Parallel between the isotopic composition of coccolith calcite and carbon levels across Termination II: Developing a new paleo-CO$_2$ probe

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Abstract. Beyond the pCO$_2$ records provided by ice core measurements, the quantification of atmospheric CO$_2$ concentrations and changes thereof relies on proxy data, the development of which represents a foremost challenge in paleoceanography. In the paleoceanographic toolbox, the coccolithophores occupy a notable place, as the magnitude of the carbon isotopic fractionation between ambient CO$_2$ and a type of organic compounds that these photosynthetic microalgae synthesize (the alkenones) represents a relatively robust proxy to reconstruct past atmospheric CO$_2$ concentrations during the Cenozoic. The isotopic composition of coeval calcite biominerals found in the sediments and also produced by the coccolithophores (the coccoliths) have been found to record an ambient CO$_2$ signal through culture and sediment analyses. These studies have, however, not yet formalized a transfer function that quantitatively ties the isotopic composition of coccolith calcite to the concentrations of aqueous CO$_2$ and, ultimately, to atmospheric CO$_2$ levels. Here, we make use of a micro-separation protocol to compare the isotopic response of two size-restricted coccolith assemblages from the North Atlantic to changes in surface ocean CO$_2$ during Termination II (ca. 130-140 ka). Performing paired measurements of the isotopic composition ($\delta^{13}$C and $\delta^{18}$O) of relatively large and small coccoliths provides an isotopic offset that can be designated as a “differential vital effect”.

We find that the evolution of this offset follows that of aqueous CO$_2$ concentrations computed from the ice core CO$_2$ curve and an independent temperature signal. We interpret this biogeochemical feature to be the result of converging carbon fixation strategies between large and small cells as the degree of carbon limitation for cellular growth decreases across the deglaciation. We are therefore able to determine a transfer function between the coccolith differential vital effects and aqueous CO$_2$ in the range of Quaternary CO$_2$ concentrations. We here consolidate a new coccolith $\Delta\delta^{13}$C proxy that overtakes the strong assumptions that have to be made pertaining to the chemistry of the carbonate system in seawater, as required in CO$_2$ proxy methods such as the boron isotope and alkenone proxies.
1 Introduction

Reconstructing the changes of atmospheric pCO₂ levels over geological timescales has been one of the major challenges in paleoenvironmental research for the last forty years (Neftel et al., 1982; Broecker, 1982; Pagani, 2013). In particular, due to its effects as a greenhouse gas, atmospheric pCO₂ is believed to have exerted a first-order control on the temperature changes at the Earth’s surface that occurred over the Phanerozoic (Berner, 1990). On shorter timescales, analyses of the concentrations of CO₂ of air bubbles trapped in Quaternary ice cores, which have provided relatively direct pCO₂ estimates, have exemplified the synchronicity of pCO₂ and glacial-interglacial changes (Petit et al., 1999). Beyond 800 kyrs, however, estimates of atmospheric pCO₂ rely on more indirect proxies, mainly derived from the marine sedimentary record (Broecker, 2018). The alkenone εₒ proxy uses the magnitude of the carbon isotopic fractionation between algal lipids produced by certain haptophytes and ambient CO₂ to derive CO₂ concentrations (see review by Pagani 2013). Other proxies alternatively derive the levels of aqueous CO₂ from a set of parameters of ocean carbonate chemistry. This is the case of the foraminifera boron isotope proxy, which aims at reconstructing values for surface ocean pH (Sanyal et al., 1995; Foster, 2008) and can further be used to derive surface ocean CO₂ (Shao et al., 2019). The 45-million-year record of atmospheric pCO₂ (Zhang et al., 2013) is a flagship outcome of the εₒ proxy in published literature and has yielded invaluable insights into the quantitative forcing of CO₂ decline on Earth cooling during the Cenozoic.

The coccolithophores, the same primary producers that synthesize the alkenones, also operate the intracellular biomineralization of calcite material, the coccoliths (Volkman et al., 1980). The geochemistry of these calcareous nanofossils, albeit ubiquitous in marine sediments since the Jurassic (de Vargas et al., 2007), has been overall set aside in paleoclimate reconstructions. This is due, in part, to the large biologically-induced offset between coccolith δ¹³C and δ¹⁸O compositions and that of inorganic calcite (or “vital effect”) which hinders their use as traditional δ¹³C paleo-thermometers (Dudley et al., 1986). Recent evidence has, however, shed light on the potential for the coccoliths to record past CO₂ concentrations. Numerical methods, with quantitative constraints stemming from culture data, have provided a valuable understanding on the factors dictating the magnitude of the vital effect (Rickaby et al., 2010; Bolton and Stoll, 2013; Hermoso et al., 2016a, b; McClelland et al., 2017). Collectively, these studies have shown that differences in metabolic rates of the coccolithophores, in particular photosynthesis and calcification, can be put forward to explain the range of vital effects measured in coccolith calcite, in particular between different coccolithophore species. In turn, the rates of photosynthesis and calcification, which therefore dictate the coccolith vital effect, are dependent on the availability of CO₂ in the environment, on cell size and growth rate, on the ratio of the production of calcite relative to organic matter and on the possible expression of CO₂ concentrating mechanisms (CCMs). As a notable corollary, the magnitude of coccolith vital effects decreases under conditions of increasing medium CO₂ (Hermoso et al., 2016b). Beyond the culture flasks of the laboratory, this result is also observed in datasets from the geological record: A global decrease in surface ocean CO₂ is believed to explain for instance why the magnitude of the vital effects of coccoliths extracted from marine sediments has increased since the Late Miocene (Bolton and Stoll, 2013; Hermoso et al.,
On shorter geological timescales, variations in vital effects have been observed throughout glacial-interglacial cycles (Jin et al., 2018), and tentatively linked to changes in surface ocean CO₂ (Hermoso, 2016). Using the inverse approach, attempts at deriving Eocene-Oligocene CO₂ concentrations from the vital effects of fossil coccoliths using transfer functions from culture data were successful in reproducing the pCO₂ curves obtained using the alkenone and boron paleobarometers (Tremblin et al., 2016).

