Experimental evaluation of oxygen isotopic exchange between inclusion water and host calcite in speleothems

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Abstract. The oxygen and hydrogen isotopic compositions of water in fluid inclusions in speleothems are important hydroclimate proxies because they provide information on the isotopic compositions of rainwater in the past. Moreover, because isotopic differences between fluid inclusion water and the host calcite provide information on the past isotopic fractionation factor, they are also useful for quantitative estimation of past temperature changes. The oxygen isotope ratio of inclusion water ($\delta^{18}O_{fi}$), however, may be affected by isotopic exchange between the water and the host carbonate. Thus, it is necessary to estimate the bias caused by this post-depositional effect for precise reconstruction of palaeo-temperatures. Here, we evaluate the isotopic exchange reaction between inclusion water and host calcite based on a laboratory experiment involving a natural stalagmite. Multiple stalagmite samples cut from the same depth interval were heated at 105°C in the laboratory for 0–80 hours. Then, the isotopic compositions of the inclusion water were measured. In the 105°C heating experiments, the $\delta^{18}O_{fi}$ values increased from the initial value by 0.7‰ and then remained stable after ca. 20 hours. The hydrogen isotope ratio of water showed no trend in response to the heating experiments, suggesting that the hydrogen isotopic composition of fluid inclusion water effectively reflects the composition of past dripwater. We then evaluated the process behind the observed isotopic variations using a partial equilibration model. The experimental results are best explained when we assumed that a thin $CaCO_3$ layer surrounding the inclusion reacted with the water. The amount of $CaCO_3$ that reacted with the water is equivalent to 2% of the water inclusions in molar terms. These results suggest that the magnitude of the isotopic exchange effect has a minor influence on palaeo-temperature estimates for the Quaternary climate reconstructions.

1 Introduction

Speleothems have provided invaluable terrestrial climate records over historical (e.g., (Zhang et al., 2008)) and glacial-interglacial (e.g., Wang (2001);Wang et al. (2017)) time scales. Among multiple climate proxies in speleothems (Fairchild et al., 2006), the stable oxygen isotope ratio of $CaCO_3$ ($\delta^{18}O_{ca}$) is the most widely used hydroclimate proxy. Changes in $\delta^{18}O_{ca}$

have been interpreted as proxies for monsoon strength (Wang, 2001), precipitation amount (Wang et al., 2017) or temperature (Dorale, 1998;Mori et al., 2018) depending on the hydroclimatic setting of each cave site.

This indeterminate nature of the $\delta^{18}O_{ca}$ interpretation is related to the fact that $\delta^{18}O_{ca}$ values are mainly controlled by two factors: the temperature in the cave and the annual mean $\delta^{18}O$ value of the rain water. To overcome this ambiguity, the isotopic compositions of water in fluid inclusions in stalagmites have been regarded as important proxies (Schwarcz et al., 1976;McDermott, 2004). Cave dripwaters are sealed in micro-scale cavities as fluid inclusions, whose $\delta^{18}O$ value is usually close to the $\delta^{18}O$ value of local rain. Generally, stalagmites contain inclusion water, which accounts for 0.05 to 0.5 wt. % of a speleothem (McDermott, 2005). Thus, the isotopic compositions of fluid inclusions of stalagmites ($\delta^{18}O_{fi}$) trapped under humid conditions should directly preserve the $\delta^{18}O$ value of dripwater in the past.

$\delta^{18}O_{fi}$ values are also useful for estimating past temperature variations. Although experimental studies suggest that the $\delta^{18}O_{ca}$ values of speleothems are affected by kinetic effects (e.g., Hansen et al. (2019);Dietzel et al. (2009)), a modelling study suggests a relatively stable value for the temperature dependence of $\delta^{18}O_{ca}$ values (Mühlinghaus et al., 2009). The combination of the two isotopic compositions, $\delta^{18}O_{fi}$ and $\delta^{18}O_{ca}$, provides a direct estimate for the oxygen isotopic fractionation between calcite and water, which is controlled mainly by the formation temperature. Thus, variations in past temperature (i.e., not absolute values but relative changes) can be estimated on the assumption that the kinetic effect is constant during the speleothem formation period.

Early studies analysed the hydrogen isotope ratio of fluid inclusions water (δD_{fi}), and the $\delta^{18}O_{fi}$ value was inferred from the modern δD vs $\delta^{18}O$ relation in meteoric water (Schwarcz et al., 1976;Harmon et al., 1979;Genty et al., 2002;Matthews et al., 2000;McGarry et al., 2004). These studies measured δD_{fi} because of the technical difficulties associated with measuring the small amounts required to estimate $\delta^{18}O_{fi}$ values and a fundamental concern about the integrity of the $\delta^{18}O_{fi}$ data, namely, oxygen isotopic exchange between inclusion water and host calcite in speleothems.

