Coccolithophores, a group of unicellular calcifying phytoplankton, have been major contributors to marine carbonate production since the calcite plates that they produce (coccoliths) first appeared in the fossil record over 200 million years ago (Ma). The response of this process to changes in environment on evolutionary timescales remains poorly understood, particularly in warm climates. Here we integrate a dataset consisting of carbon isotope ratios of size-separated coccolith calcite from marine sediments with a cell-scale model to interrogate cellular carbon fluxes and $p_{CO_2}$ through the Eocene (~55–34 Ma), Earth’s hottest interval of the past 100 million years. We show that the large coccolithophores that rose to dominate the oceans through the Eocene have higher calcification-to-carbon fixation ratios than their predecessors while the opposite is true for smaller coccolithophores. These changes, which occurred in the context of increasing ocean alkalization, may have played a role in an apparent positive carbon cycle feedback to decreasing $p_{CO_2}$. Our approach also provides independent support of multiproxy-based evidence for general $p_{CO_2}$ decline through the Eocene in step with temperature. Together, this challenges the emerging view that a general decline in $p_{CO_2}$ reduces calcification on evolutionary timescales.

Coccolith production and export removes alkalinity from the surface ocean, reducing its capacity to buffer changes in $p_{CO_2}$ (ref. 1). On geologic timescales, coccolith burial in deep-sea sediments removes carbon from the ocean–atmosphere system. From a biogeochemical perspective, carbon fixation in the surface ocean is limited by nutrient availability, temperature and light levels; the amount of calcite produced is then dictated by the particulate inorganic carbon (PIC) to particulate organic carbon (POC) ratio (PIC/POC) of biogenic material. PIC and POC are, respectively, the time-integrated rates of calcite precipitation ($R_{calc}$) and carbon fixation ($R_{fix}$). $R_{calc}/R_{fix}$ (conceptually distinct from PIC/POC but equivalent at steady state) varies between species of coccolithophore, so changes in species composition through time can alter the amount of calcite transported to the deep ocean. At the cellular level, $R_{calc}/R_{fix}$ is a physiologically important parameter because calcification and carbon fixation fluxes have opposing effects on intracellular chemistry. These fluxes also drive the carbon isotope ratio of dissolved inorganic carbon (DIC) in the intracellular environment in opposite directions, which lays the foundation for reconstructing this ratio in ancient coccolithophores through the carbon isotope compositions of their intracellularly formed coccoliths.

The prevailing view, based largely on the morphology and weight of fossil coccoliths, is that coccolithophore calcification decreased during periods of lowered $p_{CO_2}$ in the icehouse Neogene and glacial periods (although there are counter-examples (1,18,19); Supplementary Table 1). There is a degree of ambiguity in the meaning of ‘calcification’, whether this refers to the amount of calcite per cell, production rates or the rate of calcite precipitated per unit carbon fixed. In this Article, we target the last of these explicitly by constraining $R_{calc}/R_{fix}$. We take an approach that integrates carbon isotopes and morphometric data, with a cellular carbon flux model, to simultaneously constrain $R_{calc}/R_{fix}$ across different taxonomic groups and $CO_2$ levels. The fossil record captures how communities change on evolutionary timescales, and the Eocene in particular, characterized by high but declining temperature (ref. 20) and $p_{CO_2}$ (refs. 21–27), provides a unique opportunity to gain this insight in a hothouse climate.