Overall, data from the sedimentary record concord with results of culture experiments and numerical modelling as to the role of CO₂ on the isotopic composition of coccolith calcite for both the carbon and oxygen isotope systems. Discrepancies exist, however, between culture results, where CO₂ concentrations can be controlled, and sedimentary data. A core-top study for instance revealed, for instance, that the oxygen vital effects of Noelaerhabdaceae coccoliths from sediment samples differed from close-living relatives in culture experiments (Hermoso et al., 2015). A number of methodological caveats indeed hinder the comparison of culture and sedimentary data. This includes the fact that results are obtained exclusively on monoclonal populations grown in light and nutrient-replete environments, the very little results available for coccolithophore responses in periods of low CO₂, and the short duration of culture experiments, which leaves little time for cells to adapt (Lohbeck et al., 2012).

Despite recent efforts in culture and sedimentary studies, a transfer function for the emerging coccolith vital effect-CO₂ proxy is lacking. Here, we study the isotopic response of a natural assemblage of coccoliths to changes in surface ocean CO₂ during the Pleistocene. We isolate coccoliths of different size fractions from carbonate oozes of the mid-latitude North Atlantic core MD95-2037. We measure their specific isotopic composition, and study their departures from an inorganic reference – this offset being termed ‘absolute vital effect’ thereafter. We further explore the link between the expression and magnitude of the vital effects and the hypothesized forcing of CO₂, which we derive from the atmospheric pCO₂ curves available for the interval (Bereiter et al., 2015). Specifically, we focus on Termination II (130-140 ka), during which global atmospheric pCO₂ levels increased by 80 ppm (equivalent to a 1.7 µmol.kg⁻¹ increase in CO₂aq). We pay specific attention to the control of environmental forcing on the isotopic composition of coccolith micro-assemblages differing in taxonomy and size, thus exploring a ‘differential vital effect’. In this study, we aim to push forward the potential of the use of this differential vital effect, i.e. the isotopic offset between coccoliths of different sizes, as a CO₂ probe throughout the Cenozoic Era.

2 Material and Methods

2.1 Measurements of coccolith isotopic compositions

2.1.1 Ocean study site & age model

Sediment samples are from the North Atlantic core MD95-2037 (37°05’N, 32°02’W, 2630 m depth) that was retrieved during the IMAGES expedition in 1995 (Bassinot and Labeyrie, 1996). The core is located north of the Azores Current (AC), a branch
of the North Atlantic Current (NAC) (Fig. 1). It also lies north of the Azores Front (AzF), which separates the stratified and low-productivity surface waters of the subtropical gyre in the South from the higher primary productivity region in the North where the winter mixed-layer is deeper (Lévy et al., 2005). The surface water mass is currently located in a region in relative equilibrium with respect to atmospheric CO$_2$ and aqueous CO$_2$ exchanges (Fig. 1). Previous studies at the site have established δ$^{18}$O and δ$^{13}$C curves for the benthic foraminifera species Cibidoides wuellerstorfi and the planktonic species Globigerina bulloides (Villanueva et al., 2001). Together with productivity records from alkenone abundances (Villanueva et al., 2001) and UK$_3^*$-derived sea surface temperature (SST) records (Calvo et al., 2001), these studies have highlighted the large environmental changes that occurred in the surface ocean across Termination II in this region. This includes a 7°C increase in sea surface temperatures between the glacial maximum and full interglacial conditions, and the southward migration of the Arctic front, which, during glacial intervals, may have progressed as far South as 50°N, with implications on the stratification of the water column and the renewal of nutrients to the surface ocean (Pflaumann et al., 2003; Naafs et al., 2010; Zhang et al., 2017).

We established a new age model for site MD95-2037 over Termination II (Fig. S1), using the more recent reference records (such as the benthic δ$^{18}$O composite stacks, Lisiecki and Raymo 2005; Lisiecki and Stern 2016) developed since the last model for the site over Termination II was published (Villanueva et al., 2001). The benthic δ$^{18}$O signal from Cibidoides wuellerstorfi was first aligned to the regional benthic stack for the Deep North Atlantic by (Lisiecki and Stern, 2016) using the Analyseries software (Paillard et al., 1996). The age model was then refined by aligning the Globigerina bulloides δ$^{18}$O signal for site MD95-2037 to the stacked Corchia Cave δ$^{18}$O record (Tzedakis et al., 2018), as both records are expected to reflect the changes in surface temperature and ice volume that occurred across this latitudinal band (Drysdale et al., 2005). As done by Govin et al. 2015, we evaluated the accuracy of the age model by calculating the quadratic sum of the individual uncertainties deriving from i) matching errors on Analyseries; ii) the resolution of the aligned record; and iii) the dating error of the reference speleothem δ$^{18}$O ages. The resulting age uncertainties at our site are approximately 3 kyrs at the beginning of the interval of study during the Glacial (ca. 140 ka) and at the end of the interval comprising the Last Glacial Inception (ca. 118 ka; Calvo et al. 2005). They are more modest (approx. 0.8 kyrs) during Heinrich Event 11 (H11) and the Last Interglacial (LIG).

2.1.2 Purifying coccolith fractions from core sediment samples

Sediments from the studied sections of core MD95-2037 consist of calcareous nanofossil oozes with a carbonate content ranging from 73 to 89%. Purified coccolith fractions were obtained for the interval of study (~100-145 ka) at a sampling resolution of ~20 cm at the beginning and end of the interval, and ~10 cm across Heinrich Event 11 and Termination II. We applied the separation protocol described in Minoletti et al., 2009 based on a cascade of microfiltering steps eased by gentle ultrasonic treatment. About 2g of core sediments were suspended in neutralized deionized water and sieved through a 20 µm nylon mesh to isolate the coarse fraction, while the <20 µm filtrate (fine fraction) was sequentially filtered through...
polycarbonate membranes of 12, 8, 5, 3 and 2 µm nominal apertures. Smear slides were prepared from the retentates of each membrane. This was done to check the granulometry and the purity of the fractions using a Zeiss Axio Imager M1 optical microscope fitted with a circular polarizer at Sorbonne Université, Paris. From the observations made on the microscope, we selected the 5-8 µm fraction where *Calciscus* spp. coccoliths made up 60 to 70% of the total content, and the 2-3 µm size class where *Gephyrocapsa* spp. coccoliths made up 60 to 85% of the content. These fractions were further run for isotopic analysis. Additional fine-scale observations were made using scanning electron microscopy (Zeiss Supra 55VP at Sorbonne Université) on both size fractions to check for the preservation state of the coccoliths (Fig. S2). For all samples, we found preservation to be good, with sparse etching and no significant overgrowth of calcite.

