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Carbon isotope fractionation during low temperature carbonization of foxtail and common millets

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ABSTRACT

Stable carbon isotopes of organic matter and fossilized plant remains can be used to effectively reconstruct local palaeoclimate changes, especially from plants using a single photosynthetic mode. The charred grains of foxtail and common millet are chemically stable in the environment and have been preserved widely and continuously throughout the Holocene in North China. The charred remains of these species are ideal materials for reconstructing the palaeoclimate based on $\delta^{13}C$ of foxtail and common millets heated to temperatures up to around 250 °C. This study reports $\delta^{13}C$ values of modern millets carbonized at different temperatures. The results indicate that there are no significant changes in $\delta^{13}C$ of intact and charred samples of foxtail millet ($\leqslant$0.46‰) and common millet ($\leqslant$0.49‰) for temperatures below 300 °C. The $\delta^{13}C$ of charred foxtail millet formed at 250 °C were 0.2‰ higher in $\delta^{13}C$ than the source samples. In contrast, the $\delta^{13}C$ of charred common millet formed at 250 °C were 0.2‰ lighter in $\delta^{13}C$ than the source samples. The $\delta^{13}C$ values of grains were determined in part by the carbon content (i.e., starches, lignins and lipids) and the variable thermal tolerances of these compounds to heating. However, the observed ^{13}C carbonization associated with fractionation of only 0.2‰ in grains is much less than the natural variation typically found in wood. We therefore suggest that $\delta^{13}C$ measured in carbonized grains can serve as an effective indicator for paleoenvironmental and archaeological reconstructions.

1. Introduction

Stable carbon isotopes are tremendously useful proxies that have been used extensively to reconstruct past climatic and environmental changes (Marino and DeNiro, 1987; Marino and McElroy, 1991; Marino et al., 1992; Beerling, 1996; Williams and Ehleringer, 1996; Schleser et al., 1999). At present, many climate reconstructions based on $\delta^{13}C$ methods of terrigenous archives depend on changes in either C_3/C_4 abundance ratios (Swap et al., 2004; Liu et al., 2005) or the water utilization efficiency of plants (Araus et al., 1997; Ferrio et al., 2003). $\delta^{13}C$ records are usually derived from organic matter in sediments (Koch, 1998) and have only recently been extended to archaeological plant remains such as fossil charcoal (Bechtel et al., 2008). $\delta^{13}C$ studies helped provide an improved understanding of biogeochemical processes of carbon across a range of temporal and spatial scales (Turney et al., 2006), reconstruct high resolution climate history at a regional scale (Ferrio et al., 2006) and reveal the potentially important role of biomass burning to the global carbon sink (Kuhlbusch, 1998; Bird et al., 1999).

Foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*) are major crops in the rain fed agricultural region of the middle Yellow River. They originated around 10,000 BP (Lu et al., 2009) and were widely and continuously cultivated during the Neolithic in North China (An, 1988), where they are systematically preserved in different cultural layers. Although they have similar physical characteristics and ecological habitats and are cultivated in similar areas, foxtail millet and common millet are classified as different genera in botanical taxonomy. Both foxtail and common millets are C_4 grasses, but foxtail millet uses the NADP-malic-enzyme (NADP-ME) type carbon assimilation during photosynthesis while common millet use the NAD-malic-enzyme (NAD-ME) type (Hattersley, 1982) based on differences in bundle sheath permeability and a decarboxylation reaction (Farquhar, 1983; Hattersley and Watson, 1992).

