



C4 expansion in the central Inner Mongolia during the latest Miocene and early Pliocene

Chunfu Zhang^{a,*}, Yang Wang^a, Tao Deng^b, Xiaoming Wang^c, Dana Biasatti^a, Yingfeng Xu^a, Qiang Li^b

^a Department of Geological Sciences, Florida State University and National High Magnetic Field Laboratory, Tallahassee, FL 32306-4100, USA

^b Laboratory of Evolutionary Systematics of Vertebrates, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing, 100044, PR China

^c Department of Vertebrate Paleontology, Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, California 90007, USA

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ABSTRACT

The emergence of C4 photosynthesis in plants as a significant component of terrestrial ecosystems is thought to be an adaptive response to changes in atmospheric CO₂ concentration and/or climate during Neogene times and has had a profound effect on the global terrestrial biosphere. Although expansion of C4 grasses in the latest Miocene and Pliocene has been widely documented around the world, the spatial and temporal variations in the C4 expansion are still not well understood and its driving mechanisms remain a contentious issue. Here we present the results of carbon and oxygen isotope analyses of fossil and modern mammalian tooth enamel samples from the central Inner Mongolia. Our samples represent a diverse group of herbivorous mammals including deer, elephants, rhinos, horses and giraffes, ranging in age from the late Oligocene to modern. The $\delta^{13}\text{C}$ values of 91 tooth enamel samples of early late-Miocene age or older, with the exception of two 13 Ma rhino samples (-7.8 and -7.6 ‰) and one 8.5 Ma suspected rhino sample (-7.6 ‰), were all less than -8.0 ‰ (VPDB), indicating that there were no C4 grasses present in their diets and thus probably few or no C4 grasses in the ecosystems of the central Inner Mongolia prior to ~ 8 Ma. However, 12 out of 26 tooth enamel samples of younger ages (~ 7.5 Ma to ~ 3.9 Ma) have $\delta^{13}\text{C}$ values higher than -8.0 ‰ (up to -2.4 ‰), indicating that herbivores in the area had variable diets ranging from pure C3 to mixed C3–C4 vegetation during that time interval. The presence of C4 grasses in herbivores' diets (up to ~ 76 % C4) suggests that C4 grasses were a significant component of the local ecosystems in the latest Miocene and early Pliocene, consistent with the hypothesis of a global factor as the driving mechanism of the late Miocene C4 expansion. Today, C3 grasses dominate grasslands in the central Inner Mongolia area. The retreat of C4 grasses from this area after the early Pliocene may have been driven by regional climate change associated with tectonic processes in central Asia as well as global climate change.

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1. Introduction

Plants can be divided into three categories based on the photosynthetic pathways they use: C3, C4, and CAM (crassulacean acid metabolism). C3 plants include virtually all trees, most shrubs and herbs, and cool season grasses. The $\delta^{13}\text{C}$ values of C3 plants range from -20 to -35 ‰ (vs. VPDB), averaging -27 ‰. Under water-stressed conditions, C3 plants display more enriched $\delta^{13}\text{C}$ values (> -27 ‰); while under closed canopy conditions, C3 plants show more depleted $\delta^{13}\text{C}$ values (< -27 ‰). C4 plants include warm season grasses and a few shrubs in the families Euphorbiaceae and Chenopodiaceae. C4 plants have a mean $\delta^{13}\text{C}$ value of -13 ‰, and a range from -9 to -17 ‰. CAM plants include succulents such as cacti and some yuccas, and are found mostly in deserts. Their $\delta^{13}\text{C}$ values are mostly between C3 and C4 plants, but can be closer to (or overlap with) C3 or C4 plants depending on their local environmental conditions. CAM plants are rarely included in the

discussion of ecosystem changes because they are usually not important components of ecosystems outside deserts (Deines, 1980; Quade et al., 1989; Cerling et al., 1997; Tipple and Pagani, 2007; Wang et al., 2008a).

The carbon and oxygen isotopes of fossil tooth enamel of herbivorous mammals can be used to reconstruct paleodiets and paleoenvironments. This is because: (1) tooth enamel can survive most diagenesis and preserve a primary isotopic signature due to its low porosity (Wang and Cerling, 1994); (2) there is a significant difference between the $\delta^{13}\text{C}$ values of C3 and those of C4 plants, and there is a consistent ~ 14 ‰ enrichment in the tooth enamel $\delta^{13}\text{C}$ values of large mammals relative to their diets (Cerling et al., 1997); and (3) tooth enamel $\delta^{18}\text{O}$ compositions of large mammals primarily reflect local meteoric water $\delta^{18}\text{O}$ compositions, which in turn depend on local and global climate (Kohn et al., 1996; Wang et al., 2008b). In addition to mammalian tooth enamel, pedogenic carbonate in paleosols and organic compounds extracted from sediments or sedimentary rocks have been used to reconstruct paleovegetations and paleoclimates.

Based on the analyses of carbon isotopes in pedogenic carbonate, tooth enamel, or specific organic compounds, the expansion of C4

* Corresponding author. Tel.: +1 850 645-6698, +1 850 443 2889 (Mobile); fax: +1 850 644 0827.

E-mail address: cz05d@fsu.edu (C. Zhang).

grasses has been widely documented around the world, e.g., in Asia (Quade et al., 1989; Cerling et al., 1993; Morgan et al., 1994; Cerling et al., 1997; Deng et al., 2002; Wang et al., 2004; An et al., 2005; Biasatti et al., 2005; Gaboardi et al., 2005; Wang and Deng, 2005; Hou et al., 2006; Wang et al., 2006; Huang et al., 2007; Passey et al., 2009), Africa (Cerling et al., 1997; Boissarie et al., 2005; Segalen et al., 2006; Kingston and Harrison, 2007), and the Americas (Cerling et al., 1993; Wang et al., 1993; MacFadden and Cerling, 1994; MacFadden et al., 1994; Wang et al., 1994; MacFadden and Cerling, 1996; MacFadden et al., 1996; Cerling et al., 1997; Latorre et al., 1997; Cerling et al., 1998; MacFadden et al., 1999; Huang et al., 2001; Fox and Koch, 2003; Fox and Fisher, 2004; Fox and Koch, 2004; Koch et al., 2004). However, the timing and driving mechanism of C4 expansion remain contentious issues.

