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Stable isotopic investigations of modern and charred foxtail millet and the implications for environmental archaeological reconstruction in the western Chinese Loess Plateau

Cheng-Bang An [a],*, Weimiao Dong [a], Yufeng Chen [a], Hu Li [a], Chao Shi [a], Wei Wang [a], Pingyu Zhang [a], Xueye Zhao [b]

[a] *Key Laboratory of Western China's Environmental Systems (Ministry of Education), Lanzhou University, Lanzhou 730000, China*
[b] *Institute of Archaeology and Relics of Gansu Province, Lanzhou 730000, China*

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ABSTRACT

Stable isotopic analysis of carbon and nitrogen in human and faunal remains has been widely used to reconstruct prehistoric diets and environmental changes. Isotopic analysis of plant remains allows for a more extensive consideration of paleodiets and can potentially provide information about the environment in which the crops were grown. This paper reports the results of $\delta^{13}C$ and $\delta^{15}N$ analyses performed on modern and charred archaeological foxtail millet samples collected from the western part of the Chinese Loess Plateau. The $\delta^{13}C$ mean value of modern samples is lower than that of ancient samples. There is a significant difference between grain and leaf $\delta^{15}N$ values. These results challenge the standard assumption in isotope studies that the nitrogen isotope signals of the different part of plants consumed by humans and animals are the same. The 3–5‰ difference between human and animal $\delta^{15}N$ values is always regarded as an indicator of whether human diets contained considerable animal protein. The difference between grain and leaf $\delta^{15}N$ values makes this assumption problematic in a foxtail millet-dominated society.

Introduction

Stable isotopic analysis of carbon and nitrogen in human and faunal remains has been widely used to reconstruct prehistoric diets and environmental changes in the Chinese Loess Plateau (e.g., Liu et al., 2005; Pechenkina et al., 2005; Barton et al., 2009; Atahan et al., 2011). To date, the majority of isotopic environmental archaeological analyses have focused on the study of bone collagen. Despite its importance as a crop in areas such as China, there have not been any systematic isotopic studies of archaeological charred foxtail millet samples. However, a proper interpretation of collagen isotopic values must be founded on an appreciation of the significance of spatial and temporal differences in the isotopic values of plants (Tieszen, 1991; Heaton, 1999; Warinner et al., 2013). Carbon and nitrogen isotope analysis of plant remains allows for a more nuanced consideration of the contribution of plants to the human diet, and can potentially provide information about the environment in which the crops were grown (e.g., Araus et al., 1999; Bateman et al., 2005; Bogaard et al., 2007; Riehl et al., 2008; Lightfoot and Stevens, 2012; Szpak et al., 2013).

North China is one of the major regions where agriculture developed, with millets being both the major and the earliest domesticated crops (e.g., Lu et al., 2009; Yang et al., 2012; Zhao, 2011, 2014). Millets as an important protein source played an irreplaceable role in the rain-fed, agricultural societies of northern China (An, 1988; Lee et al., 2007; Barton et al., 2009; An et al., 2010). The isotopic signal of this crop must therefore be known with certainty because this forms the basis for estimates of dietary consumption.

In the last decade, we undertook extensive field investigations to study the evolution of agriculture in the western part of the Chinese Loess Plateau (WLP) (An et al., 2005, 2010; Jia et al., 2013). The charred foxtail millet collected from WLP makes their isotopic analysis viable, desirable and essential for a comprehensive understanding of paleodiet and subsistence strategy in this area.

This study aims to test whether there are changes in the carbon and nitrogen isotopic composition of millet in WLP over time, as well as whether different parts of the millet plant have different carbon and nitrogen signals. Its implications for environmental archaeological reconstruction are then discussed.

Study area

Today on the WLP, the mean annual temperature ranges from 6 to 10°C and the mean annual precipitation from 300 and 500 mm. The area is hilly with dispersed and sporadic woodlands. However, the vegetation is generally temperate steppe (Geography Department and the

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* Corresponding author.
E-mail address: cban@lzu.edu.cn (C.-B. An).

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Table 1
Detail of archaeological samples. The age of these sites is determined by cited references.