### 2.1.3 Isotopic analyses.

Between 50-80 µg of microseparated sample residues were collected for each level for both size fractions studied and introduced inside a Kiel IV Carbonate Device. The carbonate fraction was digested in ortho-phosphoric acid at 70°C, the resulting CO₂ gas was purified and run in a Delta V Advantage IRMS by Thermo Scientific for δ¹³C and δ¹⁸O analysis at Sorbonne Université. Values were calibrated relative to the Vienna Pee Dee Belemnite (‰ VPDB) via the NBS-19 international standard. Standard errors (1σ) are ±0.05‰ for δ¹³C and ±0.1‰ for δ¹⁸O values.

### 2.2 Quantification of the coccolith vital effects

Coccolithophores produce their coccoliths out of isotopic equilibrium relative to the environment in which they live (Dudley et al., 1986). As our working hypothesis is that the magnitude of the vital effect in fossil coccolith calcite conveys a pCO₂ signal, it is necessary to quantify this biologically-induced isotopic phenomenon in the fossil record and to study how its magnitude evolved with a changing (CO₂) environment.

#### 2.2.1 Absolute vital effects

Absolute vital effects are defined as the departure between the isotopic composition of the coccoliths and a theoretical inorganic calcite grown in the same conditions of temperature and isotopic composition of the medium. Previous studies have used the foraminiferal record to derive pseudo-inorganic values for the δ¹³C and δ¹⁸O compositions of calcite (Hermoso, 2016; Jin et al., 2018; Stoll et al., 2019; Hermoso et al., 2020b), as it is possible to hand-pick individuals and apply species-specific transfer functions between foraminifera test calcite and inorganic calcite (Pearson, 2012). It must be stressed that such a biogeochemical framework heavily relies on the fact that coeval coccolithophores and planktonic foraminifera have a similar ecology (thus record the properties of the same water mass) and that foraminiferal calcite has a known and constant vital effect during the investigated time slice. Due to their relatively small vital effects and both spatially-limited and shallow living environments (Rebotim et al., 2017), foraminifera species such as *Globigerinoides ruber* and *Oribulina universa* can be regarded as adequate substrates to derive the inorganic reference. In this study, in the absence of these specific foraminifera species in the sediments,
we attempt to derive the carbon isotopic composition of inorganic calcite $\delta^{13}C_{\text{inorg}}$ (in ‰ VPDB) from the composition of *Globigerina bulloides* planktonic foraminiferal calcite (Villanueva et al., 2001) using the equation in Bemis et al. 2000:

$$\delta^{13}C_{\text{inorg}} = 0.11 \times T + 2.07 + \delta^{13}C_{\text{bulloides}} \quad (Eq. 1)$$

where $T$ is the temperature in °C and $\delta^{13}C_{\text{bulloides}}$ is expressed in ‰ VPDB. Note here that the temperature effect on the *G. bulloides* vital effect is negligible (0.71‰) over the range of temperature change (7°C) at site MD95-2037 across Termination II, when compared to the $\delta^{13}C$ fluctuations observed for the coccolith fractions (see Results section). The minimal 3‰ offset between *G. bulloides* and inorganic calcite $\delta^{13}C$ is among the largest measured for foraminifera species, which studies attribute to higher metabolic rates (Kahn and Williams, 1981; Bemis et al., 2000).

On the other hand, the oxygen isotope vital effect for *G. bulloides* is more restricted and is not dependent on temperature, with a reported value relative to inorganic calcite (sensu Kim and O’Neil 1997) equal to -0.52‰ (Bemis et al., 1998). We therefore calculated the inorganic reference $\delta^{18}O_{\text{inorg}}$ as follows:

$$\delta^{18}O_{\text{inorg}} = \delta^{18}O_{\text{bulloides}} + 0.52 \quad (Eq. 2)$$

with $\delta^{18}O_{\text{inorg}}$ and $\delta^{18}O_{\text{bulloides}}$ both expressed in ‰ VPDB.

From the above, we can tentatively calculate the absolute vital effects of coccoliths:

$$\Delta^{13}C_{\text{abs}} = \delta^{13}C_{\text{coccolith}} - \delta^{13}C_{\text{inorg}} \quad (Eq. 3)$$

and

$$\Delta^{18}O_{\text{abs}} = \delta^{18}O_{\text{coccolith}} - \delta^{18}O_{\text{inorg}} \quad (Eq. 4)$$

with $\delta^{13}C_{\text{coccolith}}$ and $\delta^{18}O_{\text{coccolith}}$ the isotopic compositions of either the *Gephyrocapsa*-rich 2-3 µm or the *Calcidiscus*-rich 5-8 µm fractions (in ‰ VPDB).

### 2.2.2 Differential vital effects.

Coccolithophore cell size exerts a major control on the expression and magnitude of vital effects in coccolithophore organic compounds (alkenones) and calcite biominerals (coccoliths) (Popp et al., 1998; Hermoso, 2014). Studies of fossil coccoliths, together with results from numerical and culture experiments (Bolton and Stoll, 2013; Hermoso et al., 2016a; McClelland et al., 2017; Hermoso et al., 2020b), have shown that the degree of utilization of carbon from the internal pool tends to become more uniform between large and small cells when carbon limitation decreases in the environment, as does their composition in $\delta^{13}C$ and $\delta^{18}O$. Under replete ambient CO$_2$ concentrations, the demand in carbon for the metabolism is satisfied by the supply
also for the largest species, despite their smaller surface-to-volume ratio. Thus, between carbon limited- and carbon-replete conditions, the magnitude of the vital effect of a coccolith is a function of the concentration of CO$_2$ and of its size.

