CLUMPED ISOTOPIC EQUILIBRIUM AND THE RATE OF ISOTOPE EXCHANGE BETWEEN CO_2 AND WATER

HAGIT P. AFFEK

Department of Geology and Geophysics, Yale University, 210 Whitney Ave.,
New Haven, Connecticut, 06511, USA; phone: 203-432-3761,
email: hagit.affek@yale.edu

ABSTRACT. The reaction of CO_2 hydration/dehydration controls the oxygen isotopic composition in both carbonate minerals and atmospheric CO_2 through the exchange of oxygen isotopes with water. The use of $\delta^{18}O$ as an environmental indicator typically assumes isotopic equilibrium, namely full oxygen isotope exchange between CO_2 and water. Clumped isotopes is a new isotopic tracer that is used in both $CaCO_3$ and atmospheric CO_2 and reflects the thermodynamic preference of two heavy isotopes, ^{13}C and ^{18}O in this case (given as Δ_{47}), to "clump" together into one chemical bond at low temperatures. As such, the use of Δ_{47} as an indicator for temperature relies on the assumption of isotopic equilibrium. The experiments presented here examine the rate in which Δ_{47} of CO_2 that interact with water approaches the equilibrium values. This rate is indistinguishable between Δ_{47} and $\delta^{18}O$, suggesting that the isotope exchange with water also leads to reorganization of the isotopes among CO_2 isotopologues thus controlling the Δ_{47} values. The direct implication of the temporal link between Δ_{47} and $\delta^{18}O$ is that when one isotopic system shows disequilibrium, either in DIC or in gas phase CO_2, so will the other. As CO_2 clumped isotope values are independent of the oxygen isotopic composition of the water participating in the reaction, disequilibrium in Δ_{47} is often identified more readily than in $\delta^{18}O$. The combination of clumped isotopes and oxygen isotopes is therefore likely to elucidate cases of suspected disequilibrium also in $\delta^{18}O$ (and *vice versa*).

Key words: CO_2 hydration/dehydration, clumped and oxygen isotopes

INTRODUCTION

The carbon and oxygen isotopic composition of atmospheric CO_2 is often used to study the global carbon cycle and to balance the budget of CO_2 fluxes into and out of the atmosphere (for a review see Affek and Yakir, 2013). In particular, carbon isotopes are used to distinguish between land and ocean CO_2 fluxes (for example, Ciais and others, 1995). Oxygen isotopes are typically used within the land biosphere to partition the vegetation component of gross primary productivity from the soil respiratory component (Yakir and Wang, 1996; Ciais and others, 1997; Peylin and others, 1999; Cuntz and others, 2003; Welp and others, 2011). Isotope-based flux partitioning relies on the various fluxes having different isotopic signatures. As such, the oxygen isotope approach takes advantage of the difference in oxygen isotopic composition between soil and leaf water, with soil water reflecting the relatively ^{18}O-depleted rainfall and leaf water reflecting evaporative enrichment through transpiration (for example, Peylin and others, 1999). The water composition is imprinted on the $\delta^{18}O$ of the CO_2 through a reaction of oxygen isotope exchange between CO_2 and water in either the leaf or the soil. The extent of isotope exchange depends on the competition between isotope exchange and the removal of CO_2 through diffusion out of the soil (Miller and others, 1999; Wingate and others, 2009) or the leaf, or fixation in photosynthesis (for example, Gillon and Yakir, 2001).

Clumped isotopes have been suggested as a potential additional tracer for atmospheric CO_2 (Eiler and Schauble, 2004; Affek and Eiler, 2006; Affek and others, 2007; Yeung and others, 2009). Clumped isotopes examine the natural abundance of chemical bonds containing two heavy isotopes, so that the mass 47 anomaly parameter (Δ_{47}, defined in details below) measures the abundance of the isotopologue $^{13}C^{18}O^{16}O$

relative to that expected under random distribution of the heavy isotopes among all isotopologues. At equilibrium Δ_{47} approaches zero at high temperatures and increases at low temperatures, reflecting a slight thermodynamic preference for two heavy isotopes to bind with each other. Δ_{47} is formally defined through the self reaction of isotope exchange among CO_2 isotopologues (Eiler and Schauble, 2004; Wang and others, 2004):

$$^{12}C^{16}O^{16}O + {}^{13}C^{18}O^{16}O \leftrightarrows {}^{13}C^{16}O^{16}O + {}^{12}C^{18}O^{16}O. \qquad (1)$$

However, self isotope exchange among CO_2 molecules does not readily occur at the low temperatures that are relevant for the Earth surface and the atmosphere. Instead, exchange is mediated, or catalyzed, by interaction with water, thus linking Δ_{47} and $\delta^{18}O$ through the set of reactions:

$$^{12}C^{16}O^{16}O + H_2{}^{18}O \leftrightarrows H_2{}^{12}C^{16}O^{16}O^{18}O \leftrightarrows {}^{12}C^{18}O^{16}O + H_2{}^{16}O \qquad (2a)$$

$$^{13}C^{16}O^{16}O + H_2{}^{18}O \leftrightarrows H_2{}^{13}C^{16}O^{16}O^{18}O \leftrightarrows {}^{13}C^{18}O^{16}O + H_2{}^{16}O \qquad (2b)$$

Whereas the reaction of CO_2-H_2O oxygen isotope exchange and its relationship to the chemical reactions involved in CO_2 dissolution has been studied already in the early days of isotope geochemistry (Mills and Urey, 1940), for clumped isotopes only the self exchange has been studied (Wang and others, 2004). The understanding of clumped isotopes therefore focuses on the thermodynamically determined distribution of isotopologues at equilibrium. The work presented here explores the rate at which Δ_{47} approaches equilibrium, showing that this rate is determined by exchange with water and therefore the time required to reach Δ_{47} equilibrium is the same as that for $\delta^{18}O$.

MATERIALS AND METHODS

Definitions and Terminology

Experiments were performed to examine how the isotope exchange between CO_2 and water progresses with time, following both oxygen and clumped isotopes. CO_2 was analyzed simultaneously for carbon, oxygen, and clumped isotopic composition through dual inlet analysis using a Thermo-Finnigan (Bremen, Germany) MAT-253 gas source isotope ratio mass spectrometer (in the Earth Systems Center for Stable Isotopic Studies at Yale University). Carbon and oxygen isotopic compositions are reported using the VPDB and VSMOW scales, respectively.

