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Decrease in coccolithophore calcification and CO₂ since the middle Miocene

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Marine algae are instrumental in carbon cycling and atmospheric carbon dioxide (CO₂) regulation. One group, coccolithophores, uses carbon to photosynthesize and to calcify, covering their cells with chalk platelets (coccoliths). How ocean acidification influences coccolithophore calcification is strongly debated, and the effects of carbonate chemistry changes in the geological past are poorly understood. This paper relates degree of coccolith calcification to cellular calcification, and presents the first records of size-normalized coccolith thickness spanning the last 14 Myr from tropical oceans. Degree of calcification was highest in the low-pH, high-CO₂ Miocene ocean, but decreased significantly between 6 and 4 Myr ago. Based on this and concurrent trends in a new alkenone ip record, we propose that decreasing CO₂ partly drove the observed trend via reduced cellular bicarbonate allocation to calcification. This trend reversed in the late Pleistocene despite low CO₂, suggesting an additional regulator of calcification such as alkalinity.
Coccolithophores, a group of unicellular marine phytoplankton, are the only primary producers of biogenic calcite in the open ocean. During their diploid life-cycle stage, calcifying coccolithophores intracellularly produce calcite plates called heterococcoliths. These circular to elliptical coccoliths are extruded through the cell wall to form an exoskeleton, usually composed of a single layer of calcite plates, called a coccosphere. Coccolithophores play an important role in the carbon cycle because they promote the sinking of particulate organic carbon to the deep ocean. Changes in their production of organic carbon and calcification can alter the balance between the organic and inorganic carbon pumps, with strong feedbacks on climate and atmospheric carbon dioxide concentrations ($p_{CO_2}$) on seasonal to geological timescales\(^2\). Despite the importance of coccolithophore calcification to biogeochemical cycles and the large range in degree of cell-calcification (defined here as the amount of calcite per unit surface area of the cell) observed both among and within modern species, it is unclear whether specific factors drive changes in cell-calcification state of the ocean’s coccolithophore populations on evolutionary timescales. Rapid changes in ocean dissolved $CO_2$ concentration ($[CO_2]_{aq}$), pH, temperature and surface-water stratification in the coming centuries may exert selective pressure on coccolithophore calcification\(^3,4\). Short-term experiments reveal an array of species- and strain-specific physiological responses to elevated $[CO_2]_{aq}$\(^4-9\). However, selection experiments lasting around a year show that the negative effects of short term (<10 generations) high $p_{CO_2}$ exposure on coccolithophore calcification and growth are partly reversed for populations that have been exposed to long term (about 500 generations) high $p_{CO_2}$ conditions\(^10,11\). Such adaptability is consistent with the geological data indicating that coccolithophores were more ubiquitous and common in the warm, high-$CO_2$ ocean of the earlier Cenozoic, with larger coccoliths and cells\(^12,13\). Recent work has shown that calcification competes with photosynthesis for intracellular bicarbonate ($HCO_3^-\$) and that multiple species of coccolithophores reallocate $HCO_3^-\$ transport from calcification to photosynthesis at low $[CO_2]_{aq}$\(^14\).

Here we explore the long-term response of coccolithophore calcification and $HCO_3^-$ allocation to the evolution of ocean conditions and $[CO_2]_{aq}$ over the past 14 million years (Myr) in a key coccolithophore family, the Noëlaerhabdaceae. This family, which includes the genera *Emiliania*, *Gephyrocapsa*, *Pseudoemiliania* and *Reticulofenestra*, dominates most modern ocean coccolithophore communities as well as fossil assemblages since the Miocene. As an indicator of coccolithophore calcification, we show that coccolith thickness correlates strongly with cellular calcification per unit surface area across the range of modern Noëlaerhabdaceae. We then document changes in the size and degree of calcification of Noëlaerhabdaceae coccoliths since 14 Myr ago and in two sediment sequences from the tropical Atlantic and Indian Oceans containing well-preserved coccoliths (Ocean Drilling Program, ODP, Site 925 and Indian National Gas Hydrate Program Site NGHP-01-01A, respectively; Fig. 1). In contrast to recent studies of coccolith mass\(^15-18\), we present our data as coccolith thickness within narrow size classes to focus on changes in degree of calcification of coccoliths, allowing us to better capture the potential coccolithophore calcification response to changes in the palaeo-carbonate system\(^19\). These records are the first to document past long-term changes in coccolithophore calcification for given cell size classes in the Miocene and Pliocene, when $p_{CO_2}$ was higher than pre-industrial levels. Using the geochemical signature of coccoliths, we then assess if changes in degree of calcification correspond to changes in the allocation of intracellular $HCO_3^-$ resources to calcification. Finally, we evaluate the potential role of changing upper ocean stratification and $[CO_2]_{aq}$ on both degree of calcification and $HCO_3^-$ resource allocation, using new proxy records of foraminiferal stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) and carbon isotopic fractionation by alkenone-producing haptophyte algae during photosynthesis ($e_p$).

