A comprehensive study of stable carbon and oxygen isotopes for *Cathaica pulveratrix* and *Metodontia yantaiensis* land snails over last two glacial cycles at Beiyao site, central China: implications for paleovegetation and climate seasonality

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Key Points:

- $\delta^{18}O$ and $\delta^{13}C$ of *C. pulveratrix* and *M. yantaiensis* snails over last 20 ka were studied to trace paleoclimate and paleovegetation changes.
- $\delta^{18}O$ showed more rainfall during MIS3 and MIS7 stages and an overall 1.5 times stronger seasonality during glacial than interglacial period.
- $\delta^{13}C$ revealed more C_4 biomass during warm/humid MIS3 and MIS7 stages and *M. yantaiensis* ingested more C_4 than *C. pulveratrix*.

Abstract

Modern investigations have shown that oxygen and carbon isotopes of land snail shells are useful indicators of climate and vegetation in monsoonal region. However, stable isotope study on snail fossil shells in strata has been seldom done, and the reliability of those indicators needs further verification. Moreover, intra-shell stable isotope analysis of individual snail is rather scarce, and seasonal variation of the glacial-interglacial monsoonal climate remains unclear. In this context, we performed δ18O and δ13C analyses on fossil shells of cold-aridiphilous Cathaica pulveratrix and sub-humidiphilous Metodontia yantaiensis from the loess section over the last two glacial cycles at Beiyao site in southern Chinese Loess Plateau. The δ18O of fossil shells reflected monsoonal rainfall amount and more rainfall during MIS3 and MIS7. Meanwhile, the δ13C of fossil shells indicated relative abundance of C3/C4 plants and more C4 biomass during MIS3 and MIS7. The δ18O and δ13C of the two species from the same horizon are significantly different, reflecting differences in their growing season and/or physiological habits. Intra-shell variations of stable isotopes showed that climatic seasonality was relatively strong during the glacial periods

whereas seasonality became weakened during the interglacials. Our findings provide an environmental background for explaining past human activities at the Beiyao site. The investigation of stone artifacts showed that ancient human activities were relatively strong during MIS3 and MIS7. During these stages, the warm and humid climate with smaller seasonal contrast was favorable for the regional expansion of human activities.

1 Introduction

Land snails are ideal materials for paleoclimate studies(Goodfriend,1992; Wang et al., 2016; Wu et al., 2018). This is because they have advantages of being widely distributed, abundant and well preserved in strata. And they are relatively sensitive to climate changes. To date, researches on land snails include inferring the environmental conditions under which land snails survived through identifying faunal assemblage and living habit of each species (Gittenberger and Goodfriend., 1993; Wu et al., 2008), and reconstructing the paleoclimates through analyzing stable isotopes of land snail shells (Goodfriend and Ellis, 2002; Liu et al., 2006; Gu et al., 2009; Colonese et al., 2010; Rangarajan et al., 2013; Yanes and Fernández-Lopez-de-Pablo, 2016; Prendergast et al., 2016; Padgett et al., 2019).

Theoretically, oxygen isotope in snail shell is determined by the oxygen isotope of snail body water and the temperature under which shell carbonate precipitates. Although body water oxygen isotopes of land snails are modified to different extents by evaporation due to differences in physiological habits of various species, it can still be generally used to track changes in precipitation oxygen isotopes (Zarrur et al., 2011; Zhai et al., 2019). Therefore, in the case of little temperature change, oxygen isotopes of land snail shells mainly reflect oxygen isotopes of rainfall (Prendergast et al., 2016; Wang et al., 2016; Milano et al., 2018;

Padgett et al., 2019). The snail shell carbon isotope reflects the carbon isotope composition of food they intake, with a large proportion of dietary plants, e.g., organic food accounts for more than 70% of carbon sources of land snail shell (Xu et al., 2010). In brief, the shell carbon isotope value can provide information on the relative abundance of C_3/C_4 plants in the food(Goodfriend and Ellis, 2002; Prendergast et al., 2017).

Land snail fossils are abundant and widely distributed in the Asian monsoon region, especially in the Chinese Loess Plateau (Wu et al., 1996；Wu et al., 2002; Liu et al., 2006; Gu et al., 2009). However, researches on the stable isotopes of snail shells have mainly focused on studying modern land snails in different climatic regions (Liu et al., 2006; Wang et al., 2016; Bao et al., 2018, 2019; Wang et al., 2019; Zhai et al., 2019). In contrast, stable isotope analyses of fossil snails in strata have been inadquently done, and only a few species of land snails were studied (Gu et al., 2009; Huang et al., 2012). In this context, it is necessary to perform stable isotope analyses on shell fossils of different land snail species from strata in the different regions and compare those data with other paleoclimatic proxy indicators to confirm their paleoenvironment and paleoclimate significances. Moreover, stable isotope analysis of individual shell along shell ontogeny has the potential to provide seasonal information (Leng et al., 1998; Goodfriend and Ellis, 2002). However, the application of this type of research in paleoclimate is also less developed.

In this study, we systematically collected land snail fossils from loess-paleosol section over last two glacial-interglacial cycles at the Beiyao site in Luoyang, central China. Carbon and oxygen isotopes were measured on *Cathaica pulveratrix* (cold-aridiphilous) and *Metodontia yantaiensis* (sub-humidiphilous) land snails. We then compared these isotopic data with paleoclimate proxy indicators like grain size and magnetic susceptibility with attempt to reconstruct changes in climate and vegetation (C_3/C_4 plants) in the study area. The Luoyang Beiyao site is an archaeological site with human activities in the Paleolithic Age. Recent studies have found some lithics in strata belonging to the late glacial period and the middle and late MIS7 stage (Du et al., 2011; Du and Liu, 2014), indicating that there were human activities during those time periods. However, the climate and environmental context associated with the human activities is still unclear. This study will precisely analyze the environmental conditions for the human activities during the late glacial period and the middle and late of MIS7. At the same time, we also selected snail fossils during the typical periods of the glacials and interglacials, and analyzed intra-shell isotopic varation of each shell to obtain seasonal information during these periods, thereby helping us to understand changes in climatic seasonality from glacialto interglacial period.

