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Journal

Radiocarbon, 52(1)

ISSN

0033-8222 1945-5755

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Publication Date

2016-07-18

DOI

10.1017/S0033822200045070

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THE PHYTOLITH ^{14}C PUZZLE: A TALE OF BACKGROUND DETERMINATIONS AND ACCURACY TESTS

Guaciara M Santos[1,2] • Anne Alexandre[3] • Heloisa H G Coe[4] • Paul E Reyerson[5] • John R Southon[1] • Cacilda N De Carvalho[6]

ABSTRACT. Over the past decades, analysis of occluded carbon in phytoliths (opaline silica mineral bodies that form in and between plant cells) has become a workhorse of paleoclimate and archaeological studies. Since different plant types exhibit distinctive phytolith morphologies, their assemblages are used in identifying vegetation histories or food culture adaptations. A few direct radiocarbon AMS measurements of phytoliths have been carried out, but these measurements are difficult due to the low concentrations of phytoliths in some plant species, and the small amount of C per phytolith (<2%). In addition, no phytoliths samples of a known ^{14}C age are available to verify measurement accuracy and precision, and to check sample preparation protocols. Background corrections are also difficult to address due to the lack of suitable material. In this work, we designed a procedure to quantify a suitable blank using SiO_2 powder samples (close to the opal structure, and free of ^{14}C). The full phytolith extraction showed high carbon contamination components: a) ~3 μg of modern C and ~2 μg of dead C. We also performed accuracy tests on large phytolith-occluded carbon samples extracted from soils and harvested plants. The unexpected ^{14}C ages in some of the results triggered further investigations of possible sources of carbon contamination.

INTRODUCTION

A phytolith is a rigid, microscopic (<60–100 μm), highly microporous amorphous silica particle that forms in many living higher plants. Soluble silicon in the soil water is absorbed by the plant roots. As water passes through plant cells during metabolism, opaline silica (phytoliths) is deposited along cell walls or filling the cell lumen and the intercellular spaces (Sangster et al. 2009). The silica deposits account for a few ppm to 15% dry weight (Webb and Longstaffe 2002; Piperno 2006; Blecker et al. 2006). With plant decay, phytoliths (well preserved in oxidizing environments) are either incorporated into soils or exported via regional watersheds and sediments. Through the morphological identification of phytolith types, modern phytolith assemblages from soil tops have been calibrated as a proxy of present vegetation parameters (Bremond et al. 2005a,b, 2008a,b; Albert et al. 2006; Lu et al. 2006). Fossil phytolith assemblages are used in reconstructing either paleovegetation and related climate parameters (e.g. Prasad et al. 2005) or food culture adaptations in the archaeological context (Piperno 2006).

Phytoliths can occlude small amounts of a broad range of other elements (N, Fe, Al, etc.), including carbon. Occluded C, trapped during phytolith formation, represents less than 2% of the phytolith weight (Pironon et al. 2001). Stable carbon isotopic analyses ($\delta^{13}C$) of these carbon macromolecules suggested the presence of lipids and sugars or proteins, assumed to originate from the photosynthesized cell walls or cell lumen of the plant (Wilding et al. 1967; Smith and Anderson 2001; Elbaum et al. 2009). Other carbon isotopic studies have investigated the potential of phytolith-occluded C for providing information on the photosynthetic pathway of the vegetation sources (Kelly et al. 1991; Smith and White 2004; Carter 2009).

Despite the low C content, direct radiocarbon dating of opal phytoliths is immediately attractive because the C occluded in the silica body is fully protected from oxidizing environments. A few

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direct ^{14}C AMS measurements of phytolith carbon have been carried out, in attempts to obtain absolute chronologies (Kelly et al. 1991; Mulholland and Prior 1993; McClaran and Umlauf 2000; Piperno and Stothert 2003; Piperno 2006).

Kelly et al. (1991) were the first to explore the use of carbon isotope studies on phytoliths for paleoenvironmental reconstruction. Although the focus of their paper was on stable isotopic characterization of the C occluded within phytoliths (as a tool to differentiate between phytoliths produced from C_3 and C_4 grasses from temperate regions of North America), 6 of the 9 ^{14}C ages they obtained were chronologically inverted (i.e. [14]C ages were younger at depth and older towards the surface). Kelly et al. clearly recognized the importance of these inversions and devoted much of the latter half of their paper to attempting to explain them. They concluded that phytoliths are subject to extensive reworking or mixing processes that seriously disturb the stratigraphic record.

McClaran and Umlauf (2000) reported ^{14}C results on soil organic matter (SOM) and phytolith-occluded C from desert grassland. Overall ^{14}C results from phytolith carbon were much older than from SOM samples. In addition, the ^{14}C age of the SOM pool from the top 2 cm gave a post-bomb result (i.e. the sample contained excess ^{14}C from thermonuclear weapons testing, carried out during the late 1950s and early 1960s), while phytoliths from the same layer dated back to ~800 yr BP. Although they expected the SOM ^{14}C results to be younger throughout the soil column, due to downward percolation of young humic acids in groundwater, no explanation was provided for the remarkably old age obtained from the topsoil phytolith carbon sample. Furthermore, they had shortened the 30% H_2O_2 bath (designed to remove organic matter) from 4–6 hr to 30 min; therefore, they expected that the phytolith samples might still be contaminated by residual younger ^{14}C organic matter. If that was the case, the "non-contaminated" [14]C ages from their phytolith carbon samples (if properly processed) might have been even older.

Piperno and collaborators, mostly focusing on human exploitation and domestication of plants have reported several results on directly ^{14}C measurements of phytolith-occluded C (Piperno and Pearsall 1993; Zhao and Piperno 2000; Piperno and Stothert 2003; Piperno 2006). In Piperno and Stothert (2003), for example, one of the ^{14}C values was matched with dates obtained on charcoal and shells. The ^{14}C AMS results from the smallest samples were obtained by an AMS method that involves dilution of a relatively small CO_2 sample (<0.250 mg C after combustion) with a known quantity of ^{14}C-dead CO_2 to ensure that final graphite target sizes were in the accepted measuring range (0.5 to 1 mg C). To establish if the microsample-AMS dilution method could work (i.e. to show they could increase the CO_2 sample pool after combustion without affecting the final ^{14}C result), tests were performed using a known ^{14}C-age sample (TIRI-B Belfast pine wood with a consensus age of 4503 ± 6 yr BP). However, no phytolith or similar material was processed and measured to check phytolith extraction protocols.