**Results**

**Coccolith thickness and cellular calcification.** New culture experiments sampling the diversity of modern Noëlaerhabdaceae coccolithophores show variation in coccolith thickness both among the different species and among different strains of the same species. This variation in thickness correlates strongly with variation in cellular calcification per surface area as well as with changes in calcite/organic carbon, a measure of calcification per cell volume (Fig. 2 and Supplementary Fig. 1). The thickness of an individual coccolith is intimately linked to the degree of calcification of a cell, because it represents a key mechanism by which cells can regulate the amount of biomineral for a given cell volume. Various factors may drive cells to adjust calcite per cell surface area. In this study, we focus on changes that are occurring within narrow size classes. In addition, calcite per cell surface area varies with cell size across the modern diversity of placolith-bearing coccolithophores, where small cells are characterized by thinner coccoliths (Supplementary Fig. 2). This latter effect may be an adaptation to compensate for the higher surface area to volume ratio of small cells that, if calcification per cell surface area were constant across all cell sizes, would impose a much higher biomineral requirement relative to cell volume in small cells. While coccolith mass has been used as an indicator of cellular calcification in Pleistocene and recent sediments\(^15-18\), coccolith mass is driven by changes in cell size as well as degree of calcification. On the other hand, coccolith thickness within narrow size classes, or size-normalized coccolith thickness, represent degree of calcification and are indicators better suited to reconstructing coccolithophore calcification on long timescales over which significant coccolith and cell size changes occur. The range of coccolith thickness variation among cultured Noëlaerhabdaceae strains (Fig. 2) is consistent with previous observations that phenotypic differences in the degree of calcification between species and between strains of the same species tend to be much larger than the phenotypic plasticity of a single strain cultured under varying environmental conditions\(^3,5,20\). This may arise if coccolith morphotype or thickness is genetically regulated\(^3\). The potential for large intraspecific diversity may reflect the genetic architecture, in that the dominant modern Noëlaerhabdaceae *Emiliania huxleyi* has a pan-genome composed of core genes plus genes distributed variably amongst strains\(^21\).

**Decreasing cellular calcification since the late Miocene.** Over the past 14 Myr, the Noëlaerhabdaceae have undergone large variations in coccolith size (Fig. 3) and degree of calcification, represented by thickness (Fig. 4). Changes in coccolith thickness...
are evident in both narrowly restricted size classes, as well as in measurements of size-normalized (SN) thickness and calculated ‘shape factor’, confirming that they are not a direct result of temporal changes in coccolith and cell size (isometric scaling, that is, changes related to proportional changes in size) (Fig. 4). The quantification of thickness was not biased by variable coccolith fragmentation (Supplementary Fig. 3). Scanning electron microscope (SEM) observations confirm that in all samples the original crystal structure of the coccolith remains well defined. Only on some older coccoliths did we identify a small amount of diagenetic overgrowth (small abiotic crystals formed on the surface of the collar in the central area; Supplementary Data 1). However, the presence of this minor overgrowth does not correspond to an increase in coccolith thickness, except in the oldest 14 Myr old sample at the Indian Ocean Site. Thus, with this exception, the preservation visible under SEM makes it unlikely that middle Miocene Noëlaerhabdaceae coccoliths of a given size were originally thinner and more delicate than those present in our samples. This suggests that either (1) overgrowth was minor enough not to significantly impact mean coccolith thickness, or (2) the calcite that recrystallized on the surface of coccoliths was originally derived from dissolution of primary calcite of these same coccoliths. Between 8 and 3 Myr ago at both sites, Sponolithus and Discoaster nanoliths are abundant. These are typically more susceptible than placolith coccoliths to overgrowth due to their crystal structure, yet SEM images show that these susceptible forms exhibit excellent and constant preservation, providing supporting evidence that diagenetic overgrowth was not more significant when Noëlaerhabdaceae coccoliths showed a higher degree of calcification at 6–8 Myr ago relative to at 3–4 Myr ago (Supplementary Data 1; Supplementary Figs 4 and 5). The measured coccolith populations exhibit large variability in the morphology and degree of calcification of small coccoliths within and between each sample (Fig. 4; Supplementary Figs 4 and 5; Supplementary Data 1). For example, Gephyrocapsa protohuxleyi, a form close to E. huxleyi but with a central area bridge characteristic of Gephyrocapsa, was present in Pleistocene samples at both sites alongside much more heavily calcified Gephyrocapsa coccoliths. Despite this large diversity in morphology and thickness, there are significant changes in the dominance of more heavily calcified versus more lightly calcified forms over time, as well as the emergence during the early Pliocene of coccoliths thinner and/or with larger central area openings than those found in previous intervals. Coccolith degree of calcification was on average highest between 14 and 6 Myr ago and decreased abruptly in the late Miocene to early Pliocene (6–4 Myr ago) to low values that were maintained during the Pliocene and early Pleistocene (4–1 Myr ago). For the few sample points of the last 1 Myr ago, degree of calcification increased both in the Indian and Atlantic Ocean records relative to this Pliocene minimum (Fig. 4). However, we note that assemblages in our samples <1 Myr are dominated by Gephyrocapsa coccoliths and pre-date the emergence of the less heavily calcified E. huxleyi (see $k_s$ values, Fig. 4d,h), which is significant especially in modern high and mid-latitude regions. Large changes in degree of coccolith calcification, including the decrease from 6 to 4 Myr ago and the increase around 1 Myr ago at both sites, occurred within the dominant genus at a given time, and do not coincide with major shifts in the contribution of different genera to the Noëlaerhabdaceae (Fig. 4).

Late Miocene changes in cellular $\text{HCO}_3^-$ allocation. Geochemical records of carbon isotopic fractionation into coccolith calcite ($\epsilon_{\text{coccolith}}$) can be used to elucidate the relationship between the observed changes in degree of calcification and the resource allocation of carbon to calcification. Models of cellular carbon fluxes have shown that $\epsilon_{\text{coccolith}}$ becomes increasingly depleted if the rate of supply of $\text{HCO}_3^-$ to the site of calcification (coccolith vesicle) is reduced relative to calcification rate$^{14}$. Our new records of $\epsilon_{\text{coccolith}}$ from ODP Site 925 show that large cells begin to decrease the $\text{HCO}_3^-$ allocation to calcification at about 8 Myr ago, evidenced by decreasing $\epsilon_{\text{coccolith}}$ (Fig. 5a,b). This trend occurs shortly after a decrease in mean Noëlaerhabdaceae coccolith size (interpreted as a reduction in mean cell size$^{13}$) at both sites (Fig. 3) that is also observed in other low-latitude records$^{12,22,23}$. Reduced $\text{HCO}_3^-$ allocation to
calcification continues in large cells from 6 to 4 Myr ago, as indicated by decreasing \( e_{\text{coccolith}} \) during this interval, despite a stable trend in mean coccolith size. Although we cannot resolve changes in the degree of calcification of large coccoliths in this study (see Methods), the \( e_{\text{coccolith}} \) trend suggests that in large cells, the change in \( \text{HCO}_3^-/\text{CO}_2 \) allocation to calcification was of greater magnitude than any concurrent decrease in calcification that may have occurred. This significant reduced allocation to calcification in large cells drove a divergence in the range of vital effects among small and large coccoliths after 8 Myr ago (Fig. 5a), similar to the results from Caribbean ODP Site 999 (ref. 14).