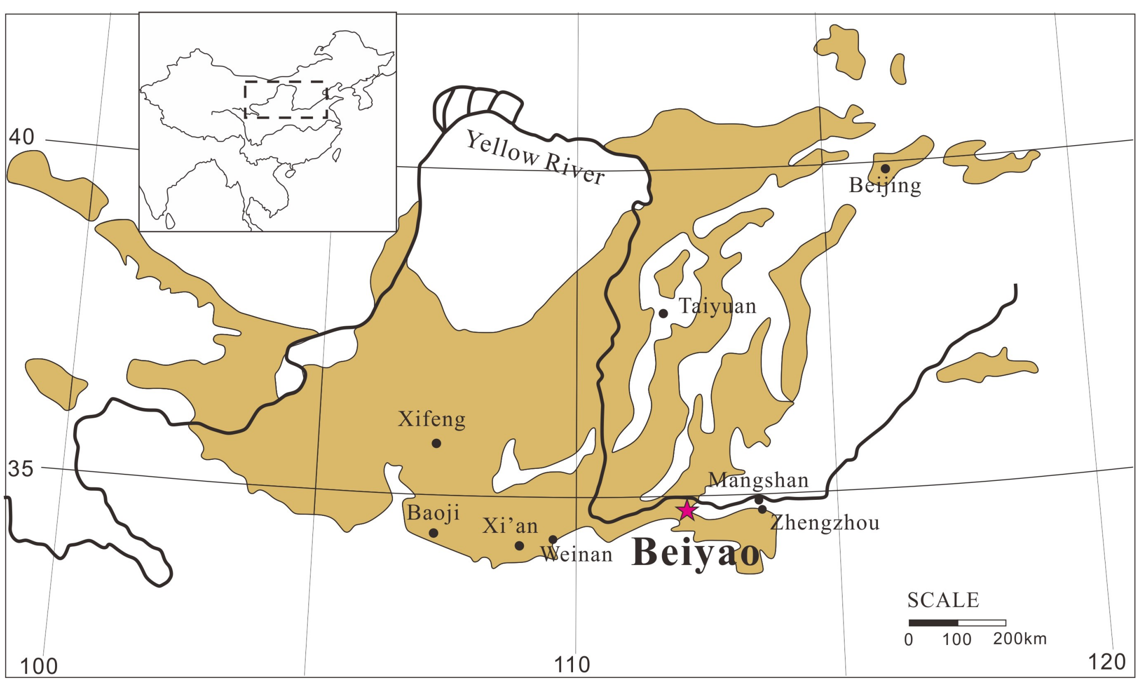

Figure 1. Location map of the study site (red star). The yellow shaded area is the distribution range of the Loess Plateau, edited from Kukla and An (1989).

2 Geological settings and sample collection

2.1 Geological settings

The loess-paleosol section is located at the Luoyang Beiyao archaeological site （34°42′24″N，112°28′46″E） on the southeast edge of the Chinese Loess Plateau (Figure 1). The Beiyao site lies on the third-grade loess accumulation terrace on the south bank of the Luo River in Luoyang. The terrace is about 20m higher than the modern river bed, and the loess section is 16.7m long from bottom to top. Grain size and magnetic susceptibility data combined with optical luminescence (OSL) and AMS ^{14}C datings showed that the loess section has covered the last two glacial-interglacial cycles (Du et al., 2011). At present, the mean annual temperature and annual precipitation are 14.2℃ and 546 mm, respectively. The study area is located in a typical monsoonal region. Northerly wind prevails and climate is cold and dry in winter, while southerly wind dominates in summer with hot and rainy condition. A large number of stone artifacts were found in the Beiyao section at depth of 6.5~7.5m and 11~13m, indicating that there were prehistoric human activities.

The magnetic susceptibility and median particle size curves showed synchronous changes, and had a good correspondence with the marine oxygen-isotope stage (MIS) curve (Tang et al., 2017). Therefore, in this study, we sub-divided the loess section to various oxygen isotope stages according to the grain size and magnetic susceptibility, referring to AMS ^{14}C and OSL datings.

2.2 Collection of land snail fossils

During the sampling process at the Beiyao site, 1m × 1m × 10cm volume loess (or paleosol) was continuously excavated downward, and the snails in each horizon were collected by screening and washing using water and a 0.5 mm sieve. The identification and statistics of the snail fossils used in this study were completed by Yan Wu. Throughout the section, there were 1911 cold-aridiphilous *Cathaica pulveratrix* (*C. pulveratrix*) and 241 sub-humidiphilous *Metodontia yantaiensis* (*M. yantaiensis*) (Wu, 2011). When the fossil fragments were counted as Quaternary loess snail individuals, the calculation method developed by Puisségur (1976) was used to convert the fragments into snail fossil individuals and sum them as the total number of individuals. The conversion formula (Puisségur, 1976) is as followed:

$$\text{Number of individuals} = \text{number of fragments}/5 - \text{number of fragments}/5 \times \text{conversion factor}$$

The conversion factor varies with the number of snail fossil fragments. When the number of snail fossil fragments is <50, 50-75, 75-100, and >100, the conversion factor is 10%, 20%, 33%, and 50%, respectively. Except for few fossils due to the strong pedogenesis at 6.8-7m, most of the section is rich in snail fossils. In this study, we used complete shell of land snails for stable isotope analysis. Totally, there are 577 *C. pulveratrix* shells from 59 horizons, and 97 *M. yantaiensis* shells from 15 horizons.

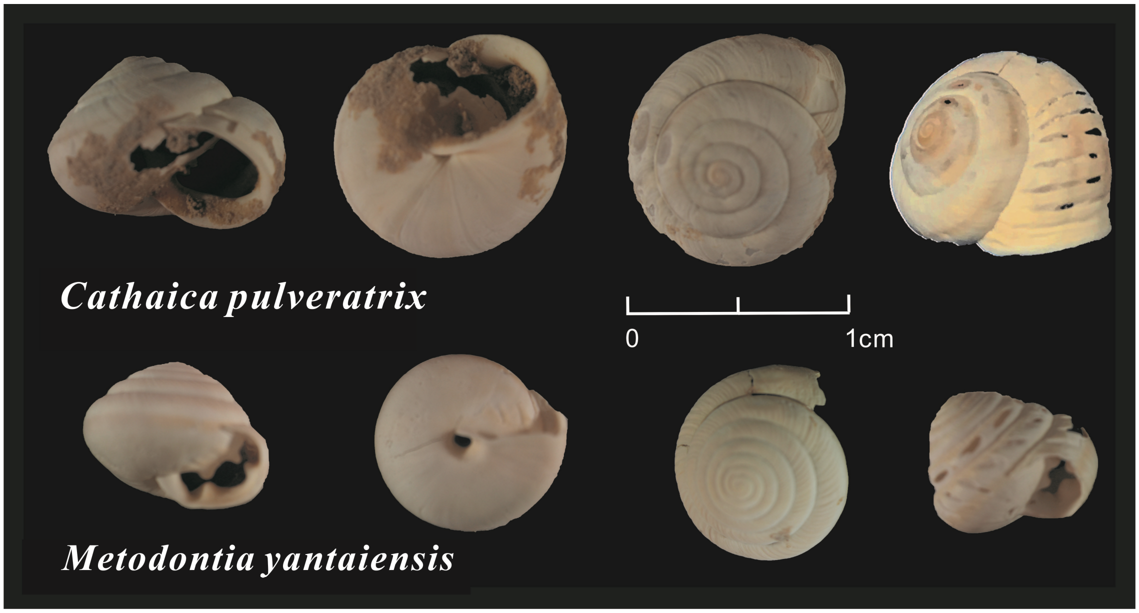

Figure 2. Photos showing shell morphology of the two species land snails. The sampling strategy along with the growth band was also shown.

