

Organic carbon isotope and molecular fossil records of vegetation evolution in central Loess Plateau since 450 kyr

ZHOU Bin^{1*}, WALI Guzalnur¹, Francien PETERSE² & Michael I BIRD³

¹ Key Laboratory of Surficial Geochemistry (Ministry of Education), School of Earth Sciences and Engineering, Nanjing University, Nanjing 210023, China;

² Utrecht University, Department of Earth Sciences, 3584 CD Utrecht, the Netherlands;

³ College of Science, Technology and Engineering and Centre for Tropical Environmental and Sustainability Science James Cook University, Cairns QLD, 4870, Australia

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Abstract Significant uncertainties remain regarding the temporal evolution of natural vegetation during the Quaternary, and drivers of past vegetation change, on the Chinese Loess Plateau (CLP). This study presents analyses of total organic carbon isotopic composition (TOC) and *n*-alkane ratios (C₃₁/C₂₇) from the Lingtai loess-palaeosol sequence on the central CLP over the last 450 kyr. The results demonstrate that the vegetation in this region comprised a mix of C₃ and C₄ plants of herb and woody growth-form. C₃ plants dominated for most of the last 450 kyr, but this did not lead to extensive forest. C₃ woody plants were more abundant in MIS9 (S3 period) and MIS5 (S1 period) during warm and humid climate conditions. Herbs increased in the region since 130 kyr, possibly as a result of increased aridity. On the orbital timescales, there was a reduction of C₃ herbal plants in MIS11 (S4) than in MIS12 (L5), and in Holocene than in the last glacial period. Our isotope and *n*-alkane proxy records are in agreement with *Artemisia* pollen changes in the region, which is/was the dominant species in this area and varying due to different heat and water conditions between glacial and interglacial periods. Though the climate in MIS1 (S0) was similar to that in MIS11 (S4), a significant increase in woody plants during the Holocene suggests the impact of human activities and ecological effects of changes in fire activity.

Keywords Carbon isotopic composition, Leaf wax lipids, C₃/C₄ variations, Late Quaternary vegetation change, Glacial/interglacial cycles

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1. Introduction

Chinese Loess Plateau (CLP) is the cradle of the Chinese nation, and over the past thousands of years, agricultural activities have almost completely destroyed all natural vegetation in the region. Today's vegetation distribution has undoubtedly been influenced by the impact of both nature

and human activities (Guo and Hou, 2010). The variety of vegetation types and the evolution of vegetation over time on the Chinese Loess Plateau is not only an important issue in the field of regional past global change, but is also of practical significance in terms of management of CLP's modern ecological environment, ecosystems and carbon sequestration potential.

Over the past few decades, researchers have studied past vegetation and climate changes throughout different periods from sediments on the Chinese Loess Plateau with various

*Corresponding author (email: zhoubinok@163.com; zhoubinok@nju.edu.cn)

methods, such as soil microstructure (Guo et al., 1993), sporopollen in loess deposits (Liu and Su, 1994; Sun et al., 1997; Wu et al., 2004; Long et al., 2011), phytoliths (Lv and Liu, 1999), and stable carbon isotopes (Ding and Yang, 2000; Gu et al., 2003; Zhang et al., 2003; Vidic and Montanez, 2004; An et al., 2005; Liu et al., 2005; Rao et al., 2006; Chen et al., 2006; Zhou et al., 2009, 2014; Yang et al., 2012). This previous research has generally found that grassland vegetation on the CLP was a mixture of C_3 and C_4 herbaceous plants, with a dominance of C_3 plants since late Quaternary, and at no previous time was the CLP dominantly forested (Gu et al., 2003; Zhang et al., 2003; Zhou et al., 2009; Zhang et al., 2013). However, a number of issues such as how vegetation evolves on different time scales, the drivers of vegetation change on the CLP and the interpretation of alternative vegetation indices, remain to be studied.

Terrestrial plants can be divided into those using C_3 photosynthetic pathway ($\delta^{13}C_{C_3}$ value = -20 – -32‰ , average -27‰) and those using the C_4 pathway ($\delta^{13}C_{C_4}$ value = -9 – -15‰ , average -13‰); terrestrial plants can also be broadly divided by their morphological characteristics into life forms such as trees, shrubs, herbs, etc. Most C_3 plants, including trees, shrubs, and herbs dominate in cold and humid environments. C_4 plants are primarily grasses that prefer warm and dry weather, being better adapted to the strong sunshine, high temperatures and arid environments. Plants deliver carbon inputs to the soil, and as a result, soil organic carbon isotopes can be used to reconstruct the isotopic abundance ratio of C_3 and C_4 throughout geological history (Bird and Grocke, 1997; Liu et al., 2002; Wang et al., 2008), with the caveat that that there is some carbon isotope fractionation when plant residues or litter are buried or decomposed (Chen et al., 2010; Wang et al., 2015). However, the plant carbon isotope may also be affected by climatic factors, and plus, plants with the same photosynthesis but different life forms, such as C_3 trees, shrubs and herbs, may also have considerable differences in terms of carbon isotopes (Keeley and Rundel, 2005; Rao et al., 2006; Zheng and Shanguan, 2007). As a result of these complications, the interpretation of vegetation change based on carbon isotopes should to be verified by other indicators such as conventional palynology and/or analysis of molecular fossils. Palynological methods can efficiently and intuitively obtain the succession information of woody and herbaceous plants in the ancient vegetation community, but the complexity of pollen extraction and issues of the identification of local pollen in loess layers makes the current pollen record with a long sequence very rare.

In recent years, molecular fossils such as n -alkanes (also called lipids, compound leaf waxes) have become a proxy with great potential in the study of vegetation evolution in loess strata (Xie et al., 2002; Liu et al., 2005; Zhang et al., 2006; Wang et al., 2007; Zhang et al., 2004; Liu et al., 2008; Bai et al., 2009; Long et al., 2011). Long-chain

n -alkanes (hereinafter referred to as n -alkanes), mostly produced by terrestrial higher plants, are more stable in the sediment after being buried or stored, and thus can provide a proxy record of original biological and climate information (Eglinton and Hamilton, 1967). Research shows that, in terms of n -alkanes, C_{27} or C_{29} are in the main peak in sediments mainly consisting of woody plants input, while C_{31} or C_{33} are in the main peak in sediments mainly consisting of herbs (Volkman et al., 1981; Cranwell et al., 1987; Zhong et al., 2009). Thus vegetation life forms can be differentiated with the help of different carbon ratio between these compounds, such as C_{31}/C_{27} or $C_{31}/(C_{27}+C_{29}+C_{31})$, which reflect the relative change in woody and herbal plants.

This project presents a combination parameter analysis on the loess-paleosol sequence from the Lingtai section on the Chinese Loess Plateau since the L5 period (450 kyr years ago) in terms of $\delta^{13}C_{TOC}$ and n -alkane content. A comprehensive comparative study on the two types of proxy data from the same period, plus $\delta^{13}C_{P_{yC}}$ records and other indicators, reveals the vegetation evolution and the possible factors influencing the climate and environment in the central Loess Plateau over the late Quaternary.