Carbon isotopic fractionation during photosynthesis can be significantly affected by both the diffusion of gaseous CO_2 through plant stomata and the carboxylation reactions that occur within plant cells. In leaves, these different activities result in varying $^{13}C/^{12}C$ signals due to thermal motion and biochemical reactions (O'Leary, 1981; Farquhar et al., 1982; Grice et al., 2008; Zhou et al., 2010). As a result, different species of plants acquire characteristic $\delta^{13}C$ values (Ascough et al., 2008). In other words, carbon isotope ratios of plants are determined by photosynthetic modes

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and physiognomic characteristics (Sternberg and DeNiro, 1983; Gleixner et al., 1998; Zhou et al., 2011), and their environmental conditions during growth (Farquhar et al., 1989). Materials used for $\delta^{13}C$ analyses usually derive from a blend of different plant fragment and charcoals recovered from sediments. Climate histories inferred from these specimens, each with different $\delta^{13}C$ values, is thus subject to species level biases. The most appropriate materials of plant for isotopic analyses are therefore those that can be identified to the species level and unambiguously assigned to a single photosynthetic mode (Marino and DeNiro, 1987). Charred cereal kernels preserved in sediment layers are potentially ideal materials for studies of palaeoclimate, palaeoenvironment and early agricultural activities because they maintain stable chemical properties while buried (DeNiro and Hastorf, 1985) and can be identified to the species level. Thus millet based analysis provides considerably more precise information than charcoal.

To properly use plant remnants to reconstruct past climate, the changes in stable carbon isotope fractionation that occur during the carbonization of plants needs to be understood. Most archaeological plant remains are burn residues. Charcoals are formed by incomplete combustion of plant materials under reducing conditions during wildfires or burning activities (Chaloner, 1989). Flame temperatures of natural fires range from 300–800 °C (Swift et al., 1993). High temperature combustion tends to cause carbon isotope fractionation in charred remains. Nineteen volatile organic compounds (VOCs) produced by Eucalyptus wood have $\delta^{13}C$ changes within ±5‰ of the original $\delta^{13}C$ of the fuel; the $\delta^{13}C$ during the flaming phase of combustion are typically 2.2 ± 1‰ more enriched than those in the smoldering phase for any given compound; increasing fractionation was observed with increasing temperatures for most VOCs (Czapiewski et al., 2002). Another study of three types of charred wood formed at different temperatures indicated that the ^{13}C and carbon content stabilized after 30 min of heating, while the heating duration had no tangible effect on the final composition of charred wood; but all three types of wood ash became progressively depleted in ^{13}C as temperature increased; when the temperatures reached values typical of low intensity natural fires (around 450 °C), the isotopic fractionation was up to 1.3‰. If measured ^{13}C values are used for paleoenvironmental reconstruction without taking into account the fractionation associated with combustion, the reconstructed record could be unreliable (Turney et al., 2006).

Charred, but still identifiable ancient seed fossils were usually formed through low temperature burning. The fossil millet kernel from the cultural layers were formed at about 250 °C by baking rather than by direct burning by fire which was confirmed by field burning experiments (Yang et al., 2011). During carbonization of foxtail and common millet, starch granules in endosperm retain their crystalline structure at temperatures below 200 °C. At 250 °C, the structure of starch granules becomes amorphous. At 300 °C the caryopses partially turn to ash and become porous, whereas the ultrastructure of the granules transforms to alveolate cavities. The heating duration from 0.5–8 h has little influence on ultrastructure characteristic of grains at the same temperature. Therefore, temperature rather than heating duration is the critical driver of the extent to which grains are carbonized. The amorphous ultrastructure of carbonized seeds recovered from archaeological layers is consistent with the expected structure of seeds carbonized at 250 °C.

To use the $\delta^{13}C$ values measured in millet grains for studies of past climatic and environmental changes, the fractionation that occurs during the carbonization process must first be quantified. Here, we report results of experiments where we carbonize modern millets at different temperatures, define and explain their trends from stable carbon isotopes. We further assess the ability of stable carbon isotopes from millets to serve as effective and reliable indicators for palaeoclimate reconstruction. Results from this study allow us to describe the development of early rain fed farming and how this could be affected by environmental changes.

2. Sampling and methods

2.1. Sampling

In 2008, modern samples of foxtail and common millets were collected from four sites on the Loess Plateau and the surrounding areas (35–40°N, 99–110°E, 900–2200 m a.s.l.). All samples were collected from flat, open and non-irrigated land located far from villages. Eighteen groups of foxtail millets and 14 groups of common millets with each group consisting of three millet plants were obtained from living plants.