Small coccoliths show evidence for decreased \( \text{HCO}_3^-/\text{CO}_2 \) allocation only since 6 Myr ago. From 11 to 6 Myr ago, \( e_{\text{coccolith}} \) and SN coccolith thickness are relatively stable (Fig. 5b,c), suggesting minimal changes in \( \text{HCO}_3^- \) allocation to calcification. In contrast, between 6 and 1 Myr ago, a near-constant \( e_{\text{coccolith}} \) indicates a stable ratio of \( \text{HCO}_3^- \) allocation to the coccolith vesicle relative to calcification rate, despite a large decrease in degree of calcification (Figs 4 and 5c). This implies a decrease in \( \text{HCO}_3^- \) allocation to calcification of comparable magnitude to the decrease in cellular calcification. In the last 1 Myr, an increase in degree of calcification in the small coccoliths with no change in \( e_{\text{coccolith}} \) suggests that allocation of \( \text{HCO}_3^- \) to calcification also increased in parallel.

**Relationship between calcification and ocean stratification.**
Water column stratification influences productivity and production depth in the tropics. Stratification can be inferred from foraminiferal \( \delta^{18}\text{O} \) gradients between the upper mixed layer and deeper waters. Changes in stratification are reflected in shifts in calcification, with increased sedimentation rates during periods of reduced stratification (Fig. 3c). This suggests a feedback mechanism where changes in calcification influence ocean productivity and vice versa.

![Figure 3](image_url)
layer (*Globigerinoides sacculifer*) and thermocline (*Globorotalia menardii*), because these reflect the upper photic zone temperature and salinity gradients. The temporal evolution of planktic foraminiferal δ¹⁸O at Sites ODP 925 and NGHP-01-01A is shown in Fig. 6. Between 3.5 and 2 Myr ago, a deep thermocline at Site 925 is inferred from independent foraminiferal assemblage indicators, potentially suggesting a deeper coccolithophore depth habitat and lower light levels. Decreased light has been shown to reduce cellular calcification (PIC/SA) twofold by a reduction in photon flux density from 80 to 15 μmol m⁻² s⁻¹ in culture, and low light levels have been proposed to decrease cellular HCO₃⁻ transport. However, neither site shows a clear decrease in δ¹⁸O gradients at this time (Fig. 6a,b), as would be expected if reduced coccolith calcification from 4 to 1 Myr ago were due to a deepening of the thermocline, resulting in a reduced temperature gradient between the two foraminifer species’ depth habitats. Proxy records suggest high productivity from 10 to 8 Myr ago in the Indian Ocean and from 6.6 to 6 Myr ago at ODP Site 925 (ref. 29). Thus, reconstructed changes in water column structure and paleoproductivity do not consistently co-vary with changes in degree of coccolith calcification.

Figure 4 | Changes in Noélaerhabdaceae coccolith thickness and ks value at two tropical sites since 14 Myr ago. (a–d) Site NGHP-01-01A, and (e–h) ODP Site 925. (a–c,e–g) Thickness data for coccoliths of 2–3, 3–4 and 4–5 μm length. Box-Whisker plots illustrate coccolith thickness data for each sample and size class (box shows median value and upper/lower quartiles, whiskers show maximum and minimum values, outliers > 1.5 x the interquartile range are shown as crosses). Also shown are mean values of raw (circles) and SN (diamonds) thickness (Supplementary Data 1). Bar graphs show the relative contribution of different genera to the Noélaerhabdaceae population in each size class and sample. (d,h) ks values (error bars are ± 2 s.e.m.). The shape factor ks, which expresses the fraction of the volume of a cube defined by the length of a coccolith that is composed of biomineral, was originally proposed to estimate coccolith mass from coccolith length and is similar to coccolith thickness. However, unlike thickness, ks does not account for variations in coccolith circularity. Pink symbols are ks for extant Noélaerhabdaceae species.
Calcification and \([\text{CO}_2{}_{aq}]\) in the Miocene–Pliocene. Carbon isotopic fractionation in phytoplankton during photosynthesis (\(\epsilon_p\)) varies directly with \([\text{CO}_2{}_{aq}]\) and has been widely applied as a CO\(_2\) proxy in the Cenozoic. However, limited data exist for the interval of major changes in calcification and HCO\(_3^-\) allocation between 14 and 5 Myr ago. In addition, the interpretation of any data is complex because of the expected influence of active HCO\(_3^-\) allocation on \(\epsilon_p\) (ref. 14). Our new record of \(\epsilon_p\) extends the published record from ODP Site 999 for the last 5 Myr\(^{30}\) back to 16 Myr ago (Fig. 7a). This extended record reveals a decrease in \(\epsilon_p\) from 16 to 8 Myr ago, an excursion to higher \(\epsilon_p\) values at 7 Myr ago, and then a continued decrease towards the present. The decline in \(\epsilon_p\) could be driven by decreasing \([\text{CO}_2{}_{aq}]\), increasing cellular growth rates that increase carbon demand relative to supply, or increasing cell sizes that reduce surface area to volume and thus diffusive supply (see ref. 31 and references therein). Following previous workers, \([\text{CO}_2{}_{aq}]\) is estimated with the formula \([\text{CO}_2{}_{aq}] = b(\epsilon_t - \epsilon_p)\), where \(\epsilon_t\) is a constant reflecting the maximum effective photosynthetic fractionation by the cell (25%), and \(b\) encompasses factors such as growth rate and cell geometry that modulate the ratio of carbon supply to demand by the cell. First, to estimate temporal variations in \(b\) due to cell size, we use previous formulations of the relationship between cell size and \(b\),\(^{22,23}\) together with our record of tropical Noëlaerhabdaceae coccolith size evolution (Fig. 7b), which shows trends similar to those at other tropical sites\(^{22,23}\). The decrease in cell size after 9 Myr ago, compared with the average between 11 and 16 Myr ago, corresponds to a 25% reduction in the \(b\) value. Second, we estimate the influence of productivity on \(b\) using proxy records from ODP Site 999 of coccolith Sr/Ca and alkenone mass accumulation rates (Fig. 7c). These records confirm that there is no long-term productivity increase, and suggest maxima from 13 to 10 Myr ago and at 8 Myr ago. Calculated \(b\) values are shown in Fig. 7d. The resulting estimates of \([\text{CO}_2{}_{aq}]\) (Fig. 7e) show a trend of continued decline over the past 16 Myr, with the exception of a local maximum at 9.3–10.3 Myr ago resulting from the unusually large cell sizes in
the geometry correction. Assuming equilibrium with the atmosphere, these results are similar in trend and magnitude to [CO2aq] predicted from the atmospheric pCO2 curve of ref. 33 derived from inverse modelling of climate data (Fig. 7e). The absolute values of [CO2aq] are subject to greater uncertainty than the trend.

