

RESEARCH ARTICLE

Assessing the effects of embedding resins on carbonate stable and clumped isotope analyses

Jingjing Guo¹  | Xiulan Zong^{1,2}  | Niels J. de Winter^{3,4}  |
 Barbara Goudsmit-Harzevoort^{1,5}  | Francien Peterse¹  | Martin Ziegler¹ 

¹Department of Earth Sciences, Faculty of Geoscience, Utrecht University, Utrecht, The Netherlands

²Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, China

³Department of Earth Sciences, Faculty of Science, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

⁴Analytical, Environmental and Geochemistry Research Group, Vrije Universiteit Brussel, Brussels, Belgium

⁵Department of Estuarine & Delta Systems, NIOZ Royal Netherlands Institute for Sea Research, 't Horntje, The Netherlands

Correspondence

Jingjing Guo, Department of Earth Sciences, Faculty of Geoscience, Utrecht University, Princetonlaan 8a, 3584CB Utrecht, The Netherlands.

Email: j.guo@uu.nl

Funding information

Dutch Research Council (NWO), Grant/Award Numbers: 016.161.365, 192.074; Flemish Research Council, Grant/Award Numbers: FWO postdoctoral fellowship 12ZB220N, 12ZB220N; China Scholarship Council, Grant/Award Number: 202104910149

Rationale: Embedding resins are widely used to fix carbonates for high-precision sample preparation and high-resolution sampling. However, these embedding materials are difficult to remove after sample preparation and are known to affect the accuracy of carbonate stable isotope analyses. Nevertheless, their impact on clumped isotope analysis, which is particularly sensitive to contamination artifacts, has so far not been tested. The observation that running resin-containing samples decreased the reproducibility of clumped isotope values for internal laboratory carbonate standards and increased the external standard deviation (SD 0.061–0.088‰) compared to the long-term observations (0.034‰), prompted us to set up an experiment to test the influence of resin addition on instrument performance.

Methods: Here we analyzed the stable and clumped isotope composition of a pure calcium carbonate standard (ETH-4) mixed with three types of embedding resins in 2:1 and 1:1 proportions. Our aim was to assess how resin addition affects isotope analyses.

Results: We found that none of the stable isotopic values were significantly different. The $\delta^{13}\text{C}$ values were $-10.22 \pm 0.07\text{‰}$ (mean \pm SD) for pure ETH-4, while the $\delta^{13}\text{C}$ values of ETH-4 mixed with embedding resins in 2:1 and 1:1 proportions were $-10.21 \pm 0.06\text{‰}$ and $-10.18 \pm 0.06\text{‰}$, respectively ($p > 0.05$). The $\delta^{18}\text{O}$ values were $-18.82 \pm 0.11\text{‰}$ for pure ETH-4 versus $-18.81 \pm 0.09\text{‰}$ and $-18.82 \pm 0.08\text{‰}$ for 2:1 and 1:1 ETH-4:resin mixtures, respectively ($p > 0.05$). Given the large uncertainty in our results, we did not find significant differences between different mixtures in the carbonate clumped isotope values (Δ_{47}), with $0.458 \pm 0.107\text{‰}$, $0.464 \pm 0.086\text{‰}$, and $0.417 \pm 0.089\text{‰}$ in pure ETH-4 and ETH-4 with 2:1 and 1:1 resin mixtures, respectively ($p > 0.05$). However, a resin-related bias in the results might be masked by the large uncertainty. The measured ETH-4 values in our study are similar to the InterCarb values ($\delta^{13}\text{C} = -10.20\text{‰}$, $\delta^{18}\text{O} = -18.81\text{‰}$, $\Delta_{47} = 0.450\text{‰}$, InterCarb-Carbon Dioxide Equilibrium Scale). However, the external

Jingjing Guo and Xiulan Zong contributed equally to this work.

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SD of Δ_{47} in sessions measuring ETH-4 with resins is higher than in sessions without deliberate resin addition for the same measuring period.

Conclusions: We find that the potential contamination from the resin addition leads to a larger variability for Δ_{47} values in sessions measuring ETH-4 including resins. We therefore recommend purification of embedded samples using a contamination trap with Porapak prior to analysis, if possible, or avoiding resins during sample preparation and workup, as well as monitoring the measurement quality during and after sessions with samples containing embedding resins.

1 | INTRODUCTION

Well-preserved carbonates, such as fossil shells formed by marine or terrestrial organisms that are preserved in sedimentary archives, corals, or cave speleothems, are at the base of several valuable paleoclimate reconstructions, as their carbon ($\delta^{13}\text{C}$) or oxygen stable isotopic composition ($\delta^{18}\text{O}$) contains information on temperature, precipitation, and vegetation composition in the past.^{1–4} The analysis of bulk carbonate isotope ratios is relatively straightforward using isotope ratio mass spectrometry; however, for high spatial resolution analysis, the carbonate often needs to be embedded to stabilize the material during cutting, polishing, and/or subsampling. Embedding resins have been extensively used for this application due to their chemical resistance, thermostability, and physical strength.⁵ After sample processing, however, it is difficult to separate embedding resins from the sample material prior to analysis, which may affect the accuracy and precision of the carbonate isotope analysis. Indeed, previous investigations on the effect of resin addition to carbonate samples yielded ambiguous results. For example, Branscombe et al. showed that the $\delta^{13}\text{C}$ value of carbonates embedded in resin (“Kleer-Set”, produced by MetPrep) can be up to 4‰ higher than the $\delta^{13}\text{C}$ in samples without resin.⁶ By contrast, Mortensen et al. found that the difference in both stable carbon and oxygen isotope values of carbonate samples embedded in resins versus those of the corresponding control samples was < 0.2‰.⁷ Similarly, a study measuring Carrara marble with and without resin suggests that two types of resins (Araldite 2020 and Kleer-SetType FF) do not influence the stable isotope values, while two quick-drying types of resin (05 Epoxy and Metal Epoxy) introduce a negative shift (up to 2‰) in both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values.⁸

Despite these initial tests on carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values,^{6–8} the influence of embedding resins on clumped isotope measurements remains unknown. The carbonate clumped isotope composition, expressed as Δ_{47} , records the occurrence of two heavy rare isotopes (^{13}C and ^{18}O) in the same carbonate molecule and is inversely thermodynamically related to the formation temperature of the carbonates.^{9,10} The Δ_{47} has evolved into a widely applied tool for the reconstruction of absolute temperatures that are independent from nonthermal effects on carbonate formation.^{11–14} For example, the application of clumped isotope thermometry to speleothem and coral

carbonates could quantitatively reproduce their past formation temperature.^{15,16} Furthermore, the reconstruction of absolute seasonal temperatures based on clumped isotopes of fossil bivalves has highlighted the crucial role of seasonal temperature differences in greenhouse climate dynamics.¹⁷ Since the isotopologue with mass 47 occurs in relatively low abundance in natural CO_2 gasses (~45 ppm),⁹ it has been demonstrated that Δ_{47} measurements can be influenced by any contaminations in samples with organic or inorganic compounds that are able to fragment into compounds with the same masses as measured during clumped isotope analysis (masses 44–49).^{18–20} To signify these potential contaminations, $\Delta_{48\text{-offset}}$ and 49 parameter based on masses 48 and 49 have been defined and monitored (Equation 1 and 2).^{18,21–23} Given the low amounts of masses 48 and 49 in natural CO_2 gas (~3.96 ppm and 44.5 ppb,⁹ respectively), the occurrence of high values for masses 48 and 49 indicates the presence of contaminants with the same mass from different sources than CO_2 from carbonate. Compounds with masses 48 or 49 could be derived from, for example, organic contaminants like hydrocarbons, chlorocarbons, or sulfur-bearing contaminants.²¹

