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EVALUATION OF THE EFFECT OF OVEN ROASTING AT 340° C, BLEACH, 30% H_2O_2, AND DISTILLED/DEIONIZED WATER ON THE $\delta^{13}C$ VALUE OF SPELEOTHEM CARBONATE

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Organic compounds derived from plants are found in many cave formations, which are collectively termed speleothems. Both the carbon in the organic compounds and the carbon in the speleothem $CaCO_3$ have distinct ratios of the stable isotopes of carbon (^{12}C and ^{13}C) that are expressed as $\delta^{13}C$ values. Values of $\delta^{13}C$ in the organic compounds are lower than $\delta^{13}C$ values of speleothem calcium carbonate and could affect the $\delta^{13}C$ values of speleothems with high organic matter concentrations, if the organic compounds were not removed. Four treatments conventionally used to destroy organic matter in carbonates prior to geochemical analysis were evaluated in this study. The treatments were oven roasting at 340° C, soaking in bleach, soaking in 30% H_2O_2, and soaking in distilled deionized water. There is no statistically significant difference between results from untreated and treated samples. These results suggest that the treatments do not affect the $\delta^{13}C$ value of speleothems' calcium carbonate. The treatments might be helpful in removing organic matter in speleothems that have high concentrations of organic matter. However, most speleothems have low organic carbon concentrations that do not affect the $\delta^{13}C$ value of the speleothem, even if left untreated. Ultimately these treatments only need to be applied to speleothems with unusually high concentrations of organic matter.

INTRODUCTION

Naturally occurring carbon consists almost entirely of two stable isotopes, ^{13}C and ^{12}C, and the ratio of these isotopes in different materials is expressed in terms of $\delta^{13}C$ values relative to the PDB standard, a sample of $CaCO_3$ from a fossil belemnite in the Cretaceous-age Pee Dee Limestone Formation of South Carolina. Paleoenvironmental studies have used $\delta^{13}C$ values of carbon derived from plants as proxies to determine the photosynthetic pathway of ancient vegetation (*e.g.*, Huang *et al.*, 2001, Dorale *et al.*, 1992, Ambrose and Sikes, 1991.) Three types of photosynthetic pathways have been recognized in modern vegetation. One is C_3 or Calvin-Benson (normal) photosynthesis, which results in $\delta^{13}C$ values of plant tissue between −22 and −35‰ (average = −26.5‰) relative to PDB. A second pathway is C_4 or Hatch-Slack photosynthesis (mostly found in tropical grasses adapted to hot and/or arid environments), which results in $\delta^{13}C$ values of plant tissue between −6 and −19‰ (average = −12.5‰) vs. PDB (Bowen, 1988). A third photosynthetic pathway is C.A.M. (Crassulan Acid Metabolism) used by desert succulents, resulting in $\delta^{13}C$ values of plant tissue between −10 and −20‰ (average = −18.0‰) vs. PDB (Attendorn and Bowen, 1997). Because the dominant plant type in a landscape is primarily a result of climate (Huang *et al.*, 2001), the difference in the average $\delta^{13}C$ values of these three kinds of plants makes carbon derived from them a useful proxy for determining the photosynthetic pathway of ancient vegetation, and thus for interpreting paleoclimate.

The C isotopic composition of plants is believed to affect the $\delta^{13}C$ value of coeval speleothem calcite, where speleothem is the term given to deposits such as stalagmites, stalactites, and flowstones formed by dripping water in caves. Speleothems, especially those found in limestone caves, commonly consist of calcium carbonate ($CaCO_3$). Some of the carbon in calcium carbonate speleothems comes from carbon dioxide (CO_2) generated in the soils above caves, where respiration by plant roots and decomposition of plant debris produce CO_2. The $\delta^{13}C$ of the CO_2 in the soil is primarily the result of the photosynthetic pathway (C3, C4, CAM) used by the plants. Because the $\delta^{13}C$ of the dissolved calcium carbonate that is contributed to a speleothem from the overlying bedrock is believed to be constant over the speleothem's growth history, the changes in the $\delta^{13}C$ of speleothem calcium carbonates have long been believed to be the result of changes in the types of plants growing on the surface above a cave. Thus, the $\delta^{13}C$ values of speleothems have been used as proxies for paleovegetation in many paleoenvironmental studies (*e.g.*, Desmarchelier *et al.*, 2000, Hellstrom *et al.*, 1998, Dorale *et al.*, 1992, Brook *et al.*, 1990). More recent work has shown that the $\delta^{13}C$ values of carbonate speleothems can also be affected by factors other than changes in the dominant vegetation over a cave. These include changes in ecosystem productivity controlling soil P_{CO2} (Genty *et al.*, 2003), changes in the $\delta^{13}C$ values of atmospheric CO_2 (Baskaran and Krishnamurthy, 1993), and changes in water/rock interactions (McDermott, 2004). In addition to these controls, factors involving precipitation of calcium carbonate in nonequilibrium may also affect the $\delta^{13}C$ value of speleothem calcium carbonate (Mickler *et al.*, 2004).