As in previous studies30, our calculations would not account for the likely increase in active carbon uptake for photosynthesis as [CO2aq] declined14,34, especially after 8 Myr ago. Because active carbon transport increases the chloroplast uptake of inorganic carbon relative to fixation, it can result in higher εp values than would be predicted from passive diffusive CO2 uptake alone35. Laboratory culture experiments suggest that active HCO3− transport to the chloroplast becomes more significant at low [CO2aq]. Simulations with the ACTI-CO model of HCO3− transport in coccolithophores14 were used to evaluate the potential impact of changes in active carbon uptake on εp and calculated [CO2aq] (Supplementary Methods; Fig. 8). In one set of simulations, we specify a logarithmic dependence of chloroplast HCO3− transport/diffusive CO2 uptake on [CO2aq] as observed in culture experiments14,27. Alternatively, if enhancement of HCO3− transport to the chloroplast is coupled, in part, to reallocation of HCO3− from the coccolith vesicle, as inferred from modelling of cultures14, our new εcoccolith and SN coccolith thickness data put additional constraints on the timing of this reallocation. Therefore in a second set of simulations, we specify chloroplast HCO3− transport based on HCO3− spared from the coccolith vesicle by the reduction in cellular calcite in the last 8 Myr. We then derive the [CO2aq] implied by measured εp for the specified parameterization of active HCO3− uptake to the chloroplast. The results in both cases indicate a greater amplitude of decline in [CO2aq] compared with that reconstructed with standard cell size and growth rate considerations only, from around 17 to 6 µM (Fig. 8).

Calcification in relation to CO2 and alkalinity since 1 Myr ago.

In the last 1 Myr, climate, the carbon cycle and ocean chemistry evolved in relation to CO2, alkalinity and coccolith morphology changes. Figure 7 | εp values and estimates of b and [CO2aq] at Caribbean ODP Site 999 and other sites for the last 17 Myr. (a) New εp data (Site 999, red diamonds; Site 925, orange diamonds; Site 902, blue diamonds) show propagated analytical uncertainty on δ13C measurements. Published εp records: Site 999 (ref. 30) (purple crosses), ODP Site 925 (ref. 37) (grey circles), DSDP Sites 588 (ref. 84) (grey triangles, maximum εp) and 608 (ref. 84) (blue squares). (b) Variations in b (Site 999) inferred to arise from changing cell size and growth rate (c) (see Methods). In c, triangles (this study) and line13 show alkenone MARs and blue shading shows Sr/Ca productivity estimates for small coccolithophores14 (all Site 999). In d, purple crosses (Site 999 (ref. 30)), grey circles (Site 925 (ref. 37)), and orange circles (Site 999, this study) show b values calculated using our new cell size correction. Red circles (Site 999, this study) show b values calculated with cell size and growth rate corrections. For error calculations, see Methods.

(e) [CO2aq] calculated using cell size (orange circles), or cell size plus growth rate (red circles), correction and εp values (Site 999, this study). [CO2aq] was also recalculated using our cell size correction for the Pli-Pleistocene at Site 999 (ref. 30) (purple crosses) and Site 925 (ref. 37) (grey circles). For all sites, reference b = 150. [CO2aq] assuming constant b for Site 999 (ref. 30) is also shown (blue crosses). Shading indicates maximum and minimum [CO2aq] estimates for all data from Site 999 (see Supplementary Methods). We do not apply our size correction to DSDP Sites 608 and 588 εp data because these sites are at significantly higher latitudes; therefore cell size history may be different compared with the tropical sites studied here. Also shown in e is the [CO2aq] expected for the Caribbean site if it were in equilibrium with the atmospheric pCO2 modelled by ref. 33 (grey line). (f) pH derived from δ18O of planktic foraminifers, for the Plio-Pleistocene30,36 and Miocene52. During the Miocene, ODP Site 999 εp values are similar to values at ODP Site 925 (ref. 37) and higher than values from DSDP Sites 588 and 608 (ref. 85). From 16 to 9 Myr ago, the maximum εp at DSDP Site 608 shows a similar trend to εp at ODP Site 999, albeit with slightly lower absolute values, suggesting that either both sites experienced similar changes in growth rates, or that a global CO2 component exerted a dominant forcing on both εp records. The temporally variable scatter to low εp values seen in the Site 608 record may result from higher frequency oscillations in growth rates at this site37. The much lower average εp at Site 588 suggest that this site experienced on average higher phytoplankton growth rates and productivity compared to Sites 925, 999 and 608.
The chloroplast on $\text{CO}_2$aq, similar to that observed in cultures (between), for $\text{CO}_2$aq implied by alkenone area. Crosses in production of thinner coccoliths (that is, a reduced PIC per cell surface second simulation (filled circles) supplements $\text{HCO}_3$.

