New radiocarbon and stable isotope data from the Usatove culture site of Mayaky in Ukraine

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Abstract

The Usatove (Usatovo) culture provides a pivotal link between farmer and forager-pastoralist cultures of the North Pontic region and adjacent areas during the Eneolithic-Early Bronze Age (EN-EBA) transition in southeast Europe. Usatove is conventionally dated to the middle of the 4th- early 3rd millennium BCE. However, the AMS dating of human remains from the Mayaky complex of the Usatove culture in Ukraine has produced dates beginning in the mid 5th millennium BCE. In this study, we report a series of new radiocarbon dates and stable isotope ratios from human remains at Mayaky and present an attempt to evaluate this data in the context of the Usatove culture chronology. The results suggest a diet of the individuals attributed to the Usatove culture at Mayaky based on aquatic resources, potentially contributing to a reservoir offset (RO) on the AMS dates obtained on human remains. The presented data indicate a change in dietary preferences of the users of the Mayaky archaeological complex in succeeding chronological periods. The presented as well as previously published AMS radiocarbon dates establish the utilization of the Mayaky archaeological site that precedes and post-dates the Usatove culture.

Key words: Eneolithic, North Pontic steppe, Usatove (Usatovo) culture, radiocarbon dating, stable isotope analysis

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1. Introduction

Usatove (Usatovo) is a southeast European Eneolithic culture distinguished by a distinctive ceramic complex, monumental burial complexes, and metalworking traditions. Usatove's rich material culture and ritual customs point to advanced social development, comparable to that of the Maykop-Novosvobodnaya cultural horizon from the Eneolithic North Caucasus (Manzura, 2020). In Ukraine, Usatove culture sites are mainly concentrated in the Dniester-Danube interfluve on the northwestern Black Sea coast (Figure 1). The two largest Usatove complexes (ritual centers) in Ukraine, Usatove-Velykyj Kuyalnik and Mayaky, contain kurgan and ground cemeteries (necropoli). The Mayaky complex contains an extensive network of ditches, characterized by some scholars as a causewayed camp (Petrenko, 2013). The "*horodyshche*" ("hillfort") term is often used in Ukrainian archaeological literature to describe the ditch network at Mayaky. We will use the term "sanctuary" in reference to the Mayaky "horodyshche" throughout this report. Other Usatove monuments in Ukraine are represented mostly by burials. Overall, about 35 Usatove-type sites are currently recognized (Manzura, 2020), but the total number of sites can be as high as 100 (Dergachev, 2021).

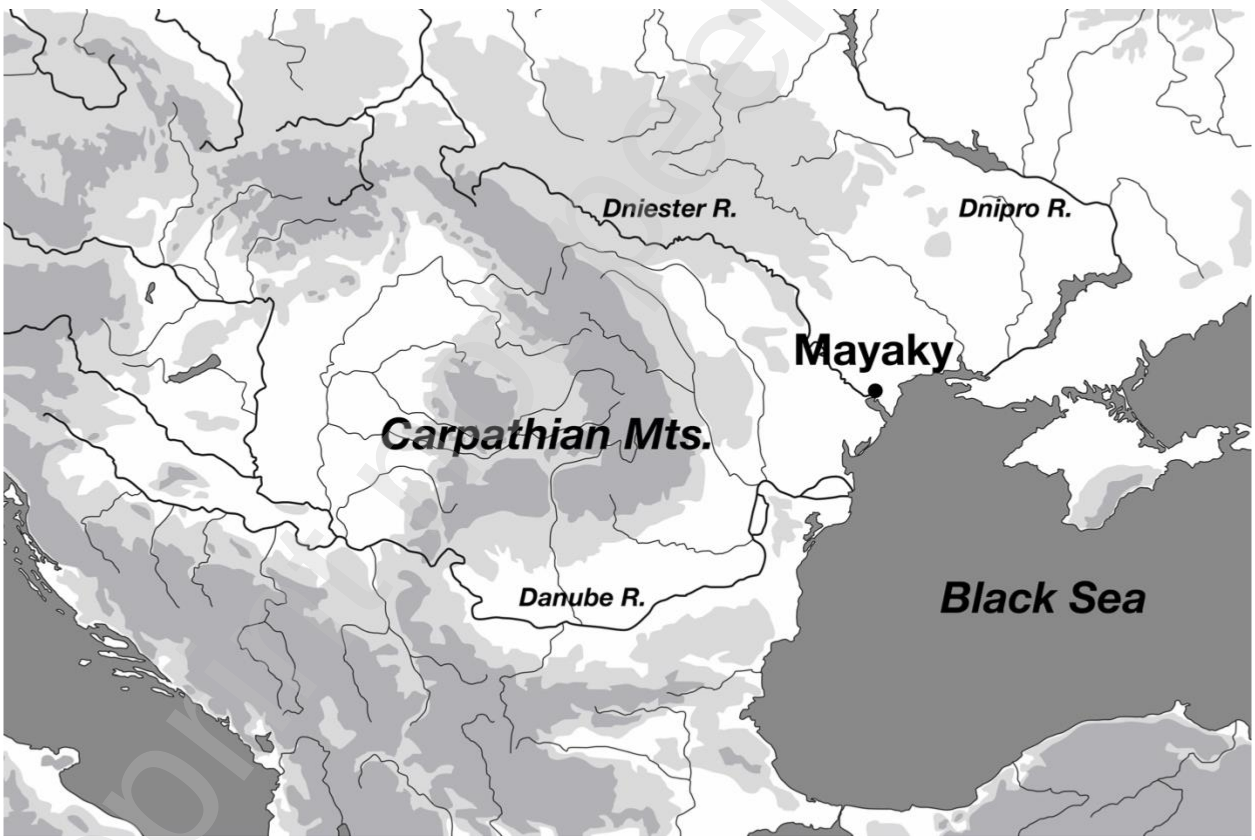

Figure 1. Map of the northwest Pontic steppe region showing the location of the Mayaky site.

It has been suggested that the origins of Usatove stem from the Cernavodă I culture of the Eneolithic (first half of the 4th millennium BCE) agrarians and stockbreeders from the lower Danube, and a northwest Pontic steppe group of the Post-Stig/Katrarzhino type, named after

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the main burial in the Katarzhino 1 kurgan (Ivanova et al., 2005). The third component of the Usatove origin and cultural traditions is considered to be Trypillia (Tripolie) (Manzura, 2020), the Eneolithic agrarians of east Europe. Based on the Trypillian pottery found at Usatove sites, Usatove is thought to have been primarily synchronous with the CII phase of the Trypillian chronology (Manzura, 2020), corresponding to *ca.* 3550-2750 BCE (Diachenko and Harper, 2016; Nikitin et al., 2010). Pottery of Eneolithic steppe (Seredny Stig) origin, the presence of cranial morphology features of the steppe inhabitants in the Usatove craniological series, as well as the potential social stratification of the Usatove burials (Patokova, 1979; Potekhina, 2019, 1989), is suggestive of steppe influences on the Usatove culture groups. This has led some scholars to consider Usatove as a Trypillia-steppe hybrid, or, rather, a Trypillian group subjected to steppe dominance (Anthony, 2008, 2007; Gimbutas, 1977; Petrenko, 2013).

Ceramic imports from the Danuban cultures of the late Eneolithic as well as the late CII stage of Trypillia are absent from Usatove sites, which helps in refining the relative Usatove chronology (Manzura, 2020). At the same time, there is a unique pottery style present at Usatove sites. The Usatove-specific pottery morphologically resembles Trypillian pottery, but it is unpainted, contains shell and grog admixture in the clay, and it is decorated with various incised and stamped decorations, including corded ornamentation (Manzura, 2020), indicative of steppe influences. The presence of a culture-specific pottery style has been used to argue that Usatove represents a separate cultural entity rather than a local Trypillian or steppe group (Petrenko, 2013). Overall, Usatove existed at the juncture of, and represented an amalgam of, cultural traditions of the farmers of "old Europe" and the new lifeways of the steppe nomadic cultures of the late Eneolithic-Early Bronze Age (EN-EBA) that brought steppe genetic ancestry and facilitated the spread of Indo-European cultural attributes in Bronze Age Europe (Allentoft et al., 2015; Haak et al., 2015; Lazaridis et al., 2022; Mathieson et al., 2015).

Established dating, based on material culture, such as the presence of imports of ceramics from securely dated chronological periods, as well as radiometric radiocarbon dates on charcoal, pottery, and animal bone, primarily from the ditches of the Mayaky sanctuary (Table S1a), place the Usatove culture in the chronological period corresponding to *ca.* 3650-2740 BCE. Some scholars argue that the Usatove chronological span is limited to the third quarter of the 4th millennium BCE (Manzura, 2020). However, Accelerator Mass Spectrometry (AMS) radiocarbon dates obtained on the Usatove-designated human remains from the Mayaky necropolis are distinctively older than the pottery and charcoal dates from the same site, obtained by radiometric methods (Petrenko et al., 2018). The discrepancy has been explained by the potential influence of a radiocarbon reservoir effect (RE), based on the high ratios of dietary nitrogen isotopes, on absolute AMS dates obtained on human specimens from Mayaky (Petrenko et al., 2018). High $\delta^{15}N$ ratios have been shown to be a result of diet consisting of aquatic resources, freshwater or marine, which has been linked to a reservoir offset (RO) of various magnitude (e.g. (Bonsall et al., 2004, 2002, 2000; Cook et al., 2002, 2001; Lillie et al., 2009; Ramsey et al., 2014; Weber et al., 2016). At the same time, the RO at Mayaky has not been quantified due to the lack of contextual faunal remains and the AMS dates on human remains from Mayaky are currently viewed as anomalous (Manzura, 2020). Yet, in our opinion, dating of the Usatove culture still requires refining as the radiometric methods of ^{14}C analysis

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used to obtain radiocarbon dates on pottery and charcoal from Mayaky primarily in the 1960s-1980s, may not be as sensitive as the AMS technique used to date human remains (Povinec et al., 2009). Furthermore, the relative typology may not be a consistently reliable tool to infer chronology (e.g., (Lillie, 1998). We set out to obtain additional AMS dates and stable isotopes from human remains from Mayaky to refine the Mayaky site chronology, and the Usatove culture chronology in general, as well as to expand our understanding of the chronological sequence and the dietary preferences, of the users of the Mayaky complex.

2. Materials and Methods

2.1. The Mayaky complex

The Mayaky sanctuary and necropolis are part of the Mayaky archaeological complex in the Belyaiivsky District of Odesa Region in southeast Ukraine (46.39, 30.27) (Figure 1). The complex is situated at the southwest side of the toponymic village at the edge of a promontory on the east bank of the Dniester River near the confluence of the Dniester River and the Dniester Estuary (Zbenovich, 1967). An area approximately 110 by 30 m of the sanctuary was preserved at the time of the excavations in the 1960s and 1980s, with most of the rest having been destroyed by landslides. The sanctuary site was enclosed, and partitioned, by a network of interconnected ditches (Petrenko and Kaiser, 2011). All ditches at the Mayaky sanctuary were infilled in layers in ancient times, but not simultaneously. This is evidenced, in particular, by the silty/mud layer at the bottom of some, but not all, ditches (Petrenko and Kaiser, 2011). The equal deflection of the layers over the entire thickness testifies to the short duration of breaks in the accumulation of filling and, in fact, to the simultaneous subsidence of the entire fill of each ditch. The longest outer ditch ran along the southeast-northwest side of the site and into a riverbank on the eastern side. At the time of its original construction, the ditch was at least 110 m long, that is, it ran the whole length of the site. The ditch was 5 m wide, but widened to 6-8 m in places, and it was a maximum of 3.7 m deep. The finds from the ditch fill included large quantities of non-human osteological material, 20% of which were fish bones (Patokova et al., 1989) as well as various material culture items including Usatove-type pottery sherds, anthropomorphic figurines, and flint flakes (Patokova et al., 1989). It has been suggested that the use of the ditch by the Usatove people might have been connected with fish smoking, based on the finds of fires/open hearths at various depth, which are regularly spaced within the ditch, as these features were covered with ash, fish bones belonging to freshwater and brackish/anadromous species such as carp, catfish, sturgeon and zander, and fish scales (Petrenko and Kaiser, 2011; Zbenovich, 1974). Overall, the ditches at the Mayaky sanctuary, in addition to layers of ash and charcoal, contained an exceptionally large amount of Usatove material culture artefacts. On average, there are 600-650 finds per cubic meter of ditch filling (Petrenko and Kaiser, 2011). The finds of large quantities of material culture artifacts, fish and animal remains throughout the ditch network at the Mayaky sanctuary suggest that the site was a center of ritual activity (Petrenko, 2013).

The Mayaky necropolis was located 200 m to the north of the ditch network and covered an area of approximately 300 X 100 m. The necropolis contained a total of 19 groupings identified

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as either kurgans or ground burial arrangements (Patokova et al., 1989; Petrenko and Kaiser, 2011), Supplementary File S1, Figure S1). Overall, the necropolis contained 47 graves containing a total of 56 skeletons (Manzura, 2020).

Orientations of the Usatove-period burials at the Usatove-Velykyj Kuyalnik complex include interments in a contracted position, both on the back and on the left side, with the head to east and northeast. Mayaky burials are predominantly on the left side with the head to northeast (Patokova, 1979; Patokova et al., 1989). Daggers made of arsenical bronze such as those found as grave goods in association with main burials of the first kurgan group of the Usatove-Velykyj Kuyalnik kurgan necropolis (Patokova, 1979), suggest that those burials date to the 4th millennium BCE (Nikitin and Ivanova, 2022; Patokova, 1979).