After being trapped in the host calcite, inclusion water may continue to exchange oxygen isotopes with the surrounding host calcite as follows:

$$3H_2{}^{18}O + CaC^{16}O_3 \rightleftarrows 3H_2{}^{16}O + CaC^{18}O_3 \tag{1}.$$

This isotopic re-equilibration effect potentially alters the $\delta^{18}O_{fi}$ values after the initial trapping of the inclusion water in the stalagmite. Such post-depositional isotopic exchange does not occur for hydrogen because of the small amount of hydrogen in the host calcite. Many studies on geothermal water have shown positive $\delta^{18}O$ shifts from the global meteoric water line (GMWL) (Clark and Fritz (1999) and references therein). These data suggest the isotopic exchange of oxygen between the geothermal water and the host aquifer rocks. This observation is one of the reasons why the earlier studies mentioned above explored the δD values of fluid inclusions rather than $\delta^{18}O_{fi}$ values (Harmon et al., 1979; Matthews et al., 2000; McGarry et al., 2004).

Recently, because of technological developments involving continuous-flow isotope ratio mass spectrometry (Vonhof
et al., 2006) and cavity ringdown spectrometry (CRDS) (Uemura et al., 2016;Arienzo et al., 2013;Affolter et al., 2014),
isotopic data from fluid inclusion have been accumulating. Interestingly, these studies suggest that post-depositional
exchange is not significant. In fact, the fluid inclusions analyses of modern stalagmites have shown that their $\delta^{18}O_{fi}$ values
are consistent with the $\delta^{18}O$ values of modern dripwaters (Griffiths et al., 2010;Dennis et al., 2001;Arienzo et al.,
2013;Uemura et al., 2016;Labuhn et al., 2015), although a post-depositional alternation induced by recrystallisation has been
suggested (Demény et al., 2016). Moreover, several studies successfully quantitatively estimated past temperatures at the
time of calcite formation (van Breukelen et al., 2008;Meckler et al., 2015;Uemura et al., 2016). Therefore, these data imply
that the oxygen isotopic exchange between inclusion water and stalagmite calcite appears to be limited and/or very slow over
natural temperature ranges.

The indirect evidence for the insignificant post-depositional effect cannot provide explanations for the mechanisms
behind this phenomenon. Thus, it is essential to investigate the isotopic exchange reaction and evaluate how much the past
temperature reconstruction would be biased by this effect. However, to our knowledge, no study has investigated the
magnitude of isotopic exchange within natural stalagmites. In addition to this natural process, the isotopic exchange reaction
may occur during the drying process of stalagmite samples at high temperatures, which was commonly conducted before
fluid inclusion measurement in the laboratory.

Here, we evaluate the isotopic exchange reaction between inclusion water and host calcite based on a laboratory heating
experiment of natural stalagmite samples. We conducted a heating experiment because a higher temperature induces an
increase in the degree of isotopic disequilibrium between water and calcite and increases the rate of isotopic exchange. As a
result, an isotopic shift caused by the exchange reaction will be easier to detect. The stalagmite samples and experimental
settings are described in Section 2. Section 3 presents the experimental results and discusses them using an isotopic
exchange model for inclusion water and host calcite. Concluding remarks are given in Section 4.

2 Methods

2.1 Speleothem samples

A stalagmite (named HSN1) was collected in Hoshino Cave, Minami-Daito Island, Okinawa, Japan (25˚ 51′ 34″N, 131˚
13′ 29″E). The stalagmite is 246 mm in length, and most parts are milky white with thin transparent layers (Fig. 1). To
compare the isotope ratios of fluid inclusions after different heating times, ten layers, A-J, were taken (2.4-3.9 mm in
thickness) from a quartered section of the stalagmite (Tables 1 and 2). Then, 3-6 wedge-shaped chipped sub-samples (51-193
mg) were taken from each layer (the shape is illustrated in Uemura et al., 2016). The sub-samples from the same depth
interval were assumed to have the same isotopic compositions.

2.2 Isotope measurements

95   The oxygen and hydrogen isotopic compositions of the water in the fluid inclusions were measured using a homemade extraction device (Uemura et al., 2016). Briefly, the speleothem sample was crushed under vacuum, and then extracted water vapour was transferred to a CRDS isotope ratio analyser (L2130-I, Picarro Inc.) at the University of the Ryukyus. Compared with the system described in (Uemura et al., 2016), the system has been improved in three ways. (1) The valves were automatically controlled using pneumatic valves. (2) The entire system was heated at 105°C because most parts were easily

100   damaged at 150°C. (3) The water vapour extracted from the stalagmite was immediately trapped cryogenically at the temperature of liquid nitrogen, thereby preventing interaction between $CaCO_3$ and water vapour. The 1σ reproducibility for the inclusion water analysis was ±0.3‰ for $\delta^{18}O$ and ±1.6‰ for δD.

Stalagmite carbonate sub-samples were measured using an isotope ratio mass spectrometer (Thermo DELTA V Advantage) equipped with a Thermo Gasbench II system at the University of the Ryukyus. Powdered sub-samples of 150-

105   200 µg were reacted with 100% phosphoric acid at 72°C in septa-capped vials before measuring the released CO_2. The 1σ reproducibility for the analysis was ±0.04‰ for $\delta^{18}O$ and ±0.03‰ for $\delta^{13}C$.

The isotopic composition is expressed in units of per mill (‰) using delta notation ($\delta = R_{sample}/R_{ref} - 1$), where R is the isotopic ratio and R_{sample} and R_{ref} are the isotopic ratios of the sample and reference, respectively. For water samples from fluid inclusions, $\delta^{18}O$ and δD data are presented relative to Vienna standard mean ocean water (VSMOW) and are

110   normalized to the VSMOW-SLAP scale. For calcite samples, $\delta^{18}O$ and $\delta^{13}C$ data are expressed relative to the Vienna Pee Dee Belemnite (VPDB) and/or VSMOW references. For clarity, the δ values of fluid inclusion water and stalagmite calcite are expressed with the subscripts "fi" and "ca", respectively. $\delta^{18}O_{fi}$, for example, indicates the $\delta^{18}O$ value of the water in fluid inclusions.

