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Stable Isotopes and Herding Strategies in Middle Uruk Period in Tell Humeida (Syrian Euphrates Valley)

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Abstract: The techniques of agriculture and animal husbandry at Tell Humeida, a Middle Uruk Period (Late Chalcolithic) site on the middle Syrian Euphrates, were studied using stable isotopes of bone collagen of domestic and wild mammals and from cereal and ruderal plant seeds. Two archaeological campaigns in 2009 and 2011 yielded a small collection of bones, most of which were taxonomically indeterminable. The work had to be interrupted due to the political conflict. The faunal study comprised collagen peptide fingerprinting for taxonomic identification, followed by isotopic analysis. Multiple ^{14}C dating were performed to date the infill to around 3600 cal BC. An isotopic analysis of the sparse plant remains suggested that irrigation and manuring were common practices. Sheep and equids predominated in the faunal assemblage. Sheep grazed on manured soils, and their diet could include millet or another C4 plant, of which, however, no carpological remains were found. The diet of equids differed from that of sheep but also that of other wild ungulates (cervids/gazelles). Their isotopic signatures indicated that they grazed in humid areas, near the watercourse. These finds indicated a settlement that was closely linked to the availability of water, which made it possible to grow crops in an almost desert-like area, and the rearing of sheep.

Keywords: Mesopotamia; livestock management; agriculture; $\delta^{15}N$; $\delta^{13}C$; peptide fingerprint

Citation: Grandal-d'Anglade, A.; García-Vázquez, A.; Moreno-García, M.; Peña-Chocarro, L.; Sanjurjo-Sánchez, J.; Montero-Fenollós, J.L. Stable Isotopes and Herding Strategies in Middle Uruk Period in Tell Humeida (Syrian Euphrates Valley). *Diversity* **2023**, *15*, 709. https://doi.org/10.3390/d15060709

Academic Editor: Ioana Nicoleta Meleg

Received: 1 February 2023
Revised: 28 March 2023
Accepted: 23 May 2023
Published: 26 May 2023

1. Introduction

1.1. Tell Humeida and Its Archaeological Context

Tell Humeida is located on the left bank of the Euphrates River, 75 km north of the Syrian city of Deir ez-Zor (Figure 1). The research at the site was part of the Middle Syrian Euphrates Archaeological Project (PAMES, acronym in Spanish), a scientific initiative coordinated and launched by the University of A Coruña and the General Directorate of Antiquities and Museums in Damascus in 2005. The geographical setting of this project was the basalt gorge of Hanuqa (Figure 1b) in the Syrian province of Deir ez-Zor [1]. The fieldwork consisted of four archaeological survey campaigns in the region around the gorge (2005–2009), as well as three excavation campaigns at Tell Qabr Abu al-'Atiq (2008–2010) and one at Tell Humeida (2011).

The work carried out showed the historical importance of the Hanuqa Gorge in both pre-classical and classical times [2]. This fact was directly related to the strategic value of this geographical feature, which marked a turning point in the Middle Euphrates basin in Syria. In the fourth millennium BC, people from southern Mesopotamia settled at Tell Humeida, the entrance to Hanuqa. Their presence must be explained in the context of the complex

expansion process of the so-called Uruk culture [3]. The actual reasons that led to this phenomenon remain a controversial question, for which various explanations have been proposed [4]. As a hypothesis, Ref. [5] proposed that the Uruk settlements documented in the Middle Euphrates Valley in Syria can be classified into three different habitat types based on their material culture, namely, colonies founded ex novo by people coming from the south (intrusion culture), trade enclaves founded in pre-existing indigenous settlements (hybrid culture), and trade sites frequented by Uruk merchants (with local cultural dominance).

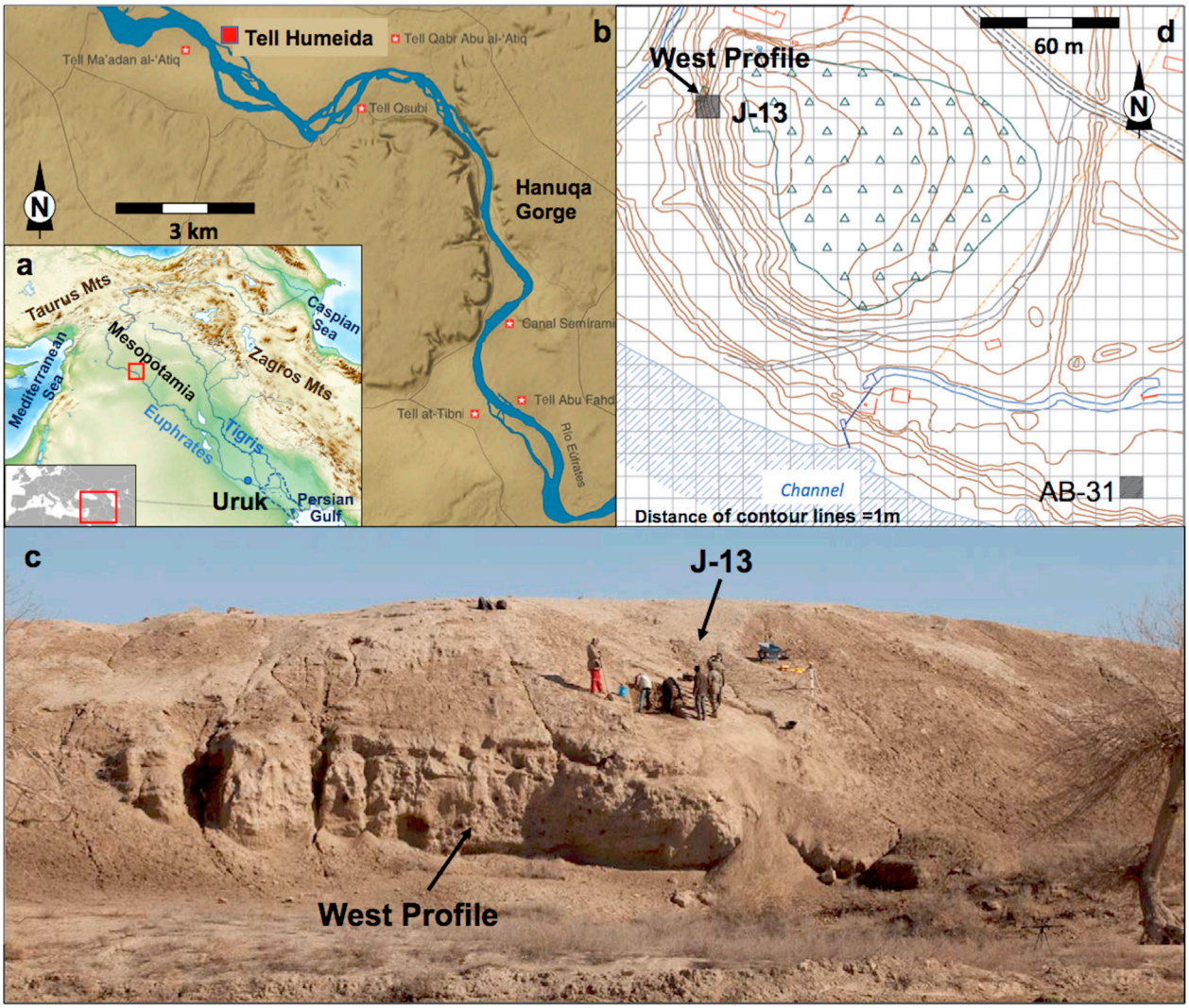

Figure 1. (**a**) General location map. The inset marks the detailed area in (**b**), the Hanuqa Gorge and Tell Humeida on the Middle Euphrates. (**c**) General view of the site from the west. (**d**) Detailed topographical map of Tell Humeida showing the excavation sites. Contour lines: 1 m.

Tell Humeida was most likely a colony of Uruk whose main purpose was to control the main waterway (the Euphrates and its tributary Habur), which provided access to raw materials that were in short supply in Lower Mesopotamia. Research at this archaeological site sheds new light on various aspects of material culture and lifestyle in the so-called Middle and Late Uruk Period, also known as Late Chalcolithic 4-5 (c. 3600–3000 BC). Noteworthy, for example, are new contributions on the production and function of beveled rim bowls (BRB), a little-known type of ceramic vessel that is nevertheless considered an identity marker of the entire culture [6–10].

Fieldwork at Tell Humeida has been interrupted since March 2011 due to the outbreak of the political conflict in Syria. Due to this suspension, we only had scarce study materials, including a series of skeletal remains of animals, most of which were difficult to identify due to their fragmented state. However, the application of analytical techniques such as taxonomic identification by means of peptide fingerprinting and stable isotope analysis allowed us to overcome this problem. In this paper we present the results of these analyses, together with the study of carpological remains and the dating of charcoals and sediments, in order to obtain as much information as possible as the inaccessibility of the site prevents progress in its investigation.

1.2. The Site and the Archaeological Surveys

The archaeological site of Tell Humeida, which occupies an area of 6 ha, consists of two sectors (Figure 1d): a roughly circular mound (150 m in diameter) and a lower area extending 260 m to the east. The western part of the mound suffered from considerable erosion, probably caused by the nearby waters of the Euphrates. Consequently, a large stratigraphic section 3 m high and 17 m long emerged, which we call the West Profile (WP). In this profile, the abundance of ceramics, including the beveled rim bowls characteristic of the Middle–Late Uruk period, was visible to the naked eye. Bone remains and charcoal were also present. The arrangement and type of material suggested that this was a midden area (Figure 2).