2. Samples and methodology

The Lingtai loess section is located in the central Loess Plateau ($107^{\circ}39'E$, $35^{\circ}04'N$, Figure 1). The average altitude of the region is 1300–1400 m and the average annual temperature is $8.8^{\circ}C$ with temperatures ranging from -4.9 to $21.2^{\circ}C$. The annual precipitation is 580 mm, which falls mostly from July to September, belonging to a temperate semi-humid climate. The surface soil in the area is cinnamon soil or black soil, supporting the growth of tree species such as *Pinus tabulaeformis*, *Platycladus orientalis*, *Ouer-*

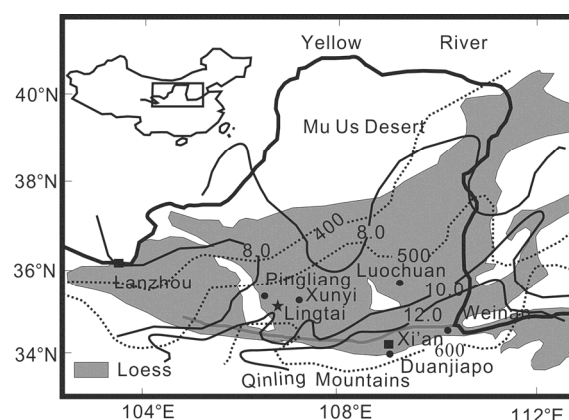


Figure 1 Sketched map of the Chinese Loess Plateau showing the location of the Lingtai loess section and other related sections. The five-pointed star shows the location of the Lingtai loess section, and other solid circles are where related sections discussed in this paper are located. The dashes indicate isohyets and solid lines isotherm. This figure is adapted from Wang and Feng (2014).

cus, *Ulmus*, and *Betula*, as part of a temperate forest steppe environment (Chen and Dong, 1990).

The Lingtai loess section, consists of a thick loess-paleosol-red clay sequence that, because its complete exposure, has been an ideal section for the study of continental dust accumulation and paleoclimate records since late Tertiary (Ding and Yang, 2000; Sun et al., 2010). The full section consists of a 168 m-thick loess-paleosol sequence of Quaternary age, overlying a 120 m thick red clay sequences from the late Tertiary. The 35 m-loess-paleosol sequence since L5 in the Lingtai section forms the focus of our present study. The uppermost 20 cm of the section is a modern cultivation layer, and therefore our sampling started from 50 cm downward at sampling intervals of 50 cm. A total of 155 samples of loess-paleosol samples were collected for this study.

Extraction of total organic carbon (TOC) and analysis $\delta^{13}\text{C}_{\text{TOC}}$: aliquots of each sample were reacted with an excess of hydrochloric acid (10% concentration), heated to accelerate the reaction and to ensure the removal of inorganic carbonates. This was followed by repeated washes until neutral at which point samples were dried at 60°C. TOC was determined by a CE440 elemental analyzer with $\delta^{13}\text{C}_{\text{TOC}}$ measured on the evolved CO_2 by a Thermo-Finnigan MAT-251 mass spectrometer adopting the static burning oxidation method (Shen and Chu, 1997). The result of repeated measurements of samples and reference materials showed that the overall estimate of the experimental error was within $\pm 0.2\%$. We took the average value of two repeated measurements as the value of sample $\delta^{13}\text{C}$.

n-Alkanes determination: about 20 g of freeze-dried sample was ultrasonically extracted (4×40 mL) with $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (3:1, V/V). The supernatant (3000 r/min, 5 min) was collected by separation and centrifugation to produce total extractable lipids (TEL) fraction, which was diluted with methanol and 6% KOH and allowed to stand overnight at room temperature. The non-acid fraction was isolated (4×3 mL) with *n*-hexane; the alkane and alcohol component with silica gel column chromatography. Analysis of this fraction by gas chromatography was conducted directly after alkane concentration and the addition of 30 μL *n*-hexane on a HP-1 capillary chromatographic column (50 m×0.32 mm×0.17 μm , J & W) and flame ionization detectors (FID) Trace GC 2000 gas chromatograph (Finnigan, Thermo Electro). The temperature of inlet and detector was 300°C. The sample was injected without being split, with

helium as the carrier gas and flow rate of 1.2 mL/min with initial temperature 80°C for 1 min ramped to 200°C at the rate of 10°C/min, and then to 270°C at the rate of 5°C/min, then to 300°C at the rate of 2°C/min maintained at 300 for 10 min, and finally ramped to 310°C at 5°C/min and maintain the temperature for 5 min. Pre-treatment and analysis of all samples were conducted in the State Key Laboratory of Loess and Environment (Xi'an) and the State Key Laboratory of Marine Geology (Tongji University).

3. Results and analysis

3.1 The timescale of the Lingtai Loess section

The CLP loess-paleosol sequence belongs to an aeolian pedogenetic sequence, with pedogenesis controlled glacial/interglacial climate cycles through the Quaternary. The climate alternated between cold and dry (glacials) and warm and humid (interglacials), dominated overall by glacials. Magnetic susceptibility is linked to pedogenesis. High magnetic susceptibility indicates a strong summer monsoon and a corresponding warm and humid interglacial period; whereas low magnetic susceptibility value indicates the weakening of the summer monsoon and a corresponding cold and dry glacial period.

The comparison between the magnetic susceptibility obtained from the CLP and the standard marines isotope stages (Imbrie et al., 1984) (Figure 2), allows determination of the depth and age of loess-paleosol boundaries since L5 (which is equivalent to MIS12, at 450 kyr) (Table 1).

3.2 Results of TOC and $\delta^{13}\text{C}_{\text{TOC}}$

TOC and $\delta^{13}\text{C}_{\text{TOC}}$ in the Lingtai loess section are shown in Figure 3b. Since 450 kyr, the average TOC abundance has been 0.20%, ranging from 0.04% to 0.7%. The TOC content of paleosol layers are higher than those in loess, and the trend of TOC over time is similar to magnetic susceptibility, indicating that TOC content is related to the degree of soil development (Figure 3a and b). In the whole sequence, TOC can be clearly divided into two sections: MIS12–MIS6 (L5–L2 period) with relatively low organic content and MIS5 (starting from S1) with relatively high organic content in loess-paleosol.

