 age control points based on first and last occurrence data (see electronic supplementary material) over a span of 40 million years. All data are calibrated to the geomagnetic polarity timescale of [65] and the integrated timescale of Berggren et al. [66].

4. Results

(a) Temperature

SSTs based on Uk′37 and TEX86 indices were reconstructed for the past 40 Ma using the linear calibration of Conte et al. [67] and the reciprocal calibration of Liu et al. [68], respectively (figure 2a). Uk′37 SST reconstructions indicate relatively constant temperatures of approximately 28°C since the Late Eocene, except for an approximately 2°C cooling during the Pliocene–Pleistocene interval. However, 28.5°C represents maximum temperatures expressed by the Uk′37 calibration (Uk′37 value reaches 1), and therefore warmer temperatures above approximately 28°C cannot be determined. By contrast, maximum temperatures associated with the TEX86 proxy are unconstrained. Reconstructed TEX86 records exhibit much larger fluctuations compared with Uk′37 with temperatures of approximately 30°C in the Late Eocene that fall to approximately 22°C during the Late Pliocene—more than 5°C cooler at the same locality today. However, the TEX86-based temperatures since Pliocene might suffer from additional GDGT inputs, as indicated by high BIT values (mean value is 0.6; electronic supplementary material) [69].

In order to estimate CO2, we do not favour one SST proxy over the other for the entire 40 million year record from Site 925. Instead, we selected the highest temperature estimates from both Uk′37 and TEX86 records to synthesize a composite temperature profile. Differences between Uk′37 and TEX86 records are considered to reflect the uncertainty of SST reconstructions at each sample, which is further used in the computation of εp37:2 and pCO2. We also realize that different
Figure 2. Time series of ODP Site 925. (a) $U^K_{37}$- and TEX$_{86}$-based SST reconstruction. (b) Adjusted carbon isotopes from foraminiferal stable isotope measurements to proximate $\delta^{13}C$ of surface water dissolved inorganic carbon. (c) Carbon isotopes of alkenones; (d) $\varepsilon_{p37:2}$ calculated from (b) and (c); (e) $pCO_2$ calculated from (d), using both $U^K_{37}$- and TEX$_{86}$-based SST estimates, and assuming $[PO_4^{3-}]$ values were 0.20, 0.14 (lower limit) and 0.39 $\mu$M (upper limit). Refer to electronic supplementary material to see details of constructing (b,d,e) and their related uncertainties; one $CO_2$ estimate at 35.52 Ma gives a value more than 2400 ppm, which is off the chart and therefore not shown in the following figures.

Calibrations for $U^K_{37}$- and TEX$_{86}$-SST conversion could introduce additional uncertainties, but they are secondary compared with the difference between the two proxies themselves.

(b) Carbon isotopes and $\varepsilon_{p37:2}$

Planktonic foraminifera are partially to fully recrystallized in the Upper Eocene–Lower Oligocene interval of Site 925. $\delta^{13}C$ measurements on the presumed surface water dwellers Turborotalia spp.
show only small differences (0.1–0.5‰) from the benthic *Cibicidoides* spp. within the same sample, suggesting that *Turborotalia* either maintained a thermocline habitat or is affected by diagenetic alteration [21]. However, agreement between *Cibicidoides* δ¹³C values at Site 925 and an Eocene–Oligocene Southern Ocean compilation of δ¹³C values from ODP Sites 689, 733, 744 and 748 suggests adequate preservation of δ¹³C values [21]. Consequently, Pagani et al. [21] reconstructed surface δ¹³C by measuring *Cibicidoides* δ¹³C values, assuming a constant offset (0.94‰) between surface and deep waters δ¹³CDIC.

Miocene mixed-layer foraminifera are better preserved than Palaeogene specimens. *Globogerinoides altiaperturus* were measured in the Early Miocene and *G. ruber* s.l. were selected in the Mid- to Late Miocene interval. A δ¹³C offset of 0.94‰ with an ascribed uncertainty of ±0.3‰ [70] was applied to both *G. ruber* and *G. altiaperturus* δ¹³C values to estimate surface water δ¹³C DIC (figure 2b). The planktic foraminifera approach in the Miocene is accompanied by benthic foraminifera *Cibicidoides* δ¹³C analyses and the applications of assumed surface–deep water isotopic offsets. Results show that the two independent approaches are within 0.20‰ from each other (see electronic supplementary material). Carbon isotope compositions on the shallow-dwelling *Globogerinoides sacculifer* since 5 Ma have been previously reported (figure 2b) [22].

