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High resolution alkenone palaeobarometry indicates relatively stable $p$CO$_2$ during the Pliocene (3.3 to 2.8 Ma)

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Abstract

Temperature reconstructions indicate that the Pliocene was ~ 3 °C warmer globally than today, and several recent reconstructions of Pliocene atmospheric CO$_2$ indicate that it was above pre-industrial levels and similar to those likely to be seen this century. However, many of these reconstructions have been of relatively low temporal resolution, meaning that these records may have failed to capture variations associated with the 41 Kyr glacial-interglacial cycles thought to operate in the Pliocene. Here we present a new, high temporal resolution alkenone carbon isotope based record of
$pCO_2$ spanning 2.8 to 3.3 million years ago from ODP Site 999. Our record is of high
enough resolution (~19 Kyrs) to resolve glacial-interglacial changes beyond the
intrinsic uncertainty of the proxy method. The record suggests that Pliocene $CO_2$
levels were relatively stable, exhibiting variation less than 55 ppm. We perform
sensitivity studies to investigate the possible effect of changing sea surface
temperature, which highlights the importance of accurate and precise SST
reconstructions for alkenone palaeobarometry, but demonstrate that these
uncertainties do not affect our conclusions of relatively stable $pCO_2$ levels during this
interval.

Keywords: Pliocene, $pCO_2$, atmospheric carbon dioxide, climate, alkenone, ODP
Site 999.

Introduction

The Pliocene was the most recent epoch in Earth history that had global temperatures
greater than today; this, coupled with the similar continental positions and vegetation
cover to the present has led to interest in the Pliocene as a possible analogue for the
warmth expected by the end of the century [1]. Research to constrain the global
temperatures of the Pliocene has been ongoing for some time, including the
significant contributions of the PRISM project and successors (e.g. [2-4]), with the
consensus that the Pliocene was globally ~ 3 °C warmer than today. Similarly, many
studies suggest that Pliocene $pCO_2$ was also higher than pre-industrial levels [5-8].
Despite these similarities, a significant difference between the Pliocene and the present day is the magnitude and pacing of Pliocene glacial-interglacial changes. Based on the foraminiferal carbonate $\delta^{18}O$ record [9], Pliocene glacial-interglacial cycles were less pronounced than those of the late Quaternary, and they were 41 Kyr long in contrast to the 100 Kyr cycles of the last 0.7 Ma. Variations in $pCO_2$ greater than the ~100 ppm fluctuations of the Pleistocene are, therefore, unexpected in a Pliocene world, especially if, as suggested by Pagani et al. [6], Pliocene Earth-system sensitivity was probably greater than 3 °C for a CO$_2$ doubling. Consistent with this, alkenone-based $pCO_2$ reconstructions have shown very little glacial-interglacial variation, especially prior to the intensification of northern hemisphere glaciation at 2.8 Ma. These records have been, in part, confirmed by boron isotope based reconstructions [7]. However, due to their low temporal resolution it is possible that they fail to capture higher frequency variability in $pCO_2$ and represent neither the mean state of the climate system nor its variability on glacial timescales well. A recent reconstruction using boron isotopes [8] has increased the temporal resolution, and in fact targeted specific glacial and interglacial peaks and troughs to attempt to resolve this. Their record exhibits fluctuations in Pliocene $pCO_2$ that are in fact larger (almost one and a half times) than those observed in Pleistocene ice core records [10-12].

To address this apparent discrepancy, we reconstruct $pCO_2$ at similarly high resolution from 3.3 to 2.8 Ma using alkenone $\delta^{13}C$ values. We apply our approach to ODP Site 999 in the Caribbean because that is the same site used by Bartoli et al. [8] and Seki et al. [7], allowing direct comparison of our records. Additionally, we also conduct a sensitivity analysis of our reconstructed $pCO_2$ levels, allowing us to constrain their potential range during this time.
Approach and Methods

Alkenone palaeobarometry

The isotopic fractionation between dissolved inorganic carbon and marine organic matter during photosynthesis ($\varepsilon_p$) is controlled by, amongst other factors, the concentration of CO$_2$ in the water in which the organism is photosynthesising ([CO$_2$]$_{aq}$). This is ultimately controlled by the concentration of CO$_2$ in the overlying atmosphere with which the ocean is in equilibrium. Other factors that can effect $\varepsilon_p$ values include physiological factors, such as cell geometry [13] and membrane permeability [14] and environmental factors, such as nutrient and light availability and their impact on carbon demand (i.e. growth rate) and carbon assimilation mechanisms [15-18].