Regarding the timing of C4 expansion, Cerling et al. (1997) concluded that it began in the late Miocene (8–6 Myr ago), earlier at lower latitudes and later at higher latitudes as the CO₂ threshold for C3 photosynthesis is higher at warmer temperatures, and that significant C4 biomass has since persisted to the present day. However, others suggested that C4 biomass was already a significant component of the local ecosystems much earlier than 8 Myr ago mainly based on $\delta^{13}\text{C}$ values of paleosol carbonates (e.g., Morgan et al., 1994; Fox and Koch, 2003, 2004), although the suitability of some of the carbonate samples used in these latter studies for vegetation reconstruction was called into question (Wang and Deng, 2005).

As to the driving mechanism, Cerling et al. (1997) proposed the CO₂ hypothesis, which states that the “abrupt and widespread increase in C4 biomass may be related to a decrease in atmospheric CO₂ concentrations below a threshold that favored C3-photosynthesizing plants” in the late Miocene. And the threshold they suggested was about 500 p.p.m.v. However, others suggested that atmospheric partial pressure of CO₂ ($p\text{CO}_2$) had been low, similar to modern levels, since the early Miocene, and that there were no significant drops in atmospheric CO₂ concentrations during the late Miocene based on proxy analyses (e.g., Pagani et al., 1999’s alkenone-based $p\text{CO}_2$ estimates, and Pearson and Palmer, 2000’s estimates based on boron-isotope ratios of ancient planktonic foraminifer shells). Consequently, other mechanisms for C4 expansion were suggested, for example, climate/hydrological change (Pagani et al., 1999; Huang et al., 2001; Wang and Deng, 2005; Huang et al., 2007).

Here we present stable carbon and oxygen isotope data of fossil and modern mammalian tooth enamel from the central Inner Mongolia, including bulk samples from 119 teeth and 144 serial samples from 7 teeth. The samples range in age from the late Oligocene (~26 Ma) to the present. Our objective of this study is to compare the paleodiet (which reflects paleovegetation) and paleoclimate record from the central Inner Mongolia area with well-established records from other parts of the world to improve our understanding of the natural variability of the Earth’s climate system and the development of C4 ecosystems.

2. Geographic and geological settings

The study area (latitude: ~41.5–44.0°N, longitude: ~112.0–116.0°E) is located in the central Inner Mongolia (Fig. 1), which is a low plateau with an elevation of slightly over 1000 m. The climate of this area is temperate continental, with an annual mean temperature range of ~–1 to 10 °C (~–23 to –10 °C in January, and ~17 to 26 °C in July). The mean annual precipitation is ~50 to 450 mm, decreasing largely from southeast to northwest, with the rains concentrating in the summer months. The sample sites lie in a roughly triangular area, with the cities Erenhot, Zhangjiakou, and Xilinhot at the apices. No samples were collected from the eastern central part of this triangle, which is occupied by the Hunshandake sand dunes.

The deposits in the study area are mainly composed of fluvial and lacustrine sediments, and their age assignments were based on a combination of faunal assemblages, paleomagnetic studies, and one

⁴⁰K–⁴⁰Ar age of 7.1 ± 0.5 Ma of the basalt capping the Baogedawula Formation (Fig. 2). The fossils were assigned ages based on the estimated ages of the strata in which they occurred in the stratigraphic column recently updated by Wang et al. (2009). The uncertainties in the fossil age estimates are less than 0.5 Ma for most fossil localities, except for Tsagan Nor, Aershan, Tairum Nor, and Yanchi where age uncertainties could be as large as 1–3 Ma. Thus, two groups of fossils from different localities having the same numerical age may not have actually been synchronous, which might confound the interpretation or comparison of the corresponding data.

3. Sampling and analytical methods

We selected 119 well-preserved fossil and modern teeth for stable carbon and oxygen isotope analyses from a diverse group of herbivorous mammals including rhinos (*Paraceratherium*, *Hispanotherium*, *Acerorhinus*, *Dicerorhinus*, and *Chilotherium*), elephants (*Anacus*, *Mastodon*, *Gomphotherium*, and *Platybelodon*), horses (*Anchitherium* and *Hipparion*), bovid (*Turcoceros*), deer (*Stephanocemas*), giraffe (*Palaeotragus*), modern horse (*Equus*), and modern cow (*Bos*). In addition, we collected modern plants in the area for stable carbon isotope analysis.

Bulk samples were prepared in either of the following two ways: (1) a piece of tooth was cut along the growth axis, the enamel was separated from dentine and other matrix with a Dremel tool, and then the clean enamel was ground into fine powder using a mortar and pestle; or (2) dentine and/or other material were removed from the surface of tooth enamel using a Dremel tool, and then fine powder was drilled/milled from the cleaned enamel surface more or less evenly along the entire growth axis to make the sample more representative of the entire tooth.

Seven teeth were serially-sampled by drilling the clean enamel surface in bands perpendicular to the growth axis. Two sets of the serial samples (IMMH and IMM2) were obtained from the same modern horse tooth from Sonid Zuoqi, but were sampled on different sides of the tooth at different densities.

The sample powder was then soaked in 5% sodium hypochlorite (NaOCl) for at least 12 h to remove organic matter. The solution was decanted after centrifugation, and the remaining powder was rinsed with distilled water at least three times. The powder was then treated with 1 M acetic acid for at least 12 h to remove non-structural carbonate, cleaned with distilled water via centrifugation at least three times, and finally freeze-dried.

Then ~2–3 mg of the enamel powder were weighed into a reaction vial (one sample per vial). Two or three sets of standards were also weighed into different vials, each containing ~100–300 µg of a selected standard. Both the samples and the standards were baked for a few hours in a drying oven set to ~71 °C to remove moisture absorbed onto the powder. Then caps and septa were screwed on the vials immediately. After flushing the vials with pure He, 100% H₃PO₄ acid was manually injected into the vials, making sure that all the powder was covered by H₃PO₄ acid. The vials were left in the sample block (25.0 ± 0.1 °C) or in a thermal bath (25.0 ± 0.5 °C) for at least 72 h. The CO₂ generated was then analyzed by a continuous flow Finnigan Delta Plus XP stable isotope ratio mass spectrometer (IRMS) at the Florida State University (FSU). Plant samples were freeze-dried and ground into powder or cut into very small pieces. Their stable carbon isotopic compositions were measured using a Carlo Erba Elemental Analyzer interfaced to the IRMS at FSU.