Clumped isotope measurements are based on the analysis of mass 47 in CO_2. It is reported using the mass 47 anomaly parameter (Δ_{47}), which is defined as the deviation of the isotope ratio R^{47} ($=[47]/[44]$) from that at a random distribution of isotopes among all isotopologues. Δ_{47} is calculated as:

$$\Delta_{47} = \left[\frac{R^{47}}{2R^{13} \cdot R^{18} + 2R^{17} \cdot R^{18} + R^{13} \cdot (R^{17})^2} - \frac{R^{46}}{2R^{18} + 2R^{13} \cdot R^{17} + (R^{17})^2} - \frac{R^{45}}{R^{13} + 2R^{17}} + 1 \right] \cdot 1000 \qquad (3)$$

where R^{13}, and R^{18} are obtained by traditional isotopic measurements of masses R^{45} and R^{46} that are measured simultaneously with R^{47}; R^{17} is estimated through its mass dependent relationship with R^{18} (Eiler and Schauble, 2004; Affek and Eiler, 2006; Huntington and others, 2009). Δ_{47} is reported here in the main text using a new, absolute reference frame (Dennis and others, 2011; and see detailed explanation in the Appendix), to allow for direct inter-laboratory comparison and for comparison with theoretical values. Note that equation 3 is independent of a reference frame. In the Appendix Δ_{47} values are reported for comparison using the traditional clumped isotope reference frame.

Experimental Approach

The experiments focused on the reaction of oxygen isotope exchange between CO$_2$ and water, and followed the change with time in both δ^{18}O and Δ_{47}, starting from a composition that is out of equilibrium with coexisting water and approaching equilibrium through oxygen isotope exchange. Approximately 100μmol of cylinder CO$_2$ with a δ^{13}C value of -10.0 permil (VPDB), δ^{18}O value of 28.1 permil (VSMOW), and a Δ_{47} value of 0.944 permil was heated at 1000 °C for 2 h in sealed quartz tubes (GM Associates, Oakland, CA). This heating leads to reorganization of the CO$_2$ isotopes among all isotopologues, resulting in nominal random distribution (Δ_{47} = 0.0266‰ in the new absolute reference frame). An unintentional outcome is a slight depletion of ^{18}O, likely due to interaction with the quartz tube, with no significant change in δ^{13}C. The offset in δ^{18}O is typically ~3 permil, but it varies depending on the amount of CO$_2$ and its initial δ^{18}O value (with a larger change when the amount of CO$_2$ is small and for higher δ^{18}O values). The quartz tubes used in these experiments were dried by flame heating under vacuum; more thorough baking for a few hours at 1000 °C does not prevent the change in δ^{18}O. These gases provided the starting point (t = 0) for the isotope exchange experiments (with a few of them analyzed to provide the starting point isotopic values).

For each experimental data point, one of these heated CO$_2$ samples was introduced into an evacuated cylindrical glass reaction vessel (20 cm long, 2 cm in diameter, sealed by a stopcock valve). CO$_2$ was allowed to adjust to the desired temperature before adding ~1 ml of de-ionized water (pH = 6) at the same temperature. Water was introduced into the vessel by filling the volume above the valve with water and slowly opening the valve to introduce the desired amount of water into the vessel, while a plug of excess water prevented introduction of air into the vessel. The large excess of water relative to CO$_2$ makes sure that the CO$_2$ δ^{18}O values when the experimental systems reach equilibrium are dictated by the water composition and are independent of the relative amounts of water and CO$_2$. The oxygen isotopic composition of the water (δ^{18}O$_w$) was -5.2 ± 0.1 permil (mean $\pm$ 1 SE, n = 4; analyzed by a GasBench system with a Thermo-Finnigan DeltaPlus-XP mass spectrometer). CO$_2$ and water were allowed to react for specific time periods, after which an aliquot of CO$_2$ was removed from contact with the water and purified on a vacuum line. Reaction times ranged from 15 minutes to ~1 or 2 days. Three data sets were obtained: an experiment at 22 °C (room temperature) was performed by interaction between CO$_2$ and water in a static reaction vessel; two experiments, at 25 °C and 42 °C, were performed in a temperature controlled shaker (Excella E24R incubator shaker, New Brunswick Scientific, Enfield, CT), and included orbital shaking of the reaction vessels at 60 RPM with the reaction vessel lying horizontally (yielding water surface area of ~3 cm^2), to reduce the effect of gas-exchange (CO$_2$ transport within the headspace of the reaction vessel and dissolution in water).

Isotope Analysis

An aliquot of CO$_2$ (~30 μmol) was sampled from each reaction vessel (in which the CO$_2$ reacts with water) by expanding the CO$_2$ into a relatively small volume of a vacuum line and using only that aliquot for further analysis. This allows for sampling the CO$_2$ in the headspace of the reaction vessel, without introducing liquid water into the vacuum line. CO$_2$ was purified cryogenically by passing twice through a trap of dry ice–ethanol in order to remove water vapor introduced into the vacuum line together with the CO$_2$ and to avoid further interaction with water, and then collected in a liquid N$_2$ trap. CO$_2$ was then cleaned of trace volatile organic compounds by passing it through a GC column (Q-Plot, 30 m long, 0.53 mm ID, Supelco, Bellefonte, PA) at -20 °C (following Affek and Eiler, 2006; Huntington and others, 2009; Zaarur and

others, 2011). CO_2 isotopic composition was determined using a Thermo MAT-253 gas source isotope ratio mass spectrometer that is designed to simultaneously measure masses 44 to 49, with masses 48 and 49 serving as indicators for sample purification. Measurements were performed in a dual inlet mode using a reference gas from a CO_2 tank (Oztech, Safford, AZ) with $\delta^{13}C$ of -3.64 permil (VPDB) and $\delta^{18}O$ of 25.02 permil (VSMOW). These calibrated values have been verified using measurements of NBS-19. NBS-19 $\delta^{18}O$ value was 28.67 ± 0.02 permil (mean $\pm$ 1 SE, n = 19 for measurements during 2010-2011) in agreement with the NIST reference value of 28.65 permil, using a fractionation factor of 10.25 permil for the phosphoric acid extraction of CO_2 from the calcite standard at 25 °C (Sharma and Clayton, 1965; Kim and others, 2007). NBS-19 $\delta^{13}C$ value was 2.05 ± 0.02 permil, slightly higher than the certified value of 1.95 permil.

