The effects of Porapak^{TM} trap temperature on $\delta^{18}O$, $\delta^{13}C$, and Δ_{47} values in preparing samples for clumped isotope analysis

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ABSTRACT

RATIONALE: The clumped isotope paleothermometer, a new proxy widely applicable in studies of paleoclimate, tectonics, and paleontology, relates the abundance of doubly-substituted isotopologues of carbonate-derived CO_2 to the temperature of formation of the carbonate phase. As this technique becomes more widely used, more is discovered about the effects of everyday laboratory procedures on the clumped isotopic composition of CO_2 gas.

METHODS: Preparation of CO_2 for clumped isotope analysis requires the removal of isobaric contaminants prior to measurement, achieved dynamically by passing the CO_2 through a gas chromatography column using a helium carrier gas or cryogenically pumping CO_2 through a static trap filled with Porapak^{TM} Q (PPQ) material. The stable and clumped isotopic compositions of carbonate standards prepared at PPQ trap temperatures between -40°C and -10°C were measured by isotope ratio mass spectrometry to evaluate potential artifacts introduced by the static PPQ trap method.

RESULTS: The stable isotopic composition of carbonates run at temperatures below -20°C were fractionated, despite achieving >99% retrieval of gas at temperatures as cold as -30°C. The $\delta^{13}C$ and $\delta^{18}O$ values decreased by ~0.01 and ~0.03 ‰/(°C below -20°C). The raw Δ_{47} values decreased by 0.003-0.005 ‰/(°C below -20°C), but the final reference-frame-corrected value ($\Delta_{47\text{-RFAC}}$) was unaffected as long as the carbonate samples and standard gases were prepared identically.

CONCLUSIONS: Preparing carbonate samples for clumped isotope analysis using a PPQ trap that is too cold can result in erroneous stable isotopic compositions. New and existing labs using the static PPQ trap cleaning procedure should determine the ideal PPQ

trap temperature for their particular system through monitoring not only yield through the PPQ trap, but also stable isotopic composition at various PPQ trap temperatures.

Introduction

The clumped isotope paleothermometer relates the abundance of doubly-substituted isotopologues of CO_2 derived from calcium carbonate to the temperature of formation of that carbonate.[1,2] This has wide-ranging applications in the fields of tectonics[3], paleoclimate[4] and atmospheric science[5]. The first theoretical papers[6,7] and calibration studies[1] were published a decade ago, and since then much about the proxy and the clumped isotope measurement has been discovered, including the development of a standardization scheme that brings measurements from all laboratories into the same reference frame[8], the discovery of the “pressure baseline effect” causing non-linearities in the mass spectrometers used[9,10], and the design of systems that can reduce the sample size requirements to less than 1.5 mg per replicate[11,12].

The carbonate clumped isotope measurement is made on CO_2 derived from calcium carbonate ($CaCO_3$), and measures the abundance of CO_2 molecules that have both ^{13}C and ^{18}O ($^{13}C\,^{16}O^{18}O$). This is described by the Δ_{47} parameter, which is defined as:

$$\Delta_{47} = \left[\left(\frac{R^{47}}{R^{47*}} - 1\right) - \left(\frac{R^{46}}{R^{46*}} - 1\right) - \left(\frac{R^{45}}{R^{45*}} - 1\right)\right] \qquad [1]$$

where R^{4X} is the observed ratio of CO_2 with mass 4X (45, 46 or 47) to CO_2 with mass 44 ($^{4X}CO_2/^{44}CO_2$) and R^{4X*} is the corresponding stochastic distribution ratio, given the bulk composition of the sample. **Equation 1** would typically be multiplied by 1000[2,9,11,12], but this factor has been removed in order to conform with recent IUPAC stable isotope notation guidelines. [13]

Unlike traditional stable isotope measurements, the clumped isotope measurement is susceptible to isobaric interference from a wider range of contaminants, including hydrocarbons, halocarbons, and sulfur compounds.[6] In addition, the abundance of these rare doubly-substituted isotopologues is low, meaning that trace amounts of contaminants can have a large effect on the measured Δ_{47} values. Contamination is revealed in the Δ_{48} parameter[14], which is defined as:

$$\Delta_{48} = \left[\left(\frac{R^{48}}{R^{48*}} - 1 \right) - 2 * \left(\frac{R^{46}}{R^{46*}} - 1 \right) \right] \qquad [2]$$

where R^{4X} and R^{4X*} are as defined in **Equation 1**. The factor of 1000 commonly found at the end of this equation[11,14] has again been removed to conform with IUPAC notation guidelines.[13] Contaminants that have a small but relevant effect on Δ_{47} will often have an order of magnitude larger effect on Δ_{48}[6], making it a good diagnostic for contamination.

To reduce the effects of these contaminants, the CO_2 is cleaned prior to sample introduction into the mass spectrometer. In initial work performed at Caltech, this involved passing each CO_2 sample through a gas chromatography (GC) column (Supelco Q-PLOT, 530μm i.d., 30m long) held at -10°C for 40-60 minutes using a helium carrier gas and baking out the column between samples.[1,14] A later design did away with the need for a carrier gas and instead used a U-trap packed with a very similar adsorbing agent, Porapak™ Q (PPQ), cooled to -12°C. This method relied on the pressure gradient created by placing liquid nitrogen on the far side of the trap to draw the CO_2 through (cryogenic pumping).[15] Porapak™ is a type of porous polymer adsorbent manufactured by the Waters Corporation, which is used to pack GC columns.

All new labs entering the field of clumped isotopes generally follow one of these two methods of cleaning CO_2 of contaminants. Currently labs at the California Institute of Technology, Johns Hopkins University, Yale University, UCLA, and the Toyko Institute of Technology use the GC cleaning method, whereas labs at Harvard, ETH Zurich, Tulane/University of South Florida, Imperial College London, University of Miami, University of East Anglia, and the University of Michigan use the static PPQ method (**Table 1**). In the labs using the static PPQ trap configuration, the dimensions of the trap, the temperature to which it is cooled, and the duration of transfer have all been empirically determined on a lab-by-lab basis. Details of this trial-and-error process rarely make it into published articles, but could be based on assessing yield (% of starting gas collected on the far side of the PPQ trap) or by testing measured Δ_{47} values of standards calibrated by other labs.

Here, we test the effects of the temperature of the static PPQ trap on the measured stable isotopic ($\delta^{13}C$ and $\delta^{18}O$) and clumped isotopic (Δ_{47}) composition of carbonate standards. We show that at colder PPQ trap temperatures the $\delta^{13}C$, $\delta^{18}O$ and raw Δ_{47} values are fractionated, but the reference-frame-corrected Δ_{47} value ($\Delta_{47\text{-RFAC}}$) is unaffected as long as standards gases and samples are prepared identically. In cases where the samples and standards are prepared at different temperatures, a simple correction can be applied to account for the fractionation.