$\delta^{13}\text{C}_{\text{TOC}}$ varied from -24.1% to -18.1% , with an average of -21.7% . Though $\delta^{13}\text{C}_{\text{TOC}}$ had no significant periodicity,

Table 1 Layer, depth, age and corresponding oxygen isotope of loess-paleosol in Lingtai section

Layer	S0	L1	S1	L2	S2	L3	S3	L4	S4	L5
Depth (m)	1	8.5	11	17.5	21	24	26.5	30.5	32.5	36.5
Age (kyr BP)	10	74	129	192	244	280	336	379.5	425	478
Oxygen isotope	MIS1	MIS2-4	MIS5	MIS6	MIS7	MIS8	MIS9	MIS10	MIS11	MIS12

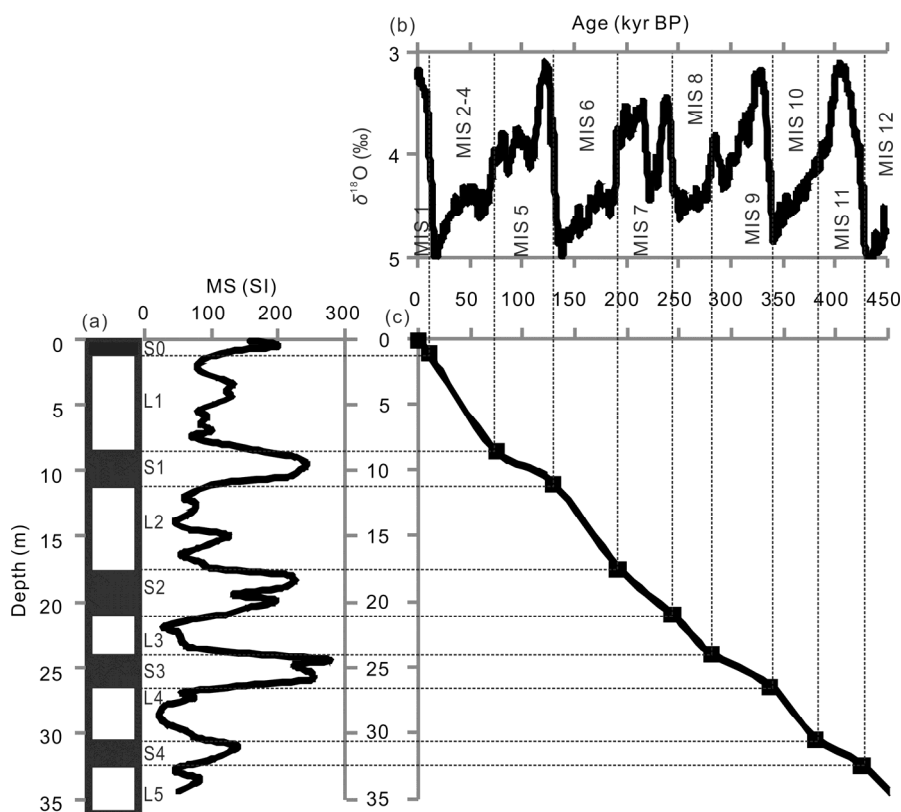


Figure 2 Time series of Lingtai loess section since L5 period. (a) Magnetic susceptibility with depth curve; (b) the standard oxygen isotope SPECMAP curve (Imbrie et al., 1984); (c) depth-age mode based on the comparison of magnetic susceptibility and oxygen isotopes.

some variations did occur between glacial and interglacial cycles. As shown in Figure 4, in MIS11/MIS12 (S4/L5) and MIS1/MIS2–4 (S0/L1), there was a positive $\delta^{13}\text{C}_{\text{TOC}}$ value in the interglacial cycle and a negative value in the glacial cycle; but in MIS9/MIS10 (S3/L4), MIS7/MIS8 (S2/L3), and MIS5/MIS6 (S1/L2), it exhibited the opposite trend, namely lower $\delta^{13}\text{C}_{\text{TOC}}$ values in the interglacials and higher in the glacials.

Based on previous carbon isotope research on modern C_3 and C_4 plants from the CLP (Liu et al., 2002; Wang et al., 2008; Liu et al., 2013), we chose -27.5‰ as the C_3 plant end-member and -12.5‰ for the C_4 plants end-member (Deines, 1980), given there could be at least a 1.57‰ increase in $\delta^{13}\text{C}_{\text{TOC}}$ during the formation and burial of organic in the paleosol (Chen et al., 2010), and an increase of approximately 1.3‰ from plants formed prior to the industrial revolution and modern plants due to the Suess effect (Marino et al., 1992). Thus, the following equation can be used to calculate the relative content of C_4 plants.

$$C_4(\%) = (\delta^{13}\text{C}_{\text{C}_3} + 2.87 - \delta^{13}\text{C}_{\text{TOC}}) / (\delta^{13}\text{C}_{\text{C}_3} - \delta^{13}\text{C}_{\text{C}_4}) \times 100\%, \quad (1)$$

where $\delta^{13}\text{C}_{\text{TOC}}$ is the carbon isotope composition of soil organic matter (‰), $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ represent the average carbon isotope value of modern C_3 and C_4 plants, respectively, and $C_4(\%)$ is the percentage of C_4 plants.

As shown in Figure 3d, the vegetation in this region contained both C_3 and C_4 plants, but was dominated by C_3 plants since 450 kyr; C_4 plants in this region, peaked at about 38% during the L4 period and over part of the Last Glacial, the region was almost occupied by C_3 plants. The average of C_4 plants in the Holocene was 25%, in line with the abundance of modern environment (Liu et al., 2002). On orbital scales, the proportion of C_3 plants declined in MIS1/MIS2–4 and MIS11/MIS12 during interglacials in comparison to that in glacials; in other glacial periods, it reflected opposite rules. In MIS5/6, C_4 plants suddenly increased in early MIS5, but C_3 plants increased slightly in MIS5 compared to MIS6.

3.3 *n*-alkanes parameters and herb/woody plant variations

Terrestrial higher plants contain abundant C27, C29, and C31 *n*-alkanes in epidermal leaf wax, reflecting evident odd-even predominance (OEP). After diagenesis and degradation, the long-chain *n*-alkanes OEP decreases, and therefore carbon preference index (CPI) can indicate the maturity and source of alkanes (Eglinton and Hamilton, 1967). A wide variety of *n*-alkanes is detected in Lingtai loess sediment samples and the carbon distribution is in the range of C15–C33. The long-chain *n*-alkanes have obvious

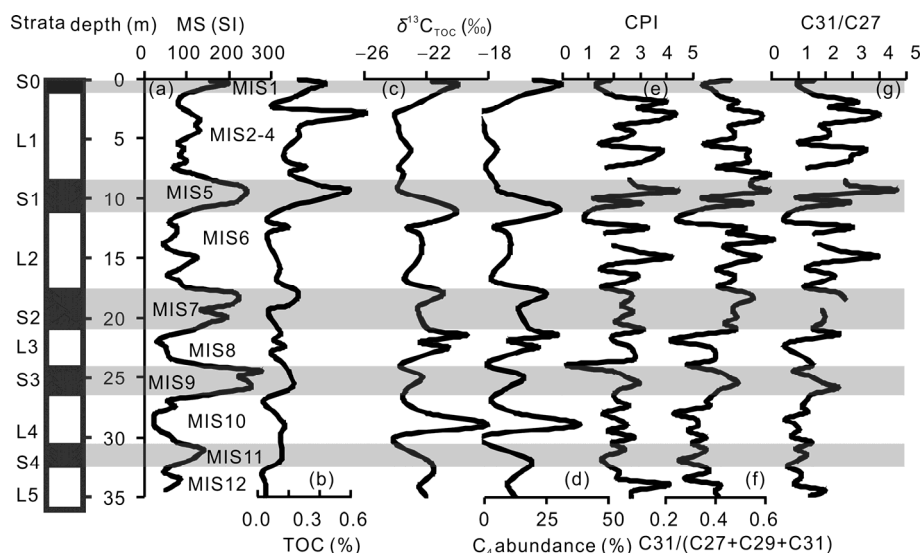


Figure 3 Magnetic susceptibility and variation in vegetation markers in the Lingtai loess section since L5. (a) Magnetic susceptibility curve; (b) total organic carbon (TOC) variation; (c) $\delta^{13}\text{C}_{\text{TOC}}$ variation; (d) percentage variation of C_4 plant obtained from end-member isotope modeling; (e) n -alkanes CPI variation; (f) n -alkane parameter $\text{C}_{31}/(\text{C}_{27}+\text{C}_{29}+\text{C}_{31})$; (g) n -alkane parameter $\text{C}_{31}/\text{C}_{27}$. Gray shaded stripes indicate interglacial periods, which corresponds paleosol layers (filled with black) in the adjacent stratigraphic column.

