



The extent of cereal cultivation among the Bronze Age to Turkic period societies of Kazakhstan determined using stable isotope analysis of bone collagen



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ABSTRACT

This paper explores the contribution of plant foods to the diet of presumed pastoral societies in Kazakhstan. Carbon and nitrogen stable isotope analysis, together with radiocarbon dating, was carried out on human and animal bones from 25 Chalcolithic, Bronze Age, Early Iron Age, Hunic and Turkic sites across Kazakhstan. We use these data to examine dietary differences across time and space within and between populations.

Our results show that at the Bronze Age sites of mountainous southern Kazakhstan people consumed C₄ plants, likely domesticated millets (*Panicum miliaceum* and *Setaria italica*) as supported by previously published archaeobotanical direct evidence. By dating individuals with high $\delta^{13}\text{C}$ values we find the earliest evidence to date of the consumption of large quantities of millet in Central Asia. By contrast, there is little input of C₄ plants to diets of individuals dating to the Bronze Age from northern Kazakhstan. Stable isotope data from later periods show that from the Early Iron Age and continuing through to the Turkic period, C₄ plants were a major component of the human food web across the region. The wide variety of stable isotope results, both within and between contemporary sites from the southern regions of Kazakhstan, indicates a diversity of food choice.

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1. Introduction

The Bronze Age in Kazakhstan has been associated with a significant transformation in the societies of the region, notably a transition from fishing and hunting to pastoralism (Kalieva and Logvin, 2011; Outram et al., 2012; Frachetti and Benecke, 2009). Pastoral nomadism as a food-producing economy based on specialized animal husbandry was considered the only form of human subsistence strategy in the grassland during the Bronze Age

(Khazanov, 1994) until recent research on past steppe societies has shown the use of a much wider range of food sources than just animal products (e.g. Leonard and Crawford, 2002; Spengler et al., 2013a; Lightfoot et al., 2014). The presence of crop species in archaeobotanical records from southern Kazakhstan show that this territory constitutes a crossroads for the movement of crops that were traded and shared by mobile pastoral societies (Frachetti, 2012; Frachetti et al., 2010; Spengler et al., 2014a). Southwest Asian crops, such as wheat and barley (*Triticum turgidum/aestivum* and *Hordeum vulgare/nudum*), were spreading eastwards to China, while the Chinese crops, such as broomcorn and foxtail millets (*Panicum miliaceum* and *Setaria italica*), arrived in Europe by the Bronze Age period (Jones et al., 2011; Motuzaitė Matuzevičiūtė et al., 2013). While great contributions have been made to the

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study of the timing and pathways of the spread of millet from China to Europe, it is still a topic of continuing research (eg. Motuzaite Matuzeviciute et al., 2013; Spengler et al., 2014a; Hunt et al., 2008).

Over the past few years, archaeobotanical studies have repeatedly provided evidence for the availability, and probable consumption, of domestic cereals among pastoralists living along the piedmont of southern Central Asia (e.g. Spengler et al., 2014a,b,c; Rouse and Cerasetti, 2014). However, despite the suggestion that during the Final Bronze Age agriculture in the region of Kazakhstan and Turkmenistan was well-established with a wide range of cereals being grown (Spengler et al., 2014a,b,c), archaeobotanical data alone cannot provide information on the extent of cereal consumption by a population or by an individual. While no stable isotope studies have been carried out in southern Kazakhstan, previous stable isotope studies from central and northern regions of Bronze Age Kazakhstan have found that only small amounts of C₄ foods were consumed by Bronze Age populations (Ventresca Miller et al., 2014; Lightfoot et al., 2014).

Stable isotope analysis is a direct method to investigate past human diets at the level of an individual. Using this technique it is possible to identify C₄ plant consumption by an individual through an analysis of their skeletal chemistry (see below). Through consideration of the existing macrobotanical evidence we can then identify which C₄ plants were available for consumption.

This paper explores the changing role of cereals in Central Asia through isotopic analysis of individuals from across Kazakhstan from the Chalcolithic to the Turkic period. Stable isotope analysis allows us to assess the proportion of C₄ plants entering the human food chain, and the analysis of animal bones allows us to consider whether those C₄ plants were eaten directly by humans or if any human C₄ signal was acquired through the consumption of C₄-eating animals. Our primary objective here is to investigate the beginning of the consumption of C₄ plants in the human diet in the present territory of Kazakhstan, by looking at isotopic evidence for changing dietary patterns through time and space. In particular, we aim to ascertain whether C₄ plants (most likely Chinese millets) were consumed on a significant scale by Bronze Age populations as a staple food. Finally, we aim to establish whether there are regional trends, by considering the human and animal data by geographic location – north and south Kazakhstan, chosen on account of their clear topographical differences. Previous research has suggested that the topography played a major role in the spread of the agricultural products (cattle and cereals) in southern Kazakhstan (Frachetti, 2012).

2. Background

2.1. Archaeobotanical evidence of broomcorn millet in Central Asia

While the dominant narrative in the literature is that the Bronze Age inhabitants of Kazakhstan were pastoralists consuming mainly animal products (Khazanov 1994; Cribb, 1991; Kuzmina, 2008), in recent years the evidence for plant cultivation and consumption has grown (e.g. Chang et al., 2003; Spengler et al., 2013a, 2014a,b,c). Sickles and grinding stones have been discovered at prehistoric sites all across Central Asia (Dakhshleiger, 1980; Volkov and Dryabina, 2001; Okladnikov, 1959), indicating human involvement in harvesting and processing plants. Ethnographic studies suggest that cereal cultivation, in particular millet (*P. miliaceum*) (Bezhkovich, 1973; Dakhshleiger, 1980), has played a very important role among the populations of Central Asia (Di Cosmo, 1994; Vainshtein, 1980; Dakhshleiger, 1980; Bezhkovich, 1973). This crop was, and still is, highly suitable for cultivation by seminomadic societies in Central Asia, as it completes its life cycle in a very short period (40–90 days) (Nesbitt and Summers, 1988) and

has the lowest water requirements among the major crops (Baltensperger, 2002; Rachie, 1975). Gaiduchenko (2000, 2002) reports the remains of “millet porridge (Panicoidae gen. sp.)” on the walls of Chalcolithic pottery from the Tersek Culture in Northern Kazakhstan, although the methodology of identification is unclear. These reports of millet residues were disputed by Frachetti et al., 2010 as they lack species identification, as well as chronological and morphological information. The earliest directly dated macroremains of a few broomcorn millet (*P. miliaceum*) grains are from the Early Bronze Age human burial at Begash (ca. 2200 cal BC) in southeastern Kazakhstan, from a sample that also included wheat grains (*T. turgidum/aestivum*) (Frachetti et al., 2010). Archaeobotanical research by Spengler and colleagues (2014a,b) report the presence of wheat, barley, broomcorn and probably foxtail millets at Tasbas in south-eastern Kazakhstan, dated to 1450–1250 cal BC (Spengler et al., 2014a) and in the Murghab delta (Turkmenistan) at site 1211/1219 which is attributed to the Late/Final Bronze Age period (ca. 1950–1300 BC) (Spengler et al. 2014c). Further to the southwest of Central Asia, broomcorn millet was found at Late Bronze Age Shortughai, Afghanistan (Spengler and Willcox, 2013) and Ojakly in eastern Turkmenistan; the grain from Ojakly was directly dated (1740–1614 cal BC) (Rouse and Cerasetti, 2014).

In neighbouring Xinjiang, China, broomcorn millet grains have been found together with wheat and barley grains at second millennium BC sites (e.g. Chen and Hiebert, 1995). For example, in the Lop Nor Desert in Xiaohe, a small vessel plaited out of willow branches, roots and grass stems and containing wheat and millet grains was placed in the burial of two wooden dolls dated to ca. 1650–1450 BC (Baumer, 2012, p. 123). Further east, broomcorn millet has been found at Majiayao culture sites in western Gansu province, China, dated to 2700–2300 BC (Dani and Masson, 1992).