2.3 Ecological habits of the two species

The two species of land snails used in this study have different living habits. *C. pulveratrix* usually lives in relatively cold and dry climates whereas *M. yantaiensis* lives in warm and sub-humid climates (Wu et al., 1996; Chen and Wu, 2008). The pictures for two species of land snails are shown in Figure 2. Both species are also living in the modern time. According to Chen (2016), *C. pulveratrix* distributes over a vast area including Shanxi, Henan, Hunan, Shaanxi, Gansu, Xinjiang provinces, and even in central Asia. The habitat for *C. pulveratrix* is usually in thick grasses or under the litter beneath trees in mountain area, on flat slope of hills as well as in ranches, orchards and crop land. *M. yantaiensis* distributes usually in northern China, i.e., Beijing, Tianjin, Hebei, Shanxi, Inner Mongolia, Shandong and Shaanxi, and also shows in area around the Yangtze River. It often lives in slightly damp bushes, grasses, under rocks and leaves in in mountainous and hilly areas.

Shell size comparion shows that *C. pulveratrix* is usually larger than *M. yantainesis* (Table 1). This morphology difference complies with their living environment conditions.According to previous studies, the large shell can reduce the ratio of surface area to volume, thereby limiting water evaporation and making it easier for the snails to survive in drier environments（Nevo et al., 1983; Yanes and Fernández-Lopez-de-Pablo, 2016）.

Table 1 Snail shell sizes of the two species at various MIS stages.

	MIS3		MIS4		MIS6		MIS7	
Genus	M. yantaiensis	C. pulveratrix	M. yantaiensis	C. pulveratrix	M. yantaiensis	C. pulveratrix	M. yantaiensis	C. pulveratrix
Spiral（number）	5	5	5	5	7	5	6	5
Height（cm）	0.55	1.5	0.5	1.2	0.95	1.3	0.8	1.1
Height of lip（cm）	0.3	0.85	0.3	0.7	0.4	0.7	0.4	0.7
Width of lip（cm）	0.35	0.7	0.35	0.6	0.5	0.7	0.5	0.8

3 Materials and Methods

3.1 Snail shells pretreatment and sampling strategy

The entire shell was firstly cleaned with distilled water, and the soil particles attached to the shell surface were brushed using a toothbrush, and then the shell was placed in a drying oven and heated at 60 °C for 12 hours. The relatively large shells were chosen for sampling along the growth band. Firstly, weremoved the residual clay cements on the surface of shells using a dental drill, then cleaned the shells using a ultrasonic utility for multiple times, and finally dried the shells in an oven. The three dimension of each shell (i.e.,shell height, width and height of shell mouth) was measured using a ruler. For intra-shell sampling, we use a micro drill to take powders from the shell lip till apex at 1-2 mm interval along the growth direction of the snail (Figure 2). The drill bit was soaked in diluted hydrochloric acid solution after each sample to remove residual carbonate powder on it.

For the carbon and oxygen isotope analyses of the whole shell, about 10 shells were combined according to the availability of snail shells in each horizon. This can ensure the measured data to represent a general and average environment condition under which land snails lived. After the shell was cleaned and dried for the first time, it was broken into fragments. The clay cement attached to each shell fragment was physically removed, and then the fragments were further cleaned using an ultrasonic utility. After very clean shell fragments were obtained, we dried them in an oven at 60 °C. Finally, we ground them into powders and homogenized using a mortar and pestle.

3.2 Stable isotope analyses

The carbon and oxygen isotopic analyses of the snail shell powder were performed on the GasBench II multifunctional gas preparation system coupled with the Delta V Plus isotope ratio mass spectrometer (Thermo Fisher). A 100μg carbonate powder reacted with 100% H_3PO_4 at 72 °C for 1 hour. The generated CO_2 passed through two NAFIONTM water traps to remove trace water and passed through a PoraPlot Q chromatography column at 45 °C to separate with other impurities. After that, the CO_2 was introduced into the isotope ratio mass spectrometer to measure the carbon and oxygen isotope ratios. Both carbon and oxygen isotope data are reported relative to the VPDB. The standards used for data correction and calibration were GBW4416 ($\delta^{13}C_{VPDB}$=1.61‰, $\delta^{18}O_{VPDB}$=-11.59‰) and NBS19

($\delta^{13}C_{VPDB}$=1.95‰, $\delta^{18}O_{VPDB}$ =-2.20‰). The analytical precision of carbon and oxygen isotopes is 0.06‰ and 0.10‰, respectively. Detailed analytical method can be found in Wang et al. (2019).

4 Results

4.1 Carbon and oxygen isotopes of whole shell for two species land snails

The variation range of $\delta^{18}O_{VPDB}$ for cold-aridiphilous *C. pulveratrix* was -2.16‰ to -8.13‰, and the average value was -5.03‰. The maximum value of $\delta^{18}O_{VPDB}$ was at the depth of 1.1 m in the profile, which corresponds to MIS2, while the minimum value of $\delta^{18}O_{VPDB}$ was at the depth of 11.7m, which belongs to MIS7. The $\delta^{18}O_{VPDB}$ value for sub-humidiphilous *M. yantaiensis* ranged from -7.34‰ to -9.71‰, with an average of -8.43‰. The maximum $\delta^{18}O_{VPDB}$ value was at 4.6 m (MIS4) and the minimum at 11.6 m (MIS7).

The $\delta^{13}C_{VPDB}$ for *C. pulveratrix* ranged from -3.17‰ to -6.62‰ with an average of -4.81‰. The maximum $\delta^{13}C$ was at the depth of 10.1 m in the profile, which belongs to MIS6 whereas the minimum $\delta^{13}C$ was at 6.6m, which corresponds to the MIS5. The range of $\delta^{13}C_{VPDB}$ for *M. yantaiensis* was between -3.05‰ and -5.03‰, and the average value was -3.95‰. The maximum $\delta^{13}C_{VPDB}$ for *M. yantaiensis* showed at 3.4 m (MIS3) whereas the minimum $\delta^{13}C_{VPDB}$ occurred at 12 m (MIS7).

4.2 Carbon and oxygen isotope changes along the growth band of individual shell

In the MIS3 and MIS5, intra-shell $\delta^{18}O_{VPDB}$ variation for *C. pulveratrix* was from -12.3‰ to 0.2‰, and the variation of $\delta^{13}C_{VPDB}$ was between -6.9‰ and -4.9‰. In contrast, of the intra-shell variation of $\delta^{18}O_{VPDB}$ for *M. yantaiensis* was relatively small, i.e., from -10.1‰ to -5.9‰. The intra-shell $\delta^{13}C_{VPDB}$ ranged from -7.7‰ to -4.8‰ (Table 1).

During the MIS4 and MIS6 stages, the intra-shell $\delta^{18}O_{VPDB}$ and $\delta^{13}C_{VPDB}$ for *C. pulveratrix* varied from -12.0‰ to -3.5‰ and from -7.8‰ to -2.6‰, respectively. The corresponding intra-shell variations for *M. yanntaiensis* were much larger, i.e., from -13.3‰ to -1.7‰ for $\delta^{18}O_{VPDB}$ and from -11.7 to -0.6‰ for $\delta^{13}C_{VPDB}$ (Table 1).

The cold-aridiphilous *C. pulveratrix* had a shell height of 1.1 to 1.5 cm, a shell lip height of 0.7 to 0.85 cm, and a shell lip width of 0.6 to 0.8 cm. In contrast, the sub-humidiphilous *M. yantaiensis* had shell height ranging from 0.55 to 0.95 cm, shell lip height ranging from 0.3 to 0.4 cm, and shell lip width ranging from 0.35 to 0.5 cm (Table 2). Obviously, the shell of *C. pulveratrix* was significantly larger than that of *M. yantaiensis*. As a result, the intra-shell sampling number for *C. pulveratrix* was larger than that for *M. yantaiensis*.