2.3 Heating experiment

115   To evaluate the effect of isotopic exchange between water and host calcite, a heating experiment was conducted. As a pretreatment process, each stalagmite sample was placed in a small glass tube (o.d. 1/2 inch and 6 cm in length) with an airtight screw cap sealed with two Viton O-rings. Then, the samples were dried in a vacuum line for 17 hours at room temperature using a turbo molecular pump (with a pressure down to 10^{-5} Pa).

After this pre-drying process for all samples, the sample tube was subjected to different temperatures of 105°C or 25°C.

120   For the 105°C heating experiment, the sample tube was heated with a silicone cable heater at 105°C for a defined time period (from 0 to 70 hours) under the same vacuum conditions (i.e., a pressure down to 10^{-5} Pa). For comparison, we also performed a non-heating experiment at room temperature (25°C) for the same time periods (from 0 to 70 hours) under the same vacuum conditions.

Then, the heated (or non-heated) sample was transferred in the closed sample tube to the fluid inclusion analysis

125   apparatus so that atmospheric exposure when introducing the sample lasted for less than 30 seconds. To evacuate the

analytical line, the samples were subjected to an additional 105°C condition for 20 minutes. Then, the sample was crushed, and the isotopic composition of the inclusion water was measured as described in Section 2.2.

3 Results and discussion

3.1 Isotope composition of inclusion water

130   The temporal variations in $\delta^{18}O_{fi}$ and δD_{fi} values resulting from the 105°C heating and non-heating experiments are shown in Fig. 2. The isotopic compositions of inclusion water were shown as a deviation from the initial value ($\Delta\delta^{18}O_{fi}$ and $\Delta\delta D_{fi}$). For the 105°C heating experiment, the $\delta^{18}O_{fi}$ values gradually increased with the heating time and then reached a constant value after ca. 20 hours (Fig. 2a). The regression curve of the data represents an exponential function (Section 3.5). The $\delta^{18}O_{fi}$ values increased by ca. 0.7‰ with respect to the initial values during ca. 20 hours from the start of heating (Fig.

135   2a). In contrast, no significant trend was found for $\Delta\delta D_{fi}$ for the 105°C heating experiment (Fig. 3b). For the room-temperature drying experiment, neither $\Delta\delta^{18}O_{fi}$ nor $\Delta\delta D_{fi}$ values showed any systematic variation (Fig. 2c and 2d).

Overall, the results suggest that the observed increase in $\delta^{18}O_{fi}$ values in the 105°C heating experiment is caused by the oxygen isotopic exchange between inclusion water and the surrounding calcite. The data from the control experiment at room temperature suggest that the oxygen isotopic exchange reaction is too small to detect under 25°C conditions. The δD_{fi}

140   data for the 25°C experiment confirms that there is no systematic isotopic variation caused by subsample cutting and length of drying time. In addition, the $\Delta\delta D_{fi}$ value for the 105°C experiment did not change because there is little hydrogen reservoir in calcite.

3.2 Evaporation during heating

Long-term heating may induce leakage from water inclusions through microscopic channels in the calcite caused by

145   decrepitation of the calcite. The measured water content (weight ratio of water in fluid inclusions to carbonate) of the experiments is shown in Fig. 3. Overall, the water content ranged from 0.05 to 0.3 wt. %, which is within the typical observed range of 0.05-0.5% (McDermott, 2005).

Although there are large variations in water content among different layers, there is no significant trend between heating time and water content (Fig. 3). This result suggests that the fluid inclusion water does not evaporate/leak even after long-

150   term heating. In addition, this finding suggests that our standard drying procedure (17 hours at room temperature, as described in Methods) is enough to remove the water adsorbed onto the calcite. Therefore, this result confirms that the increase in $\delta^{18}O_{fi}$ values (Fig. 3) in the heating experiments is not caused by evaporation due to thermal decrepitation.

3.3 Evidence of a post-depositional effect in the D-O plot

The distinct behaviour of δD_{fi} and $\delta^{18}O_{fi}$ values is clearly depicted in the δD-$\delta^{18}O$ plot (Fig. 4). The GMWL and local

155   meteoric water line (LMWL) on Okinawa-jima Island in the summer and winter seasons are shown in Fig. 4. The LMWL is

calculated based on present-day rain water data from Okinawa-jima Island (Uemura et al., 2012), ca. 400 km west of Minami-daito Island. Although the initial δD_{fi} and $\delta^{18}O_{fi}$ values in the 25°C experiment are scattered between the summer and winter LWMLs, the $\delta^{18}O_{fi}$ values of the heating experiment are systematically shifted in the isotopically enriched direction. The average $\delta^{18}O_{fi}$ value of the heating experiments (−5.4±0.4‰) is higher than that of the 25°C experiment

160 (−6.1±0.3‰) by 0.7‰. In contrast, the average value of δD does not differ between these experiments (−34.5±1.9‰ in the heating experiment, −33.9±1.3‰ in the room-temperature experiment).