From the Early Iron Age, archaeobotanical data from Kazakhstan is supported by both macrobotanical remains and phytoliths. Recent studies of phytoliths from south-eastern Kazakhstan indicated millet (Panicoid and *Setaria* sp.) and wheat (*Triticum* sp.) (Chang et al., 2003; Rosen, 2001; Rosen et al., 2000), while macrobotanical investigations of carbonised plant remains have revealed the presence of a range of domesticated cereals, including free-threshing wheat, hulled and naked barley, foxtail and broomcorn millet, and grapes (Spengler et al., 2013a).

2.2. Stable isotope research in Central Asia

The few published stable isotope studies in Kazakhstan have focused on Chalcolithic or Bronze Age sites in the northern and central territories. In combination with published zooarchaeological data (eg. Kalieva and Logvin, 2011; Frachetti and Benecke, 2009; Outram et al., 2012), they indicate a Bronze Age diet based on cattle and ovicaprid meat and milk as well as freshwater fish (Privat et al., 2006; Ventresca Miller et al., 2014; O’Connell et al., 2003; Privat, 2004). Some dietary contribution of C₄ plants is detectable, with a few human outliers from the Lisakovsk and Bestamak sites in the northern regions with higher $\delta^{13}\text{C}$ values near -18‰ (Ventresca Miller et al., 2014). The stable isotope analysis recently conducted from the central regions of Kazakhstan report the possible consumption of C₄ plants among a small part of population during the Final Bronze Age period (from ca. end of the 2nd to the beginning of the 1st millennium BC) (Lightfoot et al., 2014). From a slightly later period, stable isotope research in the Minusinsk basin of Russia, north of the Altai Mountains, has shown that C₄ plants were consumed by the local inhabitants from ca. 1400 BC onwards (Svyatko et al., 2013). Stable isotope studies of Early Iron Age Scythian populations in the Tuva region of southern Siberia (1st century BC) have shown that C₄ plants formed a

significant fraction of the dietary intake (Murphy et al., 2013). To date, no stable carbon and nitrogen isotope analyses have been published from the southern region of Kazakhstan. Therefore, despite archaeobotanical analysis of Bronze Age Begash, Tasbas or Early Iron Age sites in Semirechye we do not know the scale on which cultivated plants were consumed in this region.

2.3. Stable isotope methodology

The analysis of ancient diet through stable isotope analysis is based on the principle that “you are what you eat (plus a few ‰)” (Lee Thorp, 2008). The food that animals and humans eat is incorporated into their body tissues, and by analysing the bone chemistry we can reconstruct past human diets. For dietary analyses, the most important stable isotopes are those of carbon and nitrogen. Stable carbon isotope measurements (expressed as $\delta^{13}\text{C}$) can distinguish between diets based on plants using two photosynthetic pathways, C_3 and C_4 , and between diets based on marine and terrestrial foods (unlikely to be relevant to this study) (Vogel and Van der Merwe, 1977; Schoeninger and DeNiro, 1984; Lee-Thorp, 2008). Plants utilising the C_4 photosynthetic pathway have higher $\delta^{13}\text{C}$ as they discriminate less against ^{13}C than C_3 plants (O’Leary, 1988). C_3 plants include most of the economic plant species from the Near East and Europe, such as wheat, barley, oats and rye. Chinese millets are particularly important for this study, as they constitute the only known C_4 plants widely cultivated and consumed in Eurasian prehistory. Whilst some C_4 wild flora do grow in the steppe and deserts of Central Asia (Iacumin et al., 2004; Winter, 1981; Makarewicz and Tuross, 2006) and potentially could enter human food chain via animal milk and meat, animal stable isotope analysis along with humans will be able to tell if the C_4 plant consumptions in humans under study is direct or indirect (via animal protein). Thus, the millet consumption by humans can be investigated via the humans and animals isotopic analyses in conjunction with archaeobotanical data.

Nitrogen isotope ratios (expressed as $\delta^{15}\text{N}$) reflect the trophic level of animal and humans, where consumers at higher trophic levels have higher $\delta^{15}\text{N}$ values (Hedges and Reynard, 2007). The nitrogen isotope ratios of freshwater (and marine) fish are elevated compared to terrestrial mammals (Richards and Hedges, 1999; Schoeninger and DeNiro, 1984; Schoeninger et al., 1983). Nitrogen isotope ratios can also be affected by various environmental or climatic factors, such as salinity. Animals grazing on saline soils (Heaton, 1987; Britton et al., 2008) or living in arid environments (Heaton, 1987; Hollund et al., 2010; Ambrose and Sikes, 1991) have elevated nitrogen isotope values compared to animals who do not graze under such conditions. Suckling animals and humans also have elevated $\delta^{15}\text{N}$ values, because they are a trophic level higher than their mothers (Fogel et al., 1997).

Environmental baseline $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values vary through space and time, and it is advantageous to analyse contemporary faunal samples wherever possible. Studies have shown that carbon isotope values of C_3 plants can be higher if plants are growing in water stressed environments (Flohr et al., 2011). Environmental stress, such as aridity and salinity, can also increase $\delta^{15}\text{N}$ values of plants (Heaton, 1987; Schwarcz et al., 1999). Spengler et al. (2013b) has pointed out that at the sites of south-eastern Kazakhstan, there is a greater concentration of certain plants around old pastoralist encampments, potentially due in part to penning and high nitrogen levels from dung deposits. Animals consuming those plants would have elevated $\delta^{15}\text{N}$ values as animal dung increases the $\delta^{15}\text{N}$ value of the soil. These increased isotopic values can be reflected in both animals and human bone collagen isotope results. The elevated $\delta^{15}\text{N}$ values in combination with C_4 plant consumption were seen

elsewhere (Liu et al., 2012) and the cause of a high $\delta^{15}\text{N}$ values need to be investigated in greater details. Furthermore, the faunal carbon isotopic values in Kazakhstan could be elevated as a result of C_4 plants in the animals’ diets that today represent a minor component of the flora in Central Asia (Iacumin et al., 2004; Winter, 1981; Makarewicz and Tuross, 2006), and thus can be used to discriminate between direct and indirect C_4 input into human diet. A variety of factors for consideration when establishing a baseline for northern Kazakhstan have been outlined by Ventresca Miller et al. (2014).

Kazakhstan is one of the biggest countries in the world and contains numerous environmental niches. Populations from the Bronze Age onwards were exploring the variety of such niches (e.g. Spengler et al., 2013b). It is important to point out that vegetation across Kazakhstan generally represents a mosaic landscape with varying densities of C_4 plants. Many of the ecotonal zones in the region are dominated by a variety of C_4 grasses including *Chenopodium* spp., *Polygonaceae* (*Rumex*, *Polygonum*, etc.), as well as small Panicoid grasses (*Digitaria*, *Echinochloa*, *Panicum*, *Setaria*, etc.) which were consumed by animals (Spengler et al., 2013a). For example, *Chenopodiaceae* and *Amaranthaceae* families have C_3 and C_4 photosynthetic pathways plant species (Sage et al., 1999). One of the edible C_4 plants is *Amaranthus retroflexus* which grows across Central Asia. However, we do not have evidence for consumption of this particular plant in Prehistory as archaeobotanical research rarely identifies amaranths to species level. Archaeobotanical investigations conducted at Sintashta-Petrovka sites in the Southern Siberia, such as Kamenyi Ambar, report large quantities of *Chenopodium album* seeds were probably eaten by the local populations (Rühl et al., 2015). *C. album*, however, is a C_3 plant (Sage et al., 1999). Stipa grass, which is widely available and could also be used as a human food, is also a C_3 plant (Wittmer et al., 2008). While there are quite a lot of C_4 wild plants in the territory under study, their presence in archaeological sites is very sparse (e.g. Spengler et al., 2014b). It is therefore quite unlikely that wild C_4 plants were (directly) consumed at a high enough level to be detectable isotopically.

Finally, it is not possible to tell whether humans consumed domestic or wild C_4 plants from the stable isotope results alone. However, when these data are combined with the existing archaeobotanical data from the region, it is possible to identify the C_4 plant consumed. Here, we interpret our stable isotope results in the light of the existing archaeobotanical records from the region.