The change in relationship between degree of coccolith calcification and $\text{CO}_2$cytosol as shown in Fig. 7e (orange circles). ACTI-CO model simulation for two potential scenarios of active $\text{HCO}_3$ uptake to the chloroplast (b), and consequences for [CO2aq] implied by alkenone measurements (c). A first simulation (unfilled circles) employs a logarithmic dependence of $\text{HCO}_3$ transport to the chloroplast on [CO2aq], similar to that observed in cultures (a). A second simulation (filled circles) supplements $\text{HCO}_3$ supply to the chloroplast as a function of $\text{HCO}_3$ spared from the coccolith vesicle by the production of thinner coccoliths (that is, a reduced PIC per cell surface area). Crosses in c show [CO2aq] estimated from standard regressions between $\text{pCO}_2$ and $\text{CO}_2$aq as shown in Fig. 7e (orange circles).

The similar long-term decreases in degree of coccolith calcification at our Atlantic and Indian Ocean sites suggest a common selective pressure. This trend in calcification occurs alongside reduced $\text{HCO}_3$ allocation to the coccolith vesicle. The lack of a coherent relationship between stratification and productivity and SN coccolith thickness at both sites suggest that these factors are not strong candidates to force the common trends in degree of calcification at both sites. In contrast, while the relationship between [CO2aq] and cellular calcification has been ambiguous in clonal cell cultures (decreasing $\text{CO}_2$aq is one factor shown to reduce $\text{HCO}_3$ allocation to calcification in modern cells). Changes in [CO2aq] are expected to be globally synchronous across the stratified tropical oceans. While the magnitude of [CO2aq] decline is sensitive to the inferences about active carbon uptake by algae and detailed steps in [CO2aq] cannot be reliably identified given the resolution of our record, a progressive [CO2aq] decline since the middle Miocene is evident and correlates with a succession of adaptations in coccolithophore calcification and cell size. A decline in cell size and the first large amplitude sea level cycles accelerated erosion of shelf sediments. Estimates of alkalinity from carbon system proxies are subject to multiple uncertainties. To explore the magnitude of alkalinity change that might be possible, we compared pH estimates from boron isotopes in planktonic foraminifers with estimates of ocean [CO2aq] calculated from the cycles of atmospheric $\text{pCO}_2$ recorded in ice cores. While not diagnostic, this analysis suggests the potential for an increase in alkalinity by up to 30% during successive glacial of the last 1.5 Myr (Supplementary Methods; Supplementary Fig. 6). Such an increase would contrast with relatively stable alkalinity inferred for the previous 14 Myr from analysis of the carbonate compensation depth, although such estimates are also subject to multiple uncertainties (Supplementary Fig. 7).

Discussion

Over the past 14 Myr, selective pressure has acted on the large diversity of different degrees of calcification and morphotypes found in natural coccolithophore populations, modifying in a similar way in the tropical Atlantic and Indian Oceans the composition of the population towards better-adapted forms. A similar selective pressure has been suggested for natural populations on seasonal timescales, modulating the relative contribution of different E. huxleyi morphotypes with specific degrees of calcification. Long-term mono- and multi-clonal experiments also reveal genotypic selection, as well as beneficial new mutations, as a mechanism for adaptive evolution. Such coccolithophore species or morphotype shifts as a result of ocean changes in the future will arguably have a greater impact on carbon cycle feedbacks than direct physiological responses, highlighting the importance of studying integrated community calcification as well as species- or clone-specific responses.

The similarity in relationship between degree of coccolith calcification and [CO2aq] is even more salient when we examine which samples fall in glacial or interglacial ocean states. Planktic foraminiferal $\delta^{13}$C values from samples at Indian Ocean Site NGHP-01-01A and the orbital age model for Atlantic ODP Site 925 indicate that our youngest samples at both sites (about 0.27 Myr ago), with high SN coccolith thickness, coincide with glacial periods (Supplementary Fig. 6). The sample at 0.84 Myr ago from Site NGHP-01-01A with high SN coccolith thickness also falls during a glacial period, whereas the sample at 0.95 Myr ago from the Site 925, with lower SN coccolith thickness, falls in an interglacial. These particular sampling points therefore underscore the nature of a change in the relationship between degree of calcification and $\text{pCO}_2$, as the samples with thicker coccoliths in a given size class are from glacial periods that coincide with $\text{pCO}_2$ minima in the last 800 kyr (Supplementary Fig. 6).

In the absence of a coherent relationship with [CO2aq], we consider whether a change in ocean alkalinity may have increased cellular $\text{HCO}_3$ uptake and reduced competition for intracellular $\text{HCO}_3$, promoting the recovery of degree of coccolith calcification and $\text{HCO}_3$ allocation to calcification. No proxy record of alkalinity change has yet been produced for this time interval. Multiple lines of evidence based on geochemistry, sedimentology and modelling suggest that the rate of silicate weathering, which adds alkalinity to the ocean, accelerated around 1.5 Myr ago as the North American Precambrian basement shed regolith and experienced more intense subglacial erosion. At the same time the first large amplitude sea level cycles accelerated erosion of shelf sediments. Although our sampling resolution does not capture this higher interglacial timescales compared with the preceding 15 Myr. These particular sampling points therefore under-
the larger coccolithophores occurs several million years before the reduced allocation of HCO$_3^-$ to calcification and reduced cellular calcification in small cells.