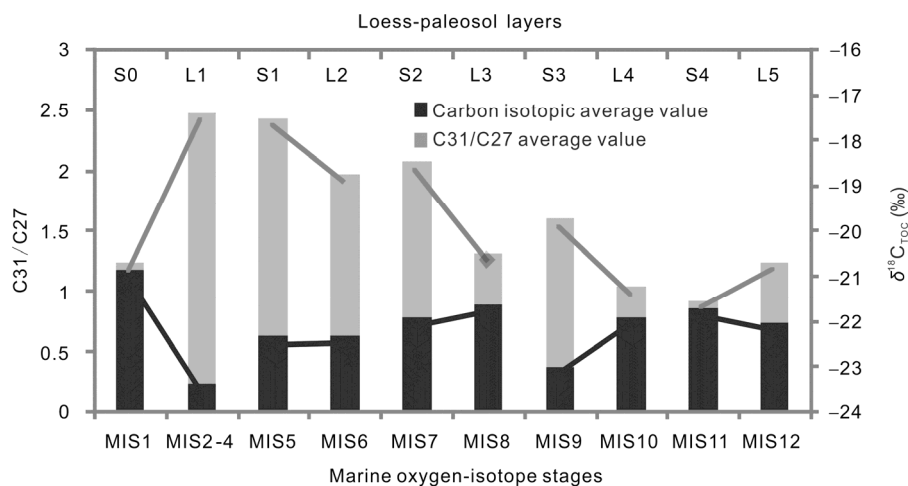


Figure 4 Variation of average value of $\delta^{13}\text{C}_{\text{TOC}}$ and $\text{C}_{31}/\text{C}_{27}$ average value of n -alkanes from Loess-paleosol layers in the Lingtai loess since L5 (corresponding to marine oxygen isotope stage MIS12-MIS1).

OEP, with C29 or C31 as the main peak. The CPI expression is:

$$\text{CPI} = 1/2 \times [(\text{C}_{25} + \text{C}_{27} + \text{C}_{29} + \text{C}_{31}) / (\text{C}_{24} + \text{C}_{26} + \text{C}_{28} + \text{C}_{30}) + (\text{C}_{25} + \text{C}_{27} + \text{C}_{29} + \text{C}_{31}) / (26 + \text{C}_{28} + \text{C}_{30} + \text{C}_{32})]. \quad (2)$$

In this sequence, the CPI average is 2.41, ranging from 0.20 to 4.41, with its lowest in the late S3. As shown in Figure 3e, CPI rose periodically in early S1, about 130 kyr BP but without changes coinciding with glacial/ interglacial cycles. The CPI of the sediments is significantly lower than that of fresh plant tissue, but larger than 1, indicating the long-chain n -alkanes mainly from higher vegetation in this region. The variation of n -alkanes ratios, $\text{C}_{31}/\text{C}_{27}$ and $\text{C}_{31}/(\text{C}_{27} + \text{C}_{29} + \text{C}_{31})$, are consistent with each other and

hence we select the $\text{C}_{31}/\text{C}_{27}$ index for detailed analysis. $\text{C}_{31}/\text{C}_{27}$ changes in the range of 0.47–4.56, with an average of 1.79, when the ratio increases, this is taken to indicate that the proportion of herbs (including grasses) increases.

$\text{C}_{31}/\text{C}_{27}$ shows periodic rise in the 130 kyr BP and greater magnitude of change. As shown in Figure 4, the $\text{C}_{31}/\text{C}_{27}$ value was low in the interglacials and high in the glacials in MIS1/MIS2–4 (S0/L1 period) and MIS11/MIS12 (S4/L5 period); it was high in the glacial cycle and low in the interglacial cycle in MIS5/MIS6 (S1/L2 period), MIS7/MIS8 (S2/L3 period), and MIS9/MIS10 (S3/L4 period). The n -alkanes $\text{C}_{31}/\text{C}_{27}$ and other parameters in the Lingtai loess section indicate a periodic increase of herbal plants in the center of the Chinese Loess Plateau since 130

kyr BP. On orbital time, the C31/C27 indicates that there were more woody plants in the last glacial cycle and MIS11/MIS12 cycle during the interglacials than that during the glacials, and there was an increase of herbs in other cycles during the interglacial cycle than during the glacial cycle.

4. Discussion

4.1 Proxy indicators of vegetation change in the Lingtai section

TOC in the Lingtai section is strongly correlated with magnetic susceptibility which itself indicates the maturity of soil. This suggests that during the interglacial periods when the paleosols developed, the climate was warm and humid, and the landscape supported abundant biomass, a proportion of which was transmuted into the soil as TOC; in contrast, during glacial periods, the climate was cold and dry, biomass of drought-tolerant shrubs was low and TOC was also low (Lin and Liu, 1992). Therefore, TOC, to some degree, can indicate past variations in vegetation and biomass. TOC increased towards the present, and particularly since 130 kyr BP, which is not consistent with the magnetic susceptibility curve. This phenomenon has also been found in other long sequences from the CLP including the Duanjiapo and Heimugou sections, and is due to the gradual degradation of more labile organic carbon over extended periods of time, the remaining carbon being of relatively higher stability (Lin and Liu, 1992; Shi et al., 2011). However, pollen content of shrub and woody plants near the cross-section (Liu

and Su, 1994), as shown in Figure 5g and h, indicates that there has been a systematic change in vegetation and biomass since S1 (130 kyr BP). Pyrogenic carbon (PyC) which is an inert component of the Lingtai loess sediments, as shown in Figure 5c, reflects an increase of biomass and regional climate aridity (Zhou et al., 2007, 2014). Thus, although the degradation of organic matter cannot be ignored as one factor driving the long-term decrease in TOC in the past, vegetation type, biomass, and climate can also lead to periodic elevation of TOC.