Δ_{47} values are reported using the newly defined absolute reference frame (Dennis and others, 2011). This reference frame is defined through the isotope exchange reaction between CO_2 and water (through equations 2) performed at several temperatures for a long enough time (at least 3 days) to reach isotopic equilibrium. As such, it enables to account for changes in Δ_{47} due to chemistry in the mass spectrometer source and allows for a direct link of measured Δ_{47} values with the theoretical values for CO_2 equilibrium (Wang and others, 2004). In order to achieve the counting statistics required for high precision Δ_{47}, each measurement consists of 90 cycles of a sample-reference comparison, with a signal integration time of either 8 or 20 seconds. Based on measurements of multiple inter-laboratory standards during 2009–2011 (Dennis and others, 2011) Δ_{47} of the reference gas is estimated as 0.943 permil, slightly higher than the value observed for CO_2 at equilibrium at 25 °C and instead equivalent to equilibrium at 21 to 22 °C. A set of standards (table S1) developed through the construction of the absolute reference frame (Dennis and others, 2011; Zaarur and others, submitted[1]) were used to test for system stability and to standardize the data (Zaarur and others, submitted[2]). Data reported in the literature has also been rescaled to the absolute reference frame (as much as possible), using values of standards measured during those earlier works (see Appendix for details).

The experiments described here follow the change with time of $\delta^{18}O$ and Δ_{47} values of CO_2, in the presence of water. The nature of these time resolved experiments prevents the replicate analysis that is usually performed in clumped isotope measurements and that is required to determine external precision. Typical uncertainty is therefore estimated based on standards. Internal mass spectrometer precision is close to that calculated for shot noise counting statistics (Merritt and Hayes, 1994), but external precision based on long-term replicate analyses of standards is approximately twice as high, ~0.02 permil for a single analysis (Zaarur and others, submitted[3]). This is the precision assumed for the analyses reported here.

RESULTS

Carbon, oxygen, and clumped isotopic compositions versus time are given in table 1 and plotted in figure 1. During each experiment, $\delta^{13}C$ did not significantly change from the starting value, with an overall mean value of -9.9 ± 0.1 permil (mean $\pm$ 1 SE, n = 29); $\delta^{18}O$ increased gradually from a starting value of 26.6 ± 0.5 permil (mean $\pm$ 1 SE, n = 5) to values of 36.3 permil after 38 h at 22 °C, 35.6 permil after 30 h at 25 °C, and 33.2 permil after 28 h at 42 °C. The starting Δ_{47} value was 0.010 ± 0.009 permil (mean $\pm$ 1 SE, n = 5), practically indistinguishable from a Δ_{47} of 0.0266 prmil that is

TABLE 1

Carbon, oxygen and clumped isotope change with time resulting from isotope exchange between CO_2 and water

Time (h)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	Δ_{47}(‰)
22°C, no shaking			
0	-10.3	26.2	0.032
0	-9.9	26.7	0.002
0.3	-10.2	26.9	0.064
1.2	-10.1	29.2	0.186
3.2	-9.8	30.5	0.331
6.3	-9.9	32.2	0.503
10.8	-10.0	34.6	0.680
21.0	-9.8	36.2	0.903
28.8	-9.8	36.6	0.912
38.3	-10.1	36.3	0.928
Equilibrium		36.5	0.941
25°C, with shaking			
0	-9.4	26.5	0.005
1.1	-9.8	30.2	0.299
2.1	-9.8	31.7	0.473
3.3	-10.1	33.3	0.805
9.5	-9.6	35.5	0.907
15.0	-9.7	35.4	0.926
24.0	-9.2	35.4	0.938
30.0	-9.9	35.6	0.918
Equilibrium		35.9	0.925
42°C, with shaking			
0	-10.5	25.2	-0.008
0	-10.6	25.9	0.017
0.5	-9.5	28.2	0.132
1.0	-9.9	29.2	0.320
2.8	-9.6	31.7	0.692
3.1	-9.7	31.9	0.663
3.7	-9.1	33.4	0.721
3.9	-10.8	30.4	0.680
6.0	-10.5	32.2	0.753
11.7	-10.3	31.0	0.882
23.5	-9.9	32.6	0.843
27.7	-9.6	33.2	0.858
Equilibrium		32.7	0.841

Starting CO_2 was heated to 1000 °C to reach random distribution ($\Delta_{47} = 0.027$‰). Water $\delta^{18}O$ was -5.2‰.

