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The influence of heat on phytolith morphology and implications for quantifying archaeological foxtail and common millets

Xin Wang[1,2*] and Xue Shang[3*]

Abstract

Phytoliths are silica bodies formed in living plant tissues, and they can be reliable indicators of their parent plants when they demonstrate characteristic phytolith morphology. As shown by the growing case studies around the globe, phytolith morphology is of significant value for identifying and even quantifying domesticated plants. However, researchers also alert that phytoliths morphology can change, for example, in alkaline solutions or heat, causing the loss of characteristic phytoliths and eventually leading to the over- or under-identification of certain phytoliths. Focusing on the degree to which heat can cause changes to the phytolith morphology of millet, the present study carried out a series of controlled heating experiments on six varieties of modern common millet (*Panicum miliaceum*) growing in different regions of Northern China. Husked grains were heated following the dry ashing method. Specimens prepared from the dry ashing method were examined under an optical microscope to quantify articulated husk phytoliths, which are characteristic millet phytoliths, and to estimate the surface area of millet grains. We estimated that 30.8% to 59.5% of the common millet phytoliths underwent morphological damage in the heating experiments. Considering our previous heating experiments on foxtail millet, we conclude that compared to foxtail millet phytoliths, common millet phytoliths experience morphological damage more quickly when exposed to heat. This observation may explain (at least partially) the contradictory results between macro- and micro-botanical results. It reminds us that common millet can be underrepresented in the micro-botanical evidence (phytoliths). We conclude the paper by discussing the potential archaeological implications of our heating experiments.

Keywords Common millet, Phytolith morphology, Heating experiment, Surface area

Introduction

Foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*) are the most ancient crops domesticated in northern China. They were cultivated and consumed as the staple food since the late Neolithic [1–3]. Macro botanical remains have been discovered at many Neolithic sites in northern China, and the results have shown that common millet was the dominant crop through the Early Neolithic, which gave way to foxtail millet beginning around 6000 BP [4].

However, microfossils such as phytoliths from the same region or site do not always reveal the same pattern as the plant macro remains. Even worse, they

*Correspondence:
Xin Wang
wangxin15@whu.edu.cn
Xue Shang
shangxue@ucas.ac.cn
[1] Department of Archaeology, School of History, Wuhan University, Wuhan 430072, People's Republic of China
[2] Archaeological Institute for Yangtze Civilization (AIYC), Wuhan University, Wuhan 430072, People's Republic of China
[3] Department of Archaeology and Anthropology, University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

could suggest the opposite and contradict the macro plant results. Case studies reporting such contradictory results are widely known in the Yellow River valley. Some of these sites are Lajia (4300–3900 BP) in the Qinghai region of Northwest China [5]; Quanhu, Yangguanzhai, Huxizhuang, Anban, Wangjiazui, Shuigou (6000–2100 BP) in the Guanzhong Basin of north-central China [6]; and Zhuzhai (8500–5000 BP) [7], Xipo, Baligang, and Huizui (6900–5000 BP) in Henan Province of the Central Plains [8]. This inconsistency, some scholars propose, may be explained by the differing preservation of foxtail and common millet grains in post-depositional processes [4]. While this is a well-educated guess, other possibilities should also be evaluated.

In particular, it is important to ask whether, or to what extent, the phytoliths of the two millets differ in their state of preservation under other conditions. It is commonly believed that phytoliths are stable and do not undergo morphological changes in the soil. However, a series of experiments have shown that the morphology of phytoliths of certain plants does change, for example, at high temperatures or in alkaline solutions [9–11]. Therefore, the influence of heat or an alkaline environment on phytolith morphology is an important topic to investigate. Unfortunately, few studies have attempted to understand the phytolith morphological changes under heat; in particular, little has been understood regarding how heat causes morphological changes or damage to the diagnostic husk phytoliths of foxtail millet and common millet. Realizing the two millets demonstrate similar phytolith morphology, our focus here is whether the two millets had the same ability to survive the heat (from the fires) related to, for example, the cooking or the waste burning.

In the present study, we aim to investigate how, or to what degree, phytoliths of the two millet differ in their ability to survive high temperatures and different durations of time. (In this study, we consider 500 ℃ or above as high temperatures because organic matters decay rapidly and some phytoliths may undergo morphological damage.) We designed and conducted a series of controlled experiments to explore this issue. Foxtail millet and common millet were heating at controlled temperatures first; then, their phytoliths were extracted for morphological examinations. We applied a quantitative approach, modified from the point counting method [12], to make estimates of the millet residues based on characteristic phytoliths surviving the heating experiments. We conclude the paper with a tentative argument that when heated common millet can change its phytolith morphology more easily than foxtail millet. We suggest that quantitative analysis should be applied to both macro and micro plant remains and the results of macro and micro plan remains must be compared for consistency.

The dry ashing method for phytolith extraction

Phytoliths are usually better preserved than macro-plant remains because they can survive for long periods of time under normal conditions. Phytoliths are the only material in certain circumstances that researchers could use or study for revealing archaeological and paleoenvironmental information. The term 'phytoliths' most often refers to the silica bodies formed by the deposition of solid silica in living plants [13]. In terms of chemical composition, phytoliths are composed mainly of non-crystalline silicon dioxide and some amount of water. Phytoliths are present in many plants, plant structures and organs (e.g., [14–16]). Most importantly, phytoliths vary in shape and size according to the species of the plant, the types of cells that deposited silica, and the location of these cells [17]. Phytolith morphology can help identify the parent plant from which phytoliths were developed and deposited [13, 17]. Researchers since the 1980s have well recognized the potential of phytolith morphology for decoding archaeological and paleoenvironmental information [14].