Site	Culture	Context	Numbers of samples	References
Qin'an 1[a]	Late Yangshao	Pit	3	An et al. (2010)
Qin'an 2[a]	Late Yangshao	Pit	3	An et al. (2010)
Shuzha[a]	Majiayao	Pit	5	Bureau of National Cultural Relics (2010)
Wenjia[b]	Qijia	Pit	1	Bureau of National Cultural Relics (2010)
Wanjiayuan[b]	Qijia	Pit	1	Bureau of National Cultural Relics (2010)
Laohuzui[b]	Qijia	Pit	1	Bureau of National Cultural Relics (2010)
Jiangjiazui[b]	Qijia	Pit	1	Bureau of National Cultural Relics (2010)
Caomaidian[b]	Qijia	Cellar	2	Bureau of National Cultural Relics (2010)
Buziping[b]	Qijia	Pit	1	Jia et al. (2013)

[a] Samples collected in excavation.
[b] Samples collected in our field survey.

Map Press, 1984). Isotopic studies show that C_3 plants have dominated the Holocene vegetation on the WLP (Liu et al., 2005, 2011; Rao et al., 2005). Pollen records revealed that during the past 6 millennia this region went through a humid-warm mid-Holocene, then changed to dry and cold at ca. 3 ka after a few fluctuations. It has not subsequently changed much up until the present (e.g., An et al., 2003; Shen et al., 2005; Cheng et al., 2010; L. Zhao et al., 2010; Y. Zhao et al., 2010).

In northern China, millets include two cereals with the same geographical distribution: *Panicum miliaceum* (broomcorn or common millet) and *Setaria italica* (foxtail millet). Archaeological studies indicate that rain-fed agriculture began in the early-mid Holocene, and foxtail millet agriculture was the backbone of early complex societies on the WLP (Li et al., 2007; An et al., 2010; Jia et al., 2013). The cultural sequence for the WLP is: Dadiwan 1 Culture (8–7.3 ka) → Early Yangshao Culture (6.3–6.0 ka) → Middle Yangshao Culture (5.7–5.5 ka) → Late Yangshao Culture (5.5–5.0 ka) → Majiaoyao Culture (5–4.5 ka) → Qijia Culture (4.3–3.8 ka) (Shui, 2001).

Material and methods

The palaeodietary study is based on the fact that the stable isotopes of carbon and nitrogen are fractionated during many biochemical reactions due to differences in atomic mass. This results in different isotope ratios, depending on the type of ecosystem (e.g., marine versus terrestrial), position in the food chain, and climatic conditions (Ambrose, 1993; Sealy, 2001).

The largest variations in the stable isotope ratios of carbon ($\delta^{13}C$) in terrestrial ecosystems are a result of different photosynthetic carbon reduction pathways in plants (C_3, C_4 or CAM plants). The variation in $\delta^{13}C$ values between each type is significant, with C_3 plants having the lowest values (~−26.5‰) and C_4 plants having the highest values (~−12.5‰), and CAM plants having varied isotopic ratios (van der Merwe, 1982; Marino and McElroy, 1991). Controlled diet experiments (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Froehle, et al., 2010) show that the $\delta^{13}C$ value of bone collagen is enriched by ca. 4 to 5.1‰ over that of the diet.

The stable isotope of nitrogen ($\delta^{15}N$) enters the biosphere from the atmosphere mainly via N-fixing soil bacteria and is then utilized by plants. It is generally believed that in temperate terrestrial ecosystems, the fractionation of $\delta^{15}N$ is dominated by a trophic-level effect. This leads to an enrichment in $\delta^{15}N$ from diet to body tissue of 2–5‰, on average 3‰, for each step in the food chain (DeNiro and Epstein, 1981; DeNiro and Hastorf, 1985; Ambrose, 1991; Hedges and Reynard, 2007). As bone collagen is a protein and therefore consists of amino acids, which are the source of nitrogen when collagen extract is analyzed, measures of bone collagen mainly reflect the isotopic composition of dietary protein intake (Ambrose, 1993). The signal of $\delta^{15}N$ is widely used to distinguish between human diets based on a large amount of animal protein and those based on a mixed subsistence strategy (Schoeninger et al., 1983; Richards et al., 2001; Hedges and Reynard, 2007).