The analytical results are reported in the standard notation as $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$: $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ (in ‰), where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{18}\text{O}/{}^{16}\text{O}$, and the reference standard is the international carbonate standard VPDB (Vienna Pee Dee Belemnite). Based on replicate analyses of the carbonate standard NBS-19 and our lab standards MERK, Roy-cc, and YW-cc-st or PDA-CaCO₃, which were all processed with each batch of samples, the analytical precision for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ is better than ± 0.1‰.

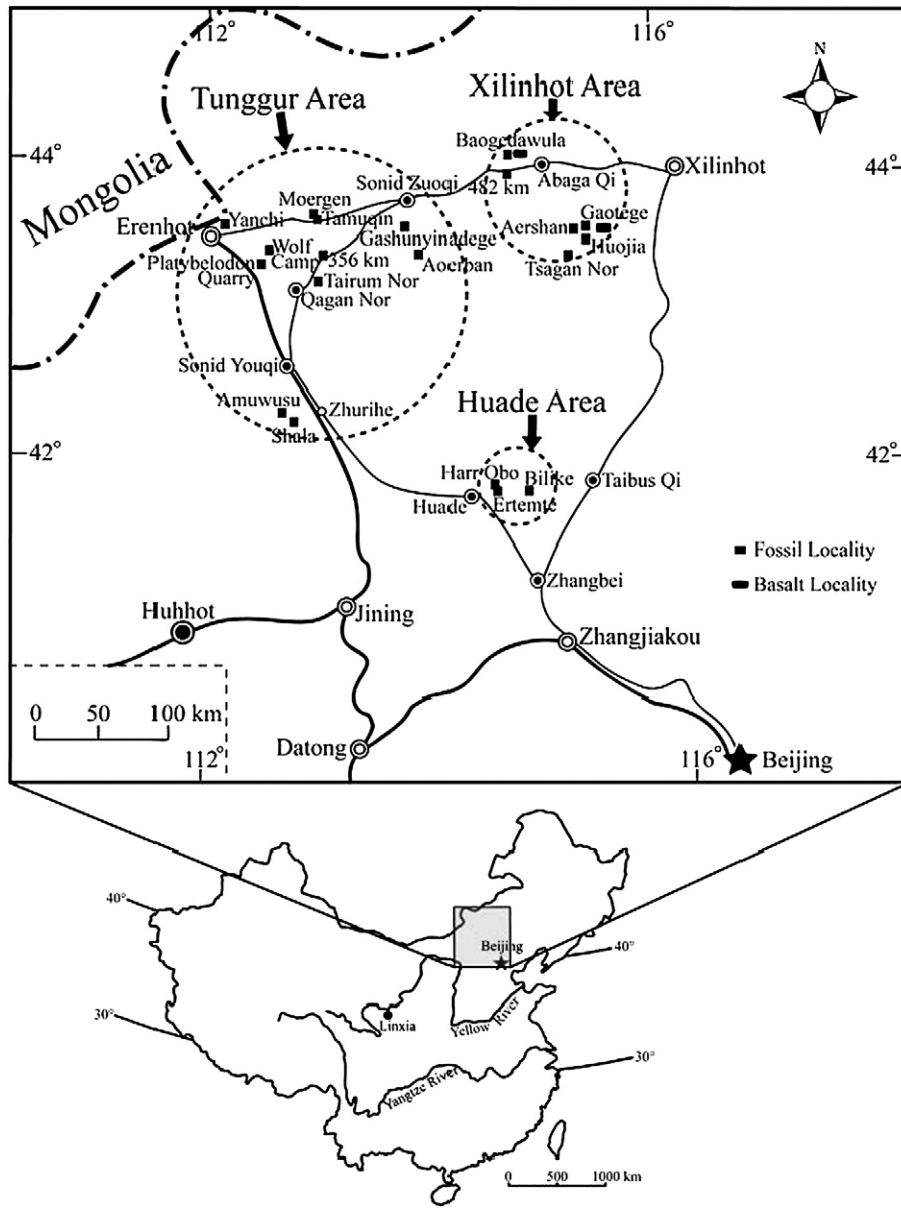


Fig. 1. Sketch map showing the fossil localities in the central Inner Mongolia area. For convenience of discussion, the localities were grouped into Tunggur Area in the northwest, Xilinhot Area in the north, and Huade Area in the south (adapted from Wang and Deng, 2005 and Qiu et al., 2006).

4. Results and discussions

4.1. Carbon isotopes, paleodiet, and paleoecology

The $\delta^{13}C$ values of tooth enamel reflect the $\delta^{13}C$ composition of the diet. For modern herbivores, an enamel- $\delta^{13}C$ value of -9% or less in general indicates a pure C3 diet, whereas enamel $\delta^{13}C$ values of -1% or higher indicate a pure C4 diet. In water-stressed environments, the conservative 'cut-off' value for a pure C3 diet is -8% for modern samples and -7% (or even higher) for fossils (Cerling et al., 1997; Wang et al., 2008a). Enamel samples from fossil herbivores in the central Inner Mongolia display significant temporal variations in $\delta^{13}C$ values reflecting changes in diets and habitats (Fig. 3 and Supplementary Table 1).