Since the 1990s, diagnostic phytoliths of millet and rice have been the focus of archaeobotanical studies in China. The growing interest in the origin and development of dryland and wet rice agriculture in North and South China led to the wide application of phytoliths analysis in archaeological studies. Many scholars interested in the origin and development of early millet agriculture in North China strongly rely on the extraction [18] and accurate identification [8, 12, 19] of the two millets' phytoliths. They believe that identifying and quantifying phytoliths lays the foundation for understanding, for example, how ancient people sustained themselves, why certain plants were domesticated and cultivated earlier than others, and what farming practices were adopted and developed to support a growing population.

Currently, phytoliths are extracted from archaeological remains mainly through dry ashing and wet oxidation [20]. Compared to wet oxidation, dry ashing has the advantage of separating phytoliths from organic matter more efficiently [20]. With a muffle furnace, researchers could process a number of samples at once. During the dry ashing process, organic matter can be removed at relatively high temperatures (for example, 500 ℃) and the ashy material can therefore be used for extracting phytoliths [20].

The articulated forms are significant for the characterization and identification of millet micro remains; thus, larger fragments of articulated phytoliths enable more accurate identification of the millets. (In the text that follows, we use 'millet phytoliths' to refer to the articulated

forms of phytoliths identified in foxtail millet and common millet.) Wang et al. [18] noticed, while comparing the dry ashing and wet oxidation methods for extracting phytoliths from archaeological soils, that articulated phytoliths are diagnostic to the two millets (foxtail and common) and they tend to be preserved better in the dry ashing method than the wet oxidation method. In the dry ashing process, charcoals can be reduced, which significantly improved the observation and identification of millet articulated phytoliths. That said, the dry ashing method is more suitable for extracting phytoliths of foxtail millet and common millet from an archaeological context.

Point counting for quantifying phytoliths

Phytolith morphology not only enables the characterization and identification of their parent plants, they can also allow for quantifying the yield of phytoliths. The fundamental principle is as follows: millet phytoliths in normal conditions exist in the form of fragments of different sizes. The quantity and size of millet phytoliths are in theory proportional to the total number of millet phytoliths or the total surface area of the phytolith's fragments. That is to say, other things being equal, more phytoliths, or a larger total surface area of all phytolith fragments, indicate a larger quantity of millet grains. Indeed, this is a semi-quantitative approach; however, it offers us a chance to compare the relative abundance of the two millets quantitatively.

The idea of making estimates of millet phytoliths above is borrowed from the point counting method, which has been widely used in environmental and geological studies as a standard approach to estimating charcoal's area and then serve as an index of charcoal's concentration [21–23]. The point counting method provides an efficient way to estimate the concentration of microscopic charcoals preserved in sediments but it can also be applied to diatoms and phytoliths [21]. Shang et al. [12] applied the point counting method to semi-quantitatively measure the surface area of articulated millet husk phytoliths, eventually making estimates of the concentration of articulated millet husk phytoliths (we will return to this topic later).

The point counting method, when applied to charcoals and phytoliths, assumes that the ratio of the number of intersecting points to the total number of points is proportional to the area [21]. The method is applied following the procedures below: First, the abundance of phytoliths is recorded by counting the number of points that phytoliths touched the eyepiece micrometer. As shown in Fig. 1, two fragments of common millet phytoliths are noticed in the field of view. The phytolith fragment P1 is off the scale; by contrast, phytolith fragment P2 touch four points of the 11-point scale. For this particular field of view, a total of four counts (3 to 6) are recorded. Second, the same counting procedure continues for different fields of view and eventually 500 different fields of view are counted. Third, a formula (Eq. 1 and Eq. 2) is used to convert the total counts of points for the 500 different fields of view into the area (S_{ph}):

$$\frac{S_{ph}}{S} = \frac{P}{M} \tag{1}$$

$$S_{ph} = S \times \frac{P}{M} \tag{2}$$

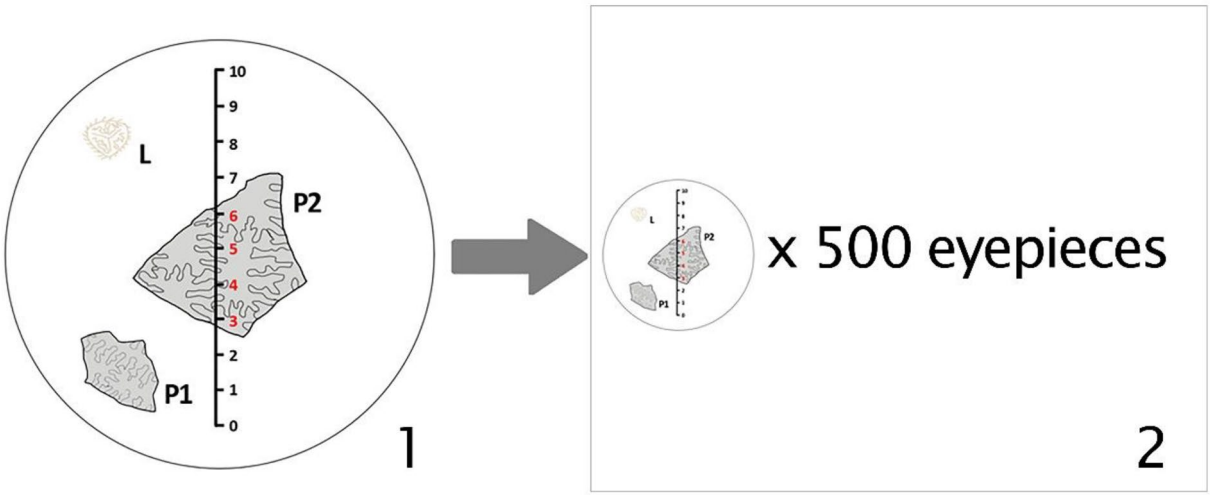

Fig. 1 Diagram of the point counting method used in this paper (P1 and P2 refer to phytolith fragments; L to *Lycopodium* spores). 1, A field of view with a 11-point micrometer and count 4 in this case; and 2, Counting points for a total of 500 different fields of view

where S_{ph} = the total area of phytoliths in 500 different fields of view;

S = the total area of the 500 fields of view;

P = the total counts of points recorded for the 500 different fields of view; and.