In addition to C in $CaCO_3$ itself, speleothems also com-

monly contain C in organic matter, including humic substances (large organic molecules) and other organic residues that are formed from the decomposition of plant litter (dead plant material) in soil (Gascoyne, 1992, Shopov *et al.*, 1994, Baker *et al.*, 1996, Baker *et al.*, 1997). The $\delta^{13}C$ values of humic substances and other organic compounds in many soils have been shown to reflect the $\delta^{13}C$ values of the plants from which they are derived (Nissembaum and Schallinger, 1974, Ambrose and Sikes, 1991, Lichtfouse *et al.*, 1995). The $\delta^{13}C$ values of humic substances are generally much lower than the $\delta^{13}C$ values of the $CaCO_3$ in which they are found (Elkins and Railsback, 2002). This difference raises concern that failure to remove organic C from speleothem carbonate might lead to erroneously low apparent $\delta^{13}C$ $CaCO_3$ values and thus to misinterpretation of the speleothem $\delta^{13}C$ record.

Various treatments have been used to remove organic matter from biogenic carbonate, such as coral, prior to geochemical analysis. This study will evaluate the effect of four such treatments by comparing the $\delta^{13}C$ values measured with and without treatment of speleothem calcite. The four treatments are: 1) oven roasting at 340° C (Boiseau and Juillet-Leclerc, 1997), 2) distilled /deionized water (DDW) (Mitsuguchi *et al.*, 2001), 3) 30% H_2O_2, (Boiseau and Juillet-Leclerc, 1997) and 4) sodium hyperchlorite (bleach) (Gaffey and Bronnimann, 1993).

Three general results are possible. In the first case, if the $\delta^{13}C$ values of treated speleothems with high organic carbon contents are greater than $\delta^{13}C$ values of untreated samples, then the treatments will seemingly have been successful at removing organic matter. In the second case, if the $\delta^{13}C$ values of treated speleothem samples show no significant difference from $\delta^{13}C$ values of untreated samples, either the treatments will seemingly have had no effect, or there will have been so little organic matter that effective treatment caused no significant change in measured $\delta^{13}C$. In the third and least likely case, if the $\delta^{13}C$ values of treated speleothem samples are less than $\delta^{13}C$ values of untreated samples, then the treatment methods will seemingly have altered the $\delta^{13}C$ values of the speleothem calcium carbonate, counter to the intent of researchers that apply such methods.

METHODS

MATERIAL

Twelve speleothems from a wide range of geographic locations were chosen for our study (Tables 1–3). Three stalagmites, two stalactites and two flowstones, all of which were recrystallized, were chosen from Caverns of Sonora in west Texas. One recrystallized stalagmite from Egypt, one stalagmite from Reflection Cave in Belize, one stalagmite from Pettijohn's Cave near LaFayette, Georgia, two recrystallized speleothems of unknown type from Carthage, Tennessee and one stalactite and one stalagmite from unknown localities were also used.

A total of 32 samples weighing 3 to 9 g were cut from the speleothems (Tables 1–3) using a diamond impregnated bandsaw blade. The samples were ground to a fine powder using a mortar and pestle.

TREATMENTS

Sub-samples weighing 10 mg were taken from each of the 32 untreated powdered samples and set aside for $\delta^{13}C$ analysis. Four sets of 40 mg sub-samples were weighed out of the 32 untreated powdered samples (a total of 128 sub-samples). One set of sub-samples was spread over clean watch glasses and roasted at 340° C in an oven for 24 hours. Samples in the remaining sets were placed in separate 40 ml amber vials (96 vials total). One set of vials was filled with 40 ml of DDW, another with 40 ml of bleach, and another with 40 ml of 30% H_2O_2. Samples were left in the solutions for 24 hours.

After treatment, the supernatant from each of the vials was carefully decanted to ensure that as few grains were lost as possible. Each vial was then filled with 40 ml of DDW. Vials sat for 10 minutes until all carbonate grains settled to the bottom. The water was then carefully decanted to insure that as few grains as possible were lost. This process was repeated 10 times to insure that none of the organic matter that might have been removed from the calcium carbonate in treatment and released into the supernatant remained in the samples. The wet samples were left in the vials and dried in an oven at 60° C for 24 hours.