In order to constrain the physiological factors, biomarkers derived from a narrow taxonomic range can be used rather than bulk organic matter. This approach also prevents terrestrial or non-photosynthetically produced organic matter from biasing the marine organic matter isotopic signature [19]. Long chain ketones (alkenones) containing 37 carbons are produced only by a restricted group of haptophyte organisms, such as Gephyrocapsaceae coccolithophores [20]. Thus, work over the past 20 years has focussed specifically on the alkenone palaeobarometer as a tool to reconstruct ancient atmospheric $\rho$CO$_2$, so long as other contributing factors (growth rate, light regime) can be constrained. In order to determine $\varepsilon_p$ values, the isotopic composition of both the dissolved inorganic carbon pool (DIC) and organic biomass must be known. The isotopic composition of the organic biomass ($\delta^{13}$C$_{org}$) is calculated from the alkenone $\delta^{13}$C ($\delta^{13}$C$_{37:2}$), corrected for a fractionation between
alkenone and haptophyte biomass by assuming a constant fractionation of 4.2 ‰ (Equation 1; [13, 16]).

\[ \varepsilon_{\text{alkenone}} = \frac{\delta^{13}C_{37:2} + 1000}{\delta^{13}C_{\text{org}} + 1000} - 1 \]

The isotopic composition of DIC is estimated by measuring the $\delta^{13}$C value of planktic foraminifera, assuming the experimentally determined temperature dependent fractionation between calcite and CO$_2$(g) ($\varepsilon_{\text{calcite-CO}_2(g)}$) shown in equation 2 [21]:

\[ \varepsilon_{\text{calcite-CO}_2(g)} = 11.98 - 0.12T \]

Where $T$ is sea surface temperature (in degrees Celsius). This fractionation factor can then be used to calculate the carbon isotopic composition of CO$_2$(g) ($\delta^{13}$C$_{\text{CO}_2(g)}$):

\[ \delta^{13}C_{\text{CO}_2(g)} = \frac{\delta^{13}C_{\text{carbonate}} + 1000}{\varepsilon_{\text{calcite-CO}_2(g)} + 1} \times 1000 - 1 \]

From this, the carbon isotopic composition of CO$_2$(aq) ($\delta^{13}$C$_{\text{CO}_2(aq)}$) can be obtained using the experimentally determined relationship of Mook et al., [22] as shown in equations 4 and 5:

\[ \varepsilon_{\text{CO}_2(aq)-\text{CO}_2(g)} = \frac{-373}{T + 273.15} + 0.19 \]

\[ \delta^{13}C_{\text{CO}_2(aq)} = \left( \frac{\varepsilon_{\text{CO}_2(aq)-\text{CO}_2(g)}}{1000} + 1 \right) \left( \delta^{13}C_{\text{CO}_2(g)} + 1000 \right) - 1000 \]

Photosynthetic fractionation ($\varepsilon_p$) can then be calculated from the determined $\delta^{13}$C$_{\text{CO}_2(aq)}$ and $\delta^{13}$C$_{\text{org}}$ (equation 6):
\[ \varepsilon_p = \left( \frac{\delta^{13}C_{CO_2(aq)} + 1000}{\delta^{13}C_{org} + 1000} - 1 \right) \times 1000 \]

and this is then used to calculate \([\text{CO}_2(aq)]\) according to equation 7:

\[ [\text{CO}_2(aq)] = \frac{b}{\varepsilon_f - \varepsilon_p} \]

where \(\varepsilon_f\) represents the isotopic fractionation during carbon fixation, assumed here to be constant and 25‰ [16]. The ‘\(b\)’ term represents the summation of physiological factors, such as cell size and growth rate. In the modern ocean this term shows a close correlation with \([\text{PO}_4^{3-}]\), allowing estimation of ‘\(b\)’ by assuming past \([\text{PO}_4^{3-}]\) was similar to that present at the site today (0.2 µM; [6, 16, 23]). Finally from \([\text{CO}_2(aq)]\), atmospheric \(p\text{CO}_2\) can be calculated, using Henry’s law (equation 8) and assuming equilibrium between the surface water and overlying atmosphere:

\[ p\text{CO}_2 = \frac{[\text{CO}_2(aq)]}{K_{Hf}} \]

The solubility coefficient \((K_{Hf})\) is salinity and temperature dependant, and calculated following the parameterization of Weiss [24, 25]. The assumptions inherent in the above treatment are discussed further below.