3. Materials and methods

3.1. Isotopic analysis

Samples from 127 human and 109 animal bones were collected from 25 sites located across Kazakhstan (Fig. 1, SOM 1). Bone samples were obtained from three institutions: Archaeological Expertise LLC archive in Almaty, Institute of Archaeology in Karaganda and Kokshe Academy in Kokshetau. Animal bones were not available from every site from which human remains were analysed. Both adult and juvenile animals were sampled from the available material, as the collection included some juvenile bones that had been placed in human graves. Most of the human samples were collected from crania, as in most cases only crania were available from the anthropological collections. Collagen was extracted following the standard method of the Dorothy Garrod Laboratory, at the, Department of Plant Sciences, Institute for Archaeological Research at the University of Cambridge. 500 mg of bone were obtained from each sample and the surfaces of the bone pieces were then cleaned by sandblasting.

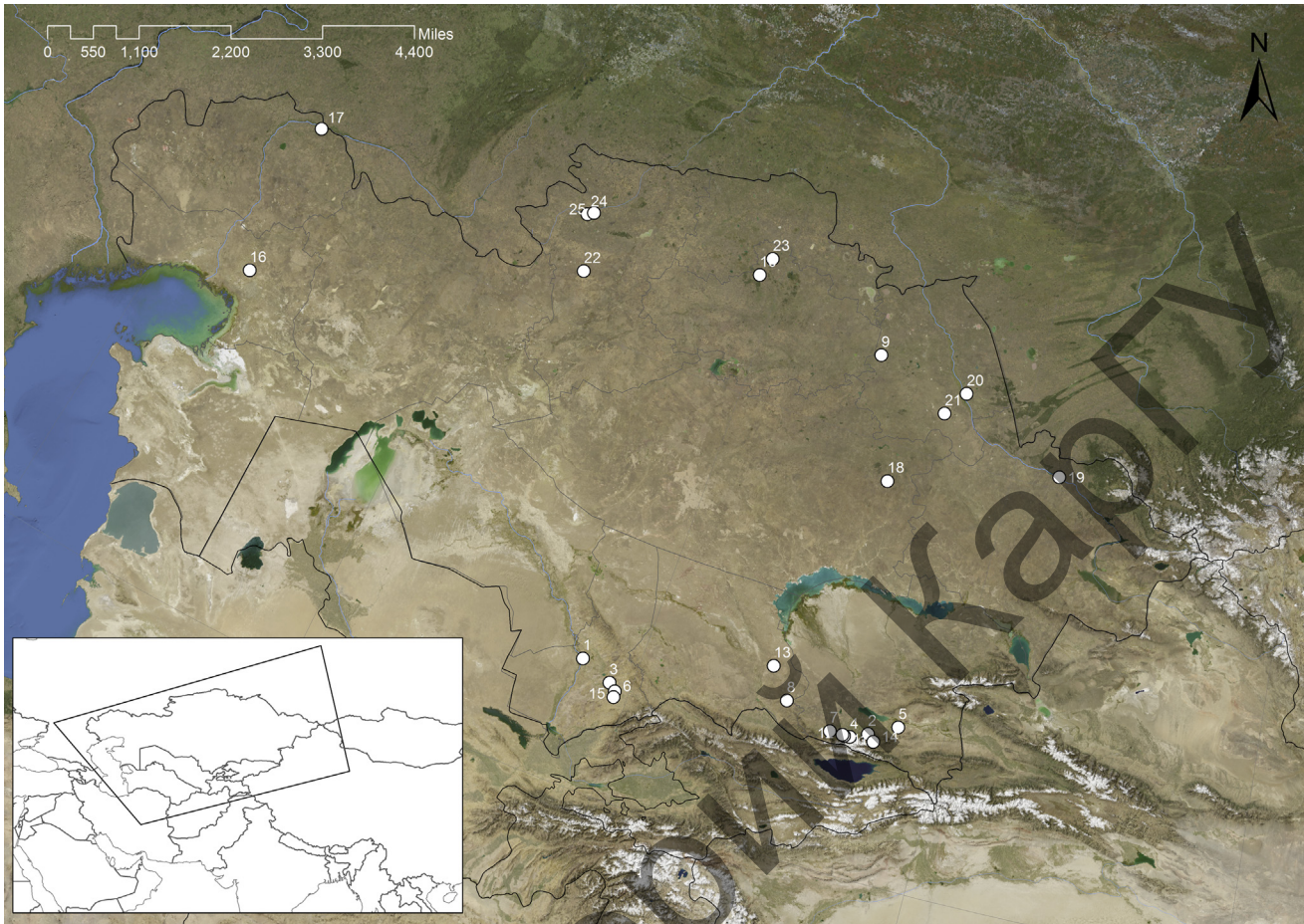


Fig. 1. The location of the sites analysed in this paper: 1. Konyr-Tobe-1, 2. Turgen-2, 3. Temirlanovka, 4. Butakty-II, 5. Karatuma, 6. Kainar Bulak-1-2, 7. Kargaly-2, 8. Oi-Dzhailau-7, 9. Bozshakol-2, 10. Ormandy Bulak, 11. Alatau-1, 12. Kamenka, 13. Khatau-1, 14. Kyzyl Bulak-1, 15. Shymkent, 16. Karabatan, 17. Kyrik-Oba-2, 18. Karagash, 19. Karatumsk, 20. Pavlodar-Semipalatinsk, 21. Zhambyl, 22. Akbidaik, 23. Kenelkel-18, 24. Lisakovsk, 25. Novoilinovka.

Bones were demineralized in 0.5 M aq. HCl at 4 °C for up to ten days, changing the acid as necessary, and after rinsing three times with distilled water, were gelatinized in an acidic solution (pH 3) at 75 °C for 48 h. The liquid fraction containing the gelatinised protein was filtered off using an Eze filter (Elkay Products) and freeze-dried. Triplicate samples of approximately 0.8 mg were used for each analysis in the Godwin Laboratory, University of Cambridge, using an automated elemental analyser (Costech, Valencia CA) coupled in continuous flow mode to a Thermo

Finnigan Delta V isotope ratio-monitoring mass spectrometer (Bremen, Germany). Stable isotope concentrations are reported relative to the internationally defined standard VPDB for carbon, and AIR for nitrogen (Hoefs, 1997). Based on replicate analyses of international and laboratory standards, measurement errors are <0.1% for $\delta^{13}\text{C}$ values and 0.2% for $\delta^{15}\text{N}$ values.

The choice of statistical tests was based on the main goal of this paper, to see if there are any dietary differences between human and animal population in space and time, as well as being based on

Table 1

^{14}C dates, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of humans from Kazakhstan. Radiocarbon data calibrated against the IntCal09 calibration curve (Reimer et al., 2013).

Site name	Mean $\delta^{13}\text{C}_{\text{VPDB}}$ (‰)	Mean $\delta^{15}\text{N}_{\text{AIR}}$ (‰)	^{14}C CHRONO lab code	^{14}C age	\pm	AMS $\delta^{13}\text{C}$	cal BC/AD 95.4 (2 σ)	Period
Karagash	-18.2	12.6	UBA-22949	4257	32	-16.4	2920–2712	Chalcolithic
Kyzyl Bulak-1	-19.	9.7	UBA-22948	3383	32	-16.5	1754–1541	Late Bronze Age
Kyzyl Bulak-1	-17.6	10.2	UBA-22947	3366	35	-17.5	1744–1536	Late Bronze Age
Oi-Dzhailau-7	-16.9	13.9	UBA-22944	3228	34	-18.2	1608–1428	Late Bronze Age
Oi-Dzhailau-7	-13.7	14.6	UBA-22943	3227	35	-12.7	1608–1427	Late Bronze Age
Karatuma	-16.4	10.6	UBA-22937	2233	33	-14.7	384–204	Early Iron Age
Karatuma	-13.2	12.4	UBA-22936	2210	33	-10.9	381–198	Early Iron Age
Kamenka	-15.6	12.3	UBA-22946	2160	27	-12	352–111	Early Iron Age
Alatau-1	-15.9	11.6	UBA-22945	2154	27	-13.6	352–96	Early Iron Age
Kargaly-1	-14.2	10.4	UBA-22940	2137	27	-13.7	351–56	Early Iron Age
Kainar Bulak-1	-11.2	12.9	UBA-22939	2082	28	-8	185–39	Early Iron Age
Kainar Bulak-1	-13.1	11.5	UBA-22938	1811	32	-10.9	126–325	Early Iron Age/Hunic
Temirlanovka	-13.7	13.2	UBA-22941	1766	27	-11.7	140–376	Hunic
Butakty-1	-10.7	11.3	UBA-22942	957	25	-6.2	1022–1155	Turkic

Table 2
Summary of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of faunal samples from the south and north of Kazakhstan.