The factors driving this differential timing and type of response between the smaller and larger coccolithophores are at this time uncertain but might include lesser plasticity of coccolith thickness in larger genera (Helicosphaera, Coccolithus and Calcidiscus), or a much stronger pressure for HCO$_3^-$ reallocation by larger cells whose diffusive CO$_2$ supply was more limited by their low surface area to volume ratio. While decreasing coccolith size has been suggested as one adaptation to decreasing CO$_2$ availability\cite{2,5,22}, changes in coccolith size, like changes in degree of calcification or changes in the allocation of available carbon to calcification, appear to be part of an array of possible adaptations that may be used simultaneously or sequentially. These strategies appear to be used to varying degrees in different cell size classes, potentially with different thresholds, as each adaptation may come with its own trade-offs. In addition to varying resource availability documented by geochemical indicators, ‘top down’ ecological pressures may contribute to changes in coccolithophore calcification, but unfortunately no proxies are yet available to evaluate their significance in the geological past.

Here, we show a new approach to exploiting independent geochemical and morphological records of coccolithophores to explore the effect of changing cellular HCO$_3^-$ allocation on the magnitude of [CO$_2$aq] change inferred from $f_{\text{CO}_2}$. This approach significantly increases the magnitude of inferred [CO$_2$aq] decline over the last 16 Myr, a result that if substantiated by future high-resolution work, would have important implications for our understanding of climate sensitivity.

The coincidence of greatest SN coccolith thickness and inferred degree of cell-calcification with the period of highest [CO$_2$aq] in the Miocene is at odds with hypotheses for less-calcified cells under future ocean acidification\cite{15,20,24}. Similar to future scenarios, proxy records of past pH derived from boron isotopes in planktic foraminifer shells suggest that high Miocene [CO$_2$aq] coincided with lower surface ocean pH compared with the Pleistocene (Fig. 7)\cite{30,53}. Although extracellular pH may influence the ease with which protons produced during calcification are exported from coccolithophore cells\cite{84}, and in some culture experiments low pH reduces cellular Particulate Inorganic Carbon (PIC) to Particulate Organic Carbon (POC) ratio and increases the incidence of coccolith malformation (refs 6,8,55–57 but also see refs 7,58), the limited phenotypic plasticity of short (<20 generations) monoclonal experiments complicates their extrapolation to real-ocean responses.

Consistent with the long-term responses to high [CO$_2$aq] and low pH since the Mocene shown here, a recent study in the Bay of Biscay found a dominance of heavily calcified E. huxleyi morphotypes during winter when [CO$_2$aq] was highest and pH and CaCO$_3$ saturation were lowest\cite{56} (Supplementary Fig. 8). In this study, the low winter calcite saturation state was driven primarily by an increase in dissolved inorganic carbon concentration and, to a minor extent, by reduced temperatures. However, preliminary results for the last 1 Myr suggest that at late Pleistocene [CO$_2$aq] levels, increased alkalinity may have favoured a higher degree of cell calcification, consistent with culture experiments at constant [CO$_2$], in which increased calcification in E. huxleyi accompanied increased alkalinity\cite{65} (Supplementary Fig. 9). A better understanding of the evolution of ocean alkalinity and [HCO$_3^-$] over the last 1.5 Myr as well as higher-resolution coccolith records may help disentangle the interplay of alkalinity and [CO$_2$aq] on coccolithophore calcification. In addition, further studies with natural populations are required to establish whether [CO$_2$aq] in the modern ocean is a significant driver of cellular calcification.

The long-term reduction in degree of cell-calcification between 14 and 1 Myr ago could potentially have influenced ocean biogeochemical cycles, if a reduction in coccolith ballast lowered the transfer efficiency of organic carbon to the deep ocean. The reduction in cell calcification we identify is coherent with a recent study documenting a global crash in coccolith CaCO$_3$ burial around 4 Myr ago\cite{55}. One consequence of reduced transfer efficiency would be a shallower mean remineralization depth of organic matter. However, $\delta^{13}$C gradients between surface and thermocline-dwelling foraminifers decrease in par with coccolith thickness from 6 to 4 Myr ago, a change driven primarily by the convergence of thermocline $\delta^{13}$C values towards surface values (Fig. 6c,d), and suggestive of a deeper depth of organic matter remineralization in the upper water column\cite{60,61}. This trend suggests that the effect of global cooling, which acts to slow remineralization rates, overrode any effect of reduced ballasting and led to a deepening of mean remineralization depth during the late Miocene\cite{60}. If future global warming likewise leads to a shoaling of mean remineralization depth, it may act to counteract and shift towards enhanced ballasting by more heavily calcified coccolithophore cells.

In summary, our observations suggest that on long timescales, increased [CO$_2$aq] and increased alkalinity may contribute selective pressures favouring thicker coccoliths of a given size and a higher degree of cell-calcification. As projected changes in surface ocean chemistry simulate increased [CO$_2$aq] but diminished alkalinity, prediction of the sign of calcification will rely on better defining the thresholds of response to each parameter. In addition, it remains to be determined whether coccolithophore responses to rapid ocean chemistry changes in the future will be analogous to the geological-timescale adaptation studied here. The plasticity of coccolith thickness and potential selective pressures in the genetically diverse modern ocean thus warrant further investigation.

