Oxygen isotope composition of waters recorded in carbonates in strong clumped and oxygen isotopic disequilibrium

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Abstract. Paleoenvironmental reconstructions, which are mainly retrieved from oxygen isotope (δ¹⁸O) and clumped isotope (δ⁴⁷) compositions of carbonate minerals, are compromised when carbonate precipitation occurs in isotopic disequilibrium. To date, knowledge of these common isotopic disequilibria, known as vital effects in biogenic carbonates, remains limited, and the potential information recorded by δ¹⁸O and Δ₄⁷ offsets from isotopic equilibrium values is largely overlooked. Additionally, in carbonates formed in isotopic equilibrium, the use of the carbonate δ¹⁸O signature as a paleothermometer relies on our knowledge of the paleowaters’ δ¹⁸O value, which is often assumed. Here, we report the largest Δ₄⁷ offsets observed to date (as much as −0.270 ‰), measured on microbial carbonates that are strongly linked to carbonate δ¹⁸O offsets (−25 ‰) from equilibrium. These offsets are likely both related to the microorganism metabolic activity and yield identical erroneous temperature reconstructions. Unexpectedly, we show that the δ¹⁸O value of the water in which carbonates precipitated, as well as the water–carbonate δ¹⁸O fractionation dependence on temperature at equilibrium, can be retrieved from these paired δ¹⁸O and Δ₄⁷ disequilibrium values measured in carbonates. The possibility to retrieve the δ¹⁸O value of paleowaters, sediments’ interstitial waters or organisms’ body water at the carbonate precipitation loci, even from carbonates formed in isotopic disequilibrium, opens long-awaited research avenues for both paleoenvironmental reconstructions and biomineralization studies.

1 Introduction

Oxygen isotope composition (δ¹⁸O) paired with clumped isotope composition (Δ₄⁷) of carbonate minerals is increasingly used for reconstructing paleoenvironmental or diagenetic conditions (Ghosh et al., 2006; Henkes et al., 2018; Mangenot et al., 2018a, b). The δ¹⁸O of carbonates depends on both the δ¹⁸O value of the water in which the carbonate precipitated and the precipitation temperature (Urey et al., 1951). Its use to reconstruct paleoenvironments can be combined with the new carbonate C–O “clumped isotope” abundance (Δ₄⁷) thermometer which depends only on the carbonate precipitation temperature (Ghosh et al., 2006). By combining the Δ₄⁷-derived temperatures and the carbonate δ¹⁸O value (δ¹⁸Ocarbonate), the δ¹⁸O value of the water (δ¹⁸Owater) in which the carbonate precipitated can be retrieved. However, this requires that solid carbonate and water reached isotopic equilibrium, which is often hard to prove. Conversely, carbonate precipitation in isotopic disequilibrium is commonly encountered (Affek et al., 2014; Loyd et al., 2016). Out of equilibrium, δ¹⁸O and Δ₄⁷ values are particularly known to occur in biogenic carbonates (Thiagarajan et al., 2011; Bajnai et al., 2018) – the most abundant carbonates in the sedimentary record. To date, the reasons for these isotopic disequilibria in carbonates remain largely under-constrained. While Δ₄⁷ compositions of carbonates seemed at first free of any biologically driven or mineral-specific fractionation known to affect δ¹⁸Ocarbonate compositions (Eiler, 2011), recently identified disequilibrium Δ₄⁷ values (Saenger et al., 2012; Affek, 2013; Tang et al., 2014; Burgener et al., 2018) shed new light on and help to unravel the mechanisms responsible for oxygen isotopic disequilibrium in carbonate minerals. Even though the identification of these vital effects does not prevent δ¹⁸O and Δ₄⁷ tools from being pow-
erful paleothermometers as empirical calibrations taking vital effects into account allow temperature reconstructions, it has become crucial to determine if the δ18O and Δ47 disequilibrium observed in carbonates as diverse as those found in coral reefs (Saenger et al., 2012), brachiopods (Bajnai et al., 2018), microbialites and methane seep carbonates (Loyd et al., 2016) along with speleothems (Affek et al., 2014) could be explained by oxygen isotope disequilibrium occurring in dissolved inorganic carbon (DIC) involved in carbonate precipitation. In this case, δ18O and Δ47 disequilibrium in biogenic carbonates would record information, however unavailable yet, on the physiological characteristics of carbonate-forming organisms.

In previous experiments we produced microbial calcium carbonates (Millo et al., 2012; Thaler et al., 2017) that recorded the strongest oxygen isotope disequilibrium ever identified between DIC and precipitation water (i.e., −25%o offset from δ18Ocarbonate equilibrium values). We used carbonic anhydrase (CA), an enzyme able to accelerate oxygen isotope equilibration between DIC and water via fast CO2 hydration and HCO3− dehydroyation. When CA was added to the precipitation water, the carbonate oxygen isotope compositions reached equilibrium with the precipitation water (Thaler et al., 2017). Here, we build on these experiments as they offer a unique opportunity to assess experimentally whether carbonates precipitated from DIC in disequilibrium with water also record Δ47 disequilibrium values and the type of information that is actually carried by these paired disequilibria. We later show how and to what extent this can be applied to previously published cases of oxygen isotopic offsets from equilibrium values in both biogenic and abiotic carbonates.

2 Materials and methods

2.1 Precipitation of microbial carbonates

Carbonates were precipitated at 30 ± 0.1°C using the procedure detailed in Millo et al. (2012) and Thaler et al. (2017) and summarized hereafter. The precipitation solution (initial pH = 6.0) was composed of ions added to Milli-Q® water (resitivity = 18 MΩ cm) by dissolving solutes in the following order: MgSO4·7H2O (16 mM), NaCl (80 mM), KCl (4 mM), urea (33.3 mM) and CaCl2 (40 mM). The aim was to mimic the ionic composition of groundwater (Millo et al., 2012). In experiments with CA whose δ18O results (but not the Δ47 ones) were recently published in Thaler et al. (2017), the precipitation solution was supplemented with CA at a concentration of 2 mg L−1. The precipitation solution (with or without CA) was then mixed at a volumetric ratio of 1:1 with the ureolytic soil bacteria Sporosarcina pasteurii (Fig. 1) suspended in Milli-Q® water, at a final optical density at 600 nm of 0.100 ± 0.010. For this study, 16 gastight Exetainer® vials were filled with the precipitation solution without CA in order to sacrifice them at regular time intervals (i.e., 30, 60, 120, 180 and 360 min and 24 h) and thus obtain information on the kinetics of the reaction, while reproducing the procedure followed for the experiment with CA (Thaler et al., 2017) consisting of 27 vials sacrificed every 10 to 30 min and after 24 h. The vials capped with rubber septa were filled up to the brim, i.e., without headspace, hence preventing any gaseous exchange with the atmosphere or headspace gases.

Ureolysis corresponds to two types of hydrolysis: (i) the hydrolysis of urea into ammonia (NH3) and carbonate (H2N-COOH) (H2N-CO-NH2 + H2O → NH3 + H2N-COOH), which is catalyzed by urease and is the rate-limiting step, and (ii) the rapid and spontaneous hydrolysis of carbamate into ammonia and CO2aq (H2N-COOH + H2O → NH3 + CO2aq + H2O) (Krebs and Roughton, 1948; Matsuzaki et al., 2013) or H2CO3 (H2N-COOH + H2O → NH3 + H2CO3) (Mobley and Hausinger, 1989; Krajewska, 2009).

Ureolysis completion was followed by evaluating the production of dissolved inorganic nitrogen (DIN = NH3 + NH4+). Determination of pH, DIN concentration and amount of precipitated carbonates (Fig. 2), as well as isotopic measurements, was performed for each vial to monitor their evolution as the ureolysis reaction progresses. The pH initially increased from 6.0 to 9.0 due to NH3 production by ureolysis and consecutive alkalinization of the precipitation solution (Fig. 2a). The subsequent carbonate precipitation (Fig. 2b) lowered pH to 8.6 (without CA) and 8.4 (with CA) and was followed by a second pH increase to 8.8 (without CA) and 8.7 (with CA) when carbonate precipitation stopped while ureolysis continued. At ureolysis completion, all the calcium initially present in solution (i.e., the limiting reagent) had precipitated whereas 35% to 45% of the DIC produced by ureolysis remained in solution. Carbonate precipitates, formed at the bottom and on the wall of the vials, were immediately rinsed with a few drops of pure ethanol in order to dehydrate bacteria and prevent further ureolysis, carbonate formation and/or dissolution–reprecipitation processes. Ethanol was then removed, and, prior to their collection, carbonates were dried overnight at 40°C in the vials placed in a ventilated oven equipped with desiccating beads. The majority of the carbonates precipitated in this study were composed of calcite with minor amounts of aragonite (1% to 4%), vaterite (2% to 4%) and magnesian calcite with low Mg content (Mg0.064, Ca0.936)CO3 (up to 8%) (Thaler et al., 2017).

All of the measured chemical parameters (pH, DIC, amount of solid carbonates, Ca2+ concentration, DIN) along with DIC and solid carbonate δ13C behave similarly with or without active CA (Thaler et al., 2017). It was not possible to measure Δ47 for all the precipitated carbonates due to their low amount, particularly for the tubes sacrificed at the beginning of the experiments (Table S1 in the Supplement).

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Figure 1. Scanning electron microscopy images of the bio-induced carbonates formed with CA (almost pure calcite and traces of vaterite and aragonite as determined using X-ray diffraction; Thaler et al., 2017). The fingerprints of *Sporosarcina pasteurii* cells are visible as black holes (a showing a cross section of carbonate) or as rods and indicated by white arrows.

Figure 2. Evolution of pH (a) and amount of precipitated calcium carbonate $C_{\text{CaCO}_3}$ (b) as a function of the production of dissolved inorganic nitrogen DIN ($\text{DIN} = \text{NH}_3 + \text{NH}_4^+$) by bacteria during ureolysis, without carbonic anhydrase (CA) (this study) and with CA (data from Thaler et al., 2017). Error bars account for 2 SD and are always smaller than symbols for pH values. The mineralogy of the carbonates precipitated with or without CA was determined by X-ray diffraction, indicating that solid carbonates are mostly composed of calcite (including up to 8 % of low-Mg calcite, (Mg$_{0.064}$, Ca$_{0.936}$)CO$_3$), vaterite (2 %–4 %) and aragonite (1 %–4 %) (Thaler et al., 2017). The influence of mineralogy on isotopic measurements and temperature estimates is discussed in the Supplement, Sect. S1.

2.2 $\delta^{18}$O and $\Delta_{47}$ measurements and associated uncertainties

All the isotopic analyses were made at Institut de physique du globe de Paris (IPGP, France). $\delta^{18}$O analyses were performed on carbonate powders of ca. 2 mg with a continuous-helium-flow isotope ratio mass spectrometer AP 2003 (Analytical Precision 2003, GV Instruments) coupled to a gas chromatograph column (GC-IRMS, Chrompac Column Type 99960), as described in Millo et al. (2012) and Thaler et al. (2017). External reproducibility on carbonate standards is $\pm 0.1$‰ (1 SD) and represents the uncertainty assigned to $\delta^{18}$O$_{\text{carbonate}}$ data.

The analytical procedure used for clumped isotope $\Delta_{47}$ measurements is only briefly presented here and detailed in Bonifacie et al. (2017). About 5 mg of carbonates was digested at 90°C for 20 min with 104 % phosphoric acid $\text{H}_3\text{PO}_4$ in a common acid bath. The produced gaseous CO$_2$ was purified with a manual vacuum line before introduction into a Thermo Scientific MAT 253 dual-inlet mass spectrometer. Each purified CO$_2$ gas was analyzed for its abundance in isotopologues with $m/z$ from 44 to 49 versus a working gas provided by Oztech Trading Corporation with $\delta^{13}$C $= -3.71$‰ Vienna Pee Dee Belemnite (VPDB) and $\delta^{18}$O $= +24.67$‰ Vienna Standard Mean Ocean Water (VSMOW), as determined with the international reference ma-
terial NBS19. One single $\Delta_{47}$ measurement corresponds to 70 cycles of 26 s integration time each (total integration time = 1820 s). Conventional $\delta^{18}O$ and $\delta^{13}C$ data were also acquired simultaneously with $\Delta_{47}$ measurements with this instrument (Tables S1 and S2). They are in excellent consistency with data obtained with the continuous-flow method on smaller samples (Table S1).

The $\Delta_{47}$ is calculated as a function of the stochastic distribution of the CO$_2$ isotopologues, as follows:

$$\Delta_{47} = \left( \frac{R_{47}^{\text{measured}}}{R_{47}^{\text{stochastic}}} - 1 \right) \times \frac{R_{46}^{\text{measured}} - 1}{R_{46}^{\text{stochastic}}} \times 1000, \tag{1}$$

where $\Delta_{47}$ is expressed in per mill ($\%$), and $R_{47}$, $R_{46}$ and $R_{45}$ are the abundance ratios of the masses 47, 46 and 45, respectively, relative to the mass 44 ($^{12}$C$^{16}$O$^{18}$O). $R_X^{\text{measured}}$ denotes measured ratios of the CO$_2$ sample. $R_X^{\text{stochastic}}$ values are calculated from the measured 44, 45, 46 and 47 abundance ratios. The number of isotopologues of mass 47 (mainly $^{13}$C$^{18}$O$^{16}$O, but also $^{12}$C$^{13}$O$^{16}$O and $^{13}$C$^{17}$O$^{17}$O) measured within the CO$_2$ sample extracted from the acid digestion of the carbonates is linked to the number of isotopologues of mass 63 (mainly $^{13}$C$^{18}$O$^{16}$O) within the reacted carbonate mineral (Guo et al., 2009). For the correction from $^{17}$O interferences, we used the $^{17}$O correction parameters from Brand et al. (2010), as recently recommended (Daëron et al., 2016; Schauer et al., 2016). In order to transfer the obtained raw $\Delta_{47}$ data into the absolute carbon dioxide equilibrium scale “CDES” ($\Delta_{47,CDES}$) being the $\Delta_{47}$ values of carbonates reacted within acid at 90 $^\circ$C, standards of CO$_2$ gases equilibrated at 25 and 1000 $^\circ$C and with bulk isotopic compositions covering the range of measured carbonate samples ($\delta^{17}$ values between $-5.0 \%_e$ and $+24 \%_e$) were analyzed interspaced with unknown samples (typically 15 equilibrated CO$_2$ gas analyses by discrete session of analysis, four analytical sessions in total; Table S5). For each analytical session, as recommended in Dennis et al. (2011), the $\Delta_{47}$ data were finally corrected with a fixed equilibrated gas line slope (only slightly varying from 0.0048 to 0.0062 over our analytical sessions) and an empirical transfer function (slopes varying from 1.0859 to 1.1344) based on the equilibrated CO$_2$ standards. Finally, the accuracy of our whole dataset and processing procedure was validated on carbonate reference material (i.e., IPGP-Carrara and 102-GC-AZ01), typically analyzed every two unknown samples (Table S5). The $\Delta_{47}$ values obtained at IPGP over the course of this study are $\Delta_{47,CDES} = 0.316 \pm 0.020 \%_e$ (1 SD, $n = 16$) for IPGP-Carrara and $\Delta_{47,CDES} = 0.620 \pm 0.010 \%_e$ (1 SD, $n = 18$) for 102-GC-AZ01. Those values are indistinguishable from the values obtained at IPGP over 4 years of analyses on the same instrument ($n > 300$) or previously reported by other laboratories (Daëron et al., 2016).

### 2.3 Temperature estimates and associated uncertainties

Apparent temperatures obtained from oxygen isotope compositions were calculated based on the measured $\delta^{18}O_{\text{carbonate}}$ values of both the precipitated carbonate and the precipitation water in each experimental vial (Table S1) and using the equation of oxygen isotopes’ fractionation between calcite and water from Kim and O’Neil (1997). Apparent temperatures issued from clumped isotope compositions were calculated from $\Delta_{47,CDES}$ data using the composite universal $\Delta_{47}$–$T$ calibration (Eq. 3 from Bonifacie et al., 2017, with $T$, the temperature). It is noteworthy that our main observations and conclusions do not change if other calibrations to temperature are used for $\delta^{18}O$ and/or $\Delta_{47}$ (e.g., Coplen, 2007; Kelson et al., 2017) (see also Table S3). For both proxies, the uncertainties on temperature estimates reported here correspond to the standard deviation of the mean of replicated isotopic measurements of the same powder propagated in the calibration equation (but the actual errors on the calibration themselves are not considered). Note that the long-term external reproducibility on homogeneous calcite reference materials found in this study (i.e., $\pm 0.020 \%_e$, 1 SD) is used for samples with only one measurement or with 1 SD lower than 0.020 $\%_e$ (Tables S1 and S5, Sect. S1 in the Supplement).

For the temperature ($T$) derived from the $\Delta_{47}$ data, we chose the calibration determined by Bonifacie et al. (2017) as it integrates a consequent number of data ($n > 300$), whose statistical weight has been properly considered and covers a wide temperature range (from 1 to 350 $^\circ$C), three characteristics that were recently shown by several teams as governing the precision on $\Delta_{47}$–$T$ calibration equations (Bonifacie et al., 2017; Kelson et al., 2017; Fernandez et al., 2017). Importantly, this calibration covers the high apparent temperature ranges reported here (i.e., low $\Delta_{47}$ values) allowing us to avoid loss of precision and accuracy when extrapolating to temperature ranges that have not been experimentally investigated. Finally the Bonifacie et al. (2017) calibration has been checked independently with other methods (Mangenot et al., 2017; Dassiè et al., 2018) on the range of temperatures ($\sim 30$ to 96 $^\circ$C) where most available calibrations diverge and/or are not well constrained. Indeed, these studies report excellent consistencies: (i) between $T$ $\Delta_{47}$ and homogenization temperatures from fluid inclusion microthermométrie (Mangenot et al., 2017) and (ii) between the $\delta^{18}O_{\text{water}}$ values directly measured in fluid inclusions by cavity ring-down spectroscopy and those calculated from combined $T$ $\Delta_{47}$ and $\delta^{18}O_{\text{carbonate}}$ of the host mineral (Dassiè et al., 2018). Though we recognize that the normalization to carbonate standards presented in Bernasconi et al. (2018) might become commonly used by the community in the future (i.e., with the ongoing intercomparison InterCarb project), we preferred not to use this correction frame here because not enough of the four carbonate standards proposed by Bernasconi et al. (2018) were run together with our samples ($n = 14$ runs in total of
ETH1, ETH2, ETH3 and ETH4 standards; Table S5), and such a normalization method will then introduce larger uncertainty than the normalization we performed with the large number of equilibrated gases ran daily together with our unknowns \( (n = 104 \) equilibrated gas; Table S5 – note also 33 secondary carbonate standards 102-GC-AZ01 and IPGP-Cararra, also run in other IPGP studies and some other laboratories). Also remarkably, \( \Delta_{47} \) values obtained here on the four ETH carbonate standards are all systematically higher than values reported in Bernasconi et al. (2018) (Table S4). Though the reason for this positive offset is still unclear, it is noteworthy that positive offsets are also observed when compiling other recent published values (Table S4; Daéron et al., 2016; Schauer et al., 2016; Fiebig et al., 2019).

3 Results and discussion

3.1 \( \Delta_{47} \) and \( \delta^{18}O \) compositions of microbial carbonates can present strongly correlated vital effects

We performed \( \Delta_{47} \) measurements on (i) microbial carbonates precipitated without CA by faithfully replicating the experiment detailed in Thaler et al. (2017) and (ii) microbial carbonates precipitated in the presence of CA remaining from these experiments. These calcium carbonates were precipitated as the result of microbially driven hydrolysis of urea into DIC and ammonia (Millo et al., 2012). They constitute a reliable model for carbonate precipitation triggered by enzymatic production or transport of DIC, as is the case for micro- and macro-skeletal carbonates common in the Phanerozoic and for microbially mediated carbonates since the Precambrian.

Without CA, the isotopic values of the very first carbonate precipitates present strong isotopic offsets from equilibrium values. The \( \Delta_{47} \) offset to equilibrium starts from \(-0.270\%e \) (the largest \( \Delta_{47} \) offset ever measured in solid carbonates), and the offset to equilibrium reaches \(-24.7\%e \) for \( \delta^{18}O_{\text{carbonate}} \) (Fig. 3 and Table S1). Both \( \Delta_{47} \) and \( \delta^{18}O_{\text{carbonate}} \) absolute values then increase as ureolysis progresses, reducing offsets from equilibrium values to \(-0.179\%e \) for \( \Delta_{47} \) and \(-15.7\%e \) for \( \delta^{18}O_{\text{carbonate}} \). In the presence of CA, the trends observed for the \( \Delta_{47} \) and \( \delta^{18}O_{\text{carbonate}} \) values are similar, but the offsets from equilibrium are drastically reduced (down to \(-0.027\%e \) for \( \Delta_{47} \) and \(-1.4\%e \) for \( \delta^{18}O_{\text{carbonate}} \) at the end of the experiment; Fig. 3), hence attesting for ongoing isotopic equilibration of DIC with water by CA enzymatic activity prior to and during carbonate precipitation. The comparable behavior of \( \Delta_{47} \) and \( \delta^{18}O_{\text{carbonate}} \) values with respect to CA suggests that both disequilibria are inherited from the \( \delta^{18}O \) and \( \Delta_{47} \) signatures of the DIC generated by the biological activity.

3.2 \( \Delta_{47} \) and \( \delta^{18}O_{\text{carbonate}} \) disequilibrium originates from the metabolic production of DIC

Here, we discuss the potential processes known to generate \( \delta^{18}O \) and \( \Delta_{47} \) isotope fractionations during carbonate precipitation and we identify the main mechanism explaining our paired \( \Delta_{47} \) and \( \delta^{18}O_{\text{carbonate}} \) disequilibria. The relatively high precipitation rate \( (R) \) in our experiments \( (\log R = -3.95 \text{ mol m}^{-2} \text{s}^{-1}; \text{Thaler et al., 2017}) \) can only account for an oxygen kinetic isotope fractionation (KIF) of about \( 1 \%e \) to \( 2 \%e \) for \( \delta^{18}O \) values (Watkins et al., 2013), while the oxygen isotope disequilibrium recorded in our carbonates reaches \(-24.7\%e \). Degassing of CO\(_2\), known to fractionate DIC oxygen isotopes (Affek and Zaarur, 2014), can be ruled out as there is no gas phase in our experiments (see Sect. 2.1). Any potential kinetic fractionation due to DIC diffusion (Thiagarajan et al., 2011) is also unlikely as precipitation occurred on the bacterial DIC-producing cells, as highlighted by scanning electron microscopy showing bacterial cells trapped within and at the surface of carbonate crystals (Fig. 1). Accordingly, the large offsets from equilibrium values observed for both \( \Delta_{47} \) and \( \delta^{18}O \) in our microbial carbonates can only result from (i) a KIF induced by CO\(_2\) hydration or hydroxylation into HCO\(_3^-\) (but only if ureolysis produces CO\(_2\) rather than H\(_2\)CO\(_3\), which has not been established yet; Matsuzaki et al., 2013) or (ii) a metabolic isotope signature of the DIC produced by the bacteria, inherited from the initial isotopic composition of urea and/or due to a KIF introduced by the urease enzyme. CO\(_2\) hydration and hydroxylation lead to the formation of HCO\(_3^-\), with two oxygen atoms coming from CO\(_2\) and the third one from H\(_2\)O (hydration) or OH\(^-\) (hydroxylation). The \( \delta^{18}O_{\text{HCO}_3^-} \) value can then be estimated using a simple mass balance calculation (Lérollé et al., 1990; Usdowski et al., 1991). The newly formed HCO\(_3^-\) is depleted in \( ^{18}O \) compared to the reacting CO\(_2\) because of the incorporation of oxygen coming from H\(_2\)O or OH\(^-\), both depleted in \( ^{18}O \) relative to \( ^{16}O \) in contrast to CO\(_2\) (Green and Taube, 1963; Beck et al., 2005). Such a low \( \delta^{18}O_{\text{HCO}_3^-} \) value, several per mill lower than the equilibrium one, can then be preserved in the calcium carbonate if precipitation occurs shortly after CO\(_2\) hydration and hydroxylation and before the full equilibration with water (Rollion-Bard et al., 2003). Regarding clumped isotopes, ab initio calculations predict that the fractionation associated with CO\(_2\) hydration and hydroxylation increases the relative abundance of \( ^{13}C-^{18}O \) bonds and thus the \( \Delta_{47} \) value (Guo, 2009). Even though this predicted fractionation trend has previously been used to explain several datasets for which CO\(_2\) hydroxylation was assumed to occur prior to carbonate precipitation (Tripati et al., 2015; Spooner et al., 2016), such a tendency can only be validated using data acquired on carbonates for which CO\(_2\) hydration and hydroxylation are demonstrated. This is the case of (i) hyperalkaline travertines (Falk et al., 2016) even though part of the reported kinetic isotope fractionation
Figure 3. Strong δ18O and Δ47 disequilibria recorded in microbial carbonates as shown by δ18OCarbonate (a) and Δ47 (b) values of calcium carbonates (CaCO3) precipitated during bacterial ureolysis at 30°C (with and without carbonic anhydrase, CA; open and solid symbols, respectively) as a function of carbonate accumulation (Ccalcite). Black symbols correspond to samples for which both Δ47 and δ18O measurements were performed. The grey horizontal lines are equilibrium δ18OCarbonate and Δ47 values at 30°C for calcite following Bonifacie et al. (2017) and Kim and O’Neil (1997) calibrations, respectively. Uncertainties (1 standard deviation, 1 SD) are smaller than the symbol for Δ47.(a) Measurements were performed. The grey horizontal lines are equilibrium δ18OCarbonate and Δ47 values at 30°C for calcite following Bonifacie et al. (2017) and Kim and O’Neil (1997) calibrations, respectively. Uncertainties (1 standard deviation, 1 SD) are smaller than the symbol for Δ47. 

in isotopic equilibrium with water, the Δ47 values affected by CO2 hydration and hydroxylation recorded in calcium carbonates should be higher than the equilibrium value, while our microbial carbonates show Δ47 values lower than equilibrium. Thus, we conclude that our low Δ47 values measured in carbonates can only be explained by a metabolic source effect. In our case it corresponds to the ureolytic production of DIC, either directly as H2CO3 or as CO2, with a Δ47 value low enough to compensate for any potentially succeeding increase due to the KIF associated with CO2 hydration and hydroxylation. Nonetheless, the slow but continuous increase observed in our experiment without CA for both Δ47 and δ18OCarbonate values more likely reflects ongoing equilibration of DIC oxygen isotopes with water at a slow rate.

Our results highlight that the isotope clumping proceeds continuously as C–O bonds are breaking and reforming in the DIC, allowing oxygen isotopes (16O, 17O and 18O) to be redistributed between H2O, OH−, H2CO3, HCO3− and CO32− species via H2O/OH− attachment to CO2 and detachment from HCO3−. In the experiment with CA, both Δ47 and δ18OCarbonate simultaneously reach values close to equilibrium, and without CA both Δ47 and δ18OCarbonate values increase simultaneously. This coevolution corroborates former observations of comparable kinetics for clumped isotopes and δ18O equilibration between DIC and water or CO2 and water once δ13C is equilibrated (Affek, 2013; Clog et al., 2015). This principle has been used to correct for the disequilibrium fractionation factor in speleothems (Affek et al., 2008).

3.3 Erroneous yet comparable temperatures reconstructed from disequilibrium Δ47 and δ18O values in carbonates

Apparent temperatures were calculated from disequilibrium Δ47 values obtained in the experiment without CA using the calibration of Bonifacie et al. (2017). Ranging from 198±21 to 115±8°C (Fig. 4), they are at odds with the actual precipitation temperature of 30±1°C (see Sect. 2.1). This shows that when carbonates precipitate from DIC in oxygen isotope disequilibrium with water, the abundance of 13C–18O bonds in carbonates does not correlate with precipitation water temperature. Conversely, the temperatures reconstructed from the Δ47 values of carbonates precipitated in the presence of CA, ranging from 47±6 to 39±2°C, are much closer to the actual precipitation temperature. Interestingly, the apparent temperatures reconstructed using Kim and O’Neil (1997) calibration from the δ18OCarbonate and δ18OWater values of the same samples show comparable offsets from the actual temperature in both experiments without CA (from 218±2 to 139±1°C) and with CA (from 39±1 to 37±1°C) (Fig. 4). Practically, this implies that similar temperatures calculated from both carbonate Δ47 and δ18OCarbonate values (in a case where the precipitation water δ18O can be determined) can neither constitute evidence against O isotope disequilibrium nor confirm that this is the true precipitation temperature.
3.4 Δ47 and δ18Ocarb paired disequilibria record the δ18O of the water in which the carbonates precipitated

The fact that both Δ47 and δ18Ocarb values permit us to calculate similarly evolving apparent temperatures along the (dis)equilibration profile recorded in carbonates as the experiment proceeds indicates that the δ18Ocarbonate, δ18Owater, Δ47 and apparent temperature values are all together linked. In a diagram of Δ47 versus δ18Ocarb, all of our data align, irrespective of the fact that they are in strong isotopic disequilibrium or close to equilibrium (Fig. 5). Their alignment is fitted with what would be expected for equilibrium Δ47 and δ18Ocarb values of calcite precipitated at various temperatures from water at a given δ18Owater value. This δ18Owater value can be calculated by combining, for the same temperature, the equations of Δ47 and δ18Ocarbonate temperature calibrations from Bonifacie et al. (2017) and Kim and O’Neil (1997), respectively (Eq. 2):

$$
\delta^{18}O_{water} = \exp \left[ -\frac{18.03}{0.0422 \times 10^{10}} + 32.42 \times 10^{-3} \right] + \ln(\delta^{18}O_{carbonate} + 1000) \right] - 1000, \quad (2)
$$

Figure 4. δ18Ocarbonate and Δ47 disequilibria in microbial carbonates induce comparable biased estimates of precipitation temperature as illustrated by apparent temperatures calculated from the carbonate δ18Ocarbonate and Δ47 signatures as a function of CaCO3 accumulation. Open and solid symbols refer to the experiments with and without CA, respectively. The dashed grey line corresponds to the actual precipitation temperature. Apparent temperatures are respectively calculated from the δ18Ocarbonate and Δ47 calibrations to the temperature of Kim and O’Neil (1997) and Bonifacie et al. (2017). Reported uncertainties were calculated as the propagation of the 1 standard deviation (1 SD) error of the isotopic data in the calibration equations (Sect. 2 and Sect. S1 in the Supplement).

Figure 5. Combined δ18Ocarbonate and Δ47 disequilibria of microbial carbonates precipitated at 30°C allow reconstruction of the δ18O of the water (δ18Owater) in which they precipitate. Solid grey curves represent the calculated Δ47 and δ18Ocarbonate compositions of carbonates precipitated at oxygen isotope equilibrium from water with fixed δ18Owater values (indicated on each curve) and variable temperatures. Horizontal dashed grey lines are calculated for fixed temperatures and variable δ18Owater. The average δ18Owater value of –6.6±0.4‰ measured in our experiments is reported using the thick solid grey curve. The solid black curve was obtained using the δ18Owater calculated with Eq. (2) (–8.0±2.8‰) with its associated errors (dashed black curves).

Despite the fact that the data present a large range of offsets from equilibrium (Fig. 5), the mean δ18Owater value calculated using Eq. (2) for each combination of Δ47 and δ18Ocarbonate values measured for our carbonates is –8.0±2.8‰ (1 SD), indistinguishable (i.e., within errors) from the δ18Owater values measured in our experiments (–6.4±0.2‰ with CA and –6.8±0.2‰ without CA; Fig. 5). Note that such precision in δ18Owater values found in disequilibrium carbonates is remarkable considering that even for equilibrium carbonates at isotopic equilibrium for both δ13C and δ18O, δ18Owater can only be retrieved from paired Δ47 and δ18Ocarbonate values with a precision of ±1‰ at best (see Fig. S1 in the Supplement). This creates the promising opportunity to retrieve the δ18O of the water in which car-
bonates precipitated out of equilibrium for both $\delta^{18}O_{\text{carbonate}}$ and $\Delta_{47}$.

In order to evaluate the applicability of such an approach to other types of carbonates, Fig. 6 compiles disequilibrium paired $\delta^{18}O_{\text{carbonate}}$ and $\Delta_{47}$ data from two previously published experimental studies (Tang et al., 2014; Staudigel and Swart, 2018). These studies were chosen to further evaluate the relevancy of our $\delta^{18}O_{\text{carbonate}}$–$\Delta_{47}$ correlation because they are the only published dataset reporting full sets of measured (rather than calculated) $\delta^{18}O_{\text{water}}$, $\delta^{18}O_{\text{carbonate}}$ and $\Delta_{47}$ values, with one or both proxies showing disequilibrium, together with precipitation temperatures. A perfect knowledge (i.e., measurements and not estimates) of these four parameters is mandatory here to adequately test whether the use of our new $\delta^{18}O_{\text{water}}$ proxy could be generalized to a large diversity of carbonates. This thus precludes plotting most published $\Delta_{47}$ studies on both natural and experimental samples, in which $\delta^{18}O_{\text{water}}$ and/or temperature were not directly measured, in Fig. 6. These two datasets are also recent enough to allow the conversion of their $\Delta_{47}$ values to the currently used normalization method (i.e., the CDES absolute reference frame). It will then allow comparison with future studies, if measuring and reporting all these four parameters together becomes the rule rather than the exception in $\Delta_{47}$ studies.

Figure 6a shows paired $\delta^{18}O_{\text{carbonate}}$ and $\Delta_{47}$ values of abiotic carbonates produced at 5, 25 and 40 °C that are known to be affected by KIF due to fast precipitation and for at least two of them by KIF due to CO$_2$ hydration and hydroxylation prior to precipitation (Tang et al., 2014). Except for these two carbonate samples, the data align on a covariation curve of $\Delta_{47}$ versus $\delta^{18}O_{\text{carbonate}}$ that cannot be explained solely by temperature variation. As for our microbial carbonates obtained with or without CA, the average calculated $\delta^{18}O_{\text{water}}$ (Eq. 2; $-11.2 \pm 1.5\%$) matches within error with the measured $\delta^{18}O_{\text{water}}$ ($-9.6 \pm 0.2\%$) (Dietzel et al., 2009).

Figure 6b shows paired $\delta^{18}O_{\text{carbonate}}$ and $\Delta_{47}$ values of abiotic carbonates that were precipitated during an initial CO$_2$ degassing + DIC equilibration phase followed by solely equilibration with water at 5, 15 and 25 °C (Staudigel and Swart, 2018). During the latter equilibration phase, even though the carbonates precipitated out of isotopic equilibrium, the paired $\delta^{18}O_{\text{carbonate}}$ and $\Delta_{47}$ values align on a covariation curve of the average calculated $\delta^{18}O_{\text{water}}$ value (Eq. 2; $-3.0 \pm 1.1\%$) close to the measured $\delta^{18}O_{\text{water}}$ ($-0.65\%$). As a major outcome of this study, we thus anticipate that reliable $\delta^{18}O_{\text{water}}$ values of precipitation water can be retrieved from carbonates presenting $\Delta_{47}$ and $\delta^{18}O_{\text{carbonate}}$ values in strong disequilibrium.

Some data presented in Fig. 6 also permit us to evaluate the conditions of applicability of our approach. In Fig. 6a, the two data points deviating from the covariation curve of $\Delta_{47}$ versus $\delta^{18}O_{\text{carbonate}}$ correspond to carbonates precipitated at pH ~ 10 and 5 °C (while the others formed at pH and...
temperatures ranging from 8.3 to 9 and 5 to 40 °C, respectively) that have recorded a KIF due to CO₂ hydration and hydroxylation prior to precipitation (Tang et al., 2014). At pH = 10, CO₂ reacts at 95 % with OH⁻, and at 5 °C DIC isotopic equilibration with water takes days. To a lesser extent, the KIF induced by CO₂ hydroxylation also seems visible at pH = 9 (and 40 °C), where CO₂ reacts at 82 % with OH⁻, but DIC isotopic equilibration with water at 40 °C only takes about 15 h. As previously detailed, the direction of these isotopic offsets from equilibrium is compatible with ab initio calculations (Guo, 2009) and can be intuitively understood as follows: in carbonates derived from CO₂ hydration, the \( R^X_{\text{stochastic}} \) term used for the \( \Delta_{47} \) calculation (Eq. 1) should be strongly modified as the \( ^18 \text{O} \) concentration in OH⁻ and H₂O is lower than in CO₂, and the reaction does not add more \(^{13}\text{C} \) than what is present in CO₂. This might explain why in the case of disequilibria acquired through CO₂ hydration, the correlation between paired \( \delta^{18}\text{O} \) and \( \Delta_{47} \) disequilibria and the precipitation water \( \delta^{18}\text{O} \) is not preserved, and \( \delta^{18}\text{O}_{\text{water}} \) cannot be reconstructed by the approach proposed here. The negative slope associated with this KIF on the \( \Delta_{47} \) and \( \delta^{18}\text{O}_{\text{carbonate}} \) diagram (Fig. 6a) is nevertheless a good tool to identify CO₂ hydroxylation reactions.

In Fig. 6b, during the CO₂ degassing phase of the precipitation experiment (Staudigel and Swart, 2018), the data also deviate from the covariation curve of \( \delta^{18}\text{O} \) versus \( \Delta_{47} \). This behavior was interpreted by the authors as a decoupling between \( \Delta_{47} \) and \( \delta^{18}\text{O}_{\text{carbonate}} \) values due to variable kinetics of \(^{12}\text{C} \)-O and \(^{13}\text{C} \)-O bonding. A known difference in equilibration kinetics takes place between C and O isotopes in the carbonate system as carbon isotopes equilibrate in seconds, while oxygen isotopes necessitate minutes to hours to equilibrate among the different oxygen-bearing species (i.e., CO₂, HCO₃⁻, CO₃²⁻, H₂O, OH⁻), depending on the pH, temperature and salinity of the solution (Zeebe and Wolf-Gladrow, 2001). However, note that in that experiment, the carbon isotope compositions evolved for several hours as a result of CO₂ degassing (Staudigel and Swart, 2018). We propose here that CO₂ degassing, because it affects both C and O isotopes, modifies the \( R^X_{\text{stochastic}} \) term (in Eq. 1), thus preventing \( \Delta_{47} \) and \( \delta^{18}\text{O} \) from varying with the proportionality that allows us to retrieve the \( \delta^{18}\text{O}_{\text{water}} \) value on a covariation plot of \( \Delta_{47} \) versus \( \delta^{18}\text{O} \). Hence, as for CO₂ hydroxylation, in the case of a KIF induced by CO₂ degassing, \( \delta^{18}\text{O}_{\text{water}} \) cannot be reconstructed exclusively from disequilibrium \( \delta^{18}\text{O}_{\text{carbonate}} \) and \( \Delta_{47} \) values.

In summary, we conclude that mechanisms that can drastically change the \( R^X_{\text{stochastic}} \) term in the \( \Delta_{47} \) calculation (such as CO₂ hydroxylation and degassing) prevent \( \delta^{18}\text{O}_{\text{water}} \) reconstructions from paired disequilibrium \( \Delta_{47} \) and \( \delta^{18}\text{O}_{\text{carbonate}} \) values. Nevertheless, these mechanisms lead to peculiar types of carbonates (e.g., speleothems that form in caves from CO₂ degassing, and travertine that forms on lands where fluids and gas escape from subsurface reservoirs, for CO₂ hydroxylation) that represent only a small fraction of all the carbonates existing on Earth. We hypothesize that ureolysis, which consists in two successive steps of urea hydrolysis, an exchange reaction with the H₂O molecule from the aqueous medium, might give a DIC whose \( R^X_{\text{stochastic}} \) term in the \( \Delta_{47} \) calculation is already close to that of DIC under equilibration with the \( \delta^{18}\text{O}_{\text{water}} \). This would explain why even our most extreme out-of-equilibrium carbonates still fall close to the covariation line of \( \Delta_{47} \) versus \( \delta^{18}\text{O}_{\text{carbonate}} \) corresponding to the real \( \delta^{18}\text{O}_{\text{water}} \) value.

3.5 Toward a better understanding of body water \( \delta^{18}\text{O} \) in biomineralizing organisms

The ability to reconstruct precipitation water \( \delta^{18}\text{O}_{\text{water}} \) from disequilibrium \( \Delta_{47} \) and \( \delta^{18}\text{O}_{\text{carbonate}} \) values further allows us to examine the origin of the vital effect observed in organisms for which (i) CO₂ degassing and hydration and hydroxylation KIF can be ruled out, and (ii) only small \( \delta^{13}\text{C} \) variations are observed, thus preserving the \( R^X_{\text{stochastic}} \) term in the \( \Delta_{47} \) calculation. We hypothesize that such an approach could help to understand how \( \Delta_{47} \) and \( \delta^{18}\text{O} \) signals are affected by kinetic effects in most of the biogenic carbonates, provided that CO₂ hydroxylation or degassing does not occur prior to carbonate precipitation. This approach could thus be applied to the vast majority of sedimentary carbonates (Milliman, 1993) (i.e., microbialsites, brachiopods, bryozoans, bi-valves, foraminifera, coccoliths) and since deep time, even when \( \delta^{18}\text{O}_{\text{carbonate}} \) variations occur in the shell of the organism. Additionally, the data presented here stand as an experimental demonstration that the mechanisms controlling carbonate \( \delta^{18}\text{O} \) equilibration with water (i.e., DIC equilibration with water) also control solid carbonate \( \Delta_{47} \) equilibrium (Watkins and Hunt, 2015). This result can be used to recover information on biomineralization mechanisms. For example, in recent coccolithophorid *Emiliania huxleyi* culture experiments, the calcitic shell produced by the organism systematically yields a 2 % positive \( \delta^{18}\text{O} \) offset from equilibrium values while its \( \Delta_{47} \) values seem to faithfully record precipitation temperature (Katz et al., 2017). These coccolithophorids were grown in waters with different \( \delta^{18}\text{O}_{\text{water}} \) compositions (i.e., measured at −6.14 ‰, −5.82 ‰ and 0.65 ‰ VSMOW that are respectively seawater A, B and C in Fig. 6c). Based on our results, which demonstrate that no \( \delta^{18}\text{O} \) disequilibrium should be recorded in solid carbonates if the associated \( \Delta_{47} \) is at equilibrium, we can assume that the coccoliths precipitated at oxygen isotope equilibrium. We also calculate the actual \( \delta^{18}\text{O} \) value of the water in which precipitation took place from Eq. (2) (respectively shifted by 1.0 ± 0.2 ‰, 2.1 ± 0.4 ‰ and 1.1 ± 0.7 ‰ towards more positive values compared to the \( \delta^{18}\text{O}_{\text{water}} \) value measured for the culture medium water; Fig. 6c). This could reflect a biologically driven difference between the \( \delta^{18}\text{O} \) of body water at the precipitation site inside *E. huxleyi* and the \( \delta^{18}\text{O} \) of ambient water (i.e., the culture medium water). This hypothesis is supported by what is known about intracellular
precipitation of coccoliths performed by coccolithophorids: each coccolith forms from the accumulation of coccolithosomes, which are vesicles containing up to approximately twelve 7 nm spherical calcium-rich granular units (Outka and Williams, 1971). Water in these ~ 100 nm vesicles can be considered a finite reservoir whose isotopic composition could be modified through isotopic exchange with DIC affected by metabolic isotope fractionation. Another mechanism that could increase the δ¹⁸O value of a finite water reservoir by equilibrating it with a comparable reservoir of DIC would be the introduction of DIC systematically as CO₂. As HCO₃⁻ and CO₃²⁻ are enriched in ¹⁶O in comparison to CO₂, the CO₂ conversion to HCO₃⁻ and CO₃²⁻ at equilibrium before precipitation would pump ¹⁶O from water.

In both case scenarios, a local change in water isotopic composition requires that the water molecules’ turnover (i.e., external inputs) in these cellular organisms is slow enough. Coccolithosomes are subunits of the Golgi complex, which is a system of flat stacked vesicles concentrating a lot of membranes in a small location (Outka and Williams, 1971). It is thus plausible that in a single-celled organism, living in seawater and performing intracellular biomineralization, specific osmolarity and water circulation regulation mechanisms are occurring. It is particularly plausible in the Golgi complex, whose water content is isolated from seawater by several membranes. We thus suggest that inside coccolithosomes, coccolith precursors precipitate in equilibrium with the body water for oxygen isotopes but that the body water has a different δ¹⁸O value than the seawater, which explains the observed δ¹⁸O apparent fractionation while the Δ₄⁷ composition reflects culture temperature (Katz et al., 2017). It has already been highlighted through geochemical analysis of coccoliths that coccolithosome water has altered pH (Liu et al., 2018) and ion concentrations (Hermoso et al., 2017) in comparison to seawater. We hypothesize that the internal δ¹⁸O water would thus be another parameter controlled by the coccolithophore algae.

3.6 Ubiquity of the observed δ¹⁸O carbonate–δ¹⁸O water–Δ₄⁷–temperature covariations in both equilibrium and disequilibrium carbonates

As shown above (Fig. 5), in a diagram of Δ₄⁷ versus δ¹⁸O carbonate, disequilibrium carbonates precipitated at fixed temperature plot on the theoretical line of equilibrium carbonates precipitated with a similar δ¹⁸O water but at a different temperature. This is illustrated in Fig. 7 where the three disequilibrium data series studied in this paper (Fig. 5 for this study and Fig. 6a and b for datasets from Tang et al., 2014, and Staudigel and Swart, 2018) align with equilibrium data series. In other words, the values of the disequilibrium 1000ln α carbonate-water for oxygen isotopes (with α = δ¹⁸O carbonate+1000 δ¹⁸O water+1000) are similar to the equilibrium 1000ln α carbonate-water for any given, and independently determined, apparent Δ₄⁷ temperature (Fig. 7). In detail, our closest-to-equilibrium data recording low apparent temperatures match better with the predicted equations from Coplen (2007) and Watkins et al. (2013), recently updated (Daëron et al., 2019). This last calibration is based on carbonates from two caves where calcite precipitates extremely slowly and is thus assumed to have precipitated at equilibrium. Note that the use of these two cave samples for determining the dependence on temperature of the equilibrium 1000ln α carbonate-water relies on the assumption that constant environmental conditions, including temperature in the two caves (7.9 and 33.7°C) and the δ¹⁸O water value of the precipitation water, prevailed over the whole period of carbonate precipitation (Coplen, 2007; Kluge et al., 2014). In Fig. 7, the disequilibrium data recording high apparent temperatures (above 100°C) match better with the predicted equation of Kim and O’Neil (1997). This 1000ln α carbonate-water dependence on temperature was established on carbonates precipitated in the laboratory at well-known δ¹⁸O water and temperatures (from 10 to 40°C) but was suspected to present a small KIF due to a high precipitation rate that lowers the value of the 1000ln α carbonate-water (Watkins et al., 2013). Despite this, we used this equation to retrieve the δ¹⁸O water from our experimental carbonates, because most of them are associated with high apparent Δ₄⁷ temperatures. Coplen (2007) or Watkins et al. (2013) equations would have 2 ‰ lower values (ca. −10 ± 2 ‰ compared to −8 ± 3 ‰ calculated with the equation of Kim and O’Neil, 1997). From our results, due to our experimental conditions and the associated error in our dataset, it is not possible and not our intention to argue in favor of one of these calibrations. This however shows how crucial it is to improve knowledge on the equilibrium 1000ln α carbonate-water at both high and low temperatures in order to improve the accuracy and precision of our new proxy for reconstructing the δ¹⁸O water from which carbonates, even disequilibrium ones, precipitated.

Importantly, here we establish a new method to determine the equilibrium 1000ln α carbonate-water, which consists in using the kinetics of Δ₄⁷ and δ¹⁸O covariations during (dis)equilibration. Notably, because of the very large range of apparent temperatures recorded by disequilibrium carbonates (between ~ 40 and 200°C; Fig. 7), this method could be particularly adapted to calibrate 1000ln α carbonate-water at high temperatures for which the differences between the two most popular equations for 1000ln α carbonate-water dependence on temperature (Kim and O’Neil, 1997; Coplen, 2007) appear larger (Fig. 7). Unfortunately, none of the three experimental setups having produced these disequilibrium carbonates (this study, as well as Tang et al., 2014, and Staudigel and Swart, 2018) were designed for the purpose of calibrating the equilibrium 1000ln α carbonate-water. It is thus not possible, using these datasets, to propose a meaningful calibration. At least in our experiment, too many phenomena including the relatively high precipitation rate, variations in δ³⁴S values (~ 3 ‰) (Thaler et al., 2017) and presence of traces of arag-
Conclusions

Our experimental results show that the information held in disequilibrium (and apparent disequilibrium) carbonates is diverse and promising. First, a paired $\Delta_{47}$ and $\delta^{18}O$ carbonate disequilibrium indicates that carbonates have precipitated in a dynamic environment where DIC and water did not reach isotopic equilibrium. In our microbial carbonate experiments, all the DIC is produced in isotopic disequilibrium with water and precipitates rapidly. Accordingly, the disequilibrium O isotope compositions recorded in those carbonates are maximized compared to what can be expected in nature where newly produced DIC is expected to be mixed with at least partly equilibrated ambient DIC before carbonates precipitate. Second, the combined use of clumped and traditional oxygen isotopic compositions allows the retrieval of the $\delta^{18}O$ of the precipitation water, i.e., organism body water or environmental water, even for carbonates presenting $\delta^{18}O$ and/or $\Delta_{47}$ disequilibria or apparent disequilibria. Hence, except in the case of processes such as CO$_2$ degassing and CO$_2$ hydration and hydroxylation, which likely modify the $R_{\text{Stochastic}}$ term in $R_{\Delta_{47}}$ calculation, paired $\Delta_{47}$ and $\delta^{18}O$ carbonates disequilibria in carbonates can be used to reconstruct the oxygen isotope composition of both DIC and water at the precipitation loci even when precipitation occurred under disequilibrium conditions. Third, the (dis)equilibration trend in a covariation diagram of $\delta^{18}O$ versus $\Delta$,$\delta^{18}O$ can be used as a new method to determine the equilibrium fractionation factor between carbonate and water for a wide range of temperatures. Altogether, this opens up new avenues to better constrain not only past climate changes through improved paleoenvironmental reconstructions but also the physiology and habitat of sea life sensitive to ocean acidification.

Data availability. All the data generated and analyzed in this study are available within the paper and in its Supplement.

Supplement. The supplement related to this article is available online at: https://doi.org/10.5194/bg-17-1731-2020-supplement.

Author contributions. CT and AK conceived the research. CT performed the microbial precipitation experiment and the $\delta^{13}C$ and $\delta^{18}O$ analyses during her PhD thesis under the supervision of MA and BM. AK performed the $\Delta_{47}$ analyses during her PhD thesis under MB’s supervision. CT took the lead in the interpretation of the results and the writing of the original draft. All authors provided critical feedback and helped shape the research, analyses and manuscript.

Competing interests. The authors declare that they have no conflict of interest.
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