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**Paleovegetation  
reconstruction using  
 $\delta^{13}\text{C}$  of SOM**

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# Paleovegetation reconstruction using $\delta^{13}\text{C}$ of Soil Organic Matter

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## Abstract

The relative contributions of  $C_3$  and  $C_4$  plants to vegetation at a given locality may be estimated by means of  $\delta^{13}C$  of soil organic matter. This approach holds great potential for paleoecological reconstruction using paleosols. However, two uncertainties exist, which limits the accuracy of this application. One is  $^{13}C$  enrichment as plant carbon becomes incorporated into soil organic matter. The other is due to environmental influences on  $\delta^{13}C$  of plants. Two types of data were collected and analyzed with an objective of narrowing the error of paleovegetation reconstruction. First, we investigated  $\delta^{13}C$  variations of 557  $C_3$  and 136  $C_4$  plants along a precipitation gradient in North China. A strong negative relationship is found between the  $\delta^{13}C$  value of  $C_3$  plants averaged for each site and the annual precipitation with a coefficient of  $-0.40\%/100$  mm, while no significant coefficients were found for  $C_4$  plants. Second, we measured  $\delta^{13}C$  of soil organic matters for 14 soil profiles at three sites. The isotopic difference between vegetation and soil organic matter are evaluated to be  $1.8\%$  for the surface soil and  $2.8\%$  for the soil at the bottom of soil profiles. Using the new data we conducted a sample reconstruction of paleovegetation at the central Chinese Loess Plateau during the Holocene and the Last Glaciation, and conclude that, without corrections for  $^{13}C$  enrichment by decomposition, the  $C_4$  abundance would be overestimated. The importance and uncertainties of other corrections are also discussed.

## 1 Introduction

Carbon isotopic ratios of soil organic matter ( $\delta^{13}C_{SOM}$ ) are close to that of the vegetation, and thus  $\delta^{13}C_{SOM}$  can be used to estimate the relative abundance of  $C_3$  and  $C_4$  biomass in the vegetation at a given locality in the past. Many researchers have used  $\delta^{13}C_{SOM}$  of paleosols and/or loess to reconstruct paleovegetation and paleoclimate (e.g. Stanley et al., 1991; Boutton, 1996; Boutton et al., 1998; Guillaume et al., 2001; Wang and Zheng, 1989; Gu, 1991; Frakes and Sun, 1994; Han et al., 1996; Wang and

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Follmer, 1998; Lin et al., 1991; Ding and Yang, 2000; Vidic and Montañez, 2004; Liu et al., 2005; An et al., 2005). These reconstructions use isotope mass-balance equations of the following:

$$C_3(\%) = (\delta^{13}C_{SOM} - \delta^{13}C_{C_4}) / (\delta^{13}C_{C_3} - \delta^{13}C_{C_4}) \times 100; \quad (1)$$

$$C_4(\%) = 100 - C_3(\%) \quad (2)$$

where  $\delta^{13}C_{C_3}$ ,  $\delta^{13}C_{C_4}$  are the mean  $\delta^{13}C$  values of  $C_3$  and  $C_4$  plants at a given locality at the time the soil under study was developed;  $C_3(\%)$  and  $C_4(\%)$  are percentages of  $C_3$  and  $C_4$  biomass in the local vegetation. The accuracy of the reconstruction depends upon the accuracy of the knowledge on the end member values of  $\delta^{13}C_{C_3}$  and  $\delta^{13}C_{C_4}$ . The use of these equations also assumes that  $\delta^{13}C_{SOM}$  represents the  $\delta^{13}C$  value of bulk local vegetation.

To acquire the  $\delta^{13}C$  values for the pure  $C_3$  and  $C_4$  plants at the time paleosols formed, isotopic values for modern plants are first obtained. However, modern plants may be isotopically different from ancient plants due to environmental changes, such as variations in the  $\delta^{13}C$  and concentration of the atmospheric  $CO_2$ , and in the amount of annual precipitation (e.g. Farquhar et al., 1982; Körner et al., 1988). Attempts have been made by several authors to correct the effects of these environmental factors before Eqs. (1) and (2) were applied (Liu et al., 2005; Chen et al., 2005).

Another source of uncertainty comes from the assumption that  $\delta^{13}C_{SOM}$  equals the  $\delta^{13}C$  of vegetation. It is known that isotopic enrichment occurs during decomposition such that the soil organic matter tends to have higher  $\delta^{13}C$  values than the vegetation (Troughton et al., 1974; Stout et al., 1975; Schleser and Pohling, 1980; Balesdent et al., 1993; Boutton, 1996; Bird and Pousal, 1997). To our knowledge, this effect has not been taken into consideration when Eqs. (1) and (2) are used for paleovegetation reconstruction. It has been argued that using specific biomarkers, such as n-alkanes, this source of error can be ignored (Zhang et al., 2004). While this statement is likely

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but yet to be demonstrated, it is certain that isotopic enrichment in the bulk soil organic matter relatively to the vegetation due to decomposition should be corrected.

This paper evaluates various corrections described above for paleovegetation reconstructions. We particularly focus on the precipitation effect on  $\delta^{13}\text{C}$  in both  $\text{C}_3$  and  $\text{C}_4$  plants, and the  $^{13}\text{C}$  enrichment of soil organic matter relative to vegetation due to decomposition. We have collected new data for quantifying these two effects.

New data is necessary because the existing data do not yield certain sensitivities for these two effects. Many studies have observed that  $\delta^{13}\text{C}$  of  $\text{C}_3$  plants decreases significantly with increases in precipitation (e.g. Ehleringer and Cooper, 1988; Smedley et al., 1991; Wang et al., 2003, 2005; Schulze et al., 2006). Liu et al. (2005) measured  $\delta^{13}\text{C}$  of three  $\text{C}_3$  species occurring in Northwest China, and found that the mean  $\delta^{13}\text{C}$  decrease is 1.1‰ with every 100 mm increase in annual