Carbon isotope results from modern phytolith assemblages (harvested plants or topsoils in paleoclimatic reconstructions), usually involve only stable isotope measurements due to the small amount of CO_2 recovered from each sample (Smith and White 2004; Carter 2009). Consequently, the chronology associated with phytoliths is generally obtained by ^{14}C AMS or independent dating techniques on different materials, or they are simply assumed to be "modern" (when samples are from topsoil or living plants). The only post-bomb ^{14}C dating of phytolith carbon that we can find was published by Piperno and Becker (1996). The phytolith assemblage came from a soil depth of 0–20 cm from a tropical forest floor, and the processed phytolith material (presumably chemically extracted by Piperno and Becker) was combusted and graphitized at University of California, Riverside, and measured at CAMS/Livermore. As far we are aware, this result is the only evidence that "modern" phytolith assemblages are in fact valid indicators of present-day [14]CO_2 atmospheric conditions.

More recent attempts to match ^{14}C date phytoliths with independent chronologies have demonstrated to be particularly difficult (Prior et al. 2005; Rieser et al. 2007). In these studies, dating specialists have tried to match ^{14}C ages of phytolith carbon with optically stimulated luminescence (OSL) dates (also from phytoliths) and tephrochronology information. C A Prior (personal communication, 2005) believed that their difficulties in matching these chronologies are mostly associated with the phytolith sample extraction methods applied for the samples undergoing ^{14}C dating.

Today, ^{14}C AMS can easily measure targets of 0.020 mg C (Santos et al. 2007b), reducing the required amount of organic material to be processed for a measurement. However, the phytolith extraction background or procedural blank is still difficult to quantify due to the lack of a suitable blank material. Since graphite targets produced are in the sub-mg range, sources of C contamination must be addressed carefully (Santos et al. 2007a). Furthermore, no phytoliths of known age are available to verify measurement accuracy and precision, or to check sample preparation protocols. The combination of an appropriate background assessment and a known-age phytolith standard are essential for quantifying realistic uncertainties associated with sample preparation and ^{14}C measurements, leading to a better interpretation of the scientific evidence. Note that none of the previous phytolith ^{14}C studies (mentioned above) have undertaken a full suite of ^{14}C measurements on blanks or phytolith secondary standards to directly evaluate extraction protocols.

With the purpose of quantifying a procedural blank and performing accuracy tests, we measured ^{14}C-free SiO_2 powder (as a first attempt to mimic the opal structure), and conducted multiple measurements of large amounts of phytoliths extracted from soils and harvested plants. The unexpected age and variability of some of the results obtained on this work triggered further evaluation of phytolith extraction procedures and investigations of possible sources of C contamination. These extra studies included ^{14}C measurements of phytoliths extracted from living plants obtained from 3 locations of the bulk plant material itself, of CO_2 from lab air (CEREGE and KCCAMS) and plant sampling site air and of solvents (methanol and ethanol—to determine sources of carbon) and dry ice used in laboratory procedures.

MATERIALS AND METHODS

Phytolith Samples

Two phytolith samples from CEREGE (France) that were available in large amounts, previously chemically treated for environmental calibration (Kandara; Bremond et al. 2005) or used as oxygen isotope laboratory standard due to its homogeneity (MSG70; Crespin et al. 2008), were selected to check reproducibility and accuracy.

The MSG70 sample originates from the Mascareignite level (10–20 cm) of a volcanoclastic soil from La Reunion (France). The morphological study suggests that phytoliths extracted are mostly from bamboo plants (Meunier et al. 1999). Two ^{14}C dates from charcoals that set the boundaries for this level were obtained by NSF-Arizona AMS Laboratory; they are 335 ± 90 ^{14}C yr for charcoals from the upper part (7.5–10 cm) of the mascareignite horizon and 3820 ± 85 ^{14}C yr for charcoals from the lower and main part (10–>20 cm) of the horizon (Ouar 1998).

The Kandara sample (Kandara 0–1–2–4; Bremond et al. 2005a) is a mixture of 4 samples collected along a forest/savanna transect (Cameroon, West Africa) from the top of an hydromorphic soil on the shoreline of a swamp (samples 0 and 1) and a ferralitic soil (samples 2 and 4). The phytoliths are mainly from palms and dicotyledon trees. This sample is assumed to be "modern" (decades or a few hundred years old) as the phytolith assemblage reflects the current vegetation assemblage (Bremond et al. 2005a).

Three phytolith samples were extracted from living grasses for the purpose of this study: a) the Grass1 sample obtained from leaves and stems of grasses harvested in 2007 from the side of a crop field on a calcisol, close to the CEREGE laboratory, Aix-en-Provence, France; b) the BioCore Prairie obtained from leaves and stems of grasses harvested in 2008 from a rural area in Madison, Wisconsin, USA. This grass was growing on about 30 cm of loess terrain, underlain by glacial till; and c) the MN sample, also extracted from leaves and stems of grasses picked in 2008 from a rural area in Minnesota, USA, underlain by till or outwash.

Bulk fractions from the living grasses and several other materials were also selected for this study. The complete sample list and its rationale are shown in Table 1, and will be discussed further below.

Phytolith samples for ^{14}C AMS measurements were subjected to the following steps: a) phytolith extraction followed by surface organic matter removal (details below), and b) combustion and graphitization (AMS target preparation).

Phytolith Extraction

Phytoliths were extracted from dry soil following the summarized protocol adapted from Kelly (1990): (a) ~20 g of soil was slightly crushed and sieved at 2 mm; (b) carbonates were dissolved using HCl (1N); (c) iron oxides were reduced with 88.4 g/L of trisodium citrate ($C_6H_5Na_3O_7$) and 1 g/L of sodium dithionite ($Na_2O_4S_2$) in H_2O; (d) organic matter was oxidized using H_2O_2 (30%) until the reaction subsided; followed by (e) deflocculation in a sodium hexametaphosphate $Na(PO_3)_6$ (5%) solution buffered at pH 7; (f) sieving of the samples at 60 μm; and (g) clay removal by sedimentation or centrifugation. Densimetric separation (h) of phytoliths was carried out using a zinc bromide heavy liquid ($ZnBr_2$) with a density of 2.3; (i) the supernatant (phytoliths) was washed with HCl (1N) and distilled water, using hydrophobic fluoropore filters (PTFE filters) built in a glass filtration unit. Phytoliths were removed from the filters using a flux of distilled water. The PTFE filters (#1.2 to 2 μm) were moistened with a few drops of ethanol before filtration (as recommended by the vendor). The phytoliths retained were then washed with HCl and distilled water; and (j) finally, phytolith samples were transferred to glass vials and dried in an oven at 110 °C for at least 24 hr.