From 26 Ma to ~ 8.5 Ma, the $\delta^{13}C$ values of enamel range from -7.6% to -13.0% (Fig. 3), with a mean of $-9.7 \pm 1.2\%$ ($n=91$, all means reported ± 1 standard deviation). These $\delta^{13}C$ values indicate that herbivores in the central Inner Mongolia were feeding predominantly on C3 plants from the late Oligocene to early late Miocene

(Fig. 3a). Only three enamel samples have $\delta^{13}C$ values slightly higher than -8.0% : -7.6% and -7.8% for two 13 Ma rhino fossils from the Platybelodon Quarry of the Tunggur Area, and -7.6% for an 8.5 Ma suspected rhino from Tsagan Nor of the Xilinhot Area (Figs. 1 and 3). These slightly higher $\delta^{13}C$ values are consistent with water-stressed C3 diets predicted for late Miocene herbivores (Fig. 3a, Passey et al., 2002, 2009), and thus likely indicate consumption of C3 plants experiencing water stress, suggesting that water-stressed conditions may have existed in the Tunggur and Xilinhot Areas prior to ~ 8.5 Ma. Alternatively, these slightly higher $\delta^{13}C$ values could indicate ingestion of a small amount of C4 plants. However, the lack of significant seasonal $\delta^{13}C$ variations within individual teeth (Fig. 4e and f) from this time period renders this latter possibility very low (Wang et al., 2008a). The pure or nearly pure C3 diets for this time period suggest that there was no significant C4 biomass in the central Inner Mongolia ecosystems before ~ 8.5 Ma. This is consistent with observations in other parts of the world (Cerling et al., 1997). The $\delta^{13}C$ variations among species suggest mixed habitats likely including forests (more negative $\delta^{13}C$ values) and C3 grasslands (less negative $\delta^{13}C$ values). At

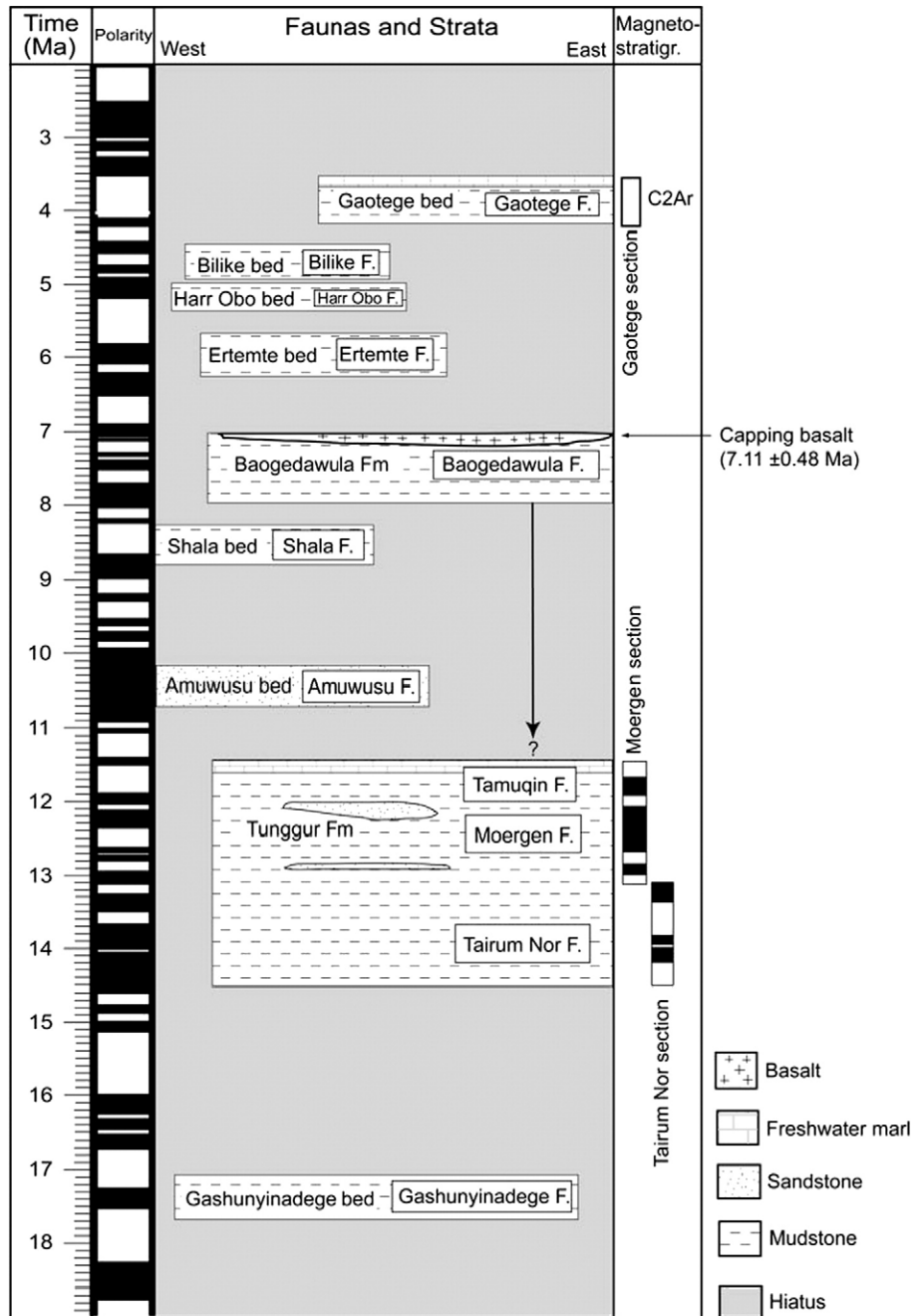


Fig. 2. Stratigraphic column showing the strata from which the fossils for this study were discovered (modified from Qiu et al., 2006 and Wang et al., 2009).

~12.5 Ma, there were two sets of samples: one from the locality Milestone 482 km (43.94°N, 114.50°E) in the Xilinhot Area and another from the Wolf Camp (43.55°N, 112.66°E) in the Tunggur Area (Fig. 1). A comparison of the $\delta^{13}\text{C}$ values of these two sets of samples reveals that the Wolf Camp samples have a mean $\delta^{13}\text{C}$ value of $-9.4 \pm 0.6\%$, significantly higher than that of the Milestone 482 km samples (Table 1), suggesting that the Wolf Camp was probably more arid than the Milestone 482 km locality. This is consistent with oxygen isotope data, which show that samples from the Wolf Camp have generally higher $\delta^{18}\text{O}$ values than those from Milestone 482 km, although the difference in the mean $\delta^{18}\text{O}$ values is not statistically significant (Table 1). Therefore, an east–west moisture gradient, similar to (but weaker than) that observed in the region today, may have existed in the area at that time. As mentioned in Section 2 above, however, the uncertainties in the age estimates might render this comparison insignificant, because these two groups of fossils may not have exactly the same age.

