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# Stable Isotopic Evidence for Human and Animal Diets From the Late Neolithic to the Ming Dynasty in the Middle-Lower Reaches of the Hulu River Valley, NW China

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The study of human and animal paleodiets, representing the unique subsistence strategies and human-environment interactions adopted over evolutionary time, has attracted intensive research attention. Historically, the western Loess Plateau (WLP) served as a key area for the evolution of human-land relationship. The human subsistence patterns in the WLP changed significantly from prehistoric to historical periods based on archaeobotanical data. However, the trajectory and influencing factors of ancient human and animal diets in the WLP remain unclear, mainly due to the lack of isotopic data in the upper reaches of the Wei River. In this paper, we reported 172 human and animal isotope samples (C and N) and 23 radiocarbon dates from three sites in the middle-lower reaches of the Hulu River Valley (HRV). At least three periods of dietary patterns for humans were observed in the WLP from the late Neolithic to Ming Dynasty. During 5300–4000 Before Present (BP), humans and domesticated animals such as pigs and dogs consumed a greater proportion of millets and millet byproducts. Between 3000 and 2200 BP, the diets of pigs and dogs remained largely comprised of $C_4$ foods, while humans consumed both $C_3$ and $C_4$ foods, which contradicted the evidence of an overwhelming proportion of wheat and barley ($C_3$ crops) from the contemporaneous cultural sediment. The contradictions between plant remains and human diets are probably related to geopolitical factors. Between 1000–500 BP, human diets were more diverse and heterogeneous in this region. Combined with environmental and archaeological evidence, the changes in diets and subsistence strategies over the three periods can be attributed to the comprehensive influence of regional cultural development, geopolitics and technological innovation. This paper not only reveals the trajectory and influencing factors of ancient human and animal diets in the middle-lower HRV, but also explores how subsistence strategies, particularly in terms of dietary structure, will change in the context of cultural exchange and diffusion, and emphasizes the important influence of geopolitical interactions in the WLP.

**Keywords: isotopic analysis, paleodiet, *trans*-Eurasia exchange, geopolitical interaction, western Loess Plateau**

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## INTRODUCTION

The study of human subsistence strategies highlights the ability of humans to adapt to changing environments, which provides an essential means to explore the mechanisms and influences of human-environment interactions, and has therefore received extensive attention across disciplines over the last few decades (von Cramon-Taubadel, 2011; Obregon-Tito et al., 2015; Marchant et al., 2018; Bleasdale et al., 2020). Historically, northwest China was easily influenced by climate change and *trans*-continental cultural exchanges, where human subsistence strategies were diverse. For example, this region served as the frontier for fishing-hunting practices and agricultural activities during the prehistoric agricultural civilization (Bettinger et al., 2007; Dong et al., 2013; Liu F. et al., 2019), and later evolved into a mixed region of crop and livestock agricultural practices (Spengler et al., 2014; Yang et al., 2019; Janz et al., 2020). Therefore, changes in human livelihoods in northwest China reflected the social, economic and political changes associated with climate change and transcontinental cultural exchanges from prehistoric to historical periods.

However, the spatial-temporal patterns of human subsistence strategies in northwest China are still not well understood. Most previous research has focused on the prehistoric period, especially the late Neolithic and Bronze Age (Chen et al., 2015; Barton et al., 2020; Dong et al., 2020a, 2022b), and a few studies have focused on the Iron Age in Xinjiang (Liu W. et al., 2010; Zhang et al., 2018; Wang X. et al., 2021), while very little attention has been paid to historical studies. Evidence from many plants, animals and isotopes has illustrated the human livelihood patterns in the Qinghai-Tibet Plateau (Du et al., 2004; Chen et al., 2015; Song et al., 2021; Wende et al., 2021) and the Hexi Corridor (Atahan et al., 2011a; Zhang et al., 2017; Yang et al., 2019, 2020). But those in the western Loess Plateau (WLP), a critical area of dramatic changes in human survival strategies, remain deficient, with only limited evidence from the Hehuang Valley (Ma M. et al., 2016; Ren et al., 2021).

Studying human and animal dietary structures can improve our understanding of human livelihood patterns (Walker and DeNiro, 1986; Lv, 2017; Hu, 2018; Krajcarz et al., 2020). Compared to indirect evidence suggested by plant remains and animal bones unearthed from archaeological sites (Flad, 2007; Zhao, 2010; Du et al., 2020), the inbuilt diet information associated with human bones and teeth present direct evidence of consumption, which can be obtained by the study of isotopes (Kohn, 1999; Wang T. et al., 2019; Wang X. et al., 2021), plant microfossils (Henry et al., 2011; Salazar-García et al., 2021; Scott et al., 2021), ancient proteins (Welker et al., 2016; Jeong et al., 2018; Wilkin et al., 2020) or ancient DNA (Weyrich et al., 2017; Wang C. et al., 2021; Wang W. et al., 2021). Carbon and nitrogen isotopes can directly and effectively reflect the dietary structures and trophic levels of humans and animals (Schoeninger et al., 1983; Kohn, 1999; Hu, 2018), and therefore have played an important role in the study of human diet reconstruction (Atahan et al., 2011b; Dong Y. et al., 2017; Liu and Reid, 2020), agricultural origins (Barton et al., 2009; Leipe et al., 2019; Liu X. et al., 2019), and animal management (Hu et al., 2014; Ma et al., 2021; Vaiglova et al., 2021).

Along with the introduction of pastoralism into China during the Late Holocene, changes of human diets and livelihood patterns in China are still unclear. In the steppe region of northern China, animal husbandry rapidly replaced the indigenous lifestyle (Yuan, 2016; Dong et al., 2021a; Zhang et al., 2021). Following the arrival of Afanasevo and Andronovo populations in northern Xinjiang, the communities relied on a mixed economy of cultivation and animal husbandry quickly shifted to the dominance of animal husbandry (Wei and Feng, 2020). In the Central Plains, pastoralism was selectively used and then indirectly contributed to the formation of early states (Han, 2015; Zhang, 2017). However, in the farming-pastoral ecotone, the change trajectory of human subsistence strategies (especially the dietary structure) is still very vague due to complex factors.

The middle-lower reaches of the Hulu River Valley (HRV), located in the upper reaches of the Wei River basin in the WLP, represent a key area relating to the origins, spread and exchange of agriculture from the Neolithic to historical period (Su, 2008; Barton et al., 2009; Atahan et al., 2011b). Here, archaeobotanical evidence indicates that the agricultural planting structures have changed dramatically (Li, 2018; Chen et al., 2020). The discovery of only a few common millets suggested that previous societies had grown crops as early as Dadiwan 1 Culture (7800–7350 BP) (Liu et al., 2004; An et al., 2010). Over the periods of Yangshao to Qijia Culture, millet-based agriculture was established and expanded rapidly in the WLP, and millets played an increasingly important role in human livelihoods (Chen et al., 2020; Wang and Cui, 2021). With the intensification of transcontinental exchange, wheat and barley were introduced into this region, although not widely used, during 4300–3600 BP (Barton and An, 2014; Liu et al., 2016a). There is a gap in human settlement intensity from 3600 to 3000 BP in the prehistoric record. After 3000 BP, agriculture once again intensified, as wheat was widely distributed, altering the original agricultural planting structure (Chen et al., 2020; Ren et al., 2021). During the Song-Qing Dynasty, wheat became the dominant staple food, with evidence of various agricultural planting patterns based on historical documents and archaeobotanical evidence (Li, 2018).