We quantify these differential vital effects (noted as the $\Delta_{\text{small-large}}$ notation) as the isotopic offsets between coccoliths differing in size, a measure directly linked to the size of the cell that produced them (Henderiks, 2008). Here, they are calculated as the difference in $\delta^{13}$C and $\delta^{18}$O compositions between the Gephyrocapsa-rich 2-3 $\mu$m and the Calcidiscus-rich 5-8 $\mu$m fractions.

$$\Delta^{13}C_{\text{small-large}} = \delta^{13}C_{\text{Gephyrocapsa-rich}} - \delta^{13}C_{\text{Calcidiscus-rich}} \quad (\text{Eq. 5})$$

and

$$\Delta^{18}O_{\text{small-large}} = \delta^{18}O_{\text{Gephyrocapsa-rich}} - \delta^{18}O_{\text{Calcidiscus-rich}} \quad (\text{Eq. 6})$$

with all fraction $\delta^{13}$C and $\delta^{18}$O expressed in ‰ VPDB.

A foremost advantage of using an isotopic offset between two “coccolith” signals is that it is not influenced by the possible biases introduced by the calculation of the isotopic composition of inorganic calcite (including temperature and the possible vital effects affecting foraminifera $\delta^{18}$O and $\delta^{13}$C). Therefore, only the measurement of the isotopic composition of two coccolith fractions of distinct sizes is needed.

### 2.3 Input CO$_2$ values used for our calibration of the coccolith vital effects

The surface ocean is in constant chemical exchange with the atmosphere with respect to CO$_2$. When the air and the ocean are at equilibrium, aqueous CO$_2$ concentrations can be computed directly from atmospheric pCO$_2$ via Henry’s Law, provided that sea surface temperatures (SSTs) and salinity are known (Zeebe and Wolf-Gladrow, 2001). We used the pCO$_2$ data from the Antarctic ice cores (Bereiter et al. 2015) to calculate equilibrium concentrations in aqueous CO$_2$ for site MD95-3037 over Termination II in the mixed-layer water mass, where coccolithophores thrive (Winter et al., 2002). SST estimates are taken from the alkenone-derived UK$_{37}^\mathrm{r}$ record for the core by Calvo et al., 2001, which documents an increase from 13 to 21°C across Termination II. Salinity estimates at our site were obtained following the method of Gray and Evans 2019 by scaling the maximum salinity changes (around $+1.5$ psu in the glacial ocean relative to modern salinity at our site) (De Vernal et al., 2005) with the sea level curve published by Spratt and Lisiecki, 2016. Salinity varies from 34.5 during the glacial to 36 during the interglacial. Calculations of the concentration of aqueous CO$_2$ were made using the “seacarb” package in R (https://CRAN.R-project.org/package=seacarb) with $K_1$ and $K_2$ constants from Lueker et al., 2000. Error estimates (1SD) for [CO$_2$] were obtained by running 10,000 Monte Carlo simulations with the following uncertainties: Vostok pCO$_2$ $\pm$ 3 ppm (Petit et al., 1999), and a conservative estimate of salinity of $\pm$ 1 psu. We considered a $\pm$ 1.2°C error on SST estimates (Conte et al., 2006).

The UK$_{37}^\mathrm{r}$ paleothermometer is overall considered to yield reliable estimates of past surface sea surface temperatures (Herbert, 2003). An overestimation of UK$_{37}^\mathrm{r}$-derived SSTs might arise in particular when C$_{37:4}$ alkenone abundances exceed 5%, which
generally occurs in locations with freshwater inputs (Calvo et al., 2002). Above this threshold, the UK37°-SST relationship breaks down. Here, while C37:4 alkenones are indeed found in larger concentrations during glacial than during interglacials at the nearby U1313 site, their concentration does not exceed 5% (Stein et al., 2009).

3. Results & Discussion

3.1 Size controls on coccolith vital effects

The relatively small coccoliths of the *Gephyrocapsa*-dominated 2-3 µm fractions exhibit δ¹³C values that span from -0.14 to 1.70‰ (Fig. 2). Larger coccoliths from the *Calcidiscus*-dominated 5-8 µm fractions exhibit more negative (=lighter) δ¹³C compositions ranging from -1.50 to -2.36‰. This 1.5 to 3‰ difference in δ¹³C between the isotopic compositions of the small versus the large coccoliths confirms previously observed biogeochemical data from culture experiments between *Gephyrocapsa* and *Calcidiscus* strains (Ziveri et al., 2003; Hermoso, 2014), and the observations made in sedimentological studies (Bolton and Stoll, 2013).

For the oxygen isotope system, the δ¹⁸O composition of the *Gephyrocapsa* fraction ranges from 0.07 to 3.47‰, while that of the *Calcidiscus* fraction is consistently more negative across the interval, ranging from -1.01 to 1.70‰. As for the carbon system, this 1.5‰ offset is within the range of the isotopic differences between cultured *Gephyrocapsa* and *Calcidiscus* coccoliths, where the δ¹⁸O of *Gephyrocapsa* coccolith calcite is typically 1 to 2‰ more positive than inorganic calcite, and 2 to 3‰ more positive than *Calcidiscus* coccolith calcite (Ziveri et al., 2003; Candelier et al., 2013; Hermoso et al., 2016a).