Increasing decomposition with time should lead to the TOC that remains having a progressively increased $\delta^{13}\text{C}$ value (Chen et al., 2010; Wang et al., 2008). However there is no evidence of a long-term trend in $\delta^{13}\text{C}_{\text{TOC}}$ in the Lingtai section since 450 kyr, which suggests that the record primarily reflects changes in vegetation, in turn influenced by changes in climate. Since the late Quaternary, the variations in $\delta^{13}\text{C}_{\text{TOC}}$ in the Lingtai section have been mirrored by variations in *n*-alkanes isotopes, but with the latter exhibiting smaller amplitude changes (Peterse et al., 2015),

Comparison of the proportion of C_4 plants obtained from the reconstruction of $\delta^{13}\text{C}_{\text{TOC}}$ and $\delta^{13}\text{C}_{\text{PyC}}$, as shown in Figure 5e, indicates the latter has a relatively large contribution in C_4 biomass, which may be linked, in the semi-arid Chinese Loess Plateau, to surface fire burning relatively more short herbs including C_4 grasses. Therefore, the contribution of woody C_3 plants to $\delta^{13}\text{C}_{\text{PyC}}$ may be underestimated in some certain periods (Zhou et al., 2009). Organic carbon isotopes in the Lingtai section indicates that the vegetation in this region was a mixed type of C_3 and C_4 plants, dominated with C_3 plants; overall, there has been no signif-

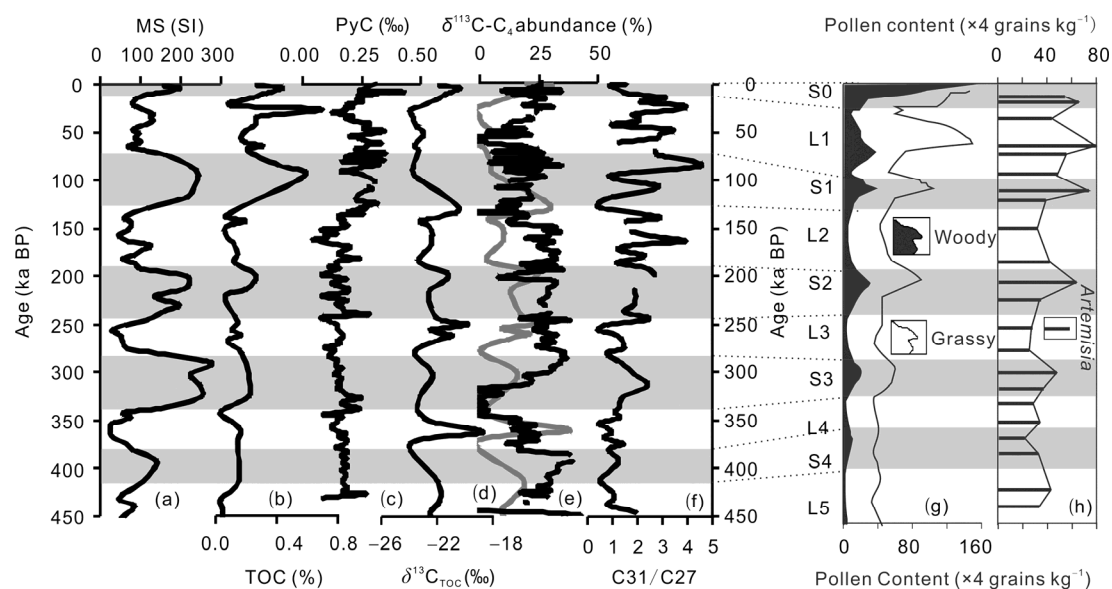


Figure 5 Variation of climate and vegetation proxies in Lingtai loess section since 450 kyr. (a) Magnetic susceptibility curve; (b) cyclic variation of TOC in glacial/interglacial period and increase since 130 kyr BP; (c) amount of PyC was high in the interglacial period and low in the glacial period and exhibits a sustained increase after 130 kyr BP; (d) no overall cyclicality in $\delta^{13}\text{C}_{\text{TOC}}$ but with some cyclic variation in the glacial periods evident; (e) percentage variation of C_4 plants obtained from the reconstruction of $\delta^{13}\text{C}_{\text{TOC}}$ and $\delta^{13}\text{C}_{\text{PyC}}$; (f) *n*-alkanes ratio C31/C27 indicates periodic increase of herbs around 130 kyr BP; (g) variation of counted particles of woody and herb pollen in the Pingliang section (Liu and Su, 1994); (h) variation of counted particles of *Artemisia* in Pingliang section (Liu and Su, 1994).

icant change in terms of the balance between vegetation photosynthesis since 450 kyr BP.

No evident glacial-interglacial cycles for *n*-alkanes's CPI from the sequence indicate a similar source of long-chain *n*-alkanes in loess accumulation and paleosol development periods and the similarity of degradation in these two periods. The variation around 130 kyr further confirms the double impact of microbial degradation and vegetation changes on organic matter (Zhang et al., 2006; Long et al., 2011). The *n*-Alkane parameters C31/C27 and C31/(C27+C29+C31), in the Lingtai loess section indicate an increase of herbs in the middle of the Chinese Loess Plateau at 130 kyr BP. The C31/C27 index shows changes in the relative proportion of woody plants but does not directly quantify the absolute value of herbaceous versus woody plants. It can be inferred that the vegetation in this region was a mixture of woody and herbaceous plants, dominated by herbs over most of the period in accord with pollen data of grass shrub/(grass shrub+needle leaves) results (Wu et al., 2004).

4.2 Variations in vegetation on orbital timescales in the Lingtai section

On an orbital timescale, $\delta^{13}\text{C}_{\text{TOC}}$ was high in MIS1/MIS2-4 and MIS11/MIS12 cycles during the interglacials in comparison to the glacials, which is consistent with the generally inferred increase in C_4 biomass in interglacial periods (Gu et al., 2003; Liu et al., 2002). However, C31/C27 indicates an increase in woody plants in these two cycles during the interglacials compared to the glacials. Most C_4 plants are herbs preferring to warm and dry climates. The increase of C_4 plants indicated by carbon isotopes contradicts the increase of woody plants indicated by *n*-alkanes. In the other three glacial-interglacial cycles, $\delta^{13}\text{C}_{\text{TOC}}$ suggests an increase in C_3 plants in the interglacials compared to the glacials while C31/C27 indicates the increase of herbaceous plants. Most C_3 herbs prefer cold and wet climates and their apparent increase in the interglacial periods also needs further verification.

The variation in *Artemisia* pollen content in this region can reconcile the apparent contradictions between $\delta^{13}\text{C}_{\text{TOC}}$ and C31/C27 during glacial cycles. Research on modern vegetation on the CLP shows that *Artemisia*, belonging to shrubby-herbaceous plant, is a dominant species of local vegetation (Cheng et al., 2011). The pollen record from a loess sequence in Ancun Village, Pingliang, close to Lingtai, indicates that *Artemisia* was abundant in the past times (as shown in Figure 5g and h) (Liu and Su, 1994). In the S0/L1 (MIS1/MIS2-4) and S4/L5 (MIS11/MIS12) cycles, the *Artemisia* pollen was less abundant in the paleosol than in the loess, which is consistent with the higher $\delta^{13}\text{C}_{\text{TOC}}$ value in the corresponding interglacial. And, the fewer herbaceous plants indicated by C31/C27 suggest that the high $\delta^{13}\text{C}_{\text{TOC}}$ value does not mean an increase in C_4 herbaceous plants in interglacial periods.