Nonetheless, archaeobotanical evidence alone cannot completely explain the contribution of crops to human and animal diets, as well as the role they play in various diet patterns. In contrast, isotopes may provide more direct and telling evidence (Hu, 2018). The only isotopic analysis performed at the Dadiwan site revealed that almost animals under investigation exhibited a $C_3$ signal, except for three dogs exposed to millets during 7900–7200 cal BP, and millets were likely a dominant crop in the diets of humans, domestic pigs and dogs from 6500 to 4900 BP (Barton et al., 2009). However, previous studies have focused on evidence from charred seeds, animals and isotopes studies are limited to the Neolithic period. Following the late Neolithic, cultural exchanges and dissemination became more frequent (Jia et al., 2013; Dong G. et al., 2017), but human and animal diets and their influencing factors remain unclear, mainly due to the lack of continuous archaeological excavation materials. Located on the southern edge of the farming-pastoral ecotone, the archaeological culture in the HRV is relatively continuous and complete (Xie, 2002; Wang, 2012), providing an excellent opportunity to investigate the variation of human livelihood patterns under the exchange and integration of agricultural and animal husbandry management.

In recent years, many human and animal bones from the late Neolithic to the Ming Dynasty have been excavated from the Yabeili (YBL, 崖背里), Wangjiayangwan (WJYW, 王家阳湾), and Zhongtianxingfucheng (ZTXFC, 中天幸福城) sites, which are distributed across the middle-lower HRV, providing important materials for exploring the dietary structure of humans and animals in this area. Using carbon and nitrogen isotopes of human and animal bones along with radiocarbon dating, this paper intends to (1) characterize human and animal diets during the period from the Late Neolithic to the Ming Dynasty in the middle-lower HRV; (2) summarize the livelihood patterns in the WLP, and reveal how human livelihood patterns, especially dietary structure, change in response to natural and social environmental changes.

## STUDY AREA AND ARCHAEOLOGICAL CONTEXT

The HRV (105.1°E–106.5°E, 34.5°N–36.5°N) is located to the west of the Liupan Mountains, and is characterized by complicated topography with elevations that gradually decreases from north to south and from east to west. Numerous rivers develop in the valley, converging and forming the Hulu River, the largest tributary of the upper Wei River (Li et al., 1993; Wang, 2018). The HRV is situated at the intersection of the southeastern monsoon and the Tibetan Plateau climate regions, hence the influences of summer monsoons on the environment in the middle-lower reaches are more obvious than that in the east and west, with a mean annual temperature of 9.68°C and mean annual precipitation of 464 mm ([1]Mo et al., 1996; Han et al., 2020). Evidence from carbon isotopes of plants and soil during the Holocene indicates that $C_3$ plants dominated the natural vegetation, with an average carbon isotope of –26.7‰ (Wang et al., 2005; Zhang et al., 2015), while the $C_4$ plants represent the minority and exhibit seasonal differences, with an average of –12.4‰, which contributes to tracing diet variations (Zhao et al., 2013; Jiang et al., 2019; Zhang D. et al., 2020).

The HRV not only nurtured early farming communities in northern China, but also displayed a continuous archaeology cultural sequence from the Neolithic to Bronze Age (Li et al., 1993; Xie, 2002). The Dadiwan is the earliest Neolithic site in this region, and is a typical site of the Dadiwan 1 Culture (∼7800–7300 BP) (Gansu Provincial Institute of Cultural Relics and Archaeology [GPICRA], 2006; Zhang et al., 2010). After that, the Yangshao Culture (∼6300–5000 BP) flourished and spread widely throughout the area to numerous sites (Dong et al., 2016). The Majiayao Cultural sites moved to a wider area in the northwest, and human activities displayed unprecedented prosperity in this period (∼5300–4800 BP) (Wang, 2012). Subsequently, the site number, distribution area, and population gradually decreased during the following lower Changshan Culture (∼4800–4400 BP) and Qijia Culture (∼4200–3600 BP) (Mo et al., 1996; An et al., 2005). Moreover, the sparse-wood grasslands and grasslands were the dominant vegetation types in the Wei River valley before the Qijia Culture, which provided excellent hydrothermal conditions for human populations shifting from the hunting-gatherers to farmers (Xia et al., 1998; Shang and Li, 2010; Sun and Feng, 2015). The archaeological culture in this area became more complex after the disintegration of the Qijia Culture. The collision-integration initially occurred between native Siwa Culture and Central Plains cultures, followed by Eurasian steppe cultures and indigenous cultures that later converged and exchanged again (Li et al., 1993; Wang, 2012).

## MATERIALS AND METHODS

### Study Sites

Three adjacent archaeological sites, namely YBL, ZTXFC, and WJYW, located in the middle-lower HRV, were excavated by the Gansu Institute of Cultural Relics and Archaeology in 2019. The YBL site (35.00°N, 105.65°E), in Qin'an County, Tianshui City, is situated on the left terrace of the lower reaches of the HRV. The ZTXFC site (35.20°N, 106.02°E) and the WJYW site (35.30°N, 105.91°E), which are 41 km away from the YBL site in the northeast, are situated along the Shuiluo River, which is a tributary of the Hulu River (**Figure 1**). Evidence from recovered pieces of pottery, human and animal bones, stone tools and bone-made artifacts from different cultural layers and relics by on-site collection and flotation across the three sites represented the period from the late Neolithic to the Ming Dynasty at least.

### Analysis of the Isotopic Composition of Bones

The $\delta^{13}C$ values of $C_3$ crops (including wheat and rice) and $C_4$ crops (including foxtail millet and broomcorn millet) are quite different due to various photosynthetic modes (Bender, 1971; Smith and Epstein, 1971), which can be used to reconstruct the paleodiet based on $\delta^{13}C$ values in bones that were absorbed from plant isotopes (Teeri and Schoeller, 1979; Tieszen et al., 1983). Similarly, the $\delta^{15}N$ values are different among animals at different trophic levels, and these differences may be amended by many factors (Hedges and Reynard, 2007; Caut et al., 2009; Jaouen et al., 2019; Ma et al., 2021).

A total of 172 human and animal samples were selected for isotopic analysis. All samples were treated following the process described by Ma M. et al. (2016) with some modifications. First, cleaned bones were placed in 0.5 mol/L hydrochloric acid (HCl) at 4°C for 2 weeks with daily replacement. Then the collagen was rinsed and the samples were then immersed in 0.125 mol/L NaOH at 4°C for 20 h, after which they were rewashed. Next, 0.5 mol/L hydrochloric acid (HCl) was added at room temperature (∼20°C) for 4 h to remove any absorbed $CO_2$ and then the samples were rinsed again. The remains were then submerged in a HCl solution (PH = 3) at 75°C for 48 h.

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[1] http://data.cma.cn/site/index.html