Experimental

Measurements were made in the University of Michigan (U of M) Stable Isotope Laboratory. Carbonate standards of 4-6 mg were prepared on a custom built off-line system described by Defliese *et al*.[16] The resulting clean CO_2 was analyzed on a ThermoFinnigan MAT 253 isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with cups to measure masses 44-49. Unless otherwise noted, samples were measured at 16 volts on the *m/z* 44 beam for 60-80 cycles (6-8 acquisitions of 10 cycles each with pressure balanced at the beginning of each acquisition). Standards including heated gases (1000°C) and gases equilibrated with H_2O (25°C) were measured alongside carbonate standards, and the raw Δ_{47} values were converted into the absolute reference frame[8], and acid-corrected using a fractionation factor of 0.067‰ for a 75°C reaction temperature[16]. The resulting final values are labeled $\Delta_{47\text{-RFAC}}$ (Reference Frame, Acid Corrected). All data discussed here can be found in **Table S1** (Supporting Information).

PPQ trap configuration

The samples presented here were cleaned through the PPQ trap in one of three configurations:

- **"ColdPPQ" configuration:** Data collected between May 2012 and October 2014, and during 3 days in the June 2015 measurement session. PPQ trap temperature at -30°C. Gas equilibrated for 5 minutes, followed by 15 minutes of cryogenic pumping and collection.[16]
- **"Stable Isotope Test" configuration**: Data collected in November 2014. PPQ trap temperature varied between -10°C and -40°C. 5 minutes of equilibration followed by 15 minutes of cryogenic pumping and collection. (Note: samples only analyzed for 20-30 cycles each instead of 60-80).
- **"WarmPPQ" configuration:** Data collected between January and June 2015 (excluding 3 days where the system reverted to the ColdPPQ configuration). PPQ trap held between -10°C and -15°C. 10 minutes of cryogenic pumping and collection (no equilibration period).

In the ColdPPQ and WarmPPQ configurations, standard gases were measured alongside carbonate samples, allowing for calculation of $\Delta_{47\text{-RFAC}}$. In the Stable Isotope Test configuration, samples were measured over a 2-day period with no accompanying standard gases, thus prohibiting the conversion of raw Δ_{47} to $\Delta_{47\text{-RFAC}}$, but still allowing the comparison of $\delta^{13}C$ and $\delta^{18}O$ values, and the internal comparison of raw Δ_{47} values.

Carbonate standards

Two in-house carbonate standards were measured in this study. Carrara Marble (CM) is a high-temperature calcite standard similar to IAEA NBS 19, and the current batch has been measured at U of M since 2011. Carrara marble powders (both in-house standards and NBS 19) have been measured in other labs and have been found to have Δ_{47} values between 0.373 and 0.404‰.[8] Joulters Cay Ooids (OO) was added in 2014 to provide a lower temperature and aragonitic standard. OO is made up of Holocene aragonitic ooids collected by lab members from the Bahamas at a site with a mean annual temperature of ~22°C[16] (which would correspond to a Δ_{47} value of 0.704‰ using a recent calibration[16]).

The CM and OO standards were independently measured on a ThermoFinnigan Kiel III carbonate device connected to the same MAT 253 mass spectrometer as performed the clumped isotope measurements and on a ThermoFinnigan Kiel IV carbonate device connected to a second ThermoFinnigan MAT 253 mass spectrometer. Their stable isotopic compositions were determined by normalization of measured values to measured values of the IAEA standards NBS 18 and NBS 19. Final values for CM were 2.05 ± 0.04‰ for δ^{13}C and -2.16 ± 0.09‰ for δ^{18}O. OO was measured to have a composition of 4.77 ± 0.09‰ for δ^{13}C and -0.16 ± 0.09‰ for δ^{18}O, all relative to VPDB. In addition, two replicates of CM and two of OO were prepared on the clumped isotope prep-line without undergoing the PPQ trap cleaning step, and the measured δ^{13}C and δ^{18}O values were within error of values measured on the Kiel III and Kiel IV devices. This was done to test for biases introduced by differences between the Kiel devices and the clumped isotope reaction system (for example, the clumped isotope system uses a common acid bath, whereas the Kiel devices use many separate reaction vessels where acid is added to each sample individually), and to identify baseline contaminant levels in an uncleaned sample.

Stable Isotope Values and Small Deltas (δ^{45}, δ^{46}, and δ^{47})

Although the ultimate result of a stable isotope measurement is a δ^{13}C and/or δ^{18}O value, the raw beam intensities (in millivolts) recorded by the mass spectrometer are first converted into “small delta” values that relate the abundance of CO_2 molecules with a given heavier mass (45, 46 or 47) in the sample gas and reference gas relative to the most common isotopologue (mass 44). These small deltas are defined following Huntington *et al.*[14] and are calculated as:

$$\delta^{4X} = \left[\frac{Rsa4X}{0.5*(Rst4X\_1 - Rst4X\_2)} - 1 \right] \qquad [3]$$

where 4X is 45, 46, or 47. Rsa4X and Rst4X are defined as the ratio of the intensity of a given mass (4X, 45-47) to the intensity of mass 44 for the sample (Rsa) or reference gas

(Rst). Rst4X_1 and Rst4X_2 are the half-cycle measurements of the reference gas preceding and following the sample gas measurement. The factor of 1000 commonly found at the end of this equation[14] has been removed to conform with IUPAC notation guidelines.[13] The $\delta^{18}O$ value can be calculated from the measured δ^{46} value, and by assuming mass-dependent behavior of ^{17}O relative to ^{18}O, and the $\delta^{13}C$ value can be calculated from a ^{17}O-corrected δ^{45} value. The δ^{47} value is a metric for the total inventory of heavy isotopes, and is a convolution of the $\delta^{13}C$ and $\delta^{18}O$ values. As the behavior of δ^{45}, δ^{46}, and δ^{47} tracks the changing isotopic composition, these direct measurements are used in place of $\delta^{13}C$ and $\delta^{18}O$ values in this study.

Results

Effect of PPQ trap temperature on yield

The custom-built reaction-extraction apparatus at the University of Michigan permits the direct quantification of yield through the PPQ trap as the sample gas is frozen into the same isolated volume equipped with an electric manometer before and after cleaning.[16] The yield is calculated as the pressure post-PPQ cleaning minus the background pressure divided by the pressure pre-PPQ cleaning minus the background pressure. The error on a single calculated yield is conservatively estimated at ±0.5% or less.

Figure 1 combines data from all three measurement configurations and relates yield to PPQ trap temperature. Yields of greater than 99% are routinely achieved for PPQ trap temperatures as low as -30°C and a duration of 5 minutes of equilibration followed by 15 minutes of cryogenic pumping/trapping. The yield drops to 97-98% between -30°C and -40°C. This indicates that gas is not being quantitatively transferred and collected. Some loss is expected (< 1%) because this cleaning step is employed to remove contaminants and residual non-condensables, but yields of less than 99% suggest that not all the sample gas is being collected after cleaning.

In setting up the static PPQ trap at U of M, the duration and PPQ trap temperature were initially determined by assessing the yield through the PPQ cleaning step. A threshold of 99% yield was deemed satisfactory, and a combination of -30°C PPQ trap temperature and 5 minutes equilibration followed by 15 minutes of collection was sufficient to routinely achieve this level of yield.

The duration of the PPQ transfer step also contributes to the overall yield but was not explicitly tested in this study. Increasing the transfer duration for the coldest PPQ trap temperatures would likely have increased the yield. The duration of the transfer step in both the ColdPPQ and WarmPPQ configurations was deemed to be long enough if the yield was above 99%.