M = the total number of scale points (11*500 = 5500).

Even better, one could record the quantity of *Lycopodium* spores while counting the points for the 500 different fields of view, and use Eq. 3 to make estimate of the concentration of phytoliths in the studied sample (in our case, each sample consists of 0.5 g foxtail millet or common millet). Here, *Lycopodium* spores are used as markers because one tablet of *Lycopodium* spores contains a fixed number of grains (in our case, 20,848 grains). When a tablet of *Lycopodium* spores is added to, and fully mixed with the extracted phytoliths (in water), one could use the quantity of *Lycopodium* spores to infer the total area of phytoliths in the 0.5-g sample. Since the total area of phytoliths is proportional to the concentration of phytoliths, one could eventually calculate the concentration of phytoliths in the 0.5-g sample of foxtail millet or common millet. The concentration of phytoliths, S_{con}, is calculated following Eq. 3.

$$S_{con} = S_{ph} \times \frac{L}{(l \times M)} = \frac{S \times P \times L}{(l \times W \times M)} \quad (3)$$

where S_{con} = the concentration of phytoliths in a unit of the sample (cm^2/g);

S_{ph} = the total area of phytoliths in 500 different fields of view;

S = the total area of the 500 fields of view;

P = the total counts of points recorded for the 500 different fields of view;

L = the number (20,848 in our study) of the *Lycopodium* spores; l = the total number of *Lycopodium* spores in the 500 different fields of view;

W = the weight of the studied sample (0.5 g in our study); and.

M = the total number of scale points (11*500 = 5500).

Materials and methods

The present study relies on the dry ashing method and the point counting method for extracting and quantifying the total area of articulated millet husk phytoliths, to eventually make estimates of the concentration of articulated millet husk phytoliths. We have published our heating experiment results and quantitative results on foxtail millet elsewhere [12]; therefore, our primary concern in this study lies in common millet phytoliths. However, we will discuss the quantitative results along with those of foxtail millet phytoliths.

Six sub-varieties of modern common millet (labeled from CM1 to CM6), growing and harvested in four provinces (Gansu, Hebei, Inner Mongolia, and Shanxi) of northern China, are selected for experiments and comparison (sample details shown in Fig. 2 and Table 1). In China, millet is mainly cultivated in northern China, and the Gansu, Hebei, Inner Mongolia, and Shanxi provinces are especially well-known for their millets. We selected millet specimens from different regions to hopefully investigate whether different cultivation regions and conditions may have been related to the loss of phytoliths in the heating experiments.

We sampled 0.5 g of each sub-variety of modern common millet and applied the dry ashing to each sample to extract the phytoliths. The general procedures we adopted in our study can be described as follows: (1) Put husked grains of the six sub-varieties into a Muffle furnace and set it to remain hot at 500 ℃ for 8 h; (2) Removed the ash of husked grains out of the Muffle furnace, sampled the ash, and put it into a centrifugal tube; (3) Added one tablet of *Lycopodium* spores (20,848 grains) to the ash in each centrifugal tube and thoroughly mixed them (in water); (4) The centrifugal tubes were centrifuged in a regular bench top centrifuge at 2500 rpm for 5 min; (5) Discarded the supernatant and leaf the centrifugal tubes overnight to dry. The extraction of phytoliths was done until this moment.

To examine and quantify the articulated millet husk phytoliths, we prepared a proper amount of the ashy sample, mixed it with Canada balsam, and mounted it on a thin slide. The microscopic observation and point counting were done under a Nikon ECLIPSELV100 POL microscope with an 11-point eyepiece micrometer at 500X. Only diagnostic or recognizable phytoliths are counted (Fig. 3a); phytoliths unrecognizable due to the morphological damage or changes are dismissed from quantification. The area of articulated millet husk phytolith were estimated by counting the number of scale points that the phytoliths touched the micrometer. The counting was made in one direction only, and the micrometer was fixed. Each counting was considered valid only when 500 different fields of view were recorded, and the same procedure was repeated three times (T_1, T_2, T_3) for each sample. The area (or concentration) of millet husk phytolith per gram, S_{con}, is calculated following Eq. 3 (S = 1 cm^2, L = 20,848, W = 0.5 g, M = 11*500 in this study).