For decades, carbon isotope ratios of coccolith calcite were ignored in the field of palaeoclimatology because they exhibited large and variable deviations from calcite precipitated at equilibrium in the same environment, making them an unreliable target to reconstruct the carbon isotopic composition of DIC. More recently, these deviations, or carbon isotope vital effects (CIVEs), have themselves been shown to contain information regarding rates of calcification (22). CIVEs are a function of coccolithophore physiology and ambient carbonate chemistry (22–27). Coccolith calcite enriched (depleted) in $^{13}C$ compared with equilibrium calcite possesses a positive (negative) CIVE (25–27). CIVE expression is driven by the molar ratio of carbon demand to carbon supply and the partitioning of carbon between calcification and carbon fixation (25). The direction of the CIVE is largely set by $R_{calc}/R_{fix}$, which differs by a factor of around four in modern species ($R_{calc}/R_{fix}$ is approximately 0.5 in *Emiliania huxleyi* and 2.0 in *Calcisicus leptoporus*). A high $R_{calc}/R_{fix}$ (typically greater than 1) produces a negative CIVE, while a low $R_{calc}/R_{fix}$ (typically less than 1) produces a positive CIVE. This difference is due to the effect of photosynthesis, which discriminates against $^{13}C$, leaving the residual intracellular pool enriched in $^{13}C$ (ref. 25). The magnitude of the CIVE is set by the molar ratio of carbon demand to supply (carbon utilization), which increases with cell size and growth rate and decreases with ambient $CO_2(aq)$. Intracellular allocation of $HCO_3^−$ (ref. 25) and variations associated with changing light conditions (25) impose secondary controls.

Cells of different sizes, and characterized by different values of $R_{calc}/R_{fix}$, express distinct CIVEs under the same $CO_2(aq)$ regime.

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Coccolith size scales with cell size, so size separation of coccoliths enables the study of different-sized cells. Analysis of multiple size fractions from the same sample also allows us to define a new parameter, \( \text{CIVE}_{\text{mean}} \), which is the isotopic composition of coccolith calcite of a specific size fraction minus the mean isotopic composition of all coccolith size fractions from that sample of sediment. This parameter is independent of equilibrium compositions and so can place constraints on \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) and \( \text{CO}_2(\text{aq}) \), two key drivers of CIVEs, without independent knowledge of the carbon isotope composition of equilibrium calcite. We present a new coccolith calcite isotope dataset comprising isotope measurements from five size fractions of coccoliths across 37 depths from the Eocene section of Ocean Drilling Program (ODP) leg 208, site 1263, 1,300 km off the coast of southern Namibia (see Methods for site description).

The carbon isotope ratios of the individual size fractions reveal rich variability compared with the bulk carbonate record (Fig. 1a). To explore the data, we used a cell-scale carbon isotopic flux model to find the optimal combination of \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) and \( \text{CO}_2(\text{aq}) \) that provides the best fit to measured isotopic CIVE\(_{\text{mean}}\). Our isotopic data for the Eocene exhibit large deviations from the bulk record at approximately 46 million years ago (Ma) (Fig. 1a). After optimizing for values of \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) and \( \text{CO}_2(\text{aq}) \), we were able to successfully model the distribution of measured CIVE\(_{\text{mean}}\) (Fig. 1b).

**Calcification across the Eocene**

Eocene coccolithophores were some of the largest of the entire Cenozoic era. Compared with modern, glacial and Miocene periods, Eocene coccolithophores grew under higher temperatures and higher \( p_{\text{CO}_2} \) (refs. 18–20). In our study, we identified four dominant genera across the Eocene: Coccolithus, Chiasmolithus, Discoaster and Reticulofenestra (Fig. 2). We found that \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) ratios across size ranges and genera were similar to those of modern-day coccolithophores; however, the existence of substantially larger cells means that more calcite was produced per cell of \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \), which we conclude was surprisingly low. Nevertheless, owing to the apparently large size of these cells, they may have produced 2.8 times more calcite per cell than modern-day Coccolithus braarudii. We have also been able to place constraints on the \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) of extinct genus Discoaster, which we conclude was surprisingly low. Nevertheless, owing to the apparently large size of these cells, they may have produced 2.8 times more calcite per cell than modern-day Coccolithus braarudii (Supplementary Information).