**Analytical**

In this study, analytical determinations of \(\varepsilon_p\) values were conducted similar to those of previous alkenone paleo-\(p\text{CO}_2\) studies (e.g. [6, 7, 23, 26-28]) from ODP Site 999 (12°44.639' N, 78°44.360' W, 2838 m water depth. Site 999 is slightly out of equilibrium in the modern ocean, with surface waters oversaturated in CO\(_2\) relative to the atmosphere, providing a small (<10 gCm\(^3\)yr\(^{-1}\); [29]) net source of CO\(_2\) to the
atmosphere. However the site has been shown to be capable of recording past changes in $pCO_2$ and the air-sea equilibrium is not thought to have changed significantly from the Pliocene to today (see discussion in [8]). Specifically, 27 samples were freeze dried, ground by hand and solvent extracted either by Soxhlet apparatus or ultrasonically. Soxhlet extractions were performed using a dichloromethane (DCM):methanol (MeOH) azeotrope (2:1, v:v), refluxing for 24 hours. Ultrasonic extractions were performed with, sequentially, DCM, DCM:MeOH (1:1, v:v) and MeOH, repeated 3 times for each solvent with each extraction taking 15 minutes in an ultrasonic bath with ~15 ml of solvent each time. Supernatants were removed and combined before reduction by rotary evaporation and finally evaporated to dryness under a stream of N$_2$. Following elution through small (4 cm) sodium sulphate columns to remove excess water, total lipid extracts were divided into apolar and polar fractions using alumina flash column chromatography using 4 column volumes of $n$-hexane:DCM (9:1, v:v) and 3 column volumes of MeOH, respectively. Alkenone concentrations were quantified by GC-FID (Hewlett Packard 5890 Series II) following trimethylsilyl derivatisation. The GC oven was programmed to increase in temperature from 70°C to 130°C at 20°Cmin$^{-1}$, then to 300°C at 4°Cmin$^{-1}$, finally being held isothermal for 25 min. The column was a CPSil-5CB (dimethyl polysiloxane equivalent), 0.12 µm film thickness, ~50 m length and 0.32 mm interal diameter with a H$_2$ carrier gas. Alkenone identification was confirmed by GC-MS (ThermoQuest Trace MS, He carrier gas). Absolute compound concentrations were quantified by reference to an internal standard (hexadecan-2-ol) added prior to column chromatography.

Sea surface temperature (SST) was reconstructed using the alkenone unsaturation index ($U_{37}^{K}$;[30, 31]; equation 9):
Eqn. 9 \[ U_{37}^{K'} = \frac{[C_{37:2}]}{[C_{37:2} + C_{37:3}]} \]

Where \( C_{37:2} \) is the di-unsaturated methyl alkenone and \( C_{37:3} \) is the tri-unsaturated compound. SSTs were then calculated using the calibration of Müller et al. [32] (equation 10):

Eqn. 10 \[ U_{37}^{K'} = 0.033T + 0.044 \]

Concerns have been raised about the use of \( U_{37}^{K'} \) as the index approaches 1 [33]. This would be of particular concern at Site 999 as over the studied interval \( U_{37}^{K'} \) is > 0.9. However the challenge of calibrating SSTs towards the upper limit of \( U_{37}^{K'} \) seems to be a problem largely restricted to sediment trap based calibrations. For core tops a linear calibration seems to hold true, and in fact the updated core-top calibration of Conte et al., [33] is essentially identical to that of Muller et al., [32] (which is more widely used and therefore our preferred). They are especially similar towards the top end of the scale. As we are dealing here with alkenones which have made it to the sea floor a core top calibration seems most appropriate.

Alkenone isotope analyses were performed on a ThermoFisher Delta V connected via a GC isolink and conflo IV to a Trace GC. The GC oven was programmed to increase in temperature from 70°C to 200°C at 20°Cmin\(^{-1}\) then to 300°C at 6°Cmin\(^{-1}\) and finally held isothermal for 25 min. Conversion to the VPDB scale was calculated by reference to a laboratory standard gas tank of known \( \delta^{13}C \). Instrument performance was monitored using an in house fatty acid methyl ester standard and long term precision is ~0.3 ‰.
Ten to fifteen specimens of the planktic foraminifera *Globigerinoides ruber* were picked from the 300-350 µm fraction for δ¹³C analysis. This was determined with a Finnigan MAT 251 with an online automatic carbonate preparation device at the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven. Calibration to the VPDB scale was performed using the international NBS19 standard. Reproducibility is better than ±0.06 ‰ over a one-year period based on repeat measurements of a laboratory standard.

The age model for ODP Site 999 is as discussed in Seki et al. [7]. Uncertainty propagation on our alkenone-derived CO₂ estimates was performed by Monte Carlo modelling (n=25000). Uncertainties of 2 °C and 0.1 ‰ were applied to temperature and foraminiferal calcite δ¹³C, (normal probability function (pdf), 2 σ error) and 2 and 0.1 to salinity and [PO₄³⁻], respectively (2 σ; uniform pdf). 2 σ errors on alkenone δ¹³C were estimated from replicate runs, calcite δ¹³C from repeat runs of an internal standard, estimated integrated analytical and calibration error for *U*37K based temperatures [32] and conservative estimates of likely variation for salinity and [PO₄³⁻]. An 11 % error on the slope of b=a[PO₄]+c was assumed [26].