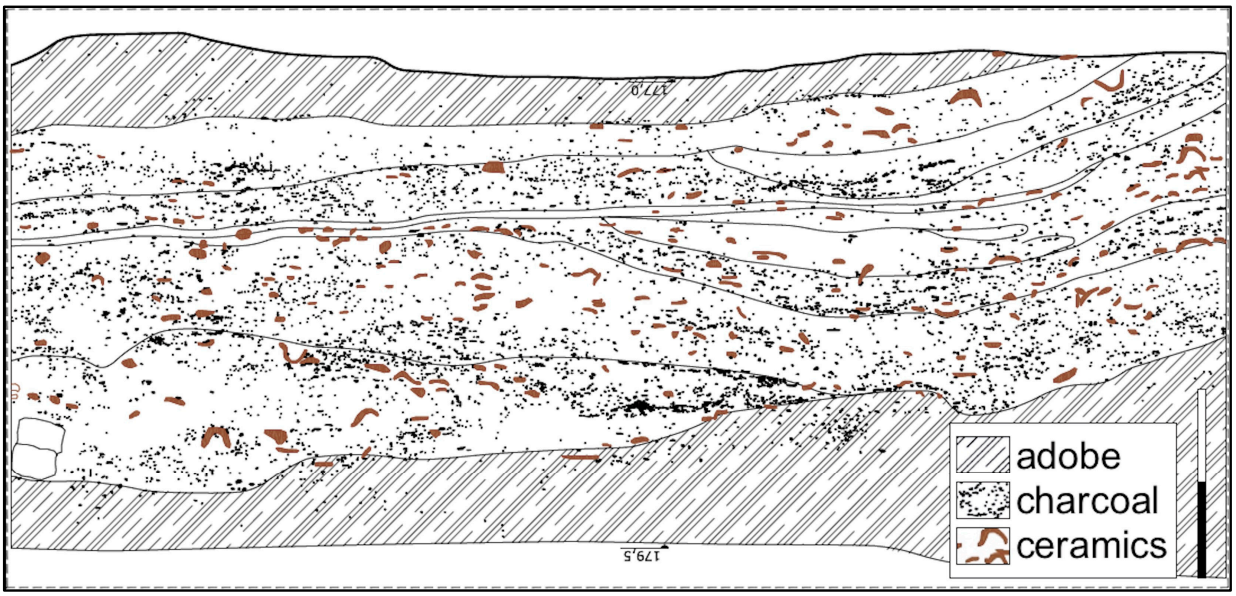

Figure 2. Section of the west profile (WP).

During the 2011 campaign, two archaeological surveys were carried out at Tell Humeida: a test sounding of 5 × 5 m in the square J-13, on the mound, just above WP (Figure 3), and AB-31, in the lower area. In J-13, two periods of occupation were excavated: Palaeobyzantine (sixth to seventh cal AD) and Middle Uruk, with three phases of occupation [11]. The most recent phase consisted of Stratigraphic Unit 1003 (SU 1003), which holds a wall or Constructive Unit 105 (CU 105), preserving three courses of mud bricks of modest size (22 × 11 × 9 cm), a type of Uruk-period brick known as *riemchen*. The second phase was SU 1004, together with SU 1005 and SU 1006, in which charcoal, ashes, animal bones, and pottery abounded, especially the characteristic beveled rim bowls. This midden-like level apparently corresponded to that revealed in WP. Finally, the remains of an adobe wall documented beneath this debris fill composed the third phase (CU 106). The second survey in the lower area (see Figure 1d), AB-31 (10 × 10 m), yielded no evidence from the Uruk period.

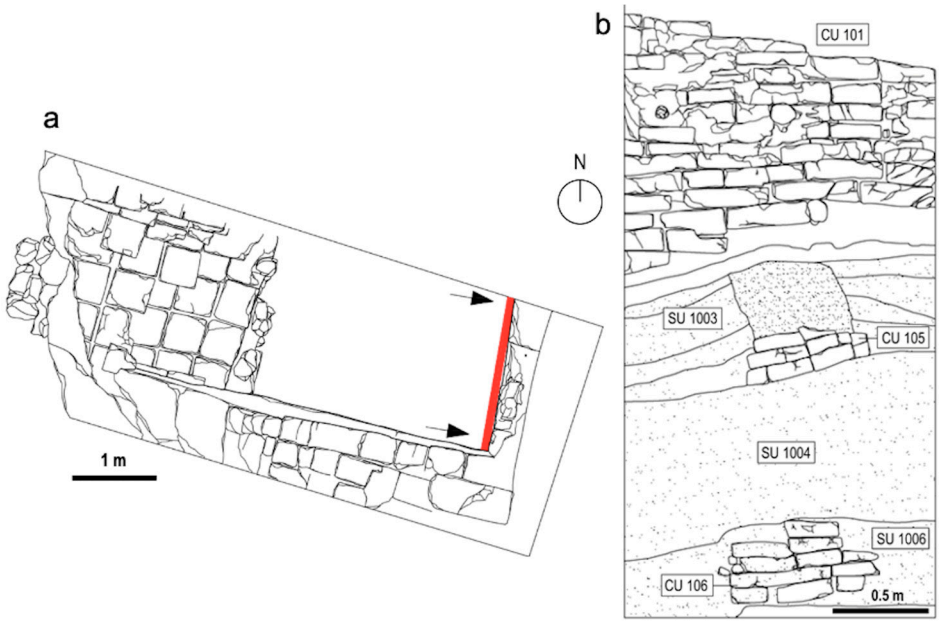

Figure 3. (**a**) Plan and (**b**) section of the west profile (WP) showing the main stratigraphic units (SU) and constructive units (CU). In this profile, SU 1005 is not visible but consists of the same materials of SU 1004 disturbed at the contact with the Byzantine wall.

1.3. Stable Isotopes Rationale

A good option for studying past economies when the bone record is poor, as in this case, is to resort to stable isotope analysis. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of bone collagen have become a routine method for the study of human and animal nutrition [12,13]. In addition, the C and N isotope signatures of plant remains (grains, seeds, chaff, etc.) reflect environmental conditions at the time, such as human-mediated or non-mediated water availability or manure fertilization [14]. Thus, the combination of the isotopic data of animals and plants from a site provides important data on the economy of ancient societies, their farming methods, and the husbandry of their animals [15]. The study of trophic relationships between organisms using stable isotopes is possible due to isotopic fractionation, the phenomenon whereby the isotopic composition of an element in a given compound changes as it transitions from one physical state or chemical composition to another [16,17]. An example is the change in isotope ratio values ($\delta^{13}C$, $\delta^{15}N$) that occurs between an organism and its diet, often referred to as the trophic offset. This is due to the preferential mobilization of the light isotope and retention of the heavy isotope during food assimilation and tissue synthesis, in this case, of bone collagen [18].

The relative composition of carbon stable isotopes in plants is determined by the values in atmospheric CO_2, which changed considerably from pre-industrial times to the present day due to the burning of fossil fuels. While the value of atmospheric CO_2 today is about −8‰, values between −6‰ and −6.5‰ were recorded in the Chalcolithic [19]. The isotope values in the plant depend directly on the type of photosynthesis carried out, which can be of the C3, C4, or CAM type [20,21]. C3 plants are herbaceous and shrubby plants of temperate or cold climates, including most cereals. When taking up CO_2, C3 plants discriminate strongly against $^{13}CO_2$ and prefer to take up molecules with ^{12}C. Therefore, the $\delta^{13}C$ values of the plant are lower than those of the air (between −12.4‰ and −37.0‰ [16]). During water stress, C3 plants close the stomata to prevent water loss through evapotranspiration. This leads to a lower discrimination ability of the plants toward CO_2 molecules with the heavy isotope and thus to an increase in the $\delta^{13}C$ signature. Thus, variations in plant $\delta^{13}C$ relative to atmospheric CO_2 levels values ($\Delta^{13}C$) reflect greater or lesser availability of water depending on climate and plant irrigation [19,22–24]. In contrast, C4 plants (warm-climate herbaceous plants, including various crops such

as millet, sorghum, sugarcane, maize, etc.) are better adapted to dry climates. Because they decouple photosynthesis from CO_2 uptake, C4 plants have systematically higher $\delta^{13}C$ values (between −2.0‰ and −14.0‰ [16]). The CAM plants, Crassulaceae, show an intermediate metabolism and intermediate $\delta^{13}C$ values. The bone collagen of primary consumers is enriched by +5‰ over the plants they feed on [25]. From herbivores and up the food chain, the trophic offset between prey and predator is lower, varying from +0.8‰ to +1.3‰, according to [26].

Several factors have a significant influence on nitrogen isotopic values in plants. Soil nitrogen synthesis is crucial because microbial activity depends on climate, i.e., on the chronology and geographical location, specifically latitude and altitude [27,28]. Plants of the family Fabaceae take up N directly from the air through symbiotic bacteria and usually have lower $\delta^{15}N$ values than the rest of the vegetation [29]. In plants from agricultural systems, fertilization of crops with manure or slash and burn agriculture produces an increase in $\delta^{15}N$ in the crop by as much as 2 to 5‰ [14,30]. The relative composition of nitrogen isotopes in bone collagen is related to the position of the individual in the food web as each trophic level is characterized by an average increase in the value between 3 and 5‰ [26]. In addition to the diet type, individual nitrogen metabolism also influences the isotopic signature recorded in mammalian bone collagen. In mammals living in arid areas, high $\delta^{15}N$ values have been reported, which have been attributed to the mammals' own physiology under water stress conditions [31,32] or to their preference for certain types of plants [33].

2. Materials and Methods

2.1. Samples

Initially, our intention was to take a series of samples for a preliminary assessment of the site. Since the investigation was cut short by political events, our work must be based exclusively on this preliminary sampling. At WP, we took three samples of charcoal for ^{14}C dating. A set of 20 bone samples also came from this profile (Figure 4). All of them were included in the isotope analysis.

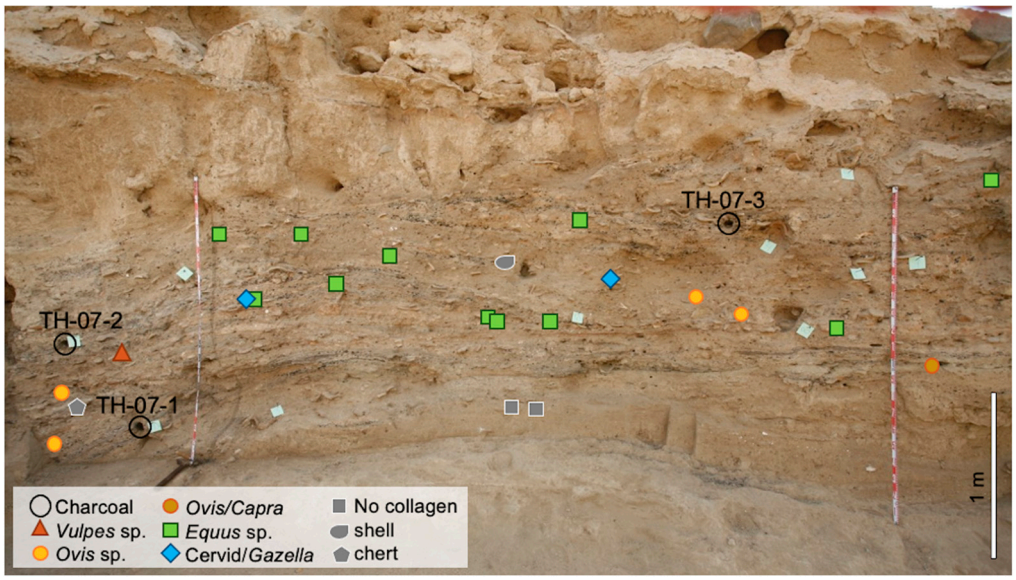

Figure 4. Photograph of the west profile (WP) showing the charcoal sampling points and the position of the analyzed bone samples and other interesting archaeological remains. Fragments of beveled rim bowls are visible all along the profile infill.