Such positive $\delta^{18}O$ shift is opposite to the negative $\delta^{18}O$ shift of inclusion water in speleothems possibly induced by recrystallisation (Demény et al., 2016). Instead, the positive shift is similar to the $\delta^{18}O$ shift from GMWL found in observations of geothermal water (Clark and Fritz, 1999). Although the magnitude of the shift is much larger for geothermal

165 water, 5-15‰, the $\delta^{18}O_{fi}$ shift found in our experiment is likely caused by the exchange of ^{18}O between the inclusion water and the host calcite. The possible reasons for the small magnitude of the $\delta^{18}O$ shift, 0.7‰, in our experiment will be discussed later (Section 3.4 and 3.5).

In fluid inclusion studies, closeness to the LMWL in δD-$\delta^{18}O$ plots has been used as proof for the validity of analytical methods and the integrity of the sample. Our result calls for caution regarding the δD-$\delta^{18}O$ plot test. Most of the inclusion

170 data from the heating and room-temperature experiments are distributed between the summer and winter LWMLs. As discussed above, the artificial increase in the $\delta^{18}O$ value by heating is systematic. However, it is difficult to detect such a shift in the δD-$\delta^{18}O$ plot because the shift is small in the scattered data points. The data from the heating experiment (more than 10 hours) plot outside the summer LMWL, but a 0.7‰ deviation could be interpreted as resulting from different climate conditions. Because the oxygen isotopic exchange results in higher $\delta^{18}O$ values without a δD shift, the isotopic exchange

175 results in a lower deuterium excess value ($d = \delta D - 8\ \delta^{18}O$), −6‰ shift in our experiment. Therefore, the low deuterium excess value of inclusion water can potentially be used as an indicator for the existence of oxygen isotopic exchange.

3.4 Partial isotopic exchange between water and calcite

To interpret the experimental result, we consider two hypotheses: (i) the oxygen isotopic exchange reaction occurred between inclusion water and the entirety of the host calcite (hereafter referred to as the "fully reacted hypothesis") and (ii)

180 the $\delta^{18}O_{fi}$ values are equilibrated with a limited amount of $CaCO_3$ (hereafter referred to as the "partially reacted hypothesis"). These hypotheses are schematically explained in Fig. 5.

For the fully reacted hypothesis, the number of oxygen atoms in calcite can be considered infinite compared with that in the water inclusions because the water content of a stalagmite is very small: 0.05-0.5 wt. % (McDermott, 2005). Thus, the $\delta^{18}O_{fi}$ is simply controlled by the $\delta^{18}O$ value of calcite and the equilibrium fractionation factor between $CaCO_3$ and H_2O at

185 the ambient temperature (Fig. 5b). At 105°C, the fractionation factor between $CaCO_3$ and water is 1.0167. Because the $\delta^{18}O_{ca}$ value of HSN1 stalagmite is 25.5‰ vs VSMOW (i.e., −5.3‰ vs VPDB), the $\delta^{18}O_{fi}$ value in equilibrium with the calcite should be 8.6‰ vs VSMOW. Thus, $\delta^{18}O_{fi}$ value should be enriched by 14 to 15‰. This simple hypothesis, however, is not

realistic because the inclusion water likely reacts only with the inner surface of $CaCO_3$ surrounding the inclusions. In fact, the results in Fig. 2a show that the actual change in the $\delta^{18}O_{fi}$ value is only 0.7‰.

190   The small $\delta^{18}O_{fi}$ shift observed in the experiment can be interpreted as the result of (i) insufficient reaction time between water and calcite and/or (ii) reaction with a limited amount of $CaCO_3$. The first hypothesis can be rejected because the $\Delta\delta^{18}O_{fi}$ enrichment plateaued within 20 hours (Fig. 2a). Therefore, the experimental results support the latter hypothesis. A model for this partially reacted hypothesis will be discussed in the following section.

3.5 Isotopic re-equilibration model

195   We describe a partial isotopic equilibration model that considers the changes in $\delta^{18}O_{fi}$ values from the time when the inclusion water was entrapped in $CaCO_3$ (time 0) to a certain time required to reach a new isotopic equilibrium (time t_1; at a certain temperature T_1). An example of this re-equilibration scenario is that inclusion waters at room temperature (time = 0, temperature = T_0) are heated to 105°C (T_1) and reach a new isotope equilibrium at time t_1.

   For the partially reacted hypothesis, we assumed that a limited amount of $CaCO_3$ in the reacted layer exchanged oxygen
200   isotopes with the inclusion water (Fig. 5a). The isotope mass balance between the initial and partially equilibrated conditions at a heating time during a course of reaction at time t can be written as follows:

$$3 \cdot \gamma \, \delta^{18}_{ca\_ra(0)} + \delta^{18}_{fi(0)} = 3 \cdot \gamma \, \delta^{18}_{ca\_ra(t)} + \delta^{18}_{fi(t)} \tag{2}$$

205   where γ is a molar ratio of $CaCO_3$ involved in the reaction to the inclusion water (i.e., $\gamma = CaCO_3$ in reacted layer / H_2O in inclusion: M_{CaCO_3}/M_{H2O}), $\delta^{18}_{ca\_ra(t)}$ indicates the $\delta^{18}O$ value of the $CaCO_3$ in the reacted layer, and $\delta^{18}_{fi(t)}$ indicates the $\delta^{18}O$ value of the inclusion water at time t. If the $CaCO_3$ does not react with the water (i.e., $\gamma = 0$), for example, the $\delta^{18}O_{fi}$ value does not change before and after the reaction.