Until now, attempts to reconstruct \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) for Discoaster with morphometric approaches have been hampered by a lack of intact coccospheres in the fossil record and the uniqueness of their star-shaped morphology. Hence, leaving the foraminifera calcite enriched in \(^{13}\)C; we investigate vital effects associated with foraminifera in the Supplementary Information. Also shown are contemporaneous bulk sediment \(^{8}\)C values, which track the total sum of coccolith and foraminifera calcite and changes depending on their proportion, vital effects and mixed-layer whole-ocean signals. Covariation between the fine fractions and bulk is interpreted to imply that the bulk is largely composed of coccoliths and so their calcite dominates the geochemistry, although we cannot rule out some overgrowth (Supplementary Information).

Our results show that there was a large shift in community composition from Discoaster to dominance by Coccolithus, Chiasmolithus and Reticulofenestra, and that the timing of this coincides with a decline in both \text{CO}_2 and temperature. Inverse modelling efforts suggest that the community shift corresponded to a change from coccolithophores with a low apparent \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) to those with a higher apparent \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) in the largest size fraction. Yet in the smallest size fraction, the community shift corresponds to a change from the genus Coccolithus to Reticulofenestra and a shift from high to low apparent \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \). There is a relative lack of studies that have focused on reconstructing \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \) across the Eocene epoch, although a morphometric study focused on the PETM suggests that species such as Coccolithus pelagicus possessed ratios of ~2 (ref. 1), similar to our findings for the Coccolithus genus \( \frac{R_{\text{calc}}}{R_{\text{fix}}} \).
It is additionally important to understand how our results fit into the wider context of the global carbon cycle. Evidence suggests that across the Eocene, the calcite compensation depth (CCD) deepened, ocean pH rose and an increase in global weathering probably drove an increased flux of alkalinity to the oceans. Carbonate mass accumulation rates at site 1263 and at other sites across the ocean appear to have declined (Supplementary Table 1). The putative deepening of the CCD implies an increase in the saturation state of the deep ocean, which suggests that there was either a whole-ocean decrease in calcite production rates or an increase in delivery of alkalinity to the deep ocean reservoir, possibly via increased respiration-driven dissolution of exported carbonate. Resolving the intensity of respiration dissolution during the Eocene could be addressed in future work by assessing the history of decoupling between the lysocline and CCD.

Implications of temperature, light and nutrients on growth rate and calcification

Growth rate is a key parameter in all phytoplankton δ13C-based investigations of palaeo-CO2, but is notoriously difficult to constrain because phytoplankton fix δ13C in the water column, and relative CO2, given here as the ratio of modelled output CO2(aq) to modelled input CO2(aq) (see Methods for further derivation of relative CO2 and reasoning for its use over absolute values), trends remained the same.

In our study, we assume that $R_{\text{fix}}$ is constant for each genus and size fraction throughout our record. Predicted $R_{\text{fix}}$ values therefore reflect the best fit for the entire Eocene. This is a limitation to our approach: the long-term environmental change across the Eocene may be associated with variations to $R_{\text{fix}}$, which, due to our assumption that $R_{\text{fix}}$ is constant across our interval, cannot be captured. Previous culture studies on multiple species have found complex responses for variations in $R_{\text{fix}}$, but the translations of these results to evolutionary timescales is not clear. Changes in $R_{\text{fix}}$ within a genus and size fraction could potentially be constrained if this modelling approach were paired with an independent high-quality CO2 record as an input and high-resolution record of $\delta^{13}$CDBP.