2.2. Experimental carbonization

The foxtail and common millets were divided into eight groups. Each group was randomly divided into six portions and each portion was composed of 3–5 grains. Five portions of samples were selected from each group. Thus, 40 portions in total were placed into finger shaped bottles to be carbonized. The millet grains were heated in a drying oven to 50 °C and 100 °C for 3 h and to 200 °C, 250 °C and 300 °C for 2 h to obtain grains carbonized under different charring temperatures (Table 1).

A previous experimental study showed that carbonized millet grains were formed at about 250 °C (Yang et al., 2011). To simulate natural oxygenated conditions, 14 portions of foxtail millet and 10 portions of common millet from different sites (each with 3–5 grains) were placed into finger shaped bottles and heated at 250 °C for 2 h in a drying oven.

2.3. Sample treatment and stable carbon isotope analysis

Carbon isotopes were analyzed in millets. One hundred sample portions were divided into three groups: 40 portions of carbonized grains were heated at different temperatures, with eight portions of natural millet in Group A, 24 portions of charred millet formed at 250 °C and 24 portions of natural millet in Group B and the remaining four portions of lemma and palea foxtail millet in Group C.

Each sample portion was placed in a beaker and covered with 1% hydrochloric acid to remove any carbonates. The samples were then washed with distilled water to pH > 5 and oven dried at 40 °C for 24 h. The dried samples were ground in an agate mortar and homogenized, then vacuum sealed in a quartz tube with copper oxide and silver foil and combusted for at least 4 h at 850 °C. The CO_2 gas from the combustion tube was extracted and cryogenically purified. The isotopic ratio of the extracted CO_2 gas was determined using a MAT-251 gas source mass spectrometer with a dual

Table 1
Experimental carbonization of eight groups of foxtail and common millet.

	50 °C (h)	100 °C (h)	200 °C (h)	250 °C (h)	300 °C (h)
Foxtail millet					
Group 1	3	3	2	2	2
Group 2	3	3	2	2	2
Group 3	3	3	2	2	2
Group 4	3	3	2	2	2
Common millet					
Group 1	3	3	2	2	2
Group 2	3	3	2	2	2
Group 3	3	3	2	2	2
Group 4	3	3	2	2	2

inlet system at the Institute of Earth Environment, Chinese Academy of Sciences.

All isotope ratios are expressed in the following δ notation:

$$\delta^{13}C\ (‰) = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{std} - 1] \times 1000$$

The isotopic standard used is Vienna Pee Dee Belemnite (VPDB) and the analytical precision at the 1σ level is reported as 0.2‰.

2.4. Statistical analysis

Analysis of variance (ANOVA) tests were performed on the experimental data to determine the effect of carbonization (fixed factor) and location (random factor) on the $\delta^{13}C$ of millet grains. Unless otherwise stated, differences were considered statistically significant when $p < 0.05$. SPSS 15.0 for Windows was used for the statistical analysis.

3. Results

3.1. $\delta^{13}C$ of bulk grains charred at different temperatures

The average $\delta^{13}C$ from intact and heated millets have less than 1‰ offset (Table 2 and Fig. 1). Mean $\delta^{13}C$ values vary slightly with temperature, becoming enriched in both types of millet at 50 °C and then becoming increasingly depleted up to 200 °C. Then $\delta^{13}C$ is enriched at 200 °C and 250 °C for both types of millet. Carbonized millets at 300 °C yield different trends; the $\delta^{13}C$ of foxtail millet is depleted by 0.46‰ whereas the $\delta^{13}C$ of common millet is enriched by 0.49‰. The $\delta^{13}C$ of lemma and palea is lighter than that of the grains by 1.27‰.

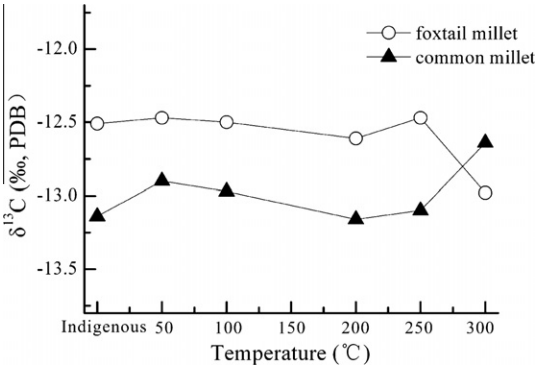

Fig. 1. Mean $\delta^{13}C$ of grain samples heated to different temperatures.