Regular quality monitoring of clumped isotope measurements based on carbonate standard ETH-3 in our laboratory revealed that the reproducibility of the measurements decreased during sessions with resin-containing samples (with resin sample proportions around 1:1), although the reproducibility of stable isotope values remained stable. This suggests that embedding resins may affect the reproducibility of clumped isotope measurements. To further assess this issue, in the present study we analyzed stable and clumped isotopes of a pure carbonate standard ETH-4 with a known isotopic composition, as well as mixtures of the same standard with different proportions of resin (2:1 and 1:1) from different brands. We aimed to determine the influence of embedding materials on the measured carbonate isotope values based on the offsets in the results for samples with and without resin. In addition, we monitored the acid reaction process and the difference in the discoloration of phosphoric acid after reaction with carbonates with and without resin to assess whether the presence of resin influences this reaction and consequently impacts the measurements. Notably, different methods exist for clumped isotope analysis (e.g., varying in sample size, acid digestion temperature, etc.), and the influence of embedding resin on the final result might differ for each of these preparation methods. In

this study, we use the Kiel device preparation method,^{24,25} which is now widely used and in particular is applied for high spatial resolution studies (e.g., mollusks, speleothems, and corals), where resin embedding is commonly used.^{12,17,26}

2 | METHODS

2.1 | Experimental setup

To assess the effect of embedding resins on carbonate isotope values, four batches of commonly used resins from three different brands were selected for this study (Figure 1 and Table S1). We considered resins with low viscosity that require curing at room temperature in around 24 h, specifically Araldite 2020, which is used at the German Research Center for Geosciences (resin A at GFZ, produced by Huntsman), Araldite 2020, which is used in the GeoLab at Utrecht University (resin B at UU, produced by Huntsman), EpoxiCure 2, which is used at the Institute of Earth Environment, Chinese Academy of Sciences in Xi'an (resin C at IEE, produced by Buehler), and EpoThin 2, which is used in the GeoLab at Utrecht University (resin D at UU, produced by Buehler). All selected resins consist of two individual components, a resin and a hardener (Table S1). For this study, fully-cured resins were subsampled using a micromotor (Strong power N90 Micromotor, 35,000 rpm) with a 0.3 mm diameter bit made by tungsten carbide.

The synthetic calcium carbonate ETH-4 with a known isotopic composition was chosen as the standard/reference material (STD, $\delta^{13}\text{C} = -10.20\%$, $\delta^{18}\text{O} = -18.81\%$, $\Delta_{47} = 0.450\%$,

InterCarb-Carbon Dioxide Equilibrium Scale [I-CDES]).²⁷ For each aliquot, 0, 50, or 100 μg of powdered resin was added to around 100 μg of ETH-4 standard. In total, 48 pure ETH-4 standards, 41 2:1 ETH-4:resin mixtures, and 25 1:1 ETH-4:resin mixtures were measured (Figure 1).

2.2 | Isotope analyses

The stable and clumped isotope analyses of all samples were done on a MAT 253 mass spectrometer (Thermo Fisher Scientific) coupled to a Kiel IV carbonate device for sample gas preparation.²⁴ In this study, the related sessions that comprise samples without resins are labeled as Session Nx (x indicates session number), sessions with resin-containing samples are labeled as Session Sx, and sessions that contain ETH-4 with resin mixtures are labeled as Session Rx (Figures 2 and 6). The three sessions comprising the resin tests (R17, 21, and 47) were carried out in June and September 2022. All samples were reacted with 103% phosphoric acid at 70°C for 360 s. The released CO₂ gas was purified by two liquid nitrogen (LN₂) traps to remove water, and a Porapak Type Q (50–80 mesh) trap was placed in between the traps to collect any organic contaminations. The Porapak Q trap ("Bishop 2.0") consists of Sulfinert 2000™ stainless-steel tubing filled with 35 mm of Porapak Q embedded in quartz wool kept at -50°C during analysis, with 1 cm of silver wool before and after to remove gaseous sulfur compounds.^{24,28} Before starting each new measurement session, the Porapak trap was baked out for at least 1 h at 150°C and the released gasses were pumped away.²⁸ The purified gasses were measured in long integration dual inlet mode

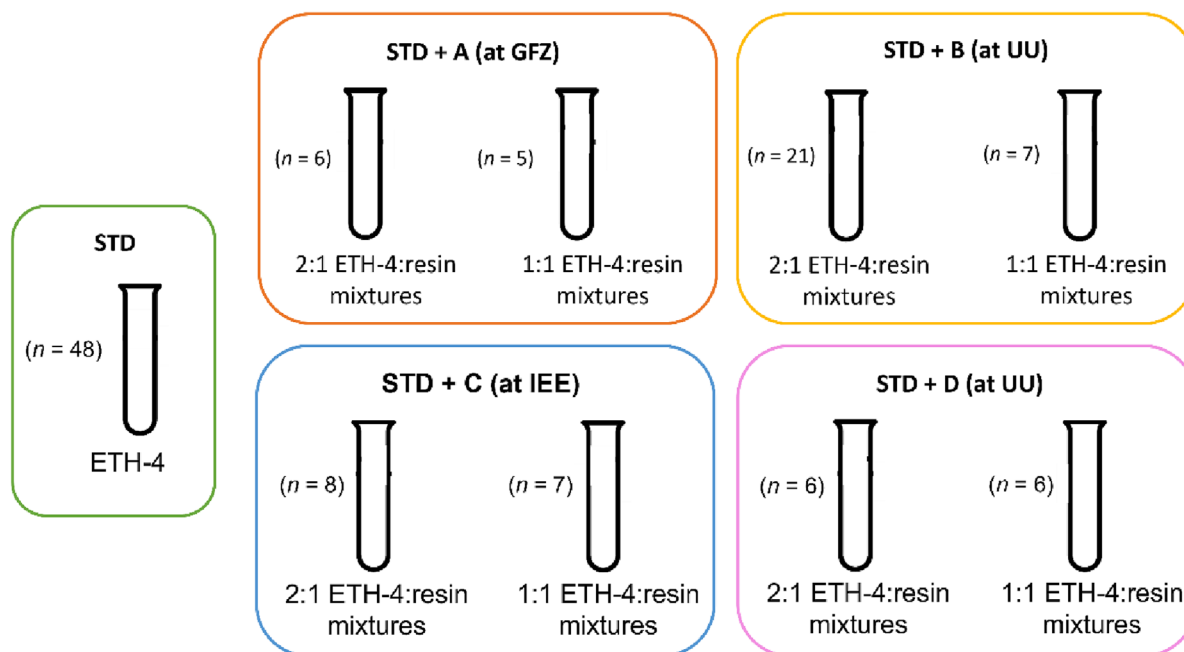


FIGURE 1 Overview of the experimental setup. Details of the brand name and main components of the embedding resins are listed in the Table S1. The number of replicate measurements for each sample is indicated in brackets in each panel. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

