Note: ***The comments of Dr Defliese are in bold italics,*** the modification in the text are in red

- ***This paper by Thaler et al. is very well written, and I think would be of interest to a large number of people in the scientific community. The finding that disequilibrium precipitation can potentially be used to track 'true' equilibrium is novel, and potentially very exciting. I find the paper well written, and I have few comments or questions that the authors do not answer. My only general comment is that I caution the authors to not declare one set of d18O calibrations/measurement to be representative of equilibrium versus another as of this point. I (and probably many others in the community) am VERY sceptical of assertions that a single datapoint represents isotopic equilibrium for d18O and that all others do not. Overall, this is a good paper and I'm interested to see where this goes next.***

We would like to thank Dr William Defliese for accepting to review our work and for his encouraging comments. We are pleased that he found our article well written and our scientific finding to be novel and promising. Dr Defliese raised some comments to which we answered both in this letter and by modifying the text of the manuscript. We hope it improved the clarity of the manuscript.

- ***Abstract: Good Introduction: Good***
- ***My only general comment is that I caution the authors to not declare one set of d18O calibrations/measurement to be representative of equilibrium versus another as of this point. I (and probably many others in the community) am VERY sceptical of assertions that a single datapoint represents isotopic equilibrium for d18O and that all others do not.***
- ***Section 3.6: I'm not sure you can really make any statements here about better matching the Coplen (2007) and Watkins et al. (2013) data at low temperature, as figure 5 does not show error bars. When the errors are plotted (particularly important for the D47-based temperatures), it looks to me like your data overlaps both d18O calibration relationship.***

-We fully agree with Dr Defliese on this point. We regret that our manuscript could give the impression that we chose some calibrations as establishing the "true" equilibrium value. In order to deliver a much clearer message on this point, we modified the manuscript as follows:

Line 457: 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 $1000\ln\alpha_{\text{carbonate-water}}$ at both high and low temperatures in order to improve the accuracy and precision of our new proxy for reconstructing the $\delta^{18}O_{water}$ from which carbonates, even disequilibrium ones, precipitated.

-Additionnally, the Δ_{47} based temperature errors are now plotted on the mentioned figure

- ***Section 2.1: I'd like to see some statement about the mineralogy of the carbonates. It looks like that is reported to some extent in the supplement, but I think a sentence on mineralogy of your precipitates should go in the methods.***

We have added a new figure 1 (formerly in the supplementary material) presenting a scanning electron microscopy (SEM) picture of the carbonates and the following lines in section 2.1:

Line 100: The major part of the carbonates precipitated in this study was composed of calcite (Thaler et al., 2017) with minor amounts of aragonite (1 to 4%), vaterite (2 to 4%) and magnesian calcite with low Mg content $(Mg_{0.064},Ca_{0.936})CO_3$ (up to 8%) (Thaler et al., 2017).

- ***Section 3.1 Lines 171 and 172: Maybe mention in the text the equilibrium value and measured value, so that readers do not think -0.27 per mill is the measured value?***

We modified the text as follows:

Line 206: The Δ_{47} offset to equilibrium starts down to -0.270‰ (the largest Δ_{47} offset ever measured in solid carbonates) and the offset to equilibrium reaches -24.7‰ for $\delta^{18}O_{carbonate}$.

- ***Section 3.2 Line 197: Do you know the isotopic composition of the urea? This should be easily testable. I realize it doesn't impact the results of this study, but would be nice to know!***

This is an interesting question to which we had to answer in Thaler et al., 2017. In this study, we measured the $\delta^{13}C$ value of urea, but we still do not know neither its $\delta^{18}O$ value nor its clumped isotope ratio. But even if we could have measured them, the system stays under constrained for the following reasons:

- If urea conversion into dissolved inorganic carbon (DIC) is total, its $\delta^{13}C$ is transferred as is to the DIC (despite the ~12 permil enzymatic fractionation associated with that conversion step), and then to the carbonates if all the DIC precipitates. It is not the case for $\delta^{18}O$ and thus for the clumped isotope ratio, due to the ureolysis reaction mechanism, which is a double hydrolysis (please see below).
- 2/3 of the oxygen atoms in the DIC produced by ureolysis come from water (with an unknown fractionation), and 1/3 from urea (with a fractionation that can be considered = 1 if all of the urea converts into DIC). Thus what matters the most is not the initial isotopic composition of urea but rather the isotopic value of the enzymatic reaction product. In our case, this product can be CO_2 which is then hydrated and/or hydroxylated, a step that adds an additional fractionation step. We unfortunately cannot measure the $\delta^{18}O$ nor the clumped composition of the CO_2 that just got produced.

We however have calculated in Thaler et al.,2017 that:

"With equilibrium initial conditions [we hypothesized here that CO_2 is beeing produced with an isotopic composition in equilibrium with water], $CO_{2(aq)}$ hydration and hydroxylation can explain a 13‰ offset from equilibrium for the $1000\ln\alpha HCO_3^-$-H_2O value whereas the initial offset from equilibrium observed in solid carbonates was -24.7‰ in the experiment with CA. This demonstrates that CO_2 cannot be in oxygen isotope equilibrium with water when it is generated by ureolysis."

Note that in response to one of Pr Bernasconi's comment, we now describe in the manuscript the ureolysis mechanism:

line 82: Ureolysis consists in 2 successive hydrolysis steps: (i) the hydrolysis of urea into ammonia (NH_3) and carbamate (H_2N-$COOH$) ($H_2N-CO-NH_2+H_2O \rightarrow NH_3+H_2N-COOH$), which is catalyzed by urease and is the rate limiting step, and (ii) the rapid and spontaneous hydrolysis of carbamate into ammonia and $CO_{2(aq)}$ ($H_2N-COOH+H_2O \rightarrow NH_3+CO_{2(aq)}+H_2O$) (Krebs and Roughton, 1948; Matsuzaki et al., 2013) or H_2CO_3 ($H_2N-COOH+H_2O \rightarrow NH_3+H_2CO_3$) (Mobley and Hausinger, 1989; Krajewska, 2009).

- ***Section 3.3 Line 239: "using the calibration..."***

Correction made

- ***Section 3.4 Lines 278-280: This is true, with the important exception of carbonates in which d13C is also out of equilibrium, which is discussed later in section 3.4.***

That is very true and needed to be highlighted in that sentence. We modified it as follows:

Line 309: “Note that such precision in $\delta^{18}O_{water}$ values found in disequilibrium carbonates is remarkable considering that even for carbonates at isotopic equilibrium for both $\delta^{13}C$ and $\delta^{18}O$, $\delta^{18}O_{water}$ can only be retrieved from paired Δ_{47} and $\delta^{18}O_{carbonate}$ values with a precision of ±1‰ at best (see Supplementary information).”

- ***Line 297: "be explained solely by temperature..."***

Correction made

- ***Line 340: "form in caves from CO2..."***

Correction made

- ***Figure 1: I’m confused by the x-axis. Why is CaCO3 being presented in units of millimolar, which is appropriate for a dissolved solution? A solid should be presented in units of mass, i.e. milligrams, or alternatively as moles precipitated. Figure 2: Same comment as per figure 1.***

This mM unit was chosen to allow comparison with the experiment presented in Thaler et al., 2017. Having the amount of carbonates in mM unit also permits to directly compare it to the amount of DIC and dissolved inorganic nitrogen (DIN) produced in the solution. This latter points out that not all of the DIC precipitates, which has implication for the expression of several fractionation factors.

The comparison between the amount of carbonate formed and the DIN is now available in the new Figure 1, that was previously in the supplementary material of the manuscript

Although we agree mM is an odd unit to use for solids, we see some interest in keeping it that way, and would prefer not to change it.

- ***Figure 5: The trendline symbols for Watkins (2013) and Kim and O’Neil (1997) are too similar, and difficult to distinguish.***

We modified the trendline used.

- ***Also I’m a bit confused by the Watkins data, why are the symbols for Watkins on the same line as Kim and O’Neil yet the trendline lies above?***

We agree this may be confusing, but that is what Watkins et al., 2013 and then Watkins and Hunt 2015 propose.

Below is displayed Figure 5 from Watkins et al., 2013 and its legend, where their data are represented along with cave samples from Coplen, 2007.

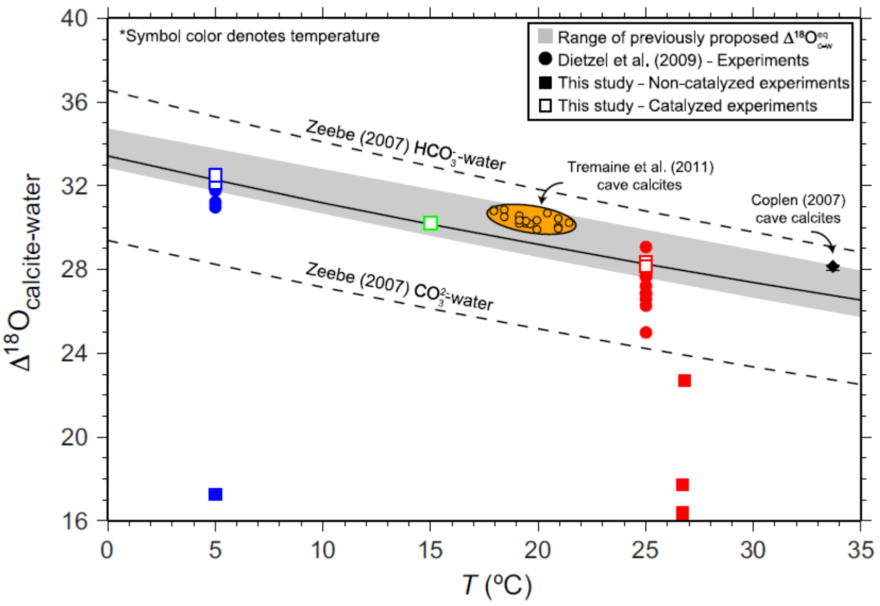


Fig. 5. Relationship between the observed oxygen isotope composition of calcite and temperature. The grey shaded area represents the range of proposed equilibrium values. The dashed lines show the equilibrium oxygen isotope composition of HCO_3^- and CO_3^{2-} relative to water. The solid line is parallel to the equilibrium CO_3^{2-}–water line to illustrate that nearly all of the temperature-dependence of oxygen isotope fractionation between calcite and water can be attributed to the temperature dependence of oxygen isotope fractionation between CO_3^{2-} (or HCO_3^-) and water. The temperature-dependence observed in the literature and in our catalyzed experiments does not necessarily imply oxygen isotope equilibrium between calcite and water. The data point from a natural cave calcite may represent thermodynamic equilibrium; it precipitated several orders of magnitude more slowly than experimental calcites (see Fig. 6).

The experiment catalyzed with CA presented in Watkins et al., 2013 falls on the solid line in their Figure 5. Even though it is not mentioned in the legend, it appears that this solid line is quite hard to distinguish from the line corresponding to Kim and O'Neil (1997) equation (i.e., 1000lna=18.03*1000/(T°C+273.15)-32.42).

As an illustration, at 0°C (where all curves intercept the Y-axis) the $1000\ln\alpha_{calcite-water}$ value calculated with Kim and O'Neil (1997) equation (in other words $\Delta^{18}O_{calcite-water}$) equals 33.6, and at 35°C, $1000\ln\alpha_{calcite-water}$ equals 26.1.

Accordingly, Watkins et al., 2013's catalyzed experiment is indeed on the same trendline as Kim and O'Neil, 1997, below the cave carbonates of Coplen, 2007.

However, we have also reproduced, again below, Figure 6 from Watkins et al., 2013 with its legend, where the model calibration equation ($\Delta^{18}Oeq_{calcite-water}$=17747/(T°C+273,15)-29.777) is given:

At 0°C, $\Delta^{18}Oeq_{calcite-water}$ = 35.2 and at 35°C, $\Delta^{18}Oeq_{calcite-water}$ = 27.8 (On Watkins et al., 2013's Figure 5, these two points are above the solid line).

So indeed, Watkins et al., 2013's model follows Coplen (2007) trendline even though the CA-catalyzed experiment datapoints fall on Kim and O'Neil (1997) curve. The catalyzed experiment datapoints fall on Watkins et al (2013) model trend line when represented against the precipitation rate (logR) that is incorporated in the calculation that produced Figure 6 lines. However, no 1000lnα equation corrected for logR value is given in the figure.

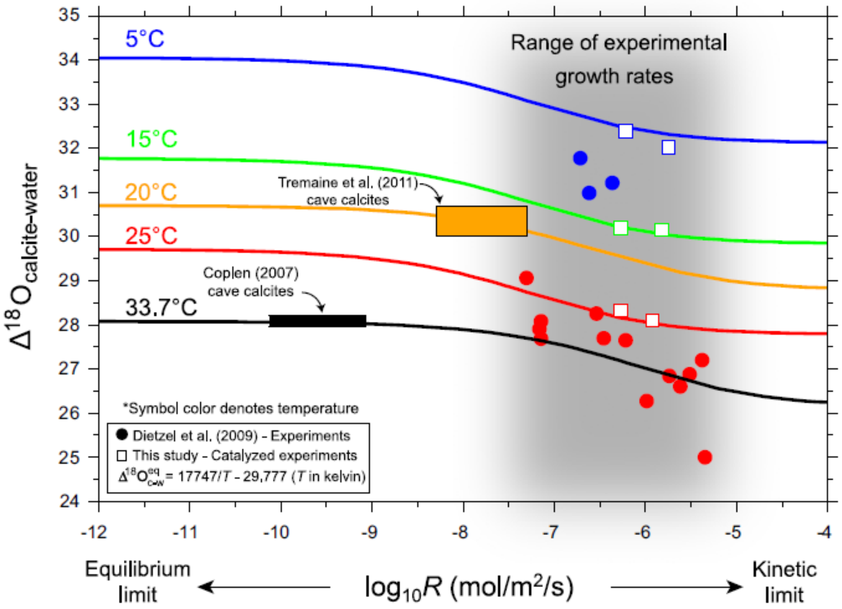


Fig. 6. $\Delta^{18}O_{c-w}$ versus precipitation rate R. The model curves describe the rate-dependence on $\Delta^{18}O_{c-w}$ while accommodating the equilibrium $\Delta^{18}O_{c-w}$ at 33.7 °C and $pH = 7.4$ inferred from natural cave calcite (Coplen, 2007). The slowly grown cave calcites from Tremaine et al. (2011) formed at about 20 °C and $pH = 7.8$. In the model, the temperature dependence arises solely from temperature-dependent partitioning of oxygen isotopes between DIC species and water. The pH dependence arises from the difference in HCO_3^- versus CO_3^{2-} participating in calcite growth as a function of growth rate. The data of Dietzel et al. (2009) are isotopically light, which may be a consequence of isotopic disequilibrium among DIC species in the bulk solution from which calcite is precipitated. Such a disequilibrium effect is not incorporated into the model. An important insight from the agreement between model and data is that direct measurement of equilibrium $\Delta^{18}O_{c-w}$ by conventional methods may require experiments that last years to decades.

As you might wonder, Coplen (2007) exact equation is:

$$\boxed{1000 \ln \alpha_{\text{calcite-water}} = 17.4(1000/T) - 28.6 \qquad (3)}$$

To confirm previous statements (that is a bit puzzling), below is Table 1 from Watkins and Hunt, 2015 with the calcite-water equilibrium equation used. Again Coplen, 2007 and Watkins et al., 2013 are undistinguishable:

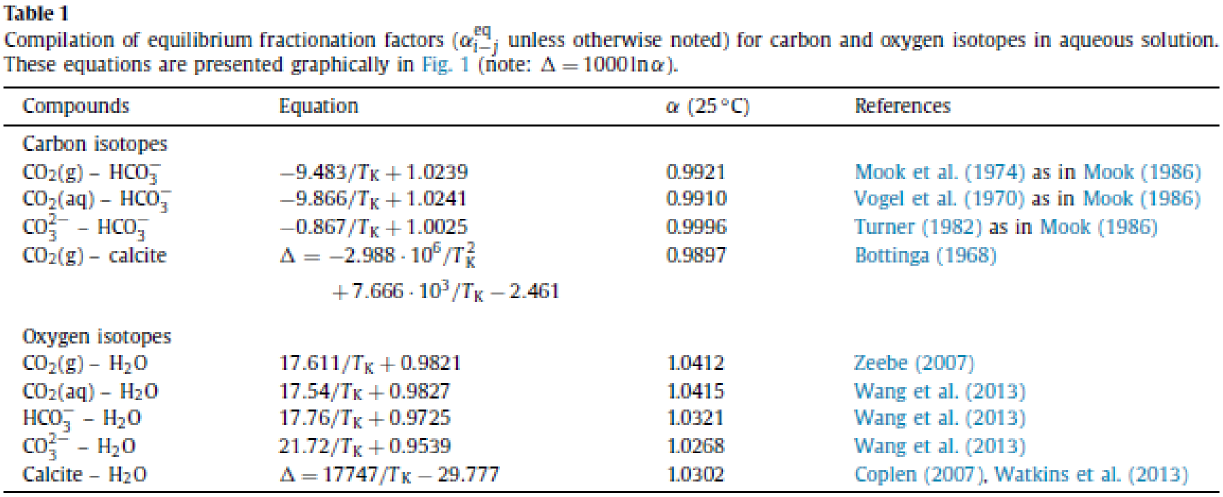


Table 1
Compilation of equilibrium fractionation factors (α^{eq}_{i-j} unless otherwise noted) for carbon and oxygen isotopes in aqueous solution. These equations are presented graphically in Fig. 1 (note: $\Delta = 1000 \ln \alpha$).