3.2. $\delta^{13}C$ of bulk grains at 250 °C

The $\delta^{13}C$ from 14 samples of foxtail millet and 10 of common millet show minimal isotopic fractionation under conditions that simulate low intensity natural burning (250 °C; Table 3 and

Table 2
$\delta^{13}C$ of foxtail and common millet samples heated at different temperatures.

Temperature (°C)	Heating period (h)	Sample no.	Foxtail millet (‰)			Common millet (‰)		
			$\delta^{13}C$	SD	$\Delta^{13}C$	$\delta^{13}C$	SD	$\Delta^{13}C$
Intact		1	−13.1			−13.4		
		2	−12.3			−12.9		
		3	−12.4			−12.9		
		4	−12.3			−13.4		
		Mean	−12.5	±0.37	0	−13.1	±0.27	0
50	3	1	−13.0			−13.0		
		2	−12.3			−13.1		
		3	−12.2			−12.6		
		4	−12.4			−12.9		
		Mean	−12.5	±0.36	0.04	−12.9	±0.22	0.24
100	3	1	−13.0			−12.8		
		2	−12.3			−13.3		
		3	−12.1			−12.6		
		4	−12.6			−13.2		
		Mean	−12.5	±0.40	0.01	−13.0	±0.32	0.17
200	2	1	−12.9			−12.7		
		2	−12.4			−13.2		
		3	−12.8			−13.4		
		4	−12.2			−13.4		
		Mean	−12.6	±0.32	−0.10	−13.2	±0.29	−0.02
250	2	1	−13.0			−13.4		
		2	−12.5			−13.4		
		3	−12.1			−12.4		
		4	−12.3			−13.2		
		Mean	−12.5	±0.40	0.04	−13.1	±0.50	0.04
300	2	1	−13.2			−12.8		
		2	−12.6			−12.7		
		3	−13.0			−12.2		
		4	−13.1			−12.9		
		Mean	−13.0	±0.25	−0.46	−12.6	±0.29	0.49
Lemma and palea		1	−13.9			n.d.		
		2	−13.4			n.d.		
		3	−13.8			n.d.		
		4	−14.0			n.d.		
		Mean	−13.8	±0.26	−1.27	n.d.	n.d.	n.d.

"n.d." represents values not determined; $\Delta^{13}C$ defines the difference between mean charred and indigenous bulk isotopic values.

Table 3
Bulk $\delta^{13}C$ of indigenous grains heated to 250 °C.

Sample no.	Foxtail millet			Common millet		
	Indigenous (‰)	Charred (‰)	$\Delta^{13}C$ (‰)	Indigenous (‰)	Charred (‰)	$\Delta^{13}C$ (‰)
1	−12.6	−12.8	−0.20	−13.0	−13.1	−0.06
2	−12.6	−12.6	−0.05	−13.5	−13.7	−0.22
3	−12.9	−12.7	0.27	−12.6	−12.9	−0.30
4	−12.2	−11.8	0.38	−12.6	−12.8	−0.18
5	−12.8	−12.3	0.51	−12.5	−12.6	−0.11
6	−12.4	−12.0	0.39	−12.9	−13.4	−0.53
7	−12.3	−11.9	0.40	−12.6	−12.7	−0.06
8	−11.6	−11.3	0.23	−13.4	−13.7	−0.31
9	−12.8	−12.5	0.38	−13.9	−14.2	−0.22
10	−12.1	−12.1	0.07	−13.9	−13.9	−0.06
11	−12.9	−12.8	0.14			
12	−13.0	−12.9	0.13			
13	***−13.01***	***−12.03***	***0.98***			
14	−12.6	−12.3	0.26			
Mean	−12.5 ± 0.40	−12.3 ± 0.45	0.22	−13.1 ± 0.54	−13.3 ± 0.56	−0.21

Values in bold and italics indicate anomalous samples.