In this study, our collection of environmental archaeological samples followed two principles: firstly, pits, cellar and other archaeological sites selected should have clear stratigraphies; secondly, in the absence of a clear stratigraphic unit, a single cultural layer should be selected for sampling. Following these principles, samples were recovered from

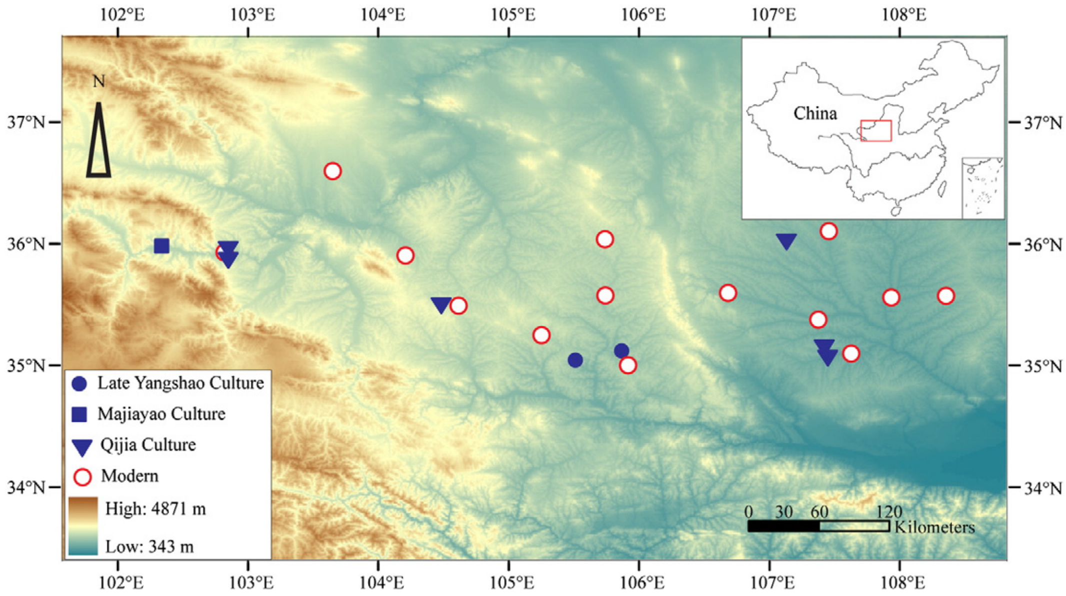

Figure 1. Map showing the study area and sample locations.

Table 2
The results of δ^{13}C and δ^{15}N analyses of modern and charred foxtail millet.

	δ^{13}C				δ^{15}N			
	Mean	Range	SD	n	Mean	Range	SD	n
Late Yangshao (grains)	−10.4	1.5	0.6	6	3.8	7.2	5.1	3
Majiayao (grains)	−10.1	1.2	0.7	3	6.0	1.6	0.8	3
Qijia (grains)	−9.4	1.1	0.4	6	4.7	5.9	2.9	6
Modern (grains)	−12.3	1.9	0.5	14	2.1	6.5	3.7	14
Modern (leaves)	−12.6	1.4	0.5	7	0.0	7.0	2.1	7

the selected units or layers using 10–20 l of sediment per sampling horizon. Details of the archaeological samples are shown in Table 1 and Figure 1.

From each sample, plant seeds were collected by flotation using 0.315-mm aperture sieves to collect the small fraction and 1.25-mm aperture sieves to collect heavy objects. After drying and sorting through 0.315-mm, 0.63-mm and 1.25-mm mesh sieves, all seeds were identified using a 40× stereo microscope. Foxtail millet and common millet were separated on the basis of comparative morphology using a microscope together with measurements of individual grains (Liu and Kong, 2004; Zhao, 2004). Separation was based on the principles that foxtail millet is smaller in length and width than common millet, the length of foxtail millet grains is usually similar to the width, and the embryo shape differs from the common millet (Liu and Kong, 2004). Other small differences were also used to help distinguish the two species (Liu and Kong, 2004), such as the proportion of embryo length in the whole length of the grain and the shape of their husks.

Modern samples were collected in WLP in the fall of 2011 (Fig. 1). All samples were selected from crops grown under uniform field conditions, such as in flat fields with uniform fertility. Neither irrigation nor routine rotation was involved. When the plants were mature and naturally dried, the whole plant was wrapped with aluminum foil. All information, including sampling sites, species (if possible) and environmental conditions, was recorded in detail. The samples were brought back unopened to the laboratory where they were stored in a cold room until analysis. Simulated-carbonization of the modern grains was conducted under 200°C in a muffle furnace for 8 h. Other modern grains are not charred.