δ¹³C₃₇:₂ values are more ¹³C depleted during the Palaeogene relative to the Neogene (figure 2c). Calculation of δ¹³C of surface seawater aqueous CO₂ by converting the mixed-layer δ¹³C DIC (figure 2b), using the composite temperature estimates and associated uncertainties from organic geochemical proxies (§4a), assuming equilibrium isotopic fractionations between different carbonate species. Aqueous CO₂ δ¹³C and δ¹³C₃₇:₂ are further used to calculate ε₃₇:₂.

Figure 2 shows that variability in δ¹³C₃₇:₂ is predominantly responsible for the character of ε₃₇:₂ trends. ε₃₇:₂ values exhibit a long-term decreasing trend, with the majority of ε₃₇:₂ varying between 21 and 18‰ during the Late Eocene to Early Oligocene, 16–13‰ in the Late Oligocene/Miocene and 13–11‰ during the Pliocene/Pleistocene (figure 2d).

(c) *pCO₂* estimates

We assume a value of 25‰ for εᵢ [41,42,44], and a range of [PO₄³⁻] from 0.14 to 0.39 μM, representing the present-day phosphate concentrations from 0 to 100 m near Site 925 [56], with 50 m (0.20 μM) to be the most representative depth for alkenone production. Estimates of *pCO₂* were then calculated assuming air–sea equilibrium and proxy temperatures (the composite temperature, see §4a) using Henry’s law (figure 2c). The uncertainty of *pCO₂* is constrained by the variability of temperature and phosphate estimates. The maximum *pCO₂* is calculated by using the upper limit of the εᵢ, highest phosphate level and highest temperature estimates; whereas the minimum of *pCO₂* is a result of the lower limit of εᵢ, lowest phosphate and lowest temperature (see §4a,b for the determination of uncertainties associated with temperature and εᵢ). The resulting uncertainty of *pCO₂* estimates averages 38%, much larger than an error propagation model through Monte Carlo procedure (11%) with assumed uncertainty of different parameters used in *pCO₂* calculations [20].

5. Discussion

(a) Comparisons between Site 925 and published Cenozoic *pCO₂* records

Our new CO₂ results from Site 925 (figures 3 and 4) broadly agree with previously published composite alkenone–*pCO₂* record for the Cenozoic [28]. Nevertheless, systematic differences are apparent with the new record indicating higher CO₂ levels after approximately 32 Ma (figure 3). This discrepancy can be explained, in part, by regional differences in oceanography and algal growth conditions, as expressed by variable εᵢ values. For example, the Miocene average εᵢ at
Figure 3. A comparison of alkenone-based $p$CO$_2$ composite from multiple marine sites as compiled in the study of Pagani et al. [28] and ODP Site 925 record since the Late Eocene. Antarctic glaciation thresholds (approx. 750 ppm) and Northern Hemisphere glaciation threshold (approx. 280 ppm) deduced from climate models [71] are marked by dashed lines.

Figure 4. Comparison between different proxy-based $p$CO$_2$ estimates for the past 40 Ma. Boron isotope, palaeosol and stomata data are summarized by Beerling & Royer [25], with additional data from Foster et al. [15].

Site 925 is approximately $15\%$, significantly higher than the mean value of approximately $10\%$ at Deep Sea Drilling Project (DSDP) Site 588 [19,20]. Furthermore, new SST estimates (e.g. [19,20]) are used in this study, and these new U$^{37}$ and TEX$_{86}$-based temperature records are considerably warmer during the Miocene to Early Pliocene, compared with foraminifer $\delta^{18}$O-based estimates (6–8°C, DSDP Site 588 [22]). Warmer SST estimates increase calculated $p$CO$_2$ levels [22].
Finally, comparison of our new record with recent CO$_2$ reconstruction efforts, with putatively improved techniques and higher quality data [25], suggests broad agreement with boron isotope-pH [13,15,16], stomata [5–9] and palaeosol estimates [10–12], although palaeosol estimates have inherently larger uncertainties (figure 4).