Table 2 Statistics for Intra-shell $\delta^{18}O$ and $\delta^{13}C$ variations of two species at various MIS stages.

Species	Depth (m)	MIS	Number of sampling	$\delta^{18}O$‰ Average (VPDB)	$\delta^{18}O$ ‰ Max	$\delta^{18}O$‰ Min	$\delta^{13}C$‰ Average (VPDB)	$\delta^{13}C$‰ Max	$\delta^{13}C$‰ Min
C. pulveratrix	3.4	3	37	-9.5	-6.4	-12.3	-5.6	-4.9	-6.5
C. pulveratrix	4.9	4	44	-4.7	0.3	-9.7	-4.7	-2.6	-6.5
C. pulveratrix	9.0	6	45	-3.6	3.5	-12.0	-5.2	-4.1	-7.8
C. pulveratrix	11.8	7	28	-5.7	-0.2	-10.9	-6.2	-5.1	-6.9
M. yantaiensis	3.4	3	12	-9.8	-8.7	-11.6	-6.4	-5.1	-7.7
M. yantaiensis	4.9	4	12	-10.5	-7.8	-12.7	-1.6	-0.6	-3.5
M. yantaiensis	9.0	6	30	-5.7	-1.7	-13.3	-10.4	-8.7	-11.7
M. yantaiensis	11.8	7	31	-10.1	-5.9	-13.8	-6.2	-4.8	-7.4

4.3 Statistics of C. pulveratrix and M. yantaiensis in loess-paleosol strata

In the Beiyao section, the maximum number of cold-aridiphilous snails *C. pulveratrix* was 70, occurring at the depth of 9.7 m (belonging to MIS6). In contrast, the maximum number of sub-humidiphilous snails *M. yantaiensis* was 34, appearing at the depth of 4.5 m (belonging to MIS3) (Figure 3). At the bottom of the interglacial paleosol S1, very few of land snail fossils were left because of the influence of strong pedogenesis. However, the other horizons in the section were rich in snail fossils. Therefore, without considering this factor, the cold-aridiphilous species *C. pulveratrix* had a certain number distributing from MIS2 to MIS7, with two most abundant horizons (with fossil number of 58 and 70) respectively in MIS4 and MIS6. The sub-humidiphilous species *M. yantaiensis* were mainly found in MIS3 and MIS7, with maximum number reaching up to 34 and 23, respectively. Moreover, when the number of *M. yantaiensis* increased in some horizon, the number of *C. pulveratrix* in the same horizon or neigbouring horizons significantly reduced. Conversely, when the number of *C. pulveratrix* reached the peak of the stage, the number of *M. yantaiensis* approached the minimum or 0.

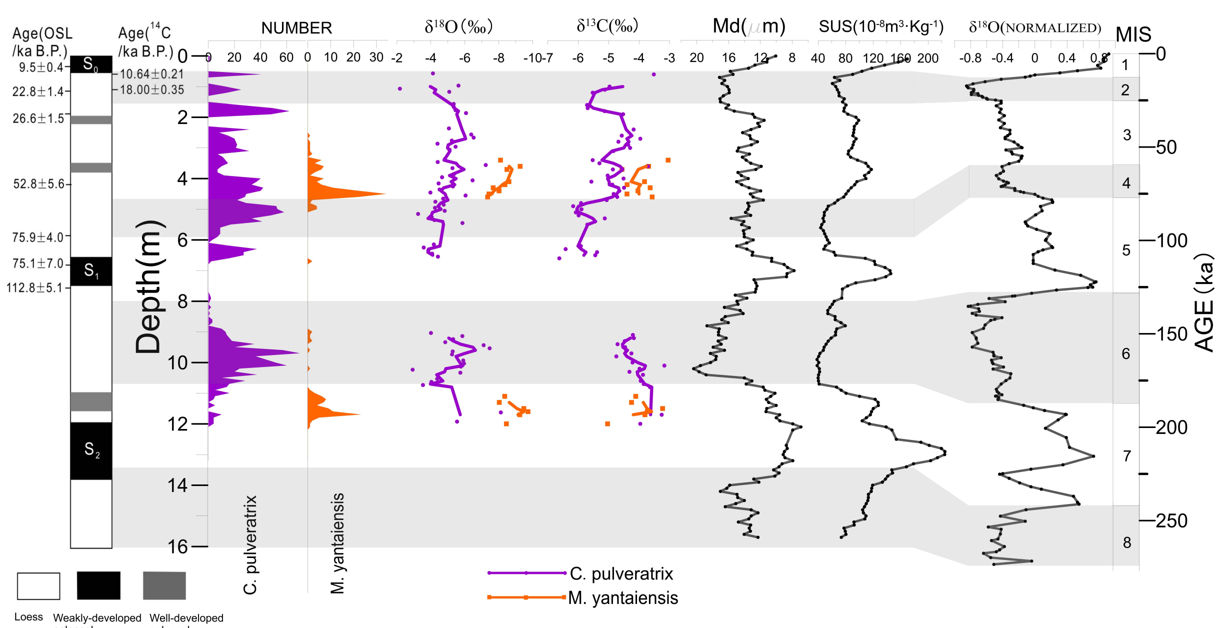

Figure 3. Changes in carbon and oxygen isotopes of C. pulveratrix and M. yantaiensis snails over the last two glacial-interglacial cycles, in comparison with median grain size (Md), magnetic susceptibility (SUS) and deep-sea $\delta^{18}O$ curve. Stages partition, age data and $\delta^{18}O$ value(standardized) of MIS were from Martinson (1987), Md data were from Tang et al. (2017), SUS data were from Du and Liu (2014), ^{14}C and OSL age data ware from Du et al. (2011).

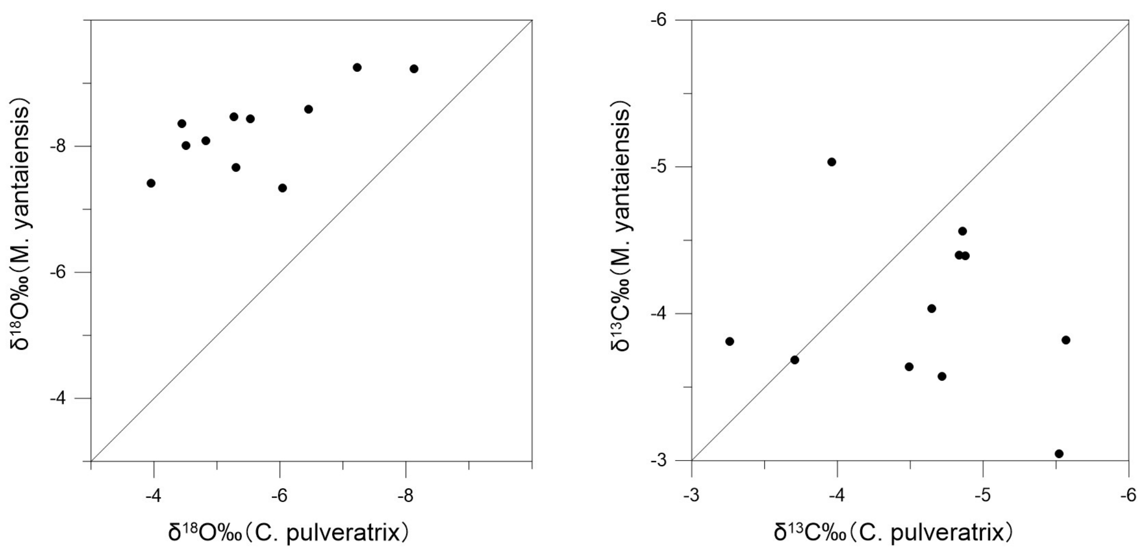

Figure 4. Comparison of carbon and oxygen isotopes between C. pulveratrix and M. yantaiensis from the same horizon. Note that the $\delta^{18}O$ value of M. yantaiensis was significantly lower than that of C. pulveratrix, while the $\delta^{13}C$ value of M. yantaiensis wasmostly higher than that of C. pulveratrix.