expected for CO_2 that has undergone heating to 1000 °C. Δ_{47} increased to 0.928 permil after 38 h at 22 °C, 0.918 permil after 30 h at 25 °C, and 0.858 permil after 28 h at 42 °C. Note that $\delta^{18}O$ values at the 42 °C experiment are nosier than those of the 22 °C and 25 °C; this is especially noticeable at the 4 data points between 3 h and 4 h that span a range of 3 permil. This level of variability is not observed in Δ_{47} of the same samples and is likely to be the result of heterogeneity in water composition within the reaction vessel due to evaporation and condensation, which is unavoidable at the high temperature experiment but not observed in the lower temperature experiments. For the direct comparison of reaction rates (see below) the mean values for the samples at the 3 to 4 h time window are used.
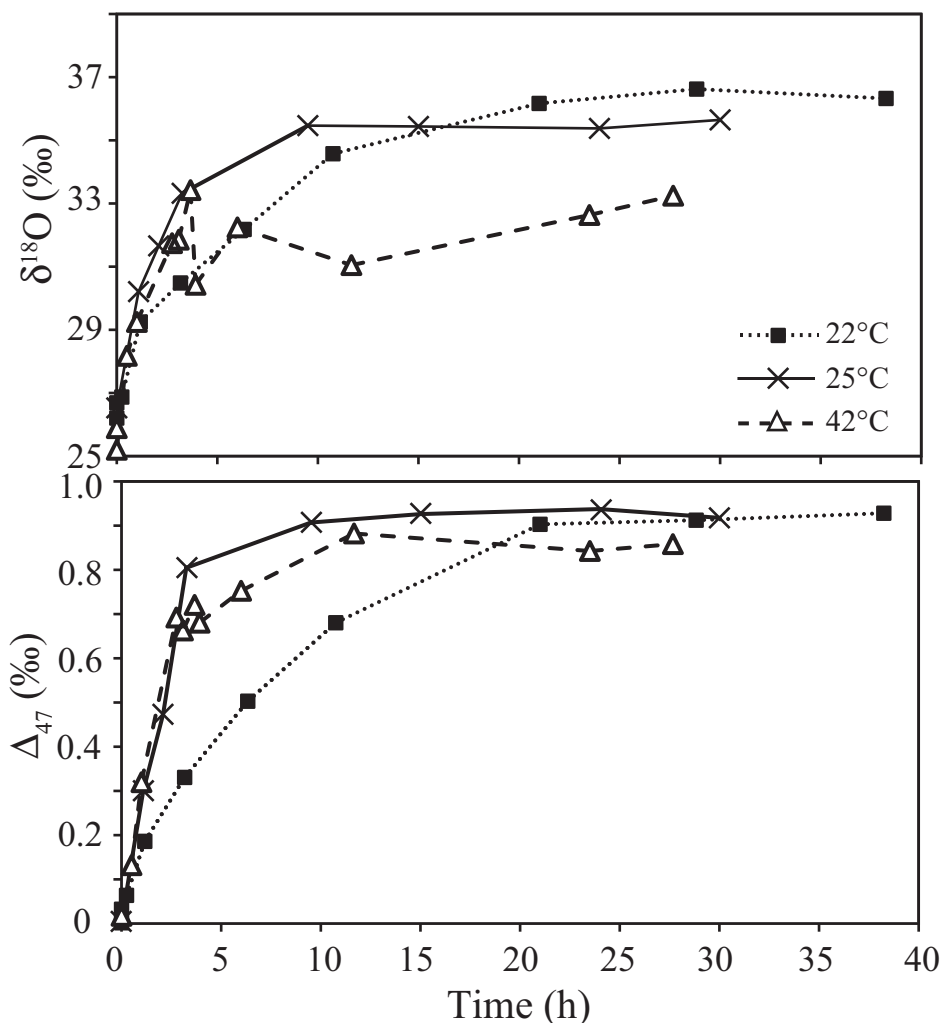
Fig. 1. Change with time of $\delta^{18}O$ and Δ_{47} following the progress of oxygen isotope exchange between CO_2 and water through the hydration/dehydration reaction. Experiments were performed at 22 °C without shaking (■) and at 25 °C (×) and 42 °C (△) with shaking, using CO_2 that is out of equilibrium ($\delta^{18}O$ = 25–26‰, Δ_{47} = ~0.027‰) with the water used in the experiment ($\delta^{18}O_w$ = −5.2‰). CO_2 isotopic composition was followed as it approaches equilibrium. Equilibrium values are given in table 1. Estimated analytical uncertainty is ±0.2‰ for $\delta^{18}O$ and ±0.02‰ for Δ_{47}.

In all 3 experiments, $\delta^{18}O$ and Δ_{47} values gradually approached the values expected at equilibrium. The final Δ_{47} values for the 25 °C and 42 °C experiments (that both included shaking) are within error from the values predicted theoretically at thermodynamic equilibrium: 0.925 permil and 0.841 permil, respectively (table 1; following Dennis and others, 2011, in using a 4th order polynomial fit to the data of Wang and others, 2004). The final $\delta^{18}O$ values are also consistent with the expected equilibrium oxygen isotope fractionation at the respective temperatures (41.1‰ and 37.9‰ for 25 °C and 42 °C, respectively; Brenninkmeijer and others, 1983) for the measured water composition of −5.2 permil. CO_2 reached complete isotope exchange (>95% of the equilibrium value) after 10 h at 25 °C and between 6 and 12 h at 42 °C.

The 22 °C experiment reflects a slower approach to isotopic equilibrium, with an expected equilibrium Δ_{47} value of 0.941 permil and oxygen isotope fractionation of

41.7 permil. CO_2 in this experiment passes 95 percent exchange only at 21 h. The slow approach to equilibrium is likely due to the slow transport of CO_2 in the absence of shaking between the solution, where reaction occurs, and the headspace of the reaction vessel from which the CO_2 sample in obtained. Whereas this stagnant experimental setup does not reflect a direct measure for the isotope exchange rate because the apparent reaction rate includes significant contributions from diffusion and dissolution, the slower reaction progress enables the early stages of isotope exchange, when the system is still far from equilibrium, to be examined in more details.

<div align="center">DISCUSSION</div>

<div align="center">*Reaction Rates Linking Δ_{47} and $\delta^{18}O$*</div>

The reaction of CO_2 hydration/dehydration has long been studied and identified as the slow step in the reaction set of the carbonate system, with a chemical equilibrium reaction rate of $0.037s^{-1}$ at 25 °C (Mills and Urey, 1940; Kern, 1960; Miller and others, 1971; Johnson, 1982). The rate of reaching isotopic equilibrium among the different species of dissolved inorganic carbon (DIC) varies depending on the isotopic system in question. Carbon isotopic equilibrium is obtained through internal rearrangement among the DIC species and therefore progresses at the same rate as the chemical equilibrium, requiring ~28s to reach equilibrium at 25 °C and pH = 8 (Zeebe and others, 1999; Dreybrodt and Scholz, 2011). The time required to reach oxygen isotopic equilibrium is significantly longer, on the order of several hours at neutral pH and room temperature (Zeebe and Wolf-Gladrow, 2001; Lécuyer and others, 2009). The reason for this is that oxygen isotopic equilibrium is obtained by direct reaction between water and CO_2 with the equilibration rate being dependent on the availability of $CO_2(aq)$; at a neutral pH $CO_2(aq)$ is present at only low concentrations, with the system being dominated by HCO_3^- (Usdowski and others, 1991; Zeebe and Wolf-Gladrow, 2001).

It is not *a priori* clear whether the time required to reach clumped isotopic equilibrium would be close to the carbon or the oxygen isotope system, or the equilibration rate may be some combination of both. Clumped isotopes may be similar to carbon isotopes, with the isotopic redistribution among DIC species leading also to internal reorganization of the isotopes within the CO_2 isotopologues. Alternatively, clumped isotopes may follow the rate observed in oxygen isotopes, with the internal rearrangement of isotopes among CO_2 isotopologues (eq. 1) being controlled by the replacement of oxygen atoms in CO_2 through exchange with water.

The experiments performed here focused on the reaction of oxygen isotope exchange between CO_2 and H_2O through CO_2 hydration/dehydration, following the time evolution of both $\delta^{18}O$ and Δ_{47} as CO_2 approaches isotopic equilibrium. The data indicate that the time required to reach equilibrium is indistinguishable between $\delta^{18}O$ and Δ_{47} (fig. 1). In this context, isotopic equilibrium is reached when isotope exchange is complete, namely when all oxygen atoms in CO_2 have been replaced by oxygen atoms originating from water (or in the case when the amount of water is not in excess with respect to CO_2, it refers to complete oxygen atom rearrangement between water and CO_2, so that $\delta^{18}O$ values in both CO_2 and water reflect the ^{18}O mass balance of the system). As such, the replacement of oxygen atoms in CO_2 not only changes the abundance of the $^{12}C^{16}O^{18}O$ isotopologue that defines $\delta^{18}O$ (eq. 2a), but also the abundance of $^{13}C^{16}O^{18}O$ that defines Δ_{47} (eq. 2b).