$\delta^{13}C$ ANALYSIS

Sub-samples weighing 10 mg were taken from each of the 128 treated samples and 32 of the untreated samples and reacted with 100% phosphoric acid at 50º C. The evolved CO_2 was isolated using a cold finger under vacuum at the University of Georgia Stable Isotope Lab and measured on a Finnigan MAT 252 stable isotope mass spectrometer. The standard used for isotopic analysis was Iceland spar and has a $\delta^{13}C$ value of −4.7‰ (vs. PDB). Two samples of gas extracted from solid Iceland spar were analyzed on the mass spectrometer 13 times, and the precision of the analysis was ± 0.1‰.

ORGANIC ANALYSIS

Sub-samples weighing 2 g were placed in 40 ml amber vials and reacted with 40 ml of 1 N HCl at room temperature to dissolve the calcite. After two days, some carbonate grains remained at the bottom of some of the vials. An additional 2–3 ml of 1 N HCl was added to all the vials, which resulted in complete dissolution of the remaining grains. No precipitated organic matter was observed on the bottom of the vials.

The resulting supernatants (with the organic matter freed in solution) were analyzed for total organic carbon (TOC) concentration on an OI 1010 carbon analyzer at the University of Ottawa's G.G. Hatch Isotope Laboratory following the method described by St-Jean (2003). During this process, an aliquot of the sample was siphoned into a reaction vessel at 100°C. Drops of phosphoric acid were added to the sample, releasing the total inorganic carbon (TIC), which was passed in a helium carrier through nafion and chemical traps to remove water and then on to a non-destructive infrared detector (NDIR) tuned to CO_2. With the TIC removed, sodium persulfate was added to oxidize the TOC to CO_2. The effluent was directed to the NDIR for determination of the TOC concentration. Standards for this analysis were sucrose1, potassium phthalate and citric acid.

Table 1. Characteristics of samples used in this study.

Sample ID	Sample Location	Speleothem ID and Type	Mineralogy	TOC Concentration of Calcite (ppm)
C.S.1-T	Sonora, Texas	C.S.-1 (stalagmite)	Calcite	165.9
C.S.-1Mi	Sonora, Texas	C.S.-1 (stalagmite)	Calcite	184.8
C.S.-1Mo	Sonora, Texas	C.S.-1 (stalagmite)	Calcite	170.1
C.S.-1b	Sonora, Texas	C.S.-1 (stalagmite)	Calcite	144.9
C.S.-2i	Sonora, Texas	C.S.-2 (stalagmite)	Calcite	123.9
C.S.-2o	Sonora, Texas	C.S.-2 (stalagmite)	Calcite	126.4
C.S.-3d	Sonora, Texas	C.S.-3 (stalagmite)	Calcite	168.4
C.S.-3L	Sonora, Texas	C.S.-3 (stalagmite)	Calcite	140.7
C.S.-T1	Sonora, Texas	C.S.-T1 (stalactite)	Calcite	130.2
C.S.-T2	Sonora, Texas	C.S.-T2 (stalactite)	Calcite	123.9
C.S.-F.D.	Sonora, Texas	C.S.-F (flowstone)	Calcite	113.4
C.S.-F.Ma	Sonora, Texas	C.S.-F (flowstone)	Calcite	65.1
C.S.-F.Mb	Sonora, Texas	C.S.-F (flowstone)	Calcite	52.5
C.S.-F.Mc	Sonora, Texas	C.S.-F (flowstone)	Calcite	56.7
C.S.-F.L	Sonora, Texas	C.S.-F (flowstone)	Calcite	48.3
C.S.-F2a	Sonora, Texas	C.S.-F 2 (flowstone)	Calcite	159.6
C.S.-F2b-R	Sonora, Texas	C.S.-F 2 (flowstone)	Calcite	161.7
C.S.-F2c-R	Sonora, Texas	C.S.-F 2 (flowstone)	Calcite	163.8
Reflx-T	Reflection Cave, Belize	Reflx (stalagmite)	Calcite	252.4
Reflx-L	Reflection Cave, Belize	Reflx (stalagmite)	Calcite	239.4
Reflx-B	Reflection Cave, Belize	Reflx (stalagmite)	Calcite	218.4
MR-68o	Carthage, Tennessee	MR-68 (type uncertain)	Calcite	182.7
MR-68m	Carthage, Tennessee	MR-68 (type uncertain)	Calcite	212.1
MR-68i	Carthage, Tennessee	MR-68 (type uncertain)	Calcite	231.4
Car-Mro	Carthage, Tennessee	Car-Mr (type uncertain)	Calcite	224.7
Car-Mri	Carthage, Tennessee	Car-Mr (type uncertain)	Calcite	224.7
P.J.Y.	LaFayette, Georgia	P.J. (stalagmite)	Calcite	203.7
P.J.O.	LaFayette, Georgia	P.J. (stalagmite)	Calcite	239.4
E.E.-12D	Wadi Sannur, Egypt	E.E.-12 (stalagmite)	Calcite	102.9
E.E.-12L	Wadi Sannur, Egypt	E.E.-12 (stalagmite)	Calcite	104.4
Unknown-1	Unknown	Unknown-1 (stalactite)	Calcite	184.8
Unknown-2	Unknown	Unknown -2 (stalagmite)	Aragonite	193.2