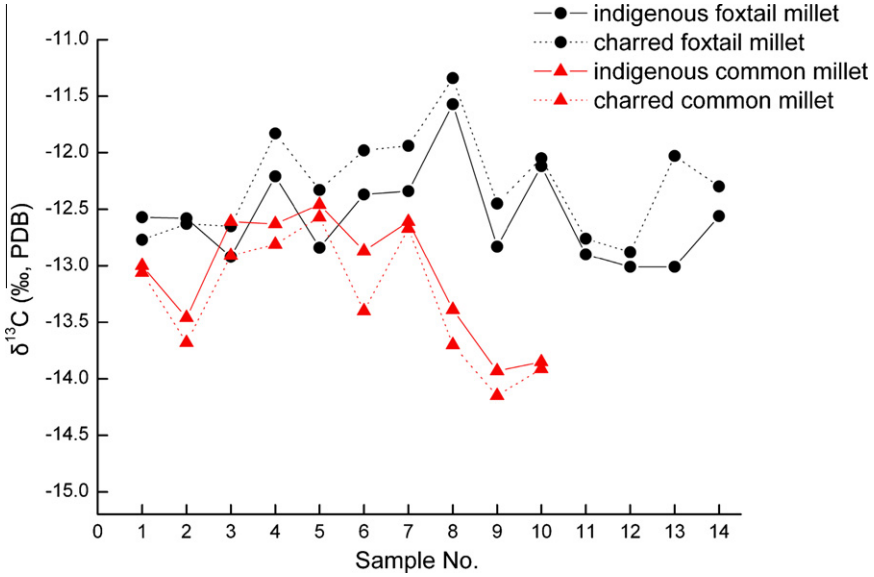

Fig. 2. $\delta^{13}C$ values of foxtail millet and common millet samples intact and charred at 250 °C.

Fig. 2). Again, there are no significant differences between intact and charred millets (Table 3 and Fig. 2). We eliminate foxtail millet sample no. 13 because the $\delta^{13}C$ value (0.98‰) caused by the heterogeneity of the analyte was found to be anomalous using the Boxplot Function in SPSS. With that sample removed, the average $\delta^{13}C$ of foxtail millet is −12.5 ± 0.40‰ for intact grains and −12.3 ± 0.45‰ for charred grains. The $\delta^{13}C$ values vary from −0.20‰ to 0.51‰ with an average of 0.22‰. For common millet, the average $\delta^{13}C$ is −13.1 ± 0.54‰ for intact grains and −13.3 ± 0.56‰ for charred grains. The $\delta^{13}C$ values for these samples vary from −0.53‰ to −0.06‰ with an average of −0.21‰ (Fig. 3).

4. Discussion

The $\delta^{13}C$ values of plants depend primarily on the plants' photosynthetic pathways. Differences in intrinsic leaf processes may lead to differences in ^{13}C fractionation. Specifically, the lack of photosynthetic carbon reduction (PCR) "suberized lamella" in NAD-ME-type species (Hattersley and Browning, 1981) may induce faster leakage of CO_2 and HCO_3^- from PCR tissue. As a result, more of the carbon released during C_4 acid decarboxylation (in PCR cells) will leak back to PAC (mesophyll) tissue in NAD-ME-type plants than in NADP-ME-type plants (Hattersley, 1982; Schulze et al., 1996). The difference will likely be reflected in the plant $\delta^{13}C$ values, and the mean $\delta^{13}C$ is expected to be greater for NADP-ME-type than NAD-ME-type plants. As expected, carbon isotopes measured in plants from the Loess Plateau are 0.6‰ heavier in $\delta^{13}C$ in modern foxtail millet than modern common millet.