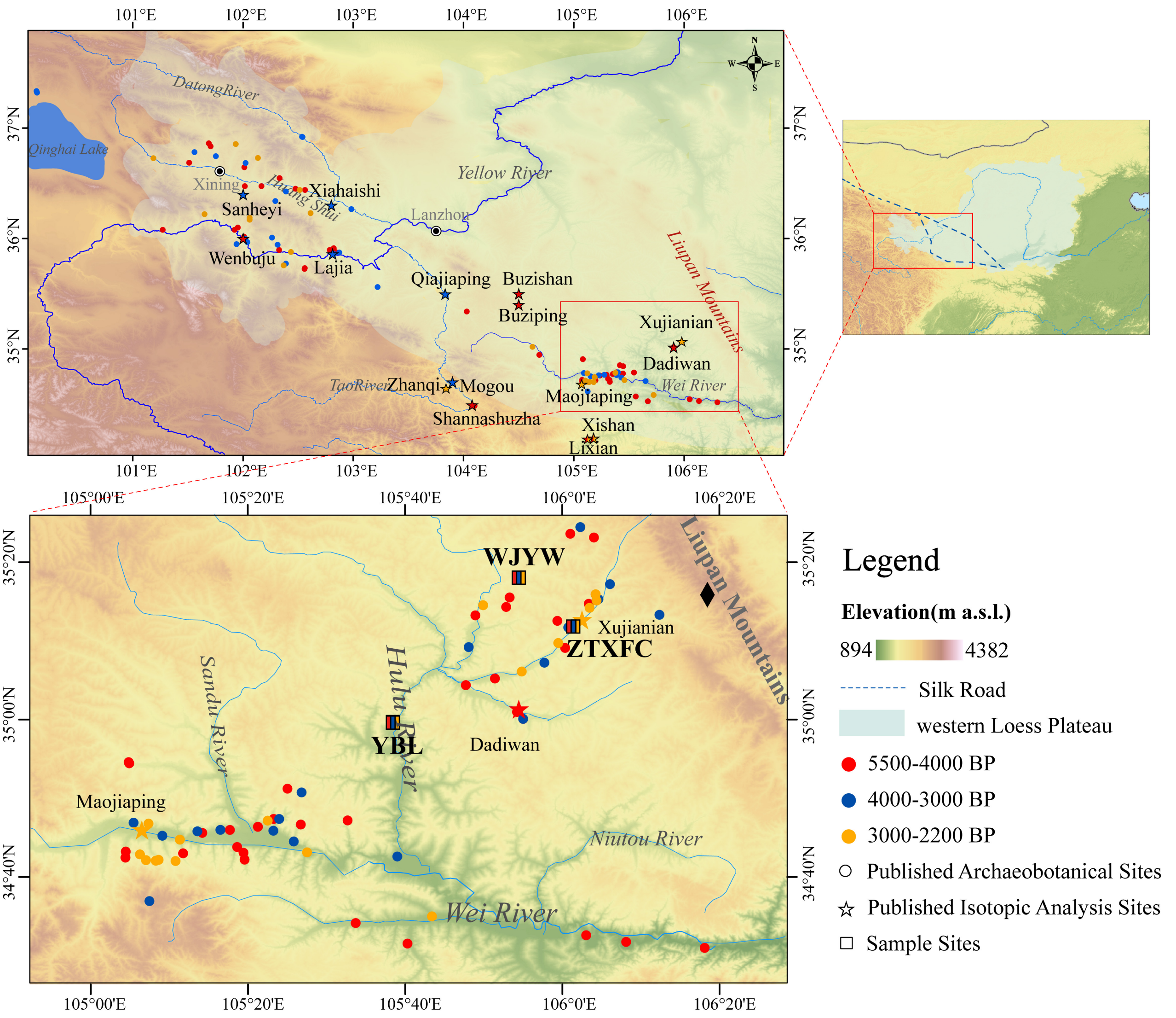

**FIGURE 1 |** Map showing the location of the study area and the distribution of sites with published archaeobotanical and isotopic analysis, as well as the locations of sample sites in the WLP.

Finally, the solution was filtered, frozen and freeze-dried to extract the collagen.

The measurement of organic carbon and nitrogen content along with the atomic C/N ratio was conducted in the State Key Laboratory of Applied Organic Chemistry at Lanzhou University. Stable carbon and nitrogen isotope ratios of bone collagen were analyzed using a Thermo Finnigan Flash DELTA plus XL mass spectrometer coupled with EA at the MOE Key Laboratory of Western China's Environmental System, Lanzhou University, China. To check the stability of the instrument system and to ease calibration, a standard and replicate were added following every ten samples (Graphite, $\delta^{13}$C: −16.0 ± 0.1‰; Wheat, $\delta^{13}$C: −27.2 ± 0.13‰; Collagen, $\delta^{13}$C: −9.0‰; Glycine, $\delta^{13}$C: −33.3‰; IAEA-600, $\delta^{15}$N: 1.0 ± 0.2‰; Protein, $\delta^{15}$N: 5.9 ± 0.08‰; Puge, $\delta^{15}$N: 5.6‰). All carbon and nitrogen isotopes were measured relative to V-PDB and AIR standard samples, respectively. The measurement analytical precision was ± 0.2‰ for both carbon and nitrogen isotopic ratios. Furthermore, 23 human and animal collagen samples were selected for radiocarbon dating using accelerator mass spectrometry (AMS), and the results were calibrated to calendar age using OxCal v.4.4.4 (Bronk Ramsey, 2021) with the IntCal 20 calibration curve (Reimer et al., 2020), and reported as "cal BP." Significant differences were examined using the Mann–Whitney U test in SPSS Statistics 22 ($P < 0.05$).

## RESULTS

### Bone Preservation Status

Overall, samples with C/N atom ratio of bone collagen between 2.9–3.6 and yield > 1% are considered to be in good condition (DeNiro, 1985; Ambrose, 1990). In this study, the C/N atomic ratios of all samples were between 2.9 and 3.6 (3.2∼3.3), and the yield ranged from 1.1% to 23.5%. Additionally, the carbon and nitrogen elements content in the samples was similar to that in modern samples. Therefore, all samples were considered uncontaminated and appropriate for further analysis.

## Chronology

The 23 AMS radiocarbon dates of human and animal collagen samples ranged from 5277 to 548 cal BP (95.4%) (**Table 1**). Combined with recovered pottery, archaeological stratigraphy and chronology results, the cultural remains in the three sites were divided into three periods: period 1 (5300–4000 cal BP), period 2 (3000–2200 cal BP), and period 3 (1000–500 cal BP).

## Human and Animal Isotopic Analysis

In this study, all samples were identified as wild herbivorous (deer), domesticated herbivorous animals (cattle, sheep, and horses), domesticated omnivorous animals (dogs and pigs) and humans for isotopic analysis (**Table 2** and **Supplementary Table 1**).

During Period 1 (5300–4000 cal BP), 18 animals (dogs, pigs, deer, and pheasants) and 4 human bone collagen samples were analyzed, of which the $\delta^{13}$C and $\delta^{15}$N values ranged from –8.0 to –21.9‰ and 2.5 to 10.9‰, respectively, indicating more significant variability in diet assemblage. As shown in **Figure 2A**, the lower $\delta^{13}$C (mean = –19.0 ± 1.9‰) and $\delta^{15}$N (mean = 4.2 ± 0.8‰) values of wild herbivorous (deer; $n = 7$) suggested a relatively stable $C_3$ diet. On the contrary, almost all samples originating from domesticated omnivorous animals (dogs and pigs; $n = 9$) exhibited higher $\delta^{13}$C (mean = –10.6 ± 3.0‰) and $\delta^{15}$N (mean = 7.4 ± 1.2‰) values, which suggested a continuous intake of more $C_4$ foods (likely millets and millet byproducts and/or millet-based animal protein), aside from one individual who ingested a greater proportion of $C_3$ plants. Furthermore, three of four human samples from this period displayed obvious $C_4$ signals, indicating that they relied heavily on $C_4$ foods. The $\delta^{13}$C values of domesticated omnivorous samples were very similar to most humans (Mann–Whitney U test, $P = 0.203 > 0.05$), revealing that domesticated pigs and dogs may have exhibited diets closely related to humans.