In addition, to understand the influence of high temperature on the morphology of common millet phytoliths, specimens of CM6, a sample of modern common millet growing in Inner Mongolia of northern China, were randomly selected. They were divided into three subgroups, then heated at 500℃ for 2 h, 4 h, and 6 h, respectively. The examination of phytolith morphology for the three subgroups follows the procedures described

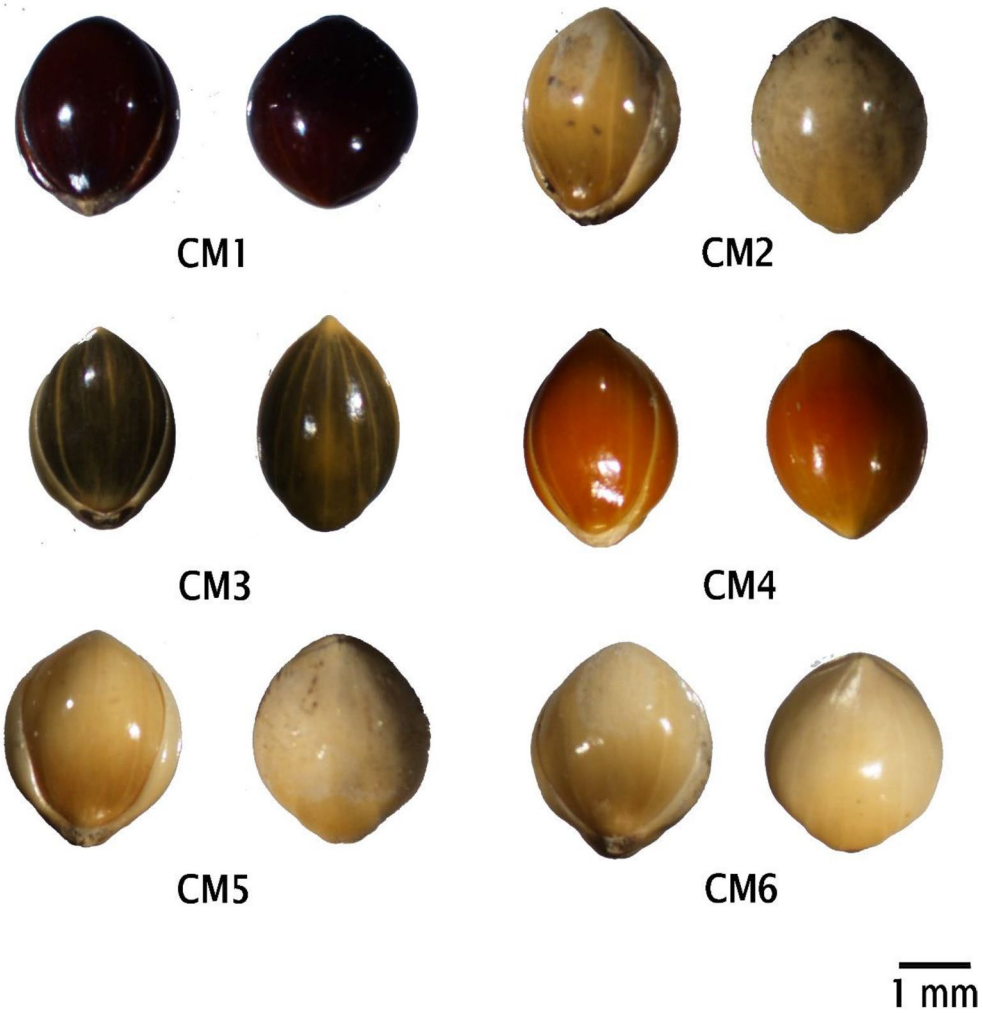

Fig. 2 Specimens of six sub-varieties of modern common millets

for CM1 to CM6, and their results were compared to that of CM6 heated at 500 ℃ for 8 h.

Next, the surface area of the husk is calculated. The millet grain was considered an ellipsoid, and the surface area was calculated using the ellipsoid formula. The length, width, and thickness of 100 grains of common millet were measured for each sample randomly selected.

The surface area of husk per unit mass (cm^2/g), S_{sur}, was calculated following Eq. 4:

$$S_{sur} = \frac{\sum_{i=1}^{100} 4\pi(a_i b_i + b_i c_i + a_i c_i)/3}{W} \quad (4)$$

where S_{sur} = the total husk area in a unit of weight of the sample (cm^2/g); a = the half value of the length of one grain; b = the half value of the width of one grain; c = the half value of the thickness of one grain; and

W = the weight of 100 grains (g).

Results

For each sample (CM1 to CM6), the mean length, width, and thickness were calculated for every 100 grains and so was the weight of the same 100 grains (see Table 2). Also, we calculated the mean area concentration of common millet husk phytoliths for each sample for three times (see Table 3). The mean area concentration of millet husk

Table 1 Information of common millet modern samples

Sample no.	Name of varieties	Producing area
CM1	Xifengyingshu	Gansu province
CM2	Yangyuan	Hebei province
CM3	Qinan	Gansu province
CM4	Jinzhong	Shanxi province
CM5	Xifengnianshu	Gansu province
CM6	Neimenggu	Inner Mongolia