The time evolution of the isotopic composition in these experiments is determined by the rate of the isotope exchange reaction, combined with the rate of a gas-exchange component of CO_2 dissolution and the diffusion of CO_2 molecules within the reaction vessel headspace. This gas-exchange and diffusive components are difficult to quantify and may vary depending on specific experimental conditions such as shape and size of the reaction vessel, or shaking rate; it therefore prevents direct

quantitative assessment of the isotope exchange reaction rates. The analysis thus focuses on a comparison between Δ_{47} and $\delta^{18}O$, relying on the well understood characteristics of oxygen isotopes in this reaction to provide a relative rate between the two isotopic systems.

Comparisons were performed for two temperatures and under two different gas-exchange regimes. The simultaneous measurement of clumped isotopes and oxygen isotopes for each CO_2 sample lends itself to examining the relative reaction rates between the two systems. The time required to reach equilibrium in the experiments that include shaking is on the order of ~10 h for both $\delta^{18}O$ and Δ_{47}, and is similar to that observed previously for oxygen isotope exchange at pH ~8 (Johnson, 1982; Zeebe and Wolf-Gladrow, 2001) or at variable salinities (Lécuyer and others, 2009), suggesting that in these experiments the contribution of the gas-exchange component to the apparent rate is small (though probably not completely eliminated) and that the rate is dominated by the isotope exchange reaction. Note that in this type of experiments, the isotopic compositions are measured in the gas phase (headspace) so that the rates always include a component of gas exchange between solution and headspace. The rate of gas exchange is likely to vary among experimental setups (and among natural systems such as exchange with leaf water versus soil films versus the surface ocean). As such, it cannot be independently constrained in the laboratory experiments described either here or in the literature. The observation, however, of constant isotopic values at long reaction times, suggests also equilibrium between solution and headspace.

Reaction rates can be explored in more detail by focusing on the early stages of the reaction, when the system is still far from equilibrium, by examining the composition change with time as the reaction progresses (fig. 2). When plotting the natural logarithm of the difference between the composition at time t and the final equilibrium value versus time, the slope gives the reaction rate (Mills and Urey, 1940; Usdowski and others, 1991). As expected, the experiment without shaking shows significantly lower apparent reaction rate compared to the experiments that included shaking, reflecting the larger contribution from CO_2 transport and dissolution that effectively slows the reaction. The rates observed, $0.12 \pm 0.003\ h^{-1}$ for Δ_{47} and $0.15 \pm 0.01\ h^{-1}$ for $\delta^{18}O$, are indistinguishable.

The experiments that included shaking progressed faster and therefore have fewer data points at the far from equilibrium stage but can nevertheless be used for comparison between the two isotopic systems. At 25 °C the rates are $0.34 \pm 0.01\ h^{-1}$ for Δ_{47} and $0.38 \pm 0.05\ h^{-1}$ for $\delta^{18}O$, faster than the no-shaking experiment due to the reduced limiting effect of gas-exchange, and indistinguishable for the two isotopic systems. Whereas the experimental time resolution (fig. 1) does not reveal a temperature effect on the time required to reach full equilibrium, the reaction rate far from equilibrium shows that at 42 °C the reaction is much faster than at 25 °C. Rates at 42 °C are $0.51 \pm 0.02\ h^{-1}$ for Δ_{47} and $0.62 \pm 0.03\ h^{-1}$ for $\delta^{18}O$.

The $\delta^{18}O$ reaction rate in all 3 experiments seem slightly higher than that for Δ_{47}, though the two systems are within error from each other. Note, however, that the uncertainty reported in the slope is 1 SE of the calculated slope, and therefore includes only the spread of data around a linear relationship; it does not include the uncertainty in estimating the $\delta^{18}O$ values of the starting CO_2 and water, and therefore the expected equilibrium values, to which the $\delta^{18}O$ slope is sensitive.

The similarity in reaction rates between Δ_{47} and $\delta^{18}O$ suggests that the internal rearrangement of isotopes among CO_2 isotopologues, that leads to clumped isotopic equilibrium, progresses through oxygen isotope exchange with water. Namely, the gradual replacement of oxygen atoms in CO_2 by those coming from water also leads to the redistribution of the ^{13}C-^{18}O bonds in CO_2. This is likely achieved through selective
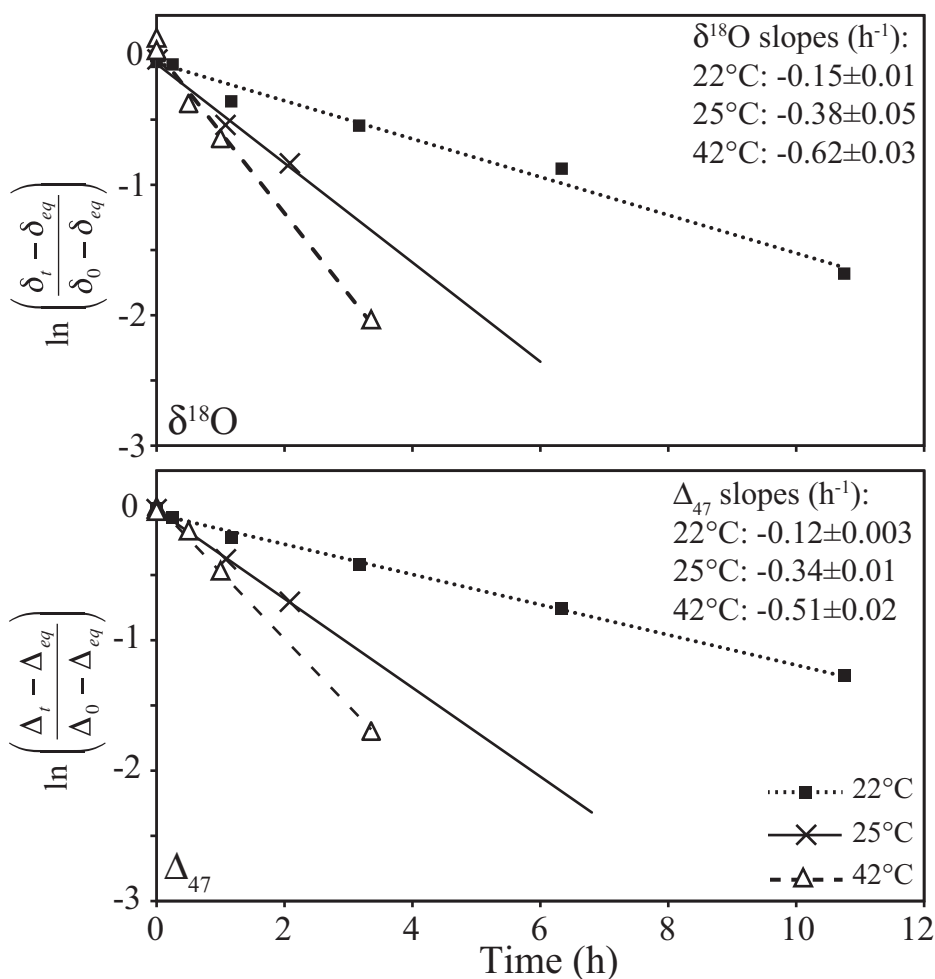
Fig. 2. Reaction progress with time at the early stages of the hydration/dehydration reaction, when CO_2 is still far from equilibrium. Experiments were performed at 22 °C without shaking (■) and at 25 °C (×) and 42 °C (△) with shaking. CO_2 isotopic composition is plotted as the natural logarithm of the distance from equilibrium, so that the slopes reflect the rate of reaction in each case.