Compounds	Equation	α (25 °C)	References
Carbon isotopes			
$CO_2(g) - HCO_3^-$	$-9.483/T_K + 1.0239$	0.9921	Mook et al. (1974) as in Mook (1986)
$CO_2(aq) - HCO_3^-$	$-9.866/T_K + 1.0241$	0.9910	Vogel et al. (1970) as in Mook (1986)
$CO_3^{2-} - HCO_3^-$	$-0.867/T_K + 1.0025$	0.9996	Turner (1982) as in Mook (1986)
$CO_2(g)$ – calcite	$\Delta = -2.988 \cdot 10^6/T_K^2 + 7.666 \cdot 10^3/T_K - 2.461$	0.9897	Bottinga (1968)
Oxygen isotopes			
$CO_2(g) - H_2O$	$17.611/T_K + 0.9821$	1.0412	Zeebe (2007)
$CO_2(aq) - H_2O$	$17.54/T_K + 0.9827$	1.0415	Wang et al. (2013)
$HCO_3^- - H_2O$	$17.76/T_K + 0.9725$	1.0321	Wang et al. (2013)
$CO_3^{2-} - H_2O$	$21.72/T_K + 0.9539$	1.0268	Wang et al. (2013)
Calcite – H_2O	$\Delta = 17747/T_K - 29.777$	1.0302	Coplen (2007), Watkins et al. (2013)

- ***Additionally, if it doesn't clutter up the plot too much it would be good to see the error bars on the carbonate datapoints.***

-We chose to only mention the error on our data point in the legend, not only to not clutter up the plot, but also because no error is given in Kim and O'Neil, 1997, which makes error comparison between datasets complicated. We thus chose to not represent any error in the first place. However, please note that the symbol size is quite big and probably encompass the error (if it is of around 0.3 or 0.4 permil, which is plausible).

-The Δ_{47} based temperature errors are now plotted on the mentioned figure. The following sentence has also been added to the associated legend:

"X-axis errors for this study are included in the symbol size. The Y-axis error for all the reconstructed temperature is given in the figure."

Bibliography

Coplen, T. B.: Calibration of the calcite–water oxygen-isotope geothermometer at Devils Hole, Nevada, a natural laboratory. *Geochim. Cosmochim. Acta* **71**, 3948–3957, https://doi.org/10.1016/j.gca.2007.05.028, 2007.

Watkins, J. M., Nielsen, L. C., Ryerson, F. J. & DePaolo, D. J.: The influence of kinetics on the oxygen isotope composition of calcium carbonate. *Earth Planet. Sci. Lett.* **375**, 349–360, https://doi.org/10.1016/j.epsl.2013.05.054, 2013.

Watkins, J. M. & Hunt, J. D.: A process-based model for non-equilibrium clumped isotope effects in carbonates. *Earth Planet. Sci. Lett.* **432**, 152–165, https://doi.org/10.1016/j.epsl.2015.09.042, 2015.

Kim, S. T. & O'Neil, J. R.: Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochim. Cosmochim. Acta* **61**, 3461–3475, https://doi.org/10.1016/S0016-7037(97)00169-5, 1997.

(The comment of Pr Bernasconi are in bold italics, the modification in the text are in red***)***

> ***This paper proposes that the deviation from equilibrium for clumped isotopes and oxygen isotopes is closely related by a constant relationship, and that due to this relationship the isotopic composition of the delta 18O value of paleowaters can be reconstructed even from carbonates precipitated out of equilibrium. This is an interesting concept, but it to be reinforced by using additional calibration curves for clumped and oxygen isotopes (see details below) before the conclusions can be considered robust. In particular the Bonifacie et al.2017 curve is not calculated with the "Brand" parameters whereas the data presented here are, thus there is the possibility of an offset (see comment below) thus alternatives have to be considered. Similarly, there are other curves for calcite-water oxygen isotope fractionation that are probably closer to equilibrium (see below) and these alternatives should also be tested to evaluate the robustness of the conclusions. In addition, there are also other datasets available in the literature on dolomite that can be used to support the conclusions of this paper, and these should be included as well.***

We would like to thank Pr. Bernasconi for agreeing to evaluate our work. His review raises important points which we are pleased to clarify in this letter. Some points also required modification that figure in the the revised manuscript, inducing minor changes that however do not change our initial conclusions.

First of all, we would like to stress out that the goal of the paper is *not* to establish equilibrium but rather to suggest, for future studies, an original method to approach it better. Indeed, future well-designed experiments associated with accurate isotopic measurements should permit to better approach equilibrium equations. Since it is crucial that our objective here is clearly understood by readers (ie. that *our paper does not aim to establish equilibrium*), we have added the following sentence:

Line 457: "From our results, due to our experimental condition 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 $1000\ln\alpha_{\text{carbonate-water}}$ at both high and low temperatures in order to improve the accuracy and precision of our new proxy for reconstructing the $\delta^{18}O_{water}$ from which carbonates, even disequilibrium ones, precipitated."

Second, we would like to stress out that, in the original submission of the manuscript, we did take into account several calibrations *both* for clumped and oxygen isotopes, arguing the choice of calibration both for Δ_{47} and $\delta^{18}O$ would not change our conclusions (e.g., Kelson 2017 versus Bonifacie 2017, former lines 150 to 152; or comparing our reconstructed $\delta^{18}O_{water}$ with several combination of $\delta^{18}O_{water\text{-}calcite}$ calibrations former lines 420 to 422, all of this text remains in the new version of the manuscricpt).
Detailed answer to this comment and to other specific comments about using a calibration calculated with Brand parameters or using dolomite calibrations are detailed underneath, after the more detailed comments made by S. Bernasconi.

- ***As the paper is not very long, it would be better to incorporate the supplementary information into the main manuscript, this would make the paper more easily readable as all the important information is in one document.***

We initially considered that the technical information necessary to the clumped isotope community scientist to evaluate and reproduce our analysis and calculation protocols are not of interest for the greater biogeosciences community and hence provided them in the supplementary material with a summary (paragraph 2.2. former lines 99 to 142) in the main text. We however decided to move the following items, of interest to the broader biogeosciences community, from the supplementary material to the main text following S. Bernasconi recommendation:

-the ureolysis mechanism, new lines 82 to 86

- Supplementary Figure 1, (now fig. 1) that helps the reader to appreciate how close to the cells precipitation is occurring

- Supplementary figure 2, (now Fig. 2) that permits to better understand how the microbial ureolysis triggers carbonate precipitation

- Supplementary text explaining the rationale for the choice of Δ_{47} calibration, new lines 168 to 193

- ***Line 29: The delta 18O of carbonate not "the delta 18O composition of carbonate"***

Correction made

- ***Line 32 Abundance not abundancy***

Correction made

- ***Line 36-37 these two papers report disequilibruium but these are extreme cases, and not so common,. In particular other occurrences of Methane seep carbonates have been shown to precipitate in equilibrium (see Zhang et al. EPSL 512, 207-213).***

We agree that strong disequilibrium is not common, however disequilibrium (strong enough to not be able to reconstruct correct precipitation temperature) is common. As highlighted recently by Daëron et al., 2019: "Most earth surface calcites precipitate out of isotopic equilibrium" which of course do not signify that no carbonates can precipitate at least close to equilibrium. However, the novelty of this article is specifically to evaluate the information that can be obtained from carbonates formed with an identifiable isotopic disequilibrium, which is why we focus on these datasets. Carbonates formed at isotopic equilibrium are already usable to reconstruct paleoclimates with traditional approaches, and are not the topic of our study.

- ***Line 38 change to "in some biogenic carbonates" Disequilibrium is found in corals and possibly in brachiopods, but other widespread carbonates do not show disequilibrium (foraminifera, Peral et al. 2019 for example are in equilibrium). As the sentence is formulated here it seems to indicate that disequilibrium is dominant in biogenic carbonates, which is not the case. This may suggest that the clumped isotope thermometer is seldom useful. Please correct.***

-The intention of the text (former) lines 36 to the end of the paragraph is to say that the vital effect issue is well known and widespread for $\delta^{18}O$ and increasingly identified for Δ_{47}. The $\delta^{18}O$ vital effect can be strong in corals and brachiopods or coralline algae but it exists as well in coccoliths or foraminifera. We thus prefer to say that disequilibrium is common in biogenic carbonates. This does not prevent $\delta^{18}O$ and Δ_{47} tools to be useful as empirical calibrations taking vital effects into account allow temperature reconstructions.

That sentence has been added in the manuscript to prevent any reader to believe that vital effect identification invalidate the use of the isotopic thermometry.
Line 44 : Even though these vital effect identification do not prevent $\delta^{18}O$ and Δ_{47} tools to be powerful paleothermometer as empirical calibrations taking vital effects into account allow temperature reconstructions, it has become crucial to determine if the $\delta^{18}O$ and Δ_{47} disequilibria 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 disequilibria occurring in dissolved inorganic carbon (DIC) involved in carbonate precipitation.

-It is however true that vital effect is mostly studied on $\delta^{18}O$ data considering the difference in the temperature precision obtained with $\delta^{18}O$ reconstruction in comparison to Δ_{47} reconstruction, due to the sensibility to temperature of the parameters and/or to the precision of the measurements. We calculated below two examples, using a reasonable 0.2 permil error on a $\delta^{18}O$ measurements (many laboratory do better) and a good 0.008 permil error on a Δ_{47} measurement (many laboratory do not reach that precision) using two calibrations which use are proposed by the reviewers (the result would be similar with other calibrations):

Used equation: Δ_{47}=0.0449*10^6/T^2+0.167 (Bernasconi et al., 2018)			
	Δ_{47} - 0.008	Δ_{47} real	Δ_{47} + 0.008
Range of Δ_{47} comprised within the error:	0.719	0.727	0.735
recalculated T°C:	12.0	10.0	8.0
	error on T°C : +/- 2°C		

Used equation: $\delta^{18}O_c$-$\delta^{18}O_w$= 17747/T –29.777 (Watkins et al., 2013)			
	$\delta^{18}O$ - 0.2	$\delta^{18}O$ real	$\delta^{18}O$ + 0.2
Range of $\delta^{18}O_c$ comprised within the error:	32.7	32.9	33.1
recalculated T°C (for a $\delta^{18}Ow$ = 0):	10.9	10.0	9.1
	error on T°C : +/- 0.9°C		

The additional error associated to the equations parameters themselves is not taken into account.

Due to that difference in precision, vital effects can be masked in many species (except in coral, brachiopods or our microbial carbonates where clumped disequilibrium was big enough to be identified) in Δ_{47} studies, but it does not mean it is not there. We believe that these vital effects deserve being studied so that reconstructions get richer in information.
We acknowledge that Peral et al., 2018 show that with a precision of 0.008 they cannot distinguish species specific differences on the Δ_{47} in several species of foraminifera (while those species specific vital effects are known for $\delta^{18}O$). We also acknowledge that the calibration obtained on foraminifera agrees well with the kele 2015 calibration recalculated by Bernasconi et al. 2018. This indicates that paleoclimate reconstruction from Δ_{47} measurement on foraminifera, or from any "close to equilibrium" carbonates, measured with a 0.008 precision, will give similar temperature reconstruction precision. The fact that any vital effect cannot be identified today does not mean it will not when the precision gets better… especially when already identified on $\delta^{18}O$. We would like to add that in Peral et al., 2019 size specific vital effect on both $\delta^{18}O$ and Δ_{47} on the species G. inflate is indeed described.

- ***Line 121 change to "…measured ratios of the sample CO2"***

Correction made

- ***Line 128 "Equilibrium Scale " not "Equilibrated Scale"***

Correction made

- ***Line 150 and supplementary information: The Bonifacie et al. 2017 is not calculated with the Brand parameters, thus it should not be used to calculate temperatures of samples whose D47 is calculated using the Brand parameters, as there could be an offset. For example the Kele et al. 2015 calibration before recalculation was indistinguishable from the Bonifacie et al. 2017 common calibration (it was also part of it). However, upon recalculation by Bernasconi et al. 2018; Geochem. Geophys. Geosys.), the intercept has changed by -38 ppm, thus the authors should be careful in establishing "equilibrium" with an equation that is not based on the Brand parameters.***

We agree with S. Bernasconi on the fact that using a calibration not calculated with the Brand parameters to convert data themselves calculated with the Brand parameters is not ideal. However, this feature is not the only one to consider for the use of a calibration compatible with the acquired data. Then, as stressed out in the supplementary discussion of the initial submission (where we detailed the rationale for our choice of calibration) there are strong arguments why we have chosen (and still want) to use Bonifacie et al. 2017 calibration in our case, which we still believe is the most valid/compatible to compare to our data. We however acknowledge that this information might have been somehow hidden in supplementary info (as also mentionned by S. Bernasconi's above comment) in our initial submission, we thus moved some explanations/clarifications in the main text (as in supplementary information). See detailed comments and changes underneath

New line 168 (in the main text):

For the temperature (T) derived from the Δ_{47} data, we chose the calibration determined by Bonifacie et al., (2017) as it integrates a consequent number of data (n > 300), which statistical weight have been properly considered, and covers a wide temperature range (from 1 to 350°C), three characteristics that were recently shown by several teams as governing the precision on Δ_{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 Δ_{47} values) allowing to avoid loss of precision/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, Dassié et al., 2018) on the range of temperatures (~30 to 96°C) where most of available calibrations are diverging and/or not well constrained. Indeed, these studies report excellent consistencies: i/ between $T\Delta_{47}$ and homogenization temperatures from fluid inclusion microthermometry (Mangenot et al., 2017), and *ii/* between the $\delta^{18}O_{water}$ values directly measured in fluid inclusions by cavity ring down spectroscopy and those calculated from combined $T\Delta_{47}$ and $\delta^{18}O_{carb}$ of the host-mineral (Dassié et al., 2018).

-For taking into account the brand parameter issue underlined here by S. Bernasconi, we still prefer to use the Kelson et al., 2017's calibration (see lines 159 to 161, present in our original submission) which is, as our data presented here, *directly* normalized with equilibrated gas standards only (that is the correction frame used by the whole community since 14 years) and with limited uncertainties because describing a large range in temperature, and acquired in a single lab (thus avoiding complications arising from comparing data from different laboratories, as shown in Petersen et al., 2019). Given the current consensus of using equilibrated gas standards, we believe that comparing our data with Kele/Bernasconi 2018 (normalized to carbonate standards) would introduce much more confusion (and likely bias – see supplementary Table S4) than the comparison we currently propose.

That being said, it is noteworthy that significant improvements are currently undertaken by the clumped isotope community to minimize bias between laboratories by using carbonate standards to normalize data instead of equilibrated gases (S. Bernasconi is leading this INTERCARB project with 4 other clumped isotope researchers including M. Bonifacie co-author of this paper). The project has gathered more than 2000 measurements on the same standards from 26 different laboratories. But the results have only been communicated as internal reports to participants and are thus not yet public before their publications.

Interestingly, the about 38 ppm difference mentioned here by S. Bernasconi between the intercepts from Bonifacie et al. 2017 and Kele/bernasconi2018 calibrations (NB slopes are similar) is similar to the average offset found between IPGP and ETH labs on the four standards ETH1, ETH2, ETH3, ETH4 analyzed in both labs (see Table below, also now added as supplementary Table S4) and used to normalize the Kele/Bernasconi calibration.

