



HAL
open science

Late Miocene threshold response of marine algae to carbon dioxide limitation

Clara Bolton, Heather M. Stoll

► **To cite this version:**

Clara Bolton, Heather M. Stoll. Late Miocene threshold response of marine algae to carbon dioxide limitation. *Nature*, 2013, 500 (7464), pp.558 - 562. 10.1038/nature12448 . hal-01667995

HAL Id: hal-01667995

<https://hal.science/hal-01667995>

Submitted on 19 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Late Miocene threshold response of marine algae to carbon dioxide limitation**

2

3 Clara T. Bolton and Heather M. Stoll

4

5 Geology Department, University of Oviedo, Jesus Arias de Velasco S/N, 33005,

6 Oviedo, Asturias, Spain

7

8 **Coccolithophores are marine algae that use carbon for calcification and**

9 **photosynthesis. The long term adaptation of these and other marine algae to**

10 **decreasing carbon dioxide levels during the Cenozoic era¹ has resulted in modern**

11 **algae capable of actively enhancing carbon dioxide at the site of photosynthesis.**

12 **This enhancement occurs through the transport of dissolved bicarbonate (HCO_3^-)**

13 **and with the help of enzymes whose expression can be modulated by variable**

14 **aqueous carbon dioxide concentration, $[\text{CO}_2]$, in laboratory cultures^{2,3}.**

15 **Coccolithophores preserve the geological history of this adaptation because the**

16 **stable carbon and oxygen isotopic compositions of their calcite plates (coccoliths),**

17 **which are preserved in the fossil record, are sensitive to active carbon uptake and**

18 **transport by the cell. Here we use a model of cellular carbon fluxes and show that**

19 **at low $[\text{CO}_2]$, the increased demand for HCO_3^- at the site of photosynthesis results**

20 **in a diminished allocation of HCO_3^- to calcification, which is most pronounced in**

21 **larger cells. This results in a large divergence between the carbon isotopic**

22 **compositions of small versus large coccoliths only at low $[\text{CO}_2]$. Our evaluation of**

23 **the oxygen and carbon isotope record of size-separated fossil coccoliths reveals**

24 **that this isotopic divergence first arose during the late Miocene to the earliest**

25 **Pliocene epoch (about 7-5 million years ago). We interpret this to be a threshold**

26 **response of the cells' carbon acquisition strategies to decreasing [CO₂]. The**
27 **documented coccolithophore response is synchronous with a global shift in**
28 **terrestrial vegetation distribution between 8 and 5 Myr ago, which has been**
29 **interpreted by some studies as a floral response to decreasing partial pressures of**
30 **carbon dioxide (*p*CO₂) in the atmosphere⁴⁻⁶. We infer a global decrease in carbon**
31 **dioxide levels for this time interval that has not yet been identified in the sparse**
32 ***p*CO₂ proxy record⁷ but that is synchronous with global cooling and progressive**
33 **glaciations^{8,9}.**

34

35 Coccolithophores are unique among algae in that they use carbon both for calcification
36 and for photosynthesis. Cultures of coccolithophores grown under ambient, CO₂-
37 limiting conditions show an unusually large array (up to 5 ‰) of non-equilibrium
38 carbon and oxygen stable isotopic fractionations ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$)^{10,11}. These isotope
39 ‘vital effects’, so-called because they are thought to result from biological processes, are
40 also evident in coccoliths from recent sediments and sediment traps. The isotopic
41 difference between small and large coccoliths diminishes in cultures grown at elevated
42 [CO₂] (increased dissolved inorganic carbon concentration at constant pH)¹² (Fig. 1b)
43 and is absent in fossil coccoliths from past Palaeocene greenhouse climates^{13,14}. We
44 assert that vital effects reflect the adaptation of cellular carbon fluxes to aqueous CO₂
45 availability, and in a new model we reveal the origin of carbon isotope vital effects. We
46 then evaluate the timing of the emergence of vital effects in the fossil record and its
47 relationship to Cenozoic climate evolution and the long-term decrease in *p*CO₂.

48

49 Photosynthesis in large cells may be more sensitive to limitation by diffusive CO₂
50 supply because of the lower ratio of surface area to volume (Supplementary Fig. 2).