The pollen spectra in Ancun Village, Pingliang section also indicates an increase of herbaceous pollen in MIS1 and MIS11 compared to the corresponding glacial periods and, the increase of C_3 trees is significant (Liu and Su, 1994). The carbon isotope composition of modern vegetation on the CLP shows that the $\delta^{13}\text{C}$ of C_3 trees and shrubs is higher than C_3 herbs (Zheng and Shangguan, 2007), and thus, the higher $\delta^{13}\text{C}_{\text{TOC}}$ values and a decline in C31/C27 in these two interglacial cycles may be a result of the relative increase in woody plants. In the other three cycles, *Artemisia* pollen content was relatively higher in the paleosols than in loess, which confirms the increase of C_3 in these cycles. In addition, the comparison between woody and herbaceous pollen contents shows the increase of herbaceous plants, C_4 herbs, and C_3 trees besides *Artemisia*, which may explain the significant increase of $\delta^{13}\text{C}_{\text{TOC}}$ in early MIS5, resulting in the less obvious negative value of $\delta^{13}\text{C}_{\text{TOC}}$ in MIS5 than that in MIS6.

Studies have shown that solar radiation was low and the climate was warm and semi-humid in MIS11 (S4) (Hao et al., 2012; Guo et al., 1993). The magnetic susceptibility and grain size indicate that the summer monsoon continued weakening after a rapid increase, while the winter monsoon was relatively weak over the whole period (Sun et al., 2010). The weak summer monsoon resulted in a rainy season concentrated in the warmer summer, and thus the cold and dry early spring would inhibit the growth of C_3 herbaceous plants, giving C_4 plants, C_3 trees, and shrubs an enhanced competitive edge (Yang et al., 2012). The variation in the amplitude of solar radiation in Holocene (S1) and MIS11 (S4) were similar over the last 450 kyr, indicating similar climate type in these two stages (Hao et al., 2012). The vegetation in the Holocene is similar to that in the S4 period, but the large number of pine tree species indicates the influence of human activities in the Holocene, especially in the middle and late Holocene. In addition, fire, as an ecological element, may have contributed to the abundance of C_4 herbaceous plants and the germination of some Pinaceae (Bond and Keeley, 2005; Zhou et al., 2014). In S3, S2, S1, and other relatively strong summer monsoon periods, the range of rainfall time was wider and the rainfall in early spring was conducive to the growth of C_3 plants, thus gaining an ecological niche advantage. During S3 and S1, generally considering the warmest and most humid periods in this region, carbon isotopes and *n*-alkanes parameters indicate that there were more woody plants for a short period, but there was not a large area of stable forests, which is not consistent with the contention that the landscape in this region was dominated by lush grassland most of time.

4.3 Comparison of $\delta^{13}\text{C}_{\text{TOC}}$ and *n*-alkanes in loess and discussion on their uncertainties

The contention that there were more C_3 plants in glacial

periods and more C_4 in the interglacial periods originates from the carbon isotope evidence over the last glacial cycle but does not appear to hold true over multiple glacial cycles. There has also been insufficient discussion of the interpretation of carbon isotopes—are they an indicator of changes in the relative proportions of plants using the two major photosynthetic pathways or do they reflect changes in life form of the vegetation with no change in photosynthetic pathway? From the existing long $\delta^{13}C_{TOC}$ record, as shown in Figure 6, the variation in different sections is consistent or obviously different, which is generally attributed to the similarities and differences of climatic factors in time and space, such as fluctuation of monsoon (Sun et al., 2012), summer monsoon evolution on an orbital timescale (An et al., 2005; Liu et al., 2005; Zhang et al., 2013), and differences in the water and heat configuration (Yang et al., 2012; Peterse et al., 2014; Zhou et al., 2014). While reconstructing vegetation evolution with different carbon isotopes in the same section will result in differences in interpretation, mainly due to different organic components with different origins and compositions (Liu et al., 2007) and different response of climate factors (Xie et al., 2004; Wang et al., 2008). Thus, detail analysis should be carried out when attempting to reconstruct past variations in vegetation from long sediment isotopic records.

n -Alkane ratios of C_{31}/C_{27} , C_{31}/C_{29} , and $C_{31}/(C_{27}+C_{29}+C_{31})$, which reflect the abundance changes of herbaceous and woody plants in the ecosystem, are widely adopted in the research of paleovegetation and paleoclimate in loess-paleosol. The consistency in n -alkanes parameters in loess sediments has contributed to some accepted fruits of the evolution on an orbital timescale or vegetation fea-

tures in some certain periods. However, the relationship between distribution characteristics of long-chain n -alkanes in modern topsoil and origin vegetation is complex. Some studies have clearly contended that the main carbon peak cannot determine the growth type of vegetation (Bi et al., 2005; Rao et al., 2011). In addition, influenced by vegetation type, biomass contribution and climate change, there is uncertainty of production rate and proportion when being stored in the soil after burial (Rao et al., 2011). Thus, when applying n -alkanes parameters, such as C_{31}/C_{27} , to indicate the relative variation of herbal/woody vegetation, other supporting evidence should also be provided.

5. Conclusion

This study, through the comparison between $\delta^{13}C_{TOC}$ and n -alkanes C_{31}/C_{27} , combined with pollen statistics, reveals the vegetation evolution in the middle of CLP since the late Quaternary.

(1) The vegetation in this region was a mixed type of C_3 and C_4 plants, or in terms of growth form, a mixed type of herbaceous and woody plants, and overall dominated by C_3 plants since 450 kyr. There was never any extensive forest in the region over this time period.

(2) Herbaceous plants increased in the region since 130 kyr; on orbital timescales, there was a reduction in C_3 *Artemisia* herbaceous plants in MIS11 (S4 period) compared with MIS12 (L5 period) and a reduction in Holocene (S0 period) compared with the last glacial period (L1 period); in other glacial cycles, C_3 *Artemisia* increased in interglacial periods compared with the glacial periods.

(3) In relatively warm and humid MIS 9 (S3 period) and MIS5 (S1 period) on the CLP, C_3 woody plants may have increased for a short time; the climate in MIS11 (S4 period) was similar to that in MIS1 (S0 period), but trees increased significantly in the Holocene.

(4) Vegetation changes on orbital time scales in the central Loess Plateau are controlled by climatic factors, such as aridity and temperature, but affected largely by human activities and/or fire activities in the Holocene.

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References

An Z S, Huang Y S, Liu W G, Guo Z T, Clemens S, Li L, Preww W, Ning

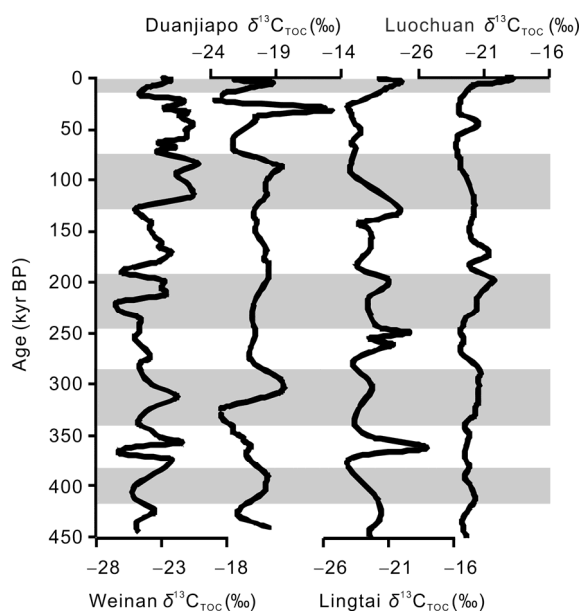


Figure 6 C_4 plants percentage curve obtained from the reconstruction of $\delta^{13}C_{TOC}$ in Weinan (Sun et al., 2012), Duanjiapo (An et al., 2005), Lingtai (this study), and Luochuan (Zhang et al., 2013).