These shifts in isotopic composition between species may be associated with biochemical differences in plant organs and tissues (O'Leary, 1988; Farquhar et al., 1989; Hayes, 2001). Starches and sugars tend to have $^{13}C/^{12}C$ ratios similar to that of the carbon initially fixed in photosynthesis, whereas cellulose and hemicellulose tend to be heavier by approximately 1–2‰ and lipids lighter by up to 15‰ (Farquhar et al., 1989; Boutton, 1991; Ehleringer, 1991). The main components of foxtail millet grains are starch, protein and lipid, while the lemma and palea are composed mainly of cellulose. The stable carbon isotopes of lemma and palea were found to be 1.27‰ heavier than those of grains. Because they are always found with kernels but have a different isotopic signature, the lem-

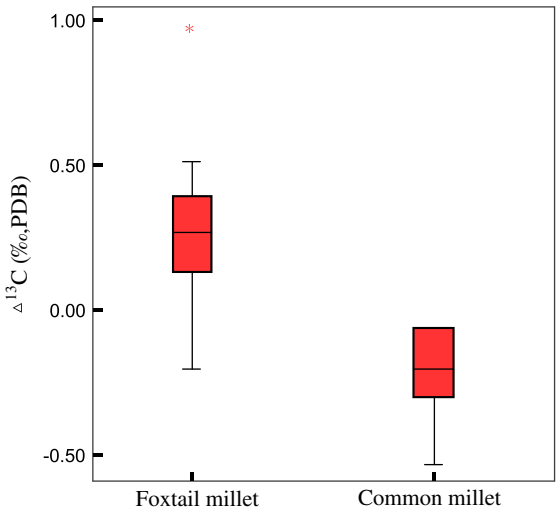

Fig. 3. Boxplot of the $\delta^{13}C$ between intact and charred grain samples at 250 °C.

ma and palea should be removed when carbonized seeds from archaeological layers are used for stable carbon isotope analysis. Analyzing only one plant tissue type, such as grains or cellulose, will help reduce error.

Carbonized cereal grains are usually formed at lower temperatures than charcoals (Marino and DeNiro, 1987; Tieszen and Fagre, 1993). The temperatures at which herbaceous plants become charred are substantially lower; for example millet chars at about 250 °C. Charring temperature is a significant factor that determines both the extent of carbonization and the change in $\delta^{13}C$ that occurs during combustion (Turney et al., 2006; Ascough et al., 2008). The carbon isotopic signatures of heated specimens depend on the variable thermal tolerances of the biochemical components that compose the source material (Turekian et al., 1998). The dynamics of carbonization, including volatilization and oxidation during the initial pyrolytic stage and preferential oxidation of isotopically enriched material, can cause significant isotopic separation (Krull et al., 2003). Stable carbon isotope composition is also altered when polymer chains break due to high temperatures. The $\delta^{13}C$ value of a given source material is a function of the fractional contributions of refractory compounds such as lipids and lignins and more oxidizable starches, proteins, sugars and holocellulose, each with their own $\delta^{13}C$ signals (Nadelhoffer and Fry, 1988).

While the composition of both foxtail and common millet grains is dominated by proteins, lipids, starches, crude fiber and ash (Table 4) (Chai, 1999), the relative proportions of oxidizable ('labile') and refractory carbon compounds differ between the two species (Guo et al., 1998; Liu et al., 2006; Chai, 2009). The signatures of stable carbon isotopes also differ from one carbon compound to another. The $\delta^{13}C$ values of millets therefore depend on both the composition and thermal tolerances of the carbon compounds that compose each species. The morphological characteristics of millet grains (Fig. 4) are visible phenomena directly reflecting changes in the components during carbonization; these characteristics can therefore be used to assess changes in carbon compounds and stable carbon isotope fractionation that occur during carbonization.

Table 4
The main nutritive components in foxtail and common millet (%).