RESULTS

The $\delta^{13}C$ values of the treated speleothem carbonate show no statistically significant difference from those of the untreated samples (Tables 1–3, Fig. 1). We follow standard statistical procedure in reporting the average of the differences between paired samples to one more significant figure than the original $\delta^{13}C$ values (Tables 1–3). The average difference between the oven-roasted samples and corresponding untreated samples is 0.00‰ (Fig. 2). The average difference between samples treated with bleach and corresponding untreated samples is 0.06‰. The average difference between the samples treated with 30% H_2O_2 and corresponding untreated samples is 0.02‰. The average difference between samples treated with DDW and corresponding untreated samples is 0.03‰ (Fig. 2). Applying *t*-tests show the mean differences between the treated and untreated samples are not significantly different from zero ($p > 0.93$ and thus very far from statistical significance for all four treatments) (Tables 1–3).

DISCUSSION

The treatments to remove organic carbon had no statistically significant effect on the $\delta^{13}C$ values of the carbonates studied (Figs. 1 and 2). As noted in the Introduction, this may be because the treatments are ineffective at removing organic carbon.

Table 2. Carbon isotope results[a].

Sample ID	$\delta^{13}C$ by Conventional Treatment	$\delta^{13}C$ with Roasting at 340°C	$\delta^{13}C$ with Bleach Treatment	$\delta^{13}C$ with H_2O_2 Treatment	$\delta^{13}C$ with DDW Treatment
C.S.1-T	–7.9	–7.9	–7.9	–7.9	–7.9
C.S.-1Mi	−8.7	–8.7	–8.8	–8.8	–8.8
C.S.-1Mo	−8.2	–8.2	–8.1	–8.2	–8.2
C.S.-1b	−6.8	–6.8	–6.9	–6.8	–6.7
C.S.-2i	–5.9	–5.9	–5.9	–5.9	–5.9
C.S.-2o	–6.1	–6.2	–6.0	–6.1	–6.0
C.S.-3d	–8.0	–7.9	–8.0	–7.9	–7.9
C.S.-3L	–6.5	–6.5	–6.7	–6.6	–6.5
C.S.-T1	–6.4	–6.2	–6.2	–6.3	–6.3
C.S.-T2	–5.7	–5.8	–5.9	–5.8	–5.7
C.S.-F.D.	–5.1	–5.3	–5.4	–5.1	–5.1
C.S.-F.Ma	–2.7	–2.7	–3.1	–2.2	–2.7
C.S.-F.Mb	–2.6	–2.4	–2.5	–2.5	–2.5
C.S.-F.Mc	–2.4	–2.4	–2.7	–2.3	–2.4
C.S.-F.L	–2.2	–2.3	–2.3	–2.2	–2.2
C.S.-F2a	–7.5	–7.5	–7.6	–7.6	–7.4
C.S.-F2b-R	–7.6	–7.6	–7.7	–7.6	–7.6
C.S.-F2c-R	–7.7	–7.6	–7.8	–7.6	–7.6
Reflx-T	–12.1	–12.0	–12.0	–11.9	–11.9
Reflx-L	–11.2	–11.2	–11.4	–11.1	–11.3
Reflx-B	–10.6	–10.5	–10.4	–10.6	–10.3
MR-68o	–8.6	–8.4	–8.7	–8.6	–8.6
MR-68m	–10.3	–10.3	–10.1	–10.3	–10.4
MR-68i	–11.1	–11.1	–11.0	–11.1	–11.1
Car-Mro	–10.6	–10.7	–10.7	–10.7	–10.6
Car-Mri	–10.6	–10.5	–10.7	–10.6	–10.3
P.J.Y.	–9.7	–9.6	–9.7	–9.7	–9.7
P.J.O.	–11.2	–11.2	–11.4	–11.0	–11.3
E.E.-12D	–5.0	–5.0	–4.9	–5.0	–4.9
E.E.-12L	−4.9	–5.1	–5.0	–4.8	–4.9
Unknown-1	−8.8	–8.8	–8.8	–8.7	–8.8
Unknown-2	−9.2	–9.1	–9.2	–9.2	–9.2

[a] $\delta^{13}C$ values are all in ‰ with respect to the PDB standard. Location, mineralogy, and TOC concentration of samples are given in Table 1.