Eocene CO2 levels

Our model implies that relative CO2(aq) was higher in the early Eocene and decreased across the epoch as CIVE mean emerge and increase in magnitude, in line with previous data (Fig. 3). Given site 1263 was probably close to equilibrium with the atmosphere (Methods), modelled CO2(aq) can be assumed to reflect atmospheric pCO2. Unlike the use of other carbon isotope-based pCO2 indices,
Without the MECO data included, our relative CO$_2$(aq) and on reconstructed CO$_2$(aq) values. The equation of the line has been overlaid the five-point moving average was used to avoid noise in the data and δ negative values of c was greater than zero. The black circles represent the median relative CO$_2$(aq) over 10,000 iterations of our Monte Carlo optimization (left-hand output, with the grey shaded region representing the moving average of plus 2 sigma and minus 2 sigma. Our modelled low relative CO$_2$(aq) during the middle Eocene coincides with a step-wise increase in proxies linked to an increase in the rate of chemical weathering and therefore CO$_2$ draw-down$^{37,40}$. The dashed black line during the MECO joins points without a moving average. Using a moving average at this time greatly skews the trend line given the large, and uncertain, deviation to higher relative CO$_2$. Also shown is the approximate interval of the EECO. The five-point moving average of relative CO$_2$(aq) is plotted against the five-point moving average of δ$^{18}$O$_{benthic}$. The five-point moving average was used to avoid noise in the data and evaluate the longer-term trend. Error bars represent one sigma uncertainty on reconstructed CO$_2$(aq) values. The equation of the line has been overlaid on the figure. The four points in red represent the points from the MECO and younger, which have been excluded from the P-value calculation (P < 0.001). Without the MECO data included, our relative CO$_2$(aq) and δ$^{18}$O$_{benthic}$ show a robust negative correlation. The warmer early Eocene, characterized by more negative values of δ$^{18}$O$_{benthic}$, has on average higher values of relative CO$_2$(aq). The slope of relative CO$_2$(aq) with δ$^{18}$O$_{benthic}$ for each of the 10,000 Monte Carlo simulations was calculated and plotted as a histogram. Only one value was greater than zero.
data and code availability are available at https://doi.org/10.1038/s41561-022-01006-0.

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Methods
Sample selection and preparation. The sediment samples were from ODP leg 208, site 1263. Site 1263 has remained at a relatively constant latitude for most of the Cenozoic. Ocean general circulation models suggest reduced Atlantic upwelling during the late Eocene, with the Walvis ridge probably outside of any significant zones of upwelling. We thus posit that site 1263 was in equilibrium with the atmosphere. Site 1263 was drilled from a current water depth of 2,717 m, with the palaeodepth during the Eocene believed to be around 1,500 m (ref. 3). Thirty-seven samples were selected at approximately 0.5 Myr intervals through the early and middle Eocene.

Sediment samples were placed in 100 ml of pH-neutral de-ionized water and placed on an orbital shaker overnight for disaggregation. Samples were then filtered through 64 μm, 38 μm and 20 μm sieves to remove large particles such as foraminifera and their fragments. The <20 μm fraction was separated into four smaller fractions with microfiltration: 3–5 μm, 5–8 μm, 8–10 μm and 10–20 μm. The 10–20 μm fraction was further separated into 10–15 μm and 15–20 μm fractions via the settling method. Through size separation and microscopic analysis, we constrained the average coccolith length for each genus across all size fractions. The coccolith length was subsequently used to determine cell size through established morphometric relationships (Supplementary Information). Nannofossil relative abundance is presented as percentage contribution of coccoliths to total counted and averaged across two counts. The microfiltration technique was assumed to be imperfect; we therefore ascribed an uncertainty to the measured coccolith carbon isotopic values relative to an international standard (δ13C) assuming contamination from neighbouring size fractions. The 15–20 μm fraction and the 3–5 μm fraction were assumed to have a 25% contamination from the 10–15 μm fraction and the 5–8 μm fraction, respectively, in line with previous studies. The remaining size fractions were assumed to have a 12.5% contamination from the bounding size fractions. Scanning electron microscope images show good preservation of coccolith material; however, we address the possibility of slight oversampling on individual coccoliths/nannoliths in the Supplementary Information.