5 Discussion

5.1 Oxygen isotopes in land snail shells and changes in summer monsoon rainfall

Many studies have shown that oxygen isotope in land snail shell carbonate is positively related to oxygen isotope in atmospheric precipitation. (Gu et al., 2009; Prendergast et al., 2016; Wang et al., 2016; Milano et al., 2018; Padgett et al., 2019; Wang et al., 2019; Zhai et al., 2019). Generally speaking, the $\delta^{18}O$ values of *C. pulveratrix* were more positive than those of *M. yantaiensis* (Figure 4a). This is consistent with the eco-physiological habits of the two land snail species. The *M. yantaiensis* snails like to live in a relatively warm and humid environment and in seasons with more abundant rainfall. Due to the rainfall effect, the summer rainfall $\delta^{18}O$ will be more negative, so $\delta^{18}O$ in shell carbonate of *M. yantaiensis* is also relatively low. In contrast, the active season of *C. pulveratrix* is relatively cool and dry with less rainfall (such as spring and autumn), so relatively more positive oxygen isotope of rainfall during this time can result in relatively high $\delta^{18}O$ in shell carbonate of *C. pulveratrix*.

Snail shell $\delta^{18}O$ can be combined with other paleoclimate indicators such as the median grain size (Md), magnetic susceptibility (SUS) of the loess and faunal assemblages of land snails to indicate the strength of the East Asian summer monsoon (Wu et al., 2018). A previous study has shown that the shell $\delta^{18}O$ of *C. pulveratrix* can be used as an indicator of summer precipitation to reflect the strength of the summer monsoon (Gu et al., 2009). Specifically, the shell $\delta^{18}O$ of *C. pulveratrix* in the monsoon region of China decreased when the summer precipitation increased. This is consistent with the $\delta^{18}O$ record of stalagmites in Hulu cave in Southern China (Wang et al., 2008).

Generally, the shell $\delta^{18}O$ of *C. pulveratrix* showed a negative correlation with SUS and a positive correlation with Md in the Beiyao loess-paleosol section (Figure 3). This is consistent with the results of Gu et al. (2009). In the middle part of MIS7, the $\delta^{18}O$ of *C. pulveratrix* exhibited a negative shift, with the minimum value being -8.13‰. Meanwhile, the Md value decreased, the number of cold-aridiphilous species *C. pulveratrix* decreased, and the number of sub-humidiphilous species *M. yantaiensis* increased (Figure 3). It suggested that the East Asian summer monsoon intensified during this period, and the $\delta^{18}O$ of precipitation became more negative due to large amount of precipitation.

At the beginning of MIS6, the $\delta^{18}O$ value of *C. pulveratrix* experienced a positive shift, while the SUS value also became lower, indicating that the climate tended to be drier. Subsequently, the $\delta^{18}O$ of *C. pulveratrix* showed a change to more negative value, with the most negative value reaching -7.5‰, and the Md value also became lower, indicating that there have been a significant increase in rainfall amount during the middle part of MIS6.

At the end of MIS5 and during MIS4, the $\delta^{18}O$ values of *C. pulveratrix* snails were generally more positive, with an average $\delta^{18}O_{VPDB}$ value of -4.2‰. At the same time, the SUS increased and the Md decreased. Collectively, it indicated a relative cold and dry climatic condition.

From MIS4 to MIS3, the $\delta^{18}O$ of *C. pulveratrix* snail showed a significant decrease, indicating that the climate has entered a humid and rainy mode. However, the oxygen isotope became more positive during middle MIS3, which corresponded to the decrease in SUS. This implied that the climate during MIS3 was variable and there was once a relatively cold and dry climate. Despite this, the $\delta^{18}O$ of *C. pulveratrix* during the middle MIS3 was still more negative than that during MIS4, indicating a slightly drying middle MIS3. The $\delta^{18}O$ values of *C. pulveratrix* during the late stage of MIS3 were -0.6‰ by average more negative than those during the early stage of MIS3, suggested a generally more humid climate during the late MIS3. But we acknowledged that the $\delta^{18}O$ during the early MIS3 was highly variable and some negative extrema that are even lower than the late MIS3 $\delta^{18}O$ also appeared during this period. This may reflect some transient stages with much humid condition also occurred during the early MIS3. The three-stage sub-division of MIS3 can be also envisaged on the SUS curve of our loess section (Figure 3). The average $\delta^{18}O$ value of *C. pulveratrix* was -5.3‰ during MIS3 stage. In contrast, the average $\delta^{18}O$ during MIS2 was much higher (-

4.2‰) and it showed a clear trend of increase, suggestive of a climatic transition from wetness to dryness.

Within MIS2 stage, the $\delta^{18}O$ values of *C. pulveratrix* increased up to -2‰ at about 21.6 ka, which marked extreme dryness during the last glacial period (LGM). Similarly, the $\delta^{18}O$ of *C. pulveratrix* from Mangshan loess section in central China also showed an extremely positive value (approximately -1‰) around 22 ka (Gu et al. 2009). The two study sites are about 100 km away. Collectively, it manifested a synchronous regional drought in central China during the LGM.

The $\delta^{18}O$ values of *M. yantaiensis* exhibited almost the same pattern of variation as those of *C. pulveratrix* did. During late MIS7 stage, the $\delta^{18}O$ of *M. yantaiensis* was more negative than that of *C. pulveratrix* and attained to the most negative of -9.71‰ when the $\delta^{18}O$ of *C. pulveratrix* dropped to its most negative one (Figure 3). In the meantime, SUS also increased its peak value. These lines of evidences corroborated abundant rainfall brought by the intensified summer monsoon during the late MIS7. During the early MIS3, the $\delta^{18}O$ of *M. yantaiensis* showed a gradually decreasing trend, which was synchronous with the changes in *C. pulveratrix* $\delta^{18}O$ and SUS. This further confirmed climate shifted to more humid condition from MIS4 to early MIS3.