   At t_1, the $\delta^{18}_{ca\_ra(t)}$ and $\delta^{18}_{fi(t)}$ reach an isotopic equilibrium state. This equilibrium state can be written as:

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$$\delta^{18}_{ca\_ra(t1)} - \delta^{18}_{fi(t1)} = \varepsilon^{18}_{T1} \tag{3}$$

   where ε^{18}_{T1} indicates the oxygen isotopic enrichment factor at the temperature T_1. Since the isotopic exchange reaction is considered to be faster when the isotopic difference, $(\delta^{18}_{ca\_ra(t)} - \delta^{18}_{fi(t)})$, differs markedly from the isotopic enrichment factor
215   at the new equilibrium state (ε^{18}_{T1}), the reaction can be written as:

$$-\frac{d(\delta^{18}_{ca\_ra(t)} - \delta^{18}_{fi(t)} - \varepsilon^{18}_{T1})}{dt} = k_{18}(\delta^{18}_{ca\_ra(t)} - \delta^{18}_{fi(t)} - \varepsilon^{18}_{T1}) \tag{4}$$

where k_{18} indicates a reaction constant. The integration of Eq. (4) yields:

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$$\left(\delta^{18}_{ca\_ra(t)} - \delta^{18}_{fi(t)} - \varepsilon^{18}_T\right) = \left(\varepsilon^{18}_{T0} - \varepsilon^{18}_{T1}\right)\exp(-k_{18}t) \tag{5}$$

where $\varepsilon^{18}_{T0} \left(= \delta^{18}_{ca\_eq(0)} - \delta^{18}_{fi(0)}\right)$ represents the oxygen isotopic enrichment factor at the initial temperature of T_0.

To fit our experimental data, in which we measured the difference between heated and initial (i.e., non-heated)

225   conditions, we took the difference between Eq. (5) and the initial condition (t = 0) of Eq. (5) $(\delta^{18}_{ca\_eq(0)} - \delta^{18}_{fi(0)} - \varepsilon^{18}_{T1} = \varepsilon^{18}_{T0} - \varepsilon^{18}_{T1})$. Thus, with Eq. (2), we obtain

$$\Delta\delta^{18}_{fi(t)} = \left(\frac{3\gamma}{1+3\gamma}\right)\left(\varepsilon^{18}_{T0} - \varepsilon^{18}_{T1}\right)\left(1 - \exp(-k_{18}t)\right) \tag{6}$$

230   where $\Delta\delta^{18}_{fi(t)}(= \delta^{18}_{fi(t)} - \delta^{18}_{fi(0)})$ represents the $\delta^{18}O_{fi}$ difference between the reacted and initial conditions. When the new equilibrium state is reached (t = t_1), Eq. (6) yields:

$$\Delta\delta^{18}_{fi(t1)} = \left(\frac{3\gamma}{1+3\gamma}\right)\left(\varepsilon^{18}_{T0} - \varepsilon^{18}_{T1}\right) \tag{7}.$$

235   This equation provides an estimate of γ based on the experimental results.

A regression curve based on Eq. (5), is shown in Fig. 2a. Based on Eq. (7), the value of γ is estimated to be 0.02. This suggests that the amount of $CaCO_3$ reacted with water is equivalent to 2% of the water inclusions in molar terms. The thickness of the reacted layer of $CaCO_3$ can be roughly estimated on the assumption that the fluid inclusions filled with water are cubic with 50 μm edges. With a calcite density of 2.71 g/cm³, the thickness of the reacted layer of $CaCO_3$ is estimated to

240   be 0.6 μm.

This result is explained schematically in Fig. 5a and 5c. The $\delta^{18}O_{fi}$ value increased by only 0.7‰. Since the equilibrium fractionation factor between $CaCO_3$ and water at 105°C is 1.0167, the $\delta^{18}O_{ca}$ of the reacted layer should be 11.3‰ vs VSMOW. Thus, in this case, the $\delta^{18}O_{ca}$ value of the reacted layer changed significantly.

3.6 Impact for palaeo-climate reconstruction

245   In this section, we estimate the impact of the isotopic exchange effect on Quaternary palaeo-climate reconstructions. First, at 105°C, detectable isotopic exchange occurred within 20 hours. This finding suggests that researchers should be aware of this effect during experimental procedures, such as the heat drying process, before fluid inclusion measurements. Second, we consider a case in which the dripwater enclosed in the fluid inclusions during a glacial period with a temperature of 15°C re-equilibrates at the modern average temperature of 25°C. The isotopic enrichment factors (ε^{18}_T) for 15°C and 25°C

250 are 30.6‰ and 28.4‰, respectively. Thus, based on Eq. (7) with a γ value of 0.02, the fluid inclusion oxygen isotope ratio $\delta^{18}O_{fi}$ would increase by only 0.1‰. With the temperature dependence of the $\delta^{18}O$ fractionation factor between water and calcite (0.2‰/°C), this isotopic exchange results in a 0.5°C bias in the palaeo-temperature estimate. We should note that we do not know the reaction rate of the isotopic exchange under normal ambient temperatures. Calcite and fluid inclusion water might not reach a new equilibrium state even after thousands of years. Thus, this estimate is an upper limit of the bias.