Culture and numerical experiments have attributed the large isotopic difference in both carbon and oxygen systems between *Gephyrocapsa* and *Calcidiscus* coccoliths to the different strategies of carbon acquisition between coccolithophore cells of different sizes. In the carbon system on the one hand, small cells are less sensitive to limitations in the diffusive supply of CO₂ to the cell due to a relatively larger surface-to-volume ratio than larger cells (Popp et al., 1998; Burkhardt et al., 1999). This enables small cells to fix more carbon through photosynthesis, and in turn, they produce coccoliths from a remaining internal carbon pool with a more positive (“heavier”) δ¹³C composition than larger cells (McClelland et al., 2017). On the other hand, the factors driving the array of oxygen vital effects amongst strains are not so well constrained. There is compelling evidence that coccoliths are isotopically mineralized from HCO₃⁻ ions that are pumped from the intracellular carbon pool into the coccolith vesicle (Nimer et al., 1994; Brownlee and Taylor, 2004). It has been hypothesized that the degree of equilibration between CO₂ and water molecules inside the intracellular carbon pool sets the amplitude of the coccoliths’ vital effect (Hermoso et al., 2016a). Hence small cells, which calcify more quickly than large cells, source HCO₃⁻ that has maintained the original “heavy” δ¹⁸O composition of the CO₂ diffused through the cell membrane, and ultimately produce coccoliths with a more positive δ¹⁸O than large cells.
Overall, coccolith δ¹³C and δ¹⁸O across Termination II confirm the size-dependence of isotopic signals observed in culture and numerical experiments. In this next part, we will try and establish how these isotopic compositions compare to the inorganic reference computed from the isotopic composition of foraminiferal tests in the sedimentary record, since, in culture and numerical experiments, the magnitude of coccolith vital effects has been tied to the degree of carbon limitation in the medium (Rickaby et al., 2010; Hermoso, 2014; Hermoso et al., 2020a).

3.2 Controls on absolute coccolith vital effects

As shown in Fig. 3, both coccolith size fractions exhibit very negative δ¹³C compositions relative to the computed values for inorganic calcite. The small 2-3 µm coccolith fraction lies 1 to 3‰ below inorganic calcite, while the offset is around -5‰ for the larger 5-8 µm fraction (Fig. 3). This offset is reduced in the oxygen system: The 2-3 µm fraction has a δ¹⁸O composition close to that of inorganic calcite (an approximate +0.2‰ offset), while the 5-8 µm fraction exhibits a mean +1.3‰ offset relative to inorganic calcite (Fig. 3).

Taking the foraminifera-derived inorganic reference at face value, it thus appears that the magnitude of Δ¹³Cabs for both size fractions is much larger than those observed for cultured coccoliths. In culture experiments, Gephyrocapsa typically displays an absolute vital effect in carbon and oxygen between -1 and 0‰ and between 1.0 and 1.3‰, respectively. Calcidiscus coccolith calcite typically lies 0.5 to 2‰ below inorganic calcite in both carbon and oxygen systems (Hermoso, 2014; Hermoso et al., 2016b; McClelland et al., 2017). We suggest that these differences result from the calculation of the inorganic reference from foraminifera isotopic records. Most empirical equations from culture experiments linking the isotopic composition of foraminifera tests to that of inorganic calcite are tested under changing medium temperature (Bemis et al., 2000, 1998). In the present study specifically, the use of the isotopic composition of Globigerina bulloides to estimate the δ¹³C and δ¹⁸O of inorganic calcite is additionally hindered by the large uncertainties surrounding this foraminifera’s vital effect and its life cycle within the water column. G. bulloides indeed has one of the largest vital effects among foraminifera: The isotopic composition of its test lies respectively 4‰ and 0.5‰ below that of inorganic calcite in the carbon and oxygen systems in the surface ocean (Kahn and Williams, 1981), an array of values consistent with results from culture experiments (Bemis et al., 1998, 2000) (Fig. 3). The -4‰ offset in the carbon system is however reduced to 2‰ at a 100 m depth where this species thrives (Kahn and Williams, 1981). Similarly, the offset in the oxygen system varies from 0.5‰ to 1.5‰ in the first 100 m (Kahn and Williams, 1981). As large uncertainties persist as to the calcification depth of this foraminifera, which migrates down the water column during its life cycle (Blanc and Bé, 1981), we are unable to derive a reliable and accurate measurement of the isotopic composition of inorganic calcite from G. bulloides. Thus overall, we deem the uncertainties surrounding the computation of coccolith vital effects too large to interpret their changes across the interval.
3.3 Controls on differential coccolith vital effects

Having highlighted the different biases affecting the interpretation of absolute vital effect results in this study, we choose to focus the rest of our interpretations on the differential vital effects, which do not require the computation of an inorganic reference. Several studies in the geological record (Bolton and Stoll, 2013; Tremblin et al., 2016; Hermoso et al., 2020b) indeed suggest their variations carry an environmental signal, which we propose to test and quantify below.

3.3.1 Covariation of differential vital effects across the interval of study

In this study, coccoliths from the *Gephyrocapsa* 2-3 μm fraction exhibit more positive vital effects for both carbon and oxygen than the larger coccoliths throughout the interval (a mean of 2.8‰ and 1.5‰, respectively; Fig. 3). This, as detailed above, is within the range of the vital effect differences observed in culture experiments between *Gephyrocapsa* and *Calcidiscus* strains. Interestingly, the isotopic offset between both size fractions is not constant through time. In detail, the largest offset for both carbon and oxygen vital effects is found at the beginning of the interval in the glacial ocean (at 3.81 and 2.38‰ respectively). Both Δ13Csmall-large and Δ18Osmall-large generally decrease across the deglaciation. They reach a minimum during the cold Heinrich Event 11 (1.57 and 0.68‰, respectively). Relatively steady during the Last Interglacial, the Δ13Csmall-large and Δ18Osmall-large increase at the onset of the last glaciation. Over the entire interval, Δ13Csmall-large and Δ18Osmall-large display a regression coefficient (r²) of 0.80 and a p-value < 10⁻⁴: When the difference in δ¹³C between small and large coccolith size fractions increases, the offset in their δ¹⁸O increases as well. Similar results were obtained by Bolton and Stoll 2013; Hermoso et al. 2020 in the fossil record in two case studies spanning intervals of the Miocene. They argue that an environmental parameter, namely CO₂, is at the origin of the variations of both carbon and oxygen differential vital effects.