51 Active transport of HCO_3^- for photosynthesis is expected to be driven by the extent of
52 diffusive CO_2 limitation, and may therefore differ between small and large cells. A new
53 model (Supplementary Discussion) reveals the active HCO_3^- fluxes to the cell, the site
54 of photosynthesis (chloroplast) and the site of calcification (coccolith vesicle, CV)
55 required to explain the observed array of carbon isotopic fractionation into organic
56 matter and coccolith calcite, ϵ_p and $\epsilon_{\text{coccolith}}$ respectively, observed in coccolithophore
57 species of different sizes grown in culture at variable $[\text{CO}_2]$ ^{12,15} (Fig. 1). The model
58 confirms that at low $[\text{CO}_2]$, active HCO_3^- transport to the chloroplast is increased at the
59 expense of active HCO_3^- transport to the coccolith vesicle. A similar competitive
60 reallocation of HCO_3^- to photosynthesis from calcification at low $[\text{CO}_2]$ has been shown
61 in the laboratory¹⁶. As a consequence, at low $[\text{CO}_2]$, a smaller proportion of calcification
62 is supported by a direct influx of HCO_3^- to the coccolith vesicle, decreasing $\epsilon_{\text{coccolith}}$.
63 This process is amplified in larger cells, which at low $[\text{CO}_2]$ feature the lowest
64 proportion of calcification supported by direct influx of HCO_3^- to the coccolith vesicle.
65 Consequently, the difference in $\epsilon_{\text{coccolith}}$ between large and small coccolithophores is
66 greater at low $[\text{CO}_2]$. Culture data and our model indicate that this relationship is non-
67 linear, with the steepest dependence of $\epsilon_{\text{coccolith}}$ on $[\text{CO}_2]$ over the range 12-19 μM (Fig.
68 1b). Vital effects in $\delta^{18}\text{O}$ have previously been ascribed to changes in the relative
69 contribution of carbonate (CO_3^{2-}) and HCO_3^- to coccolith calcite¹⁷, which produces an
70 effect analogous to that generated by variable relative influx of CO_2 and HCO_3^- to the
71 coccolith vesicle predicted by our $\delta^{13}\text{C}$ model (Supplementary Discussion).
72
73 Evaluation of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in size-separated coccoliths from five (Integrated) Ocean
74 Drilling Program sites (Supplementary Methods and Supplementary Fig. 9) shows that
75 vital effects of stable isotopes in coccoliths were minimal before and after the Eocene-

76 Oligocene (about 34 Myr ago) and Oligocene-Miocene (about 23 Myr ago) transitions,
77 and that large (more than 1‰) vital effects first appeared during the late Miocene to
78 earliest Pliocene (about 7-5 Myr ago). A striking divergence in isotopic composition in
79 different-sized coccoliths is demonstrated in records from two widely separated sites,
80 Caribbean Site 999 and sub-Antarctic Site 1088 (Figs 2 and 3). In samples pre-dating 7
81 Myr ago, only small $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ differences (less than 0.75‰) between size fractions
82 are observed. After the divergence, which begins at 6-7 Myr ago at Site 999 and 4-5
83 Myr ago at Site 1088, persistent vital effects of 1.5-3‰ in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ are recorded,
84 with large coccoliths consistently recording lighter $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ relative to smaller
85 coccoliths (Fig. 2). We interpret this diachrony as a real lag that is too large to result
86 from age model discrepancies (Supplementary Methods and Supplementary Fig. 11).
87 We note that temporal changes in mean coccolith size in the sediments do not affect our
88 data from restricted coccolith size classes.
89
90 The marked increase in vital effects in coccoliths in the late Miocene cannot reflect an
91 expansion into a wider range of depth habitats, because the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in
92 different-sized coccoliths are positively correlated (Fig. 2, Supplementary Fig. 10), not
93 negatively correlated as would be expected from depth segregation in the photic zone¹³.
94 We also find no cause to suggest that the depth habitat of all coccolithophores at both
95 sites migrated from deeper CO_2 -enriched to shallower CO_2 -depleted waters within the
96 photic zone (Supplementary Discussion). At Site 999, it is possible that circulation
97 changes associated with the gradual closure of the Central American Seaway about 14
98 to 3 Myr ago (ref. 18) stemmed the eastward flow of CO_2 -rich upwelled water from the
99 equatorial Pacific; however, the emergence of the Panama Isthmus is not modelled to
100 strongly affect circulation near Site 1088 (ref. 19). The shift to a large array of vital