(b) Palaeoclimate implications

(i) The late Middle Eocene to Early Oligocene

Coupled climate–ice sheet model simulations of the Eocene/Oligocene climate transition indicate that sudden nonlinear jumps in ice volume can be triggered by the congruence of favourable orbital cycles when pCO$_2$ concentrations decrease to 2.5–3 times pre-industrial levels (approx. 750 ppm; figure 5) [71,73]. Independent proxy reconstructions of pCO$_2$ across the Eocene–Oligocene transition, including those estimated by the boron isotope-pH methodology [16] and alkenone-based CO$_2$ records from Site 925 [21], support these model results and suggest CO$_2$ began to decrease immediately prior to the Oi-1 event and declined during the climate transition, probably falling below the approximately 750 ppm threshold.

Compared with a multi-site approach, oceanography and algal growth conditions might be less variable in a single site. However, the Late Eocene–Early Oligocene CO$_2$ record at Site 925 may still carry features that are specific to this locality. Although no major tropical upwelling has been detected throughout the entire record by calcareous nannofossil assemblages [55], plate reconstructions indicate that Site 925 tectonically moved from the Southern to the Northern Hemisphere and was positioned on the equator around 30 Ma (figure 1). This transit would have altered the expression of local sea surface CO$_2$, particularly at the equator where the convergence of trade winds drives upwelling of CO$_2$-rich deep waters [74]. A distinct
spike in $\varepsilon_p$ values and reconstructed pCO$_2$ is evident in the Middle Oligocene (approx. 29.5 Ma, figure 2d), representing about 10–30% increase in CO$_2$ from the background level. The magnitude of present-day equatorial upwelling, however, is represented by a seawater CO$_2$ enrichment in the western equatorial Atlantic by approximately 30 ppm—about 9% deviation from atmosphere–seawater equilibrium [58]. This potentially implies that equatorial upwelling only partially contributed to the Middle Oligocene pCO$_2$ spike recorded in Site 925, or that upwelling intensity was higher and/or upper water-column stratification was less intense during the Oligocene.

(ii) The Late Oligocene to Early Miocene

Climatic behaviour during the Late Oligocene/Early Miocene interval is particularly difficult to explain given existing CO$_2$ records. After a period of gradual cooling following the onset of glaciation on Antarctica near the Eocene–Oligocene climate transition at approximately 34 Ma, an approximately 0.8‰ decrease in benthic $\delta^{18}$O values (ODP 1218, eastern equatorial Pacific [75]) suggests a period of substantial warming and/or deglaciation during the latest Oligocene (approx. 27–23 Ma; figure 5). However, our corresponding CO$_2$ records indicate a long-term decrease in atmospheric carbon dioxide through the Late Oligocene (figure 5). Isotopic evidence for warming and/or partial deglaciation at the end of the Oligocene is not simply an artefact of stacked benthic $\delta^{18}$O records from different marine sites [72,75] given that the $\delta^{18}$O record from ODP Site 1218 spans the entire Oligocene and unambiguously supports the presence of a negative oxygen isotope excursion during this time [75]. Thus, the cause for this negative $\delta^{18}$O trend remains unresolved in relation to our new CO$_2$ record.

Another apparent decoupling between CO$_2$ and climate occurs near the Oligocene–Miocene boundary (approx. 23 Ma), represented by a transient, positive benthic foraminiferal $\delta^{18}$O excursion (greater than 1‰) interpreted as a period of substantial glaciation (known as the Mi-1 event) [76]. Our records suggest invariant CO$_2$ concentrations during this apparent glaciation/deglaciation, defying our current understanding of the necessary forcing required to drive Antarctic ice sheet variability.