One possible reason for them to be ineffective might be the size of humic molecules relative to the size of the $CaCO_3$ particles in which they are housed, and with the non-porous nature of that $CaCO_3$. If humic acid molecules are 1 to 10 nm in size and the $CaCO_3$ enclosing them is pulverized to 1 μm particles, most humic acid molecules will lie well inside any $CaCO_3$ particle and thus be immune to treatment prior to dissolution of that $CaCO_3$. Only permeability through micropores (pores less than 1 μm in size) would allow access by attacking fluids. It thus may not be reasonable to expect pretreatments to affect molecules or particles with sizes orders of magnitude smaller than the grain size.

The failure of the various treatments to yield $\delta^{13}C$ values significantly different from untreated values also probably results from the C isotope systematics of spelean carbonate, for three reasons. First, speleothems contain little organic carbon compared to the organic contents of brachiopod shells, many mollusk shells, and many limestones (Fig. 3). The skeletal carbonate nearest to spelean carbonate in organic content is coral aragonite, and Boiseau and Juillet-Leclerc (1997) found that H_2O_2 treatment of coral aragonite was more likely to cause a decrease in $\delta^{13}C$ because of dissolution of post-biotic cement than it was to cause the expected increase by removing significant organic carbon. Secondly, speleothems have $\delta^{13}C$ $CaCO_3$ values lower than those of marine carbonates, so that $\delta^{13}C$ of the bulk material is changed less by incorporation of organic carbon with low $\delta^{13}C$ values (Fig. 3). Thirdly, at least some speleothems

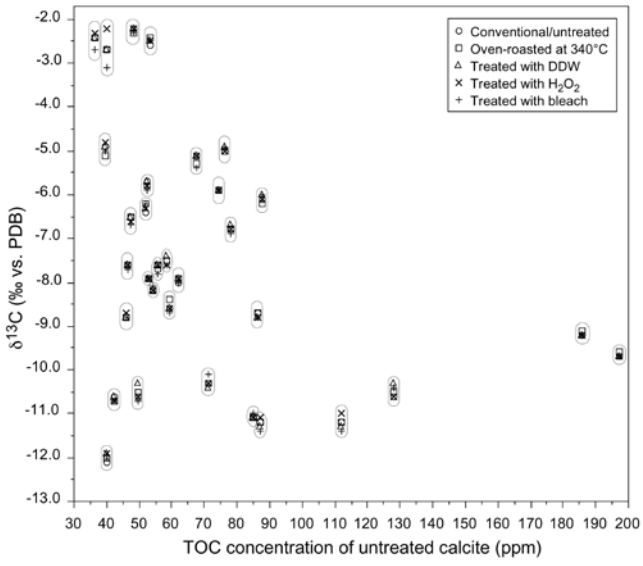

Figure 1. $\delta^{13}C$ of 32 carbonate samples, each analyzed with no treatment and with four different treatments, plotted against concentration of total organic carbon in each sample. Ovals and loops enclose the five symbols for any one sample. Uncertainty of $\delta^{13}C$ values plots within vertical extent of symbols. General overlap of symbols illustrates that treatments have little effect on measured $\delta^{13}C$.

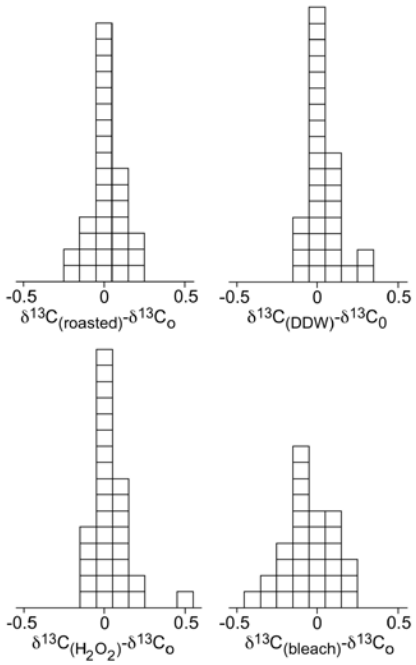

Figure 2. Frequency of differences between $\delta^{13}C$ untreated and corresponding treated speleothem carbonates. Plots show no more than a 0.5‰ change in the difference of $\delta^{13}C$ values of speleothem carbonate after treatments have been applied.