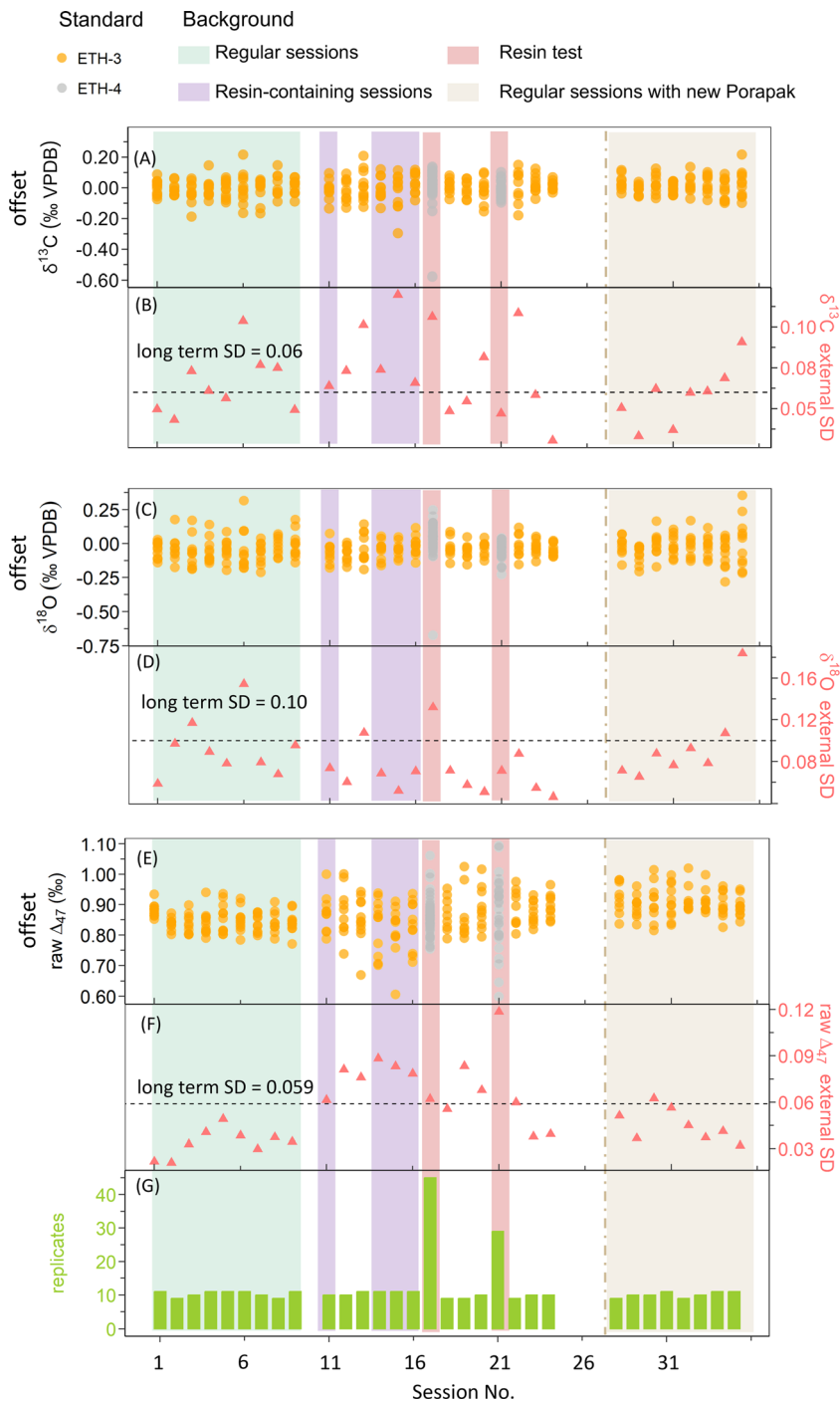


FIGURE 2 Scatter plots showing the offset (accepted InterCarb values minus measured values, ETH-3, $\delta^{13}\text{C} = 1.71\text{‰}$, $\delta^{18}\text{O} = -1.78\text{‰}$, $\Delta_{47} = 0.6132\text{‰}$; ETH-4, $\delta^{13}\text{C} = -10.20\text{‰}$, $\delta^{18}\text{O} = -18.81\text{‰}$, $\Delta_{47} = 0.4505\text{‰}$, I-CDES)²⁷ and external standard deviation of standards (ETH-3 in orange and ETH-4 in gray) for $\delta^{13}\text{C}$ (A and B), $\delta^{18}\text{O}$ (C and D) and raw Δ_{47} (E and F) during each session between April and August 2022. Sessions highlighted in light wheat are runs with a new Porapak. Color bars in the background represent regular sessions with samples without resins (Sessions N1–N9 in light green), sessions with resin-containing samples (Sessions S11, S14–S16 in purple), and sessions with resin-containing samples that are part of this study (Session R17 and R21 in red). The dashed black lines indicate the standard deviation derived from a long-term monitoring check standard IAEA-C2. The green bars in panel (G) indicate the number of standard replicates during each session [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/rcm.9597)]

with 400 s integration time, followed by a CO_2 reference gas ($\delta^{13}\text{C} = -2.82\text{‰}$, $\delta^{18}\text{O} = -4.67\text{‰}$). To eliminate the nonlinearity effects of the mass spectrometer, a pressure baseline (PBL) correction was conducted based on background scans (high voltage versus beam intensity on masses 44–49) before the start of each session.^{24,25} All samples were measured at least five times (Figure 1 and Table 1). The stable carbon and oxygen isotope ratios are expressed in δ notation and reported relative to Vienna Pee Dee Belemnite (VPDB) after correction using other ETH standards (ETH-1, -2, and -3). The Δ_{47}

value of each aliquot was individually corrected to the I-CDES with their accepted InterCarb values.²⁷ Measurements with an internal standard deviation (SD) on Δ_{47} above 0.15‰ were treated as outliers. The potential contaminations were monitored by $\Delta_{48\text{-offset}}$ and 49 parameter:²⁹

$$\Delta_{48\text{-offset}} = \Delta_{48\text{ sample}} - \frac{\sum_{i=1}^n (\Delta_{48\text{ standards}})}{n} \quad (1)$$

TABLE 1 Mean values, standard deviation (SD), and difference between ETH-4 with and without embedding resins (Δ) of stable isotopic compositions ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$), clumped isotopic compositions (Δ_{47}), internal standard deviation of Δ_{47} (Δ_{47} SD), $\Delta_{48\text{-offset}}$, and 49 parameter indicating organic contaminants for different samples

Sample	#	$\delta^{13}\text{C}$ (‰)		$\delta^{18}\text{O}$ (‰)		Δ_{47} (‰)		Δ_{47} SD (‰)		$\Delta_{48\text{-offset}}$ (‰)		49 parameter (ppm)				
		Mean	SD	Δ	Mean	SD	Δ	Mean	SD	Mean	SD	Mean	SD	Δ		
ETH-4 (STD)	48	-10.22	0.07	-	-18.82	0.11	-	0.458	0.107	-	0.113	0.018	-	0.35	0.60	-
2:1 mixture	41	-10.21	0.06	0.01	-18.81	0.09	0.01	0.464	0.086	0.007	0.112	0.016	-0.001	0.101	0.590	-0.14
1:1 mixture	25	-10.18	0.06	0.03	-18.82	0.08	0.00	0.417	0.089	-0.040	0.113	0.014	0.000	0.110	0.349	-0.24
2:1 mixture A	6	-10.20	0.04	0.02	-18.80	0.03	0.01	0.441	0.094	-0.017	0.102	0.012	-0.011	0.515	1.475	-0.66
2:1 mixture B	21	-10.22	0.05	-0.00	-18.83	0.10	-0.01	0.460	0.080	0.002	0.115	0.014	0.001	0.000	0.160	-0.19
2:1 mixture C	8	-10.24	0.04	-0.02	-18.86	0.04	-0.05	0.453	0.074	-0.005	0.107	0.020	-0.006	-0.033	0.314	-0.13
2:1 mixture D	6	-10.13	0.06	0.08	-18.69	0.09	0.13	0.519	0.111	0.062	0.120	0.018	0.006	0.221	0.267	0.54
1:1 mixture A	5	-10.18	0.05	0.04	-18.76	0.07	0.05	0.354	0.114	-0.104	0.117	0.018	0.004	0.266	0.480	-0.52
1:1 mixture B	7	-10.23	0.07	-0.01	-18.86	0.09	-0.05	0.434	0.050	-0.024	0.114	0.012	0.001	0.047	0.283	-0.41
1:1 mixture C	7	-10.16	0.06	0.05	-18.85	0.07	-0.03	0.416	0.090	-0.041	0.108	0.016	-0.005	0.196	0.425	-0.25
1:1 mixture D	6	-10.15	0.04	0.06	-18.78	0.06	0.03	0.452	0.094	-0.006	0.113	0.013	0.000	-0.047	0.140	0.22
All	114	-10.21	0.07		-18.81	0.10		0.451	0.097		0.113	0.016		0.021	0.433	0.24
Accepted value		-10.20			-18.81			0.450								0.55