101 effects in coccoliths occurs at a time when there is no evidence for large changes in
102 coccolithophore growth rate at either site, as indicated by coccolith Sr/Ca records
103 (Supplementary Methods and Supplementary Fig. 5). A shift from predominantly (more
104 than 70%) diagenetic calcite to primary coccolith calcite would be required to
105 homogenise a 1.5‰ isotopic difference in primary $\delta^{18}\text{O}$ to the less than 0.6‰ recorded
106 in older sediments (Supplementary Fig. 8). This is not consistent with the moderate to
107 good coccolith preservation throughout the Miocene-Pliocene at both sites evident in
108 scanning electron microscope images (Supplementary Figs 6 and 7), nor with Sr/Ca
109 values, which confirm biogenic rather than abiogenic (diagenetic) Sr partitioning
110 throughout the Miocene-Pliocene study interval (Supplementary Discussion). The
111 presence of vital effects at the Pliocene end of both records, and their absence at the
112 Miocene end, is unlikely to result from differences in species contributions in a given
113 size fraction over time. Counts of coccoliths in all size fractions from end-member
114 samples show that, despite changes in species composition and size distribution over the
115 16 Myr study interval, the genera or families dominating each size fraction remain
116 similar (Supplementary Table 3). For example, at Site 1088, smallest and largest
117 coccolith size fractions in both Pleistocene and Miocene end-member samples are
118 dominated (more than 70% CaCO_3) by small reticulofenestrid and *Coccolithus*
119 *pelagicus* coccoliths respectively, yet only the Pleistocene sample records a large array
120 (up to 3‰) of vital effects (Fig. 2).

121

122 Our model of coccolithophore carbon allocation suggests that the late Miocene
123 emergence of vital effects represents a modification of carbon acquisition strategies of
124 the cells as $[\text{CO}_2]$ decreased below a critical threshold (Fig. 1). We propose that a
125 decrease in $p\text{CO}_2$ caused tropical waters (Site 999) to fall below this $[\text{CO}_2]$ threshold at

126 about 7 Myr ago. Because CO₂ is more soluble in cold waters, a continued *p*CO₂ decline
127 into the early Pliocene (about 5 Myr ago) was required before a similar limiting [CO₂]
128 was reached in the cooler sub-Antarctic waters of Site 1088 (Supplementary Fig. 12).

129

130 The emergence of large-scale vital effects in coccoliths in the late Miocene, rather than
131 at earlier transitions such as the Eocene-Oligocene or Oligocene-Miocene, for which
132 important step decreases in *p*CO₂ are estimated from proxies and inferred from climate
133 records²⁰⁻²³, is consistent with culture data¹², which suggest low sensitivity of $\epsilon_{\text{coccolith}}$ to
134 [CO₂] variation above 19 μM . At typical concentrations of dissolved inorganic carbon
135 in the surface ocean (2050 μM) and estimated production temperatures for a typical
136 mid-latitude site (20 °C; Supplementary Fig. 5), the range of maximum sensitivity (12-
137 19 μM [CO₂]) corresponds to *p*CO₂ in the range 575-375 parts per million by volume
138 (p.p.m.v.). As [CO₂] decreases below 20 μM there is an exponential increase in the
139 requirement for active HCO₃⁻ transport to the chloroplast (Supplementary Fig. 4). Since
140 the late Miocene, further decreases in *p*CO₂, even to low values typical of the last
141 glacial¹³, have not resulted in a subsequent increase in the magnitude of size-related
142 vital effects. One explanation could be that further decreases in [CO₂] were
143 accompanied by a decrease in cellular calcification, thereby limiting further decreases in
144 the supply of HCO₃⁻ to the coccolith vesicle relative to calcification. Decreased
145 calcification in coccoliths of a given size over the Cenozoic could support the operation
146 of such a mechanism^{24,25}.

147 Few *p*CO₂ proxy reconstructions cover the interval leading up to the divergence of vital
148 effects in coccoliths (12-5 Myr ago). Alkenone-based records suggest low and stable
149 *p*CO₂ during this interval (Fig. 3b). However, these estimates could be too low because
150 of the nature of the applied corrections for temperature and phosphate

151 concentrations^{22,26}. New alkenone-based $p\text{CO}_2$ estimates from the western tropical
152 Atlantic covering the mid to late Miocene, although low in resolution, suggest
153 substantially higher values (400-500 p.p.m.v.)²⁷. Although uncertainties remain large,
154 stomatal proxies indicate a $p\text{CO}_2$ decrease⁷, consistent with inverse modelling of climate
155 data⁸ (Fig. 3b). Our data suggest that substantial surface ocean cooling over the last 15
156 Myr, up to 14 °C in the subtropics²⁸, may reflect an important global $p\text{CO}_2$ decrease that
157 is poorly resolved by existing $p\text{CO}_2$ proxy records, rather than a decoupling of
158 atmospheric CO_2 forcing and climate as suggested by some authors²⁸.