In square J-13, we took five charcoal samples along the profile for ^{14}C dating, three of them from UE 1006. From this same unit, we collected 55 kg of sediment for the carpological study and a set of skeletal remains, totaling 187 bones and teeth. One of them was a fish

and was not included in this work. From the set of mammal bone and dental remains in UE 1006 (Figure 5), we selected 26 samples for isotopic study, so that in total, we had 46 bone remains and six ^{14}C dates, as well as a small set of plant macro remains, to characterize the Middle Uruk occupation at Tell Humeida. Charcoal samples were dated (AMS ^{14}C) by The Tandem Laboratory at Uppsala University (Uppsala, Sweden).

Figure 5. Photograph of the J-13 square at SU 1004 depth. By the time the photograph was taken, the skeletal remains had been removed to reveal the remains of ceramics, mainly beveled rim bowls.

2.2. Separation and Identification of Plant Remains

The total of 55 kg of sediment was analyzed at the Archaeobiology Laboratory of the Institute of History of the Spanish National Scientific Council (CSIC) in Madrid, where the botanical remains were separated by flotation. The floating fraction was collected in a 250 µm sieve and, once dry, was transferred to the laboratory, where it was sieved in a column of 2 mm to 0.25 mm sieves. Once sorted, the charcoal remains were separated from the seeds into the five analyzed fractions (>4 mm, >2 mm, >1 mm, >0.5 mm, and >0.25 mm), and the latter were identified using the reference collection of the Institute of History Archaeobiology laboratory. The identifications were made with a LEICA (Wetzlar, Germany) binocular microscope.

2.3. Taxonomic Identification of Faunal Remains

The set of animal bone remains comprised a total of 209 elements, most of which were very fragmented, making their morphological identification difficult or impossible. Identifications were accomplished with the aid of the Archaeobiology Laboratory modern comparative osteological collections housed at the Institute of History and the Autonomous University of Madrid (Madrid, Spain). It was not always possible to reach specific identifications, so artificial categories of large- and medium-sized mammals were created, based on size, in which skull fragments, long bone splinters, and fragments of ribs and vertebrae were recorded. The large mammal category included equid, bovine, and deer remains, while the medium-sized mammal category grouped bones of medium-sized taxa, such as gazelle, sheep, goat, or medium- to small-sized carnivores. The bone remains of sheep and goats that could not be diagnosed at a specific level, following the characteristics indicated by [34], were grouped under the mixed category sheep/goat (OC).

Since most of the bone remains were too fragmented to be identified by their morphology, we performed identification of the taxa by collagen peptide fingerprinting, a technique currently known as ZooMS, zooarchaeology by mass spectrometry [35]. The basis of the ZooMS technique is simple. Although all vertebrates have a similar gene in their DNA

from which the bone collagen molecule is synthesized, there are small differences in the sequence of nucleotides in the DNA between different taxa and therefore small differences in the sequence of collagen amino acids. A fraction of the collagen obtained was digested with trypsin, which broke the molecule bonds between specific amino acids (after a Lysine or an Arginine, if not followed by Proline). Thus, each analyzed sample provided a set of peptides that were identified by MALDI-TOF mass spectrometry (Matrix-Assisted Laser desorption/ionization, time of flight) in a Ultraflex III MALDI-TOF/TOF mass spectrometer (Bruker, Billerica, MA, USA), in the Unit of Mass Spectrometry and Proteomics of the University of Santiago de Compostela (RIAIDT, USC, Spain). This type of mass spectrometry separates peptides according to their mass/charge, so each sample produces a spectrum of peptides that can be compared with those described in specific databases [36] paying attention to the presence/absence of peptides that differ between taxa and allow their identifications. A combination of certain of these peptide markers is generally needed to identify a particular taxon.

2.4. Isotopic Analysis of Seeds and Bone Remains

The preparation of plant samples and bone remains for isotopic analysis was carried out at the Molecular Paleontology Laboratory of the University Institute of Geology (University of A Coruña). The seed samples were placed in glass test tubes for ABA (acid-base-acid) pre-treatment as described in [37]. The first step consisted of digestion in hydrochloric acid (HCl) 0.5 M for 30 min at 70 °C to remove non-structural carbonates; the second step was incubation in sodium hydroxide (NaOH) 0.1 M at 70 °C for 60 min to remove humic acids, followed finally by a repeat of the initial acid treatment to remove the CO_2 that could have been incorporated during the second step. Each of these steps was followed by repeated rinses in distilled water. The final product, once dry, was manually ground with an agate mortar and pestle for IRMS analysis.

The study of the bone remains required the extraction of bone collagen, following a standardized protocol based on chemical and physical methods (digestion and filtration) to eliminate the non-protein components of the bone. After selecting the most compact parts of the bone remains and eliminating the surface and any remains of cancellous bone, a small fragment of about 1 to 2 g was washed repeatedly in an ultrasonic bath (alternating ultrapure water and acetone). After drying for 48 h, the samples were manually powdered in an agate mortar to a particle size between 0.3 and 0.5 mm. The collagen extraction method followed [38], with an extra step to remove humic acids or other organic contaminants [39]. First the bone powder was demineralized by solubilization in HCl 1 M for 20 min. Unwanted organics components were then solubilized in NaOH 0.125N for 20 h, at room temperature. Each incubation was followed by a filtration (5 µm) to eliminate carbonates, organic contaminants, small fragments of collagen, and other organic molecules of low molecular weight. The collagen thus obtained was diluted in mild HCl (0.01 M) for 17 h at 100 °C and filtered (5 µm). The obtained gelatin was lyophilized for further analysis.

The isotopic analysis of bone gelatin or grains was performed by combustion in a ThermoFinnigan MAT 253 isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled via a Conflo III interface (Thermo Fisher Scientific, Waltham, MA, USA) to an EA1108 elemental analyzer (Carlo Erba Instruments, Milan, Italy) in the Research Support Services of the University of A Coruña (SAI, UDC, Spain). The samples were calibrated with the VPDB and AIR standards using acetanilide as the internal standard. The analytical precision was 0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$. The results were expressed according to the delta (δ) notation, which indicates the ratio of the heavy and light isotopes of the element in the sample relative to its standard (VPDB for C and AIR for N).

The quantifications were made in duplicate, and all the obtained results were contrasted to meet three criteria that ensured the quality of the preservation of the collagen analyzed for each sample: extraction yield, expressed as the percentage of collagen in bone, $\geq$1.5%; C and N percentages in collagen of $\geq$11% and $\geq$5%, respectively; and the atomic C:N collagen ratios between 2.9 and 3.6 [40–44]. Correlation tests were also carried out in

order to verify the absence of a relationship between these different indicators and the $\delta^{13}C$ and $\delta^{15}N$ values [43].

3. Results

3.1. Chronological Framework

All the dated charcoals yielded ages congruent with the archaeological environment. Table 1 shows the ^{14}C ages BP and calibrated in OxCal 4.4 [45] against the IntCal20 curve [46]. The dates from the three WP charcoal samples were almost identical, indicating very rapid deposition. They were also similar to those obtained from the charcoals of square J-13. The combination of dates calibrated using the OxCal R-combine function yielded an age between 3637 and 3521 cal BC for WP and between 3699 and 3533 cal BC for SU 1006 from square J-13. We therefore considered that both areas were contemporary and represented the same episode of debris accumulation that occurred in a relatively short time, just 150 years.

Table 1. Results of ^{14}C dating of charcoal from Tell Humeida and calibrated ages in OxCal 4.4 [45] against IntCal20 curve [46].

Area	Depth/SU	Sample	Lab Code	^{14}C Age BP	Age Cal BC	
					From	To
WP	Bottom	TH.07.1	Ua-35246	4 820 ± 50	−3706	−3383
WP	Middle	TH.07.2	Ua-35247	4 705 ± 45	−3629	−3371
WP	Top	TH.07.3	Ua-35248	4 810 ± 45	−3697	−3385
J-13	SU 1004	TH.11.M4	Ua-42141	4 772 ± 35	−3640	−3383
J-13	SU 1005	TH.11.M5	Ua-42143	4 706 ± 42	−3629	−3371
J-13	SU 1006	TH.11.M1	Ua-42140	4 811 ± 34	−3645	−3527
J-13	SU 1006	TH.11.M2	Ua-42142	4 835 ± 35	−3698	−3527
J-13	SU 1006	TH.11.M3	Ua-42144	4 917 ± 40	−3780	−3638

3.2. Study of Plant Remains

The sediment sample from J-13 provided a total of 131 seed and plant remains (Table S1, Supplementary Materials). The small analyzed sample size did not allow us to explore aspects of agriculture in this period beyond determining the presence/absence of certain species and pointing out their correspondence with other contemporary sites.

The remains of Tell Humeida comprised both cultivated and wild plants. Most of them were grains of cultivated plants, three different species having been identified, *Triticum monococcum* (einkorn), *T. dicoccum* (emmer), and *Hordeum vulgare* (barley), as well as less defined categories such as *T. monococcum/dicoccum* or Cereal indet., which reflected the difficulty of identifying the different species at more precise levels due to the preservation of the materials. In the case of barley and emmer, in addition to cereal grains, chaff (rachis, glume bases, and spikelet forks) was identified. The dominant species in this deposit were barley and emmer, which dominated the cereal spectrum, while einkorn was less abundant. Both were the species best represented in other contemporary sites such as Tell Brak in Syria [47] or Arlanstepe in Turkey [48]. There was no evidence of C4 cereals such as millet, although the presence of foxtail millet (*Setaria italica*) is known from the Euphrates Valley sites Tell Abu Hureyra and Tell Mureybit at levels before 5000 BC [49]. Among the wild species, there was an abundance of riparian plants (*Carex* sp., *Cyperus* sp.) and ruderal flora or weeds accompanying crops (*Medicago/Melilotus, Salvia*, and *Rumex* sp.).