3.3.2 Controls of CO₂ on carbon and oxygen differential vital effects

Coccolithophores rely on available aqueous CO₂ in the surface ocean to fix carbon both through photosynthesis and calcification (Hein and Sand-Jensen, 1997; Monteiro et al., 2016). The Δ13Csmall-large and Δ18Osmall-large offsets show a statistically significant correlation with changes in the ambient CO₂ concentrations (r² = 0.44, p < 10⁻² and r² = 0.51, p < 10⁻³, respectively; Fig. 4). When the CO₂ content increases in the surface ocean, the isotopic offset between large and small coccoliths decreases. The relationship with atmospheric pCO₂ as recorded in the Antarctic ice cores is not as significant, however, with a r² of 0.27 (p = 0.02) between coccolith Δ13Csmall-large and ice core pCO₂, and a r² of 0.37 (p < 0.01) with Δ18Osmall-large (Fig. S3). The largest decoupling between atmospheric and oceanic CO₂ over the interval occurs during Heinrich Event 11. This marked cold event in the mid-latitude North Atlantic (Jiménez-Amat and Zahn, 2015; Deane et al., 2017) leads to a short-lived event of increased pCO₂ solubility and thus increased aqueous CO₂ (ca. 9 μmol.kg⁻¹). This peak in oceanic CO₂ levels, which does not appear in the global atmospheric pCO₂ record, coincides with the lowest magnitudes of differential coccolith vital effects across the interval.
Culture and numerical studies offer insight into the way that changes in CO₂ concentrations drive the variations in the difference in δ¹³C and δ¹⁸O of small versus large cells (Bolton and Stoll, 2013; Hermoso et al., 2016b; McClelland et al., 2017). In the modern ocean, phytoplankton cells experience CO₂ limitation (Hein and Sand-Jensen, 1997; Reinfelder, 2011). This is especially the case for the larger cells, which are less permeable to ambient CO₂ due to a small surface-to-volume ratio (Popp et al., 1998). Culture studies have demonstrated that when CO₂ concentrations in the culture medium increase, increasing photosynthetic carbon fixation in large cells drives their coccolith δ¹³C towards “heavier” values, thereby reducing the Δ¹³C₅₉₅ large. In the oxygen system, the reduction of Δ¹⁸O₅₉₅ large under conditions of increasing CO₂ is explained by an increase in the equilibration time of HCO₃⁻ with CO₂ in small cells, due to a larger supply of CO₂ from the environment into the intracellular pool. As a result, their coccolith δ¹⁸O evolves towards “lighter” values, similar to that of large coccoliths.

In agreement with culture results, both Δ¹³C₅₉₅ large and Δ¹⁸O₅₉₅ large scale inversely with CO₂ changes over the interval. A standard OLS regression models yields the following relationship between coccolith differential vital effects and aqueous CO₂ for aqueous CO₂ concentrations below 10 μmol.kg⁻¹ (Eqs. 7 and 8, for the carbon and oxygen isotopic systems, respectively):

\[
CO₂ (±0.51) = -0.76 \times Δ¹³C_{small-large} + 10.47 \quad (Eq. 7)
\]

\[
CO₂ (±0.48) = -1.15 \times Δ¹⁸O_{small-large} + 10.04 \quad (Eq. 8)
\]

with CO₂ in μmol.kg⁻¹, and Δ¹³C₅₉₅ large and Δ¹⁸O₅₉₅ large in ‰ VPDB. Compared to the CO₂ ranges tested in culture experiments (typically 1 to 5 times present-day concentrations; Rickaby, Henderiks, and Young 2010; Lennart T. Bach et al. 2013; Hermoso, Chan, et al. 2016), the range of the increase in surface ocean aqueous CO₂ at site MD95-2037 corresponding to the atmospheric pCO₂ change throughout Termination II is arguably small in absolute values (1.9 μmol.kg⁻¹ at core MD95-2037). However, the rise from 7.3 to 9.2 μmol.kg⁻¹ represents a 25% relative increase in CO₂ over the deglaciation in a very carbon-limited ocean, and is thus likely to trigger a strong response in coccolith metabolic rates. This is evident in Fig. 5, which shows that the sensitivity of coccolith differential vital effects to the measured changes in CO₂ in our study is twice as large as the one derived from the datasets of Bolton and Stoll 2013 for the Late Miocene on similar coccolith size fractions (see Table S1 for details on the datasets used).

The largest change in carbon and oxygen differential vital effects occurs at the end of MIS 6, from the onset to the peak of Heinrich Event 11 centred around 132 ka. In the glacial (G) ocean, during the Last Interglacial (LIG) and the Glacial Inception (GI), differential vital effects display a variability that does not seem to correspond to a change in CO₂ levels (Fig. 4). This might be due to either i) processes other than CO₂ affecting coccolithophore calcification and photosynthesis and thus vital
effects (i.e. growth rate, carbon concentrating mechanisms) or ii) a disequilibrium between atmosphere and seawater CO₂ concentrations. These points are discussed below.

3.3.3 Impact of coccolithophore physiology changes on differential vital effects

The direct comparison of coccolith differential vital effects to CO₂ concentrations can be complicated by the influence of a number of physiological factors affecting the geochemistry of the coccolithophores. In addition to the cellular surface-to-volume ratio and supply of carbon in the form of CO₂ already mentioned above, coccolith vital effects are also dependent on growth rate (µ), and the possible presence of CO₂ concentrating mechanisms (CCMs).

At a given [CO₂], larger division rates increase the demand of the cell for carbon (Popp et al., 1998). Larger division rates might therefore increase coccolith vital effects in the absence of any change of [CO₂]. However, modeling and culture results reveal an inverse relationship linking cell size (thus surface/volume ratio) and growth rate (Huete-Ortega et al., 2012; Aloisi, 2015), which suggests that any change in growth rate is theoretically compensated by a change in cell size. If across the interval there is nevertheless a shift towards coccolithophore assemblages with larger growth rates within a given size fraction, this could reduce the efficiency of coccolithophore photosynthesis and calcification. Specifically, an increase in growth rate with no observable change in cell size could explain why Δ¹³Csmall-large and Δ¹⁸Osmall-large increase between the LIG and GI despite similar [CO₂] (Fig. 5). The alkenone concentrations available at this site over Termination II (Villanueva et al., 2001), a proxy for growth rate changes, indeed report that coccolithophore productivity varied over the interval. However, they are uncorrelated to coccolithophore differential vital effects (Fig. S3). Including alkenone concentrations in Eqs. 7 and 8 relating coccolith differential vital effects to aqueous CO₂ does not lead to better regression coefficients. Overall, this suggests that growth rate changes are not responsible for the observed discrepancies between Δ¹³Csmall-large, Δ¹⁸Osmall-large and [CO₂].