159

160 The appearance of large-scale vital effects in coccoliths between 7 and 5 Myr ago is
161 synchronous with a global expansion in terrestrial C_4 plants (that is, those using the C_4
162 photosynthetic pathway; mostly tropical grasses) relative to C_3 plants (primarily trees)
163 in low-latitudes and mid-latitudes^{4-6,29} (Fig. 3a). In some regions, such as the Himalayan
164 foreland and Arabian Peninsula, it has been suggested that a shift to increasingly arid
165 conditions was the dominant driver of the late Miocene rise in C_4 plants²⁹. However, the
166 shift to C_4 dominance has also been widely interpreted as a response to decreasing
167 $p\text{CO}_2$, because at low ratios of atmospheric CO_2 to O_2 concentrations C_4 plants have a
168 competitive advantage over C_3 plants⁴⁻⁶. The presence of a biochemical carbon-
169 concentrating mechanism allows C_4 plants to decrease energetically costly
170 photorespiration rates, and also to decrease stomatal conductance (a measure of the rate
171 at which water and CO_2 can diffuse in or out of the leaf), thus decreasing water loss.
172 Conditions that favour C_4 over C_3 plants are suggested to occur below a $p\text{CO}_2$ of about
173 500 p.p.m.v. when accompanied by high temperatures during the growing season (that
174 is, at low latitudes), or at lower $p\text{CO}_2$ in cooler climates^{4,5}. Thus, both terrestrial and
175 marine photosynthesizers may be showing adaptation at a common $p\text{CO}_2$ threshold.

176

177 We show that the large array of isotopic fractionations in modern coccolith carbonate is
178 indicative of the operation of strong carbon-concentrating mechanisms in
179 coccolithophore cells, which became highly significant since the latest Miocene. We
180 speculate that this change occurred as a threshold response to increased CO₂ limitation,
181 beginning in the late Miocene in the tropical oceans and progressing to higher latitudes
182 by the earliest Pliocene. This increase in the degree of active carbon uptake by
183 coccolithophores will need to be accounted for in the application of ϵ_p to estimates of
184 [CO₂] (ref. 30). The relatively low [CO₂] threshold suggested to have driven the late
185 Miocene diversification of coccolithophore carbon acquisition strategies is consistent
186 with estimates of less than 500 p.p.m.v. $p\text{CO}_2$ required to promote the tropical C₄-
187 dominated ecosystems that also expanded over this interval⁴⁻⁶. We speculate that such a
188 low $p\text{CO}_2$ threshold, affecting both marine and terrestrial primary producers, could be
189 reversed within decades as a result of rapid anthropogenic CO₂ release and absorption
190 by the ocean.

191

192 **Methods summary**

193 We adapt a model for the $\delta^{13}\text{C}$ composition of photosynthetically fixed carbon in
194 diatoms³¹ with an additional module for the coccolith vesicle, allowing us to simulate
195 the $\delta^{13}\text{C}$ of coccolith calcite as a function of the passive and active carbon fluxes into
196 the coccolith vesicle and cell (model ACTI-CO; see Supplementary Discussion).
197 Coccolith size fractions were separated from bulk IODP sediment samples using site-
198 specific and interval-specific settling and microfiltration protocols (Supplementary
199 Methods). Coccolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were measured on a Nu Perspective dual-inlet
200 isotope ratio mass spectrometer connected to a NuCarb carbonate preparation system,