Species (n)	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)					$\delta^{15}\text{N}_{\text{NAIR}}$ (‰)				
	Max	Min	Mean	Sd	Median	Min	Max	Mean	Sd	Median
Ovicaprids South (27)	-16.7	-20.4	-18.7	0.8	-18.5	5.5	11.8	8.1	1.5	7.7
Ovicaprids North (22)	-17.6	-19.8	-19.0	0.5	-19.1	5.3	11.5	6.9	1.5	6.2
Cattle South (5)	-17.0	-19.7	-18.4	1.0	-18.1	7.0	9.9	8.4	1.1	8.6
Cattle North (29)	-18.6	-20.0	-19.4	0.4	-19.4	5.1	8.8	6.7	1.0	6.6
Horses South (4)	-18.0	-20.7	-19.4	1.2	-19.5	4.5	8.5	6.7	1.8	7.0
Horses North (15)	-19.3	-20.9	-20.3	0.4	-20.3	4.2	6.9	6.0	0.8	6.1
Dogs South (3)	-14.5	-18.1	-16.9	2.0	-18.0	8.8	13.4	11.1	2.3	11.3
Dogs North (3)	-18.1	-19.0	-18.3	0.6	-18.1	9.3	12.3	10.6	1.5	10.3
Wild herbivores south/north (2)	-19.0	-20.4	-19.7			5.3	5.9	5.6		

the sample size available. Statistical analyses were performed using SPSS 21.0 for Mac. Samples were tested for normality using histograms and Kolmogorov–Smirnov and Shapiro–Wilk tests. The parametric data were investigated using independent samples t-tests. The non-parametric tests employed were Kruskal–Wallis tests. Outliers are identified as samples that lie more than 1.5 times the inter quartile range (IQR) below quartile 1 (Q1) or above quartile 3 (Q3). The minimum sample size for statistical analysis was five. The statistical significance levels is considered to be $p < 0.05$.

3.2. AMS ^{14}C dating

A total of 14 adult human bone samples were subject to accelerator mass spectrometer (AMS) ^{14}C dating (Table 1). All samples were prepared at the CHRONO Centre for Climate, the Environment, and 14Chronology, Queen's University Belfast. The prepared collagen was sealed under vacuum in quartz tubes with an excess of copper oxide (CuO) and combusted at 850 °C to produce carbon dioxide (CO_2). The CO_2 was converted to graphite on an iron catalyst following the zinc reduction method (Slota et al., 1987). The

Table 3
Summary of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of human samples from Kazakhstan. Lisakovsk data were taken from Ventresca Miller et al. (2014) and Aymyrylg and Ai-Dai data were taken from Murphy et al. (2013).

Site name (per site-n)	Period (total-n)	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)					$\delta^{15}\text{N}_{\text{NAIR}}$ (‰)				
		Min	Max	Mean	Sd	Median	Min	Max	Mean	Sd	Median
South											
	Bronze Age (15)	-19.1	-13.3	-16.2	1.9	-16.3	9.7	14.7	13.0	1.6	13.4
Oi-Dzhailau 7 (11)	Bronze Age	-18.5	-13.3	-15.6	1.8	-14.8	12.8	14.7	13.8	0.6	13.8
Kyzyl Bulak-1 (4)	Bronze Age	-19.0	-16.3	-17.8	1.2	-18.0	9.7	12.8	10.7	1.4	10.2
	Early Iron Age (68)	-17.3	-10.5	-14.7	1.7	-15.1	8.8	17.2	12.1	1.5	12.2
Karatuma (39)	Early Iron Age	-17.0	-12.9	-15.4	1.0	-15.5	8.8	17.2	12.3	1.6	12.4
Kargaly-1 (4)	Early Iron Age	-15.5	-13.6	-14.6	0.9	-14.6	10.4	12.5	11.8	1	12.2
Kainar Bulak-1–2 (17)	Early Iron Age	-15.4	-10.5	-12.6	1.4	-12.7	10.1	14.7	12.1	1.4	12.0
Khatau-1 (1)	Early Iron Age	-15.0	-15.0	–	–	–	15.9	15.9	–	–	–
Alatau-1 (3)	Early Iron Age	-17.3	-15.9	-16.5	0.7	-16.2	10.0	11.6	10.8	0.8	10.9
Shymkent (1)	Early Iron Age	-11.3	-11.3	–	–	–	10.7	10.7	–	–	–
Kamenka (3)	Early Iron Age	-16	-15.3	-15.6	0.3	-15.6	9.7	12.3	10.8	1.4	10.3
	Medieval (26)	-19.4	-10.0	-15.3	2.6	-15.3	10.4	14.0	11.0	0.8	11.8
Temirlanovka (4)	Medieval	-14.6	-12.6	-13.5	0.8	-13.4	12.0	14.0	13.1	0.8	13.1
Butakty-1 (6)	Medieval	-15.7	-10.7	-12.7	2.2	-11.6	10.7	12.2	11.5	0.6	11.5
Turgen-2 (7)	Medieval	-18.1	-15.1	-16.2	1.2	-15.6	11.4	11.9	11.4	0.5	11.7
Konyr-Tobe-1 (9)	Medieval	-19.4	-12.4	-17.0	2.4	-17.8	10.7	12.9	11.9	0.7	12.0
North											
Karagash (1)	Chalcolithic (1)	-18.2	-18.2	–	–	–	12.6	–	–	–	–
	Bronze Age(46)	-19.7	-17.5	-18.7	0.5	-18.8	9.9	14.4	12.3	1.1	12.0
Novoilinovka (8)	Bronze Age	-19.2	18.6	-18.9	0.2	-18.9	11.2	11.4	10.8	0.2	11.2
Lisakovsk (28)	Bronze Age	-19.7	-17.5	-18.8	0.4	-18.8	9.9	14.4	12.0	1.0	12.0
Kyrik-Oba-2 (1)	Bronze Age	-17.7	-17.7	–	–	–	13.2	–	–	–	–
Ormandy Bulak (1)	Bronze Age	-19.0	-19.0	–	–	–	10.9	10.9	–	–	–
Karatumsk (1)	Bronze Age	-19.6	-19.6	–	–	–	13.9	–	–	–	–
Bozshakol (2)	Bronze Age	-18.7	-18.4	-18.6	–	–	11.5	13.8	12.6	–	–
Karabatan (1)	Bronze Age	-17.5	-17.5	–	–	–	13.6	13.6	–	–	–
Pavlodar-Semipalatinsk (1)	Bronze Age	-19.3	-19.3	–	–	–	13.9	–	–	–	–
Zhambyl (1)	Bronze Age	-18.8	-18.8	–	–	–	13.0	13.0	–	–	–
Akbidaik (1)	Bronze Age	-18.2	-18.2	–	–	–	13.5	13.5	–	–	–
	Early Iron Age (38)	-17.5	-12.8	-15.2	1.1	-15.2	9.9	14.0	12.0	1.4	12.3
Ai-Dai (18)	Early Iron Age	-16.6	-12.8	-15.1	1.0	-15.3	9.9	11.8	10.7	0.5	10.5
Aymyrylg-8 (21)	Early Iron Age	-17.5	-13.2	-15.3	1.3	-15.2	11.8	14.0	13.2	0.5	13.2

Table 4
Results of statistical analyses; (*BA – Bronze Age; **EIA – Early Iron Age).