**Methods**

**Cellular calcification and thickness in culture.** Eight clonal strains of *E. huxleyi* and *Gephyrocapsa* (Supplementary Table 1) were maintained as dilute batch cultures in natural seawater from the Cantabrian Sea (Northern Spain). Prior to experiments, seawater was sterile filtered at 0.2 μm, heated to 80 °C for 3 h, cooled overnight in a sterile hood, and pH was adjusted to 8.3 by addition of NaOH. Media was enriched with major nutrients (P, N), trace metals and vitamins according to the K/2 recipe\cite{52} modified by eliminating the Tris buffer and silicate. Media was then sterile filtered at 0.2 μm just prior to inoculation. Experiments were carried out under a 12:12 light-dark cycle of 16:8 h at a constant temperature of 16 °C under saturated light growth conditions (80–150 μmol m$^{-2}$ s$^{-1}$ photon flux). A homogeneous distribution of cells was maintained by placing the cultures on a roller system providing gentle rotation during growth. Through serial dilution, cultures were maintained in dilute cultures for 8–12 generations before sampling, to establish stable nutrient and carbon chemistry in the media. Each strain was grown in duplicate, and in some cases triplicate, culture bottles. One strain was cultured in two separate experiments and each experiment is reported separately because the two experiments had opposite trends of drift in pH. On collection, media pH and total alkalinity were measured with a Crison GLP-21pH metre calibrated with NaCl standards (National Bureau of Standards, NBS) and duplicate potentiometric titration of filtered, poisoned media samples on a Crison TitroMatic 1S, respectively\cite{36,64}. Average media alkalinity was 2.572 μmol kg$^{-1}$ (± 151, 1 s.d.), pH 8.22 (± 0.07, 1 s.d.) with drift in pH during experiments of <0.11 pH units. Cell density was maintained at biomass averaging 1.65 μg C ml$^{-1}$ and in all cases <2.5 μg C ml$^{-1}$. Cell counts were determined at harvest with a Fuchs–Rosenthal haemocytometer.

For determination of cellular carbon quota, cells were harvested on pre-combusted GF/F or QFF filters. Following acidification to remove calcite, they were analysed for carbon content by flash combustion EA (Euro Vector EA-1108) at C coupled with a Nu Instruments mass-spectrometer (Nu-Horizon). For determination of cellular calcite, cells were harvested on polycarbonate filters. Filters were acidified in 2% HNO$_3$ and Ca concentration was measured in the resultant solutions by ICP-AES (Thermo ICAP DUO 6300).

Cell size (radius) and cell surface area (SA) were derived from measurements of cellular carbon quota using the regression of Popp et al.\cite{65}, which is similar to those derived from other studies\cite{66,67}. We use PIC/SA as an optimal way to represent calcification across a range of cell sizes, because it is unaffected by the size scaling of
Coccolith taxonomy and preservation. We identified coccoliths to genus level only because species-level classification of the smallest Noelaerhabdaceae can be difficult and the light microscopy approach used (Gephyrocapsa and Emiliania huxleyi species assignments are primarily based on size)67. Although most coccoliths were complete, some were found to be missing a piece of the outer rim cycle of one or both shields when observed under the SEM. To verify that such fragmentation did not result in underestimation of coccolith mass per unit area, we quantified this potential effect from SEM images of the 2–5 μm fraction and estimated the percentage of mass loss for individual Noelaerhabdaceae coccoliths due to fragmentation (minimum 50 coccoliths per sample) using C-Calctica. In these calculations, we assumed that 50% of the total mass of each coccolith comes from the inner rim cycle and central area structure (bridge and/or grill), and that the outer rim cycle of each shield contributed 25% of the total mass. These assumptions were based on mass analyses of very well-preserved modern water column samples containing a mixture of Gephyrocapsa and E. huxleyi coccoliths. In these samples, mean contribution of inner rim plus central area to total mass was 63 ± 10% (1 s.d.), therefore our choice of 50% for fossil Noelaerhabdaceae coccoliths is conservative.

Coccolith dimensions are used to infer cell size, based on relationships between coccolith length and cell size for Noelaerhabdaceae15. On this basis, we attribute geochemical results from large coccoliths (8–10 μm) as characterizing larger cells, and those of smaller coccoliths (3–5 μm) as characterizing small cells. k values (originally devised to estimate mass from coccolith shape) were calculated from fossil coccolith mass and length data using the equation of ref. 77:

\[
k_{\text{mass}} = \frac{\pi}{2}\cdot 1.71 \cdot \text{length}^2
\]

We verify that temporal patterns in Noelaerhabdaceae coccolith mass and thickness result only from primary biomineralization and not from abiogenic post-depositional overgrowth using qualitative preservation indices and SEM images (see Results, Supplementary Data 1; Supplementary Figs 4 and 5). Noelaerhabdaceae with a closed central area occur in some samples older than 10 Myr at both sites, and some of these samples older than 10 Myr at both sites, and others of small coccoliths (3–5 μm) as characterizing small cells. k values (originally devised to estimate mass from coccolith shape) were calculated from fossil coccolith mass and length data using the equation of ref. 77:

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Stable isotopes in coccoliths. Samples were disaggregated and micro-filtered in 2% ammonia to separate coccolith size fractions (<2, 3–5, 5–8 and 8–10 μm). For one sample around 2 Myr ago, there was an insufficient number of G. menardii individuals for analysis, therefore we were unable to calculate $\delta^{13}$C of small and large coccoliths was calculated from coccolith $\delta^{13}$C relative to G. menardii $\delta^{13}$C because this foraminiferal species calcifies in equilibrium with $\delta^{13}$C DIC, and also has a similar depth habitat to the coccolithophores with maximum abundances in the chlorophyll maximum near the thermocline, such that

$$\delta_{\text{coccolith}} = \delta^{13}C_{\text{coccolith}} - \delta^{13}C_{\text{G. menardii}}$$

(3)

Propagated analytical uncertainty of $\delta_{\text{coccolith}}=\sqrt{(0.05)^2 + (0.03)^2}$. (4)

For one sample around 2 Myr ago, there was an insufficient number of G. menardii individuals for analysis, therefore we were unable to calculate $\delta^{13}$C of small and large coccoliths. See Supplementary Methods for details of carbon isotope determinations in foraminifera, $\delta^{13}$C and $\delta^{18}$O calculations, and details on ACTI-CO simulations to quantify the effect of changing active uptake on $\delta^{13}$C and $\delta^{18}$O estimates.

References

1. Klaas, C. & Archer, D. E. Association of sinking organic matter with various types of mineral ballast in the deep sea: implications for the rain ratio. Global Biogeochem. Cycles 16, 1–14 (2002).