The isotopic study of plant remains was purely testimonial given the scarcity of the sample. We were able to analyze only six samples of cereals: two of each of them. Of the weeds, two analyses were carried out for *Medicago/Melilotus*, one for *Rumex*, and four for *Salvia*. Therefore, the result of this analysis can only be interpreted qualitatively.

Table 2 shows the results of the isotopic analysis (mean values ± sd when there was more than one sample). For the interpretation, we calculated an offset of −0.11‰ for $\delta^{13}C$ and −0.31‰ for $\delta^{15}N$ to correct the charring effect, following [50]. Carbon isotope discrim-

ination ($\Delta^{13}C$), measured as a function between $\delta^{13}C$ of the plant and the atmospheric CO_2 at the time ($\delta^{13}C$ = 6.38‰ [19]), was calculated following [51] as in [19].

There are no commonly accepted criteria to ensure the validity of the isotopic values of seeds. The quality of the analyzed material was ensured by a pre-treatment to remove possible organic or inorganic contaminants. Still, certain thresholds for %C, %N, and the C:N atomic ratio ($C:N_{at}$) values of cereals and pulses have been proposed [52].

The %C and %N values of Tell Humeida cereals were consistent with those described in [52] for reliable samples. Cereal $C:N_{at}$ values fell into the interval of 20.9 and 33.3 proposed by [52] for cereals with ABA pretreatment. To our knowledge, there are no published data for comparing weed values.

The difference in $\delta^{15}N$ between grains and rachis of cereals depended largely on irrigation and manuring conditions during the growing and grain infilling seasons. Reference [30] calculated the average grain enrichment over chaff of +2.4 ± 0.8 in crops from different areas and under varying conditions of irrigation and manuring intensity. Taking as a reference only the results of current studies on wheat crops in Syria [30], the mean $\delta^{15}N$ grain–rachis offset was −1.9‰. In turn, Ref. [19] calculated $\delta^{13}C$ offsets from grain to chaff of −1.9‰ for wheat and −1.7‰ for barley.

Table 2. Results of the isotopic analysis of plant remains from Tell Humeida. %C, %N, C:Nat, mean δ values ±1 sd when more than one sample (raw data and with correction for charring effect), calculation of carbon isotope discrimination ($\Delta^{13}C$) according to [19], and isotopic values of cereal chaff calculated from grain values according to [19,30].

Taxon (Number of Samples)	%C	%N	C:Nat	$\delta^{13}C_{VPDV}$ (‰)	$\delta^{13}C$ (‰) −0.11 Offset	$\Delta^{13}C$	$\delta^{15}N_{AIR}$ (‰)	$\delta^{15}N$ (‰) −0.31 Offset	$\delta^{13}C_{VPDV}$ (‰) Chaff	$\delta^{15}N_{AIR}$ (‰) Chaff
Triticum dicoccum (2)	63.7 ± 0.5	3.4 ± 0.0	22.1 ± 0.0	−23.5 ± 0.0	−23.6 ± 0.0	17.7 ± 0.0	12.4 ± 0.0	12.1 ± 0.0	−25.4	10.5
Triticum monococcum (2)	64.7 ± 2.0	3.6 ± 0.0	20.9 ± 0.6	−24.6 ± 0.1	−24.7 ± 0.1	18.8 ± 0.1	10.4 ± 0.3	10.1 ± 0.3	−26.5	8.5
Hordeum (2)	66.9 ± 1.2	3.0 ± 0.9	26.0 ± 0.3	−24.6±0.0	−24.7 ± 0.0	18.7 ± 0.0	5.9 ± 0.1	5.6 ± 0.1	−26.3	4.0
Salvia (4)	40.7 ± 5.7	2.8 ± 0.4	17.5 ± 0.2	−22.4±0.2	−22.5 ± 0.2	16.5 ± 0.2	6.6 ± 0.8	6.3 ± 0.8	-	-
Rumex (1)	42.5	2.0	24.5	−24.7	−24.8	18.9	11.6	11.3	-	-
Medicago/Melilotus (2)	41.1 ± 1.3	5.0 ± 0.3	9.6 ± 0.8	−23.8 ± 0.1	−23.9 ± 0.1	17.9 ± 0.1	3.8 ± 0.7	3.5 ± 0.7	-	-

3.3. Study of Faunal Remains

The total number of bone fragments recovered was 207. The vast majority could not be identified morphologically to the species or even the genus level. Among the remains, there were 67 unidentifiable bone fragments (32.4%); 47.8% could only be classified as medium-sized (87 remains) or large-sized (12 remains) mammals. Among those identified to a reasonably tight taxonomic level, the most abundant were equids (5.8%). The dental morphology of four cheek teeth ruled out horses (*Equus ferus*): the lingual groove was V-shaped, and the caballine fold was absent, although this character was not always present in some primitive breeds [53]. The morphological traits suggested the presence of *Equus hemionus* (hemione or onager) or *Equus hydruntinus* (Eurasian wild ass) (Figure 6a). Among the domestic fauna, sheep represented 3.4%, and sheep/goat represented an additional 4.8%. This mixed category included a proximal metacarpal fragment from a relatively large animal (Figure 6b). Cattle (*Bos taurus*) were represented by only three remains (1.4%), while pigs were completely absent. Other taxa with a scarce presence included gazelle (two fragmented jaws and one isolated tooth), red deer (one jaw fragment), and wild carnivores: two small- to medium-sized felid bones (Figure 6c) and a fox mandible (Figure 6d).

In the Supplementary Materials, Table S2 shows the complete list of the morphologically identified skeletal remains. Table S3 shows the metric values of those remains that allowed measurements to be taken, using standardized landmarks [54–56].

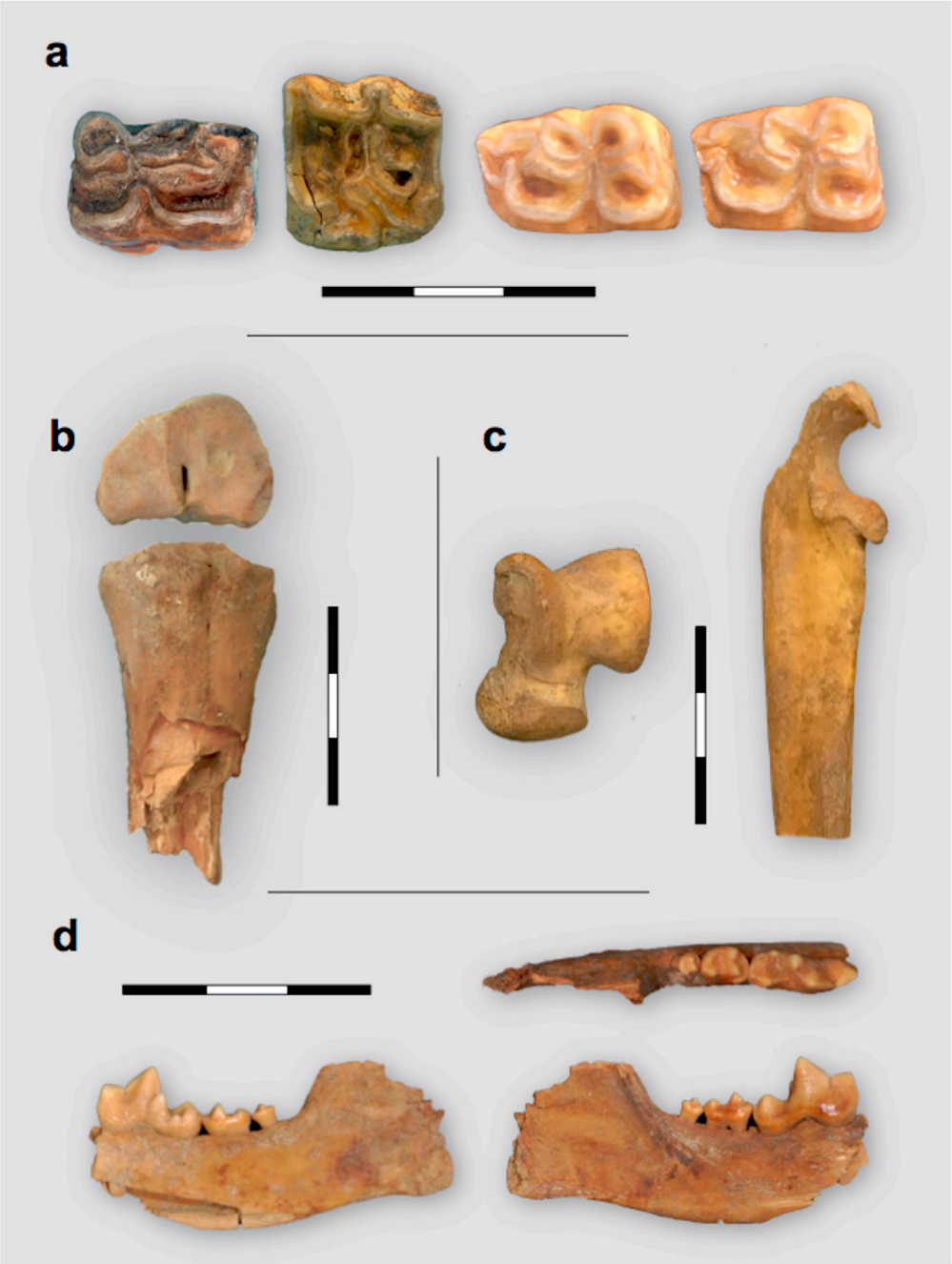

Figure 6. Some highlights of bones from Tell Humeida. (**a**) Four equid cheek teeth: from left to right, lower molar (TH09-H6), probably *Equus hemionus*; upper molar (TH09-H16) with intermediate morphology between *E. hemionus* and *E. hydruntinus;* and lower molars 1 (TH11-18) and 2 (TH11-19) from the same individual, probably *E. hydruntinus*. (**b**) Large ovine metacarpal (TH09-H20). (**c**) Feline calcaneus (TH11-30) and feline ulna (TH11-25), presumably *Caracal*. (**d**) Right hemimandible of fox (TH09-H2), probably *Vulpes cana*.