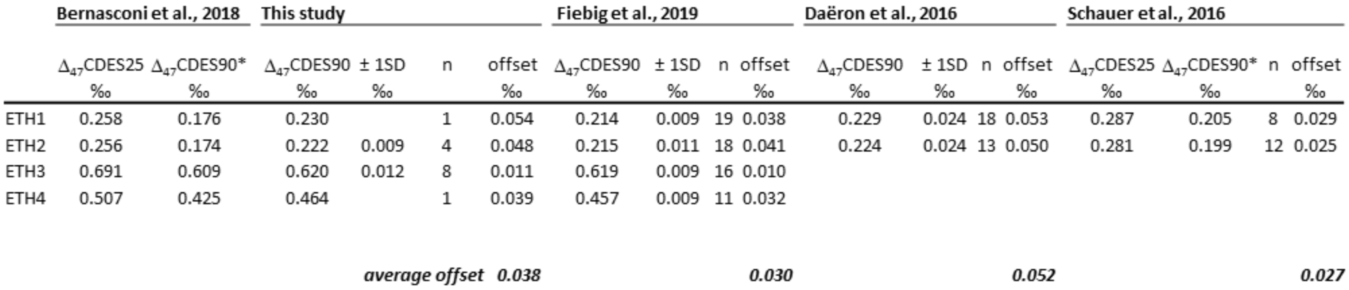
To avoid confusion of readers, and because carbonate standards will likely be increasingly (if not exclusively) used in the future Δ_{47} measurements, we decided to add in the revised documents:

New line 181 (in main text):

Thought we recognize that the normalization to carbonate standards presented in Bernasconi et al. (2018) might become commonly used by the community in the future (ie. with the on-going inter-comparison Intercarb project), we prefer 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 run in total of ETH1, ETH2, ETH3, ETH4 standards; Table S5), and such 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 ran in other IPGP studies and some other laboratories). Also remarkably, Δ_{47} obtained here on the four ETH carbonate standards are all systematically higher than values reported in Bernasconi et al., 2018 (Table S4). Thought the reason of this positive offset is still unclear, it is noteworthy that positive offsets are also observed when compiling other recent published values (Table S4; Daeron et al. 2016; Schauer et al., 2016; Fiebig et al., 2019;).

With Table S4 (in supplementary material)

Because carbonate standards will likely be increasingly used in the future for normalizing Δ_{47} measurements, we here provide inter-laboratory comparison of Δ_{47} values obtained on the four standards provided by S. Bernasconi (ETH1, ETH2, ETH3, ETH4) as supplementary Table S4. This should also allow future use of our dataset.



	Bernasconi et al., 2018		This study				Fiebig et al., 2019				Daëron et al., 2016				Schauer et al., 2016			
	Δ_{47}CDES25 ‰	Δ_{47}CDES90* ‰	Δ_{47}CDES90 ‰	± 1SD ‰	n	offset ‰	Δ_{47}CDES90 ‰	± 1SD ‰	n	offset ‰	Δ_{47}CDES90 ‰	± 1SD ‰	n	offset ‰	Δ_{47}CDES25 ‰	Δ_{47}CDES90* ‰	n	offset ‰
ETH1	0.258	0.176	0.230		1	0.054	0.214	0.009	19	0.038	0.229	0.024	18	0.053	0.287	0.205	8	0.029
ETH2	0.256	0.174	0.222	0.009	4	0.048	0.215	0.011	18	0.041	0.224	0.024	13	0.050	0.281	0.199	12	0.025
ETH3	0.691	0.609	0.620	0.012	8	0.011	0.619	0.009	16	0.010								
ETH4	0.507	0.425	0.464		1	0.039	0.457	0.009	11	0.032								
					average offset	***0.038***				***0.030***				***0.052***				***0.027***

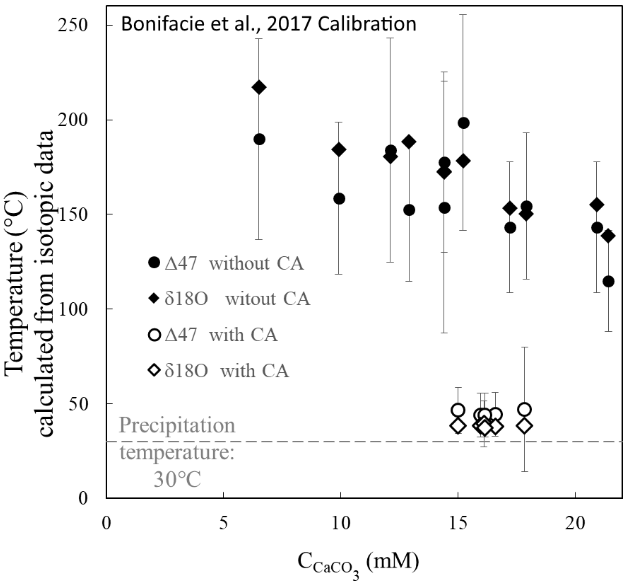
"$\Delta_{47CDES25}$" and " $\Delta_{47CDES90}$ "are Δ_{47} values reported versus the Carbon Dioxide Equilibrium Scale in the 25°C and 90°C acid digestion

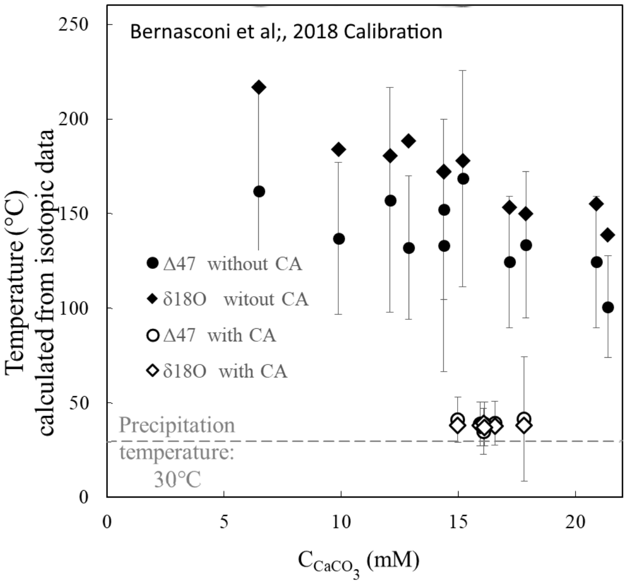
reference frame, respectively (in ‰).
" $\Delta_{47CDES90}$*" from Bernasconi et al. (2018) and Schauer et al. (2016) are calculated using the $\Delta^*_{25\text{-}90°C}$ value of 0.082‰ from Defliese et al. (2015). $\Delta_{47CDES90}$ values reported for this study are average values calculated based on Supplementary Table S5. Other $\Delta_{47CDES90}$ values are those published in Fiebig et al. (2019) and Daëron et al. (2019).
"n" is the number of replicate measurements considered.
"offset" is the calculated offset between $\Delta_{47CDES90}$ values reported in Bernasconi et al. (2018) and those from the considered studies.

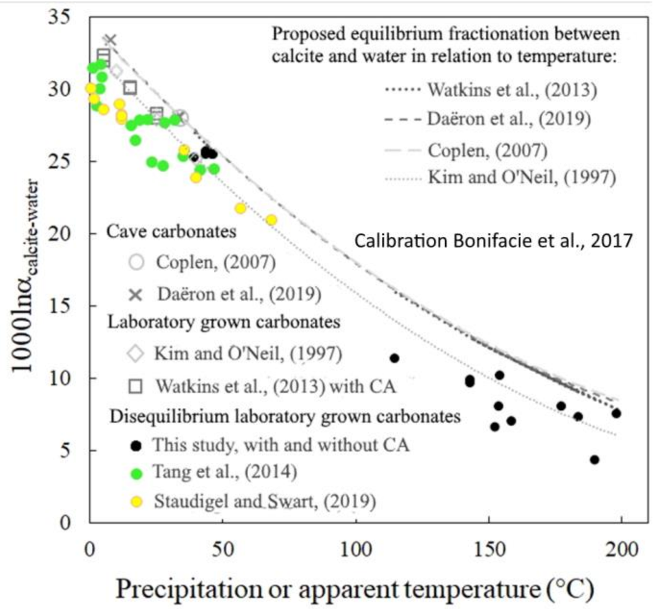
- ***The temperature difference calculated with the recalculated Kele et al.2015 instead of the Bonifacie et al. 2017 is up to 25 degrees lower for the samples with high disequilibrium. The samples produced with the carbonic anhydrase with the Bernasconi et al. 2018 calibration give yield temperatures of 35 to 40◦C about 5◦C closer to equilibrium than using the Bonifacie et al. 2017 calibration. Does this change the interpretation of the trends? This should be taken in consideration and discussed. Many of the calibrations used in the Bonifacie calibration were indeed recalculated by Petersen et al. 2019; G3) and the values of some of the datasets changed significantly. It is difficult to evaluate what difference it would make to the Bonifacie curve, but this should be checked. Due to these uncertainties, the inferences on difference to equilibrium could be biased and the calculated water compositions as well. I suggest that the temperatures should be recalculated using for example the recalculated Kele et al. 2015 and see how the interpretations may change.***

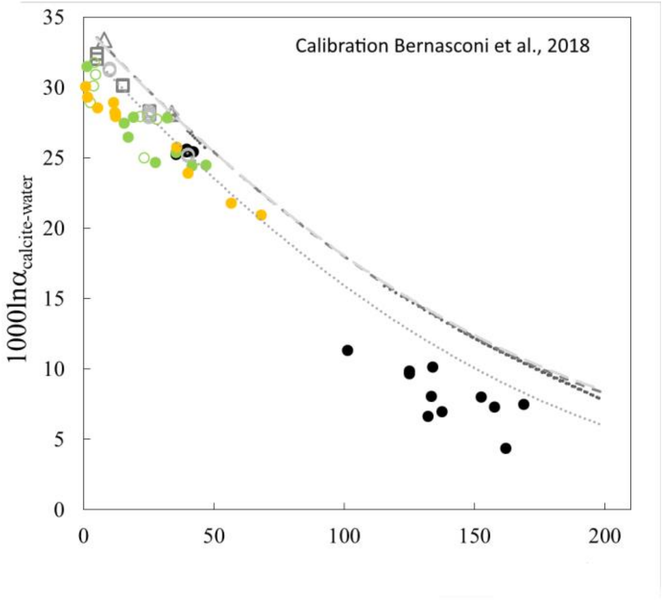
First, we would like to stress out that the Bonifacie et al. 2017 calibration was mainly based (considering the statistical weight of the respective data, and *not just the number of data* as usually made before [for more details on the statistical weight of respective data see discussion page 274 of Bonifacie et al., 2017]) on Δ_{47} data that did not significantly changed with the recalculation with the Brand parameters. Notably, the Kele 2015 data (which changed a lot with the Brand parameters as mentioned by S. Bernasconi) were only contributing to 10% to the final calibration. Thus changing to the recalculated Δ_{47} Brand values do not impact significantly the published Bonifacie et al., calibration. Importantly, datasets statistically contributing more to the calculated equations (ie. Wacker et al and Henkes et al. contributed as high as 40%) did not show large changes with the recalculations in Petersen et al (see Figure 3 of Petersen et al., 2019).

Second, we would like to show here that using kele/bernasconi calibration, as requested by S. Bernasconi, do not change our results and interpretations as shown in underneath figures.









For some points there is a better match for other there is a bigger difference, in general the agreement on the temperature recalculated on the data series with CA is indeed closer to equilibrium with Bernasconi et al., 2018 calibration, which is not in itself better or worse as, as explained in Thaler et al., 2017, the carbonates precipitated with CA corresponds to minerals that precipitate all along the equilibration, they thus should be off equilibrium a little to account for the initial "seeds" that precipitate at the beginning of the equilibration from a DIC in strong disequilibrium, and we do not know how the $\delta^{13}C$ different composition of the "seeds" impact the final Δ_{47}. For the experiment Without CA, using bernasconi's equation pushes our data even further away from Coplen/Watkins/Daeron calibration, which again is not a bad thing as long as we consider that the question of the "true" equilibrium relation is not settled yet and it is not our intention to settle it in this paper.

Anyway, the main interpretation of our data is that similar wrong temperature can be reconstructed from both d18o and Δ_{47} measurement, all the conclusions on the reconstruction of $\delta^{18}O_{water}$ comes from that point, and this does not change at the first order. It would change the isotopic composition of the calculated water, but theoretically less than using Watkins+bonifacie instead of Bonifacie and Kim and O'Neil (which would have return 2‰ lower values as already stated in the text).

- ***The same discussion is valid also for the Kim and O'Neil calibration, does that really represent equilibrium? More and more evidence is that it is not (see discussion in Daeron et al. 2019). How would the interpretations in this paper change if another oxygen isotope calibration would be used ot calculate the oxygen isotope temperature? For example the Daeron et al. 2019 or O'Neil et al. (1969)? This point should be tested and possible implications discussed also in the discussion section.***
- ***Lines 268 The Kim & O'Neil calibration is based on samples precipitated to temperatures between 10 and 40 thus not the best one for higher temperatures. Simply the fact that is the most used does not mean that it it's the best one to use.***

Our objective is not here to determine whether one calibration represents equilibrium better than another but rather to evidence that our knowledge on the equilibrium value still need to be refined, and to propose an experimental method to improve our knowledge on that topic. We thus prefer not to discuss how far from equilibrium Kim and O'Neil calibration is in comparison to other. As shown above our conclusions do not change with the calibration choice.

However, it is correct to say that the value of the reconstructed $\delta^{18}O_{water}$ changes with the calibration used. This was already discussed in the original submission and still in the manuscript with examples of effects:

"Coplen, (2007) or Watkins et al., (2013) equations would have return 2‰ lower values (ca. -10±2‰ compared to -8±3‰ calculated with Kim and O'Neil (1997) equation). From our results, it is not possible and not our intention to argue in favor of one of these calibrations "

- ***Even if this is given in detail in Thaler et al. 2017, it would be useful for this paper to put a figure with the reaction pathway for ureolysis. It would be useful to have a formula which shows where the oxygen in the carbonate molecule comes from.***

We have added the reaction mechanism in the method part:

line 82: Ureolysis corresponds to two hydrolysis : (i) the hydrolysis of urea into ammonia (NH_3) and carbamate (H_2N-COOH) ($H_2N-CO-NH_2+H_2O \rightarrow NH_3+H_2N-COOH$), which is catalyzed by urease and is rate limiting, and (ii) the rapid and spontaneous hydrolysis of carbamate into ammonia and $CO_{2(aq)}$ ($H_2N-COOH+H_2O \rightarrow NH_3+CO_{2(aq)}+H_2O$) (Krebs and Roughton, 1948; Matsuzaki et al., 2013) or into H_2CO_3 ($H_2N-COOH+H_2O \rightarrow NH_3+H_2CO_3$) (Mobley and Hausinger, 1989; Krajewska, 2009).

- ***Line 201: its better to talk about depleted in 18O rather than enriched in 16O which is the more abundant isotope***

Correction made

- ***Lines 211 -2015 Schmid 2011 (ETH dissertation, https://doi.org/10.3929/ethz-a006551449) also reported some analyses of carbonates produced by ureolytic bacteria in strong disequilibrium, and data from carbonates produced by direct hydroxylation at high pH showed the effect of hydroxylation on the clumped isotopes of carbonates. The high D47 of the carbonates formed from directly hydroxylated CO2***

can also be due to the direct inheritance of the isotopic composition of the CO2 as at high pH the equilibration time with water is very long, longer than the precipitation rate of carbonate.

We agree with that interpretation. This specific mechanism was developed in the original submission of the manuscript inside the paragraph on the effect of CO_2 hydroxylation/hydration on Δ_{47} values as illustrated by Tang et al abiotic experiment (still in the manuscript lines 342 to 358 and Figure 6).

- ***Lines 285 It is not true that the Tang and Staudigel data are the only published data reporting, water, delta 18O Water and delta 18 calcite. The authors should include the Kele et al. (2015) data with the recalculated D47 in Bernasconi et al. 2018 in their analysis as they also have all the necessary data 18 o carb 18O water and clumped isotopes to test the validity of their hypothesis.***

Agreed. We clarified the text. We wanted to say that these are the only published data with disequilibrium carbonates that present a complete set of data. Equilibrium data series indeed often present a complete set of data. However it is not the focus of our study to determine the actual value of equilibrium which is why we do not present many "equilibrium" data series, only the most "famous" that scientist may already well know and may already have compared their data to. We corrected the sentence:

Line 317" These studies were chosen to further evaluate the relevancy of our $\delta^{18}O_{carbonate}$- Δ_{47} correlation because they are the only published dataset reporting full sets of *measured (rather than calculated)* $\delta^{18}O_{water}$, $\delta^{18}O_{carbonate}$ and Δ_{47} values, with *one or both proxies showing disequilibrium*, together with precipitation temperatures."