In addition to the Usatove culture as the main component, burials from the Early Eneolithic (Novodanylivka type), early Bronze Age (Yamna and Catacomb cultures) as well as other cultures of the steppe Bronze Age have been discovered at Mayaky (Petrenko and Kaiser, 2011). The site also contains burials from the Greco-Scythian and Sarmatian periods as well as a field of ritual pits dated to the early centuries AD (Petrenko and Kaiser, 2011). Radiocarbon dates show that Mayaky was also used during the Middle Ages for burials in the Usatove-era kurgans (Petrenko et al., 2018).

2.2. Analyzed samples

Seven specimens from the Mayaky archaeological complex presented in this report came from the archeological excavations of the Mayaky necropolis in 1986 (Patokova et al., 1989), collected by V. Petrenko and one of the authors of the present study (IDP) and subsequently deposited to the Bioarchaeological Stores of the Institute of Archaeology, National Academy of Sciences of Ukraine, Kyiv, Ukraine. The remains of one sampled individual came from a burial uncovered during the 1965 excavations of the Mayaky sanctuary (Zbenovich, 1974, 1967) and subsequently deposited to the Bioarchaeological Stores. Mayaky individuals were sampled for molecular archaeology analyses at the Bioarchaeological Stores by one of the authors of the present study (AGN) and brought to the Reich Genetics Lab at Harvard University in 2014, under the permission from the Institute of Archeology and in accordance with all applicable laws of Ukraine governing the export of such archaeological materials. The specimens presented in this report were selected for radiocarbon and stable isotope analysis at the Pennsylvania State University Atomic Mass Spectrometry (PSUAMS) facility (Table 1) after they produced interpretable DNA data after a DNA sequencing screen. A detailed description of the burials can be found in (Patokova et al., 1989; Petrenko et al., 2018; Petrenko and Kaiser, 2011). Burial descriptions are also summarized in Supplementary File S1.

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Specimen ID	AMS ^{14}C Date, uncalBP, lab code; pre-treatment	Calibrated date[1], calBCE	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	C:N (atomic)
Kurgan 1 burial 9	4375±25 BP, PSUAMS-7865; XAD	3088-2911	-21.01	16.87	47.03	16.57	3.16
Kurgan 7 burial 2	5536±25 BP, weighted mean[2] of 5530±32 BP, OxA-22959[3], and 5545±40 BP, PSUAMS-7793; UF	4446-4340	-20.24	16.07	38.82	13.66	3.32
Kurgan 8 burial 4	5295±30 BP, PSUAMS-7862; XAD	4240-3997	-20.03	15.25	23.14	8.48	3.2
Kurgan 8 burial 6 skeleton 2	5330±40 BP, PSUAMS-7794; UF	4323-4047	-20.22	15.55	46.58	16.34	3.3
Kurgan 9 burial 2	5444±19 BP, weighted mean[2] of 5471±23 BP, OxA-22960[3], and 5400±30 BP, PSUAMS-7846; XAD	4346-4252	-19.77	16.37	27.46	10.02	3.2
Kurgan 10 burial 2 skeleton 2	5390±30 BP, PSUAMS-7845; XAD	4336-4065	-20.81	15.73	14.41	5.19	3.2
Sanctuary Burial 3	3445±25 BP, PSUAMS-7804; UF	1879-1642	-19.71	12.25	49.29	17.52	3.3
Kurgan 1 burial 9.1	2020±25 BP, PSUAMS-7792; UF	92 calBCE - 66 calCE	-16.5	11.36	47.27	16.91	3.26

Table 1. AMS ^{14}C dates and stable isotope $\delta^{13}C$ and $\delta^{15}N$ ratios obtained on human remains from the Mayaky archaeological complex. All specimens are from the Mayaky necropolis, except for Burial 3, which is from the Mayaky sanctuary. All specimen samplings were from bone. Estimated ages at death are presented in Supplementary File S1.

[1] Calibration was performed using IntCal20 calibration curve (Bronk Ramsey, 2009; Reimer et al., 2020).

[2] (Ward and Wilson, 1978)

[3] OxA dates are from (Petrenko et al., 2018).

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2.3. Radiocarbon Dating

Ratios of carbon isotopes ^{12}C, ^{13}C, and ^{14}C remain relatively constant during organismal lifetime. After the death of an organism, the radioactive ^{14}C decays at a predictable rate. Radiometric radiocarbon dating techniques measure the decay of radioactive carbon isotopes (^{14}C) in objects containing carbon-bearing material. Modern Atomic Mass Spectrometry (AMS) – based techniques quantify the number of atoms of the three carbon isotopes in a sample to produce an accurate date based on isotopic ratios of all three carbon isotopes. Radiocarbon dating is a useful dating tool frequently applied in archaeological studies to create and refine chronologies. Radiocarbon ages calculated from directly measured carbon isotope abundancies in carbon-containing samples are calibrated into calendar dates using calibration curves and software (Brock et al., 2010; Bronk Ramsey, 2009; Hajdas et al., 2021; Reimer et al., 2020).

For this study, radiocarbon dating was performed at Accelerated Mass Spectrometry facility at Pennsylvania State University (PSUAMS). Bone samples were processed in the Human Paleoecology and Isotope Geochemistry lab (Anthropology) following the preparation methods detailed in (Narasimhan et al., 2019) for >30kDa ultrafiltered gelatin (UF) or XAD hydrolyzed amino acids.

2.4. Reservoir Offsets

The weathering of old sedimentary rocks containing ^{14}C-free organic remains contributes to the overabundance of the two non-radioactive carbon isotopes in the environment. Water flowing over limestone or the upwelling of old organic carbonates from the deep ocean are among the major contributing factors to overabundance of "old" carbon in aquatic systems. Aquatic foodchains consequently become enriched with stable carbon isotopes, leading to the offsets on radiocarbon dating. Such reservoir offset (RO) in the East Eurasian steppe can range from hundreds to thousands of years for modern organisms (Svyatko et al., 2017). Human consumption of aquatic resources results in the presence of a RO and a need for a reservoir age correction to be applied to radiocarbon AMS-generated dates from human remains where aquatic diet can be inferred or documented through archaeological record. The presence of radiocarbon ROs in the northern Black Sea region, leading to apparent radiocarbon ages older than contemporary terrestrial dates, is well established, although the exact nature of the offset (in terms of predictable offset) remains locally uncharacterized (Bonsall et al., 2004, 2002, 2000; Budd et al., 2020; Budd and Lillie, 2020; Soulet et al., 2019).

2.5. Stable Isotope Analysis

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis of bone collagen samples are routinely used in archaeological studies to provide direct information about past human (and animal) dietary pathways (Makarewicz and Sealy, 2015). The analysis of carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) from bone collagen allows for a direct assessment of an individual's diet, particularly for the last *c*. 10 years of an individual's life (Hedges and Reynard, 2007; Schwarcz and Schoeninger, 1991). Carbon isotope measurements from bone collagen are biased towards

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the protein component of the diet, and as a result will exaggerate the contribution of animal products with respect to plant proteins (Ambrose and Norr, 1993; Jim et al., 2006).

Stable carbon isotope ratios are used to differentiate between dietary protein from marine, terrestrial, and in some circumstances, freshwater resources (Cerling et al., 1997; Eriksson et al., 2008; Schwarcz and Schoeninger, 1991). Plants species that use different photosynthetic pathways will produce markedly different carbon isotope values. Such as, those plants which follow the C_3 photosynthetic pathway (Calvin-Benson) often exhibit $\delta^{13}C$ values between − 32‰ and − 20‰, whereas plants that utilize the C_4 (Hatch-Slack) photosynthetic pathway, often fall within a comparatively enriched range of − 17‰ to − 9‰ (O'Leary, 1988). Nitrogen is only found in dietary proteins, and therefore nitrogen isotope values are especially sensitive to the protein component of diet (see Hedges and Reynard 2007 for summary). Nitrogen stable isotope ratios ($\delta^{15}N$) are used to establish the trophic level of an organism within the food web, with an approximate enrichment of *c.*3-6‰ ($\Delta^{15}N_{diet\text{-}body}$) (Hedges and Reynard, 2007; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984).

If the individuals at Mayaky were consuming a diet based entirely on C_3 terrestrial resources (plant and animal proteins), we would anticipate human isotope values around -19±1‰ for $\delta^{13}C$ and approximately 9±2‰ for $\delta^{15}N$ values. Evidence for the consumption of C_4 plant species in the region is limited, but their inclusion in the diet would result in comparatively elevated carbon isotopes values of above -18‰. For a diet formed entirely of freshwater proteins we would anticipate human $\delta^{13}C$ values of c. -22 ± 1‰ and $\delta^{15}N$ values at *c.* 12±1‰. If the diet were reliant on protein resources from brackish estuaries (living in nearshore and benthic environments), we would expect more elevated $\delta^{13}C$ and $\delta^{15}N$ values in human consumers, ca. -18 ± 1‰ and 15 ± 2‰ (Fuller et al., 2012; Guiry, 2019). Due to the location of the Mayaky site and existing dietary isotopes in the region, we anticipate the contribution of aquatic proteins in human diet (Budd and Lillie, 2020; Lillie et al., 2011).

Stable isotope analysis on pretreated >30kDa gelatin or XAD amino acids was performed at Yale Analytical and Stable Isotope Center (YASIC), following the methods detailed in (Narasimhan et al., 2019).

2.6. Statistical analysis

The Kolmogorov-Smirnov nonparametric test was performed using the AAT Bioquest online calculator at https://www.aatbio.com/tools/kolmogorov-smirnov-k-s-test-calculator. Statistical analysis of stable isotope data was generated using Microsoft Excel 2022 for Mac, version 16.63.1.

3. Results

The results of radiocarbon dating and stable isotope analyses are presented in Table 1. Five of the samples dated to 5561-5265 BP, or 4446-3997 BCE using tree ring-based calibration (Bronk Ramsey, 2009; Reimer et al., 2020), corresponding to the Eneolithic period in the East European

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prehistoric chronology (Harper, 2019). The individual from kurgan 1 burial 9 dated to 4400-4350 BP (3088-2911 calBCE), (Table 1) corresponding to the end of the Eneolithic chronology in the northwest Pontic steppe (Harper, 2019; Rassamakin, 2008). The Mayaky sanctuary burial dated to 3470-3420 BP (1879-1642 calBCE), corresponding to the Middle Bronze Age (Table 1). The bone assembly from kurgan 1 burial 9 (Burial 9.1) dated to 2045-1995 BP (92 calBCE-66 calCE), corresponding to the Late Iron Age (Table 1). The obtained dates thus appear to chronologically fall into three groups: one composed of the Eneolithic dates containing six samples, and one each belonging to the Bronze Age and Late Iron Age.

The stable isotope results for the Eneolithic group (n=6), $\delta^{13}C$ = -20.24±0.46‰ and $\delta^{15}N$ = 15.89±0.57‰, suggest a clear dietary reliance on freshwater and brackish aquatic proteins. Given the elevated nitrogen isotope values, alongside the non-human osteological remains found at the Mayaky sanctuary, it is likely that brackish/anadromous fish such as sturgeon and catfish formed the mainstay of the diet.

The Bronze Age specimen from the Mayaky sanctuary produced $\delta^{13}C$ ratios of -19.71‰ and $\delta^{15}N$ ratios of 12.25‰ suggesting a diet in which a significant proportion of the resources consumed were obtained from freshwater protein sources. The late Iron Age specimen produced $\delta^{13}C$ ratios of -16.71‰ and a $\delta^{15}N$ ratios of 11.36‰ potentially indicating a terrestrial based diet with the inclusion of C_4 resources. By the late Iron Age, the transition to C_4 species such as maize, sorghum or millet is evident in the archaeological record in eastern Europe (Filipović et al., 2020; Martinoia et al., 2021; Motuzaite Matuzeviciute et al., 2022).

To evaluate the difference in stable isotope ratios between the Eneolithic and Bronze Age populations at Mayaky, we combined the results from the Bronze Age sample with three previously published (Petrenko et al., 2018) Bronze Age samples from Mayaky. Carbon and nitrogen isotope ratios in the "Eneolithic" and "Bronze Age" group were then compared using the Kolmogorov-Smirnov (K-S) test. The difference was significant for both $\delta^{13}C$ and for $\delta^{15}N$, $p<0.0095$. There was only one additional Iron Age sample from Mayaky (Petrenko et al., 2018), thus the sample size was not sufficient for a meaningful statistical comparative analysis.

To examine dietary isotope ratios from Mayaky compared to stable isotopes from the rest of the Eneolithic-Bronze Age North Pontic steppe and adjacent areas, we combined isotope data from the Mayaky individuals presented in this report with published data from Mayaky, as well as contemporaneous populations of the North Pontic steppe and adjacent areas available from the literature (Supplementary File S2, Table S1b). The results are presented in Figure 2.

Dietary isotopes from the Eneolithic Mayaky did not cluster with any Eneolithic populations from the North Pontic region (Figure 2). The Bronze Age group from Mayaky clustered with the Eneolithic and Bronze Age pastoralists of the North Pontic steppe. The Late Iron Age specimen from this report and the Iron Age and the Middle Ages specimens from (Petrenko et al., 2018) were positioned distantly from the Eneolithic/Bronze Age group. In both Iron Age and the Middle Ages specimens, $\delta^{13}C$ ratios were more positive than any Eneolithic or Bronze Age values from the Pontic steppe.