255 Because the bias, 0.1‰, is within the typical analytical error for inclusion analyses, the post-depositional effect has little influence on the palaeo-temperature estimates for glacial-interglacial cycles.

4 Conclusions

Our experiment shows that the $\delta^{18}O$ values of fluid inclusion water in speleothems changes because of isotopic exchange reactions with the host calcite. Unlike the $\delta^{18}O$ value of inclusion water, the δD value showed no trend even after

260 prolonged heating and thus effectively reflects the original isotopic composition of past dripwater. This study is the first to present experimental data showing that such a post-depositional effect occurs in natural speleothem samples. However, the changes in the $\delta^{18}O$ values of fluid inclusion were very small, 0.7‰, in the 105°C heating experiment. Based on this result, the inclusion water reacts only with a thin layer of surrounding $CaCO_3$. The amount of $CaCO_3$ that reacted with the water is equivalent to 2% of the water inclusions in molar terms. Thus, the oxygen isotopic exchange results in a minor impact on the

265 estimation of past temperature changes: a maximum bias of 0.5°C for a 10°C climate shift. This study provides a quantitative explanation of the mechanism by which the effect of isotopic exchange appears to be insignificant in previous speleothem studies. The results also suggest that the sample treatment in the laboratory (i.e., heated drying process) should be conducted with caution because isotopic exchange may affect the $\delta^{18}O$ value of fluid inclusions.

270 **Data availability**

The data generated and used in this study are available in Tables 1 and 2 in this article.

Author contributions.

RU designed the experiment and study. YK and KO conducted the experiments. All authors contributed to the discussion. RU analysed the results, generated figures and wrote the paper.

275 **Competing interests.**

The authors declare that they have no conflict of interest.

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Tables

Table 1: Isotopic compositions of inclusion waters and calcite in HNS1 stalagmite for the 105°C experiment

ID	depth (mm)	Weight (mg)	$\delta^{18}O_{ca}$ (‰, VPDB)	water content (wt.%)	$\delta^{18}O_{fi}$ (‰, VSMOW)	δD_{fi} (‰, VSMOW)	Drying time (h)	$\Delta\delta^{18}O_{fi}$ (‰, VSMOW)	$\Delta\delta D_{fi}$ (‰, VSMOW)
A	74.2-76.8	151	-5.29	0.14	-5.88	-34.25	0.1	0.00	0.0
		97	-5.29	0.15	-5.85	-37.02	1.0	0.03	-2.8
		100	-5.29	0.14	-5.51	-35.72	7.0	0.37	-1.5
		114	-5.29	0.26	-5.00	-31.45	18.0	0.88	2.8
		51	-5.29	0.10	-5.91	-36.32	33.5	-0.03	-2.1
		97	-5.29	0.14	-4.79	-32.70	60.0	1.09	1.5
B	83.3-86.3	54	-5.53	0.12	-5.96	-35.88	0.1	0.00	0.0
		91	-5.53	0.16	-4.84	-33.69	15.0	1.12	2.2
		75	-5.53	0.08	-4.88	-34.99	68.0	1.08	0.9
C	102.0-105.9	128	-5.18	0.06	-6.41	-33.59	0.1	0.00	0.0
		193	-5.18	0.09	-6.19	-36.90	15.5	0.22	-3.3
		183	-5.18	0.09	-5.30	-32.90	16.0	1.10	0.7
		158	-5.18	0.09	-5.87	-33.73	44.0	0.54	-0.1
D*	106.3-109.0	151	-5.73	0.07	-6.04	-38.94	1.0	0.00	0.0
		156	-5.73	0.16	-5.93	-36.87	4.0	0.11	2.1
		160	-5.73	0.06	-5.29	-34.63	20.0	0.75	4.3
		192	-5.73	0.08	-5.56	-38.70	42.0	0.48	0.2
E	130.5-133.4	91	-5.31	0.23	-6.09	-32.68	0.1	0.00	0.0
		58	-5.31	0.10	-5.68	-34.24	7.5	0.42	-1.6
		56	-5.31	0.23	-5.60	-34.04	22.0	0.50	-1.4
		70	-5.31	0.19	-5.54	-34.37	43.5	0.55	-1.7

| | | 63 | -5.31 | 0.18 | -5.21 | -33.58 | 68.0 | 0.88 | -0.9 |

370 *** The initial value for sample D was one hour of drying.**

Table 2: Isotopic compositions of inclusion waters and calcite in HNS1 stalagmite for the 25°C experiment