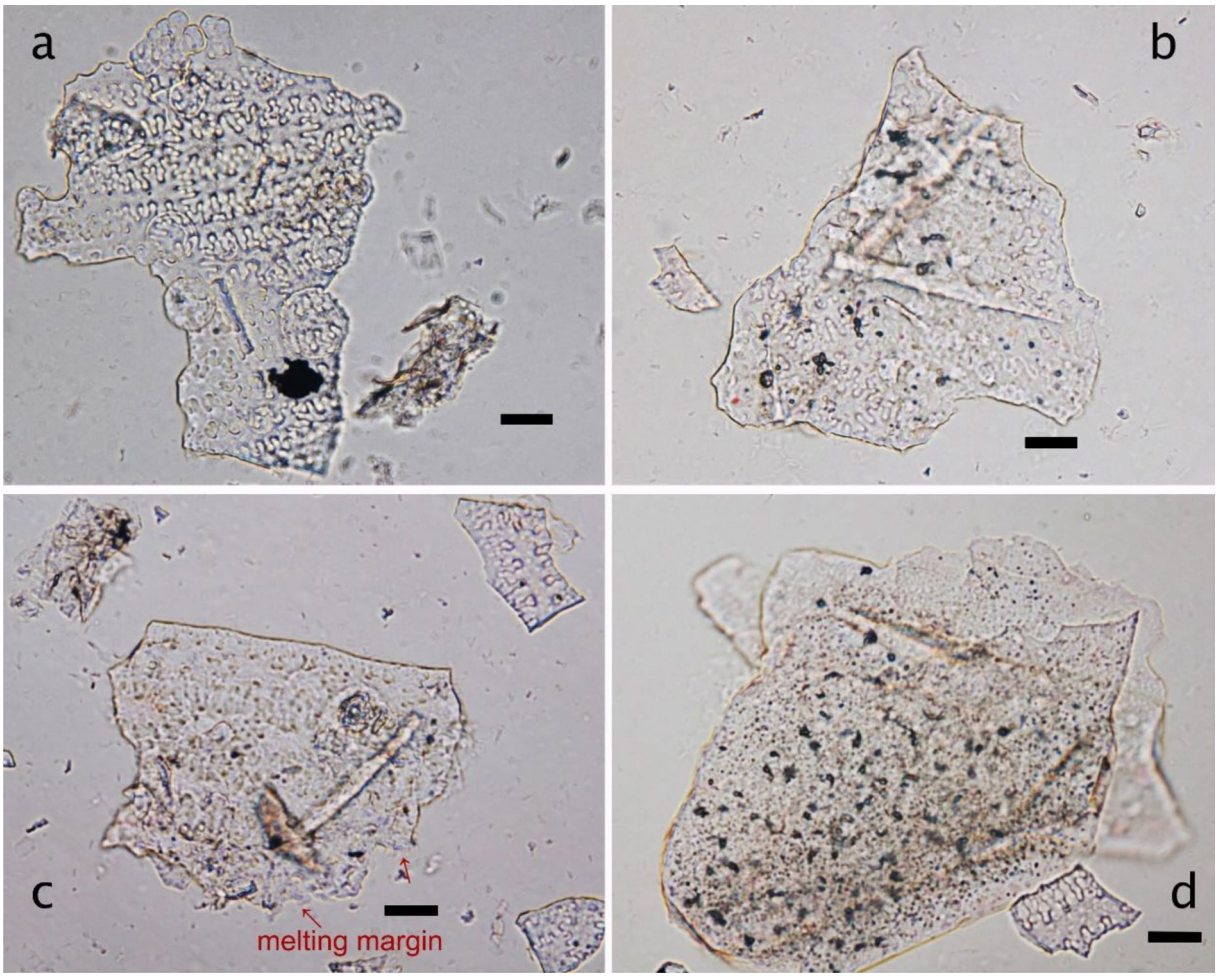

Fig. 3 Changes in phytolith morphology are observed for common millet samples heated at 500 ℃ for different durations of time (a, diagnostic phytolith, η-undulated type from the husk; b-d, phytolith with disappeared ornaments and melting margin). Scale bar = 20 μm

phytoliths (S_{con}) varies between 10.64 ± 0.95 cm^2/g (CM3) and 17.27 ± 1.99 cm^2/g (CM1). On the other hand, the surface area of husk common millet grains (S_{sur}) varies between 24.95 ± 0.97 cm^2/g (CM2) and 26.67 ± 1.52 cm^2/g (CM4). The difference between the estimated mean area concentration of articulated husk phytoliths and the surface area of husked grains is, therefore, calculated for each sample, and the value falls between 7.70 cm^2/g (CM1) and 15.65 cm^2/g (CM3).

Figure 4 and Table 4 show the mean area concentration of millet husk phytolith (S_{con}) and the surface area of husked common millet grains (S_{sur}). For comparison

Table 2 Results of measured grains of common millet

Sample no.	Mean length (mm)	Mean width (mm)	Mean thickness (mm)	Count number	Weight of 100 grains (g)
CM1	1.85	1.56	1.17	100	0.73
CM2	1.54	1.25	0.98	100	0.78
CM3	1.57	1.09	0.83	100	0.63
CM4	1.61	1.18	0.93	100	0.71
CM5	1.58	1.25	0.94	100	0.75
CM6	1.54	1.25	0.97	100	0.73

Table 3 Statistical results of common millet husk phytoliths

Sample no.	T_1	T_2	T_3	Mean area concentration (S_{con}, cm^2/g)
CM1	17.08	19.34	15.38	17.27 ± 1.99
CM2	15.05	15.88	13.20	14.71 ± 1.37
CM3	11.66	10.48	9.78	10.64 ± 0.95
CM4	12.53	13.70	13.08	13.10 ± 0.59
CM5	16.15	13.53	19.06	16.25 ± 2.77
CM6	18.12	11.33	15.05	14.83 ± 3.40

purpose, Fig. 4 and Table 4 also include S_{con} and S_{sur} for husked foxtail millet grains (data reported in Shang et al. [12]). A pattern seems clear that for all six samples, S_{con} is consistently lower than $S_{sur,}$ with an estimated loss of 30.8% to 59.5% of S_{sur}. It has been well understood that the articulated husk phytoliths, which are diagnostic in foxtail millet and common millet, are produced by the epidermal cells covering the entire husk [19]. Ideally, the surface area of husked common millet grains (S_{sur}) should be about the same as that of the articulated husk phytoliths (S_{con}). We had made this argument in our previous experimental studies on foxtail millet [12], and the results supported our assumption by demonstrating small differences between S_{sur} and S_{con} for foxtail millet (with an estimated loss of less than 15% of S_{sur} for four of the six foxtail millets).