Assuming approximately 2°C of cooling in the deep sea [77], approximately 0.5‰ of the 1‰ $\delta^{18}$O shift at Mi-1 must have been driven by an increase in ice volume. If continental ice on Antarctica had an average isotopic composition of −40‰, as indicated by isotopic modelling [71], then more than $2 \times 10^6$ km$^3$ of ice—roughly equivalent to the entire present-day East Antarctic Ice Sheet (EAIS)—must have accumulated within 400,000 years. However, CO$_2$ levels during the Oligocene appear low enough to have already maintained a fully glaciated Antarctica according to ice sheet simulations [78]. Moreover, the recovery phase of Mi-1 is even more enigmatic because models require substantially higher CO$_2$ levels—at least two times higher than the formation threshold of the EAIS (approx. 1500 ppm) to cause substantial ice sheet retreat [71,78]. Coupled climate–ice sheet simulations show that orbital forcing alone cannot cause Antarctic deglaciation once the ice sheet expands over the continent [73], and requires increases in greenhouse gas concentrations to more than four times pre-industrial levels to cause substantial ice retreat [78].

It is possible that the resolution of our new CO$_2$ record is too low to resolve large fluctuations within the body of the Mi-1 event. Still, exiting the glacial period would require substantially higher CO$_2$ levels that are not detected in our record. Alternatively, polar climate sensitivity to CO$_2$ might have been much higher than simulated in climate models, or our understanding of Antarctic ice physics is incomplete and ice sheet stability, potentially reflected by the ‘Oi’ and ‘Mi’ events (e.g. [75]), is far more sensitive and dynamic than indicated in modelling studies. Another possibility is that the assumption that benthic $\delta^{18}$O variability is representative of global temperature/ice volume is flawed. For example, Nd isotope records suggest alternating deep water sources during the Palaeogene [79,80], with the potential to alter the temperature, salinity and $\delta^{18}$O value of deep waters. Different deep water sources during the Late Oligocene and the Mi-1 event could have played a role in the magnitude of the observed benthic $\delta^{18}$O fluctuations.
Apparent discrepancies between proxy records of ice volume and CO₂ could also result if alkenone–CO₂ estimates are simply incorrect or biased for some time intervals. Various theoretical and empirical exercises have been performed to assess non-CO₂ factors that impact algal carbon isotope fractionation and uncertainties in alkenone–pCO₂ estimations [50,52]. For example, higher ambient CO₂ levels during the earlier part of the Cenozoic could have contributed to a reduction in bicarbonate uptake via β-carboxylation, resulting in εₚ values greater than 25‰ [21], as well as inhibiting CCMs. The isotopic impact of CCM upregulation during low CO₂ would arguably minimize εₚ and reconstructed CO₂ variability, which is discussed in greater detail in §5c.

(iii) The Mid- to Late Miocene

Very low Miocene pCO₂ concentrations that characterize published alkenone (figure 3) [19,20] and boron isotope [81] CO₂ records have puzzled the palaeoclimate community for nearly a decade. If atmospheric CO₂ was a key parameter forcing cryosphere expansion during the Neogene, then major Northern Hemisphere glaciation should have arguably occurred some 20 million years earlier than the accepted age of approximately 2.7 Ma (figure 3) [71,82,83]. Low pCO₂ levels are also difficult to reconcile with the well-documented warmth of the Middle and Late Miocene, which was characterized by SSTs significantly higher than today [84]. For example, subtropical east Pacific (ODP 1010) and northeast Pacific (ODP 1021) have been found to be at least 12°C warmer at 12 Ma relative to today, and 5°C warmer relative to the Early Pliocene [84]. Prior alkenone and boron isotope reconstructions have both indicated that the global warmth of the Middle Miocene climate optimum (MMCO, approx. 17–14 Ma) and the subsequent expansion and stabilization of Antarctic ice sheets (Middle Miocene climate transition (MMCT) approx. 14 Ma) were associated with relatively invariant pCO₂ [20,81] and slightly higher pCO₂ during ice expansion (figure 3) [20]. Not surprisingly, climate models applying published alkenone and boron isotope-based CO₂ records [85,86] cannot simulate Middle Miocene climate signals as determined by proxy records [19,81].