5.2 Carbon isotopes in land snail shells and vegetation changes

The carbon isotope of land snail shell is mainly related to carbon isotopes of dietary plants (Goodfriend and Ellis, 2002; Stott, 2002; Metref et al., 2003; Balakrishnan and Yapp, 2004). A previous study on modern land snails in China has shown that snail shell carbonate was enriched in ^{13}C by 14.2‰ relative to snail body that has on isotopic difference from organic diet (Liu et al., 2006). At the same time, C_3 and C_4 plants have far different carbon isotope compositions, i.e., the average $\delta^{13}C$ of C_3 plant is -27.1 ± 2.0‰ whereas the average $\delta^{13}C$ of

C_4 plant is -13.1 ± 1.2‰ (Farquhar et al., 1989; O'Leary, 1998; Cerling, 1999). Therefore, the proportion of C_3 to C_4 plants in snail food can be estimated based on the shell-diet carbon isotope fractionation and snail shell carbon isotope. Because there is a 1.3‰ decrease in the $\delta^{13}C$ of atmospheric CO_2 since the industrial revolution due to the combustion of ^{13}C-depleted fossil fuels, so-called Suess effect (Marino et al., 1992), the above two $\delta^{13}C$ end-members for C_3 and C_4 plants should be adjusted to -25.8‰ and -11.8‰, respectively, during the last two glacial-interglacial periods in our study.

The maximum $\delta^{13}C$ of *C. pulveratrix* was -7.34‰ that occurred at MIS5. Considering shell-diet carbon isotope fractionation of +14.2‰, the converted dietary $\delta^{13}C$ was -21.5‰ and the inferred proportion of C_4 plant was about 31%. The minimum $\delta^{13}C$ of *C. pulveratrix* was -9.71‰ that showed at MIS7. The estimated relative C_4 abundance was about 14%. In contrast, the most positive $\delta^{13}C$ of *M. yantaiensis* was -3.05‰ that occurred at MIS3, corresponding to a relative C_4 abundance of 61%. The most negative $\delta^{13}C$ of *M. yantaiensis* was -5.03‰ that showed at MIS7, converting to 47% of C_4 in the food. It can be seen that *M. yantaiensis* snails consumed more C_4 plants than *C. pulveratrix*. We acknowledged that the proportion of C_4 plants in snail's food was overestimated because land snails may also take in a small portion of soil carbonates that have more positive $\delta^{13}C$ than C_3 and C_4 plants. However, this does not influence our assessing the relative changes in C_4 abundances over different MIS stages.

To some extent, relative abundance of C_4 plants can reflect the climate and seasonal changes. At seasonal level, C_4 plants prefer to grow in the summer when there are more warmth and abundant precipitation whereas C_3 plants grow in spring and autumn with relatively low temperature (Sage et al., 1999; Huang et al., 2012). At glacial/interglacial time-scale, C_4 biomass tended to increase during warm/humid interglacial periods whereas C_3

biomass dominated during the cold/dry glacial periods (Liu et al., 2005; Yang et al., 2015). As shown in Figure 4, the $\delta^{13}C$ of *C. pulveratrix* was mostly more negative than that of *M. yantaiensis* at the same horizon. This may indicate that *C. pulveratrix* was more active in relatively cold/arid environments or seasons and accordingly ingested more C_3 plants. This is consistent with the phenomenon observed by Huang et al. (2012).

In general, the $\delta^{13}C$ curve of *C. pulveratrix* has a positive correlation with the SUS curve and a negative correlation with the $\delta^{18}O$ of *C. pulveratrix*. This indicates a linkage of C_3/C_4 abundance in dietary food of land snails to climate changes. Specifically, the $\delta^{13}C$ values of *C. pulveratrix* snail shell during late MIS7 were slightly more positive than those during MIS6, and the $\delta^{13}C$ of *C. pulveratrix* during MIS3 was more positive than MIS2 and MIS4 as well (Figure 3). Because the feeding habits of the same snail would not largely change, the above variation in C_4 abundance in the snail's food may reflect the changes of C_4 biomass in natural vegetation along with climate, i.e., relative abundance of C_4 plants increased during the warm/humid interglacial (or interstadial) periods. This is in accordance to the aforementioned conclusion reached by previous studies (Liu et al., 2005; Yang et al., 2015).

5.3 The relationship between snail numbers of two species and environment change

During late MIS7, the number of cold-aridiphilous *C. pulveratrix* snail was relatively lower than that of sub-humidiphilous *M. yantaiensis* and the land snail *M. yantaiensis* had reached a peak amount. At this time, Md became finer, SUS value increased, and the shell $\delta^{18}O$ values of both *C. pulveratrix* and *M. yantaiensis* shifted to more negative. These multiple proxies uniformly suggested that the warm and humid climate prevailed, which was suitable to the growth of sub-humidiphilous *M. yantaiensis*. In addition, a large number of stone artifacts were found at the depth of 11-13 m (MIS7) in the Beiyao section (Du and Liu, 2014), indicating strong human activities. The inferred warm/humid climatic condition was conducive to the intensified prehistoric human activities.

After entering MIS6, the number of cold-aridiphilous species increased and reached the peak of the whole profile at 9.7 m whereas the sub-humidiphilous species almost disappeared, which implied the climate became much colder and drier than the previous stage. In the meantime, the $\delta^{18}O$ of *C. pulveratrix* shifted to more positive value, i.e., up to -5.3‰, reflecting less monsoonal rainfall as well.

During most MIS5, land snail fossils were not preserved due to the influence of strong pedogenesis and there were only a few sub-humidiphilous snails at the depth of 6.5-7 m. At the end of MIS5, a small number of cold-aridiphilous species began to appear, indicating that the climate started to be relatively cold and dry, in accordance to the Md and SUS records.

To MIS4 stage, the number of cold-aridiphilous species significantly increased, reaching a maximum of 58, while sub-humidiphilous species rarely existed and even disappeared. The cold/dry climate as seen from the $\delta^{18}O$ of *C. pulveratrix,* Md and SUS accounted for the flourish of the cold-aridiphilous *C. pulveratrix*.

During MIS3, the numbers of *C. pulveratrix* and *M. yantaiensis* showed alternative increases, further testifying variable climatic conditions. It also indicated that the climate was of moderate conditions so that both cold-aridiphilous and sub-humidiphilous species co-existed. At the early MIS3 stage, the number of *C. pulveratrix* decreased when *M. yantaiensis* reached its peak abundance. In contrast, both the numbers of *C. pulveratrix* and *M. yantaiensis* largely reduced at the middle MIS3. To the late MIS3, *M.* yantaiensis went further reduced but the number of *C. pulveratrix* increased. This assemblage change indicated that the climate was warmer and more humid at the early MIS3 than at late MIS3. A faunal assemblage study of land snails in central Chinese Loess Plateau also suggested that the temperature and humidity were higher during the early MIS3 (Chen and Wu, 2008).

However, the δ^{18}O of *C. pulveratrix* was highly variable during the early MIS3 and was not as more negative as that during the late MIS3 (Figure 3). This reflected a variable summer monsoon and an overall less rainfall during the early MIS3.