The modern samples were first washed in an ultrasonic bath with distilled water, then air-dried, then oven-dried at 70°C for at least 48 h to a constant weight. All samples were ground with a plant sample mill into uniformly fine powder and finally sieved through a 1-mm screen. Lastly, samples were homogenized by mixing and then sealed in tin bags until examination. For the archaeological samples, the method of DeNiro and Hastorf (1985) and Aguilera et al. (2008) was used. Each sample included at least 10 grains. The δ^{13}C and δ^{15}N values of all samples were measured in the Key Laboratory of Western China's Environmental Systems (Ministry of Education), Lanzhou University. The samples were combusted to CO_2 in a Thermo Finnigan Flash EA 1112 and the isotope ratios were measured in a Thermo Finnigan DELTA plus XL isotope ratio mass spectrometer. The amounts of carbon in the combusted sample were determined from the major ion beam voltage. The carbon isotope was measured relative to V-PDB standards. All samples were measured in duplicate. The analytical precision for both carbon and nitrogen isotopes was 0.1‰.

The burning of fossil fuels, which are mainly composed of C_3 plants, beginning with the Industrial Revolution has made the modern atmospheric δ^{13}C value 1.5‰ more negative than in pre-industrial times (van der Merwe, 1989; Marino and McElroy, 1991; Tieszen and Fagre, 1993). This atmospheric variation means that the δ^{13}C values of modern plants are generally 1.5‰ lower than plants before the Industrial Revolution. The δ^{13}C values of modern samples were therefore corrected before comparison with the values of charred archaeological samples.

The δ^{13}C and δ^{15}N value of the Late Yangshao culture human and pig samples were studied in Dadiwan site (Barton et al., 2009); and the δ^{13}C and δ^{15}N value of Qijia culture human and pig were based on the study of Qijiaping site (Ma et al., 2013).

Results and discussion

Comparison between charred and modern samples

The results are given in Table 2. Temperature, latitude, altitude, the distance to the river systems, etc, have no apparent correlation with the spatial variation of modern foxtail millet isotopic values ($r^2 \approx 0$, $p > 0.05$). Combining precipitation values with the δ^{13}C results of modern foxtail millet revealed a correlation between precipitation and isotopic variation to a moderate degree ($r^2 = 0.43$, $p < 0.01$) (Fig. 2a). Generally, higher foxtail millet δ^{13}C values appear in areas with higher precipitation, but this correlation needs further testing because we have limited sample numbers and are in a small area. In the case of δ^{15}N, no such linear relationship with precipitation was detected ($r^2 = 0.05$, $p > 0.05$) (Fig. 2b).

Simulated carbonization of the grains revealed that neither the δ^{13}C nor δ^{15}N value changed much, with $\Delta = 0.2$‰, $r^2 = 0.93$, $p < 0.01$ for the former and $\Delta = -0.02$‰, $r^2 = 0.98$, $p < 0.01$ for the latter. It is well known that the chemical composition of Chinese loess is homogeneous, so the potential diagenetic alteration of archaeological samples is minute, considering that all the parts of the study area experienced similar environment changes during the Holocene (An et al., 2003, 2004; Rao et al., 2005; Shen et al., 2005; Cheng et al., 2010). This finding indicates that no significant isotopic fractionation occurred during cooking or other low-temperature carbonization and makes the direct comparison between modern samples and archaeological ones reasonable.