Steps (b) to (e) were performed in polypropylene centrifuging bottles. Between each step, the sample was rinsed with distilled water spiked with a few drops of $CaCl_2$ (to help phytoliths aggregate and sink). The supernatant was discarded after centrifugation.

Phytoliths were extracted from living grasses using the steps described below: (a) leaves and stems were broken down to small pieces and dried at 105 °C for 36 hr after having been immersed in a bath of 10% HCl; (b) organic matter was digested by H_2SO_4 heated in a water bath at 80 °C for periods of 2–6 hr. The procedure was repeated at least 3 times or until all plant material dissolved; (c) samples were immersed in H_2O_2 (30%), stirred, and returned to the water bath at 80 °C. The procedure was repeated until the sample was completely transparent; (d) to assure that samples were totally free of any organic matter, phytolith samples were rinsed (CEREGE) or immersed and heated at 80 °C (UW-Madison) with equal amounts of 8% $HClO_4$ and HNO_3 at least 3–4 times; (d) finally, samples were transferred to glass vials and allowed to dry overnight at 110 °C. Between each step, the centrifuged/diluted/decanted sample was rinsed with distilled water. For this procedure, Pyrex® beakers and glass vials (for storage) were solely used.

After extraction, the purity of all samples extracted at CEREGE was checked by observing aliquots of phytoliths mounted on slides under an optical microscope (X600). For the samples extracted at

Table 1 The sample list and its rationale.

Goal	Sample	Description
Background assessment	SiO$_2$ powder (Aldrich,–325 mesh):	Subjected to phytolith extraction procedure to quantify chemical extraction background.
	Coal (POC#3–Argonne Premium coal) A/B/A pretreated:	Used as blank to determinate the modern C contamination component (MC) for: a) combustion and graphitization background procedures, and b) phytolith extraction procedure (by spiking SiO$_2$ powder).
	OX-I (oxalic acid I):	Used as blank to determinate the dead C contamination component (DC) for: a) combustion and graphitization background procedures, and b) phytolith extraction procedure (by spiking SiO$_2$ powder). In addition of normalization of samples.
Accuracy tests	MSG70:	Phytolith extracted at CEREGE from the Mascareignite level (10–20 cm) of a volcanoclastic soils from La Reunion (France) (Meunier et al. 1999). The phytolith layer accumulated between 335 ± 90 and 3845 ± 85 yr BP(^{14}C from charcoal samples).
	KANDARA:	Phytoliths extracted at CEREGE from the humic horizon of a feralitic soil of a forest/savanna transect from Kandara, Cameroon collected in 1997 (it was thought to be modern, as the phytolith assemblage reflects the current vegetation—Bremond et al. 2005a).
Source of contamination assessment	Grass1 (bulk grass and phytoliths):	Leaves and stems of grasses harvested in 2007 from a crop field close to the CEREGE laboratory, France.
	MN (bulk grass and phytoliths):	Leaves of grasses harvested in 2008 from a rural area in Minnesota, USA. Phytoliths extracted at UW-Madison, USA.
	BioCore Prairie (bulk grass and phytoliths):	Leaves of grasses harvested in 2008 from a nature preserve in Madison, Wisconsin, USA. Phytoliths extracted at UW-Madison, USA.
	CO$_2$ extracted from air samples:	From rural areas (Madison and Minnesota), CEREGE and UCI processing labs.
	Dry ice:	Used at the KCCAMS/UCI prep laboratory.

the UW-Madison (BioCore Prairie and MN, see Tables 1, 2), we deliberately decided to skip the purity evaluation to avoid removing some of the material and consequently reducing the graphite target sizes. Previous phytolith samples extracted at the UW-Madison laboratory, following the procedure described above, were mounted on slides and checked under the microscope and considered clean of surface contamination.

Table 2 ^{14}C concentrations are given as fractions of modern C, conventional ^{14}C age and Δ^{14}C (modern results only), following the conventions of Stuiver and Polach (1977). These results were normalized to independent sets of 6 normalizing OX-I standards of ~1 mg C, and fractionated corrected using AMS δ^{13}C values. Mass balance blank subtractions have been applied to unknown samples using the quantified blank data presented in Figure 1 and formulae presented in Santos et al. (2007a). Most of the uncertainties are dominated by the scatter in the blank data.[a]

Background assessment

UCIAMS #		Sample (mg)	Spike material	Size (mg)	Graphite (mg C)	Sample yield %	Fraction modern	±	^{14}C age (BP)	±	Δ^{14}C (‰)	±
Background tests on previously combusted phytoliths												
N/A	Kandara; previously combusted @900 °C[1,2]	70	None	N/A	0.001	0.002	—	—	—	—	—	—
37843	Kandara; previously combusted @900 °C[1,2]	73	coal[3,4]	1.12	1.03	92.4	0.0023	0.0001	48,750	210	—	—
37844	MSG70; previously combusted @900 °C[1,2]	182.5	coal[3,4]	1.08	0.926	85.7	0.0016	0.0000	51,880	200	—	—
SiO$_2$ powder series												
37847	SiO$_2$[1,4]	240.0	None	N/A	0.001	0.0012	0.2883	0.0758	9990	2120	—	—
37848	SiO$_2$[1,4]	206.0	None	N/A	0.001	0.0013	0.4959	0.0649	5630	1060	—	—
48560	SiO$_2$ exposed to air (KCCAMS/UCI prep lab)[1,4]	91.5	None	N/A	0.0023	0.006	0.4392	0.0117	6610	220	—	—
48561	SiO$_2$ (phytolith extraction–CEREGE)[1,4]	109.7	None	N/A	0.0072	0.007	0.4634	0.0052	6180	90	—	—
48562	SiO$_2$ (phytolith extraction–CEREGE)[1,4]	117.0	None	N/A	0.0066	0.006	0.4462	0.0062	6480	120	—	—
48622	SiO$_2$ (phytolith extraction–CEREGE)[1]	112.5	coal[3,4]	0.39	0.289	79.0	0.0128	0.0003	35,000	170	—	—
48628	SiO$_2$ (KCCAMS/UCI prep lab)[1]	96.9	OX-I[3]	1.05	0.189	19.4	1.0405	0.0054	Modern	—	33.5	5.3
48629	SiO$_2$ (KCCAMS/UCI prep lab)[1]	117.9	OX-I[3]	0.22	0.044	21.0	1.0534	0.0262	Modern	—	45.6	24.9
48630	SiO$_2$ (phytolith extraction–CEREGE)[1]	109.6	OX-I[3]	0.66	0.125	19.6	1.0361	0.0078	Modern	—	29.1	7.8
48631	SiO$_2$ (phytolith extraction–CEREGE)[1]	118.6	OX-I[3]	0.60	0.118	20.9	1.0296	0.0083	Modern	—	22.7	8.3