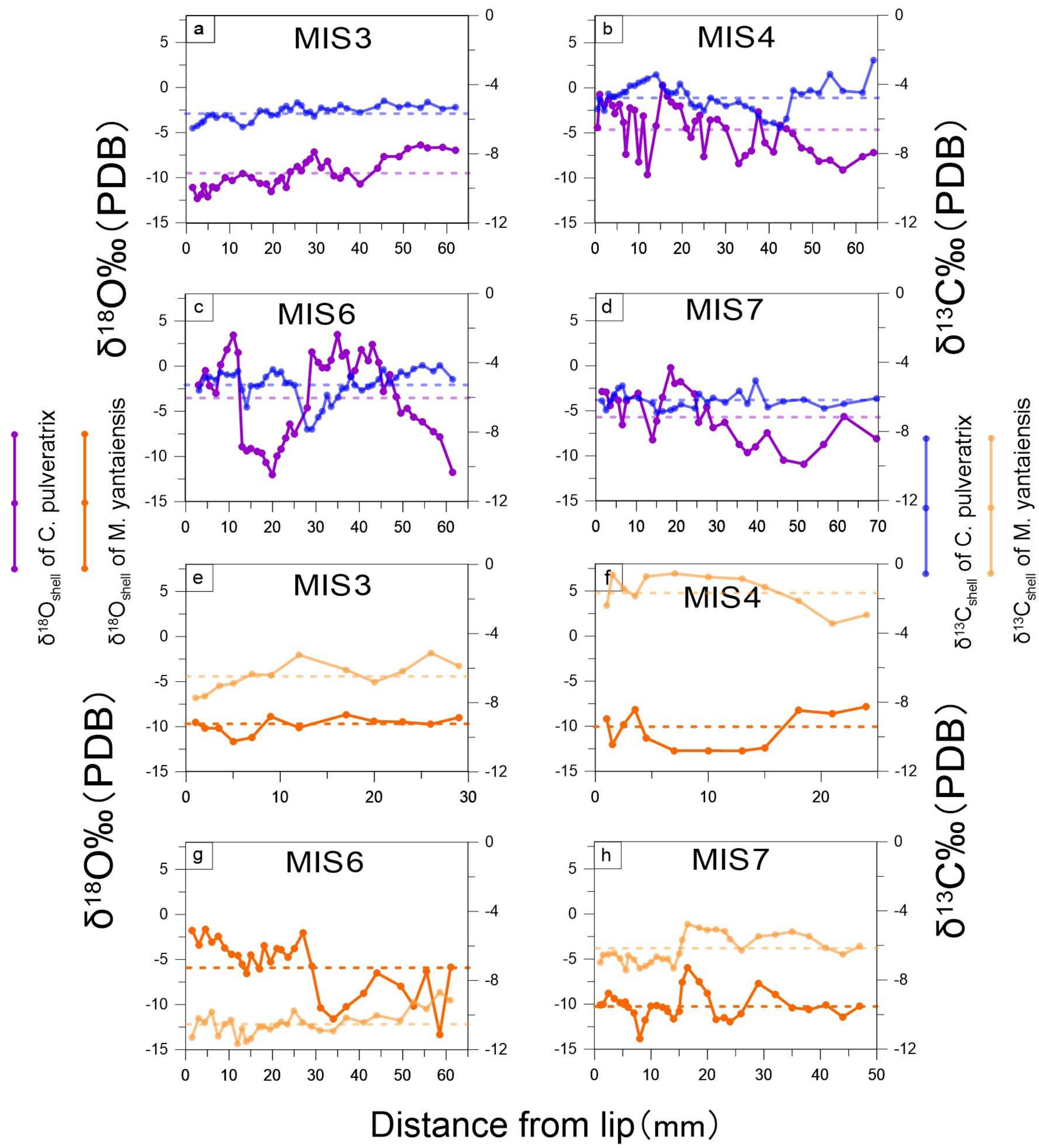

Figure 5. Intra-shell variations of δ^{18}O and δ^{13}C for the two species at various MIS stages.

5.4 Intra-shell variation of stable isotopes and climate seasonality

In this study, intra-shell stable isotope analyses were performed on both *C. pulveratrix* and *M. yantaiensis* snails at MIS3, MIS4, MIS6, and MIS7, respectively. The measured *C. pulveratrix* and *M. yantaiensis* snails were chosen from the same layer (10 cm) in each MIS stage. During MIS3 , the $\delta^{18}O$ of *C. pulveratrix* and *M. yantaiensis* were among the most negative values of the four MIS stages, with averaged $\delta^{18}O$ of -9.5.‰ and -9.8‰, respectively. Moreover, the intra-shell variations in $\delta^{18}O$ of the two snails were relatively small. For example, the $\delta^{18}O$ of *C. pulveratrix* showed a variation magnitude of 5.9‰ whereas $\delta^{18}O$ of *M. yantaiensis* only changed by 2.9‰ (Figure 5a, e). This suggested a weak seasonality during the warm/humid MIS3 stage. Padgett et al. (2019) also observed a steady trend of $\delta^{18}O$ in land snail shell in warm and humid climate. In contrast, the magnitudes of intra-shell $\delta^{18}O$ variations for *C. pulveratrix* and *M. yantaiensis* showed large increases, i.e., up to 10‰ and 4.9‰, respectively.

During MIS6, the average $\delta^{18}O$ values of *C. pulveratrix* and *M. yantaiensis* became more positive and were around -3.6‰ and -5.7‰, respectively (Figure 5c, g). Meanwhile, the intra-shell $\delta^{18}O$ of the two species exhibited largest variations during MIS6, i.e., a magnitude of 15.5‰ for *C. pulveratrix* and a magnitude of 12.1‰ for *M. yantaiensis*. These magnitudes were respectively 2.6 and 4 times of those for the same species during MIS3. It revealed extreme seasonal contrast during the cold/dry MIS6. It is worthy of mentioning that the intra-shell $\delta^{18}O$ curve of *C. pulveratrix* displayed regular seasonal changes during MIS6 (Figure 5c). Judging from the sinusoidal cycles, the *C. pulveratrix* snail may have a life span of about two years. The snail possibly started to grow from the summer of the first year to the autumn of the second year. The highest $\delta^{18}O$ values recorded in the shell growing in the spring and autumn seasons attained to ca +2‰ and the lowest $\delta^{18}O$ recorded in the shell segments in summer was about -12‰. The large seasonal contrast was unlikely only attributed to temperature changes, which would be 56 °C offset if calculating by the carbonate oxygen isotope-temperature coefficient of 1‰ per 4 °C. Obviously, seasonal changes of rainfall largely contributed to the above fluctuation of $\delta^{18}O$ of *C. pulveratrix*, that is, the negative values in shell $\delta^{18}O$ being caused by rainfall amount effect in summer. An intra-shell $\delta^{18}O$ study for the land snail collected from Ethiopia also revealed significant contribution of rainfall to the shape and amplitude of shell $\delta^{18}O$ cycles (Leng et al., 1998). Except for the shell lip part, the $\delta^{13}C$ of *C. pulveratrix* showed an overall opposite relationship with the shell $\delta^{18}O$ (Figure 5c). When the $\delta^{18}O$ was more negative in summer, the $\delta^{13}C$ became more positive, implying the snail consumed increased amount of C_4 plants in this season. In spring and autumn (at 30-45 mm from shell lip), more C_3 plants were ingested by the snail. This seasonal change of C_3/C_4 proportion in snail's food diet is consistent with the seasonal distribution of C_3 and C_4 plants in natural vegetation (Sage et al., 1999).