ID	depth (mm)	Weight (mg)	$\delta^{18}O_{ca}$ (‰, VPDB)	water content (wt.%)	$\delta^{18}O_{fi}$ (‰, VSMOW)	δD_{fi} (‰, VSMOW)	Drying time (h)	$\Delta\delta^{18}O_{fi}$ (‰, VSMOW)	$\Delta\delta D_{fi}$ (‰, VSMOW)
F	95.4-98.6	176	-5.52	0.10	-5.87	-33.01	0.0	0.0	0.0
		155	-5.52	0.07	-6.39	-34.91	19.0	-0.5	-1.9
		169	-5.52	0.06	-6.13	-32.99	42.5	-0.3	0.0
		111	-5.52	0.15	-6.40	-34.58	71.5	-0.5	-1.6
G	117.0-119.4	76	-5.35	0.22	-5.95	-32.67	0.0	0.00	0.0
		90	-5.35	0.13	-5.96	-34.24	6.0	-0.01	-1.6
		86	-5.35	0.22	-5.85	-33.49	22.5	0.10	-0.8
		79	-5.35	0.16	-6.65	-35.98	55.0	-0.70	-3.3
		92	-5.35	0.16	-6.01	-34.85	73.5	-0.06	-2.2
H	120.0_123.5	151	-5.38	0.08	-5.93	-35.17	0.0	0.00	0.0
		116	-5.38	0.06	-6.15	-34.31	27.0	-0.23	0.9
		155	-5.38	0.19	-6.44	-33.93	50.5	-0.51	1.2
		119	-5.38	0.11	-5.79	-33.39	72.0	0.13	1.8
I	148.6-152.2	84	-4.94	0.08	-6.41	-34.98	0.0	-0.12	-0.6
		113	-4.94	0.14	-6.18	-33.81	0.0	0.12	0.6
		107	-4.94	0.25	-5.94	-33.57	6.0	0.35	0.8
		95	-4.94	0.31	-5.79	-30.86	23.0	0.51	3.5
		71	-4.94	0.15	-6.77	-36.49	43.5	-0.48	-2.1
J	177.9-180.6	92	-5.30	0.16	-6.44	-34.59	0.0	0.00	0.0
		134	-5.30	0.18	-5.73	-32.05	52.5	0.71	2.5
		84.7	-5.30	0.16	-5.83	-32.23	77.0	0.61	2.4

Figures

Figure 1: Photographs of HSN1 stalagmite.

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Half section of the HSN1 stalagmite from Hoshino Cave. The horizontal bar represents 5 cm.

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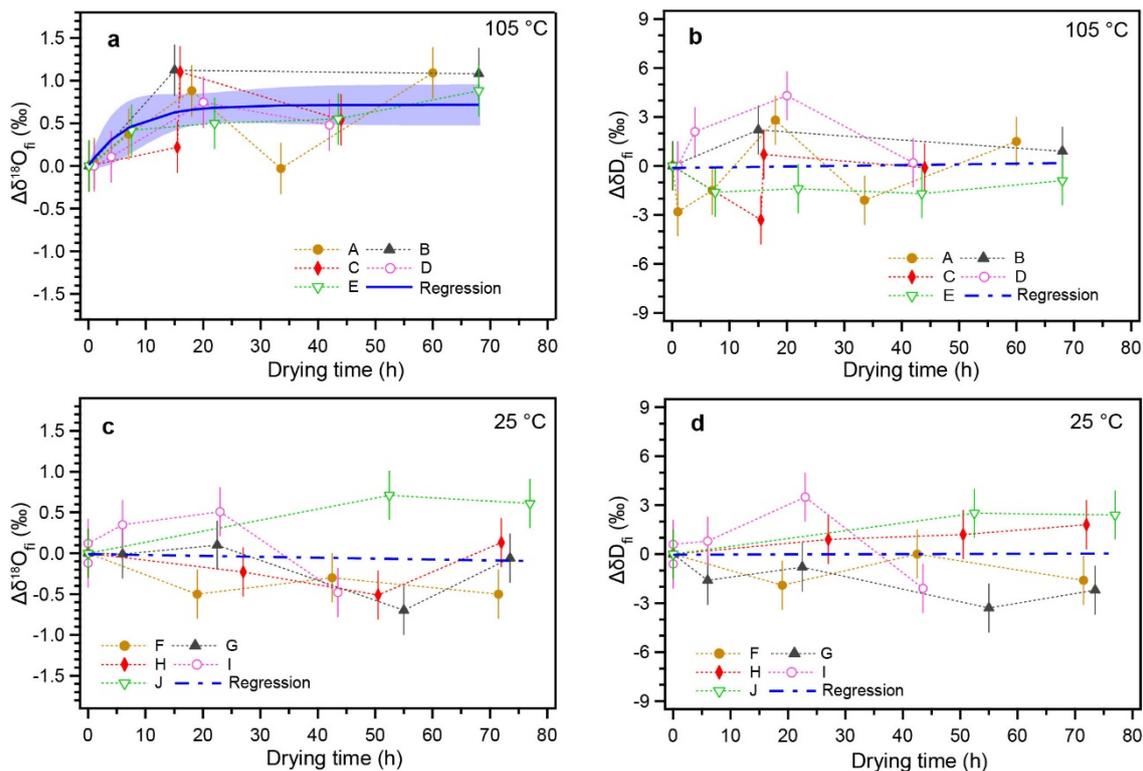
Figure 2: Changes in isotopic compositions of inclusion water with increasing drying time.