Abbreviation: SD, standard deviation.

$$49 \text{ parameter} = \left[\left(\frac{49 \text{ signal}}{44 \text{ signal}} \right)_{\text{sample}} - \left(\frac{49 \text{ signal}}{44 \text{ signal}} \right)_{\text{standard}} \right] \times 1000 \quad (2)$$

where Δ_{48} standards is the pure ETH-4 measured in each session and n is the total number of pure ETH-4 standards in each session. In our system, a $\Delta_{48\text{-offset}}$ above 1.5‰ and a 49 parameter above 0.01‰ (10 ppm) were used as the thresholds. In addition, the $\Delta_{48\text{-offset}}$ and 49 parameter of carbonate standard ETH-3 from six previous sessions measuring relatively clean samples without resin are reported for comparison (Sessions N1, N2, N5, N6, N8, and N9, indicated by the gray shaded area in Figures 4 and 5). The long-term reproducibility of the instrument was monitored using a check standard (IAEA-C2, travertine, a control material selected by International Atomic Energy Agency) from 35 sessions measured between April and August 2022 (indicated by the dashed black lines in Figure 2). Measurement quality was monitored based on the external SD of the stable and clumped isotope values of carbonate standards in each session. During the measurement of a selection of ETH-4 aliquots in the Kiel carbonate preparation device, the acid reaction was recorded by a camera to observe potential differences in the reaction between samples with and without resins (Video S1).

2.3 | Statistical analysis and data visualization

The statistical analysis and data visualization were undertaken in R version 4.2.2 (R Core Team).³⁰ Differences in the isotopic values between ETH-4 and resin mixtures as well as different brands were examined by one-way analysis of variance (ANOVA) followed by post hoc analysis (Tukey honest significant difference test). Differences were considered significant at a level of $p < 0.05$. To avoid the risk of potential contamination by resins of the Kiel system and/or the mass spectrometer, we kept the number of sample replicates relatively small ($n = 5\text{--}21$; Figure 1 and Table 1), although this potentially impacted the statistical analysis results. Due to the unequal number of sample replicates and large uncertainties introduced by the resin addition, the SD (mean \pm SD) was determined for each group of standard or mixtures to show the data dispersion. The uncertainty of the SDs (error bars in Figure 5) was calculated following Harding et al. (Equation 3).³¹

$$SE = \frac{SD}{\sqrt{2n-2}} \quad (3)$$

3 | RESULTS AND DISCUSSION

3.1 | Potential embedding resin effects on long-term instrument performance

During the regular monitoring of the measurement quality based on carbonate standard ETH-3, we found that the offset (accepted

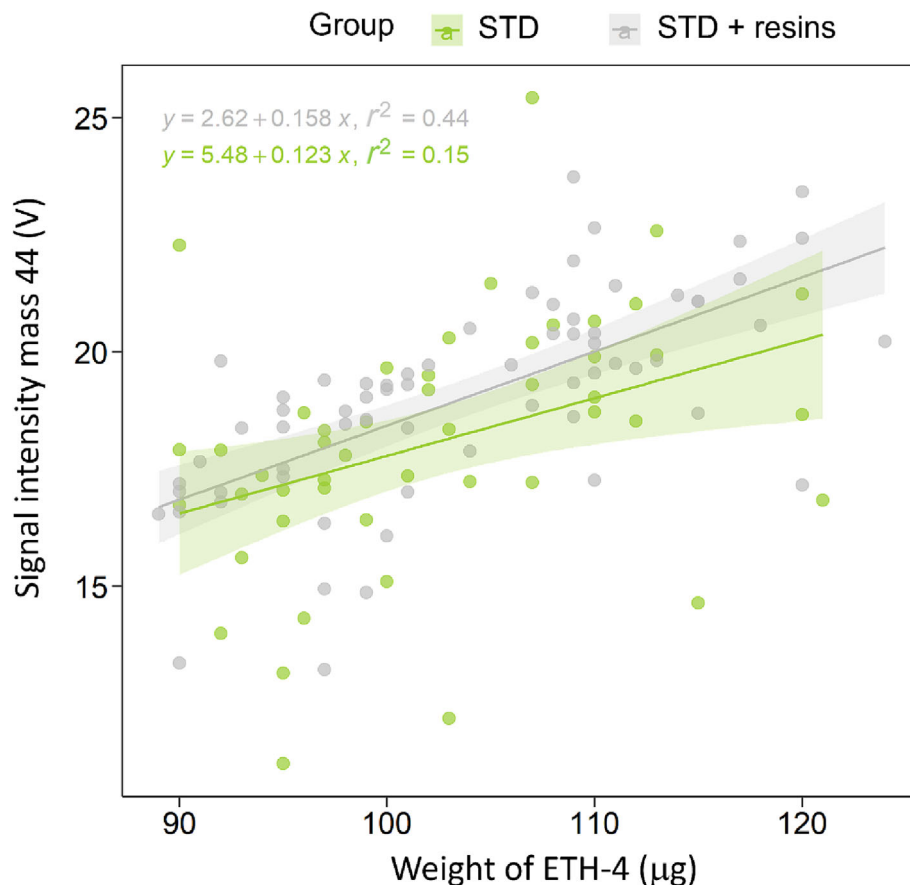
InterCarb values minus measured values²⁷) of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of ETH-3 did not show any differences between sessions without resin-containing samples and sessions with resin-containing samples (Figure 2A,C). As shown in Figure 2B, the external SD of $\delta^{13}\text{C}$ of ETH-3 standards between sessions without resin-containing samples (Sessions N1–N9, 0.04–0.10‰, Figure 2B with light green background) and sessions with resin-containing samples (Sessions S11, S14–S16, 0.06–0.12‰, Figure 2B with purple background) show a similar range that distributes around the long-term SD of IAEA-C2 (SD = 0.06‰, black dashed line in Figure 2B). The external SD of $\delta^{18}\text{O}$ of ETH-3 in sessions measuring samples embedded in resins (0.05–0.07‰) shows lower SDs than sessions without resins (0.06–0.15‰), and most of them are below the long-term SD of IAEA-C2 (SD = 0.10‰, Figure 2D). However, both the offset and external SD of raw Δ_{47} of ETH-3 standards in sessions with a batch of samples embedded in resins show values that are higher compared to the long-term external SD derived from a check standard IAEA-C2, as well as the ETH-3 standards measured in sessions with regular samples (i.e., without resin, Figure 2E,F). Specifically, the external SD of raw Δ_{47} of ETH-3 in sessions without resin-containing samples varies between 0.021 and 0.049‰ (Sessions N1–N9, Figure 2F), while it increases to 0.061‰ during the session where samples with resin are introduced (Session S11, proportion of sample and resin is around 1:1) and remains higher than the long term SD (0.059‰) in the following sessions regardless of whether samples contain resin or not (0.076–0.088‰, Figure 2F). The high external SD of raw Δ_{47} of the carbonate standards that do not contain any resin implies that the potential of cumulative effects of the resin on the equipment may exist. To disentangle the impacts of resin on stable and clumped isotopic measurements, an experiment with pure carbonate standard EHT-4 mixed with embedding resins was conducted.

3.2 | Embedding resin effects on acid reaction

There is no significant difference in the amount of CO_2 gas produced during the acid reaction of ETH-4 with or without added embedding resins based on the intensity of mass 44 (m/z , $p = 0.35$; Figure 3). This means that the embedding resins do not significantly contribute to extra CO_2 gas, which is in agreement with previous studies.^{8,32} Additionally, no clear visual difference in the gas amount was observed between acid reactions of ETH-4 with or without embedding resins (see the reaction video in Data S2).