**Results**

Alkenone and *G. ruber* δ¹³C values (Figure 1b) were used to calculate εₚ values (Figure 1a). These εₚ values are fairly stable throughout the study interval, varying between 12.2 and 9.4 ‰. This is close to the values reported by Seki et al., [7] for the same Site over this interval (12.2 - 10.9 ‰).

Using modern [PO₄³⁻] for the Caribbean Sea, εₚ values can be converted to [CO₂]ₐq (Equation 7; Figure 1b). Using our SSTs derived from *U*37K indices and assuming air-
sea equilibrium, [CO₂]_{aq} can then be used to determine atmospheric pCO₂ (Equation 8). U^{K_{37}} indices range from 0.90 to 0.99 (close to the maximum recordable value for U^{K_{37}}), resulting in SSTs at Site 999 of ~28 °C that show a slight decrease over the 500 Kyrs of our record (Figure 2b). These are ~2 °C higher than the planktic foraminifer (Globigerinoides sacculifer) Mg/Ca based SST record of Groeneveld [34] from the same site, <1 °C lower than the SSTs estimated by Bartoli et al. [8] based on a seawater Mg/Ca correction of these same data, and very similar to modern SSTs that range from 26.7 °C to 28.2 °C [35].

Our resulting pCO₂ reconstruction (Figure 2a) reveals relatively stable pCO₂ values that are within the range of previously published alkenone records from ODP Site 999 (without the lith size correction of Seki et al. [7]) and elsewhere [6]. All of our reconstructed pCO₂ (250-300 ppm) levels are similar to or slightly higher than the 240-290 ppm for Pleistocene interglacials reconstructed from ice cores [10-12] and are consistent with glacial-interglacial variability of at most 40 ppm. In fact, the entire range of determined pCO₂ values for the end of the Pliocene is less than the 80 ppm difference between the Holocene and the Last Glacial Maximum [36]. There is some variability outside of uncertainty in the ε_p record in the younger part of the record, hinting to some variability after 3 Ma, however once the full propagation of uncertainties are taken through to the CO₂ reconstruction, the variation is no longer significant. Below, we discuss the pCO₂ estimates, their variations with respect to Pliocene glacial-interglacial cycles and the potential range of pCO₂ given our assumptions of growth rate and SST.

Discussion
Glacial-Interglacial pCO$_2$ variations

We estimate absolute $p$CO$_2$ to be around $\sim$270 ppm for much of the period studied here, based on our most likely temperature, cell geometry and growth rates assumptions (see subsequent sections for sensitivity analysis of these parameters). This is similar to pre-industrial levels, and around the peak level of the Pleistocene ice core records (298.6 ppm; [10-12]). Our record is within the range of estimates given by Pagani et al., [6] (Figure 3a), although it should be noted that these authors report a broad range of absolute CO$_2$ due to differences between the sites. Our record is below the ‘CO$_2$slope’ reported in Pagani et al. [6] i.e. their extrapolated trend from the early Pliocene to the present day.

Estimating absolute $p$CO$_2$ from a single site is complicated by uncertainty as to whether the site has been in equilibrium with the atmosphere over the period of interest. As highlighted by Pagani et al. [6], different sites can exhibit very different estimates for atmospheric $p$CO$_2$, as not all of the surface ocean is in equilibrium with the atmosphere [29]. The surface ocean at Site 999 is close to equilibrium today [29] and reconstructed alkenone based $p$CO$_2$ values are similar to ice core records where they overlap in the Pleistocene [7] suggesting that the site was in equilibrium through much of this time. It is difficult to know whether this remained so in the Pliocene with different circulation in the Caribbean, so as with all single site records, our absolute $p$CO$_2$ should be treated with some care.

Our absolute $p$CO$_2$ is similar to the alkenone-based record without secondary corrections of Seki et al. [7] from the same site, although somewhat lower than both the cell size corrected alkenone record and boron isotope based records of Seki et al. [7] (Figure 3a). Bartoli et al. [8] report a broad range of $p$CO$_2$ (170-400 ppm; Figure
3b) and our record is within that range. The difference between our record and the cell size corrected record of Seki et al. [7] (Figure 4) highlights the importance of secondary corrections, particularly on alkenone based method and we explore this further below. Given the potential difficulty of assessing absolute $p$CO$_2$ levels from single site records we now focus on $p$CO$_2$ variability during this interval in the Pliocene.