Age model. The age of each sample has been calculated on the basis of three astronomically tuned age models for site 1263 that best represent the middle and late Eocene65–67. The age model from 56 Ma to 50 Ma is based on a regression model assuming contamination from neighbouring size fractions. The 15–20 μm fraction was separated into four smaller fractions with microfiltration: 3–5 μm, 5–8 μm, 8–10 μm and 10–20 μm. The 10–20 μm fraction was further separated into 10–15 μm and 15–20 μm fractions via the settling method. Through size separation and microscopic analysis, we constrained the average coccolith length for each genus across all size fractions. The coccolith length was subsequently used to determine cell size through established morphometric relationships (Supplementary Information). Nannofossil relative abundance is presented as percentage contribution of coccoliths to total counted and averaged across two counts. The microfiltration technique was assumed to be imperfect; we therefore ascribed an uncertainty to the measured coccolith carbon isotopic values relative to an international standard (δ13C) assuming contamination from neighbouring size fractions. The 15–20 μm fraction and the 3–5 μm fraction were assumed to have a 25% contamination from the 10–15 μm fraction and the 5–8 μm fraction, respectively, in line with previous studies. The remaining size fractions were assumed to have a 12.5% contamination from the bounding size fractions. Scanning electron microscope images show good preservation of coccolith material; however, we address the possibility of slight oversampling on individual coccoliths/nannoliths in the Supplementary Information.

Isotopic measurements. The δ13C was determined from ~130 μg of size-separated sedimentary material. In total, 178 samples were processed (5 size fractions × 37 depths = 185, minus 7 samples lost due to machine error). Material was analysed via a Delta V Advantage isotope mass spectrometer fitted with a Gas Bench II in the Department of Earth Sciences at the University of Oxford. An isotopic measurement was made for each of the five size fractions from each time interval. Samples were calibrated to Pee Dee Belemnite via the international NBS-19 standard. All measurements are expressed relative to Vienna PeeDee Belemnite standard, and the analytical precision was better 0.04% across repeat measurements for δ13C (1σ). The final error on isotopic measurements encompasses both analytical error and error introduced from the microfiltration technique (Fig. 1).

Development of the forward model. We developed a forward model that used coccolithophore taxonomic abundance (resolved to genus level) and a previously published cellular isotopic flux model21–23 to generate abundance-weighted predictions of size-fraction-specific CIVEmean with individual taxonomic-specific CIVEmean calculated as a function of Rfix/Rco2 and CO2(aq). Our approach separates the dependence of the CIVE from the isotopic composition of DIC by subtracting the mean CIVE for each depth (age point) from both the modelled predictions and the data. We use an iterative optimization approach to search parameter space for 20 size- and genus-specific values of Rfix/Rco2 (5 size fractions within the 4 dominant genera) and 37 (age- or depth-specific) values of CO2(aq) to maximize the fit of model predictions to the mean-normalized CIVE record (See Supplementary Fig. 5 for full work flow). Our approach allowed changes in relative CO2, modelled output CO2(aq) relative to model input CO2(aq), to be constrained independent of δ13Ccalc. We also used a foraminiferal record of δ13Ccalc to place additional constraints on absolute pCO2 (Supplementary Information). We used a Monte Carlo approach to explore the sensitivity of constrained values to model inputs (Supplementary Information).

The forward model predicts the δ13C of each size fraction as a weighted sum of the predicted δ13C for taxa that contribute to each size fraction and their observed relative abundances. Predicted δ13C values for each size and taxonomic–specific unit (STU; 4 genera across 5 size fractions giving 20 in total) were calculated from a previously published model as a function of ambient carbonate chemistry (all STUs are assumed to experience the same ambient environment at each time point) and STU-specific parameters: Rfix/Rco2 cell size and growth rate (assumed constant for each STU throughout the studied interval). Growth rate was assumed to follow an allometric scaling relationship with cell size21,22, and abundances were counted directly (model inputs are summarised in Supplementary Table 2). The assumption of constant growth rate for each STU is supported by Sr/Ca data (Supplementary Fig. 6). Parameters fitted to culture data in the original paper remain unchanged. Carbon speciation in ambient seawater was calculated via the SeaCarb package for R26 with an assumed constant calcite saturation state. The remaining parameters, CO2(aq) and Rfix/Rco2 (referred to as RR in the following mathematical representation), defined the parameter space to be explored. Initial values for CO2(aq) and Rfix/Rco2 were initialized from a random uniform distribution (Selecting initial CO2, and Rfix/Rco2).