Interestingly, phosphoric acid shows pink discoloration ~ 8 h after reaction with embedding resins at 70°C, while the acid that reacts with pure ETH-4 remains transparent (Figure S1A). The color change of phosphoric acid into pink has been reported earlier after reaction with Carrara marble mixed with resin Araldite 2020 (same brand as A and B in this study).⁸ In addition, it seems that the pink color in samples becomes darker when it is submerged in the phosphoric acid for a longer time (>24 h; Figure S1B). This suggests that the materials in embedding resin that do not react with phosphoric acid gradually leach out over time. The reaction that leads to the coloration of the

FIGURE 3 Scatter plot of the weight of ETH-4 with the signal intensity of mass 44. Pure ETH-4 shown in green and ETH-4 with embedding resins in gray. The linear regression lines of correlations with $p < 0.05$ are plotted, r^2 indicates the coefficient of determination, and the 95% confidence intervals are displayed by the shaded area. The scatter is most likely related to low precision in the weight determination [Color figure can be viewed at wileyonlinelibrary.com]



acid therefore takes place after CO_2 transfer and does not affect the analysis, as confirmed by the indistinguishable signal intensities for samples with and without resin added to the same amount of ETH-4 (Figure 3). A functionalization study of the embedding resin reported that the discoloration of the resins happens due to the change in the fluorescence of the original resin.⁵ Specifically, boronic acid with a spiropyran group acts as a dye for the embedding resin and hence shows photochromism. We assume that phosphoric acid at 70°C could play the same role as the boronic acid and therefore results in the discoloration observed here. In addition, 1:1 ETH-4:resin mixtures do not turn darker than those with a 2:1 ratio, and the coloration does not show any differences for the different brands of resins in our study (Figure S1). This suggests that the color intensity does not correlate with either the brand or the amount of resin.

3.3 | Embedding resin effects on carbonate stable isotope values

The $\delta^{13}\text{C}$ of pure ETH-4, and ETH-4 mixed with embedding resin in 2:1 and 1:1 proportions is not significantly different, with $-10.22 \pm 0.07\%$ (mean \pm SD), $-10.21 \pm 0.06\%$ and $-10.18 \pm 0.06\%$, respectively ($p > 0.05$, between green and gray boxes, Figure 4A). Comparison of the $\delta^{13}\text{C}$ values for ETH-4 and those of resin mixtures with different brands also does not show specific differences

between mixtures based on the post hoc analysis, although the p value of the ANOVA test is 0.008 (comparison without gray boxes, Figure 4A). The $\delta^{13}\text{C}$ values for the mixtures range between -10.24 and -10.13% , and are similar to that of the pure ETH-4 standard (-10.22% ; Figure 4A). Similarly, the mean $\delta^{18}\text{O}$ values of pure ETH-4, and 2:1 and 1:1 ETH-4:resin mixtures are $-18.82 \pm 0.11\%$, $-18.81 \pm 0.09\%$, and $-18.82 \pm 0.08\%$, without significant differences between mixtures ($p > 0.05$, between green and gray boxes, Figure 4B). Also, the $\delta^{18}\text{O}$ of the resin mixtures with different brands does not show a specific difference and is similar to that of the pure ETH-4, ranging from -18.86 to -18.69% versus -18.82% , except for the 2:1 mixture using brand D (EpoThin 2), which has a $\delta^{18}\text{O}$ value that is 0.13% higher than pure ETH-4 ($p = 0.026$, comparison without gray boxes, Figure 4B). The difference is slightly higher than the external SD for long-term $\delta^{18}\text{O}$ values of the check standard IAEA-C2 (0.10% for $\delta^{18}\text{O}$, based on 35 sessions measured between April and August 2022). In summary, except for the $\delta^{18}\text{O}$ offset of the 2:1 mixture with brand D resin, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of mixtures with ETH-4 and resin from different brands are similar to those of pure ETH-4 carbonate.

The external SD of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for different mixtures was used to evaluate whether the embedding resins impact the reproducibility of each measurement (Figure 5). The SD of $\delta^{13}\text{C}$ for pure ETH-4 is $0.07 \pm 0.01\%$ (SD \pm standard error of the SD; Equation 3), while that for ETH-4 with different amounts of resins

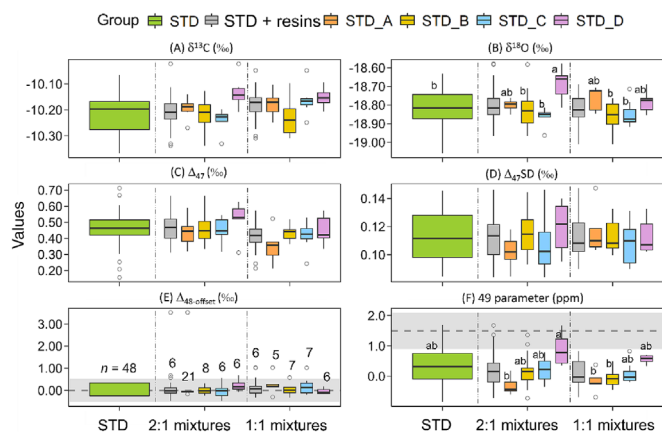


FIGURE 4 Box plots of stable and clumped isotope values, $\Delta_{48\text{-offset}}$ and 49 parameter for pure ETH-4 (STD, green box), ETH-4 with different proportions of resin mixtures (STD + resin, gray box), and different mixtures with embedding resins from different brands (brand A, orange box; brand B, yellow box; brand C, blue box; brand D, pink box). (A) $\delta^{13}\text{C}$, (B) $\delta^{18}\text{O}$, (C) Δ_{47} , (D) internal standard deviation of Δ_{47} ($\Delta_{47}\text{SD}$), (E) $\Delta_{48\text{-offset}}$ and (F) 49 parameter. The $\Delta_{48\text{-offset}}$ of the STD in panel (E) is the average Δ_{48} of pure ETH-4 in the sessions with resin measurements (green box). The gray dashed line and shaded area in (E) and (F) indicate the mean \pm SD values of ETH-3 standards in regular sessions without resin-containing samples ($0.00 \pm 0.52\text{‰}$ and 1.5 ± 0.6 ppm for $\Delta_{48\text{-offset}}$ and 49 parameter, respectively). The bold line in each box indicates the median (50th percentile), the bottom and top of the box indicate the first quartile (25th percentile) and third quartile (75th percentile) respectively, and whiskers cover the smallest and largest values within 1.5 times the interquartile range (i.e., the distance between the top and bottom of the box). Significant differences between specific samples (mixtures from different brands and STD, i.e., the gray boxes indicating ETH-4 with different proportions of resin mixtures are not included) with $p < 0.05$ are labeled using different letters. Numbers in p(E) indicate the number of aliquots per test case [Color figure can be viewed at wileyonlinelibrary.com]

from different brands ranges from 0.04‰ to 0.07‰ (Table 1 and Figure 5A). The SD of $\delta^{18}\text{O}$ for pure ETH-4 is $0.11\text{‰} \pm 0.01\text{‰}$, which is larger than that of the ETH-4 with resins, ranging from 0.03‰ to 0.10‰ (Table 1 and Figure 5B). Given the relatively low number of replicates for different resins mixtures ($n = 5\text{--}21$; Figure 1 and Table 1), it is not possible to assess if differences in the external SD are significant. Overall, the SD of most mixtures is smaller than that of pure ETH-4 for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, which indicates that resins do not affect the reproducibility of the stable isotope measurements (Figure 5). Additionally, a post hoc analysis test indicated that $\delta^{13}\text{C}$ does not show a specific difference between brands (Figure 4A). Only the $\delta^{18}\text{O}$ of brand D (EpoThin 2) was significantly less negative ($\sim 0.13\text{‰}$) than the pure ETH-4 (Figure 4A,B). Hence, our results support previous findings that resin only causes minor isotopic offsets.^{7,8} Specifically, a previous study showed that resin introduced minor isotopic effects of -0.17‰ and $+0.12\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively, which was less than the variation between natural samples in that study ($\sim 2\text{‰}$ for both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$).⁷ Moreover,

Araldite 2020, the same resin as brands A and B used in our study, was also examined in combination with Carrara marble, where it resulted in offsets in stable isotope values $< 0.01\text{‰}$.⁸ In our case, offsets introduced by this resin brand are maximum $+0.08\text{‰}$ in $\delta^{13}\text{C}$ and $+0.13\text{‰}$ in $\delta^{18}\text{O}$.