Contaminant Removal

Ultimately, the purpose of the PPQ trap is to remove contaminants such as hydrocarbons and sulfur and chlorine-containing compounds that cause isobaric interference on masses 47 and 48. One risk of using a higher PPQ trap temperature is that contaminants will not be fully removed. The presence of contaminants is usually assessed through a comparison of the Δ_{48} value of samples and pure standard gases[14]. In the case where no standard gases were run ("Stable Isotope Tests" – November 2014), relative comparison of Δ_{48} between samples run at different PPQ trap temperatures (or not through the PPQ trap at all) can suffice to assess contaminant removal. **Figure 2** shows the Δ_{48} offset (difference from the -20°C to -10°C mean value) for samples run at different PPQ trap temperatures, as well as samples where the PPQ cleaning procedure was not performed. Samples run through the PPQ trap (at any temperature) had much lower Δ_{48} offsets than those that bypassed the PPQ cleaning step. Little to no additional decrease in Δ_{48} offset occurs between -10°C and -40°C. Samples from the November 2014 "Stable Isotope Tests" were also compared with standard gases run in previous and subsequent measurement sessions. The Δ_{48} values for samples run through the PPQ trap at any temperature were within acceptable limits, whereas samples that bypassed the PPQ trap had Δ_{48} values above acceptable limits. This demonstrates that even at temperatures as high as -10°C, the PPQ trap is effective in removing contaminants.

Effect of PPQ trap temperature on δ^{45}, δ^{46}, and δ^{47}

Ideally, the PPQ cleaning step would remove contaminants while having no effect on the isotopic composition of the CO_2. **Figure 3** shows offsets in δ^{45}, δ^{46}, and δ^{47} relative to the PPQ trap temperature (with the absolute values of δ^{45}, δ^{46}, and δ^{47} on the right hand axes). The isotopic values (δ^{45}, δ^{46}, and δ^{47}) are constant for PPQ trap temperatures between -10°C and -20°C. Below -20°C, fractionation occurs, linearly driving δ^{45}, δ^{46}, and δ^{47} to lower values. The behavior of CM and OO is similar so, to combine them, the fractionation is best illustrated as the offset from the mean isotopic value of unfractionated samples (those run at temperatures of -20°C or higher). The slopes of the lines shown in **Fig.3** have units of ‰/(°C below -20°C) (abbreviated to ‰/°Cb20), and will be referred to in future as $d\delta^{4X}/dT$, where 4X is 45, 46 or 47.

The magnitude of the fractionation in δ^{45}, δ^{46}, and δ^{47} increases as the mass of the isotopologue increases ($d\delta^{47}/dT > d\delta^{46}/dT > d\delta^{45}/dT$). For the November 2014 data shown in **Fig. 3** (with CM and OO combined), these slopes are 0.0103 ± 0.0008, 0.0378 ± 0.0025, and 0.0519 ± 0.0035 ‰/°Cb20, for $d\delta^{45}/dT$, $d\delta^{46}/dT$, and $d\delta^{47}/dT$, respectively. When fitted separately, the slopes for CM are slightly steeper than for OO for all three masses (**Table S2**, Supporting Information), but agree within error. Data collected during the June 2015 "Cold PPQ Tests" are noisier but show the same pattern (**Fig. S1 and**

Table S2, Supporting Information), indicating that this behavior is consistent on a timescale of months to years.

Because δ^{45}, δ^{46}, and δ^{47} are closely related to the $\delta^{13}C$ and $\delta^{18}O$ values, the same pattern seen in **Fig. 3** can also be observed in the stable isotopic compositions (**Fig. S2**, Supporting Information). $d\delta^{13}C/dT$ and $d\delta^{18}O/dT$ are 0.0097 and 0.0392 ‰/°Cb20, respectively, for the November 2014 data with CM and OO combined.

Correction for fractionation of δ^{45}, δ^{46}, and δ^{47}

The observed fractionations in δ^{45}, δ^{46}, and δ^{47} are consistent and linear. Therefore, the "true" value of δ^{45}, δ^{46}, and δ^{47} can be reconstructed based on a linear shift of the data to a value that would have been measured if the sample were run at a PPQ trap temperature of -20°C (or higher). This can be accomplished with the following equation:

$$\delta^{4Xn} = \delta^{4X} - \frac{d\delta^{4X}}{dT} * \left(PPQtrapTemp + 20\right) \qquad [4]$$

where 4x = 45, 46, or 47 and the $d\delta^{4X}/dT$ values are taken from **Fig. 3** and have units of ‰/°Cb20. The new small delta values (δ^{45n}, δ^{46n}, and δ^{47n}) can then be used to calculate a corrected raw Δ_{47} (Δ_{47n}), as well as the new stable isotope values ($\delta^{13}C_n$ and $\delta^{18}O_n$). For samples run at -30°C, this corresponds to a shift of 0.103‰, 0.378‰, and 0.519‰ for δ^{45}, δ^{46}, and δ^{47}, respectively (10 times $d\delta^{4X}/dT$ from **Fig. 3** for the 10 degrees below -20°C at which the PPQ trap was held).

Effect of PPQ trap temperature on raw Δ_{47}

Given that δ^{45}, δ^{46}, and δ^{47} are used to calculate raw Δ_{47} (as defined by Huntington *et al.*[14]), the observed shifts in δ^{45}, δ^{46}, and δ^{47} at lower PPQ trap temperatures propagate through calculations and result in a shift in the raw Δ_{47}. Direct comparison of the raw Δ_{47} values between measurement sessions is prohibited by the continuously changing reference frame, which can shift on weekly or longer timescales. During normal measurement sessions, standard gases are run daily alongside samples to continuously assess and quantify changes in the reference frame. Even within a measurement session that might last 4-6 weeks, the reference frame may shift enough to prohibit direct comparison of raw Δ_{47} values between the beginning and end of the session.

In November 2014, we ran samples in the "Stable Isotope Test" configuration over two consecutive days, a short enough interval to consider the reference frame fixed. These samples can be used to assess the effects of the fractionations in δ^{45}, δ^{46}, and δ^{47} on the raw Δ_{47}. Each was only run for 20-30 cycles, about a third of the duration of a normal clumped isotope measurement. The long duration of typical clumped isotope measurements is necessary to reduce the standard error enough to allow for reasonable climatological interpretations of data (± 0.005-0.010‰ or ±1-2°C). In this case, the error bars are larger (± 0.02-0.04‰) than on a typical measurement, but the fractionation in raw Δ_{47} can still be seen.

Figure 4 shows the raw Δ_{47} plotted against the PPQ trap temperature for the November 2014 "Stable Isotope Test" runs. Following the pattern seen in δ^{45}, δ^{46}, and δ^{47}, a least squares regression is fitted to the data with all data measured at temperatures higher than -20°C "collapsed" to -20°C. The resulting regression line has a slope ($d\Delta_{47raw}/dT$) of 0.0037 ± 0.0019 ‰/°Cb20. For a sample run at -30°C, this would mean that the measured raw Δ_{47} would be 0.037‰ lower, corresponding to a temperature roughly 8°C higher for samples in the range of earth surface temperatures. If CM and OO are treated separately, $d\Delta_{47raw}/dT$ is 0.0032 ± 0.0033 ‰/°Cb20 for OO and 0.0046 ± 0.0024 ‰/°Cb20 for CM. As with δ^{45}, δ^{46}, and δ^{47}, CM has a larger shift in Δ_{47} per degree below -20°C than OO. This difference is investigated using idealized data in a later section.