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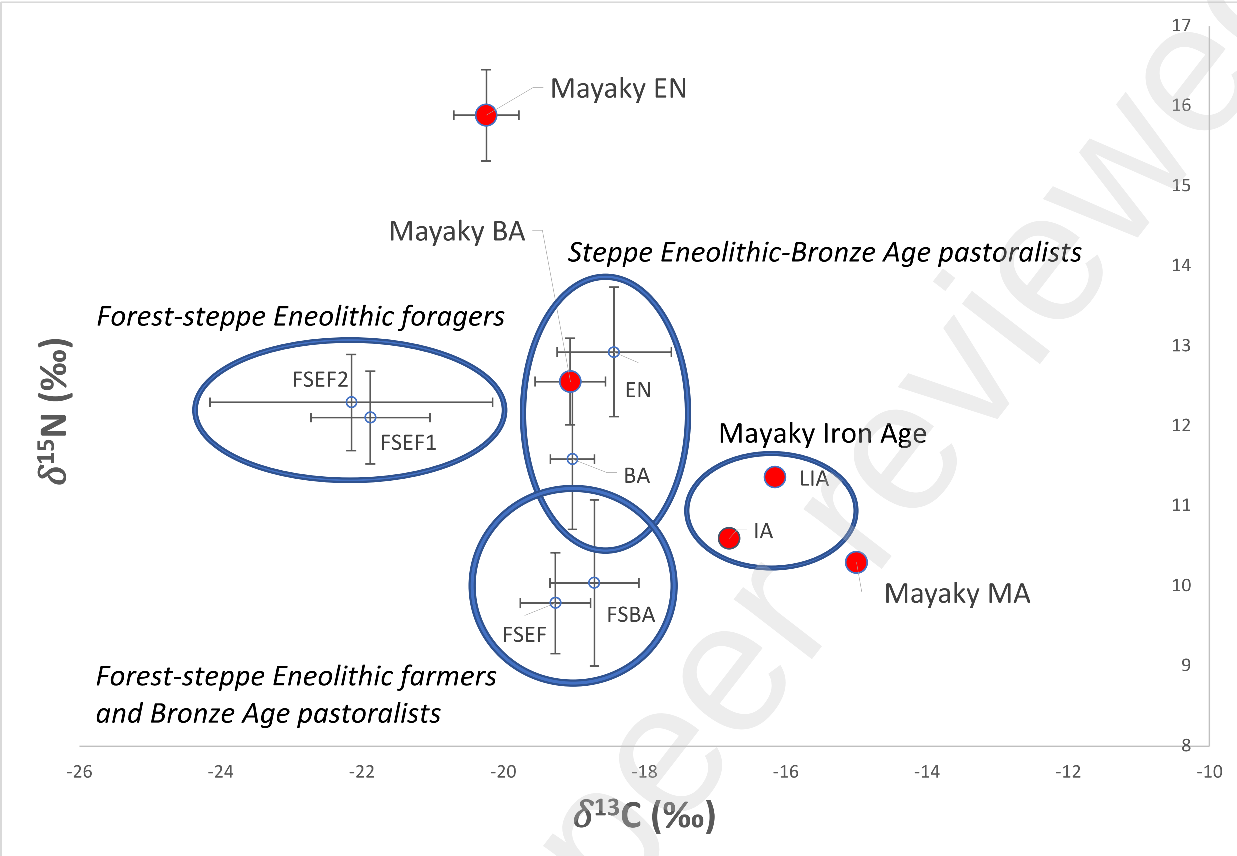

Figure 2. Comparison chart of stable isotope ratios obtained from human remains from the Mayaky archaeological complex and Eneolithic-Bronze Age populations of the North Pontic region. Stable isotope data used to construct the chart, along with sample sizes for each group as well as the data sources are presented in Tables S1b and S1d. Data points corresponding to specimens from Mayaky are shaded. FSEF, forest-steppe Eneolithic farmers (Trypillia, Middle Dniester); FSBA, forest-steppe Bronze Age pastoralists (middle Dniester); LIA, Late Iron Age; IA, Iron Age; MA, Middle Ages; BA – Bronze Age eastern Pontic steppe pastoralists; EN – Eneolithic eastern Pontic steppe pastoralists; FSEF1, FSEF2, Forest steppe Eneolithic foragers from Molukhiv Bugor and Deriivka I, respectively.

Neither the $\delta^{13}C$ nor $\delta^{15}N$ values for the Bronze Age group from Mayaky were significantly different from the Bronze Age group from eastern Pontic steppe (K-S p= 0.6247 and 0.6775 respectively). Stable isotope ratios from the Bronze Age Mayaky group were also not significantly different from those of the Eneolithic eastern Pontic steppe pastoralists: $\delta^{13}C$ K-S p= 0.4643; $\delta^{15}N$ K-S p= 0.3752. $\delta^{13}C$ ratios in the Bronze Age Mayaky group were not significantly different from those of the Bronze Age forest-steppe pastoralists from the middle Dniester (K-S p= 0.7085). However, the $\delta^{15}N$ ratios were different between the two groups (K-S p= 0.039).

4. Discussion

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The results obtained in the current study expand the understanding of the chronological history of the Mayaky complex. When combined with already published data on the Mayaky site, it becomes evident that the site was used for ritual purposes during the Eneolithic, Bronze and Iron Ages, as well as during the Middle Ages, thus being one of the longest-used burial sites in southeast Europe (Figure 3).

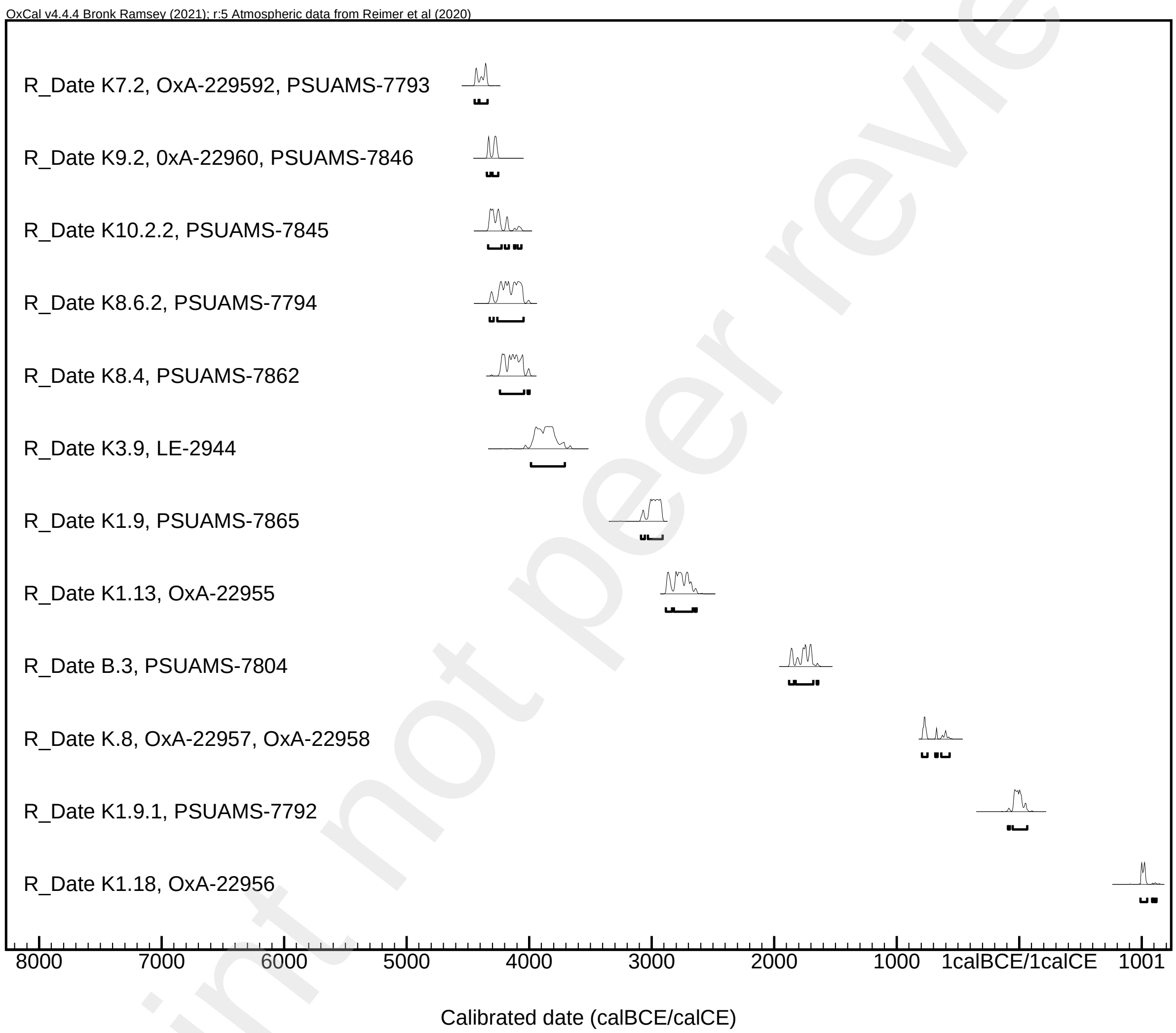

Figure 3. Multiplot of the AMS radiocarbon dates from human remains from the Mayaky archaeological complex, using new and previously published (Petrenko et al., 2018) data. The multiplot was constructed using OxCal v4.4.4 and IntCal20 calibration curve (Bronk Ramsey, 2009; Reimer et al., 2020).

After adding the previously published AMS dates from human bone to the Eneolithic dates from human remains from the Mayaky necropolis presented in this report, it becomes apparent that most of the human radiocarbon dates pre-date the conventional Usatove chronological

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periodization, which is primarily based on radiometric dating of material from the ditches at the Mayaky sanctuary, as well as comparative pottery analysis. In fact, of the seven available ^{14}C AMS dates on human remains from Mayaky, only one, Kurgan 1 burial 9, fits into the currently accepted Usatove chronology (Supplementary File S1, Figure S11). The other six Eneolithic samples fall within the 5561-5020 BP (4446-3709 BCE) chronological period. At the same time, the pottery/charcoal/animal bone ^{14}C analysis place Usatove in the 4780-4090 BP (3651-2476 BCE) chronological period (Supplementary File S1, Figure S11; Supplementary File 2, Table S1a), coinciding with the CII stage of the Trypillian chronology and corroborating comparative analysis of pottery from Usatove sites. Yet all specimens from the Mayaky site presented in this report, and those from previously published reports that produced Eneolithic dates, were archaeologically identified as belonging to the Usatove culture (Patokova et al., 1989; Petrenko et al., 2018). There is not much difference in the burial positions of those interments identified as Usatove, although the burial inventory and the degree of contraction in the burial position are somewhat different (Supplementary File S1). In the current report, only those specimens producing interpretable DNA data in a whole-genome screening assay (the results to be presented in a separate report) were chosen for radiocarbon analysis. This implies that these samples were in an overall better state of preservation. Perhaps the chronologically earlier Eneolithic burials at Mayaky were, overall, better preserved than the later-Eneolithic burials. At the same time, the reason for the earlier Eneolithic samples being better preserved than the later period Eneolithic samples remains unclear.

The existence of “archaic” (pre-Trypillia CII period) stage of Usatove has previously been suggested (Petrenko, 2013). Radiocarbon date on human remains archaeologically assigned to the Usatove period from Mayaky, obtained from the Leningrad Radiocarbon Lab in 1985 (ЛЕ-2944, 5080±60 BP), was considered anomalously old and was previously excluded from the evaluation of Usatove chronology (Petrenko and Kaiser, 2011). Taken together, the late 5th millennium Eneolithic dates obtained on human remains, whether or not affected by a RE, as well as the finds of Trypillian A-BI period ceramics at Mayaky (Petrenko, 2009), corroborate the existence of proto-Usatove burials at the Mayaky complex, not readily distinguished in the field from the late Eneolithic Usatove burials.

Stable isotope data from the Eneolithic phase at Mayaky, especially the $\delta^{15}N$ ratios, stand in isolation from the other North Pontic steppe specimens available in the literature. Comparable to the Eneolithic Mayaky $\delta^{15}N$ ratios can be found on the eastern side of the Ponto-Caspian steppe in the early Yamna population of Sharakhalsun (Knipper et al., 2020), located in the Manych steppe ecozone in the North Caucasus foothills and displaying close ecological and climatic parameters to the North Pontic steppe. Stable isotope results from Eneolithic and EBA individuals from the North Pontic and North Caucasus steppe have been shown to have similar values in previous studies (Gerling, 2015). The Manych Yamna averaged 15.88±1.57‰ for $\delta^{15}N$, compared to 15.97±0.59‰ for the Eneolithic Mayaky (Table S1b). At the same time, the Manych Yamna produced more enriched $\delta^{13}C$ ratios compared to the Eneolithic Mayaky, with -17.13±0.65‰ for the Manych Yamna vs. -20.35±0.47‰ for the Eneolithic Mayaky. Among the reasons for the higher $\delta^{13}C$ and $\delta^{15}N$ values in the prehistoric Manych steppe inhabitants, the consumption of meat from unweaned animals, the presence of C4 plants with elevated $\delta^{13}C$

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values as well as plants with elevated $\delta^{15}N$ levels such as reeds have been suggested (Knipper et al., 2020). In addition, a hot and arid environment in combination with elevated salinity in soils were listed as environmental contributors to elevated $\delta^{15}N$ ratios in the Manych steppe pastoralists (Knipper et al., 2020).