Thus, based on our results, the presence of resin does not significantly impact the stable isotope values. This contrasts with an earlier study that reported offsets of up to 4‰ in $\delta^{13}\text{C}$ values for samples with and without embedding resins.⁶ There are different possible explanations for these outcomes. First, the method of sample preparation can potentially affect the isotope measurement. In our study, we used a Kiel IV carbonate device with Porapak to prepare and purify our samples by removing possible organic contaminants. The materials in Porapak mainly consist of divinylbenzene polymers in porous beads with a high surface area that can adsorb contaminants.³³ The nonsignificant offsets that we found could be due to the use of a Porapak purification system, which removes possible contamination released from resin in carbonate samples before stable isotope analysis, and such a system has not been used in previous studies.^{6,8} Second, the brand of embedding resin may have some influence, as brands differ in composition (Table S1). The resin brand used in the study that found the largest offset (MetPrep “Kleer-Set” polyester⁶) was not tested in our study. Nevertheless, this brand was tested by Schöne et al.,⁸ who found that the stable isotope values of Carrara marble were not significantly impacted by this resin, suggesting that the anomalous $\delta^{13}\text{C}$ values reported by the previous study could also be induced by factors other than the resin.⁶ Third, the reaction temperature applied in this study is 70°C , while earlier studies used 90°C .⁶ As discussed above, the resins do not show a clear visual reaction in the gas amount with phosphoric acid at 70°C ; however, it is possible that they become more reactive at 90°C . Even a small contribution of resin in the reaction could then explain a relatively large shift in the measured $\delta^{13}\text{C}$ value if the $\delta^{13}\text{C}$ signature of the resin is very different from that of the used standard/sample, therefore the shift in $\delta^{13}\text{C}$ observed in the earlier study might be (partly) explained by the higher temperature that was used.⁶

3.4 | Embedding resin effects on carbonate clumped isotope values

The Δ_{47} values of pure ETH-4, and 2:1 and 1:1 ETH-4:resin mixtures were $0.458 \pm 0.107\text{‰}$, $0.464 \pm 0.086\text{‰}$, and $0.417 \pm 0.089\text{‰}$, respectively, which are not significantly different based on ANOVA ($p > 0.05$; Table 1 and Figure 4C). The Δ_{47} values of different brands of resins also do not show a significant difference according to ANOVA ($p > 0.05$; Figure 4C). However, for paleotemperature reconstructions, a discrepancy of 0.041‰ in Δ_{47} as observed here would correspond with a substantial difference in absolute temperatures ($\sim 13^\circ\text{C}$ based on Meinicke et al.,³⁴ the absolute value may change based on different calibrations). The large uncertainty on the Δ_{47} that masks potentially significant differences between the different standard:resin mixtures analyzed here may be attributed to

the unequal number of replicates ($n = 5-48$), which impacts the data reproducibility and makes the comparison of mean values less meaningful. Additionally, the potential contamination from resins can also contribute to the large discrepancy in Δ_{47} values. Indeed, the external SD uncertainties of Δ_{47} for mixtures with different brands (0.01–0.04‰) is higher than for the pure standard (0.01‰, error bars in Figure 5C). Regardless, the average Δ_{47} of all measurements together, including pure standards and mixtures, is $0.451 \pm 0.097\text{‰}$, which is similar to the measured value of ETH-4 previously reported (0.450‰).²⁷ The internal Δ_{47} SD on our measurements for ETH-4 with and without resin falls within a range of 0.102‰–0.120‰ regardless of the brand or mixture (Table 1 and Figure 4D). This indicates that the embedding resins do not impact the reproducibility of the clumped isotope measurements in a single aliquot. However,

the reproducibility of the Δ_{47} value for all aliquots for certain mixtures is relatively low, as indicated by the high external SD of Δ_{47} ($> 0.05\text{‰}$; Figure 5C) for all mixtures. Notably, measurements of pure ETH-4 do not have better reproducibility than measurements of mixture with resins, indicated by the average external SD of $0.107 \pm 0.011\text{‰}$ for ETH-4 and a range of 0.050‰–0.114‰ for the resin mixtures, respectively (Table 1 and Figure 5C). The Δ_{47} values of resin mixtures in 2:1 and 1:1 proportions with brand B (Araldite 2020) are $0.460 \pm 0.080\text{‰}$ ($n = 21$) and $0.434 \pm 0.050\text{‰}$ ($n = 7$), with a relatively stable mean value and lower external SD compared with other brands (Table 1, Figures 4C and 5C). The Δ_{47} values for 2:1 and 1:1 mixtures with brand A, which is the same brand as B, except it is from a different batch, are $0.441 \pm 0.094\text{‰}$ and $0.354 \pm 0.114\text{‰}$, showing a relatively higher uncertainty than for brand B, although this