The fitted line shown in **Fig. 4** was calculated following the pattern observed in **Fig. 3**, only showing a linear trend between -20°C and -40°C where fractionations in δ^{45}, δ^{46}, and δ^{47} were shown to occur. However, it appears that the trend in raw Δ_{47} continues between -10°C and -20°C. This could be due to larger error on the "Stable Isotope Test" data relative to typical clumped isotope measurements (see above). If raw Δ_{47} values are indeed being fractionated between -10°C and -20°C, it is vital that the PPQ trap temperatures are identical for all samples and standards.

Effects on $\Delta_{47\text{-}RFAC}$

In a normal measurement session, reference gases are run daily to constrain the reference frame. This reference frame takes into account changes in the non-linearity of the mass spectrometer and any stretching or compression of the Δ_{47} scale. It also takes into account variations in sample preparation through time. After the reaction step, CO_2 derived from carbonate samples is treated identically to heated and equilibrated gases. Therefore, any fractionation of gas that occurs in the cleaning step (or other preparation steps) should be fully accounted for in the reference frame correction.

If standard gases and carbonates are run at a cold temperature (e.g. -30°C), but are treated identically, a correction using **Equation 4** will shift all sample gases and standards to higher values (in both δ^{47} and Δ_{47}), keeping the unknowns in the same position in the reference frame defined by the standard gases as before the correction. As a result, there is little to no change in $\Delta_{47\text{-}RFAC}$ between the PPQ shift-corrected and uncorrected data.

Between May 2012 and September 2014, 23 analyses of CM were performed in the ColdPPQ configuration. Standard gas and CM data from these periods were corrected using **Equation 4** assuming all samples were run at exactly -30°C. **Figure 5** compares $\Delta_{47\text{-RFAC}}$ of original data ("ColdPPQ") with data corrected using **Equation 4** ("ColdPPQ corrected"). The mean $\Delta_{47\text{-RFAC}}$ value of 0.410 ± 0.006‰ (1 SE) is unchanged between the ColdPPQ configuration and the ColdPPQ corrected for the PPQ shift. Both agree well with the mean for January 2015 (WarmPPQ configuration), which is 0.414 ± 0.004‰ (1 SE) (**Figure 5, left panel**). In comparison, δ^{13}C shifts from 1.86 ± 0.10‰ (1 sd) to 1.95 ± 0.10‰ (1 sd) due to the PPQ-shift correction, bringing it into agreement with the WarmPPQ mean δ^{13}C value of 1.97 ± 0.03‰ (1sd) (**Figure 5, right panel**), and with the independent estimate of 2.04 ± 0.03‰ (1sd) from the Kiel III device. δ^{45}, δ^{46}, δ^{47}, and δ^{18}O behave in the same way as δ^{13}C, with the correction bringing ColdPPQ data into agreement with WarmPPQ data.

If, in contrast, the gases and carbonates were run through the PPQ at (even slightly) different temperatures, **Equation 4** will shift the gases and carbonates by different amounts, changing the carbonates' positions relative to the reference frame defined by the standard gases, and resulting in a different $\Delta_{47\text{-RFAC}}$ value in the PPQ shift-corrected data. Carbonates run at -30°C in June 2015 (the same temperature as the gas standards) showed an average shift of -0.037‰ in raw Δ_{47} and 0.005‰ in $\Delta_{47\text{-RFAC}}$. In contrast, carbonates run at -40°C had a shift of -0.073‰ to -0.078‰ in raw Δ_{47} and -0.026‰ to -0.022‰ in $\Delta_{47\text{-RFAC}}$ (**Tables S1c and S1d**, Supporting Information). This magnitude shift in $\Delta_{47\text{-RFAC}}$ would lead to a temperature difference of 5-6°C for samples near earth surface temperatures, a large enough difference to change climatological interpretations.

Studying the effects of the fractionation through idealized calculations

To investigate the effects of the observed fractionations in δ^{45}, δ^{46}, and δ^{47} on raw Δ_{47}, $\Delta_{47\text{-RFAC}}$ and the reference frame parameters, we can create synthetic gas and carbonate data and perturb it in different ways. We start by defining a reference frame (RF) with idealized parameters (RF1: SlopeEGL = 0.02, SlopeETF = 1.08, IntETF = 1.0), representing typical conditions for our instrument over recent measurement sessions, and create synthetic gas data that fits this reference frame (**see Supporting Information**). We also define carbonates with a range of compositions (δ^{13}C and δ^{18}O values between -30‰ and +30‰, raw Δ_{47} value between -1.5‰ and 0.5‰). For both gases and carbonates, we perturb these "true" values as if these samples were run through a PPQ trap held at -30°C. The shifted gases are then used to calculate a new reference frame (RF2: SlopeEGL = 0.02015, SlopeETF = 1.0797, IntETF = 1.0355) (**Figs. S5 and S6**, Supporting Information), which is used to calculate new $\Delta_{47\text{-RFAC}}$ values for the shifted carbonates. We assess the magnitude of the shift in raw Δ_{47} and $\Delta_{47\text{-RFAC}}$ of synthetic carbonate data through two quantities, dΔ_{47}/dT and d$\Delta_{47\text{-RFAC}}$/dT, which represent the change in raw Δ_{47} or $\Delta_{47\text{-RFAC}}$ per degree Celcius below -20°C (‰/°Cb20).

Higher (lower) dΔ_{47}/dT values are found for samples with lighter (heavier) bulk isotopic compositions (**Fig. S3**, Supporting Information). CM is lighter in δ^{13}C, δ^{18}O, and $\Delta_{47\text{-RFAC}}$ than OO, so would be expected to have a larger dΔ_{47}/dT value based on this result. At the mean compositions of CM and OO in the synthetic data, the dΔ_{47}/dT values are 0.0042 and 0.0040 ‰/°Cb20, respectively. The observed values of dΔ_{47}/dT are 0.0046 ± 0.0024 ‰/°Cb20 for CM and 0.0032 ± 0.0033 ‰/°Cb20 for OO. These synthetic dΔ_{47}/dT values were calculated using the dδ^{4X}/dT values from **Fig. 2**, where CM and OO were treated together. When CM and OO are treated separately, all three dδ^{4X}/dT values are slightly larger for CM than for OO. When these sample-specific dδ^{4X}/dT values are used instead, the newly calculated dΔ_{47}/dT values (0.00477 for CM, 0.00329 for OO) match the observed values more closely.

With the two reference frames, we can also assess the effects of the fractionation on $\Delta_{47\text{-RFAC}}$. Changes in $\Delta_{47\text{-RFAC}}$ caused by the fractionation are less than ±0.001 ‰/°Cb20 for all bulk isotopic compositions tested (**Fig. S7**, Supporting Information). This leads to a maximum shift of 0.01‰ for a sample run at a PPQ trap temperature of -30°C, but less than 0.003‰ for most common earth surface isotopic compositions (±5‰ in δ^{13}C and δ^{18}O values).