Regarding taphonomic aspects, the state of preservation of the bones was generally good. The bone surfaces were not eroded, although there was a high rate of old and recent fractures. Fragments of limb bone diaphysis and isolated teeth of the main identified species dominated. A testimonial number of remains (five samples) showed signs of thermal alteration, identifiable by the black or grayish-brown coloring affecting the surface and the entire thickness of the bone. These bones with signs of heat alteration were discarded for isotopic analysis.

Binocular examination of the bone surfaces revealed the presence of four samples with small cut marks, as shown in Figure 7. All were found to be equid remains by ZooMS identification. However, no gnawing marks were found, suggesting a rapid incorporation of the remains into the sediment once they were deposited in this context.

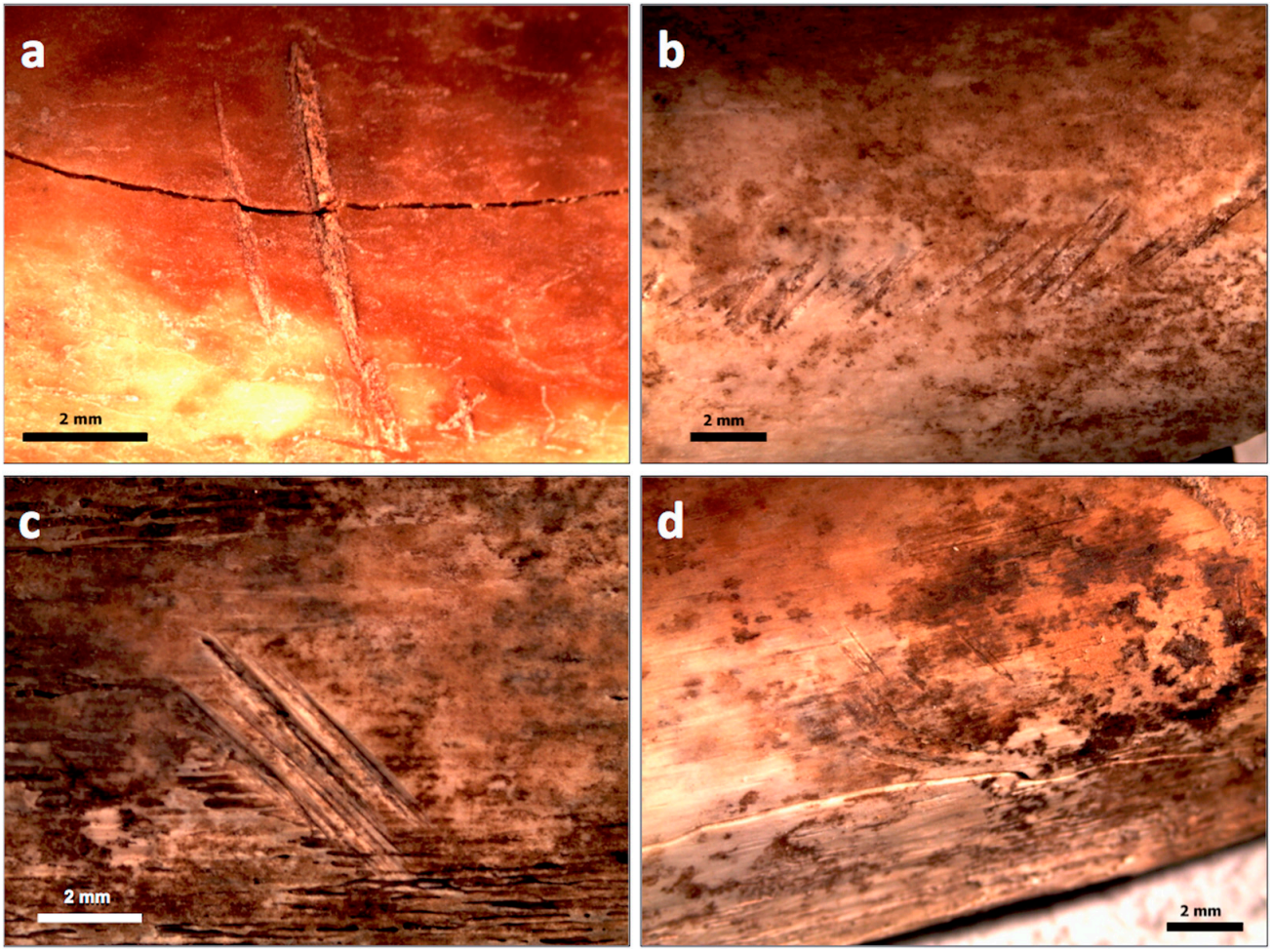

Figure 7. Cut marks on some *Equus* sp. bones. (**a**) TH09-H18; (**b**) TH09-H9; (**c**) TH09-H12b; (**d**) TH09-H4a.

For the isotopic study, we selected 46 skeletal remains, of which only 12 were taxonomically identified by their morphology (Table 3). Two of these, however, did not yield sufficient collagen for analysis. Using peptide fingerprint analysis of collagen, it was possible to identify 25 additional skeletal remains. The set of peptide markers of these samples and their taxonomic attribution can be found in Table S4 (Supplementary Materials). Of the remaining nine samples, five did not provide sufficient collagen; in three, the collagen did not meet the quality parameters; and one of the remains yielded a set of peptide markers that did not correspond to any of the taxa in the databases and was therefore discarded.

The results of the isotopic analysis are shown in Table 3 and Figure 8. The collagen quality criteria are the usual in these studies: only samples with a collagen yield (expressed as percentage of collagen in bone) of 1.5% or more with C and N contents higher than 13 and 5%, respectively, and a C:N atomic ratio ($C:N_{at}$) between 2.9 and 3.6 were considered valid [40–42]. In total, we obtained 35 taxonomically identified samples whose collagen met the quality requirements and had reliable isotopic data. This represented about 78% of the samples initially selected for the study.

Table 3. (next page). List of the samples included in the isotopic study. Morphological and peptide fingerprinting identification and $\delta^{15}N$ and $\delta^{13}C$ isotopic values are given together with the quality data of the collagen obtained (id., identification. N.I.: not identified. Fgt.: fragment).

Area	Code	Bone	Morphological id.	ZooMS id.	Yield (%)	% N_{col}	% C_{col}	C:N_{at}	$\delta^{15}N_{AIR}$ (‰)	$\delta^{13}C_{VPDB}$ (‰)
WP	TH09-H1	Rib	N.I.	*Ovis* sp.	11.2	13.3	36.9	3.2	10.5	−17.3
WP	TH09-H2	Jaw	Fox	*Vulpes* sp.	7.9	14.0	37.4	3.1	11.6	−16.1
WP	TH09-H3	Tibia fgt.	Probably *Equus*	-	<0.5					
WP	TH09-H4	Rib	N.I.	*Equus* sp.	9.4	12.9	35.3	3.2	6.5	−18.6
WP	TH09-H5	Tibia fgt.	N.I.	*Equus* sp.	2.3	13.0	35.5	3.2	5.5	−19.1
WP	TH09-H6	Lower molar	*Equus hemionus*	-	8.6	12.0	25.4	3.2	5.0	−18.5
WP	TH09-H7	Long bone	N.I.	Cervid/*Gazella*	9.3	12.0	32.8	3.2	8.6	−17.2
WP	TH09-H8	Upper molar	*Ovis/Capra*	*Ovis* sp.	11.2	12.4	26.5	3.1	12.5	−15.8
WP	TH09-H9	Long bone	N.I.	*Equus* sp.	4.6	14.6	39.9	3.2	6.2	−19.3
WP	TH09-H10	Jaw	Ungulate, medium	Cervid/*Gazella*	5.5	13.0	35.7	3.2	8.4	−19.0
WP	TH09-H11	Rib	N.I.	*Equus* sp.	10.4	13.6	37.3	3.2	5.2	−19.0
WP	TH09-H12	Long bone	N.I.	*Equus* sp.	10.3	13.1	35.9	3.2	4.4	−18.6
WP	TH09-H13	Pelvis fgt.	N.I.	-	<0.5					
WP	TH09-H14	Tibia fgt.	*Equus* sp.	*Equus* sp.	6.8	14.3	39.1	3.2	6.3	−19.2
WP	TH09-H15	Pelvis fgt.	N.I.	*Equus* sp.	3.9	14.0	37.8	3.2	3.9	−19.8
WP	TH09-H16	Upper molar	*Equus* *	-	9.8	11.2	29.2	3.0	6.7	−18.3
WP	TH09-H17	Long bone	N.I.	*Ovis* sp.	2.7	12.0	32.5	3.2	9.7	−16.1
WP	TH09-H18	Humerus fgt.	N.I.	*Equus* sp.	8.3	14.6	39.9	3.2	5.9	−20.2
WP	TH09-H19	Long bone fgt.	*Equus* sp.	*Equus* sp.	10.8	13.6	37.8	3.2	6.6	−18.6
WP	TH09-H20	Metacarpal	N.I.	*Ovis* sp.	5.6	11.9	33.1	3.2	5.8	−19.0
J-13	TH11-1	Long bone	N.I.	*Ovis* sp.	2.6	12.0	34.0	3.3	11.4	−15.9
J-13	TH11-2	Long bone	N.I.	*Ovis* sp.	1.5	11.4	32.8	3.3	12.9	−17.1
J-13	TH11-3	Long bone	N.I.	*Ovis/Capra*	1.8	11.6	32.4	3.3	9.3	−17.9
J-13	TH11-4	Long bone	N.I.	*Ovis/Capra*	2.1	13.3	37.2	3.3	9.5	−17.3
J-13	TH11-5	Long bone	N.I.	Unidentified	5.2	13.8	39.1	3.3	14.4	−17.2
J-13	TH11-6	Long bone	N.I.	*Capra* sp.	7.1	14.4	41.2	3.3	9.9	−17.7
J-13	TH11-7	Long bone	N.I.	*Ovis* sp.	1,6	9.2	26.9	3.4	11.4	−18.6
J-13	TH11-8	Long bone	N.I.	*Panthera* sp.	3.9	11.0	31.0	3.3	11.1	−17.7
J-13	TH11-9	Long bone	N.I.	no quality	2.3	10.9	34.5	3.7		
J-13	TH11-10	Long bone	N.I.	-	<0.5					
J-13	TH11-11	Long bone	N.I.	Cervid/*Gazella*	7.8	12.6	34.7	3.2	9.1	−18.1
J-13	TH11-12	Long bone	N.I.	No quality	1.6	3.8	13.4	4.1		
J-13	TH11-13	Long bone	N.I.	-	<0.5					
J-13	TH11-14	Long bone	N.I.	*Ovis* sp.	2.1	10.7	30.4	3.3	9.9	−16.8
J-13	TH11-15	Long bone	N.I.	No quality	1.8	4.0	12.3	3.6		
J-13	TH11-16	Long bone	N.I.	*Ovis* sp.	3.7	12.1	33.4	3.2	10.0	−16.0
J-13	TH11-17	Phalanx	*Bos taurus*	-	<0.5					
J-13	TH11-18	Lower molar	*Equus hydruntinus*	*Equus* sp.	1.5	13.1	35.8	3.2	7.4	−17.0
J-13	TH11-19	Long bone	N.I.	Cervid/*Gazella*	6.2	12.8	36.1	3.3	8.3	−18.5
J-13	TH11-20	Long bone	N.I.	*Ovis* sp.	7.4	10.7	29.6	3.2	13.5	−16.3
J-13	TH11-21	Long bone	N.I.	Cervid/*Gazella*	8.3	11.7	32.5	3.2	7.8	−17.9
J-13	TH11-22	Long bone	N.I.	-	<0.5					
J-13	TH11-23	Jaw	*Gazella* sp.	*Ovis/Capra*	3.8	8.5	23.5	3.2	11.3	−18.1
J-13	TH11-24	Jaw	*Ovis/Capra*	*Ovis* sp.	11.4	11.9	32.5	3.2	11.5	−17.6
J-13	TH11-25	Ulna	*Caracal*	*Felis/Lynx*	10.7	12.1	33.5	3.2	9.7	−15.9
J-13	TH11-26	Jaw	*Cervus*	-	<0.5					