Accuracy tests

UCIAMS #		Sample (mg)	Spike material	Size (mg)	Graphite (mg C)	Sample yield %	Fraction modern	±[7]	^{14}C age (BP)	±[7]	^{14}C age av.	^{14}C age st. dev.
MSG70 series												
35006	MSG70[5]	192.6	None	N/A	0.17	0.09	0.7310	0.0040	2515	45	2707	174
36291	MSG70[1]	303.0	None	N/A	0.28	0.10	0.7169	0.0023	2675	30		
38693	MSG70[1]	112.3	None	N/A	0.1	0.09	0.7337	0.0078	2490	90		
45441	MSG70 [1]	370.5	None	N/A	0.36	0.10	0.7032	0.0026	2830	30		
45442	MSG70[1]	182.0	None	N/A	0.17	0.10	0.7020	0.0050	2840	60		
45443	MSG70[1]	88.9	None	N/A	0.083	0.10	0.6976	0.0106	2890	130		
45444	MSG70; OM removed [1,6]	196.9	None	N/A	0.15	0.08	0.7185	0.0056	2660	70	2563	85
45445	MSG70; OM removed[1,6]	90.7	None	N/A	0.073	0.08	0.7330	0.0123	2500	140		
45446	MSG70; OM removed[1,6]	134.9	None	N/A	0.11	0.09	0.7299	0.0079	2530	90		
Kandara series												
35007	Kandara [5]	194.0	None	N/A	0.56	0.90	0.9478	0.0016	430	15	433	20
35008	Remaining CO$_2$ from UCIAMS35007 combustion	N/A	None	N/A	1.11	N/A	0.9498	0.0014	415	15		
36290	Kandara[1]	104.0	None	N/A	0.89	0.90	0.9448	0.0012	455	15		
38692	Kandara; OM removed; baked @500 °C (3 hr)[1,6,8]	96.9	None	N/A	0.15	0.16	0.7896	0.0050	1900	60	2163	359

Table 2 *(Continued)*[a]

UCIAMS #		Sample (mg)	Spike material	Size (mg)	Graphite (mg C)	Sample yield %	Fraction modern	±[7]	¹⁴C age (BP)	±[7]	¹⁴C age av.	¹⁴C age st. dev.
38783	Kandara; OM removed; baked @500 °C (3 hr)[1,6,8]	10.7	None	N/A	0.02	0.18	0.7483	0.0295	2330	320		
39042	Kandara; OM removed[1,6]	90.3	None	N/A	0.35	0.40	0.8052	0.0023	1740	25		
45438	Kandara; OM removed[1,6]	47.0	None	N/A	0.19	0.41	0.7743	0.0045	2055	50		
45439	Kandara; OM removed[1,6]	15.1	None	N/A	0.060	0.42	0.7611	0.0151	2190	160		
45440	Kandara; HCl rinsed[1,9]	16.5	None	N/A	0.088	0.55	0.7095	0.0100	2760	120		

Assessment of sources of contamination

UCIAMS #		Sample (mg)	Spike material	Size (mg)	Graphite (mg C)	Sample yield %	Fraction modern	±	¹⁴C age (BP)	±	$\Delta^{14}C$ (‰)	±
Solvent tests												
39906	SiO_2 + ethanol (CEREGE); not heated	97.1	ethanol/coal[3,4]	0.89	0.77	90	0.0207	0.0002	31,160	80	—	—
39907	SiO_2 + ethanol (CEREGE); not heated	105.8	ethanol/coal[3,4]	0.87	0.66	82	0.0241	0.0002	29,920	80	—	—
39908	Qtz filter + ethanol (CEREGE)	N/A	ethanol/coal[3,4]	0.97	0.83	89	0.0626	0.0004	22,260	60	—	—
39909	Qtz filter + ethanol (CEREGE)	N/A	ethanol/coal[3,4]	0.97	0.91	97	0.0555	0.0003	23,230	45	—	—
39672	MSG70 + few drops of methanol[1,7]	110.8	methanol	N/A	0.11	0.10	0.6381	0.0113	3610	150	—	—
45447	MSG70; OM removed + few drops of methanol[1,7]	94.7	methanol	N/A	0.075	0.08	0.717	0.0119	2670	140	—	—
CO_2 from laboratory air and dry ice												
47023; 24	Average of 2 targets: KCCAMS/UCI prep lab air	N/A	None	N/A	0.43	N/A	0.9817	0.0026	150	21	—	—
47513; 14	Average of 2 targets: CEREGE lab air	N/A	None	N/A	0.54	N/A	1.0419	0.0035	Modern	—	34.6	3.5
47174	CO_2 from shaved dry ice; KCCAMS/UCI prep lab[4]	N/A	None	N/A	1.03	N/A	0.0012	0.0000	53,880	240	—	—
Harvested plants (phytoliths, bulk plant material, and atmospheric CO_2)												
45448	Grass1 (phytolith); OM removed[1,7]	119	None	N/A	0.21	0.19	0.7790	0.0041	2005	45	—	—
45449	Grass1 (phytolith); OM removed[1,7]	54.8	None	N/A	0.052	0.10	0.7505	0.0178	2310	200	—	—
45450	Grass1 (phytolith); OM removed[1,7]	18.5	None	N/A	0.019	0.11	0.7306	0.0620	2520	690	—	—
47168, 69	Average of 2 targets: bulk grass–Grass1	~2.0 ea.	None	N/A	0.92	59.5	1.0490	0.0020	Modern	—	41.6	2
49703	Biocore prairie (UW-Madison phytolith extraction)[1,7]	80	None	N/A	0.047	0.13	0.3677	0.0254	8040	560	—	—
48633; 34	Average of 2 targets: bulk grass–Biocore prairie	~4.4 ea.	None	N/A	0.90	48.7	1.0546	0.0050	Modern	—	47.5	4.9
49543	Atmospheric CO_2–Biocore prairie	N/A	None	N/A	0.33	N/A	1.0517	0.0024	Modern	—	44.4	2.4
49704	MN (UW-Madison phytolith extraction)[1,7]	190	None	N/A	0.11	0.09	0.5370	0.0090	5000	140	—	—
49707, 08	Average of 2 targets: bulk grass–MN	~2.0 ea.	None	N/A	0.87	53.2	1.0605	0.0011	Modern	—	53.1	1.1
49544	Atmospheric CO_2–MN	N/A	None	N/A	0.33	N/A	1.0498	0.0021	Modern	—	42.4	2.1