removal of oxygen in the dehydration of carbonic acid back to CO_2, in which [18]O is preferentially retained in CO_2 when the carbonic acid contains [13]C (Guo, ms, 2008). This result is not overly surprising but its implications to the link between $\delta^{18}O$ and Δ_{47} in systems that are out of isotopic equilibrium (see below) has been largely ignored.

Since the early clumped isotope measurements it has been noticed that the signal is highly sensitive to interaction with water, with the presence of even trace water in a sample leading to a compromised Δ_{47} value. The results presented here clearly indicate that this sensitivity is not due to an especially fast reaction. Instead it is likely that the sensitivity is due to the catalytic role that water plays in obtaining clumped isotopic equilibrium. For CO_2 $\delta^{18}O$ the equilibrium value is determined by a mass balance of oxygen atoms coming from both water and CO_2, so that when water is present in only trace amounts it does not significantly affect the CO_2 composition. CO_2 Δ_{47}, however, is independent of the absolute $\delta^{18}O$ value and therefore at equilibrium would reach the same value (depending on temperature) irrespective of the amount of water present.

Implications of the Link Between Oxygen and Clumped Isotopes

Clumped isotopes have been developed as a thermodynamics-based tracer that reflects the formation temperature of carbonate minerals or gas phase CO_2 (Eiler and Schauble, 2004; Eiler, 2007; Eiler, 2011). Carbonate clumped isotopes are commonly used as a paleotemperature proxy (Ghosh and others, 2006; Affek, 2012), for example in paleoclimate research (Came and others, 2007; Csank and others, 2011; Finnegan and others, 2011; Keating-Bitonti and others, 2011; Suarez and others, 2011). Gas phase Δ_{47} has been used in atmospheric CO_2 as a carbon cycle tracer aiming to partition high temperature processes such as combustion from low temperature CO_2 fluxes such as respiration (Affek and Eiler, 2006; Affek and others, 2007).

The main advantage of clumped isotopes over other environmental tracers is its being based on well understood thermodynamic principles. The interpretation of measured Δ_{47} as reflecting thermodynamic characters assumes that the studied CO_2 or $CaCO_3$ has been formed under isotopic equilibrium conditions. It thus relies on $CaCO_3$ precipitating from solutions in which the DIC is at isotopic equilibrium with the water, and on atmospheric CO_2 sources (at the gross flux level) being at isotopic equilibrium. The hydration/dehydration reaction of CO_2 and its effect on Δ_{47} in both gas phase CO_2 and DIC is therefore critical for the use of clumped isotopes as a proxy for environmental conditions and in particular to the understanding of cases in which Δ_{47} deviates from equilibrium.

In the gas phase, the Δ_{47} values expected at equilibrium can be accurately predicted from theoretical calculation (Wang and others, 2004). For $CaCO_3$, the theory can give general insights but is limited from a practical perspective by difficulties in solid state *ab initio* predictions (Schauble and others, 2006; Guo and others, 2009). Nevertheless, clumped isotope values in $CaCO_3$ that formed under isotopic equilibrium conditions reflect the temperature of mineral growth, and unlike most geochemical proxies is independent of the composition of the solution from which the mineral grows. As such, an empirical calibration of carbonate clumped isotope thermometry replaces the theoretical predictions and is used to derive temperatures from carbonate clumped isotopes measurements (Ghosh and others, 2006; Zaarur and others, submitted[4]).

The empirical calibration for carbonate clumped isotope thermometry has been obtained though temperature controlled laboratory experiments of calcite precipitation (Ghosh and others, 2006; Zaarur and others, submitted[5]). Such laboratory experiments inherently reflect fast mineral growth and are therefore not *a priori* expected to reflect isotopic equilibrium. However, it is observed that a wide variety of biogenic carbonates, mostly of marine origin, conform to the same temperature dependence of carbonate clumped isotopes, leading to the assumption that this thermometer calibration reflects isotopic equilibrium (Eiler, 2001; Eiler, 2007; Tripati and others, 2010). In spite of the general conformity, there are several notable exceptions, namely groups of materials that clearly deviate from this nominal equilibrium for carbonate Δ_{47}, or processes in atmospheric CO_2 that show unexpected Δ_{47} values.

CO_2 produced by combustion in a car engine and emitted as car exhaust shows a Δ_{47} value of 0.45 permil (Affek and Eiler, 2006, with the data rescaled to the new absolute reference frame in Affek and Yakir, 2013), significantly higher than the Δ_{47} value close to zero that is expected for equilibrium at a typical engine temperature of ~900 °C. This value was interpreted together with $\delta^{18}O$ of the car exhaust CO_2 as reflecting equilibrium at a temperature of ~170 °C with the water produced in the combustion process. The finding that the reactions of approaching equilibrium progress together for both Δ_{47} and $\delta^{18}O$ implies that the values observed in car exhaust

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[4] Ibid.
[5] Ibid.

may reflect instead partial exchange at a lower temperature. A potential scenario is CO_2 produced with a Δ_{47} of 0.03 permil at $\sim$900 °C undergoing approximately 50 percent isotope exchange at a temperature of 50 °C, which is the exhaust temperature as it leaves the car tail pipe.