Species	Water	Protein	Lipid	Starch	Crude fiber	Ash
Foxtail millet	11.1	9.7	3.5	72.8	1.6	1.2
Common millet	11.4	12.2	3.8	67.1	1.9	3.8

Table 1 and Fig. 1 indicate that at temperatures below 250 °C there are no significant differences in $\delta^{13}C$ between the intact and charred samples of foxtail and common millet. Millet samples heated to 50 °C and 100 °C retain a strong resistance to charring, similar to that of indigenous grains. The endosperms of these lightly heated starch granules retain their crystalline structure but increase slightly in $\delta^{13}C$. This increase may be due to the mass differences between ^{12}C and ^{13}C. The lighter ^{12}C isotope tends to volatilize from the dissolved CO_2 more easily than the ^{13}C isotope. As the grains are heated, carbon volatilization and evaporation of water in the grains occur concurrently. At temperatures of up to 200 °C, the only morphological change in the starch granules is the appearance of submicroscopic cracks caused by evaporation of water (Fig. 4a and b). At this temperature, a minor decrease in $\delta^{13}C$ could result from changes in the chemical structure of polymolecular carbon compounds due to polymer chain breaking caused by thermal degradation.

At 250 °C, grains become substantially deformed, with bulging shapes and amorphous starch grain structures (Fig. 4c and d). The volatile loss of labile compounds and the structural destruction of polysaccharide may explain the sudden increase in $\delta^{13}C$ that occurs at 250 °C. The amount of lipid remaining in the grains may also play an important role. During heating, we observed lipid oozing from some Group A specimens (especially common millet no. 3), which also exhibited more enriched $\delta^{13}C$ values compared to other charred common millets. We therefore suggest that the change in lipid content affects the $\delta^{13}C$ values of millets. To obtain more reliable results, more data at 250 °C are needed, especially because this is the temperature at which most archeological millet seeds were formed. The $\delta^{13}C$ values obtained from the 14 samples of foxtail millet and the 10 samples of common millet in Group B show that the mean $\delta^{13}C$ values increased by 0.2‰ in foxtail millet but decreased by 0.2‰ in common millet. Foxtail millet contains a small amount of lipids and unsaturated fatty acids that can be oxidized and volatilized at 250 °C, likely causing the observed increase in $\delta^{13}C$. The decreased $\delta^{13}C$ observed in common millet was probably caused by changes in starch composition.

At 300 °C the grains become partially ash and the granules change to alveolate cavities without any observed starches (Fig. 4e and f). Foxtail millet is depleted in $\delta^{13}C$ by 0.46‰ while common millet is enriched in $\delta^{13}C$ by 0.49‰. The $\delta^{13}C$ values represent the average $^{13}C/^{12}C$ ratios of the remaining material in the grains. The opposite $\delta^{13}C$ trends observed in foxtail millet and common millet were driven by the properties of residual material for each species. The extent to which the refractory lipids decompose at 300 °C is the key determinant of the $\delta^{13}C$ value of the remaining kernel material (Turekian et al., 1998).

Vegetable oil is composed of various fatty acid glycerides. The oxidative capacity of the oil is determined by the unsaturated fatty acid (UFA) content. The crude oil content of foxtail millet is about 1.55–4.91% (dry basis). Six essential fatty acids account for 82.0–90.4% of the UFA content, with stearic acid making up 1.3–9.0% and linoleic acid up to 63.3–68.8% (Huo et al., 2006). In contrast, the average fat content of common millet is 3.6%, composed of a variety of fatty acids with stearic acid accounting for up to 12.0% and linoleic acid 5.5–7.8% of the UFA content. Although the total lipid content is similar in foxtail millet and common millet (Chai, 1999), the thermal tolerances and abundances of different UFAs differ greatly between the two species, in turn impacting their antioxidative capacities. Because there is less oxidative decomposition of lipids in foxtail millet than in common millet at 300 °C, more refractory lipids with low $\delta^{13}C$ values remain in the residual material, producing more negative $\delta^{13}C$ values in foxtail millet. In