The consumption of fish from brackish waters has been suggested to be a significant factor contributing to high $\delta^{15}N$ and variable $\delta^{13}C$ ratios in ancient humans from the Manych steppe (Knipper et al., 2020). Aquatic dietary resources are a major contributor to RE (Lanting and Van Der Plicht 1998), and fish was a significant element of the diet at Mayaky. Fish remains were abundant in the ditches of the Mayaky sanctuary. Fish bones compose 20% of all osteological remains at the Mayaky sanctuary (Patokova et al., 1989). A recent paleozoological analysis revealed that the dominant fish species that could be identified at Mayaky were Cyprinidae at 21.58% of total fish remains, followed by catfish at 16.1% and sturgeon at 8.51%. Other fish species that could not be identified comprised 49.24% (Petrenko and Kaiser, 2011). An earlier study determined that the dominant fish species at Mayaky were catfish, zander, and sturgeon, altogether comprising 85.6% of all the fish species found within the sanctuary (Zbenovich, 1974). The catfish specimens were of a particularly large size, reaching 2,7 m in length (Zbenovich, 1974). It should be taken into account that only a few fish species contain bones large enough to be archaeologically preserved and that an accurate ichthyoarchaeological quantification may not be possible without sediment sifting (Morales-Muñiz, 2014), which, to our knowledge, was not performed at Mayaky. Furthermore, remains of cartilaginous fishes such as sturgeon consist primarily of boney scutes, which can obscure the overall abundance of such fishes in zooarchaeological examinations. Therefore, previous paleozoological studies may have underestimated the actual proportion of fishes among zoological remains at Mayaky. Fish species composition at Mayaky suggests that fishing took place mainly on the Dniester and the brackish estuaries. While we cannot directly establish that fish remains from the Mayaky sanctuary ditches date to the Usatove period, the finds of mostly Usatove-type pottery in the fill of the ditches corroborate the Usatove association with fish remains.

Dietary isotope studies undertaken at the Copper Age sites of Durankulak and Varna I (Honch et al., 2006), provide a comparison base for the isotopic data in our report. Durankulak and Varna I are located on the west Pontic coast, in very similar environmental settings to Mayaky. The analysis of $\delta^{13}C$ bone collagen at Durankulak and Varna I from wild species (including *Equus*, *Cervus elaphus* and *Vulpes vulpes*) at -19.9±1‰ and domesticate species (including Bos sp., Canidae, and Ovicaprid) at -19.9±0.6‰, are typical for a C_3 terrestrial environment, and the values show limited variability between wild and domestic species. Nitrogen isotope values for wild species, recorded at 5.8±2.4‰, were slightly lower than those recorded for the domestic species at 7.5±1.8‰. One marine sample (marine turtle of unidentified species) produced a $\delta^{13}C$ value of -14.1‰ and a $\delta^{15}N$ value of 14.8‰, giving some indication of isotope values of the marine ecosystem within the Black Sea in the past. Carbon and nitrogen isotope values observed in marine foods are often elevated compared to terrestrial and freshwater ecosystems, due to extended food webs (Guiry, 2019).

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Dietary isotope studies of Eneolithic fishing populations located in the steppe and forest-steppe regions of the Dnipro (Dnieper) River, indicate similar $\delta^{13}C$ baselines of *c.* -20±2‰ for terrestrial fauna (wild species including *Cervus elaphus* (red deer), *Alces alces* (elk), *Capreolus capreolus* (roe deer), and *Sus scrofa* (wild pig), and $\delta^{15}N$ ratios at *c*. 6±1‰ (Budd et al., 2020; Budd and Lillie, 2020; Lillie et al., 2011). Freshwater isotope studies of Neolithic prehistoric populations in the Lower Dnipro Valley have produced a wide range of $\delta^{13}C$ values from freshwater fish bone collagen, ranging from -25‰ at Yasinyvatka, to -17‰ at Deriivka I, with nitrogen isotope values most often observed between 10 -14‰ (Lillie et al. 2011). Similar values are observed at prehistoric sites in the Iron Gates region, with fish collagen samples most commonly producing nitrogen isotope values at *c*. 10 ± 1‰ (Bonsall et al., 2004, 2002, 2000). These values are mirrored by research undertaken by Robson et al. (Robson et al., 2016), whose analysis of freshwater and brackish species (including pike, perch, and zander), produced $\delta^{13}C$ of − 24.2‰ to − 19.3‰, and $\delta^{15}N$ values of 5 to 10‰. However, some aquatic species, particularly cyprinids, produce noticeably lower bone collagen $\delta^{15}N$ values of *c*. 6 ± 1‰ (Naito et al., 2013; Schmölcke et al., 2016).

Of the domestic animal remains at the Mayaky site, sheep/goat dominated the assemblage (76.2%) followed by cattle (12.7%) and horse (11.1%) (Zbenovich, 1974). No domestic pig remains were recovered from the site. Wild species assemblage at the site was dominated by red deer followed by European wild ass, and included aurochs, wild boar, fox, and lion (Zbenovich, 1974). One cattle (*Bos taurus*) sample previously analyzed for carbon and nitrogen from Mayaky produced values of $\delta^{13}C$ = -18.7‰ and $\delta^{15}N$ = 6.7‰ (Petrenko et al., 2018). A cattle sample from the Eneolithic-Bronze Age site of Căplani in Moldova, across the Dniester River from Mayaky, produced $\delta^{13}C$ = -19.21‰ and $\delta^{15}N$ = 5.96‰ (Table S1c). The isotope values from both sites are in-keeping with cattle grazing on a C3 terrestrial landscape. The cattle sample from Mayaky, at $\delta^{13}C$ = -18.7‰, could be indicative of a small inclusion of C4 grasses in the diet, but it is not possible to accurately confirm this possibility with a single sample. $\delta^{13}C$ ratios of both the Căplani and Mayaky cattle samples are more positive than the average $\delta^{13}C$ for the Eneolithic human samples at Mayaky (-20.24±0.46‰), while their $\delta^{15}N$ ratios are more than 6‰ below the average human values (15.89±0.57‰), suggesting a limited input of meat derived from locally raised cattle in the diet of Eneolithic users of the Mayaky complex. Therefore, the Căplani and Mayaky cattle isotope ratios provide little utility in estimating a potential RO on AMS dates from Mayaky.

Taken together, the presented data indicate that while the animal species composition suggests a primarily terrestrial-based contribution to the diet of the Eneolithic population at Mayaky, the $\delta^{13}C$ and $\delta^{15}N$ isotope ratios are consistent with a considerable input of freshwater/brackish resources into the diets of the Eneolithic Mayaky group. This evaluation, combined with the abundance of freshwater/brackish fish remains at Mayaky, establishes a tentative link between fish consumption by the Eneolithic population at Mayaky and any potential RE that may offset the dates from the Eneolithic human remains. At this stage, however, the direct evidence for RE at Mayaky is lacking beyond the available isotope signatures. As discussed above, there is the potential for a freshwater RO on the absolute dates of the Eneolithic individuals at Mayaky, due to the freshwater resource-based subsistence of that group. However, in the absence of

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contextual animal samples from Mayaky available for AMS dating and stable isotope analysis, it is not possible to fully investigate the presence or absence of the RO at the Mayaky site. Despite this, given the date range for the earlier burials, even allowing for a 545±70-year offset, observed in the Mesolithic populations of Iron Gates with subsistence based on freshwater aquatic protein as well as anadromous/brackish fish from the Black Sea (Cook et al., 2009), the adjusted dates for a number of Eneolithic individuals at Mayaky would still largely fall within the chronological period that predates the upper range of the established Usatove chronology at ca. 4780 BP (3650 BCE), further supporting the presence of a proto-Usatove phase at Mayaky.

Dietary isotopic ratios of the Bronze Age Mayaky individuals differed significantly from the Eneolithic users of the Mayaky necropolis. This is in line with previous studies that reported significant differences in stable isotope ratios between chronological and cultural groups at Mayaky (Petrenko et al., 2018). At the same time, the Mayaky Bronze Age isotopic ratios were similar to dietary isotope ratios of the Eneolithic-Bronze Age pastoralists from the eastern part of the Pontic steppe (Gerling, 2015). It is difficult to say, given the current state of knowledge, whether steppe pastoralists from the east replaced the Eneolithic population at Mayaky, but it is apparent that dietary preferences of the pastoralist inhabitants of the eastern Pontic steppe became dominant at Mayaky during the Bronze Age. The significantly different $\delta^{15}N$ ratios between the Bronze Age group from Mayaky and contemporaneous forest-steppe pastoralists from the middle Dniester area could be the result of a greater reliance on agricultural crops in the forest-steppe zone when compared to that in the northwest Pontic steppe area.

Progressively more positive $\delta^{13}C$ ratios are evident in the Mayaky specimens from the Bronze Age towards the Iron and Middle Ages. At the same time, $\delta^{15}N$ ratios became progressively lower, indicating shifts in diet in subsequent chronological periods. The diet isotope ratios of the Iron Age Mayaky users were similar to those of the Scythian Iron Age groups in the eastern part of Ukraine, at sites such as Bil'ske Horodyshche, Medvin and Mamaj Hora (Ventresca Miller et al., 2021), particularly with respect to more positive $\delta^{13}C$ ratios. More positive $\delta^{13}C$ ratios in the Scythian populations of Ukraine have been interpreted as a result of the increased consumption of millet (Ventresca Miller et al., 2021). At Mamaj Hora, which appears to have the closest diet isotope ratios to the Iron Age Mayaky population, fish may have been a large part of the diet (Ventresca Miller et al., 2021).

5. Conclusion

Using new and published ^{14}C and stable isotope data from Mayaky, we show that the users of the Mayaky complex experienced shifts in diet from the Eneolithic to the Middle Ages. The RE offset on absolute ^{14}C AMS dates obtained on the Eneolithic human remains from the Mayaky complex is a likely factor in the discrepancy between those dates and the established Usatove chronology. At the same time, presented evidence indicates that there are pre-Usatove or "archaic" proto- Usatove burials at Mayaky that have been preferentially dated due to a comparatively better state of preservation among the available anthropological specimens from the Mayaky complex. Newly obtained dates as well as published data show that the Mayaky complex was used in the Eneolithic and throughout the Bronze and Iron Ages. Then,

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after at most a *ca*. 900-year break, the Mayaky necropolis site was used again as a burial ground during the Middle Ages. Overall, Mayaky is a unique monument of Ukrainian cultural history that contains a record of human activity spanning thousands of years. While some issues persist in terms of the absolute dating of this complex, there is significant potential for further research in the past lifeways of different culture groups at Mayaky.

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Supplementary material: Supplementary File S1, Burial Descriptions and Supplementary Figures; Tables S1a-d.

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New radiocarbon and stable isotope data from the Usatove culture site of Mayaky in Ukraine

Nikitin AG, Ivanova S, Culleton BJ, Potekhina I, Reich DE

Supplementary File S1.

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Descriptions of burials and associated finds from the Mayaky archaeological complex

(after Patokova et al., 1989; Petrenko et al., 2018; Petrenko and Kaiser, 2011; Zbenovich, 1967). Radiocarbon dates with the PSUAMS lab code - this report, OxA and ЛЕ lab codes – Petrenko et al., 2018. Images are reproduced from (Patokova et al., 1989) with the permission from authors.

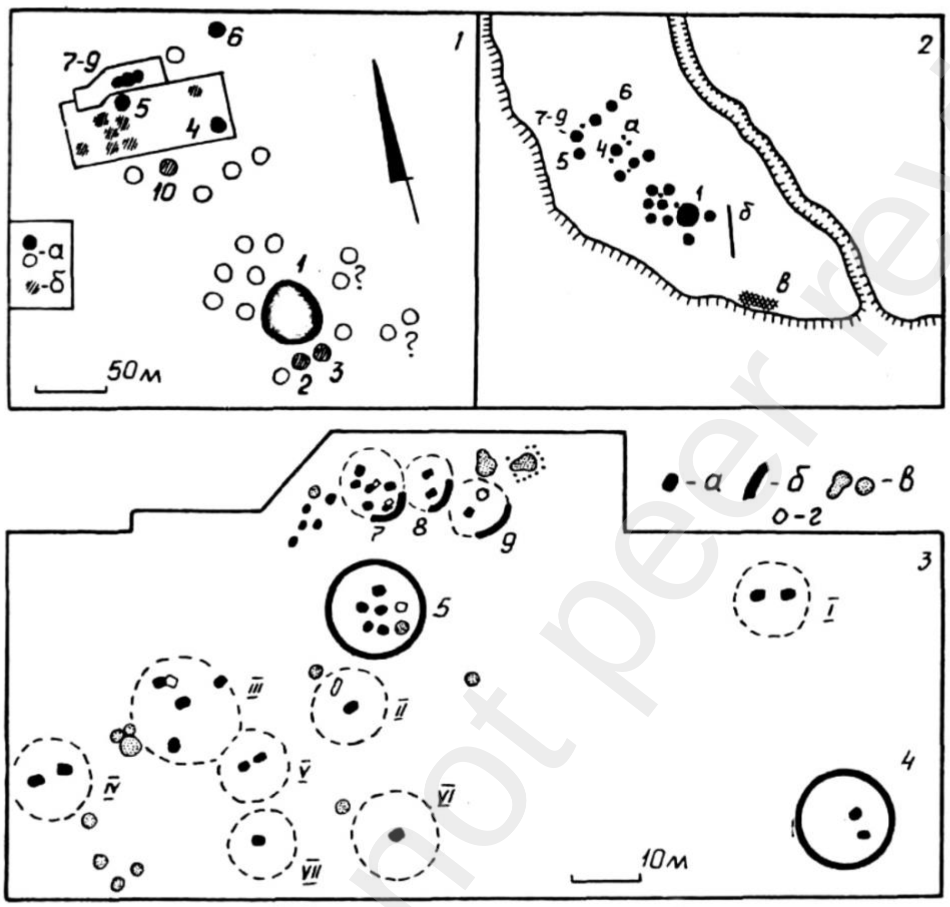

Figure S1. Layout of the Mayaky archaeological complex (after Patokova et al., 1989)**.**
1. Layout of the burial ground according to the excavation data and a topographic survey. *а*, kurgans and burial groups under possible kurgans. *б*, non-kurgan graves and grave groups. 2. Layout of the complex according to aerial photography. *а*, dwellings and pits of the first centuries AD. *б*, ancient dirt road. *в*, sanctuary. 3. Excavation plan, 1974-1975 and 1986. *а*, Usatove-designated burials. *б*, ditches. *в*, pits of the first centuries AD. *г*, non-Usatove inlet burials. Two additional burial groups (not shown) were located to the north of Kurgan 6 (Petrenko and Kaiser, 2011).