Also in low temperature CO_2 sources, particularly in respiration, Δ_{47} values that deviate from those at equilibrium have been observed. Human breath shows a Δ_{47} value of 0.81 permil which is equivalent to the equilibrium value at $\sim$50 °C, and lower than that at 37 °C (0.86‰; Affek and Eiler, 2006). A similar value was inferred for ecosystem respiration from diurnal variations in atmospheric CO_2 Δ_{47} (Affek and others, 2007), although neither soil CO_2 nor the CO_2 in the flux out of the soil has ever been directly analyzed for Δ_{47}. A mechanism that can produce this low Δ_{47} CO_2 in respiration is unknown. One potential mechanism is related bicarbonate having lower Δ_{47} than gas phase CO_2 (Schauble and others, 2006; Guo and others, 2009). Alternatively, pyruvate decarboxylation may result in a low Δ_{47} value, but has never been examined for Δ_{47}.

Additional research is clearly required in order to quantify the Δ_{47} produced in the respiration process itself, so that the extent of isotope exchange that results in the observed Δ_{47} values can be quantified. The deviation from equilibrium Δ_{47} value, however, suggests an incomplete isotope exchange between CO_2 and water in the soil. The presence of the enzyme carbonic anhydrase in soils has been suggested (Kesselmeier and others, 1999; Seibt and others, 2006; Wingate and others, 2009), mainly based on observing that $\delta^{18}O$ in the CO_2 flux out of the soil cannot be explained without a contribution from a flux of atmospheric CO_2 moving through the soil (an invasion flux) and undergoing oxygen isotope exchange with soil water. Isotope exchange in the invasion flux requires the hydration/dehydration reaction to be faster then diffusion out of the soil, leading to the inferred enzymatic catalysis. However, it does not require the reaction to be fast enough for full equilibrium (Seibt and others, 2006) and therefore does not contradict soil respiration flux having a Δ_{47} value that is lower than equilibrium. Direct measurement of CO_2 Δ_{47} in the soil profile may thus help constraining the level of enzymatic catalysis.

Disequilibrium in Δ_{47} is not likely to be associated with CO_2 fluxes into and out of leaf stomata. The Δ_{47} value of background air is close to that at equilibrium at $\sim$16 °C (0.97‰ in air from Cape Grim, Tasmania and Barrow, Alaska; Affek and others, 2007) so that the CO_2 entering the leaf during photosynthesis cannot be very far from equilibrium at the leaf temperature. In some cases the enzyme carbonic anhydrase is not fast enough to obtain full oxygen isotope exchange (Gillon and Yakir, 2000), with an estimated global vegetation mean of 80 percent exchange (Gillon and Yakir, 2001). However, the difference between background air Δ_{47} and leaf-scale equilibrium is small, so that the effect of such partial exchange is not measurable. As in soil respiration, Δ_{47} associated with leaf mitochondrial respiration is unknown. However, Farquhar and Cernusak (2012) have recently shown that when accounting for the presence of water vapor in the flux into and out of the leaf, $\delta^{18}O$ in leaf dark respiration reflects equilibrium with water.

Better understood are non-equilibrium processes affecting Δ_{47} in carbonates. Strong deviations from the nominal equilibrium calibration of the Δ_{47} temperature dependence have been observed in speleothems, with Δ_{47} values in the speleothem calcite being lower than expected based on cave temperatures (Affek and others, 2008; Meckler and others, 2009; Daëron and others, 2011; Kluge and Affek, 2012). The primary effect in this case stems from $CaCO_3$ being formed from DIC that is out of equilibrium (Muhlinghuas and others, 2009; Dreybrodt and Scholz, 2011; Kluge and Affek, 2012), and is therefore directly related to CO_2-H_2O isotope exchange. The disequilibrium results from kinetic isotope effects in the degassing of CO_2 out of the cave drip water, resulting in an increase in $\delta^{18}O$ but a decrease in ^{13}C-^{18}O bonding in

the DIC remaining in solution (Guo, ms, 2008). The observed disequilibrium in $CaCO_3$ Δ_{47} indicates that $CaCO_3$ precipitates faster than the solution DIC can be "buffered" by returning to equilibrium through CO_2 hydration/dehydration. The $CaCO_3$ therefore records this out of equilibrium DIC.

The effect on the paleotemperatures derived from clumped isotopes is large enough so that the deviation from equilibrium is easily identified. Speleothem $\delta^{18}O$ values that deviate from the commonly used oxygen isotope thermometer (such as O'Neil and others, 1969 or Kim and O'Neil, 1997) are often observed (McDermott and others, 2011; Tremaine and others, 2011). However, identifying these oxygen isotope deviations as reflecting disequilibrium is less obvious as it is often masked by uncertainties in cave drip water composition or in the choice of relevant thermometer calibration. The temporal link between Δ_{47} and $\delta^{18}O$ implies that also $\delta^{18}O$ in speleothems reflects disequilibrium, with CO_2 degassing leading to ^{18}O-enrichment.

The rate of hydration/dehydration is temperature dependent, with a faster reaction at high temperature. It is therefore likely that the extent of disequilibrium in speleothems would vary with climatic conditions, due to varying extent of "buffering." As the rate of hydration/dehydration is similar for Δ_{47} and $\delta^{18}O$ irrespective of temperature, though, it is likely that the signals of both Δ_{47} and $\delta^{18}O$ would be affected by temperature, but the link between then would be preserved. The link between Δ_{47} and $\delta^{18}O$ in CO_2 degassing was examined by an ab initio theoretical model, suggesting a Δ_{47} change of approximately 0.02 permil for every change of 1 permil in $\delta^{18}O$, with this Δ_{47}-$\delta^{18}O$ co-variance slope being almost invariant with temperature (Guo, ms, 2008). The results of this model provide the basis for the co-variance method for accounting for the effect of disequilibrium in speleothems, although the disequilibrium offset in Δ_{47} per $\delta^{18}O$ that is typically observed in caves is larger than predicted by the model (Wainer and others, 2011; Daëron and others, 2011; Kluge and others, 2013), suggesting an additional process than either amplifies the disequilibrium in Δ_{47} or somewhat counteracts the offset in $\delta^{18}O$.