In contrast to the previous period, enamel samples from ~7.5 Ma to ~3.9 Ma display a larger range of $\delta^{13}\text{C}$ variation (-2.4% to -12.6%). The mean enamel $\delta^{13}\text{C}$ value is $-8.6 \pm 2.7\%$, significantly higher (Table 1) than that of the preceding period. These $\delta^{13}\text{C}$ values indicate that herbivores had a variable diet ranging from pure C3 to mixed C3–C4 vegetation (with C4 plants comprising up to ~76% of their diet) during the late Miocene and early Pliocene. The presence of a significant C4 dietary component was detected in about half of the fossil samples from two stratigraphic levels at ~7.5 Ma (in Baogedawula) and ~3.9 Ma (in Gaotege) in the Xilinhot Area (Fig. 1). This suggests that C4 plants were an important component of the local ecosystems around 7.5 Ma and 3.9 Ma. Intriguingly, samples from two other Miocene–Pliocene fossil localities in the Huade Area in the south (i.e., Ertemte, ~6.0 Ma, 6 samples; and Bilike, ~4.7 Ma, 4 samples), yielded $\delta^{13}\text{C}$ values indicating a pure C3 diet (Figs. 1 and 3b). Because of the small sample size and the lack of samples of other ages at these southern localities as

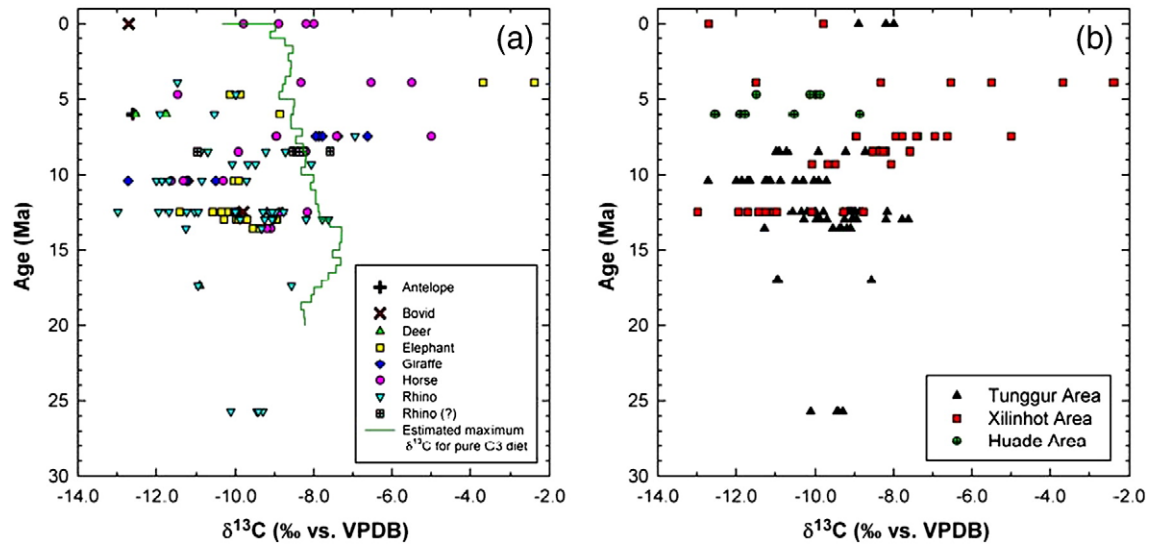


Fig. 3. Temporal variations in the $\delta^{13}\text{C}$ of bulk enamel samples from the central Inner Mongolia area: (a) arranged by taxa, and (b) arranged by grouped sites. Also shown in (a) are estimated maximum $\delta^{13}\text{C}$ values for both fossil and modern mammals that fed on C3 plants experiencing high water stress (dark green line) from Passey et al. (2002, 2009). Note that the maximum $\delta^{13}\text{C}$ value of -10.3% estimated for modern mammals by Passey et al. (2002, 2009) is too low for arid environments (Cerling et al., 1997; Wang et al., 2008b), including the central Inner Mongolia area. Therefore, an enamel- $\delta^{13}\text{C}$ of -8% was used as a conservative “cut-off” value for C3 diet for modern animals (see text for more explanation).

well as the lack of contemporary samples from other fossil localities in the Xilinhot or Tunggur Areas for comparison, we cannot determine whether the pure C3 diet indicates a retreat of C4 vegetation from the central Inner Mongolia or inadequate sampling of the populations (Fig. 3b; Supplementary Table 1). Considering that these southern fossil localities are close to the present-day boundary between temperate deciduous forest and temperate steppe, it is also possible that the southern part of the central Inner Mongolia (i.e., Huade Area) was forested without any C4 plants in the latest Miocene and Pliocene due to wetter conditions in a warmer climate. More samples are needed to resolve this issue.

Our study area is currently located in the temperate steppe vegetation zone (Ren and Beug, 2002). In contrast to the late Miocene and early Pliocene, the present-day ecosystems in the area are dominated by C3 vegetation, which is confirmed by carbon isotope analysis of plant samples collected during a recent field trip to the study area (Supplementary Table 3). Our plant survey shows that only one out of 21 species collected is a C4 plant and it is a minor component of the local ecosystem (accounting for insignificant amount of the local biomass). Although C4 plants (both monocots and dicots) have also been found in the vicinities of our study area (e.g., to the east or northeast of our study area: Redmann et al., 1995; Tang et al., 1999; Chen et al., 2007; and to the northwest: Pyankov et al., 2000), they are not a significant component of the biomass. Bulk enamel $\delta^{13}\text{C}$ values from four modern horses (three of which were calculated from serial samples) and one modern cow ranged from -8.0 to -12.7% (Supplementary Table 1), indicating a C3-based diet for these modern herbivores, consistent with the present C3-dominance in the area. The slightly enriched $\delta^{13}\text{C}$ values ($> -9\%$) could be due to an intake of a small amount of C4 plants, but the lack of significant intra-tooth $\delta^{13}\text{C}$ variations within individual teeth (Fig. 4a and b; Supplementary Table 2) indicates that these slightly enriched $\delta^{13}\text{C}$ values are most likely due to ingestion of C3 plants experiencing water stress and perhaps some CAM plants. Thus, our data suggest that significant ecological changes occurred in the central Inner Mongolia at about 7.5 Ma when C4 plants expanded into the area and also after 3.9 Ma when C4 plants either disappeared or became insignificant in the local ecosystems. However, we do not have any samples from the time interval between ~ 3.9 Ma and the present to determine the exact timing of the latter ecological change.