For each STU-specific RR (i ≤ 1; i = 20 STUs) and each age-specific CO2(aq) (k ≤ 1: K = 37 depths), the isotopic flux model was used to calculate a δ13C value, Dj (j = 1: J = 5 size fractions) then involved weighting each age and STU combination by its relative contribution to the calculated δ13C for each size fraction. To do this, D was combined with a matrix of the fractional abundance of each genus in each size fraction (B, with elements Bi), on the basis of data from microscope counts, a matrix of genus coccolith weight (C, with elements Ci). Calculated from previously published morphometric relationships27 and a sorting matrix to produce a J × K (5 × 37) matrix of age- and size-fraction-specific δ13C values (B):

\[ E = \left[ A \odot (C \odot D) \right] \odot \left[ A (B \odot C) \right] \]

\[ A = \begin{bmatrix} 11111000000000000000000000000 \ 00001111000000000000000000000 \ 00000000001111000000000000000 \ 00000000000000111110000000000 \ 00000000000000000000001111111 \ \end{bmatrix} \]

The overall result is to transform D (STU-specific δ13C × J × K matrix) to E (a J × K matrix that represents the weighted δ13C values for each size fraction that would have been measured at each time interval).

The modelled δ13C mean for each time interval was then subtracted from each size fraction δ13C at the corresponding time interval to remove the dependence on absolute isotopic compositions and generate a mean-centred value of CIVE(F) for each size fraction:

\[ F_{jk} = E_{ik,j} - E_{ik,1} / A \]

Optimization. The measured data were treated similarly to remove the dependence on δ13Ccalc and generate a mean-corrected measured isotope matrix, H:

\[ H_{ij,k} = G_{ij,k} - H_{ij,1} / A \]

where Gi,k is the absolute δ13Cvalue measured for each size fraction at each depth and δ13Ccalc is the mean δ13C across all five size fractions at each age point. F represents a J × K matrix of mean-centred model-predicted δ13C values subsequently used for the fitting of Rfix/Rco2 and CO2.

An iterative optimization approach was taken to find the best CO2(aq) and Rfix/Rco2 combination to generate model predictions (F) that fit the measured mean-centred CIVE data (H). We used a two-step iterative optimization to minimize the offset: step one consisted of optimizing for Rfix/Rco2 given an initial constant CO2(aq); step two consisted of optimizing for CO2(aq) using the values of Rfix/Rco2 determined in step one. Taking the fitted values of CO2(aq) for the new initial value, the optimization process was repeated until Rfix/Rco2 and CO2(aq) were stable. An unconstrained Nelder–Mead optimizer (fminsearch, Matlab) was used to determine fitted values of CO2(aq) and Rfix/Rco2 that reduced the misfit between the measured and modelled CIVEmean (according to equation (4)) for each iteration. Both CO2(aq) and Rfix/Rco2 were allowed to be only positive values.
Selecting initial CO2 and Rcalc/Rfix - The model outputs of Rcalc/Rfix and CO2(aq) using CIVEcalc are dependent on their initial values. However, relative change is robust across depths and across STUs (Supplementary Fig. 3). These parameters described a valley of stability in parameter space whereby the average output CO2(aq) trades off against the average output Rcalc/Rfix. We therefore present CO2 as a relative change where relative CO2 is given by the optimized CO2(aq) output divided by the initial prescribed CO2(aq) value. Differences in the trends in CO2 between different initial values were minor; however, to non-arbitrarily choose initial values of CO2 and Rcalc/Rfix, we introduced an additional optimization step that used an independent estimate of δ13Ccalc derived from coeval planktic foraminifera (Supplementary Fig. 4).

From a log-uniform distribution between 0.5 and 10, 10,000 initial values of Rcalc/Rfix were selected, while an initial 10,000 CO2(aq) values were selected from a uniform distribution between 20 and 150 μmolkg−1, with external carbon conditions allowed to reach concentrations as low as 5 μmolkg−1.