incorporate organic C processed by C_4 photosynthesis, so that the $\delta^{13}C$ of incorporated organic carbon need not cause a large decrease in $\delta^{13}C_{bulk}$ (Fig. 3). As a result of these considerations, $\delta^{13}C_{bulk}$ of speleothem carbonates can only be expected to be at most 0.05‰ less than $\delta^{13}C_{CO3}$, whereas $\delta^{13}C_{bulk}$ of mollusk shells, brachiopod shells, and limestones can be more than 1.0‰ less than the $\delta^{13}C$ of the carbonate itself (Fig. 3).

CONCLUSIONS

Conventional techniques to remove organic material from carbonates prior to isotopic analysis had no significant effect on the $\delta^{13}C$ value of the speleothems studied. These treatments could therefore be useful in removing organics from speleothems with higher organic matter content without concern that the treatments might alter the $\delta^{13}C$ value of the calcium carbonate in which the organic is contained. However, the speleothems used in this study, and by analogy most speleothems used in geologic studies, do not contain enough organic matter to affect the $\delta^{13}C$ values of speleothems even if they are left untreated. Therefore, pretreatment of speleothem samples is unnecessary unless the speleothem has unusually high organic carbon content.

Table 3. Oxygen isotope results[a].

Sample ID	$\delta^{18}O$ by Conventional Treatment	$\delta^{18}O$ with DDW Treatment	$\delta^{18}O$ with Roasting at 340°C	$\delta^{18}O$ with Bleach Treatment	$\delta^{18}O$ with H_2O_2 Treatment
C.S.1-T	–4.5	–4.8	–4.6	–4.5	–4.5
C.S.-1Mi	–5.8	–5.7	–5.8	–5.7	–5.8
C.S.-1Mo	–5.9	–5.8	–5.9	–6.1	–5.8
C.S.-1b	–5.3	–5.4	–5.4	–5.3	–5.3
C.S.-2i	–5.1	–5.2	–5.3	–5.3	–5.0
C.S.-2o	–5.3	–5.1	–5.3	–5.1	–5.2
C.S.-3d	–5.8	–5.9	–5.8	–5.7	–5.7
C.S.-3L	–4.9	–4.9	–5.0	–5.0	–5.0
C.S.-T1	–5.0	–5.2	–5.0	–5.0	–4.9
C.S.-T2	–4.9	–5.1	–5.0	–5.0	–5.1
C.S.-F.D.	–5.1	–5.0	–4.9	–5.0	–5.0
C.S.-F.Ma	–5.0	–4.9	–4.8	–5.0	–4.9
C.S.-F.Mb	–4.8	–4.7	–4.8	–4.7	–4.8
C.S.-F.Mc	–4.5	–4.6	–4.6	–4.6	–4.3
C.S.-F.L	–4.5	–4.5	–4.5	–4.4	–4.5
C.S.-F2a	–4.4	–4.3	–4.5	–4.3	–4.3
C.S.-F2b-R	–4.8	–4.8	–4.8	–4.9	–4.8
C.S.-F2c-R	–5.5	–5.4	–5.5	–5.6	–5.5
Reflx-T	–4.6	–4.5	–4.6	–4.6	–4.4
Reflx-L	–4.2	–4.4	–4.4	–4.4	–4.3
Reflx-B	–4.9	–4.8	–5.0	–4.9	–5.0
MR-68o	–4.0	–4.1	–4.2	–4.2	–4.1
MR-68m	–4.6	–4.4	–4.5	–4.4	–4.4
MR-68i	–4.2	–4.5	–4.3	–4.4	–4.2
Car-Mro	–4.2	–4.2	–4.4	–4.3	–4.3
Car-Mri	–5.0	–5.1	–5.5	–5.0	–5.0
P.J.Y.	–4.1	–4.2	–4.3	–4.4	–4.1
P.J.O.	–4.2	–4.2	–4.5	–4.3	–4.2
E.E.-12D	–11.4	–11.4	–11.2	–11.5	–11.4
E.E.-12L	–10.1	–10.2	–10.1	–10.2	–10.1
Unknown-1	–3.0	–3.1	–3.2	–3.1	–3.0
Unknown-2	–4.8	–4.9	–5.0	–4.9	–4.9

[a] $\delta^{18}O$ values are all in ‰ with respect to the PDB standard. Location, mineralogy, and TOC concentration of samples are given in Table 1.