[a]Legend: [1]Sample preloaded in combustion tube was baked at 160 °C overnight, before evacuation and combustion; [2]Melted phytoliths were recovered and recombusted to verify combustion efficiency and background; [3]Percentage yield based on weight of coal or OX-I added to sample; [4]Background samples that yielded the same ¹⁴C/C result as coal blanks of similar size were not further corrected; [5]Sample preloaded in combustion tube was baked at 200 °C for 3 hr inside a muffle furnace, before evacuation and combustion. ¹⁴C result was similar to results from samples baked at 160 °C overnight, showing that the 160 °C bake is effective in removing adsorbed CO_2; [6]Percentage yield calculations show that organic matter (OM) has been removed from the surface of opal phytoliths; [7]Errors calculated based on procedural blank, using SiO_2 to mimic opal phytolith structure; [8]Sample baked at 500 °C for 3 hr inside a muffle furnace, before evacuation and combustion. Percentage yield calculation indicates that some occluded C was lost. ¹⁴C result is similar to results from samples baked at 160 °C overnight; [9]Phytolith sample was HCl rinsed only, followed by pure water washes and drying.

¹⁴C AMS Target Preparation and Measurement

To obtain graphite targets from organic carbon, chemically pretreated material must undergo combustion at 900 °C in an evacuated, sealed 6-mm OD quartz tube (prebaked at 900 °C for 3 hr) in the presence of prebaked cupric oxide (~80 mg) and silver wire (3 mm long × 1 mm thick). After combustion, the CO_2 samples are individually released inside a vacuum line, cryogenically cleaned, and reduced to graphite, using hydrogen at 550 °C over prebaked iron powder (Santos et al. 2004, 2007a,b).

The SiO_2 powder (used for background assessment—see below), and the phytolith samples themselves, were prebaked at 160 °C overnight before evacuation for combustion, and were attached warm to the vacuum line to avoid "boiling" of the powder under vacuum due to the release of large volumes of gasses adsorbed on these fine powders. This procedure helped us reduce the backgrounds from adsorbed CO_2, as well as to minimize samples losses during evacuation, as can be noted by the consistency of the CO_2 yields (Table 2) recovered after combustion.

Atmospheric CO_2 samples were measured to evaluate the ¹⁴C levels of air at plant growth sites and in sample processing labs. CO_2 was extracted from 2-L collection flasks of whole air. The collection flask was attached to an evacuated line to allow the air to flow through it at a rate set by a mass flow controller. Water was separated from the air gas mixture by freezing in a water trap, and CO_2 was cryogenically removed. Once all of the air in the flask had been flowed through the system, the trapped CO_2 was transferred to a Pyrex tube at a flame-off port and sealed.

The dry-ice graphite target (Table 2) was produced by placing a small amount of shaved dry ice into a culture tube, attaching it to the evacuated graphitization line, and subsequently following the usual steps of cryogenically trapping the CO_2.

To evaluate ethanol and methanol C sources and whether phytoliths could potentially sorb them, quartz filters, SiO_2 powder, and phytoliths themselves (used as substrates) were deliberatively spiked with these solvents (details below). Substrates plus solvents were combusted and graphitized as described earlier.

The ¹⁴C dating of all samples was performed at the Keck-CCAMS Facility, USA, on graphite targets, pressed into aluminum target holders, mounted into the ion-source wheel and measured in the AMS (NEC 0.5MV 1.5SDH-2) compact system (Southon et al. 2004).

The uncertainties shown in Table 2 were calculated based on counting statistics and scatter in multiple measurements on each sample, along with propagated uncertainties from normalization to standards (OX-I) and secondary standards of the IAEA series from the ¹⁴C intercomparison exercises (Le Clercq et al. 1998), background subtraction of size-dependent procedural blanks (based on measurements of ¹⁴C-free coal—POC#3 from Argonne Premium Coals, Vorres [1989]—and small OX-I graphite targets; details are described below), and isotopic fractionation corrections provided by the on-line $\delta^{13}C$ AMS values, following instrumental analysis described in Santos et al. (2007a,b).

RESULTS AND DISCUSSION

Background Measurements

Phytoliths are mainly composed of amorphous (noncrystalline) silicon dioxide (SiO_2) with several percent of water (Fraysse et al. 2006). To mimic the phytolith opal structure and to quantify the procedural background, we subjected SiO_2 powder (Aldrich, −325 mesh) to the full phytolith extraction and AMS preparation procedure. We then compared the ¹⁴C results obtained from these targets

(spiked with dead coal or the modern OX-I standard) with targets of pure coal and OX-I, and with baked but otherwise untreated SiO_2 (spiked with coal) to: a) establish the background variability; b) allow comparisons between the phytolith extraction and the AMS sample preparation blank; and c) determine sources of contamination in the phytolith extraction procedure. Coal samples were pretreated by conventional acid/base/acid washes, followed by combustion and graphitization.

Figure 1 shows background results for the nominally ^{14}C-free blanks produced, i.e. pure coals, and SiO_2 powder (phytolith-extracted as well as simply baked) spiked and non-spiked with coal. No statistical differences were observed between SiO_2 powder (non-phytolith extracted, spiked with coal) and pure coal samples, indicating that our ^{14}C AMS target sample preparation blank (combustion, graphitization and graphite target pressing only) is low (<0.3 μg modern C). However, the SiO_2 powder samples subjected to the full phytolith extraction procedure (as described above) showed a higher modern carbon contamination component (MC ~3.0 ± 1.5 μg of modern C; Figure 1). Similarly, an extra correction for ~2.0 ± 1.0 μg of dead carbon (DC) was also required to bring the results for the SiO_2 powder samples spiked with small amounts of OX-I (Table 2) into agreement with the results for larger OX-I aliquots. These corrections are about 10 times higher than the corrections necessary for our ^{14}C AMS target preparation alone (Santos et al. 2007a,b). Mass balance blank subtractions using these results have been applied to the phytolith data shown in Table 2. Uncertainties of 50% were assumed for both the MC and DC components based on the scatter in the blank data, and these uncertainties are reflected in the propagated errors.