- Y F, Cai Y F, Zhou W J, Lin B H, Zhang Q L, Cao Y N, Qiang X K, Chang H, Wu Z K. 2005. Multiple expansions of C₄ plant biomass in East Asia since 7 Ma coupled with strengthened monsoon circulation. *Geology*, 33: 705–708
- Bai Y, Fang X, Nie J, Wang Y, Wu F L. 2009. A preliminary reconstruction of the paleoecological and paleoclimatic history of the Chinese Loess Plateau from the application of biomarkers. *Paleogeogr Paleoclimatol Paleocol*, 271: 161–169
- Bi X H, Sheng G Y, Liu X H, Fu J M. 2005. Molecular and carbon and hydrogen isotopic composition of *n*-alkanes in plant leaf waxes. *Org Geochem*, 36: 1405–1417
- Bird M I, Grocke D. 1997. Determination of the abundance and carbon-isotope composition of elemental carbon in sediments. *Geochim Cosmochim Acta*, 61: 3413–3423
- Bond W J, Keeley J E. 2005. Fire as a lobal 'herbivore': The ecology and evolution of flammable ecosystems. *Trends Ecol Evol*, 20: 387–394
- Chen F H, Rao Z G, Zhang J W, Jin M, Ma J Y. 2006. Variations of organic carbon isotopic composition and its environmental significance during the last glacial on western Chinese Loess Plateau. *Chin Sci Bull*, 51: 1593–1602
- Chen J, Dong X F. 1990. Agriculture, forestry and animal husbandry study of Longdong area under natural background. *J Gansu Sci*, 4: 69–73
- Chen P N, Wang G A, Han J M, Liu X J, Liu M. 2010. $\delta^{13}\text{C}$ difference between plants and soil organic matter along the eastern slope of Mount Gongga. *Chin Sci Bull*, 55: 55–62
- Cheng J, Hu T M, Cheng J M. 2011. Responses of distribution of *Artemisia sacrorum* community to climate in semi-arid and semi-humid areas of Loess Plateau. *Sci Soil Water Conserv*, 9: 51–56
- Cranwell P A, Eglinton G, Robinson N. 1987. Lipids of aquatic organisms as potential contributors to lacustrine sediments-II. *Org Geochem*, 11: 513–527
- Deines P. 1980. The isotopic composition of reduced organic carbon. In: Fritz P, Fontes J C, eds. *Handbook of Environmental Isotope Geochemistry I, the Terrestrial Environment*. Amsterdam: Elsevier. 329–406
- Ding Z, Yang S. 2000. C₃/C₄ vegetation evolution over the last 7.0 Myr in the Chinese Loess Plateau: Evidence from pedogenic carbonate $\delta^{13}\text{C}$. *Paleogeogr Paleoclimatol Paleocol*, 160: 291–299
- Eglinton G, Hamilton R J. 1967. Leaf epicuticular waxes. *Science*, 56: 1322–1335
- Gu Z Y, Liu Q, Xu B, Han J M, Yang S L, Ding Z L. 2003. Climate change as the dominant control on glacial-interglacial variation in C₃ and C₄ plant abundance in the Loess Plateau. *Chin Sci Bull*, 48: 1271–1276
- Guo Z T, Hou Y J. 2010. The general situation of natural environment changes in the Loess Plateau since the Holocene. In: Tian J L, ed. *Study on the Environmental Effects of Ecological Construction in the Loess Plateau* (in Chinese). Beijing: Meteorology Press. 1–47
- Guo Z T, Liu T S, Fedoroff N, An Z S. 1993. Shift of the monsoon intensity on the Loess Plateau at ca. 0.85 Ma BP. *Chin Sci Bull*, 38: 586–591
- Hao Q Z, Wang L, Oldfield F, Peng S Z, Qin L, Song Y, Xu B, Qian Y S, Bloemendal J, Guo Z T. 2012. Delayed build-up of Arctic ice sheets during 400 kyr minima in insolation variability. *Nature*, 490: 393–396
- Imbrie J, Hays J D, Martinson D G, McIntyre A, Mix A C, Morley J J, Pisias N G, Prell W L. 1984. The orbital theory of Pleistocene climate: Support from a revised chronology of the marine ^{18}O record. In: Berger A, Imbrie J, Hays J, eds. *Milankovitch and Climate*. Dordrecht: Reidel. 269–306
- Keeley J E, Rundel P W. 2005. Fire and the Miocene expansion of C₄ grasslands. *Ecol Lett*, 8: 683–690
- Lin B H, Liu R M. 1992. The stable isotopic evidence of the monsoon variation on Chinese Loess Plateau since the last 800 ka years. *Chin Sci Bull*, 37: 178–182
- Liu J F, Su Y. 1994. The changes of vegetation and climate in Pingliang region, Gansu Province since about 800000 yr B.P. (in Chinese). *Geograph Res*, 13: 90–97
- Liu L, Yang S, Cui L L, Hao Z G. 2013. Stable carbon isotopic composition of black carbon in surface soil as a proxy for reconstructing vegetation on the Chinese Loess Plateau. *Paleogeogr Paleoclimatol Paleocol*, 388: 109–114
- Liu W G, Huang Y S, An Z S, Wu Z H, Lu H Y, Cao Y N. 2005. Summer monsoon intensity controls C₄/C₃ plant abundance during the last 35 ka in the Chinese Loess Plateau: Carbon isotope evidence from bulk organic matter and individual leaf waxes. *Paleogeogr Paleoclimatol Paleocol*, 220: 243–254
- Liu W G, Ning Y F, An Z S, Wu Z H, Lu H Y, Cao Y N. 2002. Carbon isotopic composition of modern soil and paleosol as a response to vegetation change on the Chinese Loess Plateau. *Sci China Ser D-Earth Sci*, 48: 93–99
- Liu W G, Zhang P, Sun Y B, Huang Y S, Guo Z T, An Z S. 2008. Molecule fossil evidence for paleovegetation changes in the central of Chinese Loess Plateau during 7–2 Ma: Zhangjiachuang profile as an example. *Quat Sci*, 28: 806–811
- Liu W, Yang H, Ning Y, An Z S. 2007. Contribution of inherent organic carbon to the bulk $\delta^{13}\text{C}$ signal in loess deposits from the arid western Chinese Loess Plateau. *Org Geochem*, 38: 1571–1579
- Long L Q, Fang X M, Miao Y F, Bai Y, Wang Y L. 2011. Northern Tibetan Plateau cooling and aridification linked to Cenozoic global cooling: Evidence from *n*-alkane distributions of Paleogene sedimentary sequences in the Xining Basin. *Chin Sci Bull*, 56: 1569–1578
- Lv H Y, Liu T S. 1999. Phytolith record of vegetation succession in the Southern Loess Plateau since late Pleistocene. *Quat Sci*, 4: 336–349
- Marino B D, McElroy M B, Salawitch R J, Spaulding W G. 1992. Glacial-to-interglacial variations in the carbon isotopic composition of atmospheric CO₂. *Nature*, 357: 461–462
- Peterse F, Martinez-Garcia A, Zhou B, Beets C J, Prins M A, Zheng H B, Eglinton T I. 2014. Molecular records of continental air temperature and monsoon precipitation variability in East Asia spanning the past 130000 years. *Quat Sci Rev*, 83: 1–7
- Peterse F, Zhou B, Magill C, Eglinton T I. 2015. Combined records of monsoon precipitation, temperature, and vegetation changes in East Asia over the past 200000 years. *IMOG Meeting*. 846
- Rao Z G, Zhu Z Y, Chen F F, Zhang J W. 2006. Reviews on the stable carbon isotopic researches of organic matter of Chinese Loess (in Chinese). *Adv Earth Sci*, 21: 62–69
- Rao Z G, Wu Y, Zhu Z Y, Jia G D, Henderson A. 2011. Is the maximum carbon number of long-chain *n*-alkanes an indicator of grassland or forest? Evidence from surface soils and modern plants. *Chin Sci Bull*, 56: 1714–1720
- Shen J, Chu H. 1997. Preparation of organic carbon isotopic sample for Mass Spectrometric analysis by Static Combustion. *Anal Test Tech Instrum*, 3: 113–116
- Shi H Z, Li F C, Sun X H, Wu F, Luo X P, Li X L, Jin Z D. 2011. Distribution of organic carbon in the Luochuan Loess/paleosol and its relationship with clay minerals. *Geol China*, 2011, 38: 1355–1362
- Sun J, Lu T, Zhang Z, Wang X, Liu W G. 2012. Stepwise expansions of C₄ biomass and enhanced seasonal precipitation and regional aridity during the Quaternary on the southern Chinese Loess Plateau. *Quat Sci Rev*, 34: 57–65
- Sun X J, Song C Q, Wang F Y, Sun M R. 1997. Vegetation history of the Loess Plateau of China during the last 100000 years based on pollen data. *Quat Int*, 37: 25–36
- Sun Y B, An Z S, Clemens S C, Jan Bloemendal J, Vandenberghe J. 2010. Seven million years of wind and precipitation variability on the Chinese Loess Plateau. *Earth Planet Sci Lett*, 297: 525–535
- Vidic N J, Montanez I P. 2004. Climatically driven glacial-interglacial variations in C₃ and C₄ plant proportions on the Chinese Loess Plateau. *Geology*, 32: 337–340
- Volkman J K, Gillan F T, Johns R B, Eglinton G. 1981. Sources of neutral lipids in a temperate intertidal sediment. *Geochim Cosmochim Acta*, 45: 1817–1828
- Wang G A, Feng X B, Han J M, Zhou L H, Tan W B, Su F. 2008. Paleovegetation reconstruction using $\delta^{13}\text{C}$ of soil organic matter. *Biogeosciences*, 5: 1325–1337
- Wang G A, Jia Y, Li W. 2015. Effects of environmental and biotic factors on carbon isotopic fractionation during decomposition of soil organic matter. *Sci Rep*, 5: 11043, doi: 10.1038/srep11043
- Wang H B, Feng Z D. 2014. Geographic Differentiation of the Last Inter-