- ***Additional datasets that would be interesting to test would be the Dolomite data of Bonifacie et al. 2017 (op. cit) and the dolomite data of Müller et al. 2019 (Chem. Geol., 525, 1-17) I think this would make the message of this paper much more robust***

We disagree with this proposition that we believe is beyond the scope of our study. Plus, dolomites have a different $\delta^{18}O$ fractionation factor to water than calcite, and our samples (and the $\delta^{18}O$ calibrations used) are mostly calcites.

Bibliography

Daëron M., Blamart D., Peral M. and Affek H. P. (2016) Absolute isotopic abundance ratios and the accuracy of D47 measurements.Chem. Geol. 442, 83–96.

Daëron, M., Drysdale, R. N., Peral, M., Huyghe, D., Blamart, D., Coplen, T. B., Lartaud, F. & Zanchetta, G.: Most Earth-surface calcites precipitate out of isotopic equilibrium. *Nat. Commun.* **10**, 429, https://doi.org/10.1038/s41467-019-08336-5, 2019.

Dassié E. Genty, D., Noret, A., Mangenot, X., Massault, M., Lebas, N., Duhamel, M., Bonifacie, M., Gasparrini, M., Minster B., and Michelot, J.L. (2018) A newly designed analytical line to examine the reproducibility of fluid inclusion isotopic compositions in small carbonate samples. Geochemistry, Geophysics, Geosystems, 19 (4) 1107-1122. DOI: 10.1002/2017GC007289

Bonifacie, M. Calmels, D., Eiler, J. M., Horita, J., Chaduteau, C., Vasconcelos, C., Agrinier, P., Katz, A., Passey, B. H., Ferry, J. M., & Bourrand, J. J..: Calibration of the dolomite clumped isotope thermometer from 25 to 350° C, and implications for a universal calibration for all (Ca, Mg, Fe)CO_3 carbonates. *Geochim. Cosmochim. Acta* **200**, 255–279, https://doi.org/10.1016/j.gca.2016.11.028, 2017.

Bernasconi, S. M., Müller, I. A., Bergmann, K. D., Breitenbach, S. F., Fernandez, A., Hodell, D. A., ... & Ziegler, M. (2018). Reducing uncertainties in carbonate clumped isotope analysis through consistent carbonate-based standardization. Geochemistry, Geophysics, Geosystems, 19(9), 2895-2914.

Kele, S., Breitenbach, S. F., Capezzuoli, E., Meckler, A. N., Ziegler, M., Millan, I. M., ... & Yan, H. (2015). Temperature dependence of oxygen-and clumped isotope fractionation in carbonates: a study of travertines and tufas in the 6–95 C temperature range. Geochimica et Cosmochimica Acta, 168, 172-192.

Kelson, J. R., Huntington, K. W., Schauer, A. J., Saenger, C. & Lechler, A. R.: Toward a universal carbonate clumped isotope calibration: Diverse synthesis and preparatory methods suggest a single temperature relationship. *Geochim. Cosmochim. Acta* **197**, 104–131, https://doi.org/10.1016/j.gca.2016.10.010, 2017.

Watkins, J. M., Nielsen, L. C., Ryerson, F. J. & DePaolo, D. J.: The influence of kinetics on the oxygen isotope composition of calcium carbonate. *Earth Planet. Sci. Lett.* **375**, 349–360, https://doi.org/10.1016/j.epsl.2013.05.054, 2013.

Kim, S. T. & O'Neil, J. R.: Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochim. Cosmochim. Acta* **61**, 3461–3475, https://doi.org/10.1016/S0016-7037(97)00169-5, 1997.

Peral, M., Daëron, M., Blamart, D., Bassinot, F., Dewilde, F., Smialkowski, N., ... & Michel, E. (2018). Updated calibration of the clumped isotope thermometer in planktonic and benthic foraminifera. Geochimica et Cosmochimica Acta, 239, 1-16.Fiebig, J., Bajnai, D., Löffler, N., Methner, K., Krsnik, E., Mulch, A., & Hofmann, S. (2019). Combined high-precisionΔ 48 andΔ 47 analysis of carbonates. Chemical Geology, 522, 186-191.

Schauer A. J., Kelson J. R., Saenger C. and Huntington K. W. (2016) Choice of 17O correction affects clumped isotope (D47) values of CO2 measured with mass spectrometry. Rapid Commun. Mass Spectrom. 30, 2607–2616.

Petersen, S. V., Defliese, W. F., Saenger, C., Daëron, M., Huntington, K. W., John, C. M., ... & Olack, G. A. (2019). Effects of improved 17O correction on interlaboratory agreement in clumped isotope calibrations, estimates of mineral-specific offsets, and temperature dependence of acid digestion fractionation. Geochemistry, Geophysics, Geosystems, 20(7), 3495-3519.

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 ($\delta^{18}O$) and clumped isotope (Δ_{47}) compositions of carbonate minerals, are compromised when carbonate crystallization 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 $\delta^{18}O$ and Δ_{47} offsets from isotopic equilibrium values is largely overlooked. Additionally, in carbonates formed in isotopic equilibrium, the use of the carbonate $\delta^{18}O$ signature as a paleothermometer relies on our knowledge of the paleowaters' $\delta^{18}O$ value, which is often assumed. Here, we report the largest Δ_{47} offsets observed to date (as much as -0.270‰), measured on microbial carbonates, that are strongly linked to carbonate $\delta^{18}O$ 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 $\delta^{18}O$ value of the water in which carbonates precipitated, as well as the water-carbonate $\delta^{18}O$ fractionation dependence to temperature at equilibrium can be retrieved from these paired $\delta^{18}O$ and Δ_{47} disequilibrium values measured in carbonates. The possibility to retrieve the $\delta^{18}O$ 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 ($\delta^{18}O$) paired with clumped isotope composition (Δ_{47}) of carbonate minerals is increasingly used for reconstructing paleoenvironmental or diagenetic conditions (Ghosh et al., 2006; Henkes et al., 2018; Mangenot et al., 2018 a and b). The $\delta^{18}O$ of carbonates depends on both the $\delta^{18}O$ 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 isotopes" abundance (Δ_{47}) thermometer which depends only on the carbonate precipitation temperature (Ghosh et al., 2006). By combining the Δ_{47} derived temperatures and the carbonate $\delta^{18}O$ value ($\delta^{18}O_{carbonate}$), the $\delta^{18}O$ value of the water ($\delta^{18}O_{water}$) 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 $\delta^{18}O$ and Δ_{47} 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 Δ_{47} compositions of carbonates seemed at first free of any biologically-driven or mineral-specific fractionation known to affect $\delta^{18}O_{carbonate}$ compositions (Eiler, 2011), recently identified disequilibrium Δ_{47} values (Saenger et al., 2012; Affek, 2013; Tang et al., 2014; Burgener et al., 2018) open new perspectives to unravel the mechanisms responsible for oxygen isotopic disequilibrium in carbonate minerals. Even though these vital effect identification do not prevent $\delta^{18}O$ and Δ_{47} tools to be powerful paleothermometer as empirical calibrations taking vital effects into account allow temperature reconstructions, it has become crucial to determine if the $\delta^{18}O$ and Δ_{47} disequilibria 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 disequilibria occurring in dissolved inorganic carbon (DIC) involved in carbonate precipitation. In this case, $\delta^{18}O$ and Δ_{47} disequilibria 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‰ offset from $\delta^{18}O_{carbonate}$ equilibrium values). We used carbonic anhydrase (CA), an enzyme able to accelerate oxygen isotope equilibration between DIC and water *via* fast CO_2 hydration and HCO_3^- dehydration. 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 up on these experiments as they offer a unique opportunity to assess experimentally whether carbonates precipitated from DIC in disequilibrium with water also record Δ_{47} disequilibrium values, and the type of information that is actually carried by these paired disequilibria. We latter 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 Material 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 (resistivity = 18 MΩ·cm) by dissolving salts in the following order: $MgSO_4 \cdot 7H_2O$ (16 mM), NaCl (80 mM), KCl (4 mM), urea (33.3 mM), $CaCl_2$ (40 mM). The aim was to mimic the ionic composition of a groundwater (Millo et al., 2012). In experiments with CA whose $\delta^{18}O$ results (but not the Δ_{47} ones) were recently published in Thaler et al., (2017), the precipitation solution was supplemented with CA at a concentration of 2 mg/L. 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, 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 hydrolysis : (i) the hydrolysis of urea into ammonia (NH_3) and carbamate (H_2N-COOH) ($H_2N-CO-NH_2 + H_2O \rightarrow NH_3 + H_2N-COOH$), which is catalyzed by urease and is the rate limiting step, and (ii) the rapid and spontaneous hydrolysis of carbamate into ammonia and $CO_{2(aq)}$ ($H_2N-COOH + H_2O \rightarrow NH_3 + CO_{2(aq)} + H_2O$) (Krebs and Roughton, 1948; Matsuzaki et al., 2013) or into H_2CO_3 ($H_2N-COOH + H_2O \rightarrow NH_3 + H_2CO_3$) (Mobley and Hausinger, 1989; Krajewska, 2009).

Ureolysis completion was followed by evaluating the production of dissolved inorganic nitrogen (DIN = NH_3+NH_4^+). Determination of pH, DIN concentration and amount of precipitated carbonates (Fig. 2), as well as isotopic measurements, were 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 NH_3 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 8.5 (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) has 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 major part of the carbonates precipitated in this study was composed of calcite (Thaler et al., 2017) with minor amounts of aragonite (1 to 4%), vaterite (2 to 4%) and magnesian calcite with low Mg content $(Mg_{0.064},Ca_{0.936})CO_3$ (up to 8%) (Thaler et al., 2017).

All of the measured chemical parameters (pH, DIC, amount of solid carbonates, Ca^{2+} concentration, DIN) along with DIC and solid carbonate $\delta^{13}C$ behave similarly with or without active CA (Thaler et al., 2017). It was not possible to measure Δ_{47} for all the precipitated carbonates due to their low amount, particularly for the tubes sacrificed at the beginning of the experiments (Supplementary Table S1).

2.2 $\delta^{18}O$ and Δ_{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 ±0.1‰ (1SD) and represents the uncertainty assigned to $\delta^{18}O_{carbonate}$ data.

The analytical procedure used for clumped isotope Δ_{47} measurements is only briefly presented here and detailed in Bonifacie et al., (2017). About 5 mg of carbonates were digested at 90°C during 20 min with 104% phosphoric acid H_3PO_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 their abundance in isotopologues with m/z from 44 to 49 versus a working gas provided by Oztech Trading Corporation with $\delta^{13}C = -3.71‰$ VPDB and $\delta^{18}O = +24.67‰$ VSMOW, as determined with the international reference material NBS19. One single Δ_{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 to Δ_{47} measurements with this instrument (Supplementary Tables S1 and S2). They are in excellent consistency with data obtained with the continuous-flow method on smaller samples (Supplementary Table S1).

The Δ_{47} is calculated as a function of the stochastic distribution of the CO_2 isotopologues, as follows:

$$\Delta_{47} = \left[\left(\frac{R^{47}_{measured}}{R^{47}_{stochastic}} - 1\right) - \left(\frac{R^{46}_{measured}}{R^{46}_{stochastic}} - 1\right) - \left(\frac{R^{45}_{measured}}{R^{45}_{stochastic}} - 1\right)\right] \times 1000 \qquad (1),$$

where Δ_{47} is expressed in per mil (‰), and R^{47}, R^{46} and R^{45} are the abundance ratios of the masses 47, 46, 45 respectively, relative to the mass 44 ($^{12}C^{16}O^{16}O$). $R^{X}_{measured}$ are measured ratios of the CO_2 sample. $R^{X}_{stochastic}$ are calculated from the measured 44, 45, 46 and 47 abundance ratios. The amount of isotopologues of mass 47 (mainly $^{13}C^{18}O^{16}O$, but also $^{12}C^{17}O^{16}O$ 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 amount of isotopologues of mass 63 (mainly $^{13}C^{18}O^{16}O^{16}O$) 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

Δ_{47} data into the absolute Carbon Dioxide Equilibrium Scale "CDES" ($\Delta_{47\ \mathrm{CDES90}}$ being the Δ_{47} values of carbonates reacted within acid at 90°C), standards of CO_2 gases equilibrated at 25°C and 1000°C and with bulk isotopic compositions covering the range of measured carbonate samples (δ^{47} values between -50 and +24‰) were analyzed interspaced with unknown samples (typically 15 equilibrated CO_2 gas analyses by discrete session of analysis, 4 analytical sessions in total; Supplementary Table S5). For each analytical session, as recommended in Dennis et al., (2011), the Δ_{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 2 unknown samples (Supplementary Table S5). The Δ_{47} values obtained at IPGP over the course of this study are $\Delta_{47\ \mathrm{CDES90}} = 0.316\pm0.020$‰ (1SD, $n = 16$) for IPGP-Carrara and $\Delta_{47\ \mathrm{CDES90}} = 0.620\pm0.010$‰ (1SD, $n = 18$) for 102-GC-AZ01. Those values are indistinguishable from the values obtained at IPGP over four 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 issued from oxygen isotope compositions were calculated based on the measured $\delta^{18}O_{\mathrm{carbonate}}$ values of both the precipitated carbonate and the precipitation water in each experimental vial (Supplementary 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 $\Delta_{47\ \mathrm{CDES90}}$ data using the composite universal Δ_{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 Δ_{47} (Kelson et al., 2017) (see also Supplementary 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.* ±0.020‰, 1SD) is used for samples with only one measurement or with 1SD lower than 0.020‰ (Supplementary Tables S1 and S5, Supplementary Discussion).

For the temperature (T) derived from the Δ_{47} data, we chose the calibration determined by Bonifacie et al., (2017) as it integrates a consequent number of data (n > 300), which statistical weight have been properly considered, and covers a wide temperature range (from 1 to 350°C), three characteristics that were recently shown by several teams as governing the precision on Δ_{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 Δ_{47} values) allowing to avoid loss of precision/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, Dassié et al., 2018) on the range of temperatures (~30 to 96°C) where most of available calibrations are diverging and/or not well constrained. Indeed, these studies report excellent consistencies: i/ between T Δ_{47} and homogenization temperatures from fluid inclusion microthermometry (Mangenot et al., 2017), and ii/ between the $\delta^{18}O_{water}$ values directly measured in fluid inclusions by cavity ring down spectroscopy and those calculated from combined TΔ_{47} and $\delta^{18}O_{carbonate}$ of the host-mineral (Dassié et al., 2018). Thought we recognize that the normalization to carbonate standards presented in Bernasconi et al. (2018) might become commonly used by the community in the future (ie.

with the on-going inter-comparison 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 run in total of ETH1, ETH2, ETH3, ETH4 standards; Supplementary Table S5), and such 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; Supplementary Table S5 — Note also 33 secondary carbonate standards 102-GC-AZ01 and IPGP-Cararra, also ran in other IPGP studies and some other laboratories). Also remarkably, Δ_{47} obtained here on the four ETH carbonate standards are all systematically higher than values reported in Bernasconi et al., 2018 (Supplementary Table S4). Thought the reason of this positive offset is still unclear, it is noteworthy that positive offsets are also observed when compiling other recent published values (Supplementary Table S4; Daëron et al. 2016; Schauer et al., 2016; Fiebig et al., 2019).

3 Results and Discussion

3.1 Δ_{47} and δ^{18}O compositions of microbial carbonates can present strongly correlated vital effects

We performed Δ_{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 it 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 Δ_{47} offset to equilibrium starts down to -0.270‰ (the largest Δ_{47} offset ever measured in solid carbonates) and the offset to equilibrium reaches -24.7‰ for $\delta^{18}O_{carbonate}$ (Fig. 3 and Supplementary Table S1). Both Δ_{47} and $\delta^{18}O_{carbonate}$ absolute values then increase as ureolysis progresses, reducing offsets from equilibrium values to -0.179‰ for Δ_{47} and -15.7‰ for $\delta^{18}O_{carbonate}$. In the presence of CA, the trends observed for the Δ_{47} and $\delta^{18}O_{carbonate}$ values are similar but the offsets from equilibrium are drastically reduced (down to -0.027‰ for Δ_{47} and -1.4‰ for $\delta^{18}O_{carbonate}$ at the end of the experiment; Fig. 3), hence attesting for on-going isotopic equilibration of DIC with water by CA enzymatic activity prior to and during carbonate precipitation. The comparable behavior of Δ_{47} and $\delta^{18}O_{carbonate}$ values with respect to CA suggests that both disequilibria are inherited from the $\delta^{18}O$ and Δ_{47} signatures of the DIC generated by the biological activity.