We also tried to produce CO_2 using previously combusted phytoliths as a starting sample material (Figure 2 and Table 2). However, the amount of CO_2 produced was too small to be graphitized and measured, showing that the occluded C was efficiently extracted during the first combustion.

Precision and Accuracy Tests on Soil Phytoliths

Initial ^{14}C ages of the Kandara series (433 ± 20 yr BP; Table 2) were more reproducible than the results obtained from MSG70 samples (2707 ± 174 yr BP; Table 2). However, the mean ^{14}C age measured for MSG70 was in the range (poorly constrained) of the expected ages (335 ± 90 ^{14}C and 3820 ± 85 ^{14}C), while ^{14}C ages of the Kandara sample were slightly older than expected.

Kandara and MSG70 phytolith samples were then returned to CEREGE for further cleaning, since we believed that the older ^{14}C results were associated with an older organic matter (OM) fraction attached to the sample surface due to some incomplete chemical oxidation. To remove OM from phytolith samples, they were rinsed again with equal amounts of 8% $HClO_4$ and HNO_3 (step d, as described above). Indeed, the extra OM oxidation removed, respectively, 20% and 55% of the total C contents of the MSG70 and Kandara samples (percentage yield calculation based on the amount of CO_2 recovered after combustion; Table 2).

The average ^{14}C age of the MSG70 sample did not change significantly after the post-OM cleaning, yielding an average age of 2563 ± 85 yr BP (Table 2). However, the results from Kandara phytoliths became unexpectedly older after this extra OM cleaning, and were statistically unchanged when 2 of the samples processed was prebaked at 500 °C rather than 160 °C before evacuation and sealing for combustion at 900 °C (2043 ± 233 yr BP; Table 2). Percentage yield calculations indicate that some occluded C was lost at this high prebake temperature (500 °C) due to leakage from the melted silica body. An even older ^{14}C result was obtained when the initial Kandara material (before the pre-OM cleaning) was acid washed with HCl.

Although we successfully obtained reproducible ^{14}C results from phytolith carbon from both phytolith samples through multiple ^{14}C AMS measurements after this additional cleaning, the unex-
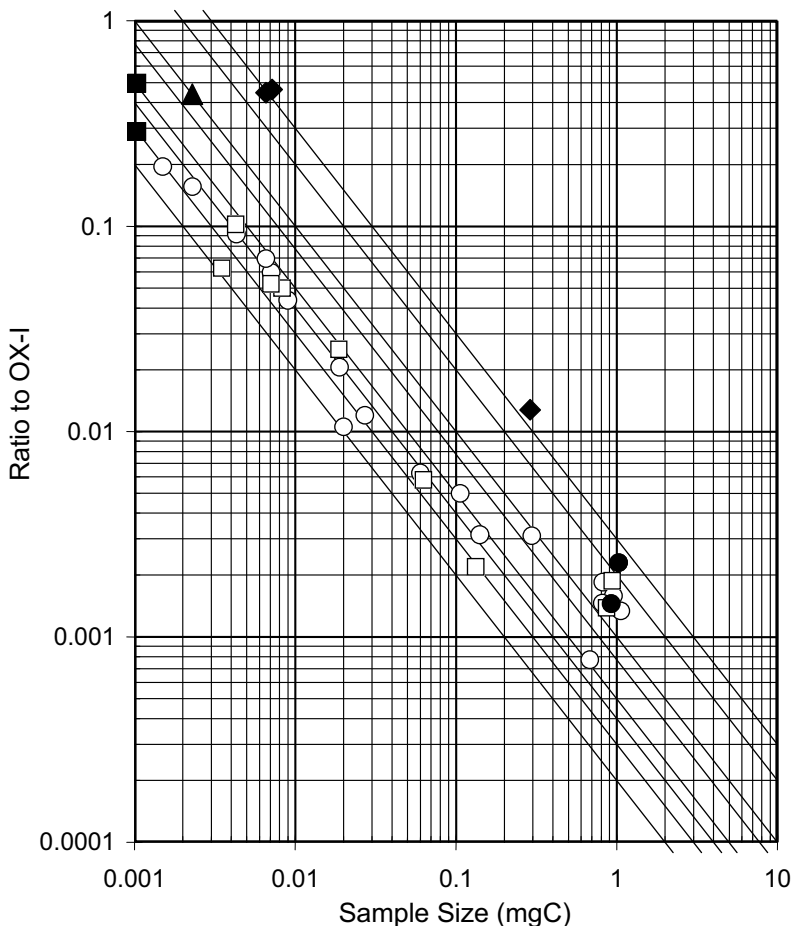
Figure 1 Blank samples from 0.001 to 1 mg C versus ratio to OX-I (modern C standard) are shown for: (■) pure SiO_2 pow-
der subjected to the ^{14}C AMS sample preparation (Table 2; target # UCIAMS37847 and 37848), (▲) pure SiO_2 powder
exposed in the lab area for 7 consecutive days and subjected to the ^{14}C AMS sample preparation (UCIAMS48560); (♦)
SiO_2 powder submitted to phytolith extraction in addition to the ^{14}C AMS sample preparation (UCIAMS48561 and 48562
[nothing added] and 48622 [spiked with coal to increase sample size]); (●) melted phytolith (previously combusted at
900 °C) and spiked with coal (UCIAMS37843 and 37844). The other sets of background samples, i.e. (□) pure coal and
(○) SiO_2 powder spiked with coal and subjected to the ^{14}C AMS sample preparation (non-phytolith extracted), are shown
for comparison and to illustrate how SiO_2 can effectively be used for background corrections when dealing with silica opal
structures (^{14}C results from these sets are not reproduced in Table 2). Note that the relationship of graphitized sample sizes
(in mg C) to ^{14}C activity (in ratio to the modern C standard) is linear (when shown in a log-log plot) and, consequently the
diagonal solid lines represent the effects of fixed amounts of modern carbon contamination from 0.2 to 3 μg C (left to right).

pected ^{14}C age shift of the Kandara material (presumably related to the extra organic matter
removal) triggered further investigations that will be addressed in the next section.