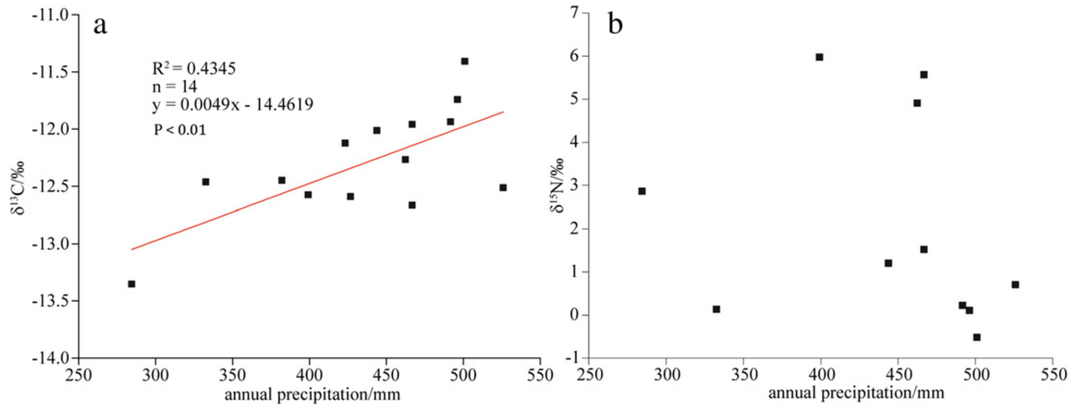

Figure 2. The relationship between modern foxtail millet and precipitation in WLP. (a) δ^{13}C value versus annual precipitation; (b) δ^{15}N value versus annual precipitation.

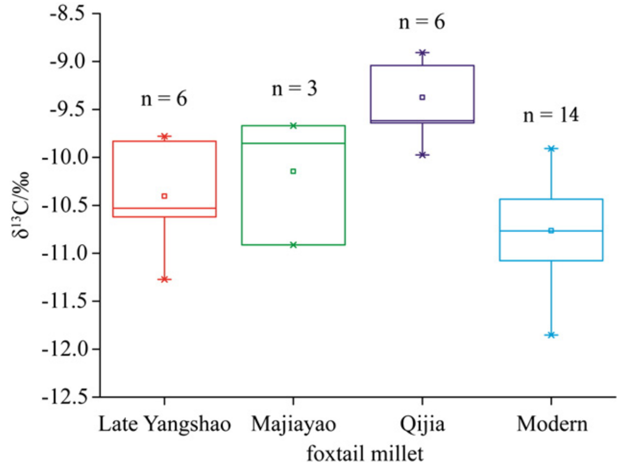

Figure 3. Boxplot of carbon isotopic results of foxtail millet, separated by different periods from Late Yangshao to modern.

The δ^{13}C mean value of modern samples is lower than that of ancient samples. If we correct the δ^{13}C value of modern samples to eliminate the effect of fossil fuels since the Industrial Revolution, the δ^{13}C mean value of modern grain samples is −10.8‰. Slight differences can be detected among different periods (Fig. 3). The Late Yangshao samples have a mean value that is slightly higher than that of modern samples, with Majiayao and Qijia samples having mean values that are also higher than that of modern samples. This difference is reflected in mean, maximum and minimum values, consistent with our previous study (An et al., 2015). This result is possibly caused by different environmental conditions and climate changes, but the exact interpretation still needs more tests.

With regard to δ^{15}N, there are differences among different periods (Table 2). This difference is reflected in both the mean values and overall ranges. However, the difference does not pass the Kruskal–Wallis test (Chi-square = 5.848, $p > 0.05$). It is suggested here that this may be attributed to a limited number of archaeological samples.

Our results show a significant difference between grain and leaf δ^{15}N values in the modern foxtail millet samples (2‰) (Table 2, Kruskal–Wallis test, Chi-square = 12.17, $p < 0.01$), just as studies on other plants (L. Zhao et al., 2010; Y. Zhao et al., 2010; Golluscio et al., 2014). The leaves have lower and more variable δ^{15}N values than the grains. This means that if livestock diets were supplemented with fodder consisting of leaves rather than grains, the difference in δ^{15}N between leaf and grain would reduce the apparent trophic level distinction between these livestock and human consumers of grain.

Comparison with bone collagen results

Compared to the previous paleo-diet reconstructions of this region (Barton et al., 2009; Ma et al., 2013), the range of foxtail millet δ^{15}N values is much larger than the range in human and pig values, while variation in foxtail millet δ^{13}C value is considerably lower than the variation in human and pig isotopic values, especially pigs, with a wide range of isotopic distribution as large as 9‰ in δ^{13}C values during the Late Yangshao period; moreover, the variation range of human δ^{13}C value apparently narrowed from the Late Yangshao to the Qijia period (Fig. 4). This finding suggests that during the Qijia period, humans did have high and less varied δ^{13}C values, indicating a higher proportion of C_4 plants in their diets compared with Late Yangshao period. This result supports the archaeobotanical data, which shows that foxtail millet dominated agriculture in the Qijia period (An et al., 2010, 2013).