Specimens from the Mayaky archaeological complex presented in this report came from the archeological excavations of the Mayaky necropolis in 1986 (Patokova et al., 1989), collected by V. Petrenko and one of the authors of the present study (IDP), and subsequently deposited to the Bioarchaeological Stores of the Institute of Archaeology, National Academy of Sciences of

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Ukraine, Kyiv, Ukraine. The remains of one sampled individual came from a burial uncovered during the 1965 excavations of the Mayaky sanctuary (Zbenovich, 1974, 1967) and subsequently deposited to the Bioarchaeological Stores. Seventeen Mayaky individuals were sampled for molecular archaeology analyses at the Bioarchaeological Stores by one of the authors of the present study (AGN) and brought to the Reich Genetics Lab at Harvard University in 2014, under the permission from the Institute of Archeology and in accordance with all applicable laws of Ukraine governing the export of such archaeological materials.

Sanctuary burials at Mayaky

Burial 3, female, 1879-1642 calBCE (3445±25 BP, PSUAMS-7804)

A Bronze-Age burial was excavated by during 1965 excavations of the Mayaky sanctuary by V. G. Zbenovich. The burial was found in the center of the western edge of exploratory trench at a depth of 0.92 m from the modern surface. The interment was placed in the mainland yellow loess (edges of the pit were not traced) contracted on the back. The legs of the interred individual were raised and bent at the knees, the arms were extended along the body, the skull was slightly tilted to the right. The orientation of the skeleton was northeast. The burial contained no funeral inventory.

Burial G5, male (18-25), 2800-2500 BCE

Grave G5 was uncovered in 2009 while digging into the Mayaky sanctuary layer. The soil reached down to the "sterile" clay. The backfill was solid and gray, like the rest of the Usatove layer in excavation section 4. Numerous finds typical of the Usatove culture were found in this backfill. The oval burial pit (1.35 × 0.8 m) was aligned to SW-NE. The walls were only up to 0.15 m high. A young male (*adultus*) laid contracted on the back, slightly turned to the left, with the head to the northeast and facing south, right forearm bent at right angles and tucked under torso, right hand grasping left forearm, which was more gently flexed, and left hand resting on pelvis. Leg bones were not preserved *in situ*. The faint reddish discoloration of the long bones, ribs and skullcap is probably due to ocher. Under the skeleton there were traces of an organic mat. Behind the back there was a vessel lying on its side. Reddish-brown pieces of ocher that laid behind the left upper arm bone and a fragment of an animal's mandible next to the left ribs were found near the bottom of the grave.

The vessel (175 mm in height) from tomb G5 was handmade. It was thick-walled, coarse-surfaced, but finely formed and well-proportioned with a high funnel-shaped neck, bulbous, with bulging shoulders and a small flat base. Two vertically perforated cylindrical eyelets ("amphora") were attached to the shoulders. The surface was smooth and evenly colored beige and light gray. The body was dark gray with tempered chamotte and sand.

In terms of cultural chronology, Grave G5, despite not being associated with a kurgan, is assigned to the late Yamna culture of the northwestern Black Sea region. Contracted on the back burial position, rectangular and oval burial pits, organic substrates and the use of small amounts of ocher are typical of the Yamna burial in the region. However, all known burials of the named type of the Yamna culture, in contrast to grave G5, were placed in kurgans. Unfortunately, no convincing comparisons could be found for the "amphora" from grave G5 in

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this group. Grave G5 is dated to the Early Bronze Age, namely to the period that directly follows the Usatove culture, which is also supported by the stratigraphic situation.

Necropolis burials at Mayaky

Burial K8, female, 795-569 calBCE (2545±19 BP) (weighted mean (Ward and Wilson, 1978)*: (2526±25 BP; OxA-22957); (2565±26 BP; OxA-22958)].*

Burial K8 was found in a shallow grave that was laid out in the south-western sector of the burial ground. Its upper part was found below the humus horizon 0.45 m below the present surface (13.88 m elevation). The burial pit had been dug into the yellow-gray sandy clay and was partly destroyed by the quarry. Therefore, the southern wall of the grave could not be reliably traced in profile as when excavating the top 0.35-0.40 m of backfill. The backfill consisted of a light-colored, humus-rich substrate, which contained many lime inclusions, was extremely compact and overall gray in color, but took on a yellow-brown color closer to the south wall, reminiscent of a transitional horizon. The burial chamber stretched WSW to ENE, had long parallel walls, the east end was rounded. The pit was 0.65 m wide and at least 1.7 m long (1.3 m was preserved). The height of the walls could be traced up to 0.5 m. The floor was 0.96 m below the surface. It was level but rose slightly towards the south side. The skeleton belonged to an adult female, around 50 years of age. The interred was laid extended on the back, the skull pointing ENE. The arms were bent to varying degrees, stretched slightly away from the shoulders and positioned asymmetrically to the body. The leg bones could only be uncovered up to the knees, the rest of the burial pit collapsed during the excavation. A tiny flake of patinated flint was found in the filling, as well as a slab of dark red ocher on the ground to the right of the skull, while otherwise no remains of ocher were observed in the grave or on the bones.

Kurgan 1

Kurgan 1 was located in the southern part of the necropolis. The kurgan was 30-50 m in diameter, covered with limestone fragments, probably from a cromlech destroyed by a plow. The mound stood 1.1 m in height from the bottom of the hollows in the place of a ditch around the embankment. The height of the mound from the horizontal surface of the field was 0.75 m. The mound's foundation, 0.3 high and 15 m in diameter, made of dense loam, probably carefully compacted during laying, was preserved under the plowed layers of the embankment. The chernozem buried under the embankment began at a depth of 0.65 m from the reper (central landmark). Limestone stones laid in a row at the same depth in the northern field of the mound, probably represent remnants of the destroyed cromlech or some other structure. The ditch in profile resembled a funnel and was filled with humus loam. It was 35 m in diameter, 3.2 m wide at the top and 1 m at the bottom, and sunk into the loam 2.3 m below the ancient surface. The mound was erected over the Usatove burial 9 and the cult pit 1, and later was used for burials of other cultures.

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Kurgan 1 Burial 7, male (18-25), 3300-2500 BCE

The grave of burial 7 (Yamna culture) had an oval shape, measured 1.8 < 1.1 m and was located at a depth of 0.35 m. A young male was buried on the back, facing east, on the level ground, his originally erected legs had fallen to his left, hands in front of his face. Near the skull there was a 150 mm long bone tube (10 mm in diameter), which had traces of ocher on the outside.

Kurgan 1 Burial 9, female, 40-45, 3088-2911 calBCE (4375±25 BP, PSUAMS-7865)

Kurgan 1, Burial 9.1, female, 92 calBCE - 66 calCE (2020±25 BP, PSUAMS-7792)

The northeastern part of the grave was destroyed by a modern dugout. The rectangular grave pit (2.2 × 1.3 m) was filled with loam and chernozem. Above the skeleton (Burial 9, Figure S2, e) laid wooden ceiling planks that collapsed into the grave after it was visited by the robbers who penetrated through the hole they made in the western part of the burial. Only the bones of the legs of a crouched skeleton, which was oriented from the left to the east, and the decay of the bedding remained in place. The bones of the pelvis were shifted and the cranium was laid on them. The rest of the bones (Burial 9.1) were in disarray at the east wall of the grave. The skull had streaks of dark purple paint. Ceramic vessels, a bowl and a pot with a pattern of cord and staple imprints found in the grave contained crushed shells admixture and had mottled surface of dark gray and light brown colors.

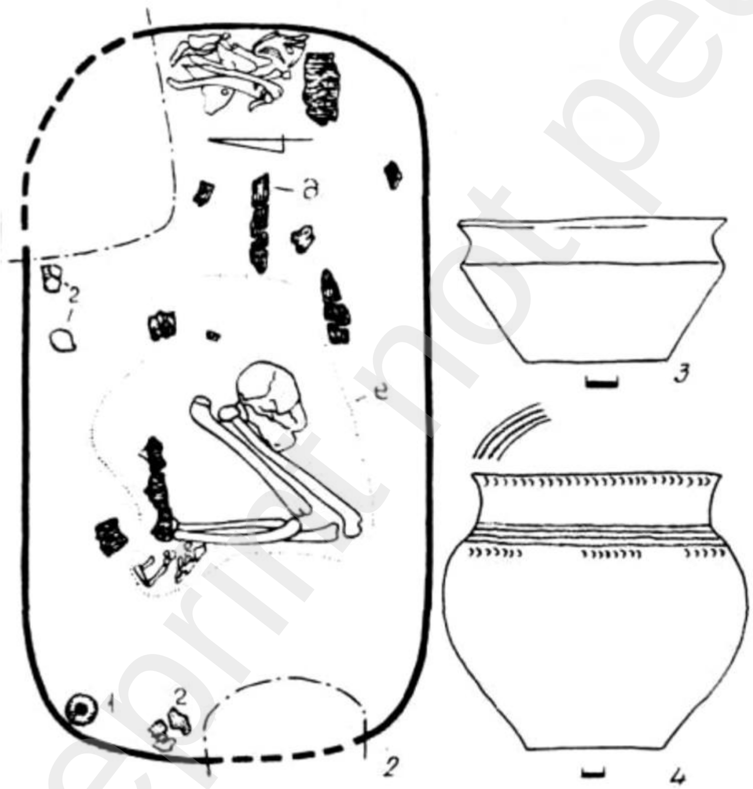

Figure S2. Kurgan 1, Burials 9 and 9.1 (after Patokova et al., 1989).

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Kurgan 1 Burial 13, female (18-25), 2885-2635 calBCE ((4175±28 BP, OxA-22955)

Burial 13 (Yamna culture) was located in the central area of the mound at a depth of 0.45 m, 1.2 m southwest of the central zero point. It was a rectangular pit with rounded corners, measuring 1.70 × 0.95 m at the top and slightly widening to 1.8 × 1.0 m at the bottom. The ground was level and at a depth of 1.7 m, the tomb walls were traced to a height of 1.25 m. The upper part of the burial pit was probably destroyed by the plow, along with the covering of the tomb. The former existence of a lateral shoulder in the upper area cannot be ruled out. Remains of the wooden covers were found in the loamy grave filling. The latter was very compact, which is why the excavators initially assumed that the grave pit had been deliberately closed in the sense of a kind of sealing. However, the remains of the wood in the backfill speak against this, as they indicate that the earth sagged into the pit. On the bottom of the grave the skeleton of a young female was laid, head to the southwest. The legs were bent and laid on the left side. The left arm was slightly bent, the hand was on the pelvis. The right forearm was not preserved. Traces of ocher were found on the skull.

Kurgan 1 Burial 15, male (mature), 3300-2500 BCE

Burial 15 (Yamna culture) was placed in the first embankment over the primary grave. The 1.5 × 1.1 m burial pit had a rectangular shape with rounded corners. The bottom was quite uneven, the depth varied between 0.70-0.85 m, measured from the central zero point. The straight walls could still be traced between 0.2 and 0.3 m in height. The deceased, a mature male, had been laid on his back with a slight twist, with the head to the north. The arms were only slightly bent and laid parallel to the body. The formerly erected legs had fallen to the left. Fragments of an S-shaped vessel (100 mm in height) with a rough, uneven, gray surface were found in the grave. The shards were tempered with fireclay and showed white mineral inclusions.

Kurgan 1 Burial 18, female (40-60), 974-1040 calCE (1020±24 BP, OxA-22956)

Burial 18 was discovered at a depth of 0.82 m, 6.5 m southeast of the reper, in a 1.2 × 0.9 m rectangular pit. The walls were preserved up to a height of 0.58 m. The bottom was found at a depth of 1.4 m. The skeleton was well preserved and belonged to a middle-aged female. It was lying on the right side with the legs drawn up, the head facing south-west. The legs were strongly bent at the knees and they laid at a right angle to the hips. The right arm was stretched out, the hand was on the knees. The left arm was bent, the hand was on the right forearm. Traces of ocher were found on the skull. Two vessels were placed on the right side of the deceased; a single-handled vessel stood by the skull and a cup-shaped container by the shoulder. The latter had asymmetrical, non-uniformly shaped walls. The rim was irregularly shaped, tapering and curving inwards. The outer surface of the gray-walled vessel (75 mm in height, base diameter 42 mm, body diameter 72 mm) was covered with an ocher slip.

The single-handled vessel had an S-shaped profile. The handle, which was oval in cross-section, connected the funnel-shaped neck with the shoulder of the vessel, the bottom was flat. The rim was flattened and slightly incurved. The sides of the handle were decorated with finger prints. The walls of the vessel, covered with a light beige engobe, were 7-8 mm thick. The fracture showed a mixture of chamotte and, probably, little limestone. It was also partly found

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on the wall. Horizontal grooves can be seen on the neck, which result from smoothing. The vessel (height 104 mm, rim diameter 115 mm, body diameter 108 mm, base diameter 60 mm) has been carefully fired, but there were also traces of a secondary fire on the base and below the handle.

Archaeologically, Burial 18 was assigned to the Bronze Age. It is possible that the sample sent for radiocarbon dating was mixed up with bone material from another grave or that the anthropological material was already mixed up when the inventory was made. At the same time, other burials from the Middle Ages (such as Burial 10) were discovered in Kurgan 1.

Burial group (kurgan) 3, Burial 9, female (20-25) and infant (ca. 1), 3986-3709 calBCE (5080±60 BP, ЛЕ-2944)

Burial group (kurgan) 3 was a 0.3 × 10 m chernozem hillock. Burials archeologically assigned to the Usatove culture (1, 2, 4, 5, 7, 9-12) were filled with unusually dense yellowish-gray loam.