During Period 2 (3000–2200 cal BP), a total of 133 animals (including dogs, pigs, cattle, sheep, horses, and deer) and 2 human samples were analyzed. The results presented in **Figure 2B** indicate that cervids ($n = 3$) exhibited more negative $\delta^{13}$C values (mean = –18.7 ± 0.3‰) and lower $\delta^{15}$N values (mean = 4.5 ± 0.6‰) than other animals, suggesting that they displayed a $C_3$ plants-based diet, which is similar to the $\delta^{13}$C ($P = 0.305 > 0.05$) and $\delta^{15}$N ($P = 0.425 > 0.05$) values of Period 1 (5300–4000 cal BP). For domestic herbivores, the isotopic values of most sheep ($n = 58$, SD = 1.0 for $\delta^{13}$C; SD = 1.0 for $\delta^{15}$N) and horses ($n = 27$, SD = 1.2 for $\delta^{13}$C; SD = 0.8 for $\delta^{15}$N) were relatively consistent, while cattle were more positive and variable ($n = 26$, mean = –16.1 ± 2.0‰ for $\delta^{13}$C; mean = 6.1 ± 1.2‰ for $\delta^{15}$N), which may be mainly due to variation in the physiological characteristics of domestic herbivores and slight differences in animal husbandry. In general, the $\delta^{13}$C values of domestic herbivores were relatively negative

**TABLE 1 |** Calibrated radiocarbon dates from three sites in the middle-lower HRV.

| Site | Lab no. | Dating material | Radiocarbon age (BP) | Calibrated age (cal BP) 2 σ | media (cal BP) | σ |
|---|---|---|---|---|---|---|
| YBL | LZU20324 | Collagen | 4440 ± 20 | 5277–4885 | 5035 | 104 |
| YBL | LZU21094 | Collagen | 4340 ± 30 | 5021–4844 | 4906 | 43 |
| ZTXFC | LZU20320 | Collagen | 4040 ± 30 | 4612–4418 | 4492 | 64 |
| ZTXFC | LZU20315 | Collagen | 4010 ± 30 | 4567–4414 | 4477 | 39 |
| WJYW | LZU20157 | Collagen | 3700 ± 20 | 4144–3976 | 4035 | 42 |
| ZTXFC | LZU20160 | Collagen | 2840 ± 20 | 3026–2869 | 2942 | 40 |
| ZTXFC | LZU20316 | Collagen | 2830 ± 20 | 2997–2869 | 2929 | 35 |
| ZTXFC | LZU20314 | Collagen | 2820 ± 20 | 2992–2860 | 2918 | 32 |
| ZTXFC | LZU20161 | Collagen | 2810 ± 20 | 2961–2855 | 2911 | 31 |
| ZTXFC | LZU20317 | Collagen | 2800 ± 20 | 2960–2850 | 2904 | 32 |
| ZTXFC | LZU20676 | Collagen | 2790 ± 20 | 2958–2800 | 2893 | 35 |
| ZTXFC | LZU21093 | Collagen | 2760 ± 20 | 2927–2780 | 2845 | 38 |
| WJYW | LZU20158 | Collagen | 2470 ± 20 | 2710–2428 | 2593 | 83 |
| WJYW | LZU20159 | Collagen | 2460 ± 20 | 2704–2369 | 2588 | 94 |
| WJYW | LZU20156 | Collagen | 2450 ± 20 | 2699–2364 | 2513 | 103 |
| YBL | LZU21433 | Collagen | 2450 ± 20 | 2699–2364 | 2513 | 103 |
| YBL | LZU20323 | Collagen | 2430 ± 20 | 2688–2358 | 2450 | 96 |
| ZTXFC | LZU20318 | Collagen | 940 ± 20 | 914–791 | 849 | 37 |
| ZTXFC | LZU20319 | Collagen | 880 ± 20 | 898–730 | 766 | 40 |
| ZTXFC | LZU20322 | Collagen | 870 ± 20 | 897–726 | 760 | 31 |
| YBL | LZU20326 | Collagen | 720 ± 20 | 684–651 | 669 | 15 |
| YBL | LZU20327 | Collagen | 650 ± 20 | 663–558 | 588 | 36 |
| YBL | LZU20325 | Collagen | 600 ± 20 | 646–548 | 605 | 28 |

**TABLE 2 |** Stable isotopic data from three sites in the middle-lower HRV.

| Date (cal BP) | Species | Sample number | $\delta^{13}C$ (‰) | | $\delta^{15}N$ (‰) | |
|---|---|---|---|---|---|---|
| | | | Range | Mean ± SD | Range | Mean ± SD |
| 5300–4000 | Dog | 2 | −11.4 to −8.8 | −10.1 ± 1.3 | 7.5–8.5 | 8.0 ± 0.5 |
| | Pig | 8 | −19.1 to −8.6 | −10.8 ± 3.3 | 4.7–9.1 | 7.3 ± 1.2 |
| | Deer | 7 | −21.8 to −15.4 | −19.0 ± 1.9 | 2.5–5.1 | 4.2 ± 0.8 |
| | Pheasant | 1 | −16.90 | −16.9 | 6.8 | 6.8 |
| | Human | 4 | −17.0 to −8.0 | −10.6 ± 3.7 | 8.6–10.9 | 10.1 ± 0.9 |
| 3000–2200 | Dog | 6 | −10.0 to −8.8 | −9.6 ± 0.4 | 8.1–8.4 | 8.2 ± 0.1 |
| | Pig | 9 | −19.4 to −7.3 | −11.9 ± 4.7 | 4.7–9.0 | 7.5 ± 1.2 |
| | Cattle | 26 | −21.1 to −13.2 | −16.1 ± 2.0 | 2.9–8.1 | 6.1 ± 1.2 |
| | Sheep/Goat | 61 | −20.4 to −7.0 | −17.6 ± 2.2 | 4.0–8.7 | 6.0 ± 1.1 |
| | Horse | 28 | −20.1 to −9.1 | −17.8 ± 2.1 | 3.4–9.3 | 5.4 ± 1.1 |
| | Deer | 3 | −19.0 to −18.4 | −18.7 ± 0.3 | 3.9–5.2 | 4.5 ± 0.6 |
| | Human | 2 | −14.4 to −11.2 | −12.8 ± 1.6 | 11.1–13.4 | 12.3 ± 1.2 |
| 1000–500 | Human | 15 | −17.8 to −8.6 | −15.4 ± 2.5 | 8.5–12.9 | 10.8 ± 1.4 |