385

a) Changes in the $\delta^{18}O$ value of inclusion water from the initial value ($\Delta\delta^{18}_{fi(t)}$) with time under 105°C heating. Time on the x-axis indicates drying time in addition to the standard 17-hour drying process at room temperature. **b)** The same as **a)** but for δD_{fi}. **c)** Changes in the $\Delta\delta^{18}_{fi(t)}$ value over time at room temperature (25°C). **d)** The same as **c)** but for δD_{fi}. The error bar of the data point represents the analytical reproducibility (1σ). The regression curve (blue) in panel **a)** was calculated with

390 IGOR (Wave Metrics Inc.) software to fit a function ($\Delta\delta^{18}_{fi(t)} = A\,(1 - \exp(-k\ t))$) (A = 0.71: k = 0.14). The blue shade indicates the 95% confidence interval. Blue dashed lines in panels **b-d** indicate linear regression lines (the correlations are insignificant).

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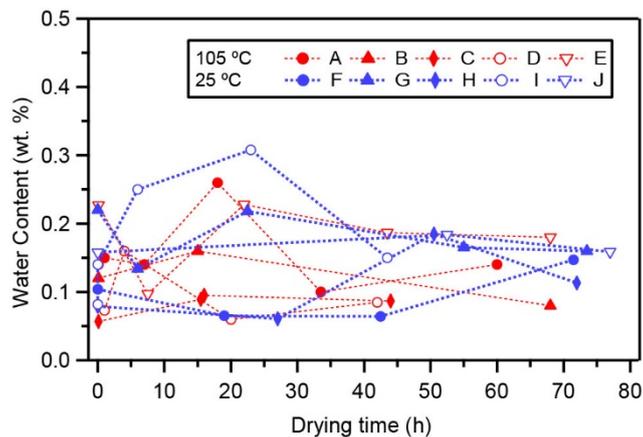
Figure 3: Changes in water content with increasing drying time.

Changes in water content (in wt.%) of each subsample with time (in hour). Symbols indicate the 105°C experiment (red,

400   layer A-E) and 25°C drying experiment (blue, layer F-J).

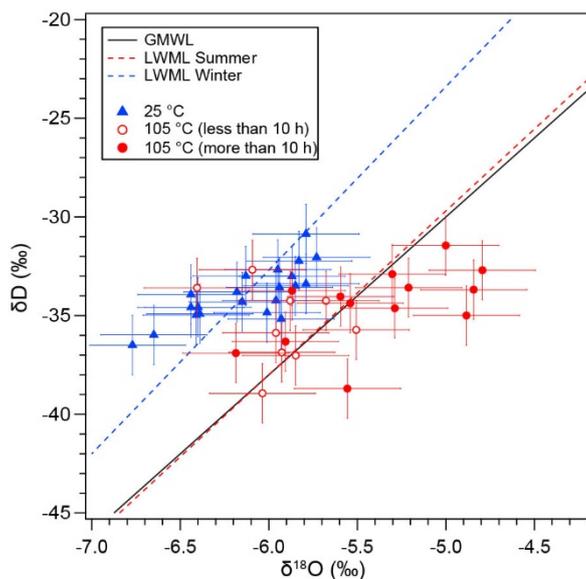
Figure 4: D-O plot of inclusion water.

405   The blue triangles indicate the isotopic compositions of the fluid inclusion in the 25°C drying experiment. The red circles indicate the isotopic compositions of the fluid inclusion in the 105°C drying experiment for < 10 hours (open red circles) and >10 hours (solid red circles). The black line indicates GMWL. Local meteoric water lines based on present-day raindata from Okinawa-jima Island for the summer season (red dotted line) and the winter season (blue dotted line) are also shown.

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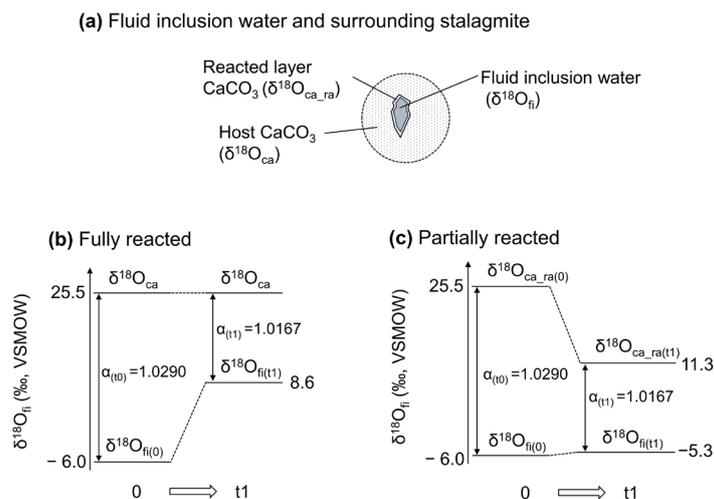
Figure 5: Schematic illustration of isotopic re-equilibration between inclusion water and stalagmite.

(a) Fluid inclusion water and surrounding stalagmite

(**a**) Schematic illustration of a water-filled fluid inclusion and surrounding $CaCO_3$. (**b**) Changes in the isotopic composition for the fully reacted hypothesis, in which the $\delta^{18}O_{fi}$ value changes in response to equilibration with an infinite amount of host

415   $CaCO_3$. In this case, the sample at room temperature (25°C at time of 0) is heated to 105°C and reaches a new isotopic equilibrium at time t_1. (**c**) The same as (**b**) but for the partially reacted hypothesis, in which the $\delta^{18}O_{fi}$ value changes in response to equilibration with a limited amount of $CaCO_3$. Note that the calcite-water fractionation factor is the same for both hypotheses.