Based on the analysis of phytolith and pollen preserved in a paleosol profile, Huang et al. (2005) suggested that C4 plants (e.g., *Aneurolepidium chinese-Stipa grandis*) expanded or retreated on a timescale of thousands of years during the Holocene in the Taipusi Banner area (Taibus Qi in Fig. 1) due to changes in temperature and/or monsoon strength (which relates to precipitation and aridity). Although this finding suggests that it is entirely possible that C4 plants have advanced and retreated many times in our study area, the low time resolution of the available fossil mammalian record precludes any meaningful discussion of such shorter time scale ecological variations.

We also analyzed serial samples from seven individual teeth, but only six of them are plotted in Fig. 4, because one tooth (sample IMM-2) from Aoerban ($43^{\circ}20'21.6''\text{N}$, $113^{\circ}54'48.6''\text{E}$) only yielded four serial enamel samples. Unfortunately we did not have enough tooth material left from the time intervals that show the C4 signals to perform serial analyses, and not surprisingly, there is not much variation in the $\delta^{13}\text{C}$ values of these serial samples. Nevertheless, the $\delta^{13}\text{C}$ values clearly show some trends: (1) In the Tunggur Area, the $\delta^{13}\text{C}$ values of a modern horse from Sonid Zuoqi ($\sim -8\%$, Fig. 4a) and those from Aoerban ($\sim -8\%$, Table 1) are $\sim 2\%$ higher than those of a fossil elephant from Tairum Nor (13.6 Ma, Fig. 4f); (2) In the Xilinhot Area, the $\delta^{13}\text{C}$ values of a modern horse from Baogedawula ($\sim -10\%$, Fig. 4b) are also $\sim 2\%$ higher than those of a rhino from Milestone 482 km (12.5 Ma, Fig. 4e); and (3) the $\delta^{13}\text{C}$ values of modern horses from the Tunggur Area are $\sim 2\%$ higher than those of a modern horse from the Xilinhot Area (Fig. 4a and b). These trends suggest that the modern C3 plants, especially those from the Tunggur Area, are experiencing more severe water stress than before, and that the modern C3 plants from the Tunggur Area are experiencing more arid conditions than those from the Xilinhot Area. The much larger ranges of $\delta^{18}\text{O}$ values (8.9‰ for the horse from Sonid Zuoqi and 9.0‰ for that from Baogedawula) may indicate that the modern day environment is much more arid than before; and the generally higher $\delta^{18}\text{O}$ values of the modern horse from the Tunggur Area than those from the Xilinhot Area indicate that the Tunggur Area is more arid than the Xilinhot Area. Both are consistent with our previous inference based on the $\delta^{13}\text{C}$ values. An opposite trend however, is observed in the Huade Area, where the $\delta^{13}\text{C}$ values of a horse from Bilike (4.7 Ma, Fig. 4c) are lower than those of an elephant from Ertemte (6.0 Ma, Fig. 4d), perhaps indicating that it had become more humid from ~ 6.0 Ma to ~ 4.7 Ma.