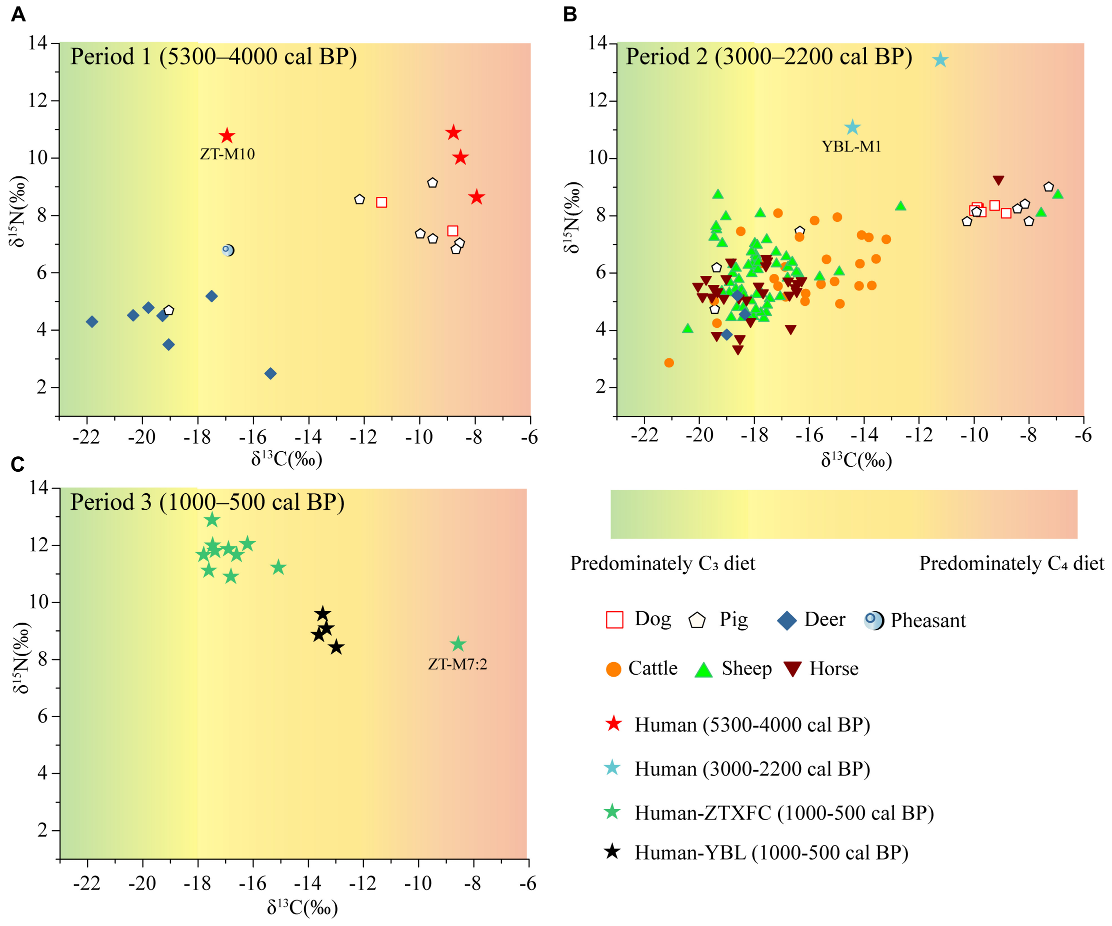

**FIGURE 2 |** Carbon and nitrogen isotopic results for humans and animals from sites in the middle and lower reaches of the Hulu River Valley during **(A)** Period 1 (5300–4000 cal BP); **(B)** Period 2 (3000–2200 cal BP) and **(C)** Period 3 (1000–500 cal BP).

and similar to those of deer (*P* = 0.165 > 0.05), except for four individuals, which were likely fed by humans. In contrast, the isotopic values of domesticated omnivorous animals exhibited more positive characteristics. Except for three pigs, which likely foraged in the wild, the remaining pigs and dogs showed higher $\delta^{13}C$ values (−10.3∼−7.3‰) and narrow ranges of $\delta^{15}N$ values

(7.8∼9.0‰), indicating a diet consisting mainly of $C_4$ foods. Two human samples exhibited very different and negative isotopic characteristics than those of the first period, one of which exhibited higher $\delta^{13}C$ and $\delta^{15}N$ values, indicating a greater proportion of $C_4$ foods in their diet. While another sample suggests that a certain amount of $C_3$ foods and many $C_4$ foods made up their diet. Unlike Period 1, significant differences were observed in $\delta^{13}C$ values between domesticated omnivores and humans ($P = 0.028 < 0.05$).

During Period 3 (1000–500 cal BP), only isotopic data from human samples were available (**Figure 2C**). All human samples demonstrated obvious differences in $\delta^{13}C$ ($P = 0.005 < 0.05$) and $\delta^{15}N$ ($P = 0.005 < 0.05$) values at the ZTXFC and YBL sites. Specifically, humans from the ZTXFC site displayed more negative $\delta^{13}C$ values than those from the YBL site, indicating a more $C_3$-based diet at the ZTXFC site and a mixed $C_3$ and $C_4$ food-based diet at the YBL site. Moreover, human $\delta^{15}N$ values during this period were significantly lower than those of the previous two periods, especially at the YBL site (**Figure 3**). An abnormal individual (ZT-M7: 2) with a more positive $\delta^{13}C$ value and a lower $\delta^{15}N$ value at the ZTXFC site was assumed to be an immigrant.

# DISCUSSION

## Human Diets and Subsistence Strategies From the Late Neolithic to the Ming Dynasty in the Middle-Lower Hulu River Valley

During Period 1 (5300–4000 cal BP), the agricultural planting structure was dominated by millets in the middle-lower HRV, according to published archaeobotanical data (An et al., 2010; Li, 2018; Chen et al., 2020). The observed $C_4$ signal in human and animal diets can be interpreted as predominating millets, or animal protein derived from millets. Slight differences in $\delta^{13}C$ values between domesticated omnivorous animals and humans support the vital role of pigs and dogs in human meat consumption from an isotope perspective. Moreover, the $C_3$ dominated diet of wild herbivores indicated that there were a few hunting games during this time and that humans ate a little wild animal production. Although the mean $\delta^{15}N$ offset of 5.9‰ between humans and wild herbivores exceeded a trophic level of $\delta^{15}N$ enrichment, the $\delta^{13}C$ values of humans and domesticated animals and the mean $\delta^{15}N$ offset of 2.6‰ between humans and domesticated animals indicate that humans consumed a limited amount of animal protein (Bocherens and Drucker, 2003; Hedges and Reynard, 2007; O'Connell et al., 2012; Liu and Reid, 2020).

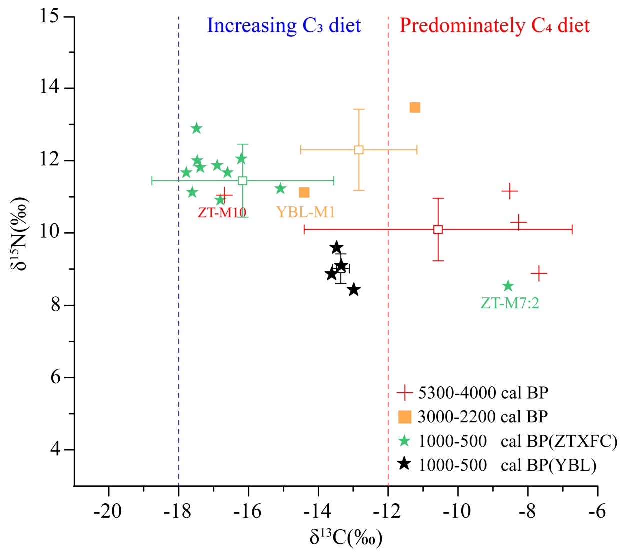

**FIGURE 3 |** Comparison of the $\delta^{13}C$ and $\delta^{15}N$ results with scatter and mean ± SD for humans from sites of the middle and lower reaches of the Hulu River Valley during three periods.

During Period 2 (3000–2200 cal BP), the similarity in isotopic values of Cervidae to those of Period 1 indicates that the environment had not changed dramatically in the middle-lower HRV. At the same time, with the widespread adoption of domesticated herbivores, the human subsistence strategy changed dramatically (Du et al., 2020). Similar to the Majiayuan cemetery, M1 is a deep vertical cave tomb with more than 200 skulls and hooves of cattle, sheep/goats, and horses at the YBL site, which suggests that the individual in M1 is likely to be a pastoralist (most likely Xirong). The $\delta^{13}C$ value for this individual is quite negative with a relatively high $\delta^{15}N$ value, indicating a diet consisting of more $C_3$ foods and animal protein. Isotopes of another individual unearthed from an ash pit, dated to 2710–2428 cal BP, suggested that this individual ingested a more significant amount of $C_4$ foods and animal protein products. During Period 2, these individuals highlighted two diets: a $C_4$-based diet and a mixed $C_3$ and $C_4$ food-based diet. According to published archaeobotanical evidence, the dependence on wheat increased significantly during this time, while the dependence on millets decreased considerably (Li, 2018; Chen et al., 2020). Therefore, it is reasonable that the $C_3$ food in the human diet was primarily from wheat or $C_3$ food-fed animal protein, while the $C_4$ food remained mainly from millets and millet byproducts. Aside from one pig, domestic omnivores showed obvious $C_4$ signals, indicating a high feeding practice. In contrast, most domestic herbivores showed high $C_3$ signals, consuming a large amount of $C_3$ food. Moreover, three sheep and one horse displayed a similar diet to domestic pigs and dogs, which might be related to animal management such as entire grazing or a combination of grazing and feeding patterns. Furthermore, the higher mean $\delta^{15}N_{\text{humans-herbivores}}$ offset of 6.5‰ and $\delta^{15}N_{\text{humans-domestic omnivores}}$ offset of 4.48‰ suggested that humans may have consumed a large amount of animal protein, including even fish and other aquatic foods. In brief, the human subsistence strategies are quite complex in the middle-lower HRV during Period 2.