3.2 Δ_{47} and $\delta^{18}O_{carbonate}$ disequilibrium originate from the metabolic production of DIC

Here, we discuss the potential processes known to generate $\delta^{18}O$ and Δ_{47} isotope fractionations during carbonate precipitation and we identify the main mechanism explaining our paired Δ_{47} and $\delta^{18}O_{carbonate}$ disequilibria. The relatively high precipitation rate (R) in our experiments ($\log R = -3.95$ mol· m^{-2}· s^{-1}; (Thaler et al., 2017)) can only account for an oxygen kinetic isotope fractionation (KIF) of about 1 to 2‰ for $\delta^{18}O$ values (Watkins et al., 2013), while the oxygen isotope disequilibrium recorded in our carbonates reaches -24.7‰. 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 Material and Methods). 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 Δ_{47} and $\delta^{18}O$ in our microbial carbonates can only result from (i) a KIF induced by CO_2 hydration/hydroxylation into HCO_3^- (but only if ureolysis produces CO_2 rather than H_2CO_3, which has not been established yet (Matsuzaki et al., 2013)) or (ii) a metabolic isotopic 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/hydroxylation leads to the formation of HCO_3^-, with two oxygen atoms coming from CO_2 and the third one from H_2O (hydration) or OH^- (hydroxylation). The $\delta^{18}O_{HCO3-}$ value can then be estimated using a simple mass balance calculation (Létolle et al., 1990b; 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_2O or OH^-, both depleted in ^{18}O relative to ^{16}O in contrast with CO_2 (Green and Taube, 1963;Beck et al., 2005). Such a low $\delta^{18}O_{HCO3-}$ value, several per mil lower than the equilibrium one, can then be preserved in the calcium carbonate if precipitation occurs shortly after CO_2 hydration/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/hydroxylation increases the relative abundance of ^{13}C-^{18}O bonds, and thus the Δ_{47} value (Guo, Ms 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/hydroxylation is demonstrated. This is the case of (i) hyperalkaline travertines (Falk et al., 2016) even though part of the reported kinetic isotope fractionation can be interpreted as resulting from CO_2 dissolution process (Clark et al., 1992) and (ii) two experimental samples (Tang et al., 2014) precipitated at high pH where CO_2 hydroxylation dominates. Both studies show, in agreement with the ab initio calculations (Guo, Ms 2009), higher Δ_{47} and lower $\delta^{18}O_{carbonate}$ values compared to equilibrium. Thus, in a case where ureolysis would produce CO_2 in isotopic equilibrium with water, the Δ_{47} values affected by CO_2 hydration/hydroxylation recorded in calcium carbonates should be higher than the equilibrium value, while our microbial carbonates are showing Δ_{47} values lower than equilibrium. Thus, we conclude that our low Δ_{47} 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 H_2CO_3 or as CO_2, with a Δ_{47} value low enough to compensate for any potentially succeeding increase due to the KIF associated with CO_2 hydration/hydroxylation. Nonetheless, the slow but continuous increase observed in our experiment without CA for both Δ_{47} and $\delta^{18}O_{carbonate}$ 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 re-forming in the DIC, allowing oxygen isotopes (^{16}O, ^{17}O and ^{18}O) to be redistributed between H_2O, OH^-, H_2CO_3, HCO_3^- and CO_3^{2-} species *via* H_2O/OH^- -attachment to CO_2 and -detachment from HCO_3^-. In the experiment with CA, both Δ_{47} and $\delta^{18}O_{carbonate}$ reach simultaneously values close to equilibrium and without CA both Δ_{47} and $\delta^{18}O_{carbonate}$ values increase simultaneously. This coevolution corroborates former observations of comparable kinetics for clumped isotopes and $\delta^{18}O$ equilibration between DIC and water or CO_2 and water once $\delta^{13}C$ is equilibrated (Affek, 2013; Clog et al., 2015). This principle has been used to correct for disequilibrium fractionation factor in speleothems (Affek et al., 2008).

3.3 Erroneous yet comparable temperatures reconstructed from disequilibrium Δ_{47} and $\delta^{18}O$ values in carbonates

Apparent temperatures were calculated from disequilibrium Δ_{47} values obtained in the experiment without CA using the calibration of Bonifacie et al., (2017). Ranging from 198±21°C to 115±8°C (Fig.

4), they are at odds with the actual precipitation temperature of 30±1°C (see Methods). This shows that when carbonates precipitate from DIC in oxygen-isotope disequilibrium with water, the abundance of $^{13}C–^{18}O$ bonds in carbonates does not correlate with precipitation water temperature. Conversely, the temperatures reconstructed from the Δ_{47} values of carbonates precipitated in the presence of CA, ranging from 47±6°C to 39±2°C, are much closer to the actual precipitation temperature. Interestingly, the apparent temperatures reconstructed using Kim and O'Neil et al., (1997) calibration from the $\delta^{18}O_{carbonate}$ and $\delta^{18}O_{water}$ values of the same samples show comparable offsets from the actual temperature in both experiments without CA (from 218±2°C to 139±1°C) and with CA (from 39±1°C to 37±1°C) (Fig. 4). Practically, this implies that similar temperatures calculated from both carbonate Δ_{47} and $\delta^{18}O_{carbonate}$ values (in a case where the precipitation water $\delta^{18}O$ can be determined) can neither constitute evidence against O-isotope disequilibrium nor confirm that this is the true precipitation temperature.

3.4 Δ_{47} and $\delta^{18}O_{carbonate}$ paired disequilibria record the $\delta^{18}O$ of the water in which the carbonates precipitated

The fact that both Δ_{47} and $\delta^{18}O_{carbonate}$ values permit to calculate similarly evolving apparent temperatures along the (dis)equilibration profile recorded in carbonates as the experiment proceeds, indicates that the $\delta^{18}O_{carbonate}$, $\delta^{18}O_{water}$, Δ_{47}, and apparent temperature values are all together linked. In a Δ_{47} versus $\delta^{18}O_{carbonate}$ diagram, all of our data align, irrespectively 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 Δ_{47} and $\delta^{18}O_{carbonate}$ values of calcite precipitated at various temperatures from a water at a given $\delta^{18}O_{water}$ value. This $\delta^{18}O_{water}$ value can be calculated by combining for the same temperature, the equations of Δ_{47} and $\delta^{18}O_{carbonate}$ temperature calibrations from Kim and O'Neil, (1997) and Bonifacie et al., (2017), respectively (Eq. 2):

$$\delta^{18}\mathrm{O}_{\mathrm{water}} = \exp\left[-\frac{18.03}{\sqrt{\frac{0.0422\times10^{6}}{\Delta_{47\ \mathrm{CDES90}}-0.1126}}} + 32.42\times10^{-3} + \ln(\delta^{18}\mathrm{O}_{\mathrm{carbonate}} + 1000)\right] - 1000 \quad (2),$$

with $\delta^{18}O_{water}$ and $\delta^{18}O_{carbonate}$ values in the same isotopic referential (here VSMOW), and Δ_{47} values reported into the absolute Carbon Dioxide Equilibrated Scale ($\Delta_{47\ CDES90}$). The calibration of Kim and O'Neil, (1997) was preferred over more recent calibration equations (e.g. Watkins et al., 2013) because it provides the best consistency for temperatures reconstructed from both the carbonate $\delta^{18}O$ and Δ_{47} values at temperatures above 100°C. Note that Kim and O'Neil, (1997) and Bonifacie et al., (2017) calibrations were developed independently, which prevents circular reasoning. Finally, as Kim and O'Neil, (1997) is the most used calcite calibration to date, it also allows for a broader comparison with previously published results.

Despite the fact that the data present a large range of offsets from equilibrium (Fig. 5), the mean $\delta^{18}O_{water}$ value calculated using Eq. 2 for each combination of Δ_{47} and $\delta^{18}O_{carbonate}$ values measured for our carbonates is -8.0±2.8‰ (1SD), indistinguishable (*i.e.* within errors) from the $\delta^{18}O_{water}$ 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 $\delta^{18}O_{water}$ values found in disequilibrium carbonates is remarkable considering that even for equilibrium carbonates at isotopic equilibrium for both $\delta^{13}C$ and $\delta^{18}O$, $\delta^{18}O_{water}$ can only be retrieved from paired Δ_{47} and $\delta^{18}O_{carbonate}$ values with a precision of ±1‰ at best (see Supplementary information). This opens the promising opportunity to retrieve the $\delta^{18}O$ value of the water in which carbonates precipitated out of equilibrium for both $\delta^{18}O_{carbonate}$ and Δ_{47}.

In order to evaluate the applicability of such an approach to other types of carbonates, Fig. 6 compiles disequilibrium paired $\delta^{18}O_{carbonate}$ and Δ_{47} data from two previously published experimental studies (Tang et al., 2014; Staudigel et al., 2018). These studies were chosen to further evaluate the relevancy of our $\delta^{18}O_{carbonate}$- Δ_{47} correlation because they are the only published dataset reporting full sets of *measured (rather than calculated)* $\delta^{18}O_{water}$, $\delta^{18}O_{carbonate}$ and Δ_{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 $\delta^{18}O_{water}$ proxy could be generalized to a large diversity of carbonates. This thus precludes plotting in Fig. 6 most published Δ_{47} studies on both natural and experimental samples, in which $\delta^{18}O_{water}$ and/or temperature were not directly measured. These two datasets are also recent enough to allow the conversion of their Δ_{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 become the rule rather than the exception in Δ_{47} studies. Fig. 6a shows paired $\delta^{18}O_{carbonate}$ and Δ_{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/hydroxylation prior to precipitation (Tang et al., 2004). Except for these two carbonate samples, the data align on a Δ_{47} versus $\delta^{18}O_{carbonate}$ covariation curve that cannot be explained solely by temperature variation. As for our microbial carbonates obtained with or without CA, the average calculated $\delta^{18}O_{water}$ (Eq. 2; -11.2±1.5‰) matches within error with the measured $\delta^{18}O_{water}$ (-9.6±0.2‰) (Dietzel et al., 2009).

Fig. 6b shows paired $\delta^{18}O_{carbonate}$ and Δ_{47} values of abiotic carbonates that were precipitated during an initial CO_2 degassing + equilibration phase followed by solely equilibration with water at 5, 15 and 25°C (Staudigel et al., 2018). During the latter equilibration phase, even though the carbonates precipitated out of isotopic equilibrium, the paired $\delta^{18}O_{carbonate}$ and Δ_{47} values align on a covariation curve of average calculated $\delta^{18}O_{water}$ value (Eq. 2; -3.0±1.1‰) close to the measured $\delta^{18}O_{water}$ (-0.65‰). As a major outcome of this study, we thus anticipate that reliable $\delta^{18}O_{water}$ values of precipitation water can be retrieved from carbonates presenting Δ_{47} and $\delta^{18}O_{carbonate}$ values in strong disequilibrium.

Some data presented in Fig. 6 also permit to evaluate the conditions of applicability of our approach. In Fig. 6a, the two data points deviating from the Δ_{47} versus $\delta^{18}O_{carbonate}$ covariation curve 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 CO_2 hydration/hydroxylation prior to precipitation (Tang et al., 2014). At pH=10, CO_2 reacts at 95% with OH^- and at 5°C, DIC isotopic equilibration with water takes days. To a lesser extent, the KIF induced by CO_2 hydroxylation seems also visible at pH= 9 (and 40°C) where CO_2 reacts at 82% with OH^- but DIC isotopic equilibration with water at 40°C only takes about 15 hours. As previously detailed, the direction of these isotopic offsets from equilibrium is compatible with ab initio calculations (Guo, Ms, 2009) and can be intuitively understood as follows: in carbonates derived from CO_2 hydroxylation, the $R^{X}_{stochastic}$ term used for the Δ_{47} calculation (Eq. 1) should be strongly modified as the ^{18}O concentration in OH^- and H_2O is lower than in CO_2 and the reaction does not add more ^{13}C than what is present in CO_2. This might explain why in the case of disequilibria acquired through CO_2 hydroxylation, the correlation between paired $\delta^{18}O$ and Δ_{47} disequilibria and the precipitation water $\delta^{18}O$ is not preserved and $\delta^{18}O_{water}$ cannot be reconstructed by the approach proposed here. The negative slope associated with this KIF on the Δ_{47} and $\delta^{18}O_{carbonate}$ diagram (Fig. 6a) is nevertheless a good tool to identify CO_2 hydroxylation reactions.

In Fig. 6b, during the CO_2 degassing phase of the precipitation experiment (Staudigel et al., 2018), the data also deviate from the $\delta^{18}O$ versus Δ_{47} covariation curve. This behavior was interpreted by the authors as a decoupling between Δ_{47} and $\delta^{18}O_{carbonate}$ values due to variable kinetics of ^{12}C-O and ^{13}C-O bounding. 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 hour to equilibrate among the different oxygen-bearing species (*i.e.* CO_2, HCO_3^-,CO_3^{2-},H_2O,

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 CO_2 degassing (Staudigel et al., 2018). We propose here that CO_2 degassing, because it affects both C and O isotopes, modifies the $R^X_{stochastic}$ term (in Eq. 1), thus preventing Δ_{47} and $\delta^{18}O$ to vary with the proportionality that allows to retrieve the $\delta^{18}O_{water}$ value on a Δ_{47} versus $\delta^{18}O$ covariation plot. Hence, as for CO_2 hydroxylation, in case of a KIF induced by CO_2 degassing, $\delta^{18}O_{water}$ cannot be reconstructed exclusively from disequilibrium $\delta^{18}O_{carbonate}$ and Δ_{47} values.

In summary, we conclude that mechanisms that can drastically change the $R^X_{stochastic}$ term in Δ_{47} calculation (such as CO_2 hydroxylation and degassing) prevent $\delta^{18}O_{water}$ reconstructions from paired disequilibrium Δ_{47} and $\delta^{18}O_{carbonate}$ values. Nevertheless, these mechanisms lead to peculiar types of carbonates (*i.e.* speleothems that form in caves from CO_2 degassing, and travertine that form on lands where fluids and gas escape from subsurface reservoirs for CO_2 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 H_2O molecule from the aqueous medium, might give a DIC whose $R^X_{stochastic}$ term in Δ_{47} calculation is already close to that of a DIC under equilibration with the $\delta^{18}O_{water}$. This would explain why even our most extreme out of equilibrium carbonates still fall close to the Δ_{47} versus $\delta^{18}O_{carbonate}$ covariation line corresponding to the real $\delta^{18}O_{water}$ value.

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

The ability to reconstruct precipitation water $\delta^{18}O_{water}$ from disequilibrium Δ_{47} and $\delta^{18}O_{carbonate}$ values further allows to examine the origin of the vital effect observed in organisms for which (i) CO_2 degassing and hydration/hydroxylation KIF can be ruled out, and (ii) only small $\delta^{13}C$ variations are observed, thus preserving the $R^X_{stochastic}$ term in Δ_{47} calculation. We hypothesize that such an approach could open perspectives to understand how Δ_{47} and $\delta^{18}O$ signals are affected by kinetic effects in most of the biogenic carbonates, provided that CO_2 hydroxylation or degassing do not occur prior to carbonate precipitation. This approach could thus be applied to the vast majority of sedimentary carbonates (Milliman et al., 1993) and since deep time (*i.e.* microbialites, brachiopods, bryozoans, bivalves, foraminifera, coccoliths), even when $\delta^{18}O_{carbonate}$ variations occur in the shell of the organism. Additionally, the data presented here stand as an experimental demonstration that the mechanisms controlling carbonate $\delta^{18}O$ equilibration with water (*i.e.* DIC equilibration with water) also control solid carbonate Δ_{47} equilibrium (Watkins et al., 2015). This result can be used to recover information on biomineralization mechanisms. For example, in recent coccolithophorid *Emiliana huxleyi* culture experiments, the calcitic shell produced by the organism systematically yields a 2‰ positive $\delta^{18}O$ offset from equilibrium values while their Δ_{47} values seem to faithfully record precipitation temperature (Katz et al., 2017). These coccolithophorids were grown in waters with different $\delta^{18}O_{water}$ 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 $\delta^{18}O$ disequilibrium should be recorded in solid carbonates if the associated Δ_{47} is at equilibrium, we can assume that the coccoliths precipitated at oxygen-isotope equilibrium and calculate from Eq. 2 the actual $\delta^{18}O$ value of the water in which precipitation took place (respectively shifted by 1.0±0.2‰, 2.1±0.4‰ and 1.1±0.7‰ towards more positive values compared to the $\delta^{18}O_{water}$ value measured for the culture medium water; Fig. 6c). This could reflect a biologically-driven difference between the $\delta^{18}O$ of body water at the precipitation site inside *E. huxleyi* and the $\delta^{18}O$ of ambient water (*i.e.* the culture medium water). This hypothesis is supported by what is known about intracellular precipitation of coccolith performed by coccolithophorids: each coccolith forms from the accumulation of coccolithosomes, which are vesicles containing up to a dozen of 7 nm spherical calcium-rich granular units (Outka and Williams, 1971). Water in these ~100 nm vesicles can be considered as a finite reservoir whose isotopic composition could be modified through isotopic exchange with a DIC affected by metabolic isotope fractionation. Another mechanism that could increase the $\delta^{18}O$ value of a finite water reservoir by equilibrating it with a comparable reservoir of DIC would be the introduction of DIC systematically as CO_2. As HCO_3^- and CO_3^{2-} are enriched in ^{16}O in comparison to CO_2, the CO_2 conversion to HCO_3^- and CO_3^{2-} at equilibrium before precipitation would pump ^{16}O 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 organites 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 mechanism 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, coccoliths precursors precipitate in equilibrium with the body water for oxygen isotopes, but that the body water has a different $\delta^{18}O$ value than the seawater, which explains the observed $\delta^{18}O$ apparent fractionation while their Δ_{47} composition reflects culture temperature (Katz et al., 2017). It has already been highlighted through geochemical analysis of coccoliths, that coccolithosomes water has altered pH (Liu et al., 2018) and ion concentrations (Hermoso et al., 2017) in comparison to seawater. We hypothesize that the internal $\delta^{18}O$ water would thus be another parameter controlled by the coccolithophore algae.