* *Equus hydruntinus/hemionus* according to its morphology, see Figure 6a.

Of the 35 samples selected, the mean yield was 6.3 ± 3.4%. The percentages of C and N were 12.4 ± 1.4% and 33.9 ± 4.2%, respectively. The mean C:N_{at} was 3.2 ± 0.1. These data indicated that the preservation of the bone collagen was quite good despite environmental conditions that might at first appear adverse due to the high temperature in the region.

Overall, the isotopic values of the analyzed fauna showed little variation, despite the fact that the sample included herbivorous and carnivorous mammals. The mean $\delta^{13}C$ was −17.8 ± 1.2‰, and the mean $\delta^{15}N$ was 8.7 ± 2.6‰. Grouping the most represented taxa, Table 4 shows the mean values, standard deviation, and range for each group. As can be

seen from Figure 8, the different herbivore taxa were partly grouped according to **δ^{13}C** and/or **δ^{15}N**. Among the sheep, one individual had a clear outlier value of δ^{15}N.

Table 4. Mean isotopic values, standard deviation, and range of Tell Humeida sheep, wild ungulates, equids, and carnivores.

		$\delta^{13}C_{VPDB}$ (‰)					$\delta^{15}N_{AIR}$ (‰)				
	n	**Mean**	**sd**	**Min**	**Max**	**Range**	**Mean**	**sd**	**Min**	**Max**	**Range**
Ovis	11	−17.0	1.1	−19.0	−15.8	4.2	10.8	2.1	5.8	13.5	7.7
Ovis (without outlier)	10	−16.8	0.9	−18.6	−15.8	2.8	11.3	1.3	9.7	13.5	3.8
Cervid/Gazella	5	−18.1	0.7	−19.0	−17.2	1.8	8.4	0.5	7.8	9.1	1.3
Equus sp.	12	−18.9	0.8	−20.2	−17.0	3.2	5.8	1.0	3.9	7.4	3.5
Carnivores	3	−16.6	1.0	−17.7	−15.9	1.8	10.8	1.0	9.7	11.6	1.9

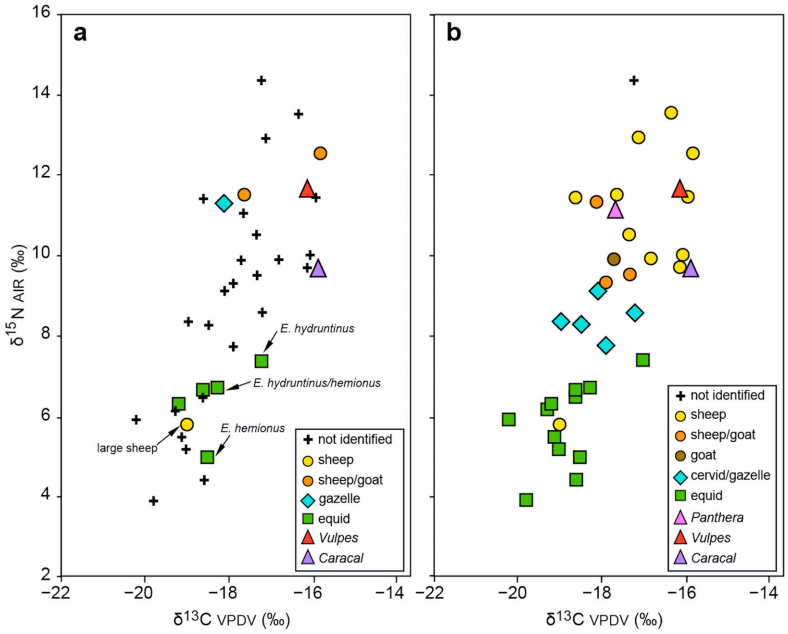

Figure 8. Animal collagen δ^{15}N and δ^{13}C values in Tell Humeida. (**a**) before identification and (**b**) after identification by peptide mass fingerprinting.

4. Discussion

4.1. Taxonomic Identification of the Fauna

Once the peptide fingerprint analysis was performed, we found that equids were present in a substantial proportion in the Tell Humeida collection. Using this technique, we were able to identify seven equid bones among the unidentifiable fragments and confirm the morphological identification of two others. They were particularly abundant in WP and hardly at all in J-13. This must be considered an artifact caused by the small number of samples because as we have seen, both sampling areas represented the same deposition of remains over a relatively short period (about 150 years).

It was difficult to determine to which equid species the remains belonged, as was common in that time and region, as the morphological and metrical characteristics of the bones varied greatly between individuals within a population [57–59]. Peptide fingerprint analysis was not helpful in taxonomic identification as it only reached the genus level (*Equus* sp.). Teeth morphology, long considered accurate, has also proven to be conflicting [60]. In the four Tell Humeida equid molars (Figure 6a), there were features indicating the Eurasian wild ass (*Equus hydruntinus*) in some cases and hemione (*Equus hemionus*) in others. The Eurasian wild ass was identified morphologically and genetically in North Syria [61,62], so it would not be surprising to find it in this area of the Middle Euphrates.

The indeterminate morphological traits of the equids could suggest that they are hybrids. The interbreeding between hemione and ass was common in Mesopotamia to produce strong and fast draught animals called *kunga*, which were highly valued as prestige goods [63,64]. According to written sources, *kunga* were mostly raised in the main breeding center at Nagar (modern Tell Brak) but in a much later chronology, in the mid-third millennium. The presence of cut marks on some Tell Humeida equid bones (see Figure 7) was also inconsistent with this scenario.

Morphologically identified sheep remains were very scarce. However, one of the most interesting applications of the ZooMS technique was the possibility to separate sheep from goats in postcranial remains due to the difference in only one marker peptide [65]. Thus, we were able to identify two of the remains morphologically classified as sheep/goat, which turned out to be sheep. We refuted the initial identification of one mandibular fragment that had been tentatively identified as *Gazella* as it presented a marker characteristic of sheep rather than that of gazelles or cervids (see Table S4). In addition, we found eight sheep remains and one goat (*Capra* sp.) among the unidentifiable remains from Tell Humeida, which allowed us to considerably enlarge the sample size of sheep for the isotope study. In two other remains, the peptide that allows differentiation between the two species was not found.

The expansion of sheep herding is considered a central feature of the Late Chalcolithic in Mesopotamia and may be associated with the expansion from the south of the Uruk culture. From the mid-4th millennium, sheep were an important source of wool as a secondary product [66].

Very few sheep bones could be measured, but specifically, there was one metacarpal of considerable size (see Figure 6b). There were few data to directly compare its dimensions with those of undoubtedly domestic sheep. For example, at Tell Halula [67], domestic sheep were smaller (Figure S1, Supplementary Materials), but it was also true that there was a long time jump between that site (7000 BC) and Tell Humeida, and the size of the domestic sheep tended to increase as husbandry and management techniques became more sophisticated [68]. This specimen, in any case, could be from a wild animal.

As with equids and sheep, other ungulates also presented identification difficulties in such a restricted and fragmented bone sample. We identified a fragment of a deer mandible that unfortunately did not produce enough collagen for isotopic study and one gazelle mandibular fragment. Among the unidentified fragments, peptide markers pointed to five other remains of ungulates such as cervids (*Cervus* or *Dama*). Another possibility was that they belonged to a gazelle, whose markers were still not well known [36] and therefore could not be differentiated from those of cervids. In any case, they were wild ungulate herbivores as sheep or goats were ruled out by the combination of their peptide markers.

Felids were represented by two bone remains identifiable by their morphology: a medium-sized ulna fragment and a medium-sized talus (see Figure 6c). Comparative metric data were scarce, but the talus of Tell Humeida was clearly larger (Figure S2 in Supplementary Materials) than those of smaller felids from Predynastic Egyptian tombs [69] such as the wild cat (*Felis sylvestris*), the sand cat (*Felix margarita*) of arid environments, or the jungle cat (*Felis chaus*) linked to watercourses. All of them are described in the region in historical times [70]. In turn, the Tell Humeida specimen was distinctly smaller than leopard tali from the Paleolithic Caucasus [71], so it would belong to a medium-sized felid.