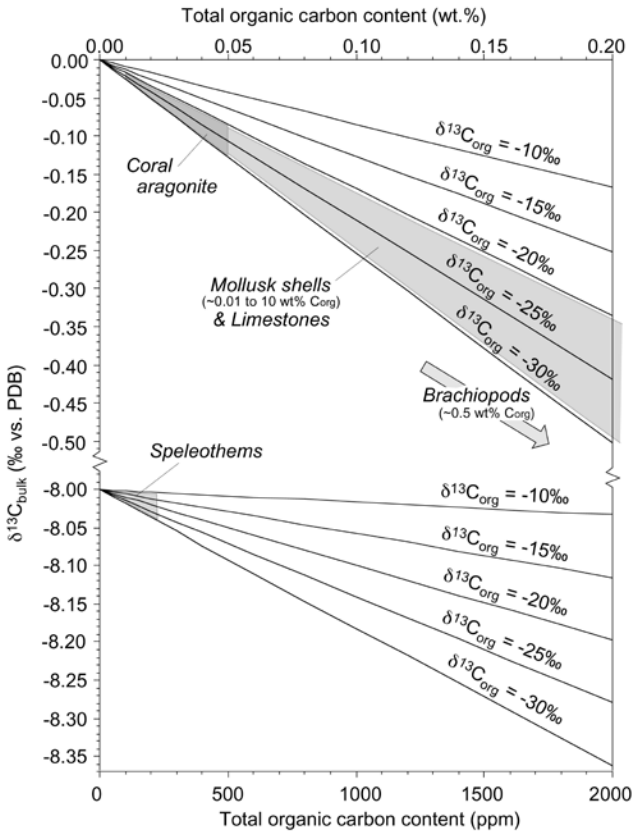

Figure 3. $\delta^{13}C_{bulk}$ of a hypothetical marine carbonate with $\delta^{13}C_{CO3}$ = 0.00 and of a hypothetical spelean carbonate with $\delta^{13}C_{CO3}$ = 8.00, as a function of total organic carbon content with various values of $\delta^{13}C_{org}$. Field for coral aragonite is from Boiseau and Juillet-Leclerc (1997), field for mollusk shells is from Weiner et al. (1983), field for limestones is from Pratt (1984) and Pancost et al. (1998, 1999), arrow for brachiopods is from Jope (1971, 1977), and field for speleothems is from this paper.

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REFERENCES

Ambrose, S., and Sikes, N., 1991, Soil carbon isotope evidence for Holocene habitat change in the Kenya Rift valley: Science, v. 252, p. 1402–1405.

Attendorn, H., and Bowen, R., 1997, Radioactive and Stable Isotope Geology, Chapman and Hall, London, 522 p.

Baker, A., Barnes. W.L., and Smart, P., 1996, Speleothem luminescence intensity and spectral characteristics: Signal calibration and a record of paleovegetation change: Chemical Geology, v. 130, p. 65–76.

Baker, A., Emi, I., and Smart, P., 1997, Elevated variable values of ^{13}C in speleothems in a British Cave system: Chemical Geology, v. 130, p. 263–270.

Baskaran, M., and Krishnamurthy, R. V., 1993, Speleothems as proxy for the carbon isotope composition of atmospheric CO_2: Geophysical Research Letters, v. 20, p. 2905–2908.

Boiseau, M., and Juillet-Leclerc, A., 1997, H_2O_2 treatment of recent coral aragonite: oxygen and carbon isotopic implications: Chemical Geology, v. 143, p. 171–180.

Bowen, R., 1988, Isotopes in the Earth Sciences, Elsevier Applied Science Publishers LTD, Essex, 647 p.

Brook, G., Burney, D., and Cowart, J., 1990, Desert paleoenvironmental data from cave speleothems with examples from the Chihuahuan, Somali-Chalbi, and Kalahari deserts: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 76, p. 311–329.

Desmarchelier, J., Goede, A., Ayliffe, L., McColloch, M., and Moriarty, K., 2000, Stable isotopic record and its palaeoenvironmental interpretation for a late Middle Pleistocene speleothem from Victoria Fossil Cave, Naracoorte, South Australia: Quaternary Science Reviews, v. 19, p. 763–774.

Dorale, J., Gonzales, L., Reagan, M., Pickett, D., Murrell, M., T., and Baker, R., G., 1992, A high resolution record of Holocene climate change in speleothem calcite from Cold Water Cave, Northeast Iowa: Science, v. 258, p. 1626–1630.