3.6 Ubiquity of the observed $\delta^{18}O_{carbonate}$- $\delta^{18}O_{water}$-Δ_{47}-temperature covariations in both equilibrium *and* disequilibrium carbonates

As shown above, in a Δ_{47} versus $\delta^{18}O_{carbonate}$ diagram, disequilibrium carbonates precipitated at fixed temperature plot on the theoretical line of equilibrium carbonates precipitated with a similar $\delta^{18}O_{water}$ 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 Figs. 6a and b for datasets from Tang et al., (2014) and Staudigel et al., (2018)) align with equilibrium data series. In other words, the values of the disequilibrium $1000\ln\alpha_{carbonate\text{-}water}$ for oxygen isotopes (with $\alpha = \frac{\delta^{18}O_{carbonate}+1000}{\delta^{18}O_{water}+1000}$) are similar to the equilibrium $1000\ln\alpha_{carbonate\text{-}water}$ for any given, and independently determined, apparent Δ_{47} temperature (Fig. 7). In details, our closest to equilibrium data recording low apparent temperatures match better the predicted equations from Coplen, (2007) and Watkins et al.,(2013), recently updated (Daëron et al., 2019). This latter calibration is based on carbonates from two caves where calcite precipitate extremely slowly and is thus assumed to have precipitated at equilibrium. Note that the use of these two cave samples for determining the dependence to temperature of the equilibrium $1000\ln\alpha_{carbonate\text{-}water}$ relies on the assumption that constant environmental conditions, including temperature in the two caves (7.9 and 33.7°C) and the $\delta^{18}O_{water}$ 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 the predicted equation of Kim and O’Neil, (1997). This $1000\ln\alpha_{carbonate\text{-}water}$ dependence to temperature was established on carbonates precipitated in the laboratory at well-known $\delta^{18}O_{water}$ and temperatures (from 10 to 40°C), but suspected to present a small KIF due to a high precipitation rate that lowers the value of the $1000\ln\alpha_{carbonate\text{-}water}$ (Watkins et al., 2013). Despite this, we used this equation to retrieve the $\delta^{18}O_{water}$ from our experimental carbonates, because most of them are associated with high apparent Δ_{47} temperatures. Coplen, (2007) or Watkins et al., (2013) equations would have return 2‰ lower values (ca. -10±2‰ compared to -8±3‰ calculated with Kim and O'Neil (1997) equation). From our results, due to our experimental condition 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 $1000\ln\alpha_{\text{carbonate-water}}$ at both high and low temperatures in order to improve the accuracy and precision of our new proxy for reconstructing the $\delta^{18}O_{\text{water}}$ from which carbonates, even disequilibrium ones, precipitated.

Importantly, we here establish a new method to determine the equilibrium $1000\ln\alpha_{\text{carbonate-water}}$, which consists in using the kinetics of Δ_{47} and $\delta^{18}O$ 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 $1000\ln\alpha_{\text{carbonate-water}}$ at high temperatures for which the differences between the two most popular $1000\ln\alpha_{\text{carbonate-water}}$ dependence to temperature equations (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 et al., 2018) were designed for the purpose of calibrating the equilibrium $1000\ln\alpha_{\text{carbonate-water}}$. 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 $\delta^{13}C$ values (~3‰) (Thaler et al., 2017), and the presence of traces of aragonite and vaterite in our carbonates (Supplementary Information) lower the accuracy of the reconstructed equilibrium $1000\ln\alpha_{\text{carbonate-water}}$ values.

As a broader perspective, we anticipate that such an approach will help in determining critical equilibrium fractionation factors for other gaseous isotopic systems (such as isotopologues of molecules containing S-O bounds) or minerals of prime interest in biology and geology if clumped isotopes measurements expand further beyond gaseous mass spectrometry (*e.g.* bounding between Fe-O, Fe-S, Ca-C).

4. Conclusions

Our experimental results show that the information held in disequilibrium (and apparent disequilibrium) carbonates is diverse and promising. First, a paired Δ_{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 retrieving 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 Δ_{47} disequilibria or apparent disequilibria. Hence, except in the case of processes such as CO_2 degassing and CO_2 hydration/hydroxylation, which likely modify the $R^{X}_{stochastic}$ term in Δ_{47} calculation, paired Δ_{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 Δ_{47} versus $\delta^{18}O_{carbonates}$ covariation diagram 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 open 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 Supplementary Information.

Author contributions

C.T. and A.K. conceived the research. C.T. performed the microbial precipitation experiment and the $\delta^{13}C$ and $\delta^{18}O$ analyses during her PhD thesis under M.A. and B.M. supervision. A.K. performed the Δ_{47} analyses during her PhD thesis under M.B. supervision. C.T. took the lead in the interpretation of the results and the writing of the original draft. All authors provided critical feedback and helped shaping the research, analyses and manuscript.

Competing interests The authors declare no competing financial interests.

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References

Affek, P. A., Bar-Matthews, M., Ayalon, A., Matthews, A. & Eiler, J. M.: Glacial/interglacial temperature variations in Soreq cave speleothems as recorded by “clumped isotope” thermometry. *Geochim. Cosmochim. Acta* **72**, 5351–5360, https://doi.org/10.1016/j.gca.2008.06.031, 2008.

Affek, H. P.: Clumped isotopic equilibrium and the rate of isotope exchange between CO_2 and water. *Am. J. Sci.* **313**, 309–325, doi: 10.2475/04.2013.02, 2013.

Affek, H. P. Matthews, A., Ayalon, A., Bar-Matthews, M., Burstyn, Y., Zaarur, S., & Zilberman, T.: Accounting for kinetic isotope effects in Soreq Cave (Israel) speleothems. *Geochim. Cosmochim. Acta* **143**, 303–318, https://doi.org/10.1016/j.gca.2014.08.008, 2014.

Affek, H. P. & Zaarur, S.: Kinetic isotope effect in CO_2 degassing: Insight from clumped and oxygen isotopes in laboratory precipitation experiments. *Geochim. Cosmochim. Acta* **143**, 319–330, https://doi.org/10.1016/j.gca.2014.08.005, 2014.

Bajnai, D., Fiebig, J., Tomašových, A., Garcia, S. M., Rollion-Bard, C., Raddatz, J., Löffler, N.,Primo-Ramos, C. & Brand, U.: Assessing kinetic fractionation in brachiopod calcite using clumped isotopes. *Sci. Rep*. **8**, 533, https://doi.org/10.1038/s41598-017-17353-7, 2018.

Beck, W. C., Grossman, E. L. & Morse, J. W.: Experimental studies of oxygen isotope fractionation in the carbonic acid system at 15, 25, and 40 °C. *Geochim. Cosmochim. Acta* **69**, 3493–3503, https://doi.org/10.1016/j.gca.2005.02.003, 2005.

Bernasconi, S. M., Müller, I. A., Bergmann, K. D., Breitenbach, S. F., Fernandez, A., Hodell, D. A., Jaggi, M., Meckler, A. N., Millan, I., & Ziegler, M. Reducing uncertainties in carbonate clumped isotope analysis through consistent carbonate-based standardization. *Geochem. Geophy. Geosy.* **19**, 2895-2914, https://doi.org/10.1029/2017GC007385, 2018.

Bonifacie, M. Calmels, D., Eiler, J. M., Horita, J., Chaduteau, C., Vasconcelos, C., Agrinier, P., Katz, A., Passey, B. H., Ferry, J. M., & Bourrand, J. J..: Calibration of the dolomite clumped isotope thermometer from 25 to 350° C, and implications for a universal calibration for all (Ca, Mg, Fe)CO_3 carbonates. *Geochim. Cosmochim. Acta* **200**, 255–279, https://doi.org/10.1016/j.gca.2016.11.028, 2017.

Brand, W. A., Assonov, S. S. & Coplen, T. B.: Correction for the ^{17}O interference in $\delta^{13}C$ measurements when analyzing CO_2 with stable isotope mass spectrometry (IUPAC Technical Report). *Pure Appl. Chem.* **82**, 1719–1733, https://doi.org/10.1351/PAC-REP-09-01-05, 2010.

Burgener, L. K., Huntington, K. W., Sletten, R., Watkins, J. M., Quade, J., & Hallet, B.: Clumped isotope constraints on equilibrium carbonate formation and kinetic isotope effects in freezing soils. *Geochim. Cosmochim. Acta* **235**, 402–430, https://doi.org/10.1016/j.gca.2018.06.006, 2018.

Clark, I. D., Fontes, J. C. & Fritz, P.: Stable isotope disequilibria in travertine from high pH waters: laboratory investigations and field observations from Oman. *Geochim. Cosmochim. Acta* **56**, 2041-2050, https://doi.org/10.1016/0016-7037(92)90328-G, 1992.

Clog, M., Stolper, D. & Eiler, J. M.: Kinetics of CO_2 (g)–H_2O (1) isotopic exchange, including mass 47 isotopologues. *Chem. Geol.* **395**, 1–10, https://doi.org/10.1016/j.chemgeo.2014.11.023, 2015.

Coplen, T. B.: Calibration of the calcite–water oxygen-isotope geothermometer at Devils Hole, Nevada, a natural laboratory. *Geochim. Cosmochim. Acta* **71**, 3948–3957, https://doi.org/10.1016/j.gca.2007.05.028, 2007.

Daëron, M., Blamart, D., Peral, M. & Affek, H. P.: Absolute isotopic abundance ratios and the accuracy of Δ_{47} measurements. *Chem. Geol.* **442**, 83–96, https://doi.org/10.1016/j.chemgeo.2016.08.014, 2016.

Daëron, M., Drysdale, R. N., Peral, M., Huyghe, D., Blamart, D., Coplen, T. B., Lartaud, F. & Zanchetta, G.: Most Earth-surface calcites precipitate out of isotopic equilibrium. *Nat. Commun.* **10**, 429, https://doi.org/10.1038/s41467-019-08336-5, 2019.

Dassié E. Genty, D., Noret, A., Mangenot, X., Massault, M., Lebas, N., Duhamel, M., Bonifacie, M., Gasparrini, M., Minster B., and Michelot, J.L.**:** A newly designed analytical line to examine the reproducibility of fluid inclusion isotopic compositions in small carbonate samples. *Geochem. Geophy. Geosy.* **19**, 1107-1122. https://doi.org/10.1002/2017GC007289, 2018.

Dennis, K. J., Affek, H. P. & Schrag, D.: Defining an absolute reference frame for ‘clumped’ isotope studies of CO_2. *Geochim. Cosmochim. Acta* **75**, 7117–7131, https://doi.org/10.1016/j.gca.2011.09.025, 2011.

Dietzel, M., Tang, J., Leis, A. & Köhler, S. J.: Oxygen isotopic fractionation during inorganic calcite precipitation—Effects of temperature, precipitation rate and pH. *Chem. Geol.* **268**, 107–115 , https://doi.org/10.1016/j.chemgeo.2009.07.015, 2009.

Eiler, J. M.: Paleoclimate reconstruction using carbonate clumped isotope thermometry. *Quat. Sci. Rev.* **30**, 3575–3588, https://doi.org/10.1016/j.quascirev.2011.09.001, 2011.

Falk, E. S., Guo, W., Paukert, A. N., Matter, J. M., Mervine, E. M. & Kelemen, P. B.: Controls on the stable isotope compositions of travertine from hyperalkaline springs in Oman: Insights from clumped isotope measurements. *Geochim. Cosmochim. Acta* **192**, 1-28, https://doi.org/10.1016/j.gca.2016.06.026, 2016.

Fernandez, A., Müller, I. A., Rodríguez-Sanz, L., van Dijk, J., Looser, N., & Bernasconi, S. M.. A reassessment of the precision of carbonate clumped isotope measurements: Implications for calibrations and paleoclimate reconstructions. *Geochem., Geophy., Geosystems* **18**, 4375–4386, https://doi.org/10.1002/2017GC007106, 2017.

Fiebig, J., Bajnai, D., Löffler, N., Methner, K., Krsnik, E., Mulch, A., & Hofmann, S. Combined high-precision Δ48 and Δ47 analysis of carbonates. *Chem. Geol.*, **522**, 186-191, https://doi.org/10.1016/j.chemgeo.2019.05.019, 2019.

Ghosh, P., Adkins, J., Affek H., Balta B., Guo W., Schauble E., Schrag D., & Eiler J.: ^{13}C–^{18}O bonds in carbonate minerals: a new kind of paleothermometer. *Geochim. Cosmochim. Acta* **70**, 1439–1456, https://doi.org/10.1016/j.gca.2005.11.014, 2006.

Green, M. & Taube, H.: Isotopic fractionation in the OH–H_2O exchange reaction. *J. Phys. Chem.* **67**, 1565–1566, https://doi.org/10.1021/j100801a507, 1963.

Guo, W. Carbonate clumped isotope thermometry: application to carbonaceous chondrites and effects of kinetic isotope fractionation. Ph. D. thesis, California Institute of Technology, 261 p. (2009).

Guo, W., Mosenfelder, J. L., Goddard, W. A. III & Eiler, J. M.: Isotopic fractionations associated with phosphoric acid digestion of carbonate minerals: insights from first principles theoretical modeling and clumped isotope measurements. *Geochim. Cosmichim. Acta* **73**, 7203–7225, https://doi.org/10.1016/j.gca.2009.05.071, 2009.

Henkes, G. A. Passey, B. H., Grossman, E. L., Shenton, B. J., Yancey, T. E., & Pérez-Huerta, A.: Temperature evolution and the oxygen isotope composition of Phanerozoic oceans from carbonate clumped isotope thermometry. *Earth Planet. Sci. Lett.* **490**, 40–50, https://doi.org/10.1016/j.epsl.2018.02.001, 2018.

Hermoso, M., Lefeuvre, B., Minoletti, F., de Rafélis, M.: Extreme strontium concentrations reveal specific biomineralization pathways in certain coccolithophores with implications for the Sr/Ca paleoproductivity proxy. *PLoS ONE* **12**, e0185655, https://doi.org/10.1371/journal.pone.0185655, 2017.

Katz, A., Bonifacie, M., Hermoso, M., Cartigny, P. & Calmels, D.: Laboratory-grown coccoliths exhibit no vital effect in clumped isotope (Δ_{47}) composition on a range of geologically relevant temperatures. *Geochim. Cosmochim. Acta* **208**, 335–353, https://doi.org/10.1016/j.gca.2017.02.025, 2017.

Kelson, J. R., Huntington, K. W., Schauer, A. J., Saenger, C. & Lechler, A. R.: Toward a universal carbonate clumped isotope calibration: Diverse synthesis and preparatory methods suggest a single temperature relationship. *Geochim. Cosmochim. Acta* **197**, 104–131, https://doi.org/10.1016/j.gca.2016.10.010, 2017.