The optimization outlined in equations (1)–(3) was undertaken for each of the 10,000 combinations, and a fitted array of CO2(aq) and Rcalc/Rfix values was generated for each combination. The fitted values of CO2 and Rcalc/Rfix were then passed to the forward model to generate 10,000 E matrices of modelled absolute CIVEs.

The δ13C of planktic foraminifera were analysed for ten depths with the associated δ13Ccalc, determined assuming a constant vital effect (P, Supplementary Information). The δ13Ccalc was added onto Ei, for the corresponding depths, and the misfit between the mean raw size fraction data (G) and the predicted mean of the E matrices was the vector of δ13C calculated via Seacarb in R68 would be ~29 μmolkg−1.

The total number of size-fraction-specific δ13C values from the ten samples where we analysed coeval foraminifera.

Comparison of the modelled CIVEs with δ13Ccalc provides constraints on the absolute magnitude of Rcalc/Rfix and CO2(aq) (and pCO2; Supplementary Fig. 4). However, estimating δ13Ccalc from planktic foraminifera relies on poorly constrained vital effects, which compromises the elegance of our approach. We thus present relative CO2(aq) changes based on an internally consistent analysis of coccolith calcite (independent of δ13Ccalc) and consider estimates of absolute CO2(aq) to be only loosely constrained by the additional consideration of the δ13C of foraminiferal calcite.

Monte Carlo exploration of model output - A Monte Carlo approach was employed to further explore how uncertainties in growth rate, coccolith abundance data, coccolith weight and external carbonate saturation state impact the results over 10,000 runs. For each iteration, values were resampled from their uncertainties (Supplementary Table 2). In addition, the initial target value for H was selected from the uncertainty ascribed due to contamination during the filtration process (Sample selection and preparation). The distribution was assumed normal around its mean. Initial Rcalc/Rfix was fixed at 1.45, and the initial CO2(aq) at 29 μmolkg−1, the values that provided the best fit of measured CIVEs with modelled CIVEs relative to δ13Ccalc. Assuming an average pCO2 of 1.000 ppm, pH of 7.7 and temperature of 25 °C across our time interval^{1–3}, the corresponding average [CO2(aq)] calculated via Seacarb in R would be ~29 μmolkg−1.

Further validates our choice. Final values of Rcalc/Rfix and relative CO2(aq) presented in Figs. 2 and 3 represent the output of Rcalc/Rfix from the Monte Carlo simulation over 10,000 iterations.

We also performed a sensitivity analysis on our Monte Carlo simulation to determine the degree of uncertainty that each of our input variables had on our modelled data. For each of the sensitivity analyses, uncertainty on all but the target variable was assumed to be 0, while the uncertainty on the target variable was ascribed as listed in Supplementary Table 2. For the initial selection of model inputs, we used the mean of our outputs to variations in our model assumptions such as the validity of assumptions made regarding minimal carbonate within the size-fraction δ13C signal. The robustness of our outputs to variations in our model assumptions such as the validity of assumptions made regarding minimal carbonate within the size-fraction δ13C signal.

The model produced a narrow range of fitted values of Rcalc/Rfix when the genus contributed a large proportion to the total sediment size fraction (Fig. 2). If a genus is underrepresented in a specific size fraction, the possible range of Rcalc/Rfix is much wider since it contributes minimal carbonate within the size-fraction δ13C signal.

Data availability - Supplementary data have been made available via EarthChem online repository (https://doi.org/10.26022/IEDA/112365).

Code availability - Code is available on GitHub at https://github.com/hmcclelland/Coccolith_CIVEs.

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Author contributions - L.M.C. and R.E.M.R. conceived the study. L.M.C. conducted the sedimentary analysis with guidance from M.H. H.L.O.M. and L.M.C. wrote the optimization and Monte Carlo procedures. L.M.C. interpreted the results and wrote the paper with comments and guidance from H.L.O.M., M.H. and R.E.M.R.

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