However, in the present study, the area of the articulated husk phytoliths of common millet was significantly smaller than the surface area of husked common millet grains. We propose this was due to the loss of common millet phytoliths during the phytolith extraction process. As shown in Table 4, the difference between S_{con} and S_{sur} for the six samples varies from 7.70 cm^2/g (CM1) to 15.65 cm^2/g (CM3), corresponding to the loss of 30.8% to 59.5% of the original surface area of husked grains in the extraction process.

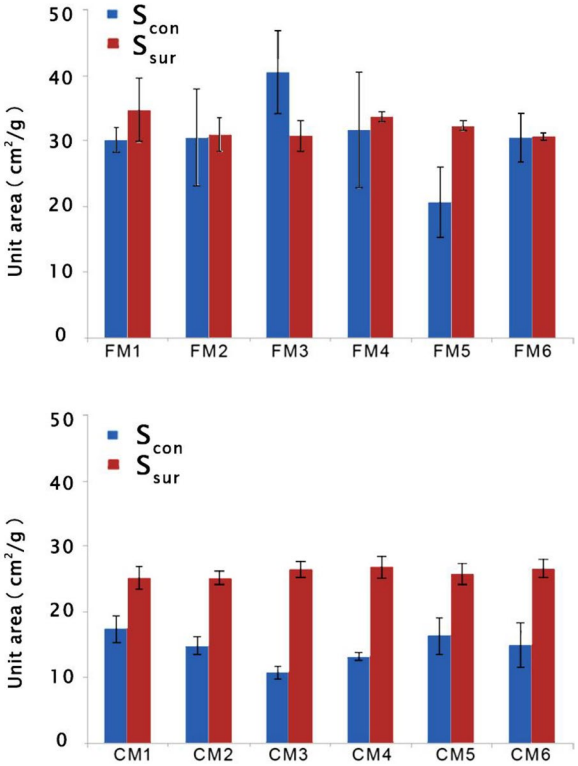

Fig. 4 Comparison between S_{con} and S_{sur} of foxtail millet and common millet (*FM* Foxtail millet, *CM* Common millet) (foxtail millet data reported in Shang et al. [12])

Table 4 Compares measured values of millet husk phytoliths (S_{con}) and the area concentration of millet husk (S_{sur})

Sample no	Mean value of S_{con} (cm^2/g)	Mean value of S_{sur} (cm^2/g)	The absolute value of differences (cm^2/g)	Proportion of differences (%)
CM1	17.27 ± 1.99	24.97 ± 1.77	7.70	30.8
CM2	14.71 ± 1.37	24.95 ± 0.97	10.24	41.0
CM3	10.64 ± 0.95	26.29 ± 1.19	15.65	59.5
CM4	13.10 ± 0.59	26.67 ± 1.52	13.57	50.9
CM5	16.25 ± 2.77	25.62 ± 1.62	9.37	36.6
CM6	14.83 ± 3.40	26.43 ± 1.39	11.60	43.9
FM1	30.18 ± 1.88	34.70 ± 4.90	4.53	13.05
FM2	30.55 ± 7.33	31.02 ± 2.60	0.48	1.54
FM3	40.49 ± 6.33	30.81 ± 2.33	9.68	31.43
FM4	31.70 ± 8.85	33.74 ± 0.80	2.03	6.02
FM5	20.67 ± 5.36	32.29 ± 0.76	11.62	35.99
FM6	30.51 ± 3.72	30.71 ± 0.56	0.20	0.64

CM common millet, *FM* foxtail millet

Specimens of CM6 were selected and heated in controlled experiments, hoping to evaluate the effect of heat on the phytolith morphology of common millet. Table 5 lists the value of S_{con} and the mean of S_{con} for three independent specimens, which were heated at 500℃ for different durations of time (2 h, 4 h, and 6 h). The results were compared to those of the mean value of S_{con} for the specimen of CM6 that was previously heated at 500℃ for 8 h.

As shown in Table 5 and Fig. 5, the area of articulated husk phytoliths (S_{con}) tended to decrease as the duration of heating increased, which fell from 25.47 ± 5.43 cm^2/g (2 h) down to 14.83 ± 3.40 cm^2/g (8 h). It is also interesting to notice that S_{con} (25.47 ± 5.43 cm^2/g, see Table 5) of CM6 heated for 2 h is about the same as the surface area of the husk common millet grains (S_{sur} = 26.43 ± 1.39 cm^2/g, see Table 4), suggesting that heating at a shorter duration of time caused a lesser loss of the area of phytoliths. We infer that when common millet was heated at high temperatures, its husk phytoliths underwent morphological changes or the loss of characteristic features (Fig. 3), eventually leading to a decrease in the identifiable phytoliths and also to a smaller area of husk phytoliths than the surface area of husked grains. Compared to foxtail millet, we suggest the morphology of phytoliths of common millet can be less resistant and less stable to heat at high temperatures (Fig. 4).

Discussion

The influence of heating on phytolith morphology and its archaeological implications

Our experiments show that prolonged heating at 500 °C can cause damage to the phytolith morphology, leading to a decrease in the area (or concentration) of articulated husk phytoliths of common millet. The combination of the heat and the duration of heating, instead of heat alone, seems responsible for the morphological changes or the loss. A good question is how reliable or to what extent can our observations on controlled experiments hold for archaeological millet (maybe common millet in particular)? We must address this issue before we argue about our methodology's applicability and interpretations to archaeological plant materials. As heat is the factor we are most concerned about, we draw our attention to fires and fire-induced impacts.