Discussion

Effects on previously published data from U of M

The discovery that δ^{13}C, δ^{18}O, and raw Δ_{47} values are fractionated when CO_2 gas is passed through a PPQ trap at -30°C is initially worrisome for a lab that has published several manuscripts using this procedure over the last three years.[16-19] However, as demonstrated above, the $\Delta_{47\text{-RFAC}}$ values are unchanged (<0.005‰ difference), as long as gas standards and samples are treated identically, which has been the practice. Therefore, any published $\Delta_{47\text{-RFAC}}$ data or calculated temperatures are unaffected by the discovery of this fractionation.

The δ^{13}C and δ^{18}O data measured in the ColdPPQ configuration, on the other hand, are affected by the PPQ trap fractionation. For a PPQ trap temperature of -30°C, the δ^{13}C and δ^{18}O values, respectively, become 0.10‰ and 0.36‰ higher than if the sample was run at -20°C or warmer. This translates to a difference of 0.34-0.36‰ in the $\delta^{18}O_{\text{water}}$ value, depending on the isotopic composition and calibration used. All samples run in the ColdPPQ trap configuration are equivalently affected, so any interpretation of relative changes in δ^{18}O, δ^{13}C, or $\delta^{18}O_{\text{water}}$ values would not be compromised. A change of 0.35‰ in the absolute value of $\delta^{18}O$ or $\delta^{18}O_{water}$, or a change of 0.10‰ in the absolute value of $\delta^{13}C$ is small enough that it does not change any published conclusions.

Lessons for using static PPQ traps in future

As the field of clumped isotope paleothermometry continues to expand, more and more labs are independently beginning to make this challenging measurement. Some of those labs will choose to use the "static PPQ" set up, and will need to determine the ideal PPQ trap temperature for their individual sample preparation device. This study serves to demonstrate the need for care when initially choosing PPQ trap temperature. High yield (>99%) alone is not sufficient. In this study, samples run at PPQ trap temperatures between -20°C and -30°C had yields above 99% but were still fractionated in δ^{45}, δ^{46}, and δ^{47}. For samples run at the coldest PPQ trap temperatures, increasing the duration of the transfer step would probably have increased the yield, but would not be guaranteed to avoid the observed fractionation, as seen in the -25°C and -30°C samples. In order to determine the ideal PPQ trap temperature and duration, it must be determined if the sample CO_2 is faithfully maintaining its isotopic composition through the cleaning step.

To determine the proper PPQ trap temperature, we recommend measuring samples at a variety of temperatures while monitoring yield, δ^{45}, δ^{46}, δ^{47}, Δ_{47}, and Δ_{48}. The ideal temperature is one where all contaminants are removed (low Δ_{48}), but δ^{45}, δ^{46}, δ^{47} and Δ_{47} fall within a plateau. That way, there is a buffer such that if the PPQ trap temperature changes by ±1-2°C, it would still be safely out of the range of temperatures at which fractionation occurs. Duration of the transfer step is also a factor. Due to the lengthy sample preparation process for clumped isotopes (~1hr/sample), saving time (even a few minutes per sample) is prized. Therefore, within a plateau of temperatures at which there is no fractionation, but contaminants are still effectively removed, we prefer the higher end (-15°C to -10°C in this case) to allow for quicker transfer of gas. It is possible that PPQ trap temperatures higher than -10°C would also be acceptable, but such temperatures were not tested in this study.

Conclusions

The clumped isotope paleothermometry technique requires additional cleaning steps beyond those used for traditional stable isotope studies in order to remove molecules that cause isobaric interferences on masses 47 and 48. This is often accomplished by passing CO_2 derived from calcium carbonate through a U-trap (or equivalent) packed with Porapak^{TM} Q material, a porous polymer adsorbent that traps contaminants but allows CO_2 to pass. Here, we demonstrate that, if the PPQ trap temperature is too cold, $\delta^{13}C$, $\delta^{18}O$ and raw Δ_{47} values will be fractionated with the magnitude of the offset increasing as the PPQ trap temperature decreases below -20°C. The yield of gas through the PPQ trap, on the other hand, is maintained at >99% for PPQ trap temperatures as low as -30°C, resulting in an interval between -20°C and -30°C where yields are high, but isotopic fractionation is occurring. If samples and standards are prepared identically, the reference frame correction will account for fractionations in δ^{47} and Δ_{47}, and $\Delta_{47\text{-RFAC}}$ will be unaffected. If samples are prepared at (even slightly) different PPQ trap temperatures from standard gases, the disparity in fractionation between the two will cause a shift in $\Delta_{47\text{-RFAC}}$ at a rate of 0.004-0.005‰/°Cb20. This can be corrected using a simple linear correction of δ^{45}, δ^{46}, and δ^{47} that is then translated through to the $\Delta_{47\text{-RFAC}}$, δ^{13}C, and δ^{18}O values. This study acts as a guide to other labs using or planning to use the static PPQ trap cleaning method in the preparation of samples for clumped isotope analysis, and puts forward a procedure by which the ideal PPQ trap temperature can be determined. Our results suggest that PPQ trap temperatures should be kept in the range of -10°C to -15°C for the sample preparation system at U of M, although each lab should individually determine the ideal PPQ temperature for their preparation system by varying the PPQ trap temperature and selecting a temperature range at which contaminants are completely removed and fractionation of stable isotope values does not occur.