Elkins, J., and Railsback, L.B., 2002, $\delta^{13}C$ Value of Soil Organic Matter in Speleothems: a New Proxy to Determine Paleovegetation and Interpret Paleoclimate: http://gsa.confex.com/gsa/2002NC/finalprogram/abstract_32537.htm [accessed September 28, 2006].

Gaffey, S., and Bronniham, C., 1994, Effects of bleaching on organic and mineral phases in biogenic carbonates: Journal of Sedimentary Petrology, v. 63, p. 752–754.

Gascoyne, M., 1992, Palaeoclimate determination from cave calcite deposits: Quaternary Science Reviews, v. 11, p. 609–632.

Genty, D., Baker, A., Blamart, D., Gilmour, M., Jouzel, J., Ouahdi, R., and van Exter, S., 2003, Precise dating of Dansgaard-Oeschger climate oscillations in Western Europe from stalagmite data: Nature, v. 421, p. 833–837.

Hellstrom, J., Malcolm, M., and Stone, J., 1998, A detailed 31,000-year record of climate and vegetation change, from the isotope geochemistry of two New Zealand speleothems: Quaternary Research, v. 50, p.167–178.

Huang, Y., Street-Perrott, A., Metcalfe, S., Brenner, M., Moreland, M., and Freeman, K., 2001, Climate change as the dominant control on glacial-interglacial variations in C_3 and C_4 plant abundance: Science, v. 293, p. 1647–1651.

Jope, M., 1971, Constituents of brachiopod shells, in M. Florkin and M.H. Stotz, eds., Comprehensive Biochemistry, v. 26C, p. 749–784.

Jope, M., 1977, Brachiopod shell proteins: their functions and taxonomic significance: American Zoologist, v. 17, p. 133–140.

Lichtfouse, E., Dou, S., Houot, S., and Barriuso, E., 1995, Isotopic evidence for soil organic carbon pools with distinct turnover rates; II, humic substances: Organic Geochemistry, v.23. p. 845–847.

McDermott, F., 2004, Paleo-climate reconstruction from stable isotope variations in speleothems: a review: Quaternary Science Reviews, v. 23, p. 901–918.

Mickler, P.J., Banner, J.L., Stern, L., Asmerom, Y., Edwards, R. L., and Ito, E., 2004, Stable isotope variations in modern tropical speleothems: Evaluating applications to paleoenvironmental reconstructions: Geochimica et Cosmochimica Acta, v. 68, p. 4381–4393.

Mitsuguchi, T., Uchida, E., Matsumoto, E., Isdale,P., and Kawana, T., 2001, Variations in Mg/Ca, Na/Ca, and Sr/Ca ratios of coral skeletons with chemical treatments: Implications for carbonate geochemistry: Geochemica et Cosmochimica Acta, v. 65, p. 2865–2875.

Nissenbaum, A., and Schallinger, K., 1974, The distribution of the stable carbon isotope ($^{13}C/^{12}C$) in fractions of soil organic matter: Geoderma, v. 11, pp. 137–145.

Pancost, R. D., Freeman, K. H., Patzkowsky, M. E., Wavrek, D., and Collister, J. W., 1998, Molecular indicators of redox and marine phytoplankton composition in the late Middle Ordovician of Iowa, U.S.A.: Organic Geochemistry, v. 29, p. 1649–1662.

Pancost, R. D., Freeman, K. H., and Patzkowsky, M. E., 1999, Organic-matter source variations and the expression of a Middle Ordovician carbon isotope excursion: Geology, v. 27, p. 1015–1018.

Pratt, L.M., 1984, Influence of paleoenvironmental factors on preservation of organic matter in Middle Cretaceous Greenhorn Formation, Pueblo, Colorado: American Association of Petroleum Geologists Bulletin, v. 68, p. 1146–1159.

Ramseyer, K., Miano, T, Valeria, D., Andres, W., Wagner, T., and Geister, J., 1997, Nature and origin of organic matter in carbonates from speleothems, marine cements and coral skeletons: Organic Geochemistry, v. 26, p. 361– 378.

Shopov, Y., Ford, D., and Schwarcz, H., 1994, Luminescent microbanding in speleothems; high resolution chronology and paleoclimate: Geology, v. 22, p. 407–410.

St-Jean, G., 2003, Automated quantitative and isotopic ($\delta^{13}C$) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyzer: Rapid Communications in Mass Spectrometry, v. 17, p. 419–428.

Weiner, S., Traub, W., and Lowenstam, H.A., 1983, Organic matrix in calcified exoskeletons, *in* P. Westbroek and E.W. de Jong, eds., Biomineralization and Biological Metal Accumulation: Dordrecht, D. Reidel Publishing Company, p.205–224.