Such other process that can potentially lead to $CaCO_3$ precipitating out of isotopic equilibrium is related to observations that fast growing minerals are depleted in ^{18}O as compared to slow growing $CaCO_3$ (Coplen, 2007; Dietzel and others, 2009; Gabitov and others, 2012). This non-equilibrium effect likely reflects an isotopic fractionation between DIC and the growing mineral due to the dynamics of attachment/detachment of the ions to the mineral (for example, DePaolo, 2011; Raynard and others, 2011) or due to the light oxygen isotopes that preferentially reside at the mineral surface being entrapped within the bulk of a fast growing mineral (for example, Watson and Müller, 2009; Gabitov and others, 2012), or both. These types of non-equilibrium may occur in fast growing minerals even when the DIC is at isotopic equilibrium with water. As such it is not directly affected by the isotope exchange reaction between CO_2 and water that is examined in this study, and reflects an additional, independent process. The effect of mineral growth rate on Δ_{47} through this process has not been thoroughly examined. However, following Coplen's (2007) observations of ^{18}O-enrichment in slow growing calcite in Devils Hole (Nevada), no disequilibrium effect has been observed in Δ_{47} in Devils Hole (Kluge and others, 2012) suggesting that unlike the CO_2-H_2O isotope exchange reaction, $\delta^{18}O$ and Δ_{47} are not coupled in the fractionation between DIC and $CaCO_3$ mineral.

CONCLUSIONS

The reaction of CO_2 hydration/dehydration and the oxygen isotope exchange between CO_2 and water that is associated with it have been studied since the early days of isotope geochemistry. This included characterization of the oxygen isotopic fractionation between CO_2 and water as well as the rate of reaction and the time required to reach full isotope exchange. For clumped isotopes, only the thermodynamic depen-

dence of the distribution of isotopologues at different temperatures—a parameter that is equivalent to the isotopic fractionation in oxygen isotopes—has been determined so far. The experiments described here complement the thermodynamic parameterization by examining the rate of the hydration/dehydration reaction with respect to clumped isotopes. The results show that the reaction progresses in parallel with oxygen isotopes, so that the reaction rates for both isotopic systems are indistinguishable. The temporal link between the two systems implies that when one isotopic tracer shows disequilibrium, so will the other. The independence of equilibrium Δ_{47} of the composition of water with which CO_2 interacts implies that Δ_{47} is a less ambiguous indicator for incomplete exchange when compared with $\delta^{18}O$ so that it can be used to identify and potentially quantify the extent of disequilibrium in either gas phase CO_2 or in $CaCO_3$ minerals.

APPENDIX

Absolute Reference Frame for Δ_{47}

Atmospheric CO_2 Δ_{47} data in the literature (Eiler and Schauble, 2004; Affek and Eiler, 2006; Affek and others, 2007; Yeung and others, 2009) has been reported using a reference frame that assumes that mass spectrometric measurements do not affect the distribution of ^{13}C-^{18}O bonds in the CO_2. However, it was recently recognized (Huntington and others, 2009; Dennis and others, 2011) that dissociation and recombination of CO_2 in the ion source of the mass spectrometer lead to a reduced measured Δ_{47} value when compared to theoretical prediction (such as Wang and others, 2004). This effect is in the order of ~10% of the signal but varies with ion source conditions. The parameters used for transforming measured Δ_{47} to values in the absolute reference frame can therefore vary among mass spectrometers and over time. It is therefore required to account for this effect in order to be able to compare data among laboratories and

[6] Ibid.
[7] Ibid.

TABLE A1

Δ_{47} values of working standards used to convert data from the original reference frame to the absolute reference frame

Working standard	Δ_{47} original (‰)	Δ_{47} absolute (‰)
Yale laboratory (2009-2012 data)		
Heated gas	0	0.0266
YCM (Carrara marble)	0.358	0.395
CO_2-H_2O 25°C	0.851	0.9252
Corn cylinder CO_2	0.872	0.944
Caltech laboratory (2004-2006 data)		
Heated gas	0	0.0266
NBS-19	0.352	0.392
BOC cylinder CO_2	0.871	0.925

Values are based on Dennis and others (2011) and Zaarur and others (submitted)[6]. Yale laboratory data refers to the experimental data in this study (details of standards data is given in Zaarur and others (submitted)[7]); Caltech laboratory data refers to literature data of atmospheric CO_2 Δ_{47}.

Δ_{47} change with time resulting from isotope exchange between CO_2 and water

Time (h)	Δ_{47} original (‰)	Δ_{47} absolute (‰)
22°C, no shaking		
0	0.009	0.032
0	-0.020	0.002
0.3	0.039	0.064
1.2	0.154	0.186
3.2	0.291	0.331
6.3	0.454	0.503
10.8	0.622	0.680
21.0	0.832	0.903
28.8	0.841	0.912
38.3	0.856	0.928
25°C, with shaking		
0	-0.007	0.005
1.1	0.261	0.299
2.1	0.425	0.473
3.3	0.739	0.805
9.5	0.836	0.907
15.0	0.854	0.926
24.0	0.865	0.938
30.0	0.846	0.918
42°C, with shaking		
0	-0.005	0.017
0	-0.030	-0.008
0.5	0.103	0.132
1.0	0.281	0.320
2.8	0.633	0.692
3.1	0.605	0.663
3.7	0.660	0.721
3.9	0.622	0.680
6.0	0.690	0.753
11.7	0.813	0.882
23.5	0.775	0.843
27.7	0.790	0.858

The data from table 1 in the main text is given here in both the original and the absolute reference frames.

compare measured to theoretical equilibrium values. This is done through a new, absolute, reference frame developed by Dennis and others (2011).

This reference frame was determined for the Yale clumped isotope laboratory using a set of CO_2 that was fully equilibrated with water (through CO_2-H_2O isotope exchange for at least 3 days) at different temperatures and through inter-laboratory comparison (Dennis and others, 2011). Standard materials that were calibrated in this reference frame using a long term data set (Zaarur and others, submitted[8]), were used to convert the measured data into the absolute reference frame and to account for changes over time. Standards data is given in table A1. The experimental data described in the main text are reported in the

[8] Ibid.

absolute reference frame; these data in the original reference frame, that have been used in previously published work, are given in table A2.

Also literature data that is used here for comparison (in particular atmospheric CO$_2$ and associated fluxes; Affek and Eiler, 2006; Affek and others, 2007) is corrected in the main text to account for the reference frame effect. This is done using materials that were measured with these literature data and later became working standards (table S1). Given that these materials were not measured originally as standards, their values defined in the absolute reference frame are estimates.

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