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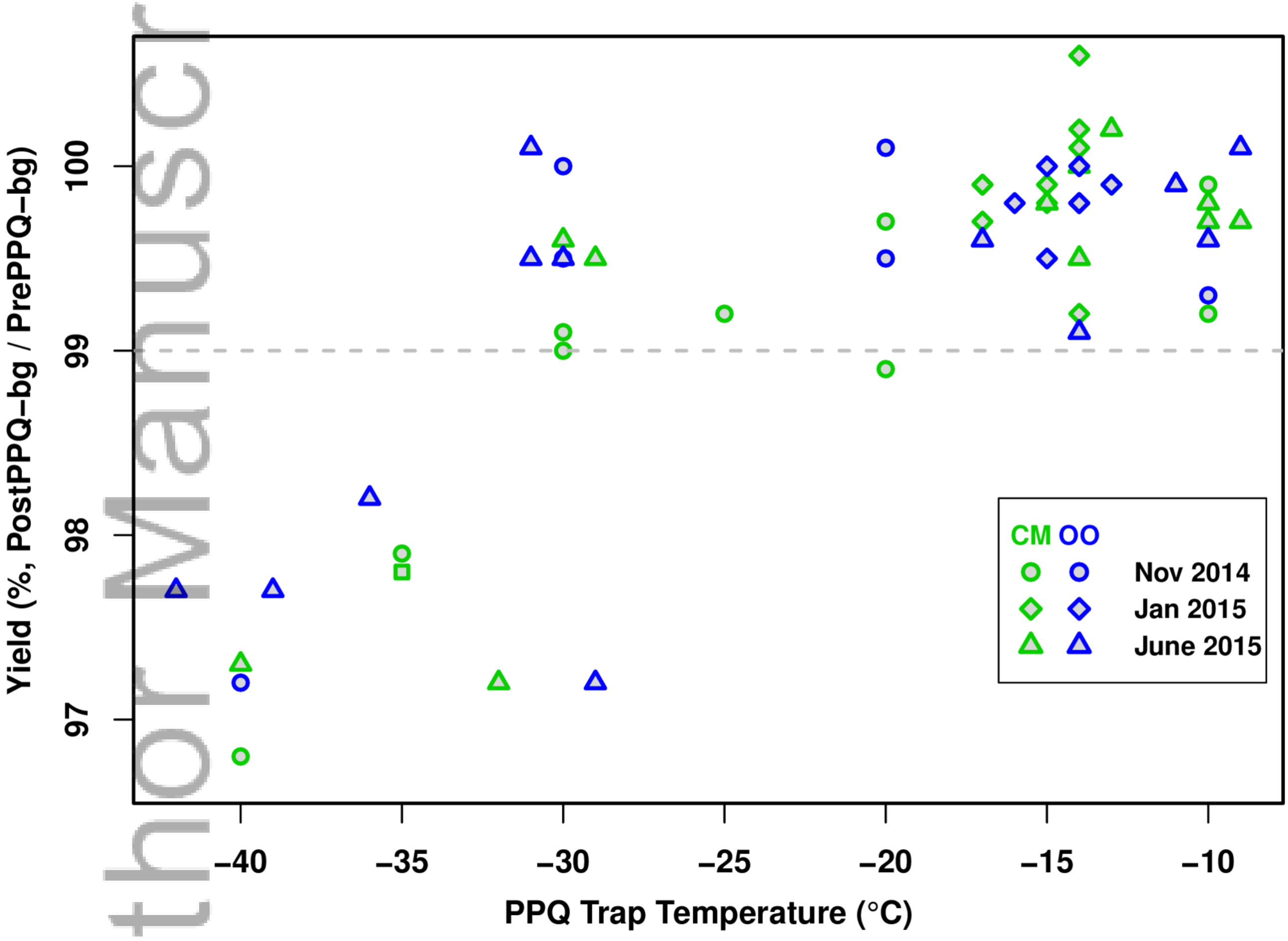
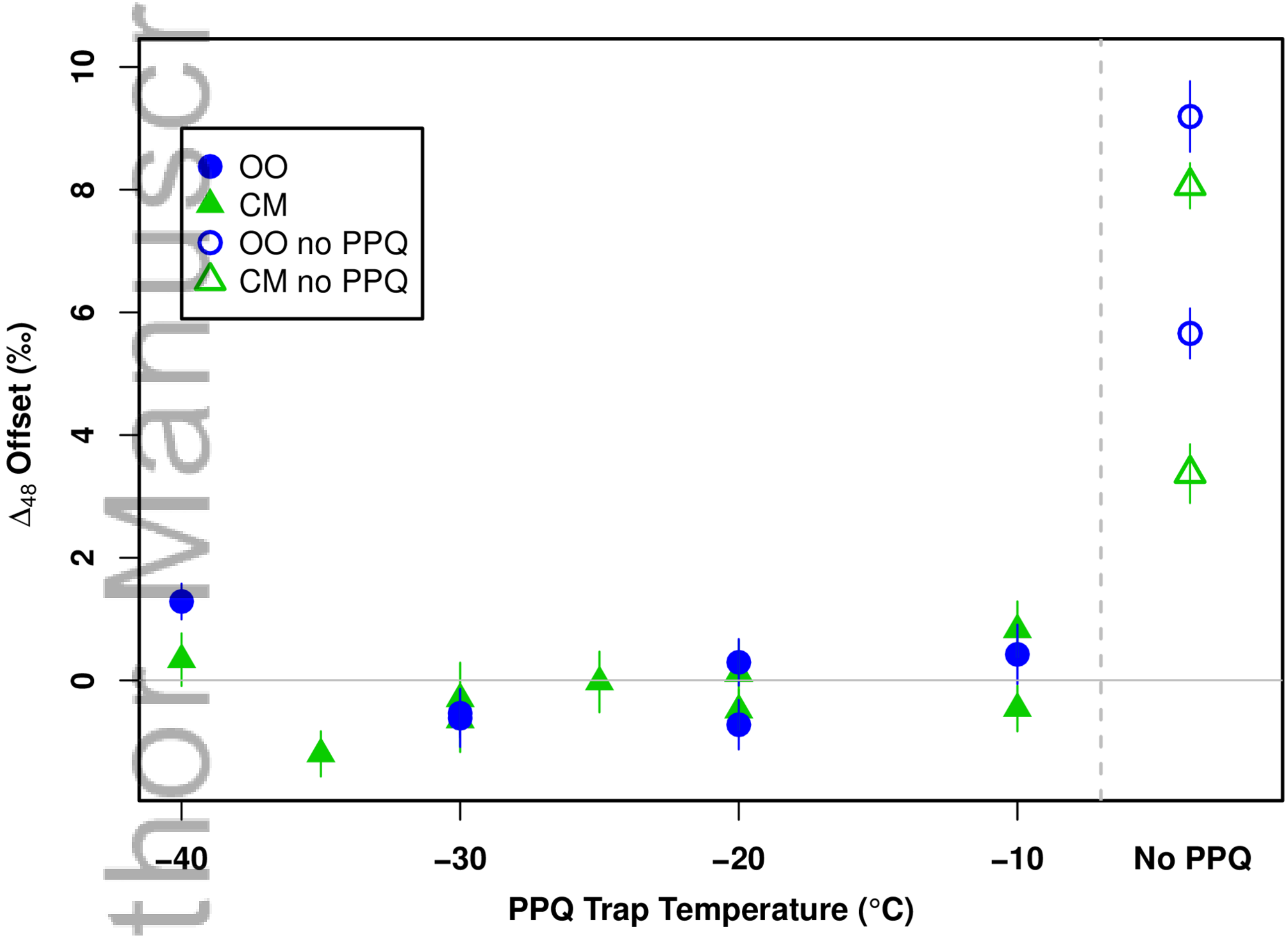
University	Method	Column?	Temp.	Duration	Reference
Caltech (off-line prep)	GC	Supelco Q-PLOT, 30 m x 530 μm i.d.	-10°C	40 min	[14]
Harvard University (off-line prep)	Static PPQ trap	Porapak^{TM} Q in glass U-trap	-12°C	unkn	[15]
Caltech, Johns Hopkins University, UCLA (automated sample prep device)	GC	Porapak^{TM} Q 120/80 mesh, 122 cm length, 2.15 mm i.d.	-20°C	15 min	[20–22]
Yale University	GC	Supelco-Q-PLOT, 530μm i.d., 30m long	-20°C	unkn	[23]
Tokyo Institute of Technology	GC	PoraPLOTQ, 25m x 0.32mm i.d.	-10°C	40 min	[24]
University of Michigan (pre July 2011) (GC method)	GC	Supel-Q-Plot, 30m x 0.53 mm i.d.	-20°C	45 min	[17]
ETH Zurich	Static PPQ trap	Porapak Q, 1.5cm filled, 6mm o.d.	-17°C	unkn	[25]
Goethe University	GC	Porapak^{TM} Q 80/120, 1.20 m x 2.15 mm i.d.	-20°C	30 min	[26]
Tulane University (now University of South Florida)	Static PPQ trap	Porapak^{TM}, 0.5” o.d., 185 mm filled	-15°C	unkn, passed through twice	[27]
Imperial College London	Static PPQ trap	Porapak^{TM} Q	-35°C	unkn	[28]
Harvard University (in-line small sample prep)	Static PPQ trap	Porapak^{TM} Q, 0.5” o.d. pyrex U-trap	-10°C to -12°C	4-7 min	[12]
University of Michigan (July 2011 – Dec. 2014) (static PPQ trap method)	Static PPQ trap	Porapak^{TM} Q material, 50-80 mesh	-30°C	5 min equilibration + 15 min transfer	[16]
University of Miami	Static PPQ trap	Porapak^{TM}	-27°C to -30°C	unkn, <45 min	[29]
University of East Anglia	Static PPQ trap	Porapak^{TM} Q	-20°C to -30°C	80 min	[30]
University of Michigan (new)	**Static PPQ trap**	**Porapak^{TM} Q, ~3” filled length, 6mm o.d., 4mm i.d.**	**-10°C to -15°C**	**10 min**	**This Study**