The evidence of burning has been noticed at many Neolithic sites globally, in the form of, for example, hearths, ash, daubs, charcoals, and charred bones, seeds, and grains [24]. In the reconstruction of fire histories, researchers have revealed that fire at archaeological sites may owe its origin to two agents, anthropogenic or natural [24, 25]. Although the distinction between the two agents is not always clear as perceived and can be hardly identifiable archaeologically, the Neolithic settled way of life left more evidence of the controlled and intentional use of fire by man, including (but not limited to) the fixed position of hearth or fireplace inside or outside the living room, the making, and firing of pottery in bonfire or kilns, the daily food cooking, the hard and burnt floor. Some of the activities above left dense and thick layers of ash or burnt debris at specific loci which, judged by their contents, were created from the long-term occupation and human activities that repeatedly occurred.

The firing of pottery may present the best example of how Neolithic humans could use fire to produce containers and tools with intended shapes, forms, functions, and styles. It is not unwise to consider the firing of pottery vessels as direct evidence of high-fired events. Modern scientific investigations have confirmed that the Neolithic pottery could be fired at temperatures up to 950 °C in the combustion zone of kilns [26, 27]. Even an open fire could secure a firing temperature (namely, the highest temperature that could be attained in the firing) of 600 °C [26, 27]. That said, there was a good chance that the occupants of Neolithic sites had access to, and the ability to manage, fires and high temperatures in their daily routines. If the occupants of Neolithic settlements kept a fire alight in the hearth or

Table 5 Results of S_{con} of CM6 during different heating times

Heating time	T_1	T_2	T_3	Mean values of S_{con} (cm^2/g)
2 h	30.64	19.82	25.94	25.47 ± 5.43
4 h	18.73	20.26	14.92	17.97 ± 2.75
6 h	17.92	25.67	22.58	22.06 ± 3.90
8 h	18.12	11.33	15.05	14.83 ± 3.40

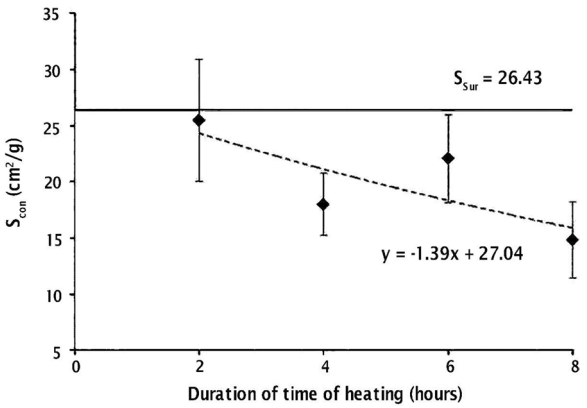

Fig. 5 Scatter diagram of S_{con} of CM6 heated for different durations of time

fireplace on purpose, which is widely noticed among modern ethnic groups, plants (either used as fuels or consumed as food) may be added to the fire. If this were the case, the microfossils, such as phytoliths and starch grains, would have inevitably heated up repeatedly over time.

Fires of a natural origin, such as forest fires and wildland fires, are believed to have a firing temperature of 300 °C or below; wild fires could not attain a firing temperature as high as artificial fires [27]. However, we could not exclude the possibility that natural fires, when they occurred in storage pits of grains and plant materials, may last longer and attain temperatures high enough (500 °C or higher) to cause morphological damage to phytoliths of the plants.

Given the discussions above, we argue that high temperatures (500 to 950 °C) could indeed be available to, and used by, the occupants of many Neolithic sites; and that the observations made on our controlled experiments may hold for archaeological sites.

One of the most interesting observations we have made in our controlled experiments (this study and those reported in Shang et al. [12]) is that the quantity of articulated husk phytoliths of common millet decreased more significantly than that of foxtail millet phytoliths when exposed to high temperatures (500 °C) and for prolonged duration of time. We argue that it has several archaeological implications.

Firstly, it revealed to us that the number or quantity of diagnostic articulated husk phytoliths of common millet and foxtail millet archaeobotanists reported for an archaeological feature (hearths, burnt floor, and storage pits, in particular) may not reflect the actual abundance of the two millets. These archaeological contexts are often reported for heat-induced morphological changes of phytoliths [28–30]. As discussed above, the phytoliths of common millet may not survive as well as those of foxtail millet in fires, other things being equal. Secondly, since the number or quantity of the diagnostic articulated husk phytoliths was sharply decreased for common millet, it may be problematic to use phytolith alone as a reliable index of the relative abundance of common millet versus foxtail millet. This could explain the contradictory results where the macro and micro plant remains do not match well. Thirdly, it alerts us that it may be risky to use phytoliths for the comparative quantification of different species of plants unless the morphological characteristics and changes of plants under different temperatures are comprehensively studied and compared.

In short, the microfossils of archaeological plants should be examined and evaluated carefully. We also point out the necessity of caution in comparing the quantitative data of phytoliths from different species of plants.

We shall also mention that other factors, which may have caused or contributed to the loss or decrease in phytoliths of archaeological common millet, shall be examined and verified case by case. In our experiments, we used six varieties of modern common millet, and our results show that, no matter which regions they grow or to what extent they differ in chemical composition, the same pattern persists: there is a trended decrease in the area (or concentration) of articulated husk phytoliths after the husked grains were heated at high temperatures and over a more extended period of time. This suggests that the loss did not correlate with millets' varieties, provenance, or chemical composition. Of course, whether our conclusion holds for archaeological millets awaits further investigation.

Contradictory results between phytoliths and plant macro remains: A new explanation?

As cooking is widespread across regions, populations, and cultures, the chance to be heated or burnt remains high for both phytoliths and macro plant remains [31]. But, the microfossils and macro plant remains differ in their state of preservation, and often they entered the archaeological record in very different ways [32]. Only under some special conditions (waterlogged and desiccated can macro plant remains be well preserved when they were charred at relatively lower temperatures (usually not exceeding 400 °C) [33, 34]. Phytoliths, by contrast, can enter the archaeological record at any point, with or without any particular treatments [32].