During Period 3 (1000–500 cal BP), according to the proportion of main crops recorded in the literature and unearthed plant remains in the middle-lower HRV, the agricultural planting structure exhibited diverse characteristics, with wheat as the main cultivated crop, and millets and other crops (such as buckwheat and oats) as the secondary subsistence crop (Local Chronicle Compilation Committee of Zhuanglang County, 1998; Local Chronicle Compilation Committee of Qin'an County, 2001; Local Chronicle Compilation Committee of Tianshui City, 2004; Local Chronicle Compilation Committee of Pingliang City, 2011; Li, 2018). Based on the observed differences in isotopes, it can be seen from **Figure 4A** that the $\delta^{13}$C values of humans were negative and presented two assemblages with different diets and livelihood patterns. Humans consumed a majority of wheat and animal protein at the ZTXFC site, while a mixture of wheat, barley, and millets played a critical role in human diets at the YBL site. The lowest $\delta^{15}$N values of humans at the YBL site suggested that they consumed the lowest amount of animal protein production. From the isotope perspective, this study provides direct evidence of human diets during the Song-Ming Dynasty in the middle-lower HRV, where wheat was the staple food, millets and other crops were supplementary food. Overall, human diets showed obvious diversification and regional characteristics during Period 3.

## Complex Diet Patterns From the Late Neolithic to the Ming Dynasty in Western Loess Plateau

To better understand the dietary trajectory, the results of this study were compared with those from other sites in the WLP (**Figures 4B,C**). Since the diets of domestic pigs and dogs were closely related to human diet patterns (Hou, 2019; Robinson, 2019), it is reasonable to combine the results of humans and domestic pigs and dogs.

As one of the earlier independent agricultural centers in northern China during the Neolithic, the Dadiwan site represents the earliest research on human and animal isotopes in the WLP (Barton et al., 2009; Zhang et al., 2010; Leipe et al., 2019). Most of the animal diets during the Dadiwan 1 Culture were $C_3$-based (mean = −19.0 ± 1.4‰ for $\delta^{13}$C), which shifted to a $C_4$-based diet (probably millets and millet byproducts) in humans and domestic pigs and dogs between 6500 and 4900 cal BP (mean = −10.0 ± 2.7‰ for $\delta^{13}$C). Domestic animals outnumbered wild animals based on zooarchaeology data during 5600–5000 BP at most contemporaneous sites, except for the Shannashuzha site, which was abundant with wild animals (Huang, 2000; Qi et al., 2006; Flad, 2007; Yu et al., 2011; Ma et al., 2021). Humans, domestic dogs and pigs consumed millets primarily at the Shannashuzha site (mean = −10.8 ± 4.3‰ for $\delta^{13}$C), which was coincident with unearthed plant remains (Hu, 2015; Ma et al., 2021). During 5300–4000 BP, the isotopic results suggested that a $C_4$-based diet was most prevalent across all archaeological sites of the WLP (Barton et al., 2009; Ma, 2013; Atahan et al., 2014). Humans heavily relied on millet agriculture during this period, which was also consistent with our data (**Figure 4B**). Generally, a strong mutualism of millet agriculture and animal husbandry or hunting games composed the core part of the human subsistence strategy in the WLP during the Neolithic period (Pechenkina et al., 2005).

With the introduction of exotic cereals (wheat and barley) and livestock (cattle and sheep/goats), the structure of crop cultivation and animal utilization changed tremendously (Dong et al., 2021a; Ren et al., 2021), and the human subsistence strategy changed accordingly during 4000–2200 BP (Zhou and Garvie-Lok, 2015). Although wheat and barley were introduced between 4300 and 3600 BP (Chen et al., 2020; Ren et al., 2021), the human subsistence strategy still heavily relied on millets and domesticated omnivores in the WLP (Flad, 2007; Ma M. et al., 2016), such as at the Xiahaishi site (mean = −8.4 ± 2.3 for $\delta^{13}$C), the Sanheyi site (mean = −9.1 ± 0.5 for $\delta^{13}$C), and the Lajia site (mean = −7.9 ± 0.3 for $\delta^{13}$C). Later, the communities of the WLP selectively incorporated exotic crops and livestock (such as wheat, cattle, sheep, and goats), and the subsistence strategy diversified in the WLP (Jaffe et al., 2021). Around 3600 cal BP, humans of the Qijiaping site (mean = −9.1 ± 1.7 for $\delta^{13}$C) maintained a millet-based diet (Ma et al., 2015; Zhang et al., 2016), while the isotopes in bone collagen (mean = −14.3 ± 1.8‰ for $\delta^{13}$C) and starch grains in dental calculus of humans from the Mogou site suggested that wheat and barley were incorporated into the staple diet (Li et al., 2010; Liu et al., 2014; Ma M. et al., 2016). Therefore, there was a significant dietary shift favoring more $C_3$ foods (likely wheat, barley and $C_3$ food-fed animals) post-3600 cal BP (Ma M. et al., 2016). Human settlement intensity decreased significantly during 3600–3000 BP (Chen et al., 2020). After pastoralism or nomadism was widely diffused in Eurasia, the utilization of the domestic animals played a more important role in the human subsistence strategy (Yi, 2012; Ren and Dong, 2016; Jeong et al., 2020). The isotopic evidence from various sites suggested different agricultural patterns during 3000–2200 BP. Previous studies have shown that the carbon isotopes of humans, pigs and dogs are more negative, such as the individual (YBL-M1) observed in this study and the Zhanqi site (mean = −16.0 ± 1.6‰), the Xishan site (mean = −12.9 ± 3.7‰), the Lixian site (mean = −12.9 ± 3.7), all of which suggest that wheat crops and their byproducts made up a large component of human and domestic herbivore diets (Ling, 2010; Atahan et al., 2014; Liu et al., 2014; Ma Y. et al., 2016). However, other isotopic data from studies at the Maojiaping site (mean = −10.4 ± 1.5‰) and this study (mean = −11.0 ± 3.7‰) still maintain the $C_4$-based diet (Wang Y. et al., 2019). Based on the general isotopic features found in the WLP, two or more groups occupied this region from 3000 to 2200 BP. Specifically, there were likely both individuals who consumed primarily millets (e.g., the Maojiaping site and this study) and individuals who subsisted on a mixed diet of wheat and millets (e.g., the Zhanqi, Xishan, and Lixian sites as well as YBL-M1). However, archaeobotanical evidence from this period shows that wheat and barley ($C_3$ food) dominated the agricultural cropping structure in the WLP (74%), and even accounted for up to 84% of the charred seeds in Gangu County, Tianshui City, while the proportion of millets ($C_4$ food) was substantially reduced (**Figure 4C**; Li, 2018; Chen et al., 2020; Ren et al., 2021). Overall, the human subsistence strategy was diverse and complex from the Bronze Age to the early Iron period.