Previous alkenone-based palaeobarometry has been at a relatively low temporal resolution, and given the 41 kyr glacial-interglacial variability in the Pliocene world, it is possible that these records do not capture rapid changes in $p$CO$_2$ [6, 7]. Our new record increases the resolution of the alkenone-based records, but unlike the boron record of Bartioli et al. [8] shows virtually invariant $p$CO$_2$ within the precision of the alkenone palaeobarometer (Figure 4). The differences between these two records cannot be due to differences in ocean-atmosphere equilibrium, as both are based on Site 999. The magnitude of variability in our record is similar to that seen in previous, low resolution records (i.e. the boron and alkenone records of Seki et al. [7], and the alkenone records of Pagani et al. [6]) which may suggest that these records have captured $p$CO$_2$ variability despite their lower resolution, and the small estimated range of Pliocene $p$CO$_2$ is a feature of Pliocene climate dynamics rather than a sampling artefact. An alternative hypothesis is that the alkenone palaeobarometer underestimates variability for an as yet unknown reason.

It should perhaps not be surprising that Pliocene $p$CO$_2$ appears to be relatively stable; the large, 100 Kyr glacial-interglacial cycles of the Pleistocene are associated with ~100 ppm of change in $p$CO$_2$ [10-12], and it is likely that the smaller amplitude variations in the Pliocene would be associated with significantly smaller $p$CO$_2$. 
changes. The large amplitude changes of Bartoli et al. [8] are therefore somewhat surprising. Given that the full uncertainty envelope for our alkenone $p$CO$_2$ records is approximately ±40 ppm, it is plausible that smaller, Pliocene variations in $p$CO$_2$ would be below the detection limit of our methods.

Cell size and productivity corrections

Atmospheric $p$CO$_2$ reconstructions from alkenone isotopes can be affected by cell size and productivity, i.e. growth rate variations. Seki et al. [7] applied a conceptual cell size correction to the alkenone data from Site 999, based on the low resolution lith size record of Kameo and Bralower [37]. However, recent high resolution data shows no evidence of changes in coccolith size over the time interval of interest [38]. Crucially, there are no changes in coccolith size – and thus, inferred coccolithophorid cell size – on glacial-interglacial timescales, and hence it is unlikely that they could account for the low variability observed here. It remains possible, however, that they could account for the relatively low absolute $p$CO$_2$ values determined for the Pliocene at Site 999, compared to $\delta^{11}$B-based estimates (~400 ppm; [7, 8]).

There is not yet a consensus approach to the application of a cell geometry correction (see discussion in Seki et al. [7] and Henderiks and Pagani [39]), however attempts have been made to correct for cell size changes by adjusting the ‘$b$’ term in equation 7. Henderiks and Pagani [39] adjusted the ‘$b$’ term based on the ratio of “fossil” haptophyte cell volume:surface area ($V:SA_{fossil}$) to that of the modern *Emiliania huxleyi* ($V:SA_{E.hux}$) used for modern culture studies. (equation 11).
Eqn. 11  \[ b' = b \left( \frac{V:SA_{fossil}}{V:SA_{E.hux}} \right) \]

Popp et al., 1998 determined \( V:SA_{E.hux} \) to be 0.9 ± 0.1\( \mu \)m and the value of \( V:SA_{fossil} \) can be estimated using the relationship between cell diameter (\( D_{cell} \)) and <i>Reticulofenestra</i> coccolith length (\( L_{coccolith} \); [39]; equation 12):

Eqn. 12  \[ D_{cell} = 0.55 + 0.88 \times L_{coccolith} \]

Reticulofenestrads (<i>Noelaerhabdaceae</i>) are thought to be important alkenone producers in the past [39], although there is some evidence that this may not be the case for some earlier parts of the Neogene [40].

The cell size correction results in a linear correction to \( pCO_2 \), the gradient of which is temperature dependent (Figure 5), where a larger coccolith length results in higher reconstructed \( CO_2 \). This effect is increased at higher SSTs. Approximately 1 \( \mu \)m of change in \( L_{coccolith} \) would be required to alter \( pCO_2 \) beyond our uncertainty envelope. This represents a size change of ~25\%, and there is no evidence for such a change on glacial-interglacial timescales at Site 999 [38].

Similarly, there is no evidence that growth rate changed on glacial-interglacial timescales at Site 999 through the interval studied. Seki et al. [7] noted that there could have been changes in the oceanography of the site as the shoaling of the Panama isthmus isolated the Caribbean from the Pacific. O’Dea et al. [41] had argued that these changes could have influenced the nutrient regime at Site 999; however, the closure of the strait to deep water is thought to have been complete by 4.6 Ma [42]. Moreover, alkenone and other biomarker concentrations and mass accumulation rates are low and relatively invariant over the studied interval [7]. Other indicators of
productivity (organic carbon mass accumulation rates) have not shown evidence of significant changes in productivity [43]. We have improved the resolution of these analyses to 16-kyr and no systematic variation is apparent, suggesting that on glacial-interglacial timescales no correction to our $p$CO$_2$ reconstruction is justified.