Comparison	n	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$			
		Test	Test statistic	Test statistic	p value	Test	Test statistic	Test statistic	p value
Statistical tests outputs of fauna									
1. North v South ovicaprid results	27, 27	Mann–Whitney	(U) 253	(Z) –1.930	0.054	Mann–Whitney	(U) 173	(Z) –3.313	0.001
2. Ovicaprid v cattle results (north only)	27, 30	Mann–Whitney	(U) 230	(Z) –2.798	0.005	Mann–Whitney	(U) 392.5	(Z) –0.200	0.842
Statistical tests outputs of human data									
3. BA* North v South human data (excluding: GM_28 and GM_29)	13, 45	Kolmogorov–Smirnov	(D) 0.779	(Z) 2.476	<0.001	Kolmogorov–Smirnov	(D) 0.503	(Z) 1.596	0.012
4. North v south EIA* humans	68, 39	Mann–Whitney	(U) 1140	(Z) –1.204	0.228	Mann–Whitney	(U) 1320.5	(Z) –0.036	0.972
5. Karatuma v Kairnar Bulak	39, 17	t-test	(df) 54	(t) –8.578	<0.001	t-test	(df) 54	(t) 0.485	0.649
6. Southern humans, BA** v EIA**	13, 68	Kolmogorov–Smirnov	(D) 0.347	(Z) 1.147	0.144	Kolmogorov–Smirnov	(D) 0.475	(Z) 1.570	0.014

graphite was then pressed to produce a “target,” which was then subject to AMS dating. The ^{14}C age mean and 1 standard deviation were calculated using the Libby half-life (5568 yr), following the conventions of Stuiver and Polach (1977). Calibration of the ^{14}C dates was undertaken using the IntCal04 calibration curve (Reimer et al., 2013). All calibrated ^{14}C ages are given at 95.4%, i.e. 2 σ probability.

A reservoir effect could be present in the dated samples, as high $\delta^{15}\text{N}$ values (see below) suggest possible fish consumption (fish are often at a higher trophic level than terrestrial food) (Richards and Hedges, 1999; Schoeninger and DeNiro, 1984; Schoeninger et al., 1983). Previous studies on the reservoir effect in the Samara region of southwest Russia and Ukraine found a ca. 400 year reservoir effect in dated individuals linked to fish consumption (Anthony, 2007; Lillie et al., 2009). On the other hand, Svyatko et al. (2009) found no evidence for a reservoir effect in Chalcolithic and Bronze Age human samples from the Minusinsk Basin in Siberia. Cook et al. (2002) suggested using a linear correlation between the $\delta^{15}\text{N}$ values and the ^{14}C dates to check for the presence of the reservoir effect in dated humans. This method is based on the assumption that if there is a marked reservoir effect, those individuals with higher $\delta^{15}\text{N}$ values would on average be expected to exhibit older ^{14}C ages than those with lower values. Unfortunately, our sample size is too small to apply a linear correlation method, however no obvious relationship can be seen between the human ^{14}C and $\delta^{15}\text{N}$ data from Kazakhstan.

4. Results and discussion

In total of 127 of the 170 humans (74.7% success rate) and all 109 animals sampled produced collagen with C:N values within the acceptable quality range of 2.9–3.6 (De Niro, 1985). While presenting the data we focus on the $\delta^{13}\text{C}$ as the most relevant for the main questions of our paper. The statistical outputs of $\delta^{15}\text{N}$ and other statistical tests can be found in Table 4 and the Supplementary Material (Som 1 A,B).

4.1. Faunal results

The isotopic data from all fauna are summarised in Table 2 and presented in detail in the SOM 1A. The distribution of the faunal dataset limits the comparisons that can be made by period. For fauna from the south of Kazakhstan, there are 4 samples from the Bronze Age (3 ovicaprids and 1 deer), 26 samples from the Early Iron Age (14 ovicaprids, 5 cattle, 4 horses and 3 dogs) and 10 from

the Medieval period (all ovicaprids). The faunal data from north Kazakhstan all date to the Late Bronze Age. In total there are 68 individuals (22 ovicaprids, 29 cattle, 14 horses, 3 dogs and 1 saiga antelope).

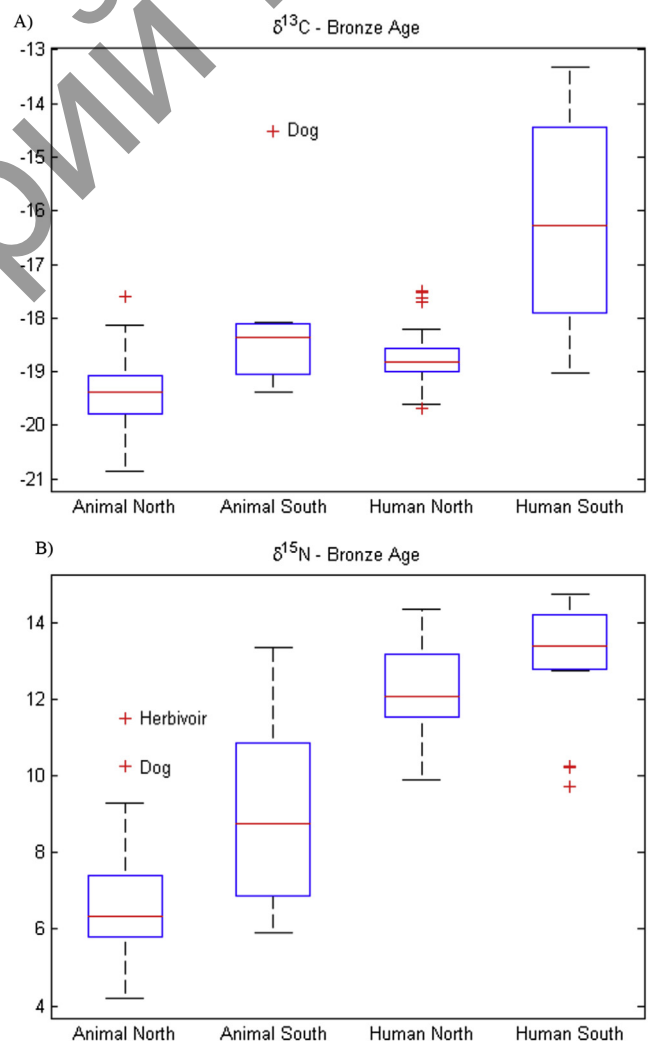


Fig. 2. The boxplot showing the $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values of Bronze Age humans and animals from the southern and northern Kazakhstan. The human data from the north Kazakhstan is supplemented by Ventresca Miller et al., (2014).

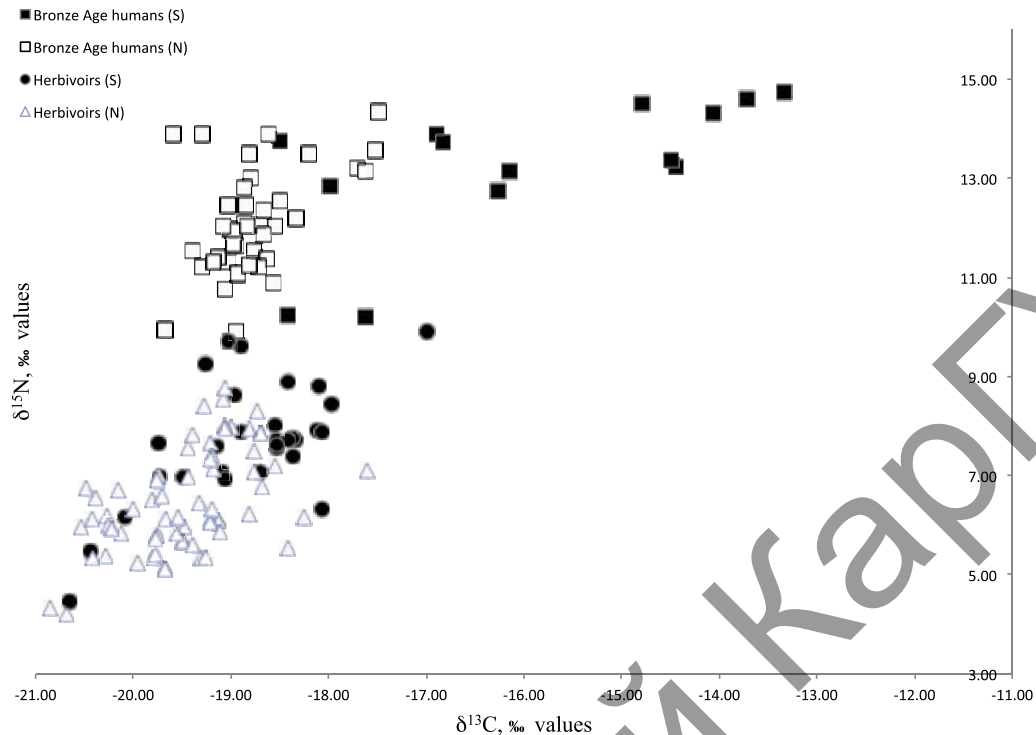


Fig. 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values of the Bronze Age southern and northern humans and fauna. The human data from the north Kazakhstan is supplemented by Ventresca Miller et al. (2014).