However, archaeologists have noticed that sometimes the two lines of evidence, phytoliths, and macro plant remains, contradict each other [4, 8]. He et al. [4] recently discussed this issue, arguing that depositional and preservation biases are responsible for the contradictory results. They [4] based their arguments partly on modern experiment studies by Märkle et al. [35], who argued that compared to foxtail millet, common millet could be charred only within a relatively narrower range of temperatures; that said, many common millet grains either remained uncharred or were overfired and deformed, therefore more difficult to be preserved in the archaeological record. He et al. [4] also noticed that phytoliths of common millet were often overestimated due to their relatively larger fragments than foxtail millet phytoliths and they went on by proposing that common millet was overestimated in microfossils (phytoliths) but underestimated in macro plant remains. He and colleagues believe that different behaviors of common millet in micro and macro plant remains lead to contradictory results.

While we acknowledge He and colleagues for their acute perception of the different behaviors of common millet in micro and macro plant remains, we propose another explanation based on our controlled heating experiments on common millet. He and colleagues suggest that husk phytoliths of common millet often exist in larger fragments than those of foxtail millet, implying that common millet phytoliths are more likely to survive, and be better preserved, in the depositional or phytolith extraction process [4]. Our heating experiments here showed that husk phytoliths of common millet are less heat-resistant than those of foxtail millet.

Combining the results of charring experiments by Märkle et al. [35], we propose that common millet, especially when exposed to heat at high temperatures and for longer periods of time, is less well-preserved in the form of phytoliths and charred grains. Our results caution that in terms of millets, neither their macro plant remains (husked grains) nor diagnostic articulated husk phytoliths are reliable for quantifying the relative abundance of the two millets. Results from both lines of evidence must be cross-checked and reevaluated case by case. If the results are contradictory, all possible causes must be examined including (but not limited to) heat (or fire histories), depositional process, and contextual use.

Quantification of archaeological millets using phytoliths

Last but not least, we would like to return to the methodology we adopted for quantifying the volume (or to some extent, the surface area) of millet, simply because we found it convenient to apply to millet and easy to produce comparable values.

For those who focus on the origin and development of early millet agriculture, it is interesting to know how much grain may have been yielded to support how many populations. Technically speaking, this is a tough and complicated issue. It is well-known that foxtail and common millet differ in grain sizes (grain lengths of 1.44 to 1.81 mm for foxtail millet and 2.25 to 2.58 mm for common millet, measurements based on modern millet grains [36]). That said, the same quantity of grains of the two millets offers different calories and must have differed in volume and weight. Therefore, the direct comparison of the absolute number of grains of the two millets makes little sense. Rather, the relative abundance (say, percentage) of the two millets more likely discloses the actual consumption of the two millets in human diet and therefore creating more meaningful insights.

Scholars have used two variables, mass and volume, to make estimates of relative abundance of the two millets [6, 37, 38]. We emphasize that volume is more important than mass for the purpose of quantitative analysis. We propose that since the total area of husk phytoliths is proportional to the surface area (or size) of the millet grain, it is theoretically feasible and possible to make estimates of the proportions of the two millets by calculating the ratio of the concentration (S_{con}) of articulated husk phytoliths for the two millets. Introducing the ratio as a new variable, we believe, can more accurately illustrate the proportions of the two millets, which eventually leads to a fuller understanding of the temporal and spatial changes of the dry farming strategy.

We have demonstrated in our experiments that the two millet grains share a similar morphology, being ellipsoidal, and their surface area is, ideally, proportional to volume. The surface area, which can be seen as an index of the volume, is a more reasonable variable to consider if one attempts to make estimates of the relative abundance of the two millets. Taking our experimental studies as an example, according to the surface area of foxtail millet (mean value of $S_{sur} = 32.2$ cm^2/g) and common millet (mean value of $S_{sur} = 25.8$ cm^2/g), the phytoliths of foxtail millet is 1.25 times higher than that of common millet, other things being equal. In other words, we may argue that phytoliths concentration of foxtail millet is 1.25 more than that of common millet. The calculation itself, we must admit, is in relative terms and must be used wisely.

Conclusion

We carried out a series of controlled heating experiments, to measure and compare the diagnostic articulated husk phytoliths, as well as the surface area, of different varieties of modern common millet. We noticed that the area (or concentration) of husk phytoliths was significantly reduced at high temperatures over longer periods of time; that is, the longer common millet was exposed to heat from high temperatures, the more likely the area (or concentration) of their husk phytoliths is reduced. We argued that this morphological change was caused because husk phytoliths of common millet are more easily damaged or lost when exposed to heat than those of foxtail millet. This could have resulted in underestimating the proportion of common millet in the form of articulate husk phytoliths in prehistoric agricultural studies. Our explanation, if supported by more extensive studies, may shed new light on the phytolith-based study of the origin and spread of millet.

Given our discussion above, the contradictory results between macro and micro millet remains previously reported could have resulted from the different preservation conditions. Attention should be paid to this issue in future studies. We suggest that during phytolith extraction using the dry ashing method, a lower temperature and shorter time (e.g., 500 °C, 2 h) should be used whenever possible to secure greater preservation for

husk phytolith of common millet. We also propose that the surface area, which can be seen as an index of the volume, is a better variable to consider if one attempts to make estimates of the relative abundance of the two millets.

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Author contributions
XW and XS designed the research and analyzed the data. XW performed experiments. XW and XS wrote and approved the final version of the paper.

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Availability of data and materials
All data supporting this study's findings are presented in the manuscript. Data and results from controlled heating experiments on foxtail millet have been published in Shang et al., but they can be obtained from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

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