Burial 9 was located in an oval pit 1.9 × 1.6 m. The bottom of the pit was at a depth of 1.37 m from the surface. The walls were sheer, traced to a height of 0.57 m. In the northeastern wall there was a step 0.37 m high from the bottom of the pit. The burial contained two individuals. Skeleton I (female, 20-25 years old) was laying crouched on the back with a turn to the left side, skull to the east (95"). The right arm was bent at the elbow at a right angle, the forearm laid across the abdomen. The left arm was probably also bent, the forearm laid across the chest. With the back of the head to the woman, on the left side of her, a one-year-old child (skeleton II) was buried, crouched on the right (?) side, the cranial crown towards the east. Burial inventory was represented by three stucco vessels, spotted, gray-brown, with crushed shell admixture. Side by side stood a so-called amphora, with traces of burnishing and a pattern of cord and round stamp imprints, with an imprint of a mat on the bottom, and an unornamented bowl. Among the tibia bones of skeleton I, a fragmentary miniature rectangular bowl with notches along the rim was found.

Radiocarbon analysis was conducted at the Leningrad Radiocarbon Lab in 1985.The material for radiocarbon analysis, 300 g total, was composed mostly from the bones of skeleton I with an addition of some tubular bone fragments from skeleton I.

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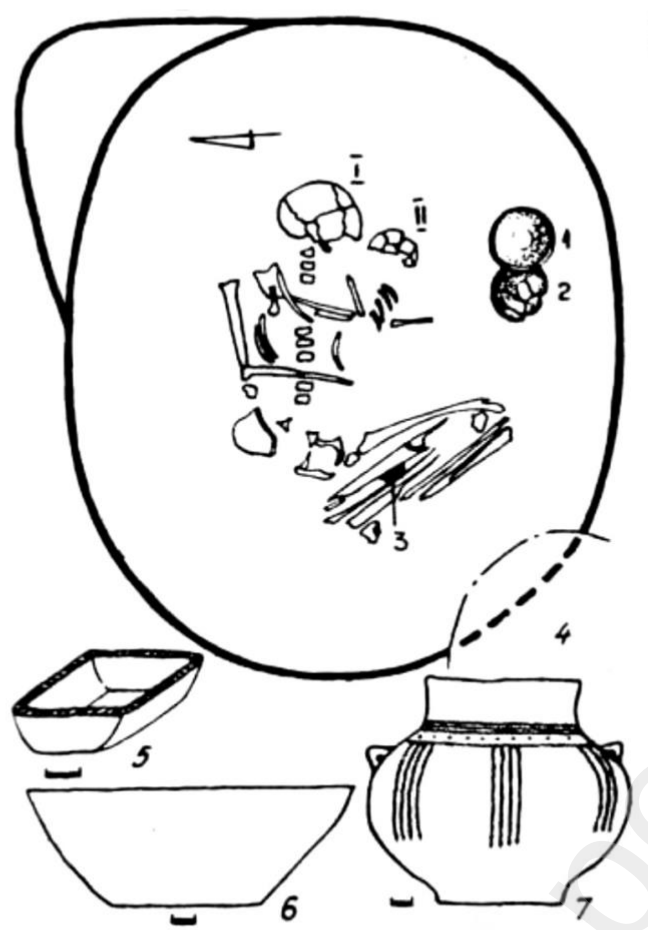

Figure S3. Kurgan 3 Burial 9 (after Patokova et al., 1989).

Burial groups (kurgans) 8, 7 and 9

Burial groups designated as kurgans 8, 7 and 9 were arranged side-by-side in the northern part of the Mayaky necropolis (Figure S4). Their mounds were not preserved. Each burial group had an arcuate ditch 0.4-0.5 m-wide and 0.6-0.65 m deep on the southeast side. Burial groups 8, 7 and 9 were formed in a chronological succession, in which the central group 7 was followed by group 9, with group 8 subsequently added on the west side of group 7 (Figure S5).

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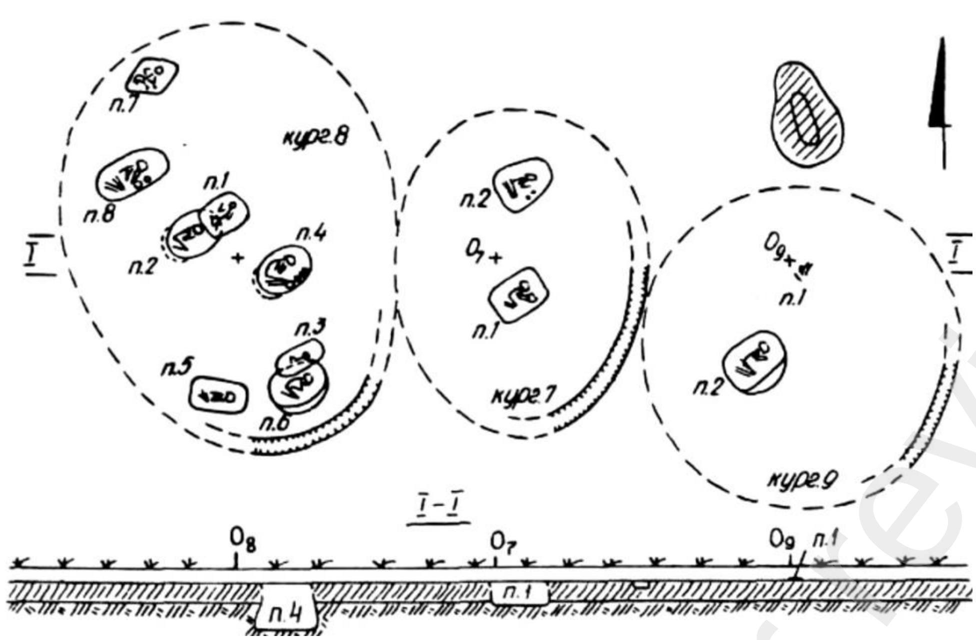

Figure S4. Burial groups (kurgans) 8, 7 and 9 (after Patokova et al., 1989).

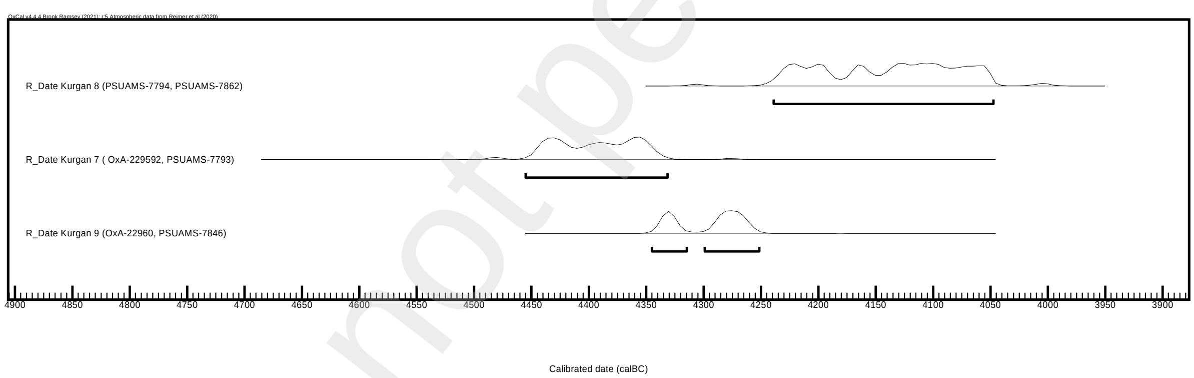

Figure S5. Multiplot of radiocarbon dates from burial groups (kurgans) 8, 7 and 9. The multiplot was constructed using OxCal v4.4.4 and IntCal20 calibration curve (Bronk Ramsey, 2009; Reimer et al., 2020).

Burial group (kurgan) 7, Burial 2, male (25-30), 4446-4340 calBCE (5536±25 BP) (weighted mean (Ward and Wilson, 1978)*: (5530±32 BP, OxA-22959); (5545±40 BP, PSUAMS-7793)]*

Burial 2 of burial group (kurgan) 7 (Figure S6) was archaeologically assigned to the Usatove culture. The grave was identified as a discoloration at the transition to the sandy loam 0.65 m below the current surface. The bottom was at a depth of 1.25 m, the walls could be traced up to 0.6 m. The oval burial pit was aligned from SW to NE and measured 1.4 × 1.15 m, it was slightly longer at the bottom (1.55 × 1.15 m). The backfill was compact and consisted of three layers: up to a depth of 1.05 m there was black earth with lenses of sandy loam, underneath there was a 0.1 m thin layer of sandy loam, in the bottom area there was black earth with many

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lime inclusions. The massive skeleton of a 25–30-year-old male laid crouched on the left side, skull facing east and turned with the face up. The arms were bent and crossed in front of the chest. The left hand was under the skull, the right by the facial bones. Spots of raspberry-colored ocher were found on the skull and on the joints of the thighs and lower legs, and the remains of organic material in the form of brown mold were also found on the lower legs. Between the upper ribs were crumbs of dark red ocher. In front of the arms were two vessels. One of these was a beige beaker (height 46 mm, rim diameter 45 mm, body diameter 72 mm, base diameter 48 mm) with a squat body and low neck. Four horizontal rows of fine cord encrusted with red ocher were applied around the neck. The body was decorated with incised lines encrusted in white, in the upper area with a zigzag pattern forming a six-pointed star, with three parallel grooves at regular intervals at the bottom. The clay was tempered with finely crushed shells.

The other vessel (height 72 mm, rim diameter 114 mm, bottom diameter 38 mm) was a heavily fragmented miniature bowl typical of the Usatove culture. It had an S-shaped profile, was thin-walled, decorated with round cord indentations along the rim, with three rows of thin cord indentations running underneath. The vessel was coated with red ocher on the inside. The shard was gray-brown, the temper consisted of finely crushed shells and grains of sand.

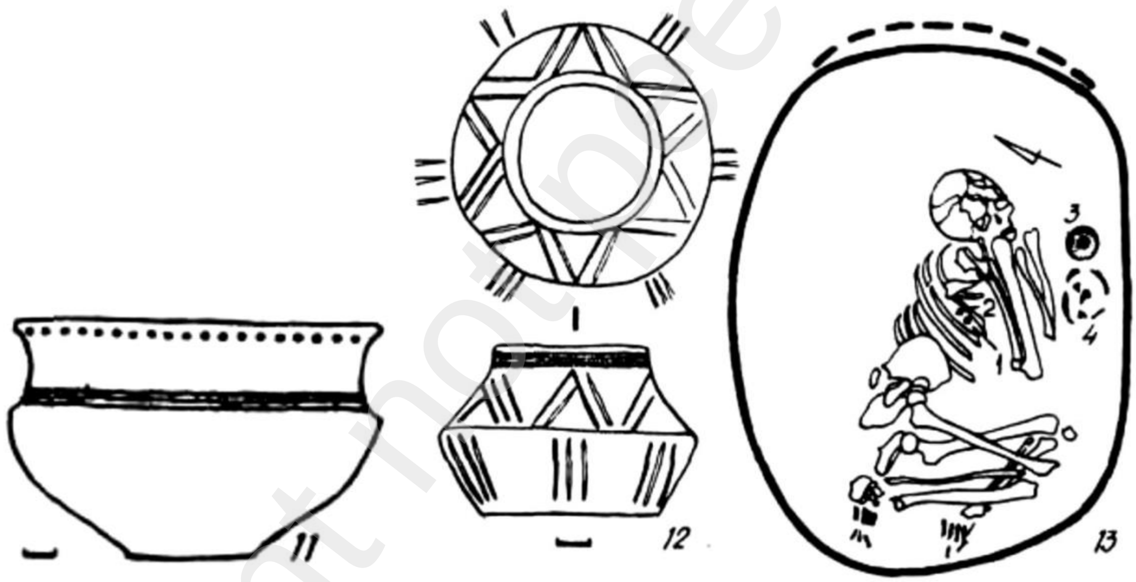

Figure S6. Kurgan 7 Burial 2 (after Patokova et al., 1989)

Burial group (kurgan) 8, Burial 4, female (ca. 60), 4240-3997 calBCE (5295±30 BP, PSUAMS-7862)

Burial 4 of burial group (kurgan) 8 was found in an oval pit with a 0.30 m-high lining and the depth of 0.8-1.6 m. The size of the grave at the top was 1.2 × 0.9 m, and 1.3 × 0.9 m at the bottom. The fill of the grave was two-layered and consisted of dense sierozem with calcareous nodules and underlying layer of continental loess-like loam. Fragments of the bones of a horse, a cow and a sheep, painted with dark red ocher, two shards and a broken light brown Usatove bowl of coarse molding with notches, impressions of a round-ended stamp and a cord, with

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grains of sand in the clay mix (Figure S7, 4(1), 6) were found 0.4-0.5 m above the bottom of the grave.

The crouched skeleton of a ca. 60-year-old female rested at the bottom of the pit on the left side, skull facing north-northeast at 33°, hands bent. The left hand was brought to the face, the right was under the cervical vertebrae. On the vault of the skull there were stripes of crimson ocher, spots of the same paint were on the pelvic bones. Brown decay was identified on the bones of the wrist and lower epiphyses of the forearm of the right hand, in the region of the left elbow joint. To the right of the deceased and on the shins, there were five ceramic vessels. 1) The bottom part of a vessel made of clay mixed with crushed shells, with soot stains on the walls (Figure S7, 10). 2) A polished pot with imprints of a cord and a pipe, with holes under the rim, and grains of sand in the clay mix (Figure S7, 7). 3) A polished bowl with finely ground shells and grains of sand in the clay mix (Figure S7, 5). 4) A polished amphora with holes under the rim and a corded ornament (Figure S7, 8). 5) A pot of crumbly clay mix with crushed shells and a pattern of pipe imprints (Figure S7, 9). The walls of vessels 3-6 were spotty, light brown and dark gray.