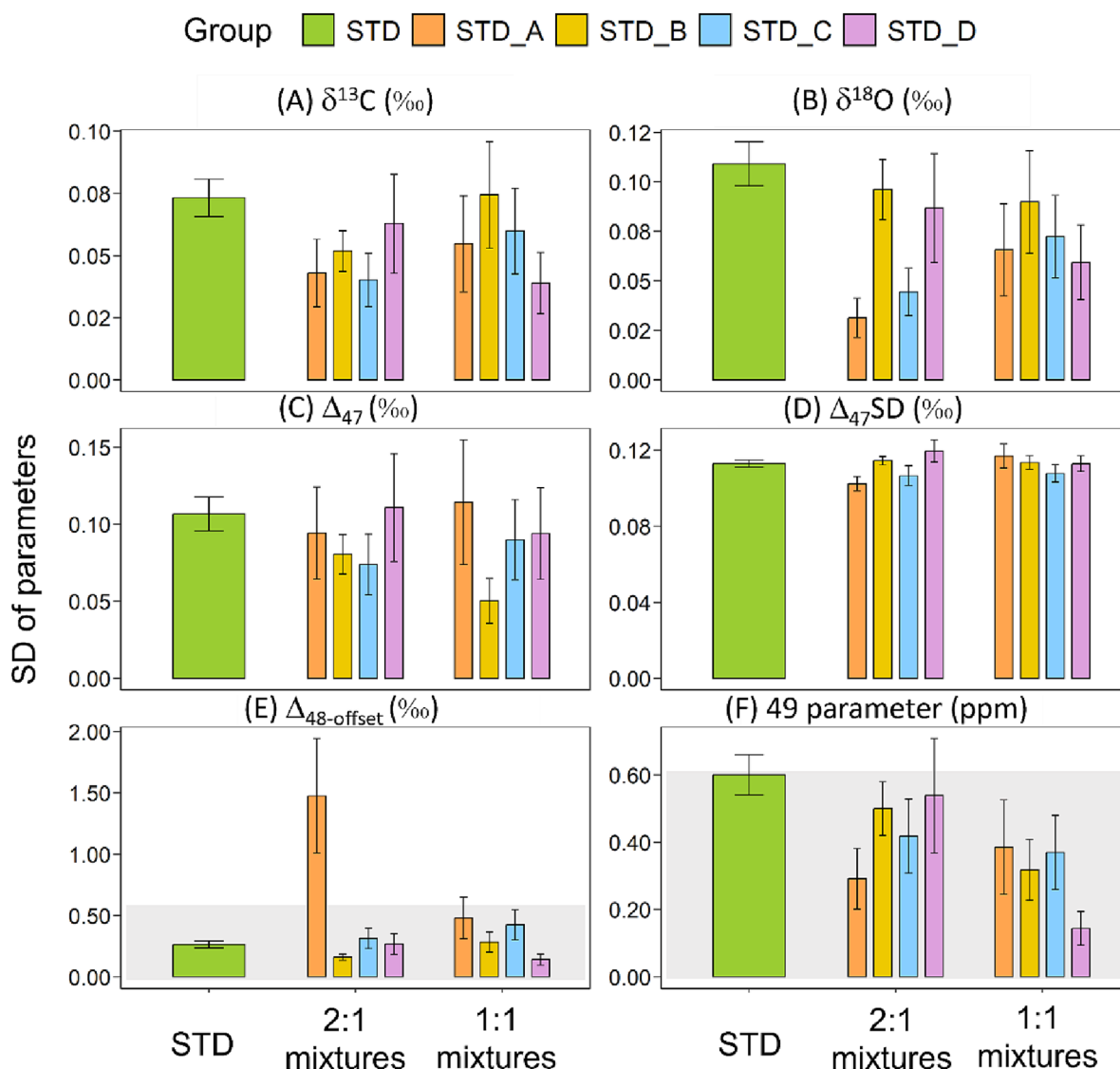


FIGURE 5 Bar plots showing the standard deviation of (A) $\delta^{13}\text{C}$, (B) $\delta^{18}\text{O}$, (C) Δ_{47} , (D) internal standard deviation of Δ_{47} (Δ_{47} SD), (E) $\Delta_{48}\text{-offset}$, and (F) 49 parameter in pure ETH-4 (STD) and ETH-4:resin mixtures in 2:1 and 1:1 proportions with different brands. The error bars indicate the uncertainty of the standard deviation (Equation 3). The gray shaded area in (E) and (F) indicates the standard deviation of ETH-3 standards in regular sessions without resin-containing samples (0.52‰ and 0.6 ppm for $\Delta_{48}\text{-offset}$ and 49 parameter, respectively). [Color figure can be viewed at wileyonlinelibrary.com]

might be an artifact of fewer replicates for brand A ($n = 6$ and 5 for 2:1 and 1:1 mixtures, respectively). Notably, the instrument used for our study shows better long-term accuracy and reproducibility from May to November 2021 (0.046%)³⁵ than during April to September 2022 when the resin test was performed (0.059% , derived from check standard IAEA-C2).

The average $\Delta_{48\text{-offset}}$ for different brands of resins ranges from -0.047% to 0.515% with a significant difference based on ANOVA ($p = 0.02$), and is always lower than the threshold (1.5% in our laboratory²⁹; Table 1 and Figure 4E), while no specific difference is observed between different mixtures according to the post hoc analysis (Figure 4E). The uncertainty on the $\Delta_{48\text{-offset}}$ is lower than 0.50% for most of the mixtures and is also within the range of $\Delta_{48\text{-offset}}$ for ETH-3 standards in sessions without resin-containing samples ($SD = 0.52\%$; Table 1 and Figure 5E). However, the external SD for $\Delta_{48\text{-offset}}$ of brand A (Araldite 2020) is the highest of all tested brands, with an SD of 1.475% and 0.480% for the 2:1 and 1:1 mixtures, respectively. This could be an artifact of relatively fewer replicates being run for this brand ($n = 6$ and 5 for 2:1 and 1:1 mixtures, respectively), but the high $\Delta_{48\text{-offset}}$ for this resin indicates that the reproducibility of the measurements may be compromised. Despite this, other parameters for brand A do not show extreme values or larger uncertainties compared to those for the other brands. Furthermore, the 49 parameter is always lower than the threshold (0.01% or 10 ppm in our laboratory),²³ and is in the same range for sessions with and without resin (Figures 4F and 5F), indicating that it is not affected by the resin. Nevertheless, the difference in 49 parameter between Araldite 2020 (brands A and B, 1:1 mixtures) and EpoThin 2 (brand D) is significant ($p < 0.05$; Table 1 and Figure 4F). Despite the high SD of the $\Delta_{48\text{-offset}}$ in brand A, the absolute values of both $\Delta_{48\text{-offset}}$ and 49 parameter are below the threshold (1.5% and 10 ppm, respectively) in most of our measurements, indicating that the resin does not impact the absolute values, but it might reduce the reproducibility.

3.5 | Purification with Porapak

To find the source of the low reproducibility of clumped isotope values in resin-containing sessions and sessions after measuring samples with resin, we evaluated the performance of the Porapak. The Porapak plays a key role in purifying the sample gas by removing allochthonous organic contributions in our system and is a crucial step for clumped isotope measurements that are sensitive to potential contaminations. We assessed the efficiency of the Porapak in removing contaminants, in this case from resin, by comparing the stable and clumped isotope measurements of pure ETH-4 at the start and end of Sessions R17, R21, and R47, and pure ETH-3 from Sessions N1, N2, N8, and N9 measuring relatively clean samples without resin (Figure 6). The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of the ETH-4 standard are around 0.3% and 0.2% less negative at the end of the session compared to at the start of Sessions R17 and R47. Interestingly, these sessions contain >40 measurements ($n = 46$ and 41 , respectively),

whereas Session R21, which contains fewer measurements ($n = 30$) only shows a slight shift in $\delta^{13}\text{C}$ (around 0.1% less negative) and no clear change in $\delta^{18}\text{O}$ between the end and the start of the session (Figure 6B). The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of the ETH-3 standard in sessions measuring relatively clean samples without resin are randomly distributed over the entire session with a range of $\sim 0.2\%$ and 0.3% , respectively (Figure 6A). Theoretically, the adsorption capacity of Porapak decreases with the accumulation of contaminants, therefore the Porapak is heated to get rid of potentially accumulating contaminants before the start of each new session. Thus, the shift towards more positive values for $\delta^{13}\text{C}$ during resin-containing sessions with many measurements can be explained by an increasingly reduced adsorption capacity of the Porapak. In contrast, the Δ_{47} and the internal SD of the Δ_{47} are randomly distributed over the session for all seven sessions regardless of whether they contain samples with or without resin (Figure 6A,B), and the 49 parameter also remains stable during each session (Figure 6A,B). Only the Δ_{48} value shows an increase of 3.99% during Session R21, but it is stable for the other sessions (Figure 6B). Nevertheless, given the shift in stable isotope values, we suggest keeping measurement sessions short(er) when measuring resin-containing samples with the Kiel system or purifying the sample gasses in an offline system with cleaning traps before transferring them to the mass spectrometer.

Although the average values of Δ_{47} do not show a significant difference between the samples with or without embedding resins ($p > 0.05$; Table 1 and Figure 4D), the reproducibility of Δ_{47} , indicated by the external SD of the different mixtures, is low compared to the check standard IAEA-C2 during the resin test period (external $SD = 0.059\%$), as well as a period in 2021 when no resin-containing samples were measured (external $SD = 0.046\%$)³⁵. This confirms our hypothesis that the addition of resin may be the source of deviating clumped isotope values observed in Sessions S11, S14–S16 (purple background in Figure 2). The external SD remains high ($0.062 \pm 0.022\%$) in the period when samples with resin were measured, and the largest SD is reached during the second resin test session (Session R21, external $SD = 0.119\%$). To improve the results, the Porapak with supposedly reduced adsorption capacity was replaced several sessions after Session R21, which led to an improvement of the Δ_{47} external SD of ETH-3 to 0.051% (light wheat background in Figure 2), indicating that the new Porapak with enough capacity indeed contributes to the improved reproducibility of Δ_{47} . Taken together, although the resin does not affect the absolute values of Δ_{47} , the reproducibility of Δ_{47} that has low abundance in natural CO_2 gasses (44.4 ppm⁹) is decreased by the resin in the measurement system.