No proxy exists to directly reconstruct the growth rate conditions at the site during the Pliocene. Our approach is to use the relationship between $'b'$ and [PO$_4^{3-}$] which has been calibrated globally [6, 23, 44] and appears to be a proxy for growth limiting nutrients [23]. Our favoured assumption is to use a modern day value for surface water [PO$_4^{3-}$] at Site 999 [45] but here we explore the possibility that this assumption is incorrect. To this end, we have performed a sensitivity test, and apply a [PO$_4^{3-}$] which represents an oligotrophic site (0.05 $\mu$M), a high nutrient area similar to present day eastern equatorial Pacific (0.6 $\mu$M) and an extreme case representative of an active upwelling region (0.9 $\mu$M)[45]. The resulting $p$CO$_2$ reconstructions vary from $\sim$230 ppm for the oligotrophic model, $\sim$390 ppm for the eastern equatorial pacific model, and $\sim$480 ppm for the extreme case (Figure 6). The sensitivity tests demonstrate that our favoured assumptions that result in $pCO_2$ of $\sim$270 ppm may be a lower bound, and if the nutrient regime was significantly different in the Pliocene then our record may be an underestimate. However, as discussed above, we have no direct evidence for such a change, and critically, no evidence of changes on glacial-interglacial timescales that may explain the low variability of our record.

The importance of accurate and precise temperature determinations

Critical to the validity of alkenone isotope $pCO_2$ reconstructions is the accurate and precise determination of sea surface temperature. Whilst some of the previously
discussed parameters (such as an evolutionary change in haptophyte cell size, or significant changes in oceanographic regime leading to changes in growth rate) are likely stable over short periods of time, sea surface temperatures can change on glacial-interglacial timescales and affect $pCO_2$ estimates. SST is involved three times in the reconstruction of $pCO_2$ from alkenone $\delta^{13}C$ values, in the conversion of $\delta^{13}C_{\text{calcite}}$ to $\delta^{13}C_{\text{CO}_2(\text{g})}$ values (equations 2 - 3), $\delta^{13}C_{\text{CO}_2(\text{g})}$ to $\delta^{13}C_{\text{CO}_2(\text{aq})}$ values (equations 4 -5) and the calculation of the solubility coefficient (equation 8). This results in a non-linear temperature dependence of the $\delta^{13}C_{\text{alkenone}}$-$pCO_2$ relationship (Figure 7). The size of this effect can be important both in terms of accuracy and precision of alkenone-based reconstructions. Proxies that show potential to reconstruct SST suitable for alkenone palaeobarometry include those based on alkenone unsaturation indices [30, 31] and planktic foraminiferal Mg/Ca ratios [46].

Estimates of uncertainty in the measurement of SST using either Mg/Ca or alkenone unsaturation suggests a combined analytical and correlation error of approximately 2 °C (2σ; [47, 48]).

The relationships between alkenone $\delta^{13}C$, $\varepsilon_p$, $[CO_2]_{\text{aq}}$ and $pCO_2$, and the effects of SST are shown in Figure 7. Higher reconstructed SST results in higher apparent $\varepsilon_p$ for a given $\delta^{13}C$ value (Figure 7a) and higher $pCO_2$ for a given $[CO_2]_{\text{aq}}$ (Figure 7b). Higher reconstructed SST, therefore, results in higher apparent $pCO_2$ for a given $\delta^{13}C$ value by integrating these two effects (Figure 7d). The magnitude of this effect is more pronounced at higher $pCO_2$ and more negative alkenone $\delta^{13}C$ values (Figure 7d), and also as $\varepsilon_p$ approaches $\varepsilon_f$ (see the discussion in Pagani et al. [27]).

For example, for an alkenone $\delta^{13}C$ value of -25 ‰ (which gives a representative $pCO_2$ of 300 ppm in this sensitivity test), the 2 °C analytical and calibration error in Mg/Ca
or \( U_{37}^{K} \) SST estimates would result in a error of \( \sim 23 \) ppm in \( pCO_2 \); at a more negative \( \delta^{13}C \) value of -28 \( \% \) (\( pCO_2 = 400 \) ppm) the same error in SST results in an error of \( \sim 34 \) ppm in \( pCO_2 \) (Figure 7d). One result of this is that an incorrect or overestimated decline in SST can lead to an artificial apparent decline in \( pCO_2 \). This requires careful consideration if estimating climate or earth system sensitivity from coupled alkenone \( pCO_2 \) and SST records. To apply this directly to the data presented here, SSTs 2 °C cooler than our data suggests (ie at the edge of the quoted uncertainty for alkenone unsaturation based temperatures) would result in average reconstructed \( pCO_2 \) over the interval studied of 255 ppm, a 15 ppm reduction. Conversely SSTs 2 °C warmer would give average reconstructed \( pCO_2 \) 20 ppm higher, at 290 ppm.