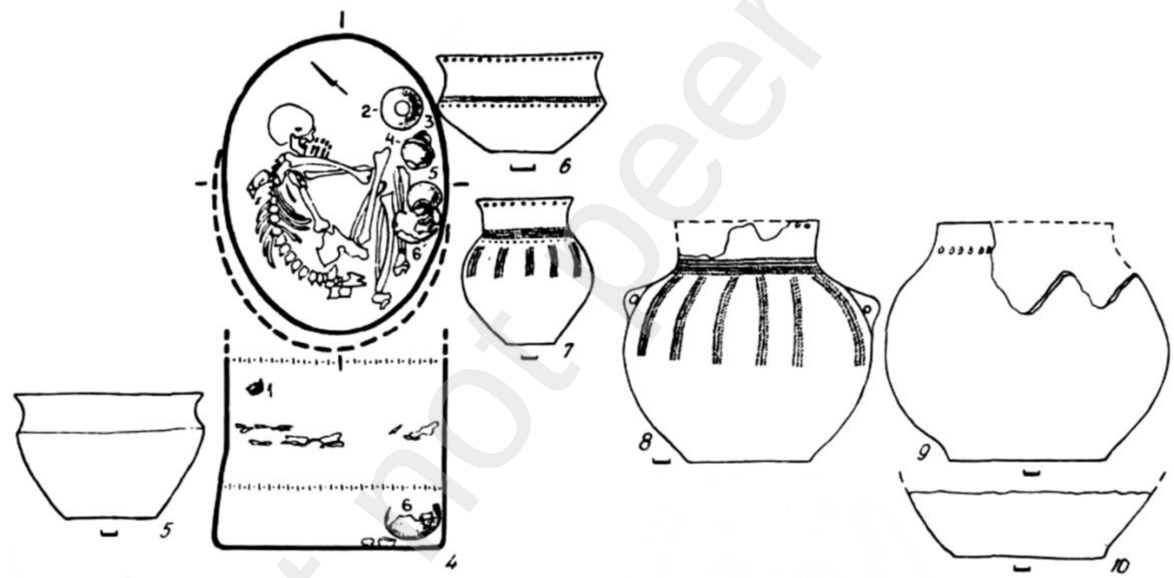

Figure S7. Kurgan 8 Burial 4 and associated finds (after Patokova et al., 1989).

Burial group (kurgan) 8, burial 6 skeleton 2, female (25-35), 4323-4047 calBCE (5330±40 BP, PSUAMS-7794)

The lower of the two skeletons in the grave (Figure S8, 11 (II)).

The grave pit, 0.75–1.50 m deep, had a lining and a ledge. The burial chamber was oval, its size at the top was 1.3 × 0.9 m, at the bottom – 1.35 × 1.10 m. Two individuals, a 40–55-year-old male (first (upper) skeleton) and 25-35 year-old female (second (lower) skeleton), were buried in the grave. The first skeleton was located 0.30 m above the bottom of the pit, under a layer (0.45 m) of unusually dense grayish-brown loam with calcareous nodules Figure S8, 11 (I)). The skeleton laid crouched on the left side, hands bent, with the skull facing east-northeast at 76°. The left hand was in the pelvis, the right hand was under the skull. On the vault of the skull

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there were stripes and spots of bright crimson ocher. A continuous thin layer of the same paint was on the long bones of the limbs, the bones of the pelvis and on the ground under them. To the left of the deceased laid a sheep's scapula.

At the bottom of the grave, under a layer of yellow loess-like loam (0.3 m) indistinguishable from the matrix, a second skeleton was found, which was laid crouched on the left side, with the skull to the east at 85° (Figure S8, 11 (II)). The spine of the skeleton was strongly curved, the femurs were pressed to the chest, the knees were brought to the shoulder joints, the feet were to the pelvis. In the hands of the buried was a pot (Figure S8, 2, 12). On the bones of the postcranial skeleton and on the ground beneath them, there were stripes of decay with spots of black paint. There were traces of bright crimson ocher on the right parietal bone. Inventory (associated with skeleton II): 1) A pot of gray crumbly clay mix with a pattern of flat stamp impressions, with a flint flake inside (Figure S8, 12). 2) A vessel with two protruding protrusions on the body, the ornament is imprints of a cord and a round-ended stamp (Figure S8, 1). The clay mix was loose, the firing was weak. 3) A bowl covered on the outside with a layer of greasy soot (Figure S8, 13). The clay mix of vessels contained crushed shells. The latter two vessels were spotted, gray and light brown in color.

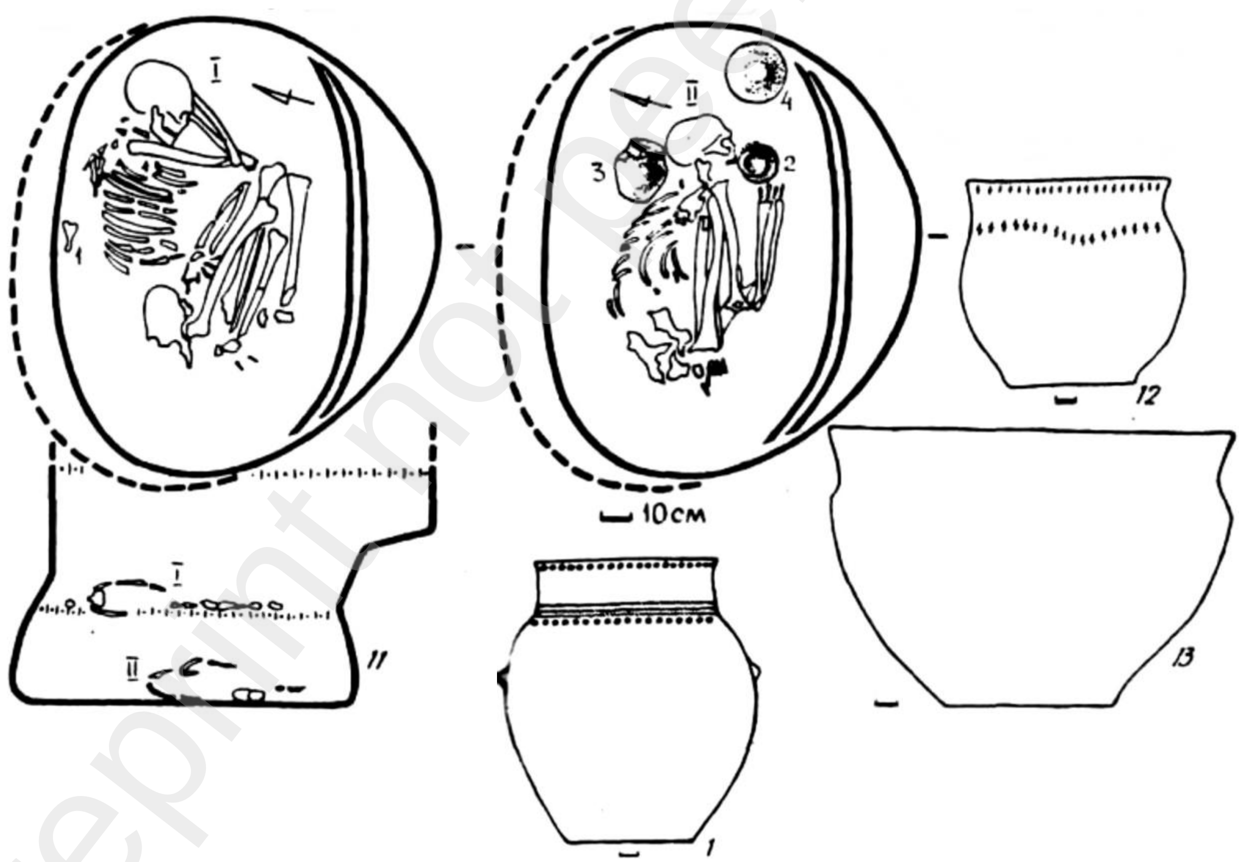

Figure S8. Kurgan 8 burial 6 skeleton 1 (I) 2 (II) and associated finds (after Patokova et al., 1989).

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Burial group (kurgan) 9 burial 2, female (mature), 4346-4252 calBCE (5444±19 BP) (weighted mean (Ward and Wilson, 1978)*: (5471±2 BP, 0xA-22960); (5400±30 BP, PSUAMS-7846))*

Two burials were discovered during the 1986 excavations of the burial group (kurgan) 9. Burial 2 was provisionally assigned to Usatove. Burial 1 belonged to a different period. Subsequent excavations produced additional burials, 3 and 4, the latter a double burial.

Burial 2 was discovered as a discoloration at the transition to the sandy loam 0.45 m below today's surface. A rectangular pit had such strongly rounded corners that it was almost oval. The SW-NE pit measured 1.6 × 1.5 m. The walls could be traced to a height of 0.6 m. The bottom was at a depth of 1.05 m The south-east pit wall had a step 0.5 m deep and 0.3 m wide. The backfill was compact, gray-brown, sandy-loamy with lime sprinkled in. A mature adult was buried lying on the left side in a contracted position, skull to NE, arms flexed, hands in front of the face. Traces of raspberry-colored ocher on right shoulder bone, lower epiphysis of right shoulder bone, pelvis, and legs. Patches of organic material of brown color were found on the long bones. This grave was only provisionally assigned to the Usatove culture due to a lack of grave goods.

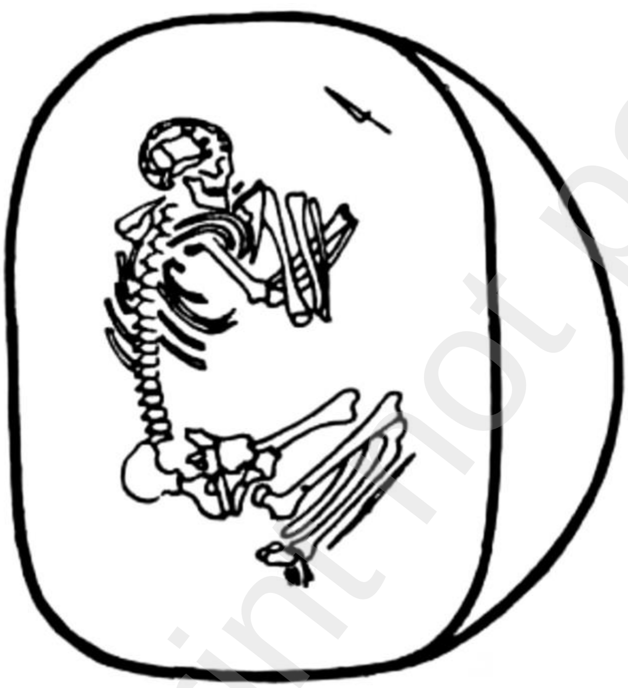

Figure S9. Kurgan 9 burial 2 (after Patokova et al., 1989)

Burial group (Kurgan) 10

Burial group (kurgan) 10, a chernozem hillock 0.15 m high and about 20 m in diameter, was located 14 m south of the 1975 excavation. Of the five Usatove-designated burials, two were damaged by pits made in the early centuries AD. The graves were filled with dense humus loam.

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Burial group (kurgan) 10, burial 2 skeleton 2, male (50-55), 4336-4065 calBCE (5390±30 BP, PSUAMS-7845)

An oval grave pit, 1.3-0.85 x 0.5-0.65 x 0.8 m. Two individuals were buried – a female over 60 years old (skeleton 1, Figure S10, I) and a male 50-55 years old (skeleton 2, Figure S10 II). Both skeletons were crouched on their left side and laid one on top of the other, with their skulls facing east-southeast. The skull of the male rested between the shoulder bones and the chest of the female. The arms of the female were folded. Her left hand was in front of her face, her right hand was near her knees. The arms of the male were bent, the hands were raised to the face. Skeleton 1 inventory - a fragment of a coarse bowl made of dark gray clay with sand (Figure S10, 1, 2). Inventory of skeleton 2 - a brown vessel with imprints of a semicircular stamp, loose ceramic mix containing grains of sand (Figure S10, 3).