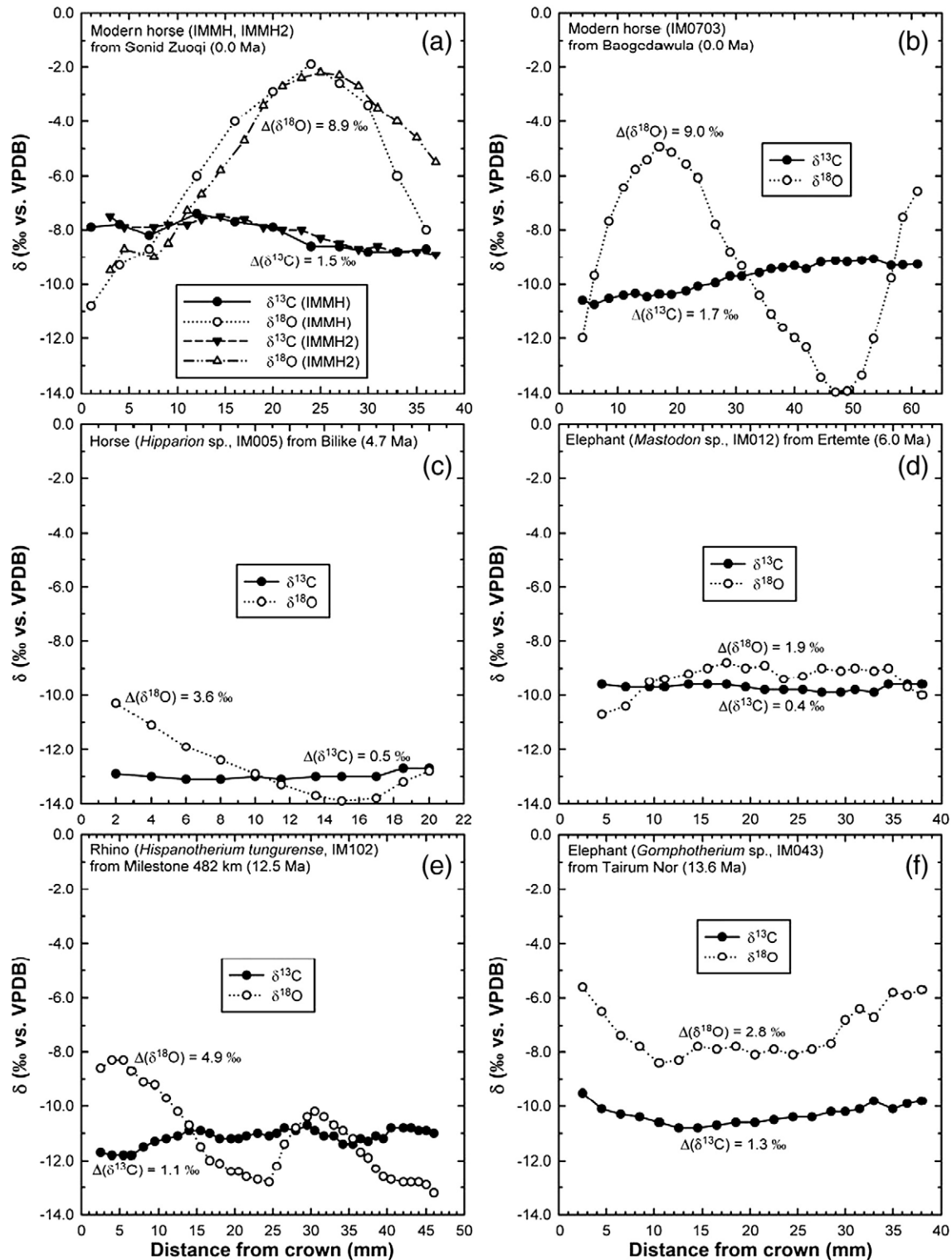


Fig. 4. Intra-tooth $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variations in modern and fossil herbivore teeth from the central Inner Mongolia area. IMM1 and IMM2 are two sets of serial samples taken from the same horse tooth but on opposing sides of the tooth with different sampling densities.

4.2. Comparison with records from other regions

Our tooth enamel C isotope data show that the first appearance of significant C4 component in the diets of herbivores in the central Inner Mongolia occurred in the late Miocene, at about 7.5 Ma, which is synchronous with the C3–C4 dietary change observed in East Africa, Indian subcontinent, and the Americas (Cerling et al., 1997). This provides support for a global factor as the driving mechanism for the late

Miocene C4 expansion (Cerling et al., 1997). However, the $\delta^{13}\text{C}$ record from the central Inner Mongolia also displays striking differences from the other records.

First, the expansion of C4 plants in the central Inner Mongolia appears to be earlier than that observed at similar latitudes in North America (Cerling et al., 1997; Passey et al., 2002). A possible explanation is that the central Inner Mongolia was warmer than similar latitudes in the central North America in the late Miocene and early

Table 1
Student *t* test for unpaired data with unequal variance.

	Mean difference (%)	df	<i>t</i>	<i>p</i>	Significant difference at 95%?
$\delta^{13}\text{C}$ (482 km) vs. $\delta^{13}\text{C}$ (Wolf Camp) (~12.5 Ma)	-1.6	11	-3.8062	<0.003	Yes
$\delta^{18}\text{O}$ (482 km) vs. $\delta^{18}\text{O}$ (Wolf Camp) (~12.5 Ma)	-0.9	24	-1.5784	0.1273	No
$\delta^{13}\text{C}$ (~7.5 to ~3.9 Ma) vs. $\delta^{13}\text{C}$ (26 to ~8.5 Ma)	1.2	27	2.1860	0.0374	Yes
$\delta^{18}\text{O}$ (~7.5 to ~3.9 Ma) vs. $\delta^{18}\text{O}$ (26 to ~8.5 Ma)	-0.7	70	-1.8595	0.0671	No

Pliocene due to its proximity to the oceans. Sufficient growing season precipitation and warmer temperatures (in addition to low atmospheric $p\text{CO}_2$) would have provided favorable conditions for the late Miocene expansion of C4 plants in the central Inner Mongolia.

Secondly, in many regions around the world where C4 expansion occurred, significant C4 biomass has persisted to the present day (Cerling et al., 1997) despite variations in their abundance in response to changes in temperature and/or precipitation on millennium or glacial–interglacial time scales (e.g., Huang et al., 2001; Gabori et al., 2005). In contrast, C4 grasses expanded into the central Inner Mongolia during the latest Miocene and Pliocene but have since become insignificant in local ecosystems, although the exact timing of C4 retreat from this area has yet to be determined. Furthermore, the C4 expansion in the central Inner Mongolia also occurred much earlier than that in the Linxia Basin and that in the Chinese Loess Plateau (CLP), both of which are located to the southwest of our study area. In the CLP (~400 to 500 km SW of our study area), C4 first expanded at ~2.9–2.7 Ma (An et al., 2005). Farther west in the Linxia Basin, C4 grasses became an important component of local ecosystems only after ~2.5 Ma (Wang and Deng, 2005). Passey et al. (2009) recently reported that C4 vegetation has been present since the latest Miocene in Baode and Yushe on the eastern edge of the CLP, south of our study area. This east–west time transgression in C4 expansion provides supporting evidence for the hypothesis of Wang and Deng (2005) regarding the role of the East Asian monsoon system in controlling the development of C4 ecosystems in northern China. Our data suggest

that, in agreement with Wang and Deng (2005), perhaps the Tibetan Plateau had not reached its present-day elevations across its vast extent until more recent time than generally thought (e.g., Rowley and Garzone, 2007; Royden et al., 2008). Consequently, the East Asian summer monsoon was not strong enough during much of the Neogene to affect the CLP, which is over 1500 km away from a moisture source, let alone the Linxia Basin, which is farther west of the CLP by a few hundred kilometers. However, with the further significant growth/uplift of the Tibetan Plateau, the East Asian summer monsoon was strengthened and the dominant wind direction in the summer shifted slightly toward the CLP and away from the central Inner Mongolia area, thus diverting some moisture away from the central Inner Mongolia and to the CLP and later also to the Linxia Basin. This resulted in the retreat of C4 plants from the central Inner Mongolia area due to a decrease in temperature, and the subsequent expansion of C4 plants in the CLP and later the Linxia Basin, due to increased seasonality with precipitation concentrating in summer months as it is today. This hypothesis appears to be supported by climate models. For example, an earlier model simulation revealed a significant drop in temperature of 5 to 6 °C in both July and January in Northeast Asia (including our study area in Inner Mongolia) from no mountain to mountain cases, with a simultaneous but statistically insignificant increase in precipitation (Kutzbach et al., 1993). A recent simulation study with higher resolution models showed a systematic change in the precipitation pattern on the Asian continent with the uplift of the Tibetan Plateau: in summertime, precipitation area moves inland, and in our study area the June precipitable water decreases in both the CGCM (Coupled General Circulation Model) and AGCM (Atmospheric General Circulation Model) simulations (Kitoh, 2004).