In addition to growth rate changes, the presence of CO₂ concentrating mechanisms (CCMs) can also affect the sensitivity of the vital effect to changing CO₂ concentrations. These mechanisms, which might include the excretion of carbonic anhydrase (CA) or dissolved inorganic carbon transporters (Reinfelder, 2011), increase the carbon supply, either from the environment to the cell, or from the cytoplasm to the chloroplast. Over our interval of study, the presence of CCMs in coccolithophores of both coccolith size fractions might explain why some Δ¹³Csmall-large and Δ¹⁸Osmall-large values of the glacial maximum are low, despite low ambient CO₂. Previous studies have invoked the presence of CCMs to explain why the transfer function established for the alkenone ε4-barometer is not suitable for periods with low surface ocean CO₂ levels such as glacial maxima (Stoll et al., 2019; Badger et al., 2019). Here, only a fraction of the Δ¹³Csmall-large and Δ¹⁸Osmall-large data points in the low-CO₂ surface ocean lie aside the general trend, indicating no threshold value below which coccolithophore inducible CCMs might be upregulated. This is compatible with observations from culture experiments performed on Emiliania huxleyi that report that CCMs are upregulated in the cell when [CO₂] falls below 7.5 µmol.kg⁻¹ in the medium (Bach et al., 2013). It is also compatible with results from other culture experiments which find no CCM upregulation in Gephyrocapsa cells in conditions of carbon
limitation (Nimer et al., 1997). Moreover, under the standing hypothesis, CCM upregulation must impact both photosynthesis and calcification rates in order to explain why glacial values for both $\Delta^{13}C_{\text{small-large}}$ and $\Delta^{18}O_{\text{small-large}}$ lie off the general trend, respectively. However, previous gene sequencing and modelling studies suggest that cellular CCM upregulation acts predominantly at the chloroplast level to boost photosynthesis, with little effect on calcification (Bach et al., 2013). On the whole, we deem the presence of CCMs to be unlikely across the interval.

3.4 Impact of air-sea gas exchange on $[\text{CO}_2]$ calculations

The surface ocean $\text{CO}_2$ values calculated from the ice core atmospheric $p\text{CO}_2$ records and alkenone UK37' cover two discrete ranges of carbon levels that do not overlap: A “glacial” $\text{CO}_2$ range centered around 7.5 $\mu\text{mol.kg}^{-1}$ and an “interglacial” $\text{CO}_2$ range centered around 9 $\mu\text{mol.kg}^{-1}$ (Fig. 5). Coccolith differential vital effects, on the other hand, paint a more nuanced picture over the interval, with no notable gap in values over the interval in either $\Delta^{13}C_{\text{small-large}}$ or $\Delta^{18}O_{\text{small-large}}$. Having exposed in the previous section the different uncertainties affecting coccolith differential vital effects, we review below the potential biases likely to affect the reconstruction of the $[\text{CO}_2]$ figures, and therefore the input values of our calibration.

The largest uncertainty when deriving surface ocean $\text{CO}_2$ values from atmospheric $\text{CO}_2$ arises from the chemical disequilibrium existing at the air-sea interface. In the modern ocean, surface ocean $\text{CO}_2$ concentrations might lie as high as 100 ppm yearly above atmospheric $p\text{CO}_2$ in equatorial regions or as low as -50 ppm in the Arctic (Takahashi et al., 2011). The range of air-sea disequilibrium is more restricted in the mid-latitude North Atlantic. To the north of the Azores Front, the existence of a deep winter mixed layer enables the cycling of nutrient at the surface and promotes primary productivity, leading surface $\text{CO}_2$ to be slightly lower than atmospheric $\text{CO}_2$. This disequilibrium promotes the dissolution of atmospheric $\text{CO}_2$ in the surface ocean, creating a 15 ppm yearly sink for global $p\text{CO}_2$ at site MD95-2037. Coccolith-derived $[\text{CO}_2]$ would indicate a value of ~ 0.8 $\mu\text{mol.kg}^{-1}$ (around 20 ppm) below those computed from Vostok $p\text{CO}_2$ records during the glacial inception. This is equivalent to the disequilibrium at the site in today’s “glacial inception” surface ocean. However, the existence of the 15 ppm sink cannot account for the coccolith-derived $[\text{CO}_2]$ values of the glacial maximum. Instead, when taking coccolith $[\text{CO}_2]$ at face value, surface ocean conditions exceed Vostok $p\text{CO}_2$ by 20 ppm in the glacial ocean.

There is compelling evidence that air-sea $p\text{CO}_2$ disequilibrium ($\Delta p\text{CO}_2$) varied in the past (Skinner et al., 2010; Martínez-Botí et al., 2015; Skinner et al., 2017). Data from boron isotopes, for instance, reveal that a widespread outgassing event, produced by the renewed upwelling of carbon-rich deep waters, enriched the surface ocean by as much as 60 ppm relative to the atmosphere over the last deglaciation (Shao et al., 2019). The existence of a high surface ocean $\text{CO}_2$ event at the onset of deglaciations had previously been hypothesized and put forward to explain the higher than expected $\epsilon_{\text{r}}$ measured during glacial maxima (Jasper et al., 1994). While the composite $\Delta p\text{CO}_2$ plot obtained by Shao et al. 2019 cannot account for regional disparities, the existence of an outgassing event could explain the 20 ppm excess of glacial coccolith-derived $\text{CO}_2$ relative to
the Antarctic record. An alternative hypothesis might include a weakening of the soft-tissue pump relative to the carbonate counter pump during the glacial maximum (Duchamp-Alphonse et al., 2018), leading to the formation of an oceanic pCO$_2$ source to the atmosphere. This could result from a stronger stratification of the water column during glacial maxima caused by the expansion of polar (melt)waters as low as 50°N (Villanueva et al., 1998; Pflaumann et al., 2003). The poor renewal of surface ocean nutrient concentration during the glacial maximum (Skinner et al., 2010), together with low glacial SSTs, may have weakened the biological pump. At the same time, both modelling and sedimentological results suggest that coccolithophore calcification spiked during glacial maxima in the midlatitude North Atlantic (Beaufort et al., 2011; Omta et al., 2013). This may have strengthened the carbonate counter pump, and made the mid-latitude North Atlantic glacial ocean a weak source for atmospheric carbon immediately before the deglaciation.