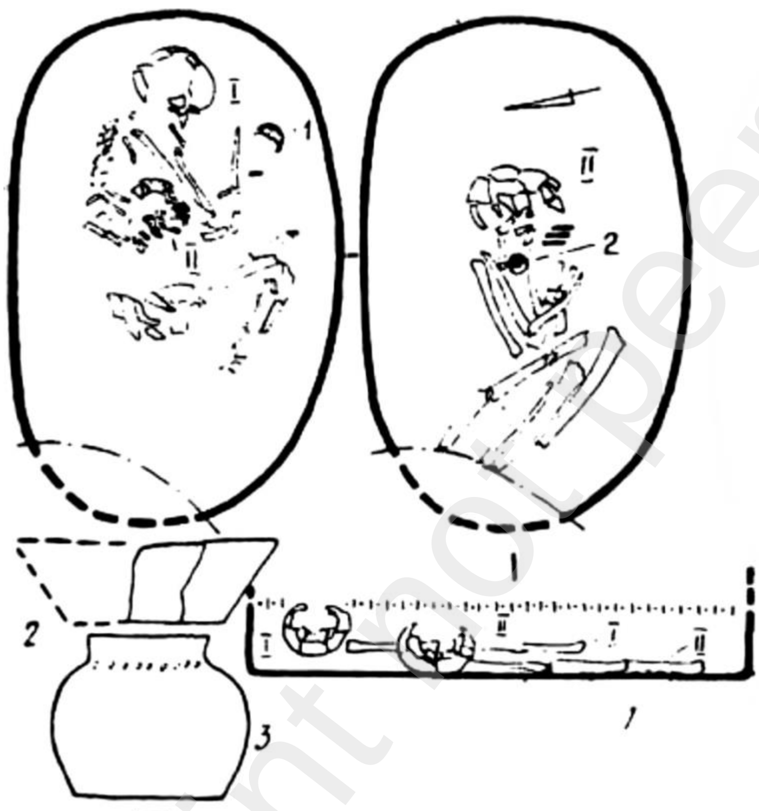

Figure S10. Burial grouping (kurgan) 10 burial 2 (after Patokova et al., 1989)

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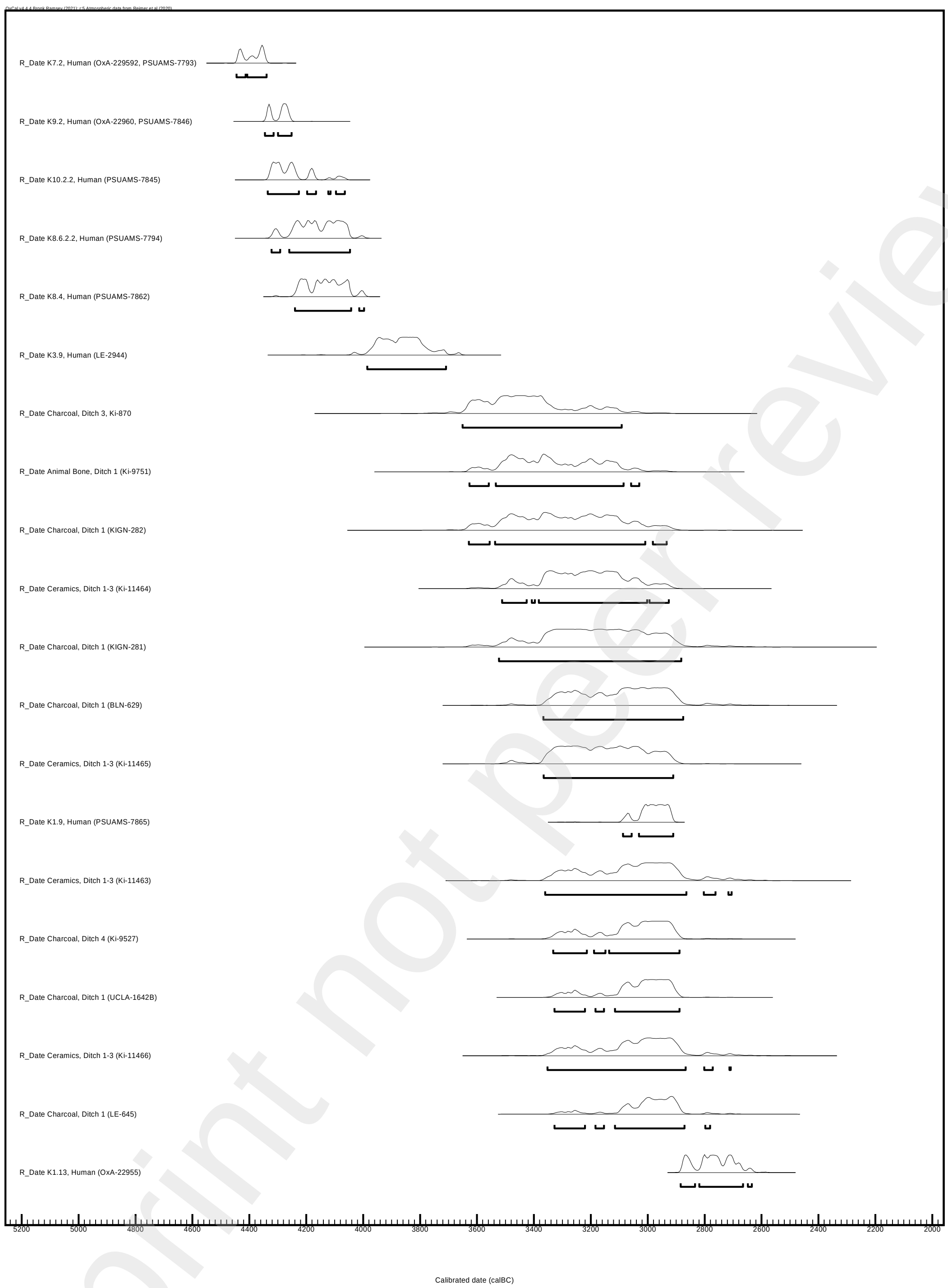

Figure S11. Multiplot of radiocarbon dates (Eneolithic-Early Bronze Age) from human remains (AMS) as well as charcoal, pottery, and animal bone (radiometry) from the Mayaky archaeological complex.

The multiplot was constructed using OxCal v4.4.4 and IntCal20 calibration curve (Bronk Ramsey, 2009; Reimer et al., 2020). Specimen description, data and sources are listed in Tables S1a, S1b and S1d.

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Table S1a. Radiocarbon dates from pottery, chacoal and animal bone from the Mayaki site.

Date, uncalBP (Lab Code)	Date, calBCE	Material	Source of Material	Reference
4670±110 (Ki-870)	3651-3092	charcoal	Ditch 3	Kovaluykh, Videiko, Skripkin 1995
4580±120 BP (KIGN-282)	3629-2934	charcoal	Ditch 3	Patkova et al., 1989
4600±90 (Ki-9751)	3627-3030	animal bone	Ditch 1	Ковалюх, Скрипкін, Відейко 2007
4475±130 (KIGN-281)	3523-2883	charcoal	Charcoal samples from the 1986 excavations	Patkova et al., 1989
4530±90 (Ki-11464)	3512-3003	ceramics	Ditch 1-3	Ковалюх, Скрипкін, Відейко 2007
4490±90 (Ki-9752)	3491-2914	animal bone	Ditch 1	Ковалюх, Скрипкін, Відейко 2007
4400±100 (BLN-629)	3367-2876	charcoal (Ulmus sp)	Sample from Ditch 1, depth 2.8m	Quitta and Kohl 1970
4460±90 (Ki-11465)	3366-2911	ceramics	Ditch 1-3	Ковалюх, Скрипкін, Відейко 2007
4370±100 (Ki-11463)	3361-2706	ceramics	Ditch 1-3	Ковалюх, Скрипкін, Відейко 2007
4360±90 (Ki-11466)	3353-2709	ceramics	Ditch 1-3	Ковалюх, Скрипкін, Відейко 2007
4475±30 BP (KIGN-280)	3341-3029	charcoal	Ditch 3	Kovaluykh, Videiko, Skripkin 1995
4380±70 (Ki-9527)	3333-2889	charcoal	Ditch 4 (Feature 1/1990)	Petrenko and Kovaluykh, 2003
4375±60 (UCLA-1642B)	3328-2889	charcoal	Ditch 1	Gimbutas 1973
4340±65 (LE-645)	3328-2782	charcoal	Ditch 1	Sementsov et al., 1969
4180±90 (Ki-9753)	3005-2476	animal bone	Trench 4-5	Ковалюх, Скрипкін, Відейко 2007
3490±35 (GrN-5126)	1920-1694	charcoal	Ditch 1	Breunig 1987

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Table S1b. Combined radiocarbon and stable isotope data for Mayaki and adjacent Eneolithic-Bronze Age sites in the Ponto-Caspian steppe and forest stepp

ID	Location (sample size)	Location	Date	δ13C (‰)	δ15N (‰)	C:N	Period-Culture	SD 13C	SD 15N	Reference
Mayaki, Middle Ages										
MA 41	Kurgan 1 burial 18	Mayaki necropolis	974-1040 calCE	-15	10.3	3.2	Middle Ages			Petrenko et al., 2018
Mayaki, Iron Age										
I12700	Kurgan 1 burial 9.1	Mayaki necropolis	92 calBCE - 66 calCE	-16.15	11.36		Late Iron Age			This report
K8	Burial 8	Mayaki necropolis, ground burial	795-569 calBCE	-16.8	10.6	3.1	Iron Age			Petrenko et al., 2018
	Mayaki Iron Age average			**-16.475**	**10.98**					
Mayaki, Bronze Age										
MA 39	Kurgan 1 burial 7	Mayaki necropolis	3000-2600 BCE	-19.1	12.8	3.4	Bronze Age-Yamna			Petrenko et al., 2018
MA 40	Kurgan 1 burial 13	Mayaki necropolis	2885-2635 calBCE	-18.9	12	3.3	Bronze Age-Yamna			Petrenko et al., 2018
MA 67	Kurgan 1 burial 15	Mayaki necropolis	3300-2500 BCE	-18.5	13.2	3.2	Bronze Age-Yamna			Petrenko et al., 2018
I12839	Burial 3	Mayaki sanctuary	1879-1642 calBCE	-19.71	12.25		Bronze Age			This report
G5	Burial 5	Mayaki sanctuary	2800-2500 BCE	-19.5	10.7	3.1	Bronze Age-Yamna			Petrenko et al., 2018
	Mayaki Bronze Age average			**-19.142**	**12.19**			**0.480437301**	**0.955510335**	
Mayaki, Eneolithic										
21	Kurgan 3 Burial 9	Mayaki necropolis	3986-3709 calBCE	n/a	n/a		Eneolithic-Usatovo			Petrenko et al., 2018
I12229	Kurgan 1 burial 9	Mayaki necropolis	3088-2911 calBCE	-21.01	16.87		Eneolithic-Usatovo			This report
I12704	Kurgan 7 burial 2	Mayaki necropolis	4446-4340 calBCE	-20.24	16.07		Eneolithic-Usatovo			This report
I12710	Kurgan 8 burial 4	Mayaki necropolis	4240-3997 calBCE	-20.03	15.25		Eneolithic-Usatovo			This report
I12707	Kurgan 8 burial 6 skeleton 2	Mayaki necropolis	4323-4047 calBCE	-20.22	15.55		Eneolithic-Usatovo			This report
I12706	Kurgan 9 burial 2	Mayaki necropolis	4340-4071 calBCE	-19.77	16.37		Eneolithic-Usatovo			This report
I12705	Kurgan 10 burial 2 skeleton 2	Mayaki necropolis	4336-4065 calBCE	-20.81	15.73		Eneolithic-Usatovo			This report
MA 69	Kurgan 8 Burial 2	Mayaki necropolis	3400-2900 BCE	-19.7	15.2	3.2	Eneolithic-Usatovo			Petrenko 2011; Petrenko et al., 2018
MA 66	Kurgan 10 Burial 2	Mayaki necropolis	3400-2900 BCE	-20.1	16.1	3.3	Eneolithic-Usatovo			Petrenko 2011; Petrenko et al., 2018
	Mayaki Eneolithic average		3400-2900 BCE	**-20.235**	**15.8925**			**0.462014224**	**0.572482065**	
Forest steppe Eneolithic farmers (Trypillia)	Verteba, Yampil (11)	Middle Dniester	3911-3095 BCE	-19.26	9.79		Eneolithic-Trypillia	0.5	0.63	Goslar et al., 2017; Lillie et al., 2017
Eneolithic Pontic steppe	Peschanka, Ozera, Vinogradnoye (3)	Eastern Pontic steppe	4241-2915 calBCE	-18.43	12.93		Eneolithic-steppe	0.81	0.81	Görsdorf et al., 2004; Gerling, 2015; Mathieson et al., 2018
Bronze Age Pontic steppe	Peschanka, Sugokleya, Vinogradnoe (12)	Eastern Pontic steppe	3000-2500 BCE	-19.02	11.59		Early Bronze Age-Yamna, Catacomb	0.31	0.88	Gerling, 2015
Bronze Age forest steppe pastoralists	Yampil (8)	Middle Dniester	2895-2636 calBCE	-18.71	10.04		Early Bronze Age-Yamna	0.63	1.04	Goslar et al., 2017
Eneolithic forest steppe foragers 1	Molukhiv Bugor (5)	Middle Dnieper	3951-3640 calBCE	-21.88	12.11		Eneolithic-Srednij Stog	0.84	0.58	Lillie et al., 2011
Eneolithic forest steppe foragers 2										
I3719	Deriivka I, Burial 102	Middle Dnieper Valley	4949-4799 calBCE	-20.11	12.32		Eneolithic-Dnieper-Donets			Mathieson et al., 2018
I4111	Deriivka I, Burial 123	Middle Dnieper Valley	4722-4548 calBCE	-24.12	11.69		Eneolithic-Dnieper-Donets			Mathieson et al., 2018
I4110	Deriivka I, Burial 9	Middle Dnieper Valley	3634-3377 calBCE	-22.22	12.88		Eneolithic-Dnieper-Donets			Mathieson et al., 2018
	Deriivka I Eneolithic foragers 2 average	Middle Dnieper Valley		**-22.15**	**12.29666667**			**2.005916249**	**0.595343038**	
Bronze Age Manych steppe	Sharakhalsun (6)	Manych steppe	2900-2680 BCE	**-17.1333**	**15.88333**		Bronze Age-Yamna	**0.653197265**	**1.568969938**	Knipper et al., 2020

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Table S1c. Stable isotope ratios (δ13C and δ15N) from animal bones from the sites of Mayaki and Căplani.

ID	Site	Animal	Age	δ13C (‰) (vs. V-PDB)	S.E.	%C	δ15N (‰) (vs. AIR)	S.E.	%N	C/N	% collagen	Reference
CG 64	Mayaki (sanctuary), Ukraine	Cattle (*Bos taurus*)	3900-3300 BC	–18.7	< 0.1		6.7	< 0.1		3.2	5.2	Gerling, 2015; Petrenko et al., 2018
Căplani_F1	Căplani, Moldova	Cattle (*Bos taurus*)	3900-3300 BC	-19.21		41.83	5.96		15.1	3.2		This study

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Tables S1a-c References

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