In the present-day environment, there were no clear C4 signals detected in the mammalian diets in the central Inner Mongolia area. On the contrary, there are still significant C4 components in the local ecosystems of the CLP (An et al., 2005) and the Linxia Basin (Wang and Deng, 2005). A comparison of 57-year weather data (Fig. 5) reveals that (1) Linxia Basin's mean monthly temperature is above freezing (0 °C) from March to November, two months longer than in the central Inner Mongolia (Erenhot, Xilinhot, and Zhurihe), which is above freezing from April to October; and (2) the monthly mean precipitations were much higher from April to October in the Linxia Basin than those in the central Inner Mongolia area, and the mean cumulative

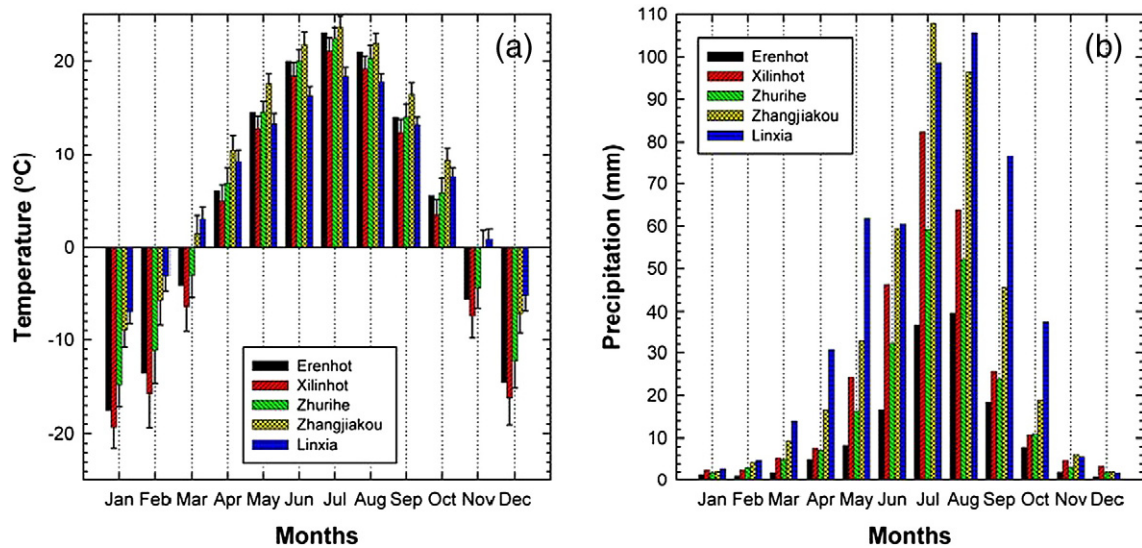


Fig. 5. Modern weather data from the weather stations in the vicinity of the study area (Erenhot, Xilinhot, and Zhurihe), from Zhangjiakou (SE of the study area), and from Linxia (which have C4 plants nowadays) for comparison. It seems that Linxia has more equable temperature (a higher mean annual temperature with higher temperatures in winter and lower temperatures in summer), longer growing season (2 more months), and more precipitation during the growing season than the study area. The data of Xilinhot, Zhurihe, Zhangjiakou, and Linxia are from: <http://bcc.cma.gov.cn/Website/index.php?ChannelID=43&WCHID=5>, and the data plotted are the mean values from 1951 to 2007. The data for Erenhot are from: <http://www.wunderground.com/NORMS/DisplayIntNORMS.asp?CityCode=53068&Units=both>. Error bars represent one standard deviation (1σ).

precipitation from April to October from the year 1951 to 2007 was also much higher in the Linxia Basin than that in the central Inner Mongolia (Linxia: 471 mm; Xilinhot: 261 mm; Zhurihe: 202 mm; and Erenhot: 132 mm. The data for Erenhot are from a different data source, <http://www.wunderground.com/NORMS/DisplayIntNORMS.asp?CityCode=53068&Units=both>, which only lists the averages). A combination of a longer growing season, more growing season precipitation and a higher mean annual temperature is probably why C4 plants are still a significant ecosystem component in the Linxia Basin now but not the case in the central Inner Mongolia area. Our data suggest that the present-day differences in precipitation and temperature regimes between the central Inner Mongolia and the CLP and the Linxia Basin were established after the early Pliocene, and were probably linked to the evolution of the Tibetan Plateau. A reduction in moisture in the central Inner Mongolia is also supported by both C and O isotope data of the serial samples, which suggest that the modern day environment is more arid than before.

5. Conclusions

Stable carbon and oxygen isotopic analyses of herbivorous mammalian tooth enamel from the central Inner Mongolia area reveal significant changes in vegetation and climate since the late Oligocene (~26 Ma). Prior to ~8 Ma, the diets of various herbivores were composed of pure or nearly pure C3 plants, suggesting the local ecosystems were dominated by C3 plants. From ~7.5 to ~3.9 Ma, there were significant C4 plants in the diets of a variety of herbivorous mammals (up to ~76% of C4 plants), implying that C4 plants were a significant component of the local ecosystems. However, the modern day ecosystem in our study area is dominated by C3 grasses. The expansion of C4 grasses in the late Miocene and Pliocene and the subsequent retreat of C4 plants from this area appear to be driven by a combination of global and regional factors. The synchronism of C4 expansion in the central Inner Mongolia and in many other places around the world at about 7–8 Ma provides support for a global factor in causing this late Miocene ecological change (Cerling et al., 1997); whereas subsequent C4 retreat from the central Inner Mongolia suggests that changes in regional temperature and precipitation regimes associated with the growth of the Tibetan Plateau may have played a major role in the evolution of C4-containing ecosystems in northern China. In comparison with the timing of C4 expansions in the CLP and the Linxia Basin, we suggest that a more recent phase of surface uplift or growth of the Tibetan Plateau during the Plio-Pleistocene (An et al., 2001) further strengthened the East Asian monsoon system, increased seasonality in northern China, and diverted some of the summer moisture to the CLP and later to the Linxia Basin away from the central Inner Mongolia area. These changes, in conjunction with a reduction in temperature in the area as suggested by climate model simulations, were responsible for the retreat of C4 grasses from the central Inner Mongolia area after perhaps the early Pliocene and for the expansion of C4 plants in the CLP and later the Linxia Basin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.epsl.2009.08.025](https://doi.org/10.1016/j.epsl.2009.08.025).

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