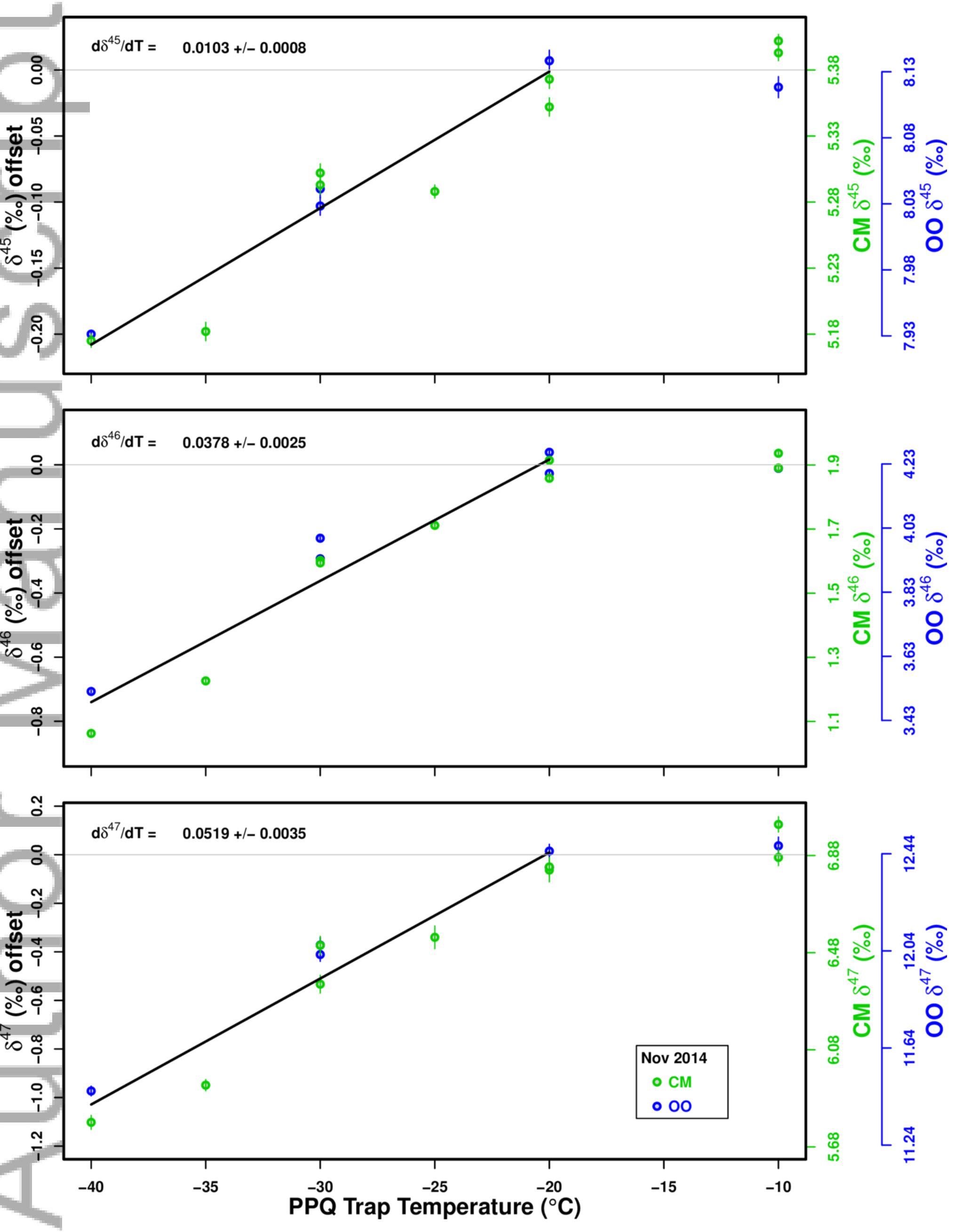
Table 1. Summary of CO_2 cleaning procedures currently and previously being used in labs internationally, taken from the published literature. unkn = unknown. GC = gas chromatography.


Yield (%, PostPPQ–bg / PrePPQ–bg)
97
98
99
100
–40
–35
–30
–25
–20
–15
–10
PPQ Trap Temperature (°C)
CM
OO
Nov 2014
Jan 2015
June 2015