Although the faunal dataset from the Bronze Age period from the south is limited, some patterns can be observed (Figs. 2 and 3). According to O'Leary (1988), in a pure C3 ecosystem, plant $\delta^{13}\text{C}$ values typically range between -22 and -34‰ . The $\delta^{13}\text{C}$ values of herbivores from southern Kazakhstan range between -17 and -21‰ , indicating that they were grazing probably in predominantly C3 environment with some intake of C4 plants, seen on some individuals. Although, as it has been already mentioned, aridity and environmental stress could also increase the $\delta^{13}\text{C}$ values in C3 plants (Flohr et al., 2011). A comparison of Bronze Age ovicaprids from north with the ovicaprids from all periods from south Kazakhstan shows that there is no significant difference by region in $\delta^{13}\text{C}$ values (Student's t-test, $p = 0.054$), but there is a difference in $\delta^{15}\text{N}$ values (Student's t-test, $p = 0.001$) (see Table 4, output 1). Also, comparison of Bronze Age ovicaprids and cattle from the north shows a significant difference in $\delta^{13}\text{C}$ ($p = 0.005$), but no difference in the $\delta^{15}\text{N}$ values ($p = 0.842$) (see Table 4, output 2), suggesting possible differences in grazing strategies.

Generally, the Iron Age animal data from south Kazakhstan show a dominant C3 plant consumption with some C4 plants in a diet (Figs. 4 and 5). Two juvenile ovicaprids from Karatuma (GM_F76, F77) and one dog sample from Kainar Bulak (GM_F20) have $\delta^{13}\text{C}$ values indicative of C4 consumption ($\delta^{13}\text{C} = -16.7$ and -16.8‰ , and -14.5‰ , respectively).

4.2. Human results

The isotope data from all humans, together with information on sample size and dating, are summarised in Tables 1 and 3 and described in detail in the SOM 1B. We consider the human data by geographical region – 'south' (the piedmont areas of the Tian Shan and Dzungaria) and 'north' (mainly lowland) – and by period (Chalcolithic, Bronze Age, Early Iron Age and Medieval) (Table 3, Figs. 2–5).

For the south of Kazakhstan human data are available from the Bronze Age ($n = 15$), Early Iron Age ($n = 68$) and Medieval ($n = 20$) periods. Isotopic data from the humans from the two Bronze Age sites in southern Kazakhstan (Kyzyl Bulak and Oi-Dzhailau-7) range in $\delta^{13}\text{C}$ values from -19.1 to -13.3‰ suggesting a substantial input of C4 into the diet, though to varying degrees, as there is significant variation within and between these sites. Our data indicate that the individuals at Oi-Dzhailau-7 in general consumed a higher proportion of C4 plants (mean $\delta^{13}\text{C} = -15.6\text{‰}$, $n = 11$) than those at Kyzyl-Bulak (mean $\delta^{13}\text{C} = -17.8\text{‰}$, $n = 4$), where three (out of four) individuals have low $\delta^{13}\text{C}$ and low $\delta^{15}\text{N}$ values, indicating that they primarily relied on C3 foods, with little or no C4 in the diet (Fig. 3, SOM 3). Human collagen $\delta^{13}\text{C}$ is expected to be 0 – 2‰ higher than the collagen carbon isotopic values of the fauna that they consume (Bocherens and Drucker, 2003). The average human-faunal offset values seen here for the Oi-Dzhailau site (3.45‰ in $\delta^{13}\text{C}$ and 6.1‰ in $\delta^{15}\text{N}$) and for Kyzyl-Bulak site for one individual with elevated $\delta^{13}\text{C}$ values (GM_036) (2.78‰ in $\delta^{13}\text{C}$ and 5.06‰ in $\delta^{15}\text{N}$) suggest that people consumed C4 plants directly. Two individuals (GM_021 & GM_030, one of which have elevated $\delta^{13}\text{C}$) from Kyzyl-Bulak have been dated to between 1754 and 1541 cal BC, and two with high $\delta^{13}\text{C}$ (GM_26 & GM_25) from Oi-Dzhailau-7 have been directly dated to 1608–1428 cal BC (Fig. 6; Table 1). The isotopic data show that C4 consumption was a significant dietary component for a number of individuals. Archaeobotanical research from the Bronze Age south-eastern Kazakhstan indicates that the only C4 plant present in anthropogenic contexts was (domesticated) broomcorn millet (Frachetti et al., 2010; Spengler et al., 2014b). Wild plants were present, but the three most abundant species (*C. album*, *Galium* and *Stipa*) all use the C3 photosynthetic pathway. Therefore, it is reasonable to link the C4 signal in humans from the south with millet consumption. These results constitute the earliest-to-date directly dated isotopic signals of C4 plant consumption outside of China where we see enriched $\delta^{13}\text{C}$ values at the samples from ca.

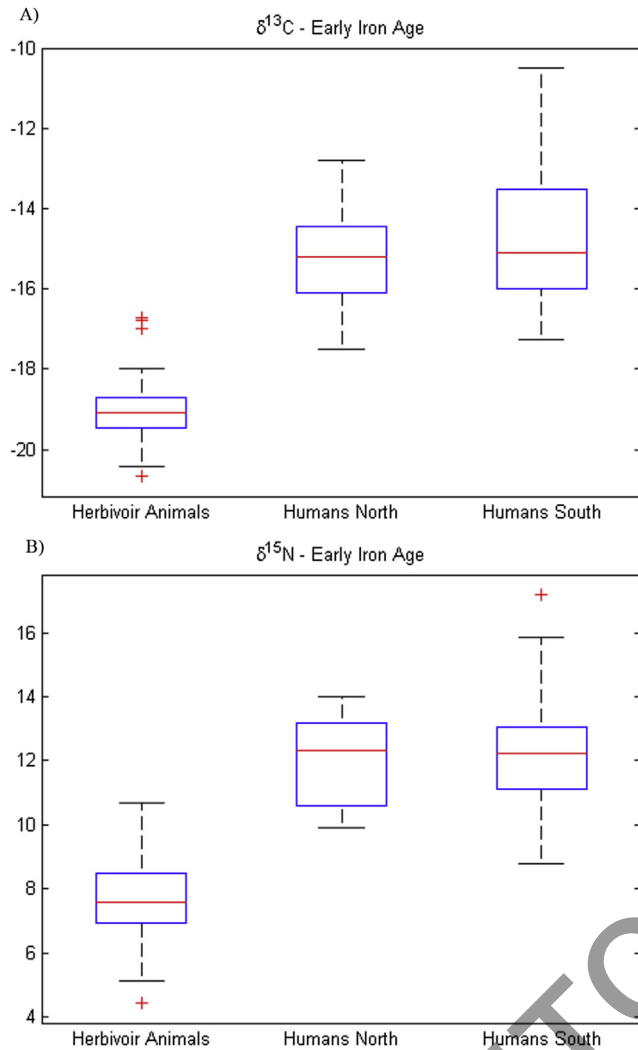


Fig. 4. The boxplot showing the $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values of Early Iron Age humans and animals from the southern Kazakhstan and Russian Altai (after [Murphy et al., 2013](#)).

1800 BC onwards. Previous research conducted by [Lightfoot et al. \(2013\)](#) has shown earlier isotopic evidence for C₄ plant consumption outside of China, such as that in Hungary during the 5th millennium BC and Greece during the 4th millennium BC. However, these individuals have not been directly dated and may well be intrusive.

The data presented here show good chronological agreement with the archaeobotanical discoveries from the Murghab delta sites in Turkmenistan and the Tasbas site in Kazakhstan showing the existing established agriculture starting from 1700 cal BC ([Spengler and Willcox, 2013](#); [Rouse and Cerasetti, 2014](#); [Spengler et al., 2014a,b,c](#)).