After the Han Dynasty, the system of dryland agriculture in the north and rice cultivation in the south was gradually improved, and wheat planting became widespread (Wei, 1988;

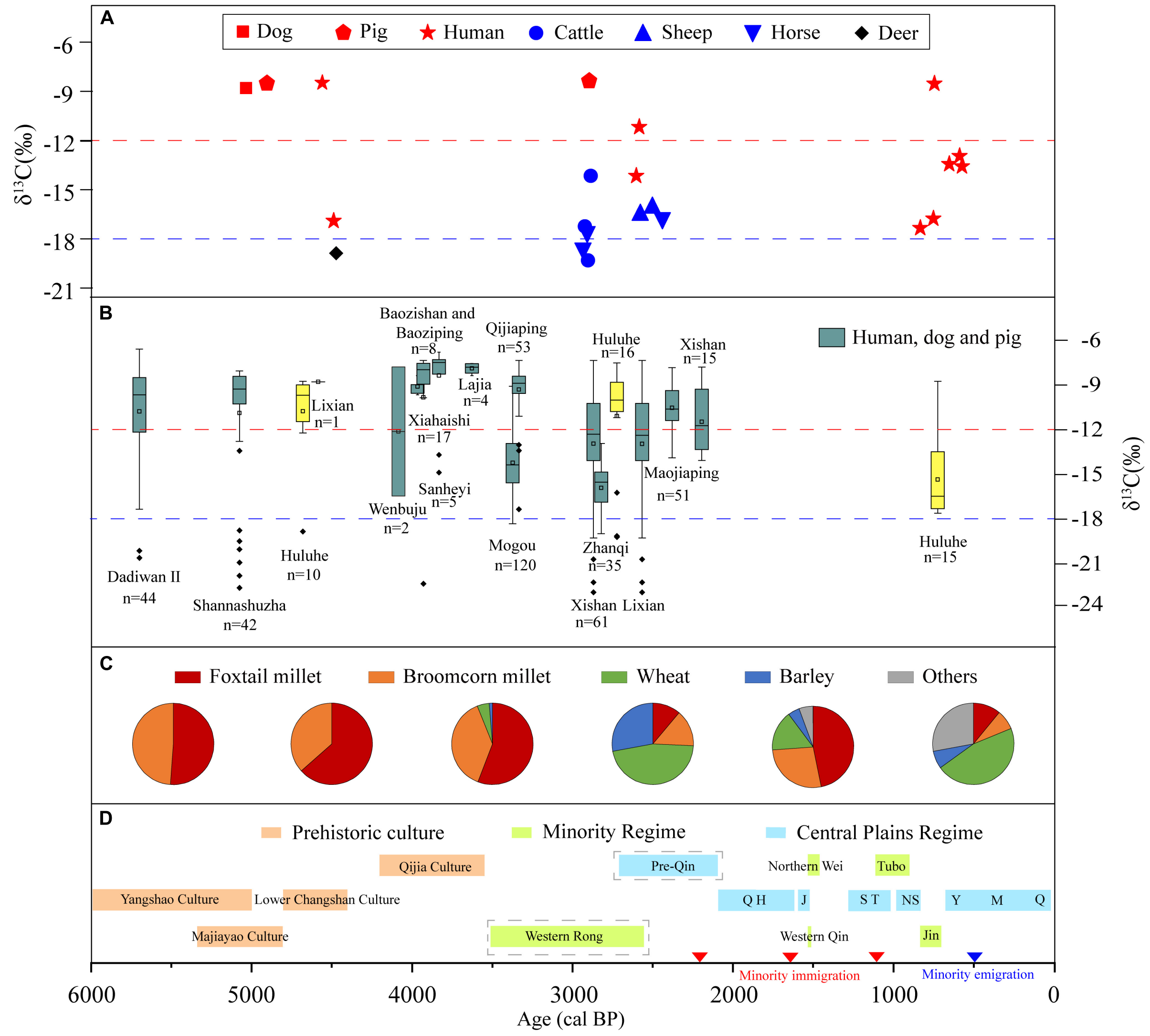

**FIGURE 4 |** Comparison of isotopic and archaeobotanical results in the middle-lower HRV and WLP. **(A)** Carbon isotopic results with $^{14}$C dates from sites in the middle-lower HRV. **(B)** The $\delta^{13}$C values from sites in the WLP (yellow boxes represent the site in the middle-lower HRV) (Cai and Qiu, 1984; Barton et al., 2009; Ling, 2010; Ma, 2013; Atahan et al., 2014; Liu et al., 2014; Ma Y. et al., 2016; Zhang et al., 2016; Ren, 2017; Wang Y. et al., 2019). **(C)** The weight percentage of crops in the WLP (Liu et al., 2004; Yang, 2014; Hu, 2015; Li, 2018; Chen et al., 2019, 2020; Ren et al., 2021). **(D)** Regime change between Han and Minority and events of Minority immigration and emigration (Li et al., 1993; Local Chronicle Compilation Committee of Zhuanglang County, 1998; Local Chronicle Compilation Committee of Qin'an County, 2001; Xie, 2002; Local Chronicle Compilation Committee of Tianshui City, 2004; Local Chronicle Compilation Committee of Pingliang City, 2011; Wang, 2012; Local Chronicle Compilation Committee of Gansu Province, 2017).

Zhao, 2015; Zhou et al., 2017). According to the literature, millets dominated the agricultural planting structure, with wheat as a supplementary crop during the Han-Tang Dynasty (2152–1043 BP). Millets and wheat were broadly planted with a set of ingenious planting methods and became the staple crop of human diets in arid and semi-arid areas of northern China during the Western Han Dynasty (2152–1942 BP) (*Book of Han·Shihuozhi; Fanshengzhishu*). In addition to millets and wheat, other crops (e.g., flax) were also valued in the Northern Wei Dynasty (1564–1407 BP) (*Qiminyaoshu*). Moreover, this pattern has been confirmed by historical archaeobotanical evidence in the WLP (Li, 2018). Based on charred seeds and chorography evidence, agricultural planting structures diversified during the Song-Ming Dynasty (990–306 BP). Humans primarily planted wheat, supplemented with millet, sorghum, barley, beans, and buckwheat in this period (**Figure 4C**; Local Chronicle Compilation Committee of Gansu Province and Agricultural Chronicle Compilation Committee of Gansu Province, 1995; Shi, 1995; Li, 2018). Although a few historical documents (such as the "*Tiangong Kaiwu*" and "*Shihuozhi*") recorded the general agricultural cropping patterns throughout Gansu Province and northern China, these documents may not accurately capture the human dietary structures in the WLP, as these grains may be used to feed livestock or trade-related activities, and so on. Therefore, our understanding of human livelihoods in the WLP remains fragmented and indirect, based solely on historical documents or limited archaeobotanical data. While evidence from human bone isotopes can directly reflect consumption patterns. Our study shows that some groups mainly ate wheat and other $C_3$ foods, while another individual (ZT-M7: 2) may have consumed multiple crops. From an isotopic perspective, this paper provides additional evidence that multiple lifestyles existed during the Song and Ming dynasties, which makes up for the lack of historical documents and archaeobotanical evidence regionally. It reveals the complexity and diversity of the human diet structure.

## Influencing Factors of Human and Animal Diets in Western Loess Plateau

Foxtail and broomcorn millet, as well as pigs and dogs, were domesticated and spread around 10,000 years ago, and millet agriculture was established under intense human activities and favorable ecological conditions during 6500–6000 BP in Northern China (Qin, 2012; Zhao, 2014; Dong et al., 2016; Wang and Cui, 2021). The agricultural population diffused widely to the surrounding areas, which directly promoted cultural development during the Neolithic (Jia et al., 2013; Leipe et al., 2019; Dong et al., 2020b, 2022a). Agriculture based on the farming millets combined with raising pigs and dogs or hunting animals was established and developed rapidly in the WLP after the middle-Yangshao Culture (Dong et al., 2016; Li, 2018). In Period 1 (5300–4000 cal BP), the occurrence of millet-based agriculture thrived in a relatively stable and suitable environment, along with abundant fresh water and fertile soil brought by the numerous tributaries of the Yellow River (Liu F. et al., 2010; Zhang et al., 2019; Zhang C. et al., 2020). Therefore, prehistoric cultures based on millet agriculture dominated human and animal diets during this period in the WLP.