- glacial Paleosol S1 in the Chinese Loess Plateau—Significance of Soil Genesis and Soil Morphology (in Chinese). Lanzhou: Lanzhou University Press. 37
- Wang Y L, Fang X M, Bai Y, Xi X X, Yang S L, Wang Y X. 2007. Distribution of lipids in modern soils from various regions with continuous climate (moisture-heat) change in China and their climate significance. *Sci China Ser D-Earth Sci*, 50: 600–612
- Wu F L, Fang X M, Ma Y Z, An Z S, Li J J. 2004. A 1.5 Ma sporopollen record of paleoecologic environment evolution in the central Chinese Loess. *Chin Sci Bull*, 49: 295–302
- Xie S C, Wang Z Y, Wang H M, Chen F H, An C B. 2002. The occurrence of grassy vegetation over the loess plateau since the last interglacier: the molecular fossil record. *Sci China Ser D-Earth Sci*, 45: 53–62
- Xie S, Guo J, Huang J, Chen F H, Wang H B, Farrimond P. 2004. Restricted utility of $\delta^{13}\text{C}$ of bulk organic matter as a record of paleovegetation in some loess-paleosol sequences in the Chinese Loess Plateau. *Quat Res*, 62: 86–93
- Yang S L, Ding Z L, Wang X, Tang Z H, Gu Z Y. 2012. Negative $\delta^{18}\text{O}$ – $\delta^{13}\text{C}$ relationship of pedogenic carbonate from northern China indicates a strong response of C_3/C_4 biomass to the seasonality of Asian monsoon precipitation. *Paleogeogr Paleoclimatol Paleoecol*, 317–318: 32–40
- Zhang H C, Yang M S, Zhang W X, Lei G L, Chang F Q, Pu Y, Fan H F. 2004. Molecular fossil and paleovegetation records of paleosol S4 and adjacent loess layers in the Luochuan loess section, NW China. *Sci China Ser D-Earth Sci*, 51: 321–330
- Zhang P, Liu W G, Qiang X K. 2013. Vegetation coverage and monsoon variation recorded by stable carbon isotope of Loess since 2.5 Ma. *Mar Geol Quat Geol*, 2013, 33: 137–143
- Zhang Z H, Zhao M X, Eglinton G, Lu H Y, Huang C Y. 2006. Leaf wax lipids as paleovegetational and paleoenvironmental proxies for the Chinese Loess Plateau over the last 170 kyr. *Quat Sci Rev*, 25: 575–594
- Zhang Z H, Zhao M X, Lu H Y, Faiia A M. 2003. Lower temperature as the main cause of C_4 plant declines during the glacial periods on the Chinese Loess Plateau. *Earth Planet Sci Lett*, 214: 467–481
- Zheng S X, Shanguan Z P. 2007. Foliar $\delta^{13}\text{C}$ values of nine dominant species in the Loess Plateau of China. *Photosynthetica*, 45: 110–119
- Zhong Y X, Xue Q, Chen F H. 2009. Alkane distributions in Modern vegetation and surface soil from western Loess Plateau. *Quat Sci*, 29: 767–773
- Zhou B, Shen C D, Sun W D, Zheng H B, Yang Y, Sun Y B, An Z S. 2007. The history of wildfire in the Chinese Loess Plateau during the last 420 ka and its implications to environmental and climate changes. *Paleogeogr Paleoclimatol Paleoecol*, 252: 617–625
- Zhou B, Shen C D, Sun W D, Bird M I, Ma W T, Taylor D, Liu W G, Peterse F, Yi W X, Zheng H B. 2014. Late Pliocene–Pleistocene expansion of C_4 vegetation in semiarid East Asia linked to increased burning. *Geology*, 42: 1067–1070
- Zhou B, Shen C D, Zheng H B, Sun Y M. 2009. Vegetation evolution on the central Chinese Loess Plateau since late Quaternary evidenced by elemental carbon isotopic composition. *Chin Sci Bull*, 2009, 54: 2082–2089