The Early Iron Age humans from seven sites in the south of Kazakhstan have a mean $\delta^{13}\text{C}$ value of $-14.7 \pm 1.7\text{‰}$, and a mean $\delta^{15}\text{N}$ value of $12.1 \pm 1.5\text{‰}$, range in $\delta^{13}\text{C}$ values from -17.3 to -10.5‰ suggesting C₄ dietary intake ([Tables 1 and 3](#); [Figs. 4 and 5](#)). Six individuals with elevated $\delta^{13}\text{C}$ values were dated, and the dates range from 384 to 39 cal BC, confirming an Iron Age date for high C₄ consumption ([Table 1](#)). It is interesting that despite the broad geography of the sites, all analysed populations consumed C₄ plants ([Fig. 4](#)). The archaeobotanical investigations in southern Kazakhstan support the stable isotope data, showing that the C₄ plants consumed were probably broomcorn and foxtail millets (eg.,

[Spengler et al., 2013a](#)). The importance of C₄ crops is indicated not only by the extent of its consumption, but also by its inferred use in ritual practices, such as seen in the Karatuma child's burial with a millet seed bedding found in kurgan 35 ([Fig. 7](#)). The isotope data demonstrate that this child also ate C₄ plants in large quantity ($\delta^{13}\text{C} = -14.9$; $\delta^{15}\text{N} = 9.0\text{‰}$). Whilst C₄ plants (most likely millets) were clearly an important dietary resource at this time, there are dietary differences visible between the sites ([Figs. 4 and 5](#)). A comparison of the two Early Iron Age sites with large sample sizes, Karatuma and Kainar Bulak-1, shows that there is a significant difference between them in $\delta^{13}\text{C}$ (mean $\delta^{13}\text{C} = -15.4 \pm 1.0\text{‰}$ and $-12.6 \pm 1.4\text{‰}$, respectively: $p < 0.001$), but not in $\delta^{15}\text{N}$ (mean $\delta^{15}\text{N} = 12.3 \pm 1.6\text{‰}$ and $12.1 \pm 1.4\text{‰}$, respectively: $p = 0.649$) ([Table 4](#), output 5), suggesting that the proportion of C₄ plants in the diet varied between these sites. The individuals with the highest $\delta^{13}\text{C}$ values at Kainar Bulak-1 (i.e. up to $\delta^{13}\text{C} -10.5\text{‰}$) must have consumed diets in which the majority of their protein derived from C₄ plants. Such a large variation in $\delta^{13}\text{C}$ values may indicate real differences in human food choice, with some of the communities eating substantially more C₄ plants than other groups.

A comparison of Bronze Age humans (excluding twin infants GM_28 and GM_29) with the Early Iron Age humans from southern Kazakhstan shows no statistical difference between these populations in $\delta^{13}\text{C}$ values (mean $\delta^{13}\text{C}$ values of $-16.2 \pm 1.9\text{‰}$ and $-14.7 \pm 1.7\text{‰}$ respectively: $p = 0.144$), suggesting minimal overall change in C₄ input over time ([Table 4](#), output 6).

The human samples from the four Medieval sites in southern Kazakhstan have a mean $\delta^{13}\text{C}$ value of $-15.3 \pm 2.6\text{‰}$ and a mean $\delta^{15}\text{N}$ value of $11.0 \pm 0.8\text{‰}$ ([Table 3](#)). Two individuals with elevated $\delta^{13}\text{C}$ values from Temirlanovka and Butakty-1 (UBA-22941 and UBA-22942) were dated to Hunic and Turkic periods ranging from AD 140–1155. One sample from Kainar Bulak-1 site (UBA-22938) also fell into the Late Hunic period dated to AD 126–325 (see [Table 1](#); [Fig. 6](#)). The range of human $\delta^{13}\text{C}$ value -19.4 to -10‰ and the mean of human-faunal offset values (3.3‰ in $\delta^{13}\text{C}$ and 3.0‰ for $\delta^{15}\text{N}$) suggests that people consumed C₄ directly. There is great variability in the $\delta^{13}\text{C}$ values between the sites, ranging from -19.4 to -10.0‰ . This suggests that as with the Early Iron Age populations, some people ate little or no C₄ plants, while other individuals had diets that were dominated by C₄ food sources. Again we can suggest that these results indicate the role of human choice in the consumption of different foodstuffs.

For the north of Kazakhstan, the one Chalcolithic period human from the site of Karagash in north-central Kazakhstan was analysed isotopically; the $\delta^{13}\text{C}$ value is -18.2‰ and $\delta^{15}\text{N}$ is 12.6‰ . These data are very similar to another northern Kazakhstan individual from the Chalcolithic settlement of Botai ($\delta^{13}\text{C} = -18.1\text{‰}$; $\delta^{15}\text{N} = 12.4\text{‰}$), which was interpreted as a result of consumption of C₃ plants and substantial amounts of meat/dairy and fish foods ([O'Connell et al., 2003](#)). The radiocarbon date of the Karagash burial attributed it to the early wave of the Yamnaya culture ranging from 2920 to 2712 cal BC ([Table 1](#)).

Eighteen individuals from nine Late Bronze Age sites of northern Kazakhstan are discussed here, plus twenty-eight individuals from Lisakovsk (1860–1680 BC) ([Ventresca Miller et al., 2014](#)). The mean northern Bronze Age human values are $\delta^{13}\text{C} = -18.7\text{‰}$ and $\delta^{15}\text{N} = 12.3\text{‰}$. The mean $\delta^{13}\text{C}$ human value is only 0.3‰ higher than that of the associated ovicaprids and 0.6‰ higher than that of the cattle ([Tables 2 and 3](#)), indicating that the human diet during the Bronze Age in northern Kazakhstan consisted of C₃ plants and animals that ate C₃ plants, with little or no C₄ in the diet of most individuals.

When considering the Bronze Age data by region (including that from Lisakovsk), statistical comparison indicates that Bronze Age southern and northern human samples are only marginally

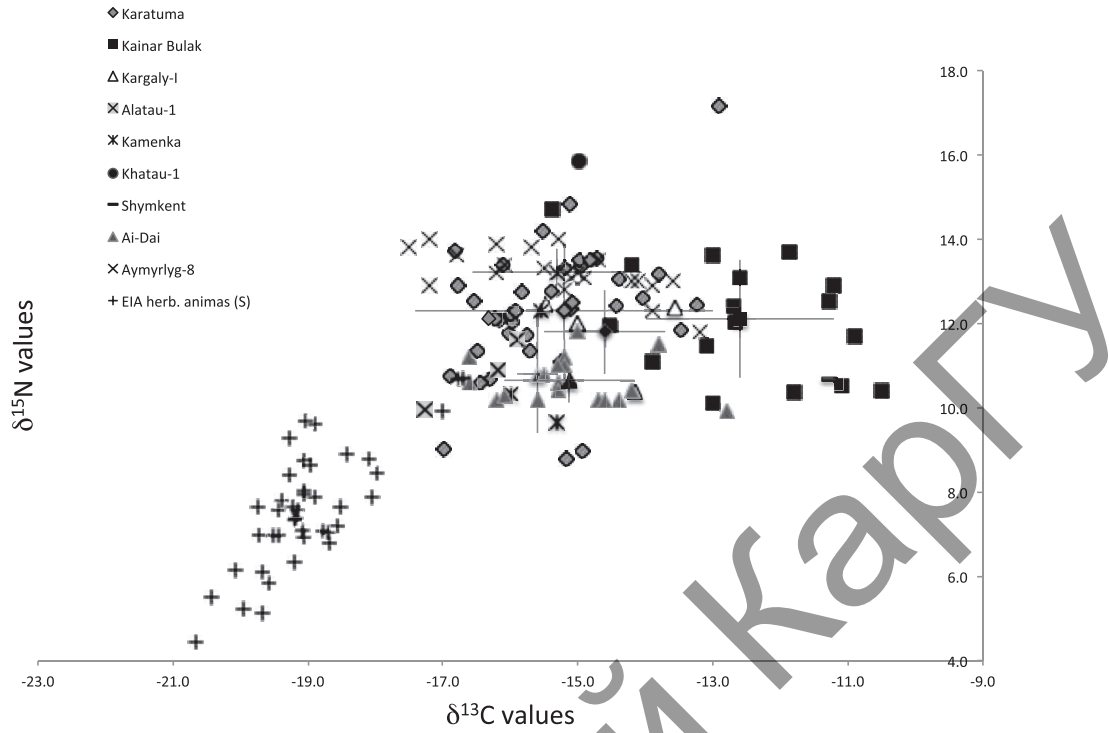


Fig. 5. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values Early Iron Age humans and fauna from the southern Kazakhstan showing the diversity of human diet between and within the same site; (EIA- Early Iron Age).