With the strengthening of *trans*-Eurasian exchange, exotic crops and livestock (including wheat, barley, cattle, goats, and sheep) were extensively distributed across Northern China around 4000 BP, leading to a massive restructuring of the agricultural structure during this time (Yuan, 2010; Dodson et al., 2013; Long et al., 2018). Wheat was introduced into the Hexi Corridor and northeast Qinghai around 4000 BP, and spread into the WLP about 3600 BP (Li et al., 2007; Yang, 2014; Dong et al., 2020a). At the same time, the human survival pressure increased, as demonstrated by the rapidly increasing population and the obvious cold-dry climate (Sun et al., 2018; Chen et al., 2020). Wheat and barley, which were more adaptable (frost-tolerant) and high-yielding, were selectively planted as the complementary crops, coupled with cattle and sheep/goat pastoral practices in the WLP (Ceccarelli and Grando, 2000; Raina et al., 2016; Jaffe et al., 2021). The prosperous millet-based agriculture was gradually lost and was instead replaced by animal husbandry with small-scale farming (An et al., 2003, 2005).

Changes in agricultural systems can often reflect social development (Fuller and Stevens, 2009). Following the establishment of the powerful state, which brought about fundamental changes in the organizational structure of human society, human survival strategies were primarily affected by geopolitical interactions, with environmental factors playing an auxiliary role (Li et al., 2020; Dong et al., 2021b). The unified cultural pattern was broken and transformed into multiple cultures after the Qijia culture (Li et al., 1993; Wang, 2012). The Siwa Culture, which formed in the middle-upper Tao River valley, collided with the Zhou Dynasty, which originated in the Central Plains, and archaeological evidence showed the coexistence of the two cultures in the WLP (Wang, 2012). In the middle of the Spring and Autumn Period, the indigenous Xirong community related to the Siwa Culture, experienced a decline following their fall to the Qin community. The northern steppe community then widely spread southward along the Great Wall and became a new Xirong community, which faced an intense conflict with the Qin community in the WLP, especially in the middle-lower HRV from 3000 to 2200 BP (**Figure 4D**; Chen, 2011; Liang, 2016). Cultural and commercial contacts were frequent as a result of the collision and fusion of the Qin and Rong communities in the WLP, which was widely manifested in cemetery characteristics, funerary rituals, and burial objects (Zhu, 2004; Cao, 2018; Wang, 2020). For instance, the Majiayuan cemetery represented a synthesis of Xirong, Qin, Central China, northern steppe and Mediterranean cultures (Li, 2009; Ma, 2018; Guo, 2019).

Complex geopolitical backgrounds inevitably provoked sophisticated diet patterns. The early Qin community moved westward into the WLP, under the jurisdiction of the Shang or Zhou Dynasty (Li, 2011). The isotopic results suggested that a millet-based diet was dominant in the Qin communities (such as those buried at the Maojiping site, the Sunjianantou site, and the Jianhe grave) (Ling, 2010; Wang Y. et al., 2019), which was consistent with the diet patterns of the unidentified individual and almost all pigs and dogs in this study, indicating that human and animal diets in the WLP were influenced by the Qin communities. In addition, historical documents such as the "*Book of Rites*" recorded that Xirong societies "wear furs with their hair down and do not eat grains" (被发衣皮,有不粒食者矣) (Huang, 2017), which is consistent with the subsistence strategy of northern nomads, consuming large quantities of wheat and keeping domestic herbivores (Machicek, 2012; Fenner et al., 2014; Hermes et al., 2018). Furthermore, another human diet (YBL-M1) in this study resembled that of the northern steppe. According to these results, the Xirong communities, which likely originated in the northern steppe, might maintain their dietary patterns and exhibit a multicultural integration burial form, similar to the Majiayuan cemetery. During the Zhou Dynasty, the Xirong community converged part of the Central Plains culture and other foreign cultures, but their diet structure probably remained unchanged (Liu C, 2012; Cao, 2018; Wang, 2020). In general, human and animal diets in the WLP were influenced by Qin and Central Plains cultures as well as Xirong culture, especially under the complex geopolitical backgrounds formed in the process of intense collision and integration between the Qin and Rong communities.

The innovation of agricultural technology further promoted the development of agricultural civilization in the Iron Age (Liu X, 2012). Wheat cultivation was encouraged by governments during the Qin-Han Dynasty. However, wheat consumption was generally hindered by the traditional dietary habits until the popularization of handle mills and the maturity of wheat processing technology at the end of the Han Dynasty (Wei, 1987; Fuller and Rowlands, 2011; Chen, 2016; Liu et al., 2016b; Dong G. et al., 2017; Zhou et al., 2017). After the Han Dynasty, with the establishment of water conservancy facilities, the emergence of winter wheat and the improvement of wheat production tools (Chen, 1981), agriculture in northern China shifted from millet-based cultivation to the agricultural cultivation mode combining millets with wheat (Zhao, 2015). Meanwhile, the human diet changed accordingly (Tao et al., 2020). Human diets heavily relied on both millets and wheat, and showed obvious diversity and regional characteristics during 1000–500 BP, filling a current gap in the archaeobotanical results. Therefore, the human subsistence strategy in this period was dominated by advanced agricultural civilization and diverse dietary habits in a complex social background. In addition, migration and integration were frequent during the Song-Ming Dynasty, especially in the communities of the Central Plains and ethnic minorities with a suitable climate and prosperous Silk Road (**Figure 4D**; Zhou and Ding, 2006; Zhang C. et al., 2020), which also promoted the diverse livelihood patterns in the WLP.

## CONCLUSION

This study analyzed the carbon and nitrogen isotopes of humans and animals from the Neolithic to the Ming Dynasty in the middle-lower HRV, and investigated a long-term diet trajectory and influencing factors of human subsistence strategies in the WLP. Our results suggest that: (1) during 5300–4000 BP, human and animal diets heavily relied on millet-based agriculture. From 3000 to 2200 BP, dietary structures tended to be complex. During this time, some people and animals still relied on $C_4$-based foods (likely millets and millet byproducts and/or millet-based animal protein), while others consumed a certain amount of wheat and barley. Between 1000 and 500 BP, human diets were dominated by wheat and supplemented with millets and other crops. (2) The region carries dramatic cultural integration and environmental changes from the Neolithic to historical periods in the WLP, where we propose that the dietary pattern from unification to complexity and diversification was influenced by regional cultural development, complex geopolitics and agricultural technological innovation.

Based on relatively continuous isotopic evidence during the past 5,000 years in the middle-lower HRV, this study further assessed the changing patterns of human and animal diets in response to natural and social environmental changes in the WLP. This study is the first to record the human dietary structure from the perspective of isotopes during the Song-Ming Dynasty, and emphasizes geopolitics as an important influencing factor on the dietary patterns in the WLP.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

GD and MM designed the study. JD, SW, GC, and WW conducted field works and sample collection. JD, WW, and LD completed experiments and data correction. JD, YX, and MM analyzed data and designed the figures. JD, SW, GC, WW, LD, YX, MM, and GD wrote the manuscript. All authors discussed the results and commented on the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.905371/full#supplementary-material

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