The ulna (see Figure 6c) was identified by ZooMS as *Felis* or *Lynx*. This is consistent with our identification as *Caracal* as the latter is phylogenetically very close to these genera. Moreover, an unidentified bone fragment yielded peptide markers compatible with the genus *Panthera*. We could not know if it was a large felid or if it could represent the possible serval (*Leptailurus serval*), since, as with the caracal, no specific markers have been published for this genus. Although the serval is most closely related to the caracal, the amino acid sequence of collagen, responsible for the mass/loading of peptide markers, did not offer the same detailed taxonomic resolution as DNA in terms of phylogenetic topology. Caracal and leopard were present in Syria at least until the 20th century [70]. The set of

peptide markers of these samples and their taxonomic attribution can be found in Table S4 (Supplementary Materials).

A fragment of a right fox mandible (see Figure 6d) with teeth (P3-M3) was recovered (TH09-H2). The small size of this specimen (Figure S3, Supplementary Materials) excluded it being from red fox (*Vulpes vulpes*). In their study on the faunal remains from Neolithic Tell Mureybet [72], the authors report on the difficulty of identifying the small foxes present in Syria due to the lack of morphometric studies on species such as Rüppell's fox (*Vulpes rueppellii*) and Blanford's fox (*Vulpes cana*). Whereas the former prefers sandy or rocky deserts, the latter inhabits semiarid hilly areas surrounded by steppes [73]. Considering such environmental grounds, these authors suggested that most probably, *V. cana* was the species present at Tell Mureybet. A comparison between the Tell Humeida and Tell Mureybet lower carnassial (m1) measurements (Figure S3, Supplementary Materials) showed that the specimen from Tell Humeida was on the upper part of the cluster of *Vulpes cana*, clearly separated from the sample of *V. vulpes*. This species was not found among the historical carnivores from Syria, although the Rüppell's fox was found in archaeological excavations at Mari, downstream of the Euphrates [70].

4.2. Manuring and Watering of Crops?

The $\delta^{15}N$ isotopic values of the wheat were unusually high (10.1 and 12.1‰), double the value of the barley. A first interpretation was that the wheats, and not the barley, were intensively manured. We could compare the Tell Humeida values with the contemporary ones (between 3900 and 3600 cal BC) from the Syrian site of Tell Brak, located north of the country [74], observing higher $\delta^{15}N$ in the einkorn and especially in the emmer from Tell Humeida, while the barley did not differ appreciably (Figure S4 in Supplementary Materials).

However, Tell Humeida is located in a more arid region, at least at present, than Tell Brak. where annual precipitation is estimated to have been between 363 and 413 mm in that period. Since aridity could increase $\delta^{15}N$ values in soils and plants due to an effect on the nitrogen cycle [75,76], it was necessary to look at $\Delta^{13}C$ values and their relationship to water input. Decreased water availability causes stomatal closure, decreased discrimination against ^{13}C, and the subsequent decrease in $\Delta^{13}C$ of grains [23]. A comparison of $\Delta^{13}C$ of cereal grains and wood from trees not from riverine forests in the ancient Near East showed that the growing conditions were in general far wetter than for present-time rainfed crops in the region [23,24]. According to [19], well-watered wheat grains showed $\Delta^{13}C$ values over 17.0‰, while for barley grains, the value was 18.5‰. All Tell Humeida samples showed values within the range of well-watered grains (Figure 9).

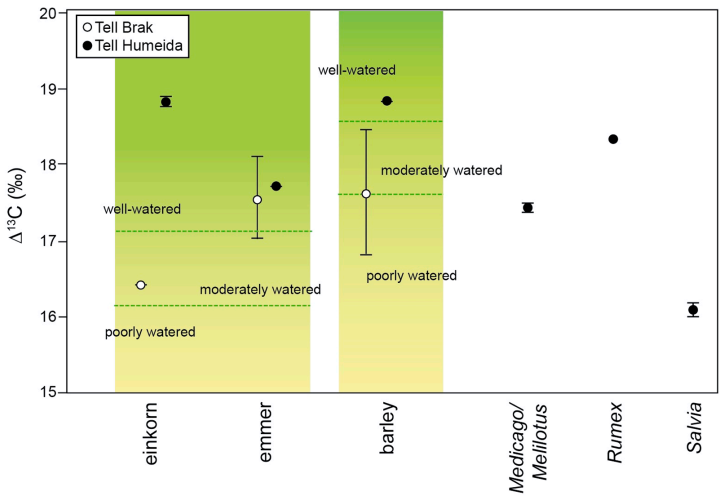

Figure 9. $\Delta^{13}C$ of wheats and barley from Tell Humeida compared with those from Tell Brak of the same chronology [74] and weeds from Tell Humeida. For wheats and barley, shaded areas represent a gradient of watering, with categories established by [19].

As reported by their isotopic values, therefore, Tell Humeida wheats received sufficient water, either by irrigation or rainfed. According to [24], whereas the Mesopotamian lowlands became the focus of irrigation-based riverine civilizations, the settlements of Upper Mesopotamia such as Tell Brak were primarily based on rainfed agriculture. In Tell Humeida, in an intermediate position in the Middle Euphrates, it seems that irrigation could had been a common cultivation strategy. If aridity was not responsible for the high $\delta^{15}N$ values in wheat, the explanation could be the abundant use of manure to fertilize the fields. Experimental studies on modern crops have shown that fertilization with animal manure produces an elevation in the $\delta^{15}N$ values of cereals, an effect often claimed to explain the high isotopic values of cereals in Mesopotamian sites [14,30].

However, there are other natural processes that have to be taken into account: the seasonal river floods characteristic of Mesopotamia would provide silty sediments and organic matter that would enrich the cultivated soils; the denitrification of sediments in wetlands, where nitrate is converted by heterotrophic, facultative anaerobic bacteria into nitrogen gas. Microbial N transformations discriminate against the heavy N isotope, leaving the substrate enriched in ^{15}N [76]. Rarely considered so far is the role of aquatic insects, which spend their larval stages in lake sediments and emerge as adults to mate over land and can result in an appreciable fertilization effect on the river flood plain with consequences for plant $\delta^{15}N$ values [77]. In any case, the intentional or unintentional manuring with manure and the effects of these other natural processes mentioned above seem to have mainly affected wheat and not barley, whose $\delta^{15}N$ values were comparatively low. This difference could have been related to the intended use of each type of cereal.

However, we must bear in mind that our data were very scarce and that in more extensive works on cereal stable isotopes from this period in the Middle East, both barley and wheat $\Delta^{13}C$ values were highly varied [19,74], so the interpretation of the limited values obtained at Tell Humeida was a conjecture. It would be necessary to analyze a much larger number of samples of each type of cereal in order to draw more reliable conclusions.

The analyzed crop plants showed isotopic and $\Delta^{13}C$ values in accordance with their ecology and physiology. *Medicago*/*Melilotus* is a legume, and therefore, its $\delta^{15}N$ was low. *Salvia* had an intermediate value and a $\Delta^{13}C$ value typical of a drier environment, and *Rumex*, a ruderal plant, resembled wheat, among which it probably grew.

4.3. Animal Diet and Husbandry

In order to interpret the type of diet of the domestic animals of Tell Humeida and in the absence of sufficient data on the various plant ecotypes of the area, we performed a relative evaluation of their isotopic values with those of the wild herbivores and carnivores (Figure 10, see also Table 4).

Although with few remains, the deer or gazelles analyzed showed intermediate isotopic values among all the specimens. Not reaching a trophic level above these wild herbivores were the carnivores ($\delta^{15}N$ offset carnivores–wild ungulates = +2.4‰), while their $\delta^{13}C$ values were considerably far from what would be expected for a trophic level ($\delta^{13}C$ offset carnivores–wild ungulates = +1.5‰). This indicates that carnivores had different food sources. In the case of the fox, either both possible species had omnivorous diets, including fruits and insects [78,79] that could contribute to elevated $\delta^{15}N$ values. The caracal fed on small mammals, mostly rodents and hares, but also ground birds and only sporadically young ungulates [80,81]. We did not have isotopic data for this small mammal fauna, which, in any case, must have had very diverse food sources. Finally, the isotopic signature of the sample identified as *Panthera* sp. fit better with a diet based on wild ungulates ($\delta^{13}C$ offset *Panthera*–wild ungulates = +2.7‰).

In contrast to the isotopic values of wild herbivores, domestic herbivores showed different and extreme values. Sheep showed very high $\delta^{15}N$ values, even higher than those of coeval carnivores. Only the large specimen mentioned above had low isotopic values that deviated markedly from the whole, again pointing to its wild origin. The high $\delta^{15}N$ values of the sheep could respond to completely different scenarios. On one hand, they

could indicate that sheep fed by grazing in the arid steppe. Aridity has been described as producing a metabolic effect consisting in the recycling of water and nitrogen compounds, resulting in high $\delta^{15}N$ values [31,32]. However, it would have to be a more marked aridity than in the case of wild ungulates. The $\delta^{13}C$ values of sheep were slightly less negative than those of cervids/gazelles (difference of 1.2‰), suggesting that sheep may have fed on plants with lower water input than wild ungulates. It is difficult to imagine, however, what could be a more arid environment than one inhabited by wild ungulates in the region. Nor do we know whether sheep grazing in such an arid landscape would be efficient.