A change in the value of air-sea disequilibrium over the deglaciation can thus provide a likely explanation for the discrepancies between the ice core pCO$_2$ and coccolith vital effect records across the deglaciation. This bias is common to the different pCO$_2$ proxies calibrated using ice core data, because the extent of the change in the disequilibrium, which remains difficult to quantify, can, in all likelihood, represent up to half of the atmospheric pCO$_2$ changes across a deglaciation.

4 Conclusions

The paleobiogeochemical records presented in this study throw light on the nature of the factors forcing both the isotopic composition of the coccoliths and the magnitude of the vital effects in relatively small versus large coccoliths. We take advantage of the major and well constrained pCO$_2$ rise that occurs over the Penultimate Deglaciation between 140 and 130 kyrs to study the response of the differential coccolith vital effects to ambient CO$_2$ levels. We validate results from previous culture and numerical experiments, in that the difference between the isotopic composition between small and large coccolith size fractions responds to a forcing exerted by the availability of CO$_2$, which sustains both cellular photosynthesis and calcification. We confirm the potential of the differential coccolith vital effects ($\Delta^{13}$C$_{\text{small-large}}$ and $\Delta^{18}$O$_{\text{small-large}}$) to be used as proxies for past CO$_2$ concentrations and provide a new quantitative biogeochemical framework transferable to low CO$_2$ periods, such as Pleistocene glacial-interglacial cycles. This works sets the foundations for the use of coccolith differential vital effects to extend the ice core pCO$_2$ records into Quaternary time periods of particular interest. This includes target intervals such as the Mid-Pleistocene Transition (ca. 1250-700 ka), over which no ice-derived CO$_2$ record exists, and during which variations in atmospheric pCO$_2$ concentrations are believed to have caused the changes of the glacial-interglacial pace from 40 to 100-kyr-cyclicity.

440 Data availability

The data used will be available from the Pangaea data repository upon acceptance of the publication.
Author contributions

M.H. and F.M. designed research; C.G. prepared samples; C.G., F.M., F.B. and M.H. analyzed data; and C.G. wrote the paper with inputs from F.M., F.B. and M.H.

Competing interests

The authors declare that they have no conflict of interest.

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Figure 1: Location and hydrological settings of core MD95-2037. The core lies north of the Azores Current (AC) and the associated Azores Front (AzF), a branch of the North Atlantic Current (NAC). It is located in the transitional waters delimited to the north by the Portugal Current (PC). Site location overlays a map of mean annual $\text{ApCO}_2$ (in ppm, Takahashi et al 2011) compiled with the Ocean Data View software (Schlitzer, 2018, http://odv.awi.de). Regions with positive (negative) values are sources (sinks) for atmospheric CO₂.
Figure 2: Deglacial changes in the isotopic composition of coccolith size fractions, sea surface temperatures and aqueous CO$_2$ concentrations. Panel A: the aqueous CO$_2$ curve is derived from the ice core pCO$_2$ (Bereiter et al., 2015) and sea surface temperature (SST) records from Calvo et al., 2001 (see Methods section). Panel B: changes in coccolith δ$^{13}$C and δ$^{18}$O per size fraction considered across the interval. Also reported are the values of the isotopic composition of foraminifera G. bulloides calcite (Villanueva et al, 2001) from which we tentatively derived values for the δ$^{13}$C and δ$^{18}$O of inorganic calcite (see Methods section). Age uncertainties over the interval of study are shown (bottom grey points).
Figure 3: Expression of the coccolith vital effects, both absolute (A) and differential (B), across Termination II. A: the magnitude of the coccolith absolute vital effect seems to be dependent on coccolith size. Arrows in panel A represent the differential vital effects represented in the associated panel B: the differential vital effect in both carbon and oxygen isotopes is less marked during the interglacial with high aqueous CO$_2$ concentrations than during the glacial maximum.
Figure 4: Scatter plots of the differential vital effects in carbon and oxygen vs. aqueous CO$_2$ concentration. $\Delta^{13}$C$_{\text{small-large}}$ and $\Delta^{18}$O$_{\text{small-large}}$ correlate with changes in the ambient CO$_2$ concentrations ($R^2 = 0.44$, $p < 10^{-2}$ and $R^2 = 0.51$, $p < 10^{-3}$ respectively). Data points are colored according to the time period considered: the glacial maximum (green), Heinrich Event 11 (orange), the interglacial (red) and glacial inception (black). Inset are the surface ocean CO$_2$ concentrations across the deglaciation (calculations are detailed in the Material and Methods section).
Figure 5: Coccolith differential vital effect sensitivity to changes in aqueous CO$_2$ since the Miocene. Differential vital effects are more important in the Late Pleistocene and modern-day ocean than during the Miocene. The correlation between aqueous CO$_2$ and differential vital effects in carbon ($R^2 = 0.71$) or oxygen ($R^2 = 0.73$) suggests that an increase in carbon limitation might be responsible for the observed change. Coccolith vital effect datasets are from Bolton and Stoll 2013 for the Miocene and Pliocene, and Candelier et al. 2013 and Hermoso et al. 2015 for the North Atlantic core-tops. The pCO$_2$ records used to compute aqueous CO$_2$ for the Miocene and Pliocene datasets are from Y. G. Zhang et al. 2013. SST data for sites 1088 and 999 are from Herbert et al. 2016 and Seki et al., 2010 respectively. Core-top CO$_2$ concentrations were calculated using a pre-industrial pCO$_2$ of 280 ppm and SST data from WOA13 (Locarnini et al., 2013).