201 with an analytical precision of 0.06‰ for $\delta^{18}\text{O}$ and 0.05‰ for $\delta^{13}\text{C}$ (1σ), at Oviedo
202 University. Mean reproducibility, based on duplicate analyses of splits of 21 random
203 samples from Sites 999 and 1088, is 0.08‰ for $\delta^{18}\text{O}$ and 0.06‰ for $\delta^{13}\text{C}$ (1σ). Sr/Ca
204 was determined in two coccolith size fractions at both Sites 999 and 1088. Reducing
205 and ion-exchange treatments were first applied to clean the samples, followed by gentle
206 dissolution in acetic acid with an ammonium acetate buffer for 12 h. Calcium content
207 was measured on a split of all samples, which were then diluted to constant calcium
208 concentrations for Sr/Ca analysis by inductively coupled plasma optical emission
209 spectroscopy on a Thermo ICAP DUO 6300 at Oviedo University. Sr/Ca data were
210 corrected for site-specific variations in sea surface temperature (Supplementary
211 Methods). All coccolith counts were performed on standard smear slides with a light
212 microscope under cross-polarized light at x1250 magnification. To assess preservation,
213 coccolith samples on polycarbonate filters were mounted onto a stub, coated with gold
214 and imaged on a JEOL 6610LV scanning electron microscope.

215

216 **References**

- 217 1 Tortell, P. D. Evolutionary and Ecological Perspectives on carbon acquisition in
218 phytoplankton. *Limnology and Oceanography* **45**, 744-750 (2000).
- 219 2 Raven, J. A., Giordano, M., Beardall, J. & Maberly, S. C. Algal and aquatic
220 plant carbon concentrating mechanisms in relation to environmental change.
221 *Photosynthesis Research* **109** (2011).
- 222 3 Reinfelder, J. R. Carbon Concentrating Mechanisms in Eukaryotic Marine
223 Phytoplankton. *Annual Review of Marine Science* **3**, 291-315 (2011).

- 224 4 Cerling, T. E. *et al.* Global vegetation change through the Miocene-Pliocene
225 boundary. *Nature* **389**, 153-158 (1997).
- 226 5 Ehleringer, J. R., Cerling, T. E. & Helliker, B. R. C₄ photosynthesis,
227 atmospheric CO₂, and climate. *Oecologia* **112**, 285-299 (1997).
- 228 6 Zhang, C. *et al.* C₄ expansion in central Inner Mongolia during the latest
229 Miocene and early Pliocene. *Earth and Planetary Science Letters* **287**, 311-319
230 (2009).
- 231 7 Beerling, D. J. & Royer, D. L. Convergent Cenozoic CO₂ history. *Nature*
232 *Geoscience* **4**, 418-420 (2011).
- 233 8 van de Wal, R. S., de Boer, B., Lourens, L. J., Köhler, P. & Bintanja, R.
234 Reconstruction of a continuous high-resolution CO₂ record over the past 20
235 million years. *Climate of the Past* **7**, 1459-1469 (2011).
- 236 9 Zachos, J. C., Dickens, G. R. & Zeebe, R. E. An early Cenozoic perspective on
237 greenhouse warming and carbon-cycle dynamics. *Nature* **451**, 279-283 (2008).
- 238 10 Dudley, W. C., Blackwelder, P., Brand, L. & Duplessy, J. C. Stable isotopic
239 composition of coccoliths. *Marine Micropaleontology* **10**, 1-8 (1986).
- 240 11 Ziveri, P. *et al.* Stable isotope “vital effects” in coccolith. *Earth and Planetary*
241 *Science Letters* **210**, 137-149 (2003).
- 242 12 Rickaby, R. E. M., Henderiks, J. & Young, J. N. Perturbing phytoplankton:
243 response and isotopic fractionation with changing carbonate chemistry in two
244 coccolithophore species. *Climate of the Past* **6**, 771–785 (2010).

- 245 13 Bolton, C. T., Stoll, H. M. & Mendez Vicente, A. Vital effects in coccolith
246 calcite: Cenozoic climate- $p\text{CO}_2$ drove the diversity of carbon acquisition
247 strategies in coccolithophores? *Paleoceanography* **27** (2012).
- 248 14 Stoll, H. M. Limited range of interspecific vital effects in coccolith stable
249 isotopic records during the Paleocene-Eocene thermal maximum.
250 *Paleoceanography* **20**, PA1007 (2005).
- 251 15 Rost, B., Zondervan, I. & Riebesell, U. Light-dependent carbon isotope
252 fractionation in the coccolithophorid *Emiliana huxleyi*. *Limnology and*
253 *Oceanography* **47**, 120-128 (2002).
- 254 16 Bach, L. T. *et al.* Dissecting the impact of CO_2 and pH on the mechanisms of
255 photosynthesis and calcification in the coccolithophore *Emiliana huxleyi*. *New*
256 *Phytologist* **199**, 121-134. doi: 10.1111/nph.12225 (2013).
- 257 17 Ziveri, P., Thoms, S., Probert, I., Geisen, M. & Langer, G. A universal carbonate
258 ion effect on stable isotope ratios in unicellular planktonic calcifying organisms.
259 *Biogeosciences* **9**, 1025-1032 (2012).
- 260 18 Kameo, K. & Sato, T. Biogeography of Neogene calcareous nannofossils in the
261 Caribbean and the eastern equatorial Pacific- floral response to the emergence of
262 the Isthmus of Panama. *Marine Micropaleontology* **39**, 201-218 (2000).
- 263 19 Schneider, B. & Schmittner, A. Simulating the impact of the Panamanian
264 seaway closure on ocean circulation, marine productivity and nutrient cycling.
265 *Earth and Planetary Science Letters* **246**, 367-380 (2006).
- 266 20 Kürschner, W. M., Kvacek, Z. & Dilcher, D. L. The impact of Miocene
267 atmospheric carbon dioxide fluctuations on climate and the evolution of