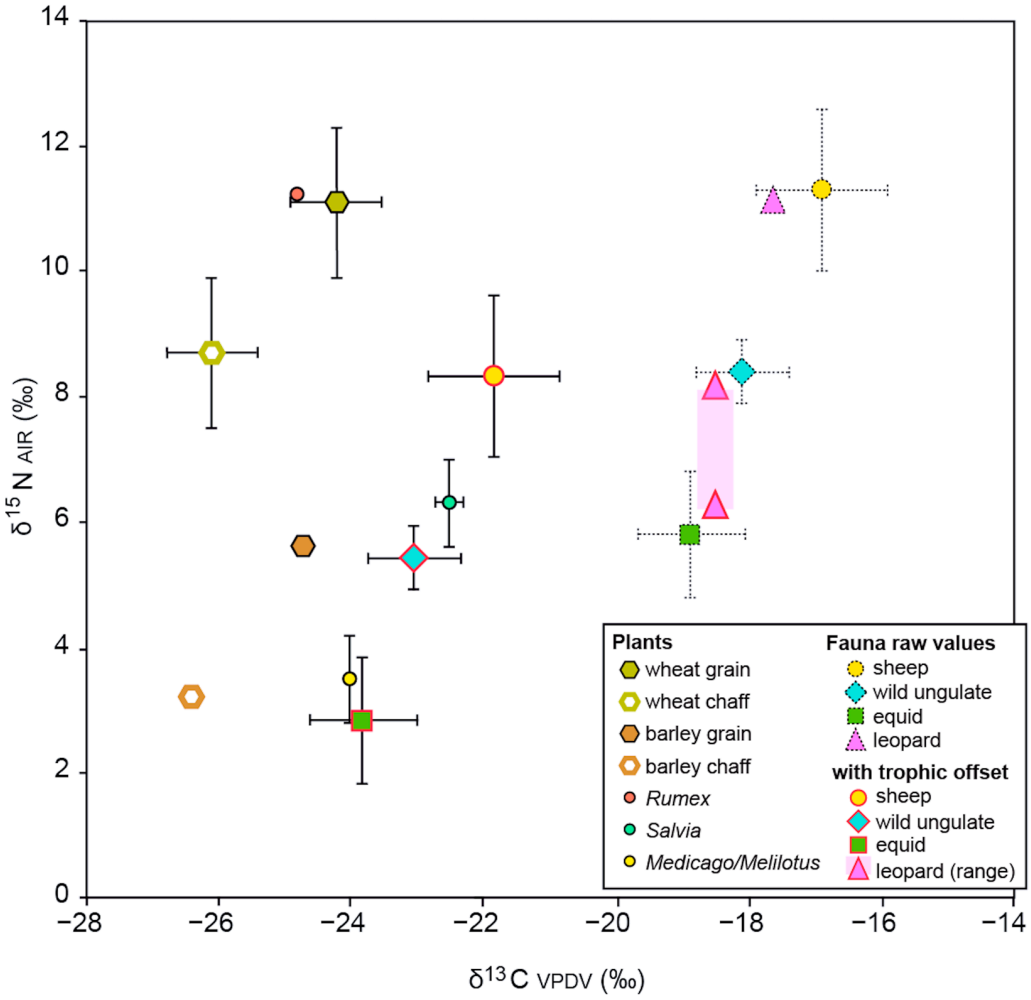

Figure 10. Mean isotopic values of plants and main animal taxa (raw values and corrected for trophic offset) from Tell Humeida with error bars representing the standard deviation, one sigma. In sheep, the outlier specimen has been omitted.

In a second scenario, grazing on highly fertilized land or feeding on forage grown under these conditions could produce the elevated $\delta^{15}N$ values recorded in sheep. According to this scenario, we could propose that the ewes fed on cereal straw grown on well-watered, highly fertilized land, such as wheats. However, the $\delta^{13}C$ values were well above the expected trophic offset of 5‰ between plant and consumer bone collagen and were not sufficiently negative to indicate that this type of feeding was preferred [25].

It would be possible for both scenarios to coexist, for example, with seasonal variation. A serial analysis of the dentine could reveal whether there were seasonal variations in the diets of these animals [82].

Finally, the carbon isotopic signal slightly deviated toward higher values compared with that of wild herbivores, which could be compatible with a C4 plant input. This could

be millet, which has been cultivated in the region since before 5000 BC according to evidence from Tell Abu Hureyra or Tell Mureybit [49]. However, recent studies re-examined the westward spread of millets that originated in North China and directly dated the millet remains. It showed that millets were only introduced into this region after 2500 BC [83–85]. Although this type of cereal never seems to have been an important food, its consumption, or at least that of C4 plants, was detected in some Middle Bronze Age humans from Tell Barri, NE Syria [86]. If there were no appreciable contributions of millet in the diet of Tell Humeida sheep, we would have to resort to wild C4 plants. Plants with this type of photosynthetic metabolism exist in the region, both in the steppe and in wetlands [87–89]. However, we could not explain why their consumption was not reflected in the isotopic values of wild ungulates.

Equids also did not seem to have fed on the same type of plant as wild herbivores. This could indicate that these animals were under human control, rather than wild animals, at least those with lower $\delta^{15}N$ and $\delta^{13}C$ values. The low $\delta^{15}N$ values of the studied equids seemed to indicate a well-watered plant-based diet, not influenced by manure, and with a significant input of legumes, which contributed to lowering the $\delta^{15}N$ value. This description was consistent with the barley analyzed at Tell Humeida, if, in addition, leguminous plants were added to the diet. From archaeological sources, we knew that barley was the most frequent food for domestic equids in Mesopotamia [63,90]. Alfalfa was also a common forage in this region, where it may have been cultivated as early as 5000 years BC [91], although the earliest recorded reference to alfalfa as a forage dates from only 3300 yr BP [92]. The few plant remains from Tell Humeida included seeds of the legume *Medicago*/*Melilotus*, but their morphology made them more likely to be the latter wild plant. Interestingly, it has been observed that Przewalski's horse preferentially consumes Fabaceae plants during the summer [93]. If this behavior is generalized to equids, it would explain their lower $\delta^{15}N$ values compared with other wild ungulates.

Equids were not abundant in other fourth millennium sites in the region. For example, among the fauna from level 4 at Tell Ramadi, located downstream along the west bank of the Euphrates River [94], sheep and pigs (absent at Tell Humeida) accounted for almost 85% of the faunal remains, but equids had only a testimonial presence (3.5%).

This scarcity of equid remains was more evident in the few isotopic studies of fauna from Mesopotamian sites, which tended to focus more on plants and secondarily on domestic fauna. We only had data from the Late Neolithic levels at Tell Sabi Abyad, which included a few gazelle and equid remains, as well as sheep and goats [95]. At this site, the equids also showed the lowest $\delta^{15}N$ values, but the values overlapped with those of the gazelles and even some caprines. The differences in the mean $\delta^{15}N$ values of these three groups at Tell Sabi Abyad [95] were several times smaller than those observed at Tell Humeida. For all these reasons, we proposed that either the Tell Humeida equids had a particular diet, controlled by humans, notably preceding the written sources, or they came from areas close to the river but without direct anthropogenic influence. Interestingly, the isotopic values of the equid molar most clearly identified as *E. hydruntinus* differed markedly from those of the other equids and were closer to those of the wild ungulates analyzed, suggesting a more steppic environment.

5. Conclusions and Future Directions

A first conclusion of this study is to emphasize the value of the peptide fingerprint analysis technique or ZooMS to identify taxa, either from unidentifiable remains or to differentiate between sheep and goats in the case of partial skeletal remains. Even though an arid climate is not usually the most conducive to the preservation of bone collagen, we found that at Tell Humeida, there was a notable proportion of remains with well-preserved collagen. Even so, we are aware that the number of samples studied was small and that it will be long and difficult to obtain more samples from this promising and, for the moment, inaccessible site.

Such a restricted sample set could only provide partial information on the way of life of the Late Chalcolithic inhabitants of Tell Humeida. The isotopic analysis of cereal grain and wild plant seeds has proved very informative at other sites with a larger number of available remains. In the case of Tell Humeida, the conclusions that could be drawn were only tentative, but we considered them interesting as they represented a novelty for Middle Euphrates sites of this age.

The isotopic signal recorded in sheep bones may have been due to contributions from various cultivated or uncultivated plants. Serial analysis of the dentine could reveal whether there were seasonal variations in the diets of these animals, and would contribute to the ongoing debate on the type of pastoralism in Late Chalcolithic Mesopotamia, i.e., whether sheep grazed on agricultural fields near the sites or grazed on distant pastures on a nomadic or seasonal basis. In the case of the equids, their particular isotopic signatures could indicate either a strong preference for legumes or a certain human control, long before the dates revealed by the written record, for the use of these animals to obtain highly valued hybrids. It would be worthwhile to check whether the equid isotopic signatures at other sites contemporary with Tell Humeida also reflect such a particular diet. From a diachronic perspective, isotopic analysis could help to establish when equids in these regions were undoubtedly tamed, or at least controlled, by humans.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d15060709/s1: Table S1: Tell Humeida plant macroremains; Table S2: Tell Humeida faunal remains; Table S3: Osteometrical data of Tell Humeida faunal remains; Table S4. Peptide markers in bone collagen and taxonomic identification by ZooMS; Figure S1. Measurements of metacarpals from sheep at Tell Halula and the specimen from Tell Humeida; Figure S2: Greatest length of felid astragalus from Egyptian tombs, Pakistan, Pleistocene leopards from Kudaro cave, and the specimen from Tell Humeida; Figure S3: Measurements of m1 from foxes recovered at Tell Mureybet and the specimen from Tell Humeida; Figure S4: $\delta^{13}C$ and $\delta^{15}N$ values of wheats and barley from Tell Humeida compared with those from Tell Brak of the same chronology.

Author Contributions: Conceptualization, A.G.-d. and J.L.M.-F.; funding acquisition, A.G.-d. and J.L.M.-F.; investigation, A.G.-d., A.G.-V., M.M.-G., L.P.-C. and J.S.-S.; methodology, A.G.-d. and J.L.M.-F.; writing—original draft, A.G.-d., M.M.-G., L.P.-C. and J.L.M.-F.; writing—review and editing, A.G.-d., A.G.-V., M.M.-G., L.P.-C., J.S.-S. and J.L.M.-F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the projects "Investigaciones Arqueológicas en el Medio Éufrates" (HAR2010-15866 of the Ministry of Economy and Competitiveness, Spain) and "Expedición arqueolóxica da UDC no Medio Éufrates Sirio" (10PXIB 167197PR, Xunta de Galicia, Spain) to J.L.M.F. The Institute of Geology of the University of A Coruña (Spain) received support from the Xunta de Galicia through the program "Consolidación y estructuración de unidades de investigación competitivas: Grupos de potencial de crecimiento" (ED431B 2021/17, Xunta de Galicia, Spain) to A.G.d.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data generated in this work can be found in the text or in the Supplementary Materials.

Acknowledgments: The authors would like to thank the General Directorate of Antiquities and Museum of Damascus and the Museum of Deir ez-Zor for their cooperation in the fieldwork. Marta Pérez Rama is credited for her collaboration in the initial lab work; María Lema Grille of the Instrumental Analysis Techniques Unit (SAI, UDC) and Esteban Guitián (RIAIDT-USC) are thanked for the use of the analytical facilities for which they are responsible. Constructive comments from three anonymous reviewers helped to improve the manuscript, for which we are grateful.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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