Intriguingly, our new record implicates a more important role for pCO₂ in the climatic variability of the Early to Middle Miocene. The partial pressure of carbon dioxide rises to 400–500 ppm during the climatic optimum of the Middle Miocene followed by an approximately 100 ppm decline during MMCT (figure 5), which better agrees with recent stomatal index [6] and boron isotope estimates (figure 4) [15]. Comparing the Site 925 record with the earlier alkenone–pCO₂ record from DSDP Site 588 [19,20], we found that the isotopic fractionation εₚ37:2 is larger (15‰ versus 10‰) and the SST estimate is higher by 6–8°C, both of which could have contributed to higher Miocene CO₂ levels in the new assessment. Although we cannot rule out the possibility that CO₂ air–sea disequilibrium at Site 925 contributed to higher local CO₂ estimates, three independent methods agree that pCO₂ during the MMCO was higher, and then declined during the MMCT cooling, implying that CO₂ was closely linked to major Miocene climate events. Importantly, Miocene CO₂ levels now appear higher than Pliocene and Pleistocene concentrations, consistent with the appearance of Northern Hemisphere glaciation during the Pliocene (figures 3 and 5).

(c) Assessing the effect of bicarbonate–CO₂ conversion

The capacity for active carbon transport is common among marine and fresh water algae [48,49,51]. This is because ribulose-1,5-bisphosphate carboxylase/oxygenase, the primary carboxylase, has a relatively low affinity for CO₂ and, for most algal species, is less than half saturated under current CO₂ levels [51]. CCMs increase carbon availability and reduce rates of photorespiration that impact rates of carbon fixation [51].

Whether or not CCMs were an important aspect of ancient alkenone producers remains speculative. Whereas some organisms, such as diatoms, show highly efficient and active CCMs [48,87], CCMs in modern E. huxleyi and other coccolithophores appear weakly expressed [53,88]. Physiological parameters of extinct alkenone-producing species are unknown. Since CCMs
Figure 6. Comparison between the spontaneous bicarbonate–CO$_2$(aq) conversion (green symbols) and the CCM potential of alkenone producers (orange bands) for the past 45 Ma. HCO$_3^-$–CO$_2$(aq) conversion ($r/r_k$) based on the model of Riebesell et al. [90] and Wolf-Gladrow et al. [91], using seawater pH and SST data provided by boron isotope studies [13,15,16,18], and a constant radius of 2.67 $\mu$m for alkenone-producing haptophyte algae. Computation of CCM potential is based on the model of [44,50], using previously published cell size [21,37,89,92] and existing palaeo-CO$_2$ estimates [18,21,28]. CCM potential $\left(\left([\mu/\zeta]/([P/C])\right)100 – 100\right)$ represents an arbitrary, qualitative scale of CCM activity.

are energetically expensive, ancient haptophytes probably lacked operational CCMs under the significantly higher CO$_2$ levels of the past [89]. However, the presence of CCMs could have become more pronounced during the Neogene due to the considerable decline in atmospheric CO$_2$ (figures 3–6). If this perspective is valid, then quantitative CO$_2$ reconstructions could be compromised during periods of relatively low $p$CO$_2$ levels.

Various theoretical models have been developed to understand the factors that would drive upregulation of CCMs [47,50,90,91]. One model assumes that active transport occurs when diffusive flux achieves a minimum threshold of intracellular CO$_2$ necessary for adequate growth. Minimum diffusive flux estimates depend on ambient [CO$_2$(aq)] and constraints imposed by cell size. Assuming spherical geometry, minimum [CO$_2$(aq)] can be defined as [47,90,91]

$$[CO_2]_{\text{min}} = \frac{F_{\text{in}}}{4\pi rD_T(1 + r/r_k)},$$

(5.1)

where $F_{\text{in}}$ is the diffusive influx of CO$_2$(aq), $D_T$ is the diffusion coefficient of CO$_2$, $r$ is the ‘surface area equivalent’ spherical cell radius [91], $r_k$ is the reacto-diffusive length (i.e. length of the boundary layer where HCO$_3^-$ has the opportunity to convert to CO$_2$(aq)), and the term $(1 + r/r_k)$ represents the contribution of extracellular, uncatalysed and spontaneous HCO$_3^-$–CO$_2$ conversion to the total supply of CO$_2$ [47,90].