Source of Contamination Assessment

Several lab procedures to extract phytoliths from soil and plant matrices have been published for
numerous applications in archaeology and environmental sciences. In some of these procedures, sol-
vents (ethanol, methanol, or acetone) have been used as a final chemical step to remove bromides,
to accelerate the drying process before storage, or even to store extracted material (Twiss et al. 1969;
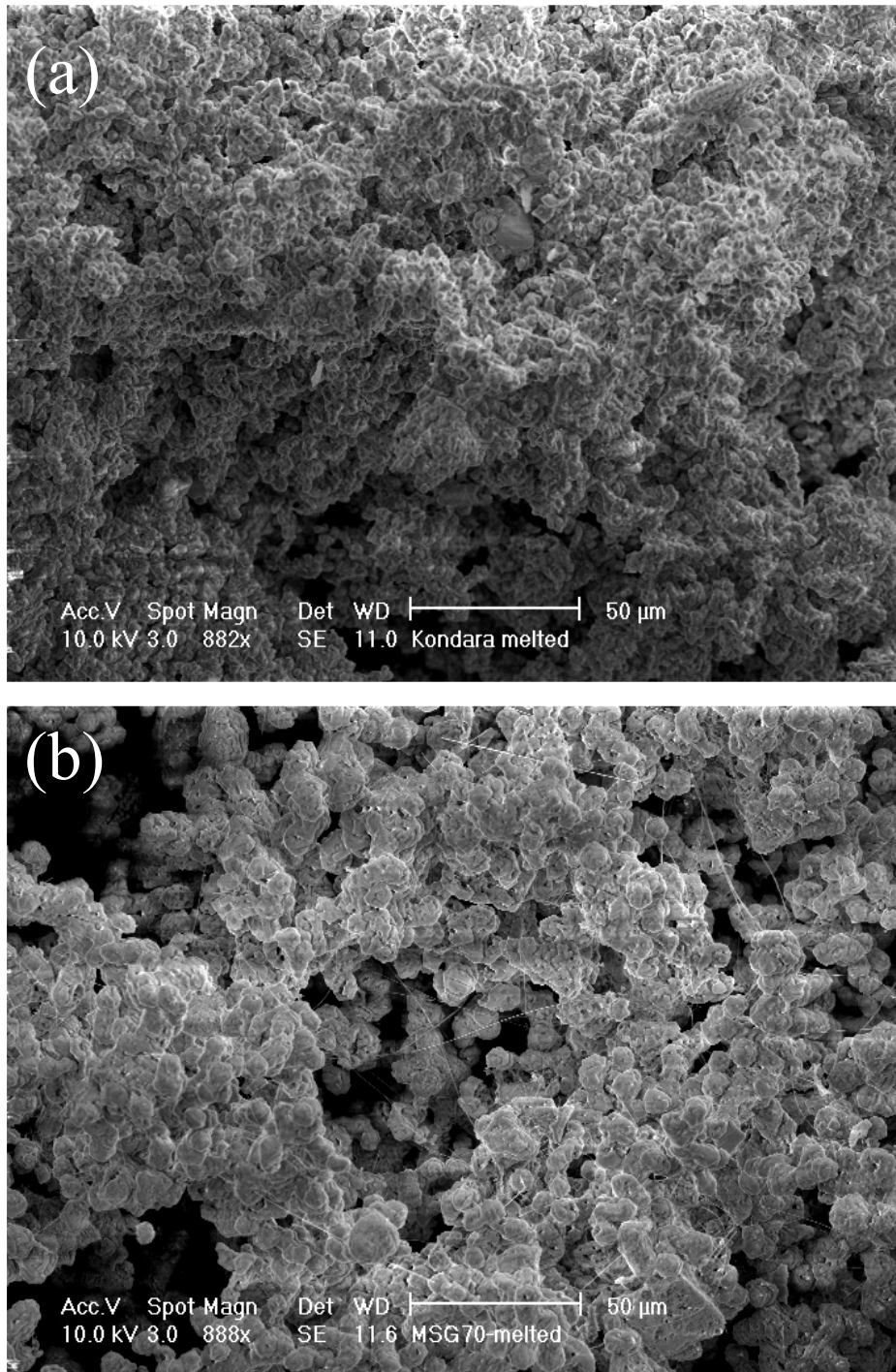
Figure 2 Scanning electron microscopy (SEM) pictures show how phytoliths were effectively melted after combustion at 900 °C: a) Kandara and b) MSG70. Pictures were taken at the Materials Characterization Facility, UCI, using a Schottky thermal field emission FEI/Philips XL-30 SEM with back-scattered electron detector. Scale is 50 μm.

Geis 1973; Carbone 1977; Madella 1996; Madella et al. 1998; Parr 2002; Coil et al. 2003). The use of solvent should not be a problem if the focus of the study is morphology, phytolith production, or morphological classification schemes. However, chemical phytolith extractions for ^{14}C measurements should always avoid any source of extraneous carbon (Piperno 2006, 2009). In a different study, lipid extraction methods for diatom samples (silicate with similar structure to opal phytoliths) were evaluated with and without the use of solvents (Schlechtriem et al. 2003). Their findings suggested that carbon stable isotopic signatures were somehow altered when chloroform was used during extraction, which we speculate may have been due to strong adsorption of the solvent on silica and incomplete subsequent removal.

In our case, a few drops of ethanol from CEREGE were used to moisten the hydrophobic PTFE filters that received MSG70 and Kandara phytoliths densimetrically separated at CEREGE. Although the amount of ethanol used was extremely small, it raised concern since in the past we have seen solvent bonding to surfaces on quartz filters (Santos et al. 2007b). In the USA, ethanol is usually made from corn (i.e. contains contemporary levels of ^{14}C), whereas methanol and acetone are usually ^{14}C-free. Since the C source from the CEREGE ethanol was unknown, quartz filters and SiO_2 were spiked with large amounts of this solvent and measured. The ^{14}C results compared with results from pure coal samples show that the ethanol was modern (Table 2), i.e. the ^{14}C background from quartz filters and SiO_2 powder exposed to ethanol and then spiked with coal increased 20 and 60 times, respectively, compared with unexposed coal-spiked samples. Therefore, the ethanol used in the CEREGE phytolith filtration was not the reason for the Kandara age increase.