For the Pliocene, the choice of SST record is important, given the uncertainty as to the possible effects of changing Mg/Ca\(_{sw}\) on Mg/Ca palaeothermometry. Recent reconstructions of Pliocene Mg/Ca\(_{sw}\) suggest that the Pliocene value could have been more than 1 mol/mol lower [49], which could change the reconstructed SST by as much as 6 °C. Our preferred \( U_{37}^{K} \) temperatures for Site 999 lie between the Mg/Ca\(_{sw}\) corrected and uncorrected records for the same time period [8, 34]. The uncorrected SST estimates of Groeneveld [34] are \( \sim 3.8 \) °C lower and would result in \( pCO_2 \) ~29 ppm lower if applied to our records, whereas the up to 2.5 °C higher temperatures of Bartoli et al. [8] would increase our estimates by ~24 ppm. At Site 999 today SSTs vary between 26.7 °C and 28.2 °C whereas temperatures at the habitat depth likely for \( G. sacculifer \) it is 24.2 – 26.6 °C [35], and so the cooler temperatures of Groeneveld [34] may be due to differences in depths of the recording organism. Crucially however, none of the SST records for Site 999 indicate glacial – interglacial
variability that would result in \( p\text{CO}_2 \) variations with a magnitude similar to those of the Pleistocene, or as recorded by Bartoli et al., [8].

**Synthesis**

We reconstruct atmospheric \( p\text{CO}_2 \) for the Pliocene (3.3 to 2.8 Ma) of \( \sim 270 \pm 40 \) ppm (2\( \sigma \)) similar to Pleistocene interglacials. We record little or no variability suggesting \( p\text{CO}_2 \) was persistently at about Pleistocene interglacial values. Only at the outer bounds of our uncertainty envelope would we record Pleistocene glacial levels of \( p\text{CO}_2 \). Uncertainty in our assumptions for productivity, SST and cell size all result in a broad uncertainty envelope around our preferred parameterization, with our best estimate suggesting \( p\text{CO}_2 \) was between \( \sim 230 \) and 300 ppm. These absolute values are lower than those derived from other approaches and this could reflect a combination of local paleoceanographic conditions and the impact of secondary effects on alkenone \( \delta^{13}\text{C} \) values. However, we see no evidence that such secondary effects were varying on glacial-interglacial timescales, and consequently, our data collectively indicate that \( p\text{CO}_2 \) at this point in the Pliocene was relatively stable. We see no evidence for glacial-interglacial changes larger than the fundamental precision of the proxy method of 40 ppm, and our record supports the idea that minimal \( p\text{CO}_2 \) variability was associated with the small glacial-interglacial climate variability of the Pliocene. However, further work is needed to improve the precision of all proxy methods, and to reconcile differences between records of Pliocene \( p\text{CO}_2 \).

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Figure Captions

Figure 1a. $\varepsilon_p$ values (filled square symbols and fine black line) are shown with the epibenthic foraminiferal oxygen isotope record for the site (Grey/heavy solid line;[42]) for comparison. For $\varepsilon_p$ the plotted uncertainty envelopes represent maximum and minimum estimates based on 2$\sigma$ extremes of the $\delta^{13}$C$_{37:2}$ measurement (dashed line) and a full monte carlo propagation of associated uncertainties (dotted line; see main text for details). b. G. ruber (open diamonds) and alkenone $\delta^{13}$C values (filled circles); error bars are 2$\sigma$ analytical errors based on replicate measurements.

Figure 2a. pCO$_2$ reconstruction based on our new high resolution $\delta^{13}$C$_{37:2}$ records (filled squares and line); as in Figure 1, the dashed line shows an uncertainty envelope representing minimum and maximum estimates based on 2$\sigma$ extremes of the $\delta^{13}$C$_{37:2}$ measurement (dashed line) and a full monte carlo propagation of associated uncertainties (dotted line; see main text for details). b. Sea surface temperature estimates based on the alkenone unsaturation index. Calibration and measurement uncertainties are approximately 2 °C and are omitted for clarity. c. Benthic foraminiferal oxygen isotope stack LR04 (Grey/heavy solid line; [9]) for comparison.