The term $r_k$ is calculated by

$$r_k = \sqrt{\frac{D_T}{k'}}$$

(5.2)

where $k'$ is the rate constant for conversion of HCO$_3^-$ and H$_2$CO$_3$ to CO$_2$(aq). An approximation for the temperature dependence of $D_T$ is given by [93]

$$D_T = 5.019 \times 10^{-6}e^{-\left(E_d/RT_k\right)},$$

(5.3)
with activation energy \( E_d = 19510 \text{ J mol}^{-1} \), gas constant \( R = 8.3143 \text{ JK}^{-1} \text{ mol}^{-1} \) and \( T_K \) is temperature in kelvin. For the rate constant \( k' \), Gavis & Ferguson [94] demonstrated that

\[
k' = k_1[\text{OH}^-] + k_2, \tag{5.4}
\]

where \( k_1 \) and \( k_2 \) are the rate constants of formation of CO\(_2\) from HCO\(_3^-\) and H\(_2\)CO\(_3\), respectively, and \([\text{OH}^-]\) is the hydroxyl ion concentration. At 25°C, \( k_1 = 8500 \text{ m}^3 \text{ mol}^{-1} \text{ s}^{-1} \) and \( k_2 = 3 \times 10^{-5} \text{ s}^{-1} \) [94].

Given the equilibrium constant for seawater \( K_w = [\text{H}^+][\text{OH}^-] \), \([\text{OH}^-]\) can be calculated if seawater pH is known. At atmospheric pressure [95],

\[
\ln K_w = 148.96502 - 13847 \times \frac{26}{T_K} - 23.6521 \ln T_K
+ \left[ \frac{118.67}{T_K} - 5.977 + 1.0495 \ln T_K \right] S^{1/2} - 0.01615S. \tag{5.5}
\]

Therefore, the portion of fixed CO\(_2\) contributed by the conversion of bicarbonates in the geological past can be calculated if seawater temperature (\( T_K \)), pH, salinity (\( S \)) and the cell size (\( r \)) of ancient alkenone producers are constrained.

Ocean pH estimates and SST reconstructions are available for the Eocene–Oligocene climate transition [16], Middle Miocene [15], Pliocene [18] and the Pleistocene [13], and ancient cell sizes for assumed alkenone producers (e.g. Reticulofenestra, Dictyococcales and Cyclicargolithus) can be estimated using coccolith size measurements [21,37,89]. However, the available coccolith length data and boron-based pH estimates are not derived from the same marine sites. Cell sizes of assumed alkenone producers show large spatial variability. For example, Pagani et al. [21] demonstrated that Reticulofenestrid coccoliths from the Southern Ocean sites (e.g. ODP 1090) were two times as large as those in the tropical Atlantic (ODP 925, 929) during the Eocene–Oligocene transition. This apparent cell size variability of alkenone producers hampers the application of available coccolith data to the HCO\(_3^-\)–CO\(_2\) conversion evaluation of the Cenozoic. Therefore, given that cell size data for the past 40 million years are not available for Site 925, we assume a constant cell radius of 2.67 \( \mu \) m, which is the average value estimated from coccolith dimensions for the Cenozoic [21,37,89].

Boron isotope-derived seawater pH and coeval Mg/Ca-based SST, with the assumption of a constant salinity of 35 and 2.67 \( \mu \) m cell radius of alkenone producers, enables an assessment of the impact of bicarbonate–CO\(_2\) conversion on alkenone–pCO\(_2\) estimates during the Cenozoic. Temporal patterns in the ratio \( r/r_k \) indicate that the percentage of fixed carbon derived from bicarbonate conversion is generally low, of the order of 1.4–2.3% (figure 6), with a trend of elevated bicarbonate contribution by the Pleistocene (approx. 40% more relative to the Late Eocene; figure 6). Since we assume constant cell size, this increase is primarily due to an approximately 0.5 pH drop from approximately 7.7 during the latest Eocene [16] to 8.2 today. Alternatively, CCMs could have become increasingly important [50,96] to counter low CO\(_2\). However, accurate quantitative correction for the effect of HCO\(_3^-\)–CO\(_2\) conversion on alkenone–pCO\(_2\) estimates will require more detailed site-specific data on cell size of the alkenone producers and pH history of the region.