- 268 terrestrial ecosystems. *Proceedings of the National Academy of Sciences USA*
269 **105**, 449-453 (2008).
- 270 21 Pagani, M., Arthur, M. A. & Freeman, K. H. Miocene evolution of atmospheric
271 carbon dioxide. *Paleoceanography* **14**, 273-292 (1999).
- 272 22 Pagani, M. *et al.* The role of carbon dioxide during the onset of Antarctic
273 glaciation. *Science* **334**, 1261-1264 (2011).
- 274 23 Pearson, P. N., Foster, G. L. & Wade, B. S. Atmospheric carbon dioxide through
275 the Eocene-Oligocene climate transition. *Nature* **461** (2009).
- 276 24 Preiss-Daimler, I., Baumann, K.-H. & Heinrich, R. Carbonate budget mass
277 estimates for Neogene discoasters from the Equatorial Atlantic (Ceara Rise:
278 ODP Site 927). *Journal of Micropaleontology* **31**, 169-178 (2012).
- 279 25 Stoll, H. M. *et al.* Insights on coccolith chemistry from a new ion probe method
280 for analysis of individually picked coccoliths. *Geochemistry Geophysics*
281 *Geosystems* **8**, doi:doi:10.1029/2006GC001546 (2007).
- 282 26 Pagani, M., Liu, Z., LaRiviere, J. & Ravelo, A. C. High Earth-system climate
283 sensitivity determined from Pliocene carbon dioxide concentrations. *Nature*
284 *Geoscience* **3**, 27 - 30 (2009).
- 285 27 Zhang, Y. G., Pagani, M., Liu, Z., Bohaty, S. M. & DeConto, R. A 40 million-
286 year history of atmospheric CO₂. *Philosophical Transactions of the Royal*
287 *Society A* (In press).
- 288 28 LaRiviere, J. P. *et al.* Late Miocene decoupling of oceanic warmth and
289 atmospheric carbon dioxide forcing. *Nature* **486**, 97-100 (2012).

- 290 29 Huang, Y., Clemens, S. C., Liu, W., Wang, Y. & Prell, W. L. Large-scale
291 hydrological change drove the late Miocene C₄ plant expansion in the
292 Himalayan foreland and Arabian Peninsula. *Geology* **35**, 531-534, doi:
293 10.1130/G23666A (2007).
- 294 30 Laws, E. A., Popp, B. N., Cassar, N. & Tanimoto, J. 13C discrimination patterns
295 in oceanic phytoplankton: likely influence of CO₂ concentrating mechanisms,
296 and implications for palaeoreconstructions. *Functional Plant Biology* **29**, 323-
297 333 (2002).
- 298 31 Hopkinson, B. M., Dupont, C. L., Allen, A. E. & Morel, F. M. M. Efficiency of
299 the CO₂-concentrating mechanism of diatoms. *Proceedings of the National*
300 *Academy of Sciences USA* **108**, 3830-3837 (2011).

301

302 **Supplementary Information** is linked to the online version of the paper at

303 www.nature.com/nature.

304

305 **Acknowledgements** We thank L. Abrevaya and A. Mendez for laboratory assistance
306 and K. Lawrence for access to unpublished data. This work used samples provided by
307 the (Integrated) Ocean Drilling Program (IODP). The IODP is sponsored by the U.S.
308 National Science Foundation and participating countries under management of the
309 IODP Management International, Inc (IODP-MI). Funding for this research was
310 provided by European Research Council grant UE-09-ERC-2009-STG-240222-PACE
311 (H.M.S.) and a DuPont Young Professor Award to H.M.S.