To further demonstrate that solvents can sorb to phytoliths (as expected due to their very large surface area and the strong absorptive properties of clean silica; Hatté et al. 2008) similar investigations were conducted directly on extracted phytolith samples. The ^{14}C age results were also consistent with the source of C in solvent spiking, with ^{14}C-free methanol shifting the ^{14}C results to older values. The MSG70 that initially yielded ^{14}C age of 2707 ± 174 yr BP ($n = 6$) shifted to 3610 ± 150 yr BP, while the MSG70-OM removed shifted from 2563 ± 85 ($n = 3$) to 2670 ± 140 yr BP (Table 2). Note that phytolith samples after being spiked with solvent were subjected to the same AMS sample preparation procedure as previously, i.e. baking at 160 °C before sealing and combustion at 900 °C. The baking may have helped to evaporate some of the solvent residue; however, it was clearly insufficient to remove all of it. Solvent-contaminant contributions can impact the ^{14}C results significantly, and cannot be properly quantified for background subtraction because the amount of sorbed solvent may be highly variable (as shown above). Although we did not obtain stable isotope δ^{13}C measurements from the phytolith carbon contaminated with solvent, caution is advised when using solvents in phytolith extraction procedures even when it is only for stable isotope measurements.

The ^{14}C analyses of air samples (Table 2) showed that the laboratories involved in the sample processing were clean. The KCCAMS/UCI prep lab air is 98% modern (slightly lower than clean 2008 air, probably due to the presence of dry ice used during graphitization procedures), while the CEREGE lab is 104% modern (very close to 2008 air). A longer-term air contamination study was carried out by measuring SiO_2 powder that was exposed to the KCCAMS/UCI prep lab environment for 7 consecutive days (note that as well as atmospheric CO_2, this material would sorb any "old" non-CO_2 gas contaminants that might affect the phytolith samples). A target with 0.0023 mg C was produced from 91.5 mg of SiO_2 powder, following the sample preparation described above (except baking it at 160 °C to avoid removing contaminants). Its ^{14}C value (UCIAMS48560, Table 2) does not indicate that the air in the lab is ^{14}C-depleted enough to affect the ^{14}C results obtained for Kandara samples.

In summary, no sources of old C contaminant that could have been added during the chemical extraction procedure were found.

Accuracy Tests on Harvested Plants Phytoliths

Since no evident sources of old C contaminant were found, we decided to measure ^{14}C in phytolith carbon from harvested plants (which are expected to reflect contemporary atmospheric ^{14}C levels). These samples were collected from rural areas (Table 1) and chemically processed in CEREGE and UW-Madison, using the protocol described above. Air samples from the US localities were also checked to rule out the possibility of any urban CO_2 contamination. Samples from the bulk harvested plants were also processed and measured, showing that they are indeed modern, but the phytolith carbon extracted from these plants gave ages of several thousand years (Table 2). The SiO_2 exposed to the full chemical extraction at CEREGE did pick up significant carbon contamination (~3 μg of modern C and ~2 μg of dead C) from the chemicals or apparatus. However, this is far too little to explain the systematic age offset, and the phytolith results have been corrected for these blanks. The phytolith extraction protocol used on harvested plants did not involve filtration; therefore, no solvent entered into contact with samples. Furthermore, Pyrex beakers and glass vials were solely used. Consequently, polypropylene contamination from centrifuge tube inner surfaces cannot be blamed for the age shift to older values. In addition, since samples were fully processed in different labs, the age offset could not be attributed to possible lab contamination. These ^{14}C AMS age discrepancies thus suggest that the extraction procedure alone allows the preservation of an old C residue on phytolith samples, strongly resistant to oxidation and high temperatures. As mentioned earlier, 2 of the Kandara phytolith samples measured were prebaked at 500 °C in a furnace before they were vacuum sealed for combustion. The C occluded was partially lost by leakage as was demonstrated by the CO_2 yield calculation, but the average ^{14}C result did not change (Table 2).

In ^{14}C dating, a mass-dependent fractionation effect determined from the measurement of $\delta^{13}C$ values of the sample has to be taken into account to correct the final ^{14}C results. Piperno (2009) suggested that age errors of a few years could be introduced if not enough phytolith material is available for both ^{14}C AMS and stable isotope measurements, and estimated rather than measured $\delta^{13}C$ values are used for the correction. However, the age offsets observed here cannot possibly be associated with isotopic fractionation, because: a) the age offset is far too large, and b) our AMS system measures $\delta^{13}C$ on-line, so that we correct for any "machine" isotopic shifts as well as the natural and sample preparation fractionation combined.

SUMMARY AND FUTURE WORK

In this work, we demonstrated that it is possible to obtain reproducible ^{14}C results from phytolith carbon by multiple ^{14}C AMS measurements from graphite targets produced from large pools of phytoliths. The results obtained show <1% precision. The CO_2 yield from phytolith combustion was also very consistent when samples were prebaked at 160 °C overnight before evacuation and attached warm to the vacuum line to avoid "boiling" of the powder under vacuum. Tests using recombusted phytoliths show that the combustion yields are close to 100%. In addition, SiO_2 powder was successfully used for background assessment of both AMS target sample preparation and phytolith extraction procedures.

Although the extraction procedure seems robust and the ^{14}C dates on phytolith carbon samples were close to those expected (for MSG70 samples), the age of the only sample thought to be modern (Kandara sample) was unexpectedly old and was apparently biased by an unknown old C source. ^{14}C results from recent harvested plants from 3 locations were also inexplicably old, though bulk

material from the same plants gave contemporary ^{14}C values. The labs involved in the phytolith extraction and AMS sample preparation were checked for potential sources of old carbon contaminants using CO_2 extracted from lab air and SiO_2 powder exposed to the lab environment; none were found. Some areas of extreme concern include: a) how can extraction procedures (without involving solvents) can affect the final results, and b) how can phytolith carbon be contaminated by any old C fractions?

As mentioned earlier, the only post-bomb ^{14}C result on phytolith carbon that we can find was published by Piperno and Becker (1996). No other published material from the last 2 decades is available showing ^{14}C results from phytolith carbon associated with "modern" vegetation that can help us to elucidate the ^{14}C age shifts to older results seen in this work.

In future work, we will evaluate other extraction protocols (Prior et al. 2005; Piperno 2006) as well as the one used in this study (Kelly et al. 1990), utilizing phytolith samples obtained from harvested plants grown within a FACE (Free-Air Carbon Dioxide Enrichment) experiment. In these experiments, controlled plots are exposed to enriched CO_2 concentrations with known ^{14}C and $\delta^{13}C$ values. We plan to extract phytoliths from living plants in both enriched and control plots and apply a coupled isotope signature approach (AMS ^{14}C and stable isotope $\delta^{13}C$) to determine the sources of dead carbon in phytoliths.

ACKNOWLEDGMENTS

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