An alternative model to estimate CCM activity [44,50] considers both diffusion and active uptake of inorganic carbon to describe the nonlinear culture results of \( P. \) tricornutum:

\[
\epsilon_P = \epsilon_f + \epsilon_t - \epsilon_{-t} - \left( \frac{1}{1 + C_C/P/\mu C(1 + \beta)} \right) \left( \frac{\epsilon_f - \epsilon_{-t}}{\beta + 1} \right). \tag{5.6}
\]

Here, \( \beta \) is a constant equivalent to the ratio of CO\(_2\) loss (leakage by diffusion) to carbon fixation, \( P \) is membrane permeability, \( C \) is cell carbon content and \( \epsilon_{-t} \) is the isotopic fractionation
associated with the inorganic carbon diffusion back from the cell to the surrounding seawater. As detailed in Laws et al. [50], the value of $\varepsilon_p$ becomes insensitive to $\mu/[CO_2(aq)]$ when

$$\frac{\mu}{C_e} \geq \frac{P}{C(1 + \beta)}.$$  

(5.7)

Empirical relationships between $P$ and $C$ show its dependence on the cell radius: $P/C = 0.285 r^{-1}$. Minimum $[CO_2(aq)]$ that would trigger active transport occurs when the term $(1 + \beta)$ approaches unity, or leakage is zero. Laws et al. [50] calculate that $\varepsilon_p$ for *E. huxleyi* would become a nonlinear function of $\mu/[CO_2(aq)]$ as photoperiod growth rate exceeds 1.1 d$^{-1}$ for a cell radius of 2.6 $\mu$m, comparable with the culture results of *E. huxleyi* [42].

Growth rates in the natural environment for *E. huxleyi* and other alkenone-producing haptophytes are generally low, below 1 d$^{-1}$, ranging from 0.1 to 1 d$^{-1}$ [53,97]. Cell dimensions of alkenone-producing algae since Middle Eocene [18,21,37,89] used in conjunction with estimates of $pCO_2$ provide a means to identify geologic intervals when upregulation of CCMs was potentially necessary. For example, cell radii for alkenone producers range from approximately 4 to 2.4 $\mu$m during the Eocene–Oligocene transition [21], and cell radii were about 50% smaller during the Early Miocene [37]. Knowledge of cell radius allows calculation of the ratio $P/C$ [50] and the ratio $\mu/[CO_2(aq)]$ can be estimated from alkenone-based $CO_2$ reconstructions and a range of modern haptophyte growth rates, assuming alkenone-based $CO_2$ estimates are valid even if CCMs were active. Using $pCO_2$ data from earlier results [19,20,28], figure 6 shows that for low to moderate growth rates, the operation of CCMs is expected during the Miocene, particularly during the climatic optimum of the late Early Miocene, suggesting that the alkenone–$CO_2$ estimates may be compromised during this time interval. Obviously, this result is speculative given the necessary assumptions. For example, $CO_2$ concentrations used in this exercise derive from alkenone measurements and if CCMs were active, then these $CO_2$ values would appear artificially lower than actual concentrations and further exaggerate the potential influence of CCMs.

6. Conclusions

An alkenone-based $pCO_2$ record spanning the past 40 million years is presented for ODP Site 925—representing the first long-term Cenozoic $pCO_2$ record constructed from a single site. Facilitated by improved methodology and careful consideration of assumptions, this record provides refined $pCO_2$ estimates from a site that is characterized by limited long-term variability in oceanographic conditions. This record, therefore, reflects our most up-to-date effort to better constrain the Cenozoic history of atmospheric $CO_2$. This new record confirms predictions from climate models regarding the role of $CO_2$ in the cryosphere evolution in both hemispheres at several key time intervals (e.g. Eocene–Oligocene transition, MMCT, Plio–Pleistocene). This record also suggests that Miocene $CO_2$ levels were higher than earlier estimates, which better reconciles a long-standing data–model discrepancy for the Miocene. However, outstanding issues remain which include the presumed warming or deglaciation in the Late Oligocene and the abrupt and transient oxygen isotope excursion at Mi-1, both of which challenge our understanding of $CO_2$ climate and ice sheet sensitivity.

We also evaluate spontaneous $HCO_3^––CO_2(aq)$ conversion, which is one variable that could impact $CO_2$ estimates from the alkenone–$pCO_2$ method. We conclude that the contribution from $HCO_3^––CO_2$ conversion to the carbon fixed by alkenone-producing algae is minor, although this potential has increased by 40% from Late Eocene to the Quaternary. Finally, an exercise to evaluate potential CCM activity in haptophyte algae suggests that low $pCO_2$ levels in the Neogene are more likely to trigger CCMs.

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