Kim, S. T. & O'Neil, J. R.: Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochim. Cosmochim. Acta* **61**, 3461–3475, https://doi.org/10.1016/S0016-7037(97)00169-5, 1997.

Kluge, T., Affek, H. P., Dublyansky, Y. & Spötl, C.: Devils Hole paleotemperatures and implications for oxygen isotope equilibrium fractionation. *Earth Planet. Sci. Lett.* **400**, 251-260, https://doi.org/10.1016/j.epsl.2014.05.047, 2014.

Krajewska, B. Ureases I. Functional, catalytic and kinetic properties: A review. *J. Mol. Catal.kel B: Enzymatic*, **59**, 9-21, https://doi.org/10.1016/j.molcatb.2009.01.003, 2009.

Krebs, H. A., & Roughton, F. J. W. Carbonic anhydrase as a tool in studying the mechanism of reactions involving H_2CO_3, CO_2 or HCO_3^-. *Biochem. J.*, **43**, 550, https://doi.org/10.1042/bj0430550, 1948.

Létolle, R., Gegout, P., Gaveau, B. & Moranville-Regourd, M.: Isotope fractionation of 18O during precipitation of carbonates at very high pH. *C. R. Acad. Sci. II* **310**, 547–552, 1990b.

Liu, Y. W., Eagle, R. A., Aciego, S. M., Gilmore, R. E. & Ries, J. B.: A coastal coccolithophore maintains pH homeostasis and switches carbon sources in response to ocean acidification. *Nat. Commun*. **9**, 2857, https://doi.org/10.1038/s41467-018-04463-7, 2018.

Loyd, S. J. Sample, J., Tripati, R. E., Defliese, W. F., Brooks, K., Hovland, Torres M., Marlow, J., Hancock, L.G., Martin, R. & Lyons, T.: Methane seep carbonates yield clumped isotope signatures out of equilibrium with formation temperatures. *Nat. Commun.* **7**, 12274, https://doi.org/10.1038/ncomms12274, 2016.

Mangenot, X., Bonifacie, M., Gasparrini, M., Götz, A., Chaduteau, C., Ader, M., & Rouchon, V.: Coupling Δ_{47} and fluid inclusion thermometry on carbonate cements to precisely reconstruct the temperature, salinity and δ^{18}O of paleo-groundwater in sedimentary basins. *Chem. Geol.* **472**, 44–57, https://doi.org/10.1016/j.chemgeo.2017.10.011, 2017.

Mangenot X., Gasparrini M., Rouchon V., Bonifacie M. Basin-scale thermal and fluid-flow histories revealed by clumped isotope (D47) - Middle Jurassic reservoirs of the Paris Basin. *Sedimentology* **65**, 123-150. DOI: 10.1111/sed.12427, (2018a).

Mangenot X., Gerdes A., Gasparrini M., Bonifacie M., Rouchon V. An emerging thermo-chronometer for carbonate-bearing rocks : Δ47/(U-Pb). *Geology* **46**, 1067-1070. Doi:10.1130/G45196.1, (2018b).

Matsuzaki, Y. Yamada, H., Chowdhury, F. A., Higashii, T., Kazama, S., & Onoda, M.: Ab initio study of CO_2 capture mechanisms in monoethanolamine aqueous solution: reaction pathways from carbamate to bicarbonate. *Energy Procedia* **37**, 400–406, https://doi.org/10.1016/j.egypro.2013.05.124, 2013.

Milliman, J. D.: Production and accumulation of calcium carbonate in the ocean: budget of a nonsteady state. *Global Biogeochemical Cycles*, **7**, 927-957, https://doi.org/10.1029/93GB02524, 1993.

Millo, C. Dupraz, S., Ader, M., Guyot, F., Thaler, C., Foy, E., & Ménez, B.: Carbon isotope fractionation during calcium carbonate precipitation induced by ureolytic bacteria. *Geochim. Cosmochim. Acta* **98**, 107–124, https://doi.org/10.1016/j.gca.2012.08.029, 2012.

Mobley, H. L., & Hausinger, R. P.. Microbial ureases: significance, regulation, and molecular characterization. *Microbiol. Mol. Biol. R.*, **53**, 85-108, 1989.

Outka, D. E., & Williams, D. C.: Sequential coccolith morphogenesis in *Hymenomonas carterae*. *J. Protozool.* **18**, 285–297, https://doi.org/10.1111/j.1550-7408.1971.tb03319.x, 1971.

Rollion-Bard, C., Chaussidon, M. & France-Lanord, C.: pH control on oxygen isotopic composition of symbiotic corals. *Earth Planet. Sci. Lett.* **215**, 275–288, https://doi.org/10.1016/S0012-821X(03)00391-1, 2003.

Saenger, C. Affek, H. P., Felis, T., Thiagarajan, N., Lough, J. M., & Holcomb, M.: Carbonate clumped isotope variability in shallow water corals: Temperature dependence and growth-related vital effects. *Geochim. Cosmochim. Acta* **99**, 224–242, https://doi.org/10.1016/j.gca.2012.09.035, 2012.

Schauer A. J., Kelson J. R., Saenger C. and Huntington K. W. Choice of 17O correction affects clumped isotope (D47) values of CO2 measured with mass spectrometry. *Rapid Commun. Mass Spectrom.* **30**, 2607–2616, doi: 10.1002/rcm.7743, 2016.

Spooner, P. T. Guo, W., Robinson, L. F., Thiagarajan, N., Hendry, K. R., Rosenheim, B. E., & Leng, M. J.: Clumped isotope composition of cold-water corals: A role for vital effects?. *Geochim. Cosmochim. Acta* **179**, 123–141, https://doi.org/10.1016/j.gca.2016.01.023, 2016.

Staudigel, P. T., & Swart, P. K.: A kinetic difference between ^{12}C-and ^{13}C-bound oxygen exchange rates results in decoupled δ^{18}O and Δ_{47} values of equilibrating DIC solutions. *Geochem. Geophys. Geosystems* **19**, 2371–2383, https://doi.org/10.1029/2018GC007500, 2018.

Tang, J., Dietzel, M., Fernandez, A., Tripati, A. K. & Rosenheim, B. E.: Evaluation of kinetic effects on clumped isotope fractionation (Δ_{47}) during inorganic calcite precipitation. *Geochim. Cosmochim. Acta* **134**, 120–136, https://doi.org/10.1016/j.gca.2014.03.005, 2014.

Thaler, C. Millo, C., Ader, M., Chaduteau, C., Guyot, F., & Ménez, B.: Disequilibrium δ^{18}O values in microbial carbonates as a tracer of metabolic production of dissolved inorganic carbon. *Geochim. Cosmochim. Acta* **199**, 112–129, https://doi.org/10.1016/j.gca.2016.10.051, 2017.

Thiagarajan, N., Adkins, J. & Eiler, J.: Carbonate clumped isotope thermometry of deep-sea corals and implications for vital effects. *Geochim. Cosmochim. Acta* **75**, 4416-4425, https://doi.org/10.1016/j.gca.2011.05.004, 2011.

Tripati, A. K., Hill, P. S., Eagle, R. A., Mosenfelder, J. L., Tang, J., Schauble, E. A., Eiler, J. M., Zeebee, R. E., Uchikawa, J., Coplen, T. B., Ries, J. B. & Drew, H.: Beyond temperature: Clumped isotope signatures in dissolved inorganic carbon species and the influence of solution chemistry on carbonate mineral composition. *Geochim. Cosmochim. Acta* **166**, 344–371, https://doi.org/10.1016/j.gca.2015.06.021, 2015.

Urey, H. C., Lowenstam, H. A., Epstein, S. & McKinney, C.R. Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and the south-eastern United States. *Geol. Soc. Am. Bull.* **62**, 399–416, https://doi.org/10.1130/0016-7606(1951)62[399:MOPATO]2.0.CO;2, 1951.

Usdowski, E., Michaelis, J., Bottcher, M. E. & Hoefs, J.: Factors for the oxygen isotope equilibrium fractionation between aqueous and gaseous CO_2, carbonic-acid, bicarbonate, carbonate, and water (19 °C). *Z. Phys. Chem.* **170**, 237–249, 1991.

Watkins, J. M., Nielsen, L. C., Ryerson, F. J. & DePaolo, D. J.: The influence of kinetics on the oxygen isotope composition of calcium carbonate. *Earth Planet. Sci. Lett.* **375**, 349–360, https://doi.org/10.1016/j.epsl.2013.05.054, 2013.

Watkins, J. M. & Hunt, J. D.: A process-based model for non-equilibrium clumped isotope effects in carbonates. *Earth Planet. Sci. Lett.* **432**, 152–165, https://doi.org/10.1016/j.epsl.2015.09.042, 2015.

Zeebe, R. E. & Wolf-Gladrow, D.: *CO_2 in Seawater: Equilibrium, Kinetics, Isotopes*. Elsevier Oceanography Book Series,. **v. 65**. 346 pp. 2001

Figures & Figure Legends

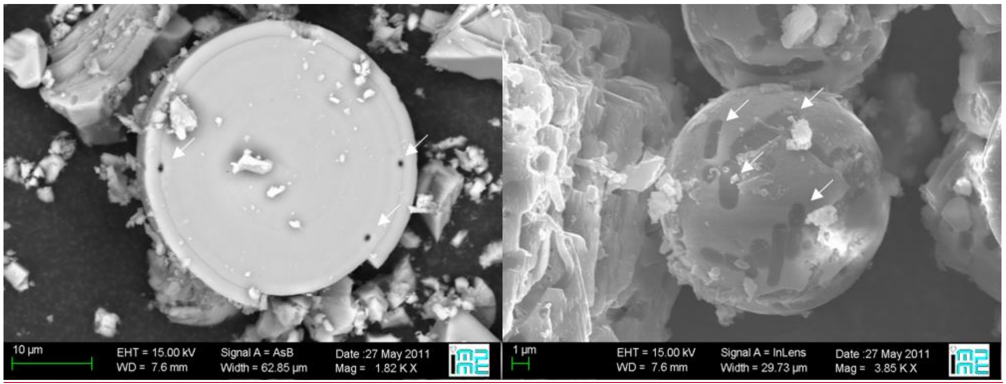

Figure 1 | Scanning electron microscopy images of the bio-induced carbonates formed with CA (almost pure calcite and traces of vaterite and aragonite as determined using X-ray diffraction (Thaler et al., 2017). The fingerprintings of *Sporosarcina pasteurii* cells are visible as black holes (on the left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

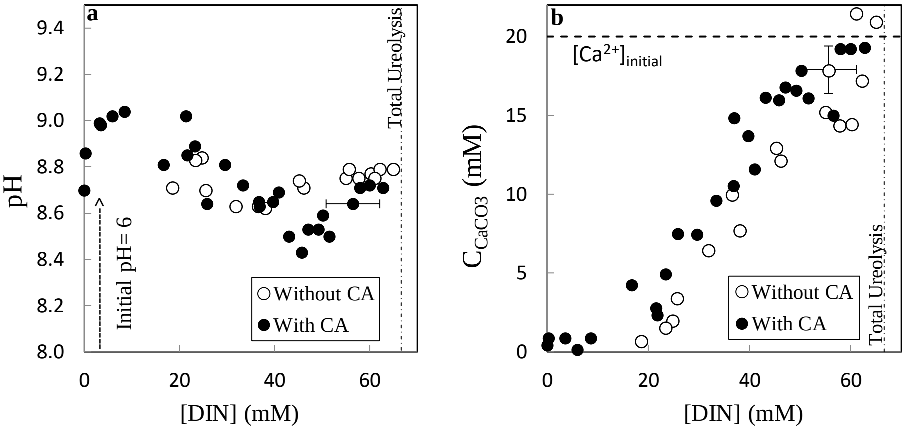

Figure 2 | Evolution of pH (**a**) and amount of precipitated calcium carbonate C_{CaCO3} (**b**) as a function of the production of dissolved inorganic nitrogen DIN (DIN = NH_3+NH_4^+) by bacteria during ureolysis, without carbonic anhydrase (CA) (this study) and with CA (data from ref. (15)). Error bars account for 2SD 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 Supplementary Discussion.

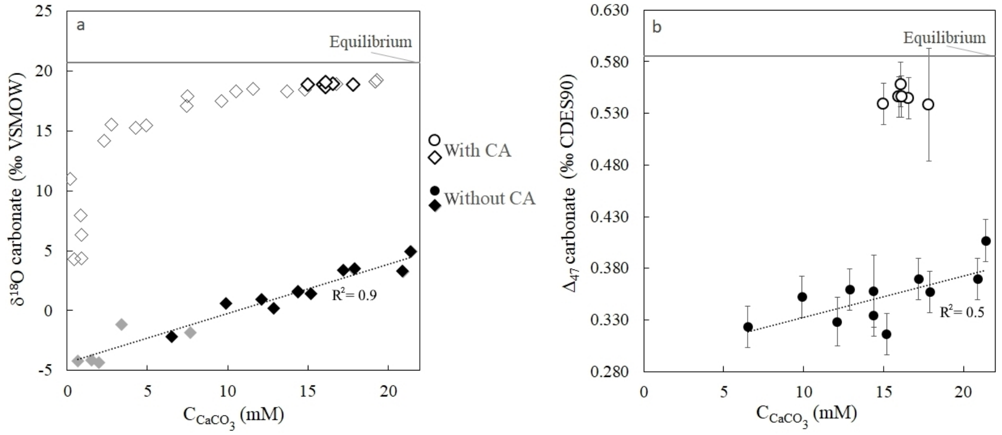

Figure 3 | Strong δ^{18}O and Δ_{47} disequilibria recorded in microbial carbonates as shown by $\delta^{18}O_{carbonate}$ (**a**) and Δ_{47} (**b**) values of calcium carbonates ($CaCO_3$) precipitated during bacterial ureolysis at 30°C (with and without carbonic anhydrase, CA; open and solid symbols, respectively) as a function of carbonate accumulation (C_{CaCO3}). Black symbols correspond to samples for which both Δ_{47} and δ^{18}O measurements were performed. The grey horizontal lines are equilibrium $\delta^{18}O_{carbonate}$ and Δ_{47} values at 30°C for calcite following Bonifacie et al., (2017) and Kim and O'Neil, (1997) calibrations, respectively. Uncertainties (one standard deviation, 1SD) are smaller than symbol for δ^{18}O and C_{CaCO3} values (Supplementary Table ~~1~~S1). Reported Δ_{47} uncertainties are detailed in Methods and Supplementary Discussion.

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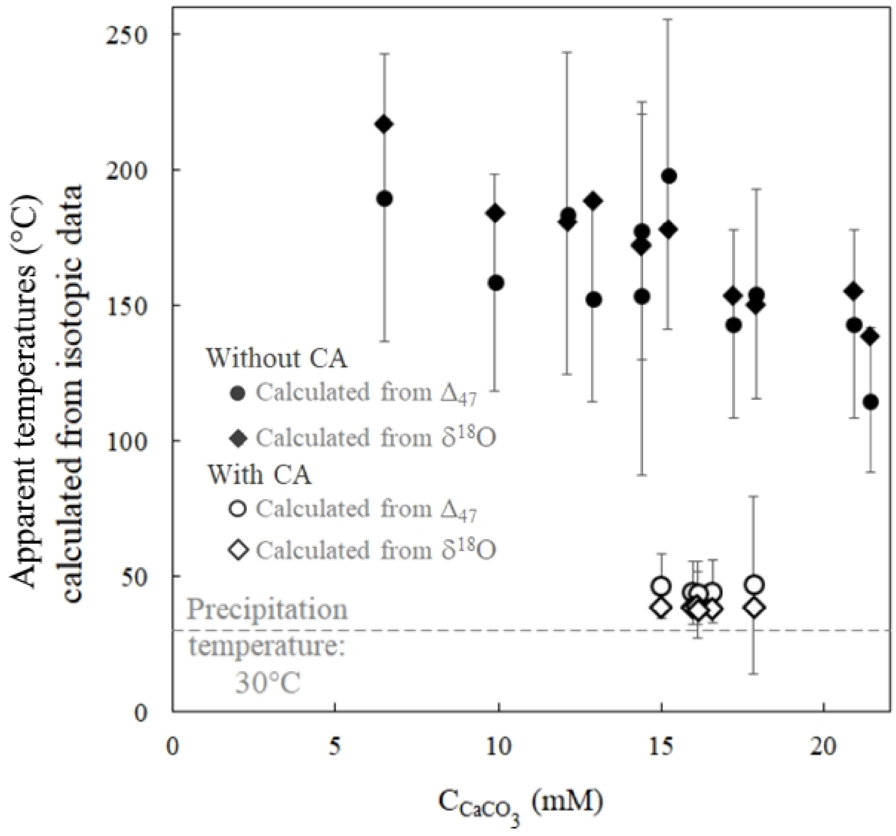

Figure ~~2~~4 | $\delta^{18}O_{carbonate}$ and Δ_{47} disequilibria in microbial carbonates induce comparable biased estimates of precipitation temperature as illustrated by apparent temperatures calculated from the carbonate $\delta^{18}O_{carbonate}$ and Δ_{47} signatures as a function of $CaCO_3$ 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 $\delta^{18}O_{carbonate}$ and Δ_{47} calibrations to temperature of Bonifacie et al., (2017) and Kim and O'Neil, (1997). Reported uncertainties were calculated as the propagation of the one standard deviation (1SD) error of the isotopic data in the calibration equations (Supplementary Information).