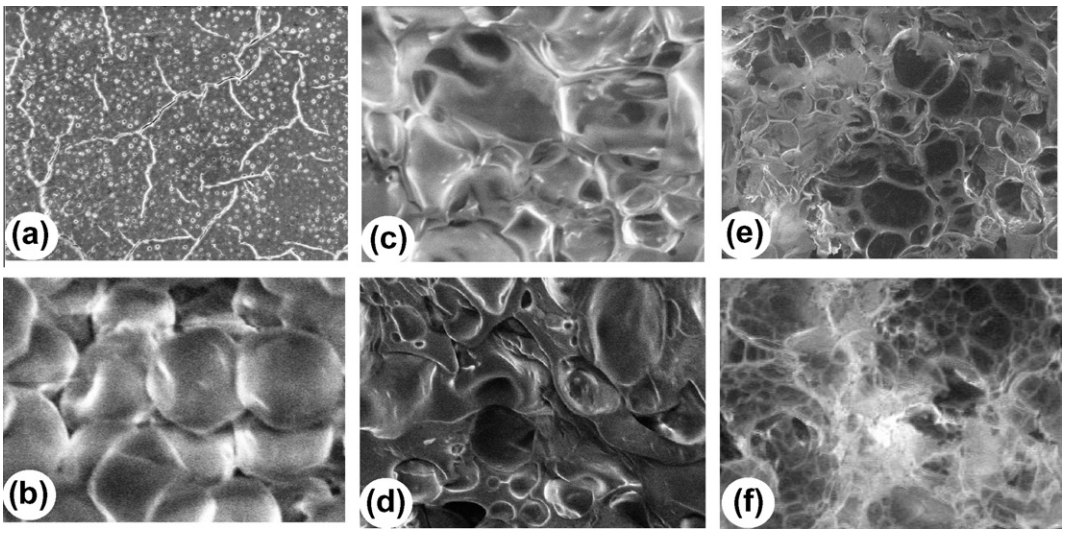

Fig. 4. SEM photographs of cross-sections of foxtail millet (top row) and common millet (bottom row) at different temperatures. (a) Submicroscopic cracking appears at 200 °C; (b) the crystalline conformation of starch grains in embryo at 200 °C; (c and d) the amorphous conformation of starch grains at 250 °C; (e and f) the development of porosity at 300 °C.

contrast, the depleted ^{13}C lipids in common millet decompose and volatilize at 300 °C, resulting in more positive $\delta^{13}C$ values in the remaining material.

The grains of foxtail and common millet contain labile proteins, starches and holocellulose and refractory lipids, each with distinct $\delta^{13}C$ signals. The high temperatures characteristic of carbonization cause polymer chains to break, resulting in changes in the relative abundances of labile and refractory compounds and the associated carbon isotope signatures. At temperatures below 250 °C, the limited variation in $\delta^{13}C$ values and the similarities between foxtail millet and common millet suggest that carbon isotopic fractionation is minimal during low temperature carbonization. At higher temperatures (e.g., 300 °C), changes in the properties of the residual material lead to intensified carbon isotopic fractionation, with $\delta^{13}C$ changes of different sign for foxtail and common millets.

Temperatures of natural fires can range from less than 300 °C to more than 1000 °C (Braithwaite and Estbergs, 1985; Whelan, 1995). Many wood carbonization experiments have demonstrated that isotopic fractionation of up to 1‰ can take place even at temperatures typical of natural fires (400 °C) (Jones and Chaloner, 1991; Turekian et al., 1998; Czimczik et al., 2002; Ferrio et al., 2006; Turney et al., 2006; Ascough et al., 2008). The duration of heating has little influence on $\delta^{13}C$ because ^{13}C composition stabilizes after 30 min of heating (Turney et al., 2006). Foxtail and common millet carbonization experiments conducted at similar temperatures have shown that the duration of heating has essentially no impact on the ultrastructure and components of foxtail and common millet. We therefore suggest that temperature rather than heating duration is the critical driver of changes in $\delta^{13}C$ in carbonized grains.

5. Conclusions

Carbonized grains found in cultural archaeological layers have generally been formed at low temperatures (250 °C) and deposited in strata over long time periods during which they had limited interaction with the buried environment. The observed ^{13}C carbonization fractionation of only 0.2‰ in grains is much less than typically found in wood. The $\delta^{13}C$ signatures conserved in carbonized grains are thus more reflective of true environmental conditions and can serve as effective indicators for paleoenvironmental and archaeological reconstructions without requiring artificial correction.

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