It should also be noted that there is a significant difference between grain and leaf δ^{15}N values. This finding complicates the use of ideal δ^{15}N trophic step models in a foxtail millet-based society. The leaves have lower and more variable δ^{15}N values than the grains. This means that if livestock diets were supplemented with fodder consisting of leaves rather than grains, the difference in δ^{15}N between leaf and grain would reduce the apparent trophic-level distinction between these livestock and human consumers of grain. Thus, it will be difficult to distinguish between humans eating foxtail millet grain and humans eating pigs fed on foxtail millet leaves. For example, humans with a diet based on modern foxtail millet grains should have a δ^{15}N value signal around ~5‰, whereas pigs fed on foxtail millet leaves should have δ^{15}N value around ~2.9‰. Therefore, humans eating such pigs should have a δ^{15}N signal of ~5.9‰. We find that there is a varied trophic level between foxtail millet and pigs or humans from the Late Yangshao period to the Qijia period (Fig. 4).

Most dietary studies based on isotope analyses assume that the nitrogen isotope signals of the different part of plants consumed by humans and animals are the same and that the plants have values 2–5 ‰ lower than the herbivores. It then follows that a 3–5‰ difference between human and animal δ^{15}N values is always regarded as an indicator of whether human diets contained considerable amounts of animal protein (Richards and Trinkaus, 2009; Price et al., 2010). The difference between leaves and grains of foxtail millet δ^{15}N value makes this assumption problematic in a foxtail millet-based society. It is therefore not surprising that in most paleodietary reconstruction in northern China, there is no clear 3–5‰ difference among foxtail millet, pig and

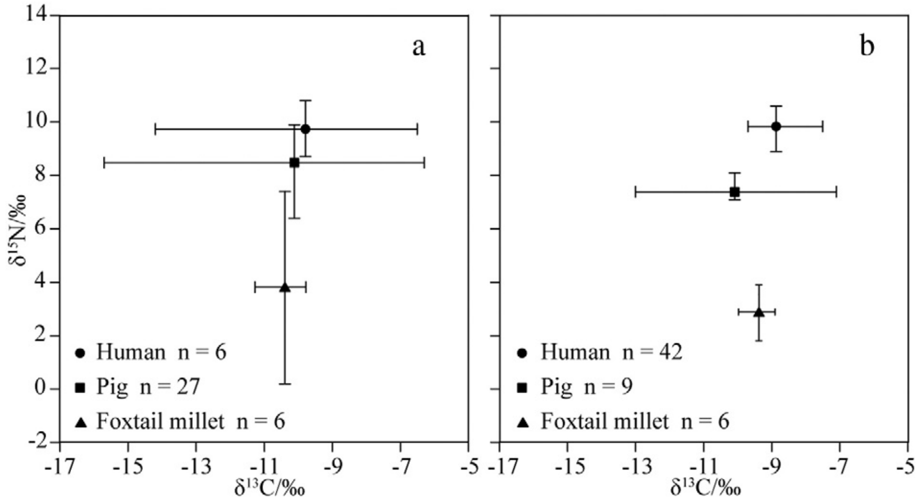

Figure 4. Mean and standard deviation of isotope results for human, pig and foxtail millet in different periods. (a) Late Yangshao period (Barton et al., 2009); (b) Qijia period (Ma et al., 2013).

human δ^{15}N values (e.g., Liu et al., 2005; Pechenkina et al., 2005; Barton et al., 2009; Atahan et al., 2011).

More work is needed to address what underlies the temporal and spatial variation in millet δ^{15}N over time. Many social and environmental factors, such as fertilization and climate change, must be included in future studies.

Conclusions

1) The δ^{13}C mean value of modern samples is slightly lower than or similar to that of ancient samples, even after correcting the δ^{13}C value of modern samples to eliminate the effect of fossil fuels since the Industrial Revolution. Simulated carbonization of the foxtail millet grains revealed that no significant isotopic fractionation occurred during low-temperature carbonization.
2) There is a significant difference between grain and leaf δ^{15}N values. This result challenges the standard assumption in isotope studies that the nitrogen isotope signals of the different part of plants consumed by humans and animals are the same and that the plants have values 2–5‰ lower than the herbivores.
3) Furthermore, the 3–5‰ difference between human and animal δ^{15}N values has always been regarded as an indicator of whether human diets contained considerable animal protein. The great difference between grain and leaf δ^{15}N values makes this assumption problematic in a foxtail millet-dominated society.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yqres.2015.04.004.

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