Similarly, the influence of the Porapak on the reproducibility of stable isotopic values was examined and compared to the check standard IAEA-C2. The external SD of $\delta^{13}\text{C}$ for sessions containing resin (Sessions S11, S14–S16, R17, R21, and R47) is $0.08 \pm 0.03\%$, which is slightly higher than the long-term external SD based on IAEA-C2 (0.06% ; Figure 2A,B), while the external SD of $\delta^{18}\text{O}$ for sessions containing resin is $0.08 \pm 0.03\%$, which is lower than the

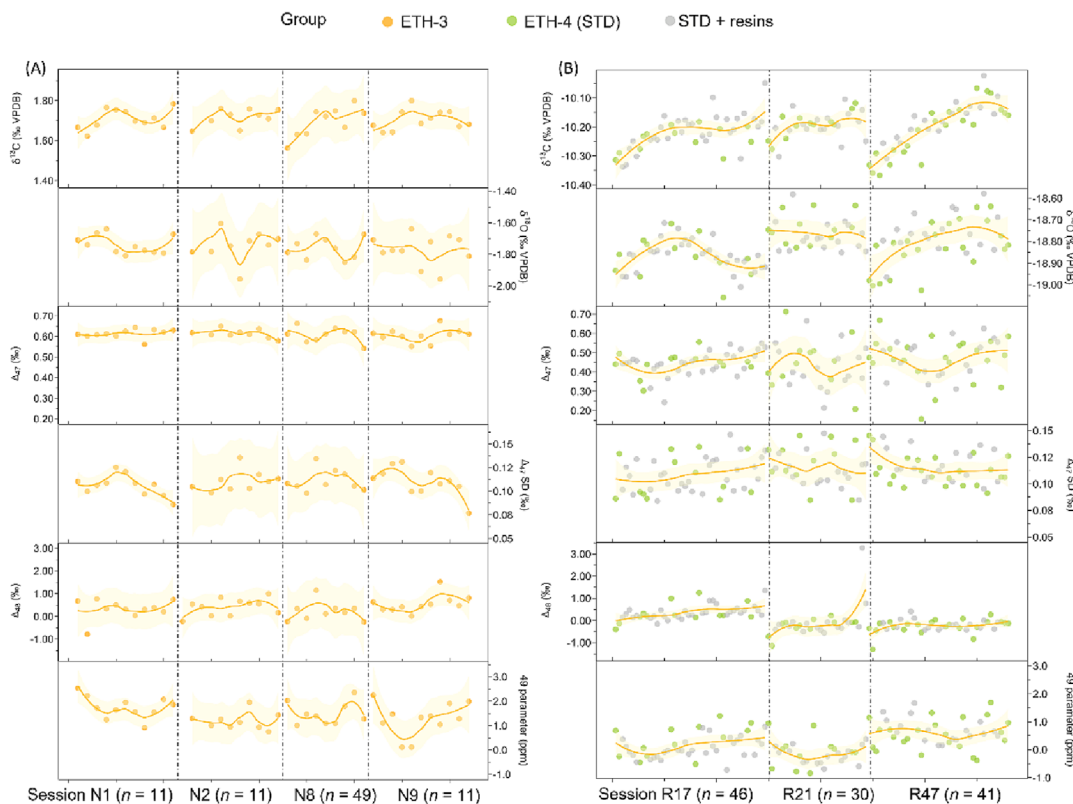


FIGURE 6 Scatter plots showing the evolution of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Δ_{47} , internal standard deviation of Δ_{47} (Δ_{47} SD), Δ_{48} , and 49 parameter over time in (A) sessions without resin-containing samples (N1, N2, N8, and N9) and (B) resin test sessions (R17, R21, and R47) for this study. Pure ETH-3 in regular sessions shown in orange, ETH-4 (STD) in resin test sessions shown in green, and ETH-4 with embedding resins in gray. The orange smoothing line indicates the trend inside per session using the locally estimated scatterplot smoothing method; the 95% confidence intervals are shown by the light-yellow shaded area. Numbers in parentheses indicate the number of aliquots per session [Color figure can be viewed at wileyonlinelibrary.com]

long-term external SD based on IAEA-C2 (0.10‰; Figure 2C,D). In general, given the relatively high abundance of stable isotopes compared to that of clumped isotopes, the comparatively low amount of potential contaminant from resin does not impact the absolute values and also the reproducibility is unaffected (Figure 4A,B).

Based on our results, we suggest that the quality of stable and clumped isotope measurements can be monitored by checking the external SD of a carbonate standard in each session and replacing the Porapak materials periodically (e.g., every 6 months in our Kiel device) to guarantee its adsorption ability, especially when samples with resin are measured. Given the cumulative impact of resins on the Kiel system and/or mass spectrometer, we suggest avoiding the addition of resin to samples as much as possible. Recently, a new system for carbonate clumped isotope analysis has been introduced, namely tunable infrared laser differential absorption spectrometry (TILDAS). This system measures the exact molecular species rather than a certain mass (m/z), and is therefore less affected by potential contaminants.³⁶ Hence, this system could provide a solution for future analysis of samples that contain large amounts of (un)known contaminants.

4 | CONCLUSIONS

After the observation of deviating clumped isotope values for carbonate standards run as part of sessions containing resin-embedded samples, an experiment was set up to examine the effects of embedding resins on stable and clumped isotope analyses. We found no detectible increase in CO_2 gas production from resins after reacting with phosphoric acid at 70°C. Although there is pink discoloration in phosphoric acid added to samples that contain resin, the discoloration only occurs after the sample gases have been transferred for analysis and thus has no influence on the measurements. We found that $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are not significantly impacted by resins. Although the absolute values of Δ_{47} are also not significantly different for samples with and without resin addition, the reproducibility of Δ_{47} appears to decrease for samples with resin. We therefore recommend avoiding resins during sample preparation and workup as much as possible. In addition, purification of CO_2 gas using a contamination trap with Porapak to adsorb potential contaminants is suggested. To optimize the adsorption capacity of Porapak, sessions with resin-containing samples should be kept shorter in a Kiel system and the Porapak materials should be

replaced periodically to warrant the adsorption capacity. After measuring the sample with resin, the Porapak should be heated to remove accumulated contaminants. At the same time, it is important to monitor the measurement reproducibility by checking the external SD of standards (e.g., ETH-3) in each session and comparing them to the long-term measurement reproducibility derived from the check standard (e.g., IAEA-C2).

ACKNOWLEDGMENTS

Arnold van Dijk and Desmond Eefting in GeoLab at Utrecht University are acknowledged for technical support. We thank Rik Tjallingii (GFZ Potsdam) for providing the Araldite 2020 (brand A). Two anonymous reviewers and handling editor Roland Bol are thanked for their constructive comments, which helped to improve this paper. This project received funding from the Dutch Research Council (NWO, Vidi grant no. 192.074 to FP and Vidi grant no. 016.161.365 to MZ). X.Z. acknowledges the funding from the China Scholarship Council (CSC, grant no. 202104910149). N.J.W. acknowledges support from the Flemish Research Council (FWO postdoctoral fellowship; 12ZB220N).

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/rcm.9597>.

DATA AVAILABILITY STATEMENT


Supporting information are deposited on Open Science Frame (<https://doi.org/10.17605/OSF.IO/8M7YD>). The original data produced for this study can be accessed through the EarthChem Library (<https://doi.org/10.26022/IEDA/112912>).

ORCID

Jingjing Guo  <https://orcid.org/0000-0002-1215-1215>

Xiulan Zong  <https://orcid.org/0000-0001-7640-1044>

Niels J. de Winter  <https://orcid.org/0000-0002-1453-5407>

Barbara Goudsmit-Harzevoort  <https://orcid.org/0000-0002-5038-9697>

Francien Peterse  <https://orcid.org/0000-0001-8781-2826>

Martin Ziegler  <https://orcid.org/0000-0003-3198-6434>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Guo J, Zong X, de Winter NJ, Goudsmit-Harzevoort B, Peterse F, Ziegler M. Assessing the effects of embedding resins on carbonate stable and clumped isotope analyses. *Rapid Commun Mass Spectrom.* 2023;37(17): e9597. doi:10.1002/rcm.9597