2. Hain, M., Sigman, D. & Haug, G. in Treatise on Geochemistry 2nd edn, Vol. 7, 1–19 (2002).

3. Feely, R. A. NATURE COMMUNICATIONS| 7:10284 | DOI: 10.1038/ncomms10284 | www.nature.com/naturecommunications

4. Rost, B., Zondervan, I. & Wolf-Gladrow, D. Sensitivity of phytoplankton to changing carbonate chemistry on morphology and weight of coccoliths formed by Emiliania huxleyi. Geobiology 18, 349–360 (2015).

5. Bemitch, J., Hemmings, N. G., Archer, D., Siddall, M. & McManus, J. F. Atmospheric carbon dioxide concentration mechanism of diatoms. Prog. Natl Acad. Sci. USA 108, 3830–3837 (2011).

6. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

7. van de Wal, R. S., de Boer, B., Lourens, L. J., Kohler, P. & Bintanja, R. Reconstruction of a continuous high-resolution CO2 record over the past 20 million years. Clim. Past 7, 1459–1469 (2011).

8. Hopkinson, B. M., Dupont, C. L., Allen, A. E. & Morel, F. M. Efficiency of the CO2-concentrating mechanism of diatoms. Prog. Natl Acad. Sci. USA 108, 3830–3837 (2011).

9. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

10. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

11. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

12. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

13. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

14. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

15. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).

16. Laws, E. A., Popp, B. N., Cassar, N. & Taninoto, J. $\delta^{18}$O discrimination patterns in oceanic coccolithophorids: significance of coccolithophore cell size for foraminiferal $\delta^{18}$O records. Paleoceanography 22 (2007).
42. Beaufort, L., Couipel, M., Buchet, N., Claustre, H. & Goyet, C. Calcite production by coccolithophores in the south east Pacific Ocean. Biogeosciences 11, 1101–1108 (2014).
43. Cubillos, M. et al. Calcification morphotypes of the coccolithophorid Emiliania huxleyi in the Southern Ocean: changes in 2001 to 2006 compared to historical data. Mar. Ecol. Prog. Ser. 348, 47–54 (2007).
44. Henderiks, J. et al. Environmental controls on Emiliania huxleyi morphotypes in the Benguela coastal upwelling system (SE Atlantic). Mar. Ecol. Prog. Ser. 448, 51–66 (2012).
45. Poulton, A. J., Young, J. R., Bates, N. R. & Balch, W. M. Biometry of detached Emiliania huxleyi coccoliths along the Patagonian Shelf. Mar. Ecol. Prog. Ser. 443, 1–17 (2011).
46. Smith, H. E. K. et al. Predominance of heavily calcified coccolithophores at low CaCO3 saturation during winter in the Bay of Biscay. Proc. Natl Acad. Sci. USA 109, 8845–8851 (2012).
47. Poulton, A. J. et al. Coccolithophores on the north-west European shelf: calcification rates and environmental controls. Biogeosciences 11, 3919–3940 (2014).
48. Ridgwell, A. et al. From laboratory manipulations to Earth system models: scaling calcification impacts of ocean acidification. Biogeosciences 6, 2611–2623 (2009).
49. De Bodt, C., Van Oostende, N., Harlay, J., Sabbe, K. & Chou, L. Individual and interacting effects of pCO2 and temperature on Emiliania huxleyi calcification: study of the calcite production, the coccolith morphology and the coccosphere size. Biogeosciences 7, 1401–1410 (2010).
50. Iglesias-Rodriguez, M. D. et al. Phytoplankton calcification in a high-CO2 world. Science 320, 336–340 (2008).
51. Langer, G. & Bode, M. CO2 mediation of adverse effects of seawater acidification in Calcidiscus leptoporus. Geochem. Geophys. Geosyst. 12, Q00501 (2011).
52. Henderiks, J. & Pagani, M. Coccolithophore cell size and the Paleogene decline in atmospheric CO2. Earth. Planet. Sci. Lett. 269, 576–584 (2008).
53. Foster, G. L., Lear, C. H. & Rae, J. W. B. The evolution of pCO2 ice volume and climate during the Middle Miocene. Earth. Planet. Sci. Lett. 341–344, 243–254 (2012).
54. Taylor, A. R., Chrachri, A., Wheeler, G., Goddard, H. & Brownlee, C. A voltage-gated H+ channel underlying pH homeostasis in calcifying coccolithophores. PLoS Biol. 9, e1001085 (2011).
55. Langer, G., Probert, I., Nehrke, G. & Ziveri, P. The morphological response of Emiliania huxleyi to seawater carbonate chemistry changes: an inter-strain comparison. J. Phycol. 48, 225–265 (2012).
56. Taylor, A. R., Chrachri, A., Wheeler, G., Goddard, H. & Brownlee, C. A voltage-gated H+ channel underlying pH homeostasis in calcifying coccolithophores. PLoS Biol. 9, e1001085 (2011).
57. Gundersen, J. H. & Christiansen, K. N. The relationship between pCO2 and calcification in four species of calcifying planktonic diatoms and an estimate of the true carbonate ion concentration. Mar. Ecol. Prog. Ser. 41, 197–208 (1987).
58. Young, J. & Ziveri, P. Calculation of coccolith volume and its use in calibration of Oligocene-Miocene time. Phil. Trans. Soc. London Ser. A 357, 1907–1929 (1999).
59. Flores, J. & Sierro, F. Revised technique for calculation of calcareous nannofossil accumulations rates. Micropaleontology 43, 321–324 (1997).
60. O’Dea, S. A. et al. Coccolithophore calcification response to past ocean acidification and climate change. Nat. Commun. 5, 5363 (2014).
61. Young, J. in Calcification: From laboratory manipulations to Earth system models: scaling calcification impacts of ocean acidification (eds Castrillejo, A., Apruzzese, D. & Oliver, R.) 243–254 (2012).
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