312

313 **Author Contributions** C.T.B. and H.M.S. designed the study and wrote the paper.

314 C.T.B. separated coccoliths and performed stable isotope, light microscope and

315 scanning electron microscope analyses. H.M.S. designed and ran the model.

316

317 **Author Information** Reprints and permissions information is available at

318 www.nature.com/reprints. The authors declare no competing financial interests. Readers

319 are welcome to comment on the online version of this article at www.nature.com/nature.

320 Correspondence and requests for materials should be addressed to C.T.B.

321 (cbolton@geol.uniovi.es).

322

323 **Figure captions**

324 **Figure 1: HCO₃⁻ allocation to the chloroplast and coccolith vesicle inferred from**

325 $\epsilon_{\text{coccolith}}$ **measured in culture. a**, simplified modelled coccolithophore carbon fluxes

326 (details in Supplementary Fig. 1). CV, coccolith vesicle, CHL, chloroplast. Dashed

327 black arrows represent passive fluxes, and solid black arrows represent active fluxes. **b**,

328 $\epsilon_{\text{coccolith}}$ as a function of [CO₂] (data from ref. 12; propagated analytical uncertainty

329 0.1‰). **c**, Coccolith vesicle HCO₃⁻ influx relative to calcification, **d**, Coccolith vesicle

330 HCO₃⁻ influx relative to chloroplast HCO₃⁻ influx, **e**, Chloroplast HCO₃⁻ influx relative

331 to diffusive CO₂ uptake by cell. Data in **c-e** are inferred from inverse model

332 (Supplementary Information) using default parameters (Supplementary Table 1).

333 Symbols in **b-e**: diamonds, *Gephyrocapsa oceanica*; squares, *Coccolithus pelagicus*

334 *subsp. braarudii*. Blue shading indicates the range of steepest dependence of $\epsilon_{\text{coccolith}}$ on

335 [CO₂].

336

337 **Figure 2: Divergence of vital effects in coccoliths. a**, Benthic foraminiferal $\delta^{18}\text{O}$ (ref.
338 9) (data points in light grey, smoothed with seven-point running mean) and $\delta^{13}\text{C}$ of
339 smallest and largest coccoliths (coloured circles). All $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values are
340 measured against Vienna Pee Dee Belemnite (VPDB). See Supplementary Fig. 10 for
341 complete size fraction data. Bubble size scales with approximate coccolith size. For the
342 Neogene, mean values for 3-Myr time windows are shown from Sites 999 and 1088.
343 The grey box denotes the time interval in **b-e** (16-0 Myr ago). **b, c**, $\delta^{18}\text{O}$ (**b**) and $\delta^{13}\text{C}$ (**c**)
344 of different-sized coccoliths from Site 999. **d, e**, $\delta^{18}\text{O}$ (**d**) and $\delta^{13}\text{C}$ (**e**) of different-sized
345 coccoliths from Site 1088. To remove secular trends and highlight differences between
346 size fractions, all coccolith isotopes are normalized to the smallest coccolith size
347 fraction in each sample. Note the different scales of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ axes.

348

349 **Figure 3: Evolution of vital effects in coccoliths, C_4 photosynthesis, and $p\text{CO}_2$ since**
350 **16 Myr ago. a**, $\delta^{13}\text{C}$ difference between smallest and largest coccolith size fractions at
351 Sites 999 (red) and 1088 (orange) and the range of tooth enamel $\delta^{13}\text{C}$ values (blue
352 shading; data from ref. 4; only North American data $<37^\circ$ plotted; however other
353 regions show a similar pattern). The propagated analytical uncertainty on coccolith $\delta^{13}\text{C}$
354 differences is 0.07‰. **b**, Estimates of $p\text{CO}_2$ from various proxies: foraminifer boron
355 isotopes (blue and yellow horizontal crosses), stomata (red diagonal crosses), alkenone
356 $\delta^{13}\text{C}$ maximum and minimum estimates (pink, green, grey and orange shading), and
357 inverse modelling of deep-sea $\delta^{18}\text{O}$ (black line). Note the change in scale at 500
358 p.p.m.v. Vertical error bars represent the uncertainty reported in published $p\text{CO}_2$
359 estimates. See Supplementary Information for $p\text{CO}_2$ data references and details of
360 uncertainty derivation for each reference.

361