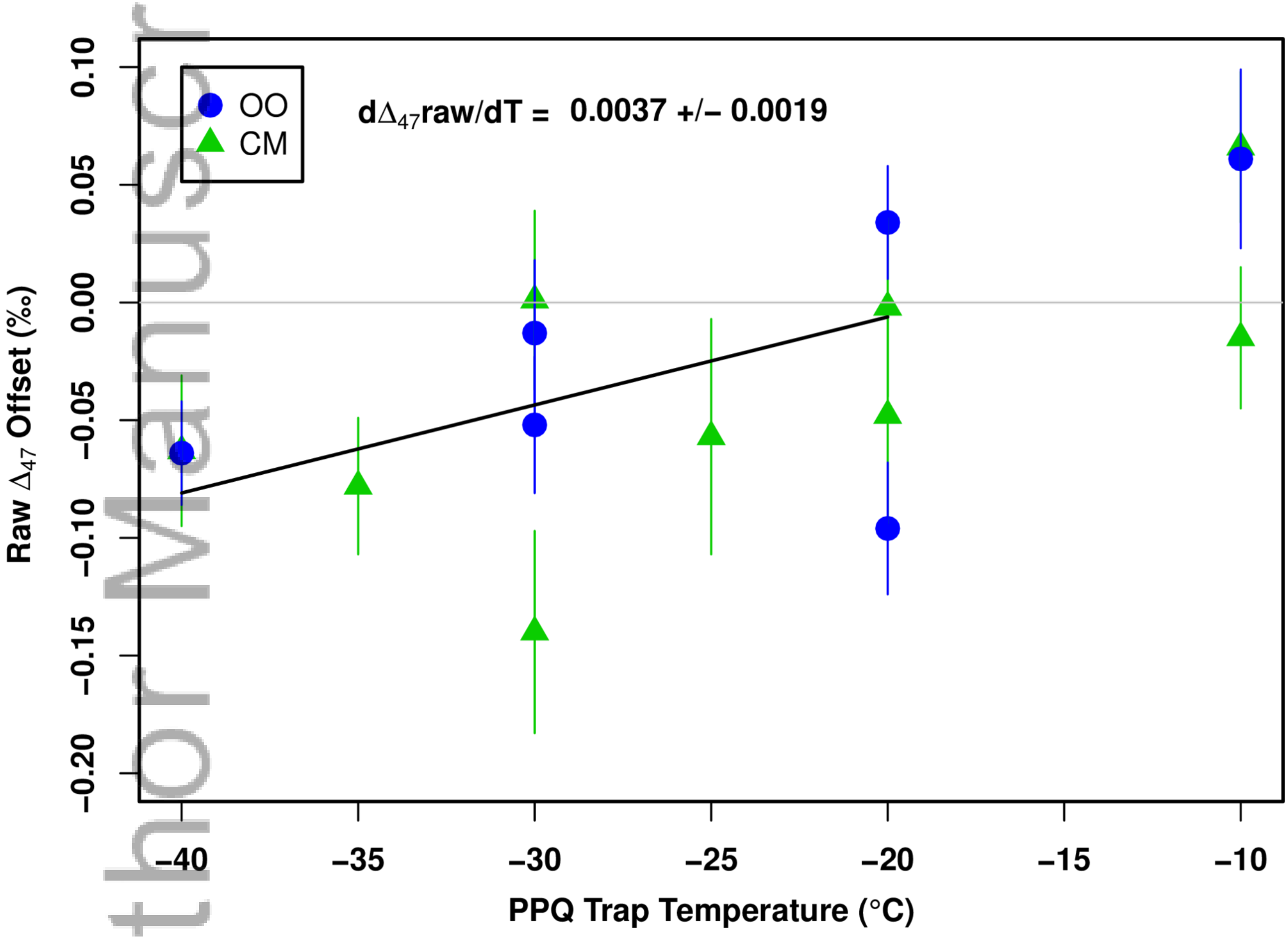
rcm_7438_f1


OO
CM
OO no PPQ
CM no PPQ
Δ_{48} Offset (‰)
0
2
4
6
8
10
−40
−30
−20
−10
No PPQ
PPQ Trap Temperature (°C)

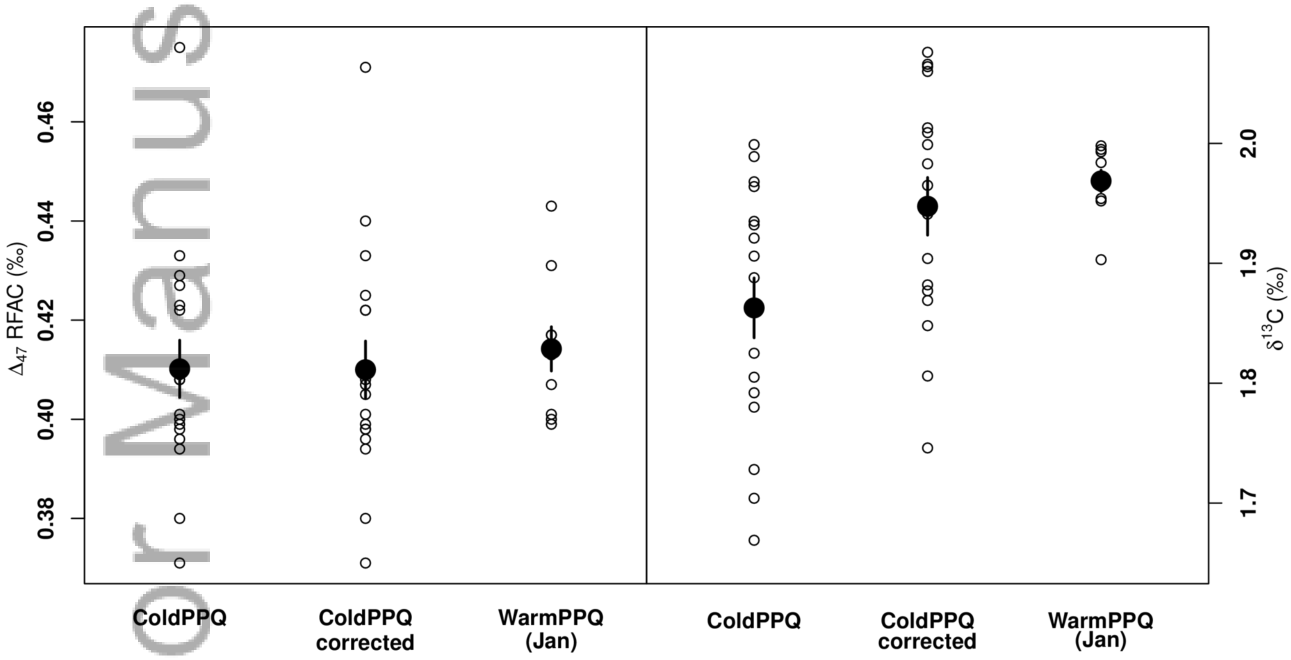
rcm_7438_f2


$d\delta^{45}/dT$ = 0.0103 +/− 0.0008
δ^{45} (‰) offset
0.00
−0.05
−0.10
−0.15
−0.20
CM δ^{45} (‰)
5.38
5.33
5.28
5.23
5.18
OO δ^{45} (‰)
8.13
8.08
8.03
7.98
7.93
$d\delta^{46}/dT$ = 0.0378 +/− 0.0025
δ^{46} (‰) offset
0.0
−0.2
−0.4
−0.6
−0.8
CM δ^{46} (‰)
1.9
1.7
1.5
1.3
1.1
OO δ^{46} (‰)
4.23
4.03
3.83
3.63
3.43
$d\delta^{47}/dT$ = 0.0519 +/− 0.0035
δ^{47} (‰) offset
0.2
0.0
−0.2
−0.4
−0.6
−0.8
−1.0
−1.2
CM δ^{47} (‰)
6.88
6.48
6.08
5.68
OO δ^{47} (‰)
12.44
12.04
11.64
11.24
Nov 2014
CM
OO
−40
−35
−30
−25
−20
−15
−10
PPQ Trap Temperature (°C)

rcm_7438_f3


OO
CM
$d\Delta_{47}raw/dT$ = 0.0037 +/– 0.0019
Raw Δ_{47} Offset (‰)
0.10
0.05
0.00
−0.05
−0.10
−0.15
−0.20
−40
−35
−30
−25
−20
−15
−10
PPQ Trap Temperature (°C)

rcm_7438_f4


Δ_{47} RFAC (‰)
0.38
0.40
0.42
0.44
0.46
$\delta^{13}C$ (‰)
1.7
1.8
1.9
2.0
ColdPPQ
ColdPPQ corrected
WarmPPQ (Jan)
ColdPPQ
ColdPPQ corrected
WarmPPQ (Jan)

rcm_7438_f5