During MIS7, two individual shells for intra-shell isotope study were taken from the depth of 11.8 m, which happened to be within the period of strong prehistoric human activities (Du and Liu, 2014). Based on the previous discussions on $\delta^{18}O$ of *C. pulveratrix* and *M. yantaiensis*, the climate was generally warm and humid during this time. The intra-shell $\delta^{18}O$ variations for *C. pulveratrix* and *M.* yantaiensis were at amplitudes of 10.7‰ and 10.9‰, respectively. The variations were smaller than those during MIS6. This overall small seasonal contrast was conducive to regional spread of human activity.

In summary, the average amplitude of intra-shell $\delta^{18}O$ variations for *C. pulveratrix* was about 8.4‰ during the interglacial periods (i.e., MIS3 and MIS7), whereas it was 12.75‰ during the glacial periods (i.e., MIS4 and MIS6). In the same manor, the intra-shell $\delta^{18}O$ of

M. yantaiensis varied by 10.8‰ and 16.5‰, respectively, during the interglacial and glacial periods. Regardless of which species, the changing amplitude was 1.5 times larger during the glacial periods. Therefore, if the intra-shell variation of $\delta^{18}O$ can be used to quantify the seasonal changes, the climatic seasonality during glacial periods would be about 1.5 times stronger than that during interglacial periods.

To explore the stable isotope differences among individual shells of each snail species from the same sampling horizon (10 cm layer), we analyzed $\delta^{13}C$ and $\delta^{18}O$ on *C. pulveratrix* from 7 layers and *M. yantaiensis* from 3 layers. The carbon and oxygen isotope data were shown in Table 3. Firstly, within the same MIS (i.e., MIS3 or MIS7), the $\delta^{18}O$ of sub-humidiphilous species (*M. yantaiensis*) showed little change, whereas the $\delta^{18}O$ of cold-aridiphilous species (*C. pulveratrix*) distributed much discretely. This may indicate that sub-humidiphilous species have a more strict requirement on climate conditions, i.e., only grow during the period of abundant rainfall, while cold-aridiphilous species had strong adaptability and can survive under large range of climate conditions. Secondly, for the cold-aridiphilous species, the shell $\delta^{18}O$ changes during the even-numbered MIS (i.e., MIS2, MIS4, and MIS6) were larger than those during the odd-numbered MIS (i.e., MIS3 and MIS7). Since the snail shells collected each sampling layer may not strictly come from the same time year, the above phenomenon may indicate that the climates within the time-span of each sampling layer during glacial periods (even-numbered MIS) were very unstable, whereas the climates during interglacial periods (odd-numbered MIS) had relatively stable and uniform conditions within the time period of each sampling layer. Previous studies have shown that climate during the last glacial period was quite unstable, with climate oscillations at centennial to millennium scales (Ren et al., 1996; Ding et al., 1998). This is in accordance to the large intra-species variation of shell $\delta^{18}O$ in each sampling layer.

Table 3 Statistics for intra-species $\delta^{18}O$ and $\delta^{13}C$ variations of two species at various MIS stages.

Species	Depth (m)	MIS	Shell number	$\delta^{18}O$ S.D.	$\delta^{18}O$ Max (VPDB)	$\delta^{18}O$ Min (VPDB)	$\delta^{13}C$ S.D.	$\delta^{13}C$ Max (VPDB)	$\delta^{13}C$ Min (VPDB)
C. pulveratrix	0.60	2	10	2.85	1.34	-7.65	0.99	-1.09	-4.54
C. pulveratrix	1.80	3	10	2.47	-2.51	-10.02	0.74	-3.68	-5.83
C. pulveratrix	3.60	3	3	1.84	-5.93	-9.33	1.52	-2.22	-5.27
C. pulveratrix	4.6	3	10	2.11	-2.69	-9.13	1.42	-2.62	-7.06
C. pulveratrix	6.60	4	10	2.56	1.10	-6.71	0.83	-4.96	-7.63
C. pulveratrix	9.10	6	10	1.98	-1.77	-7.18	1.27	-1.22	-5.88
C. pulveratrix	11.70	7	7	2.02	-5.32	-10.55	2.34	0.20	-6.29
M. yantaiensis	3.60	3	5	1.26	-8.08	-10.90	1.65	-1.93	-6.31
M. yantaiensis	4.60	3	10	1.18	-5.60	-8.92	2.20	1.18	-5.78
M. yantaiensis	11.70	7	10	1.13	-7.53	-11.38	0.62	-2.70	-4.80

6 Conclusion

In this study, we systematically analyzed stable carbon and oxygen isotopes on cold-aridiphilous *C. pulveratrix* and sub-humidiphilous *M. yantaiensis* snail shell fossils from the Beiyao loess-paleosol section in southeastern Chinese Loess Plateau. Stable isotopes were measured on both the mixed multiple shells and the single shell along the growth band. The obtained $\delta^{13}C$ and $\delta^{18}O$ data were compared with Md and SUS from the same profile and deep-ocean $\delta^{18}O$ curve to verify the reliability of snail shell stable isotopes for paleoclimate reconstruction. We reached the following conclusions:

1. $\delta^{18}O$ of snail shells in strata can be used to indicate the intensity of summer monsoon rainfall. During MIS7 and MIS3 stages, the shell $\delta^{18}O$ was more negative, indicating strong monsoonal rainfall, which showed a good correlation to Md, SUS, and deep-sea $\delta^{18}O$ curve. Meanwhile, the shell $\delta^{13}C$ can reflect the proportion of C_4 plants in snail's food and ultimately trace the relative abundance of C_4 plants in contemporary vegetation. The results showed that the relative abundance of C_4 plants increased during the warm/humid MIS7 and MIS3.

2. The stable isotopes of *C. pulveratrix* and *M. yantaiensis* from the same horizon were largely different, reflecting differences in their eco-physiological habits. The $\delta^{18}O$ of *M. yantaiensis* was significantly lower than that of *C. pulveratrix*, indicating that *M. yantaiensis* lived in warmer and more humid conditions than *C. pulveratrix*. The $\delta^{13}C$ of *M. yantaiensis* was mostly higher than that of *C. pulveratrix*, suggesting that *M. yantaiensis* ingested more C_4 plants than *C. pulveratrix*.

3. Intra-shell $\delta^{18}O$ variations revealed that there was a significant difference in the climatic seasonality between glacial and interglacial periods. During the glacial periods (even-numbered MIS), the seasonal contrast was large, whereas the seasonal contrast was small during the interglacial periods (odd-numbered MIS). Stable isotope analyses of multiple shells of the same snail species within each sampling layer showed that intra-species isotope data were largely scattered during the glacial periods, indicative of highly unstable climates change at sub-millennial scale, whereas intra-species isotopic difference was relatively small during the interglacial periods, suggestive of a steady and uniform climatic condition within millennium.

4. During MIS3 and MIS7, there were evidences of human activities around the Beiyao site, but the corresponding climate background remained unclear. By analyzing whole-shell and

intra-shell $\delta^{18}O$ and faunal assemblage of the two species snails, we concluded that the climates were relatively warm and humid with a weak seasonality. This stable climatic condition was conducive to the regional expansion of prehistoric human activities.

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