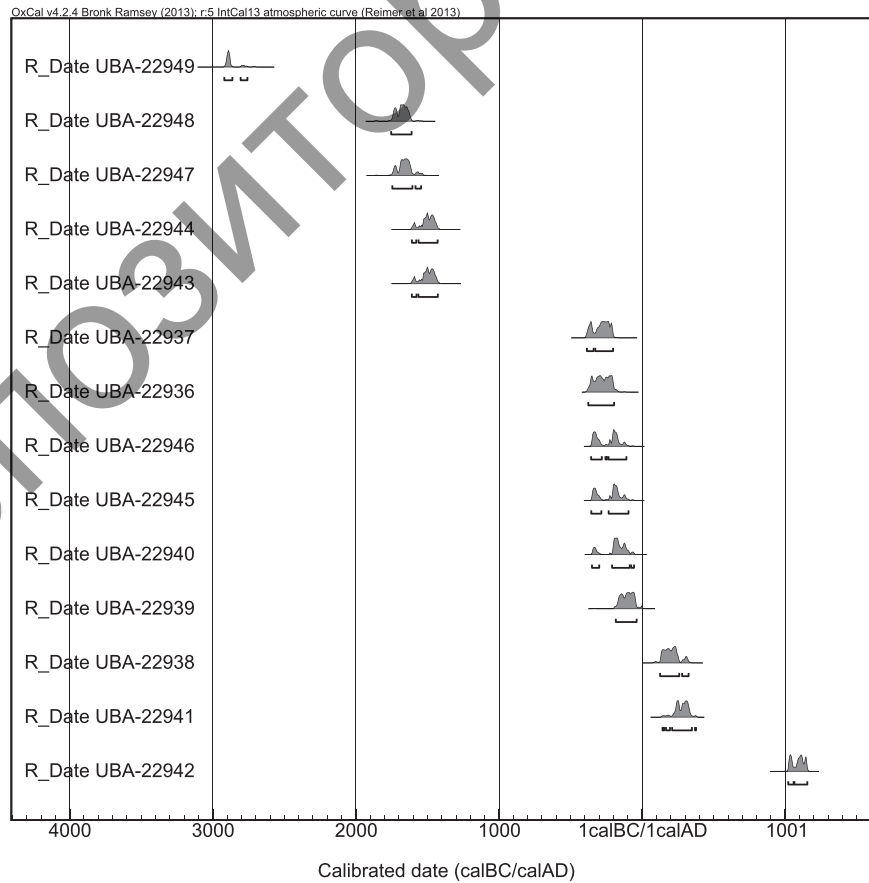


Fig. 6. Calibrated radiocarbon age ranges from the 10 sites. Horizontal bars below each distribution represent the 68.2% and 95.4% highest probability density (hpd) ranges.



Fig. 7. Wusun child's grave number 4 of kurgan 35 (GM062) in Karatuma burial ground. The child was placed on the layer of millet grain (Baipakov et al., 2010). Stable isotope results suggest subsistence based of millet ($\delta^{13}\text{C} - 14.9$; $\delta^{15}\text{N} 8.98\%$).

different in $\delta^{15}\text{N}$ ($p = 0.012$, excluding infants GM_28 and GM_29), but are significantly different in $\delta^{13}\text{C}$ ($p = 0.001$), with mean $\delta^{13}\text{C}$ values from the south much higher than those from the north ($\delta^{13}\text{C} = -16.2$ vs. -18.7% ; Table 4, output 3). Given the difference in $\delta^{15}\text{N}$ values in the limited faunal samples it is likely that any difference in human $\delta^{15}\text{N}$ values reflects ecological differences between north and south (Table 4, output 4; Fig. 2B). However, the $\delta^{13}\text{C}$ analyses show that both the ecology and the diet in these two regions was probably different, with the southern populations consuming more C_4 plants than the northern populations.

There are no available isotope data from the Iron Age of northern Kazakhstan, but published data exist from the contemporaneous sites of Ai-Dai and Aymyrlыg-8 (Murphy et al., 2013) in the Altai region of southern Siberia. Whilst the distance between these two regions is over 1000 km, both the southern Kazakh sites and those in Siberia are located in close proximity to mountain ranges and are environmentally similar. A comparison of the data shows that there is no significant difference in $\delta^{13}\text{C}$ ($p = 0.228$) between the Iron Age populations from southern Kazakhstan and southern Siberia (mean values are -16.2 and -15.2% respectively; Table 4, output 4), and both sets of data are indicative of C_4 plant consumption in these two regions.

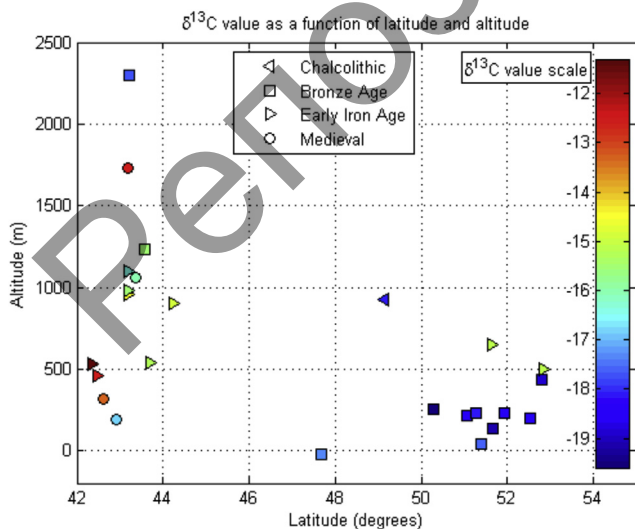


Fig. 8. The graph indicates human $\delta^{13}\text{C}$ mean value from sites across Kazakhstan relative to latitude and altitude. Bronze and Iron Age data from the north is taken from Ventresca Miller et al. (2014) and Murphy et al. (2013).

As we can see from the data presented above, a pattern of C_4 plant consumption emerges in space and time. It appears that mountain slopes (higher altitude areas) have the earliest signs of C_4 crop consumption. Previous research has shown that upland areas of the Inner Mongolian loess foothills in China were used for the earliest millet cultivation during the Neolithic period (Liu et al., 2009). In Kazakhstan, the earliest evidence for millet use are also located in the foothills of south eastern Kazakhstan, while during the Final Bronze Age and the Early Iron Age millet consumption appears in the mountainous regions of Siberia. The lower altitude human populations inhabiting the lowland steppe do not seem to have any evidence of agricultural crops (Fig. 8). This observation, however, is based on rather small sample size and more research should be done in the future. Nevertheless, our isotope data in agreement with the theory proposed by Frachetti (2012) and Spengler et al. (2014a) that millet spread following the mountain ranges as one of a group of innovations.

5. Conclusion

This paper provides a perspective on how populations in Kazakhstan used C_4 plants across space and time. The stable isotope and radiocarbon dating results presented in this study reveal that C_4 plants became established foodsources in southern Kazakhstan from the Middle-Late Bronze Age onwards (ca. 1800 cal BC), and were likely eaten directly by humans. Given the archaeobotanical evidence, it is reasonable to attribute the C_4 signal to the consumption of millet. These results constitute the earliest-to-date directly dated isotopic signals of millet consumption outside of China. The isotopic data suggests that millet was a significant dietary component for a number of individuals, such that it is detectable in stable isotope analysis of human bone collagen. While it is possible, indeed likely, that millet was consumed on a smaller scale at an earlier date, the early evidence for millet consumption in southern Kazakhstan (absent in the north during the same time period) suggests that this area represents a potential route for the spread of the millets from China, and warrants further archaeobotanical research.

This study has also shown great variation in human diet in the southern Kazakhstan within the same period and region, even between individuals from the same sites. This suggests diversity in food choices with some people eating more C_4 plants, and others more C_3 plants.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jas.2015.03.029>.

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