Figure 3 Selected records of pCO$_2$ and climate from the Pliocene to present. a. Alkenone-based pCO$_2$ records from Seki et al. [7] corrected for cell geometry (dashed line; minimum and maximum estimates) and uncorrected (purple filled squares and line; minimum and maximum estimates) and Pagani et al. [6] shown here as their reconstructed ‘CO$_{2slope}$’ (from top to bottom: from ODP sites 1012 [khaki heavy line]; 1208 [green heavy line]; 982 [cyan heavy line]; 1012 with an alternative nutrient scenario [heavy dashed line]; and 806 [lilac heavy line]. Our record is shown as open
squares and line with the error envelope from the monte carlo model (see text). b. $\delta^{11}$B-based $pCO_2$ records from Seki et al. [7] calculated using modelled [CO$_3^{2-}$] (blue filled diamonds) and assuming modern total alkalinity (TA; red filled circles). Error bars are ±25 ppm and the error envelope is based on varying TA by ±5%. The records of Bartoli et al. [8] are shown in grey, (open grey circles and line, with $2\sigma$ uncertainty envelope) along with that of Hönisch et al. [50] (filled grey circles and line, with $2\sigma$ uncertainty envelope). Also shown is the Antarctic ice core record of $pCO_2$ over the last 800 Kyrs for comparison ([12] and references therein). c. Benthic foraminiferal oxygen isotope stack LR04 (Grey/heavy solid line; [9]) for comparison (Online version in colour.)

Figure 4 Pliocene $pCO_2$ records from ODP Site 999. a Alkenone-based $pCO_2$ records from Seki et al. [7] corrected for cell geometry (dashed line; minimum and maximum estimates) and uncorrected (purple filled squares and line; minimum and maximum estimates) and our new higher resolution record (filled squares and line); the dashed line shows an uncertainty envelope representing minimum and maximum estimates based on $2\sigma$ extremes of the $\delta^{13}C_{37:2}$ measurement (dashed line) and a full monte carlo propagation of associated uncertainties (dotted line; see main text for details). b $\delta^{11}$B-based $pCO_2$ records from Seki et al. [7] calculated using modelled [CO$_3^{2-}$] (blue filled diamonds) and assuming modern total alkalinity (TA; red filled circles). Error bars are ±25 ppm and the error envelope is based on varying TA by ±5%. The record of Bartoli et al. [8] is shown as grey open circles and line with $2\sigma$ uncertainty envelope (grey lines). c. Benthic foraminiferal oxygen isotope stack LR04 (Grey/heavy solid line; [9]) for comparison (Online version in colour.)
Figure 5 The effect of the coccolith length ($L_{coccolith}$) correction on reconstructed $pCO_2$. Lines represent the correction applied at different sea surface temperatures at 3 °C intervals (as labelled). White circles represent the uncorrected values (where the coccolith length equals that of modern cultured coccolithophores). These were calculated based on representative values of $\delta^{13}C_{37:2} = -20 \%o$ and $\delta^{13}C_{cc} = 2 \%o$. $[PO_4^{3-}] = 0.25 \mu M$ and $S = 35$ psu.

Figure 6 Phosphate concentrations are closely correlated with apparent ‘$b$’ term, most likely due to a linkage to growth rate. Our favoured assumption uses the modern day concentration for the site ($0.2 \mu M$; black filled squares and line) with minimum and maximum estimates based on 2 $\sigma$ extremes of the $\delta^{13}C_{37:2}$ measurement (dashed line) and a full monte carlo propagation of associated uncertainties (which includes a ± 0.1 $\mu M$ on $[PO_4^{3-}]$; dotted line; see main text for details). We also include reconstructions with $[PO_4^{3-}]$ representative of an oligotrophic site ($0.05 \mu M$; open squares), a high nutrient area similar to the eastern equatorial Pacific ($0.6 \mu M$; open triangles) and an extreme case representative of an active upwelling region ($0.9 \mu M$; open circles)[45]. The appropriate ‘$b$’ term according to the relationship of Pagani et al. [6] is shown for each reconstruction.

Figure 7 The relationship between $\varepsilon_p$ and alkenone $\delta^{13}C$ (a); $[CO_2(aq)]$ and $pCO_2$ (b); $\varepsilon_p$ and $pCO_2$ (c); and alkenone and $\delta^{13}C$ (d) are all affected by SST. Lines are calculated at varied SST at 3 °C intervals (as labelled), and were calculated based on representative starting values of $\delta^{13}C_{37:2} = -20 \%o$; $\delta^{13}C_{cc} = 2 \%o$. $[PO_4^{3-}] = 0.25 \mu M$, $S = 35$ psu.

Short title: Stable Pliocene $pCO_2$
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