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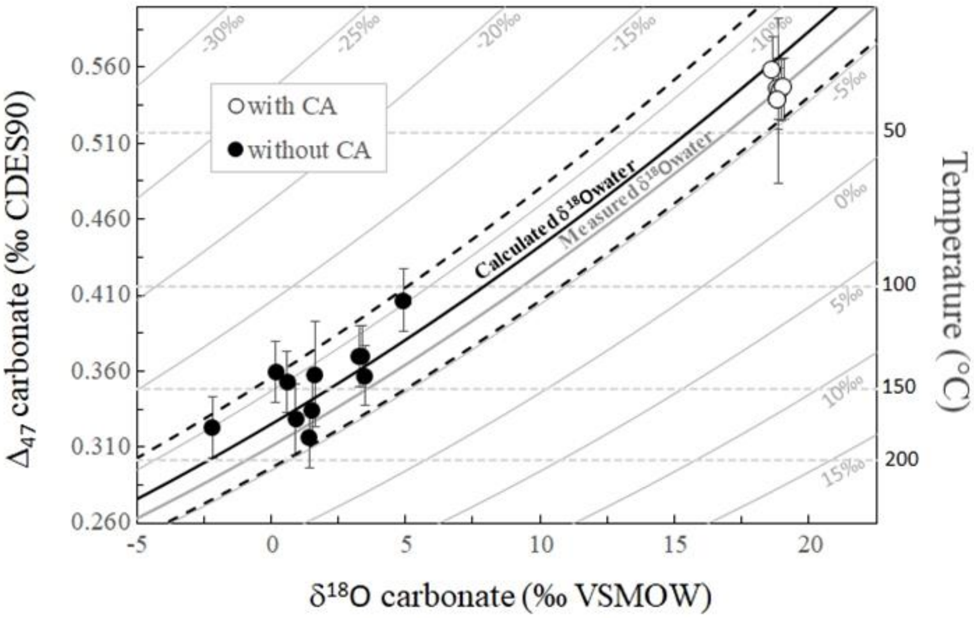

Figure ~~3~~5 | Combined $\delta^{18}O_{carbonate}$ and Δ_{47} disequilibria of microbial carbonates precipitated at 30°C allow reconstruction of the $\delta^{18}O$ of the water ($\delta^{18}O_{water}$) in which they precipitate. Solid grey curves represent the calculated Δ_{47} and $\delta^{18}O_{carbonate}$ compositions of carbonates precipitated at oxygen isotope equilibrium from water with fixed $\delta^{18}O_{water}$ values (indicated on each curve) and variable temperatures. Horizontal dashed grey lines are calculated for fixed temperatures and variable $\delta^{18}O_{water}$. The average $\delta^{18}O_{water}$ 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 $\delta^{18}O_{water}$ calculated with Eq. 1 (-8.0±2.8‰) with its associated errors (dashed black curves).

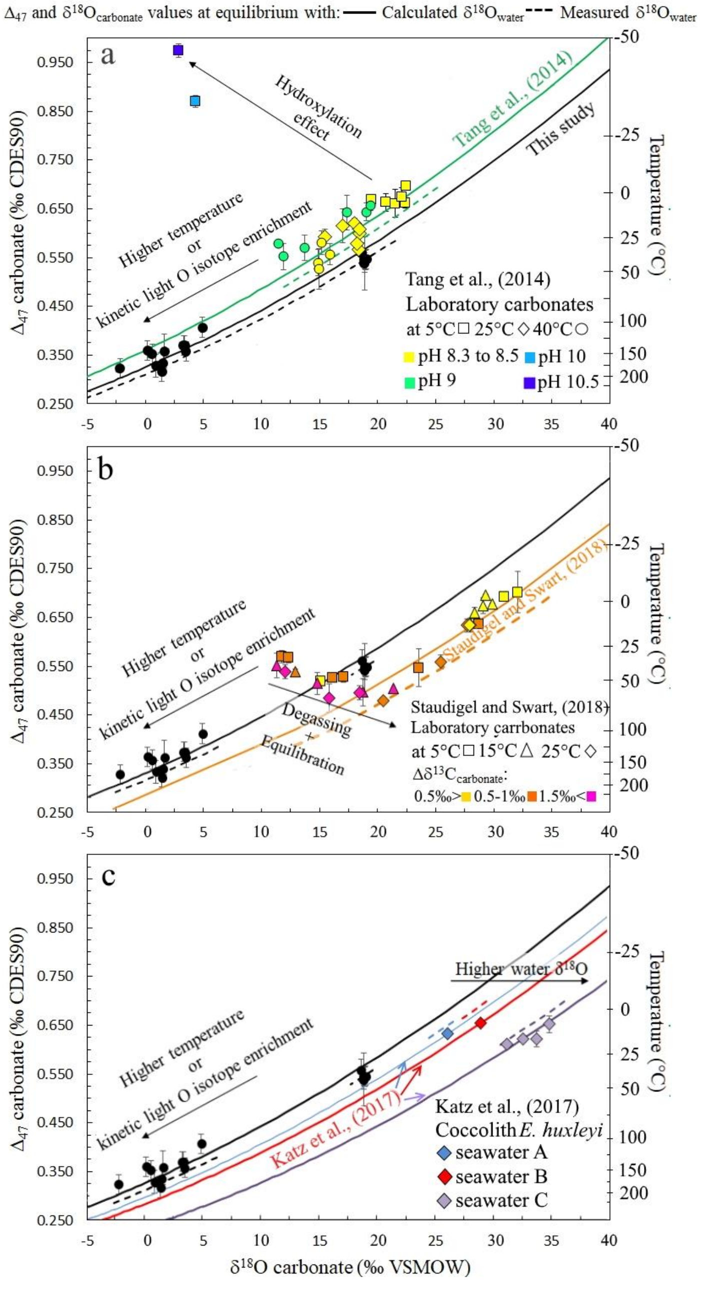
Δ47 and δ18Ocarbonate values at equilibrium with: Calculated δ18Owater --- Measured δ18Owater
a
Hydroxylation effect
Tang et al., (2014)
This study
Higher temperature or kinetic light O isotope enrichment
Tang et al., (2014)
Laboratory carbonates
at 5°C □ 25°C ◇ 40°C ○
pH 8.3 to 8.5
pH 10
pH 9
pH 10.5
b
Higher temperature or kinetic light O isotope enrichment
Degassing + Equilibration
Staudigel and Swart, (2018)
Staudigel and Swart, (2018)
Laboratory carrbonates
at 5°C □ 15°C △ 25°C ◇
Δδ13Ccarbonate:
0.5‰> 0.5-1‰ 1.5‰<
c
Higher water δ18O
Higher temperature or kinetic light O isotope enrichment
Katz et al., (2017)
Katz et al., (2017)
Coccolith E. huxleyi
seawater A
seawater B
seawater C
Δ47 carbonate (‰ CDES90)
Temperature (°C)
δ18O carbonate (‰ VSMOW)
0.950
0.850
0.750
0.650
0.550
0.450
0.350
0.250
-50
-25
0
25
50
100
150
200
-5
0
5
10
15
20
25
30
35
40

Figure ~~4~~6 | Δ_{47} and $\delta^{18}O_{carbonate}$ relationship to precipitation water $\delta^{18}O_{water}$ for other solid carbonates presenting oxygen isotope disequilibria. In (**a**) to (**c**) black data series (this study, performed at 30°C) shows how kinetic oxygen isotope fractionation in the DIC prior to carbonate precipitation can be mistaking for high temperature isotopic equilibrium. Similarly to Fig. 3, the solid curves were obtained using the $\delta^{18}O_{water}$ calculated with Eq. 1. (**a**) Abiotic carbonates from Tang et al., (2014) illustrating the effect of CO_2 hydroxylation on Δ_{47} and $\delta^{18}O_{carbonate}$ values (various pH plotted with different colors, various temperatures plotted with different symbols). (**b**) Abiotic carbonates from Staudigel and Swart, (2019) illustrating the effect of CO_2 degassing and DIC oxygen isotope equilibration with water on Δ_{47} and $\delta^{18}O_{carbonate}$ values. $\Delta\delta^{13}C_{carbonate}$ stands for the difference between the $\delta^{13}C$ value measured in carbonates and the final $\delta^{13}C$ of the data series at the end of equilibration (various $\Delta\delta^{13}C$ ranges plotted with different colors, various temperatures plotted with different symbols). (**c**) Coccolithophorid *E. huxleyi* grown at 7, 10, 15, 20 and 25°C from Katz et al., (2017) showing how coccoliths with equilibrium Δ_{47} values record the equilibrium $\delta^{18}O_{water}$ of their body water, which differs from that of the culture medium (*i.e.* artificial seawaters A, B, and C plotted with different colors).

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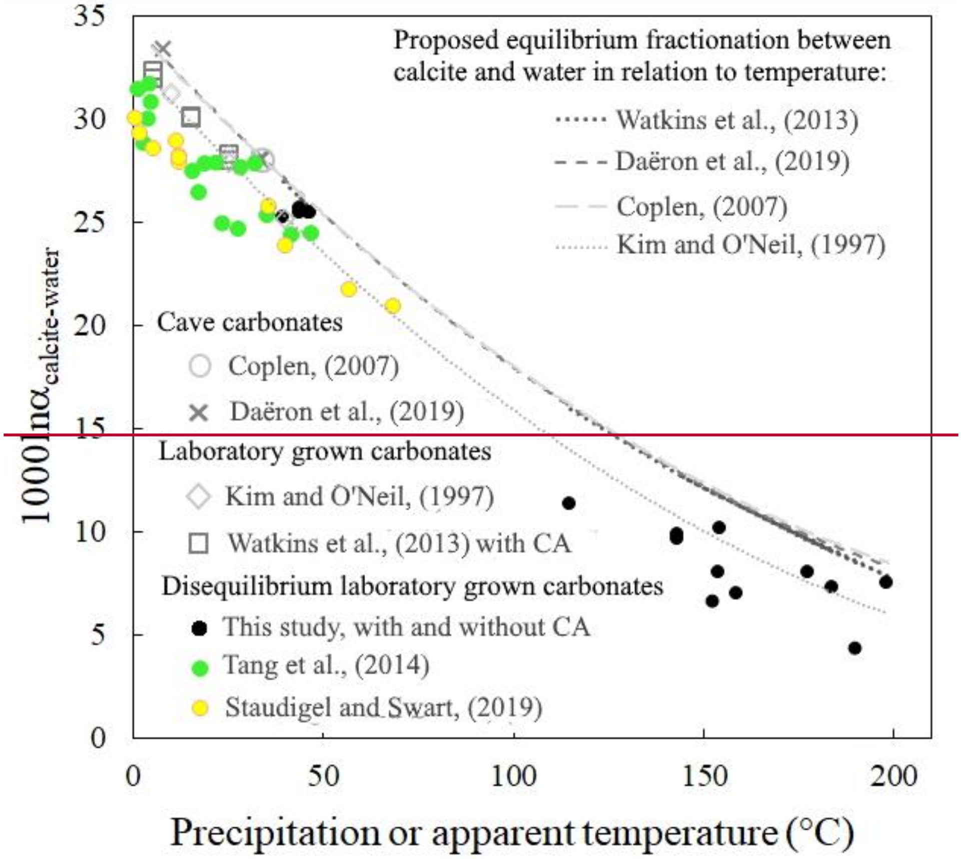
35
30
25
20
15
10
5
0
0
50
100
150
200
1000lnα calcite-water
Precipitation or apparent temperature (°C)
Proposed equilibrium fractionation between calcite and water in relation to temperature:
Watkins et al., (2013)
Daëron et al., (2019)
Coplen, (2007)
Kim and O'Neil, (1997)
Cave carbonates
Coplen, (2007)
Daëron et al., (2019)
Laboratory grown carbonates
Kim and O'Neil, (1997)
Watkins et al., (2013) with CA
Disequilibrium laboratory grown carbonates
This study, with and without CA
Tang et al., (2014)
Staudigel and Swart, (2019)

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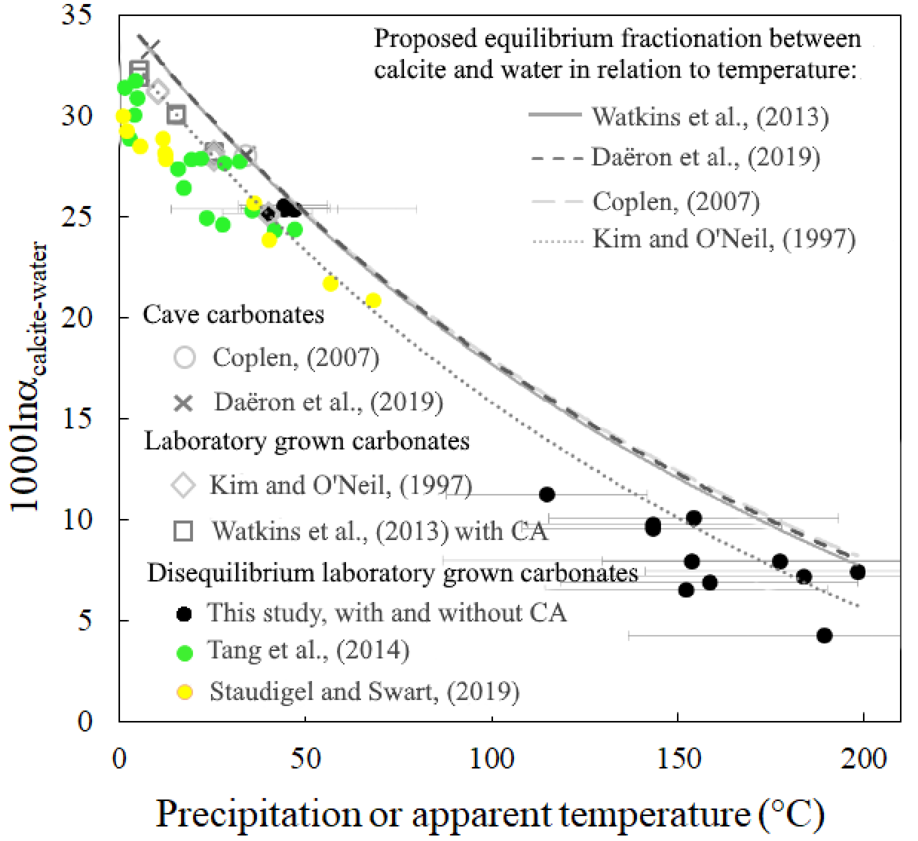

Figure 7 | The relation to temperature of equilibrium oxygen isotope fractionation factor between calcium carbonate and water ($1000\ln\alpha_{calcite\text{-}water}$) appears to be retrievable from solid carbonates (mainly calcites) in strong clumped and oxygen isotope disequilibrium such as our microbial carbonates (black dots, precipitated at 30°C) and two additional data series of laboratory grown carbonates showing disequilibrium fractionation (Tang et al., 2014; Staudigel and Swart, 2019) (green and yellow dots, respectively). The data points affected by CO_2 hydroxylation (Tang et al., 2014) or CO_2 degassing (Staudigel and Swart, 2019) (see Fig.6) are not included. Grey symbols correspond to cave carbonates precipitated at or near equilibrium (Coplen, 2007; Daëron et al., 2019) or laboratory experiments (Kim and O'Neil, 1997; Watkins et al., 2013). Those grey data series are usually considered as representative of the equilibrium fractionation factor between calcium carbonate and water whose relations to temperature, extrapolated at high temperature, are illustrated by the different dashed curves. Plotted temperatures corresponds to precipitation temperatures except for disequilibrium carbonates for which apparent temperatures have been calculated based on Δ_{47} values. X-axis errors for this study are included in the symbol size. The Y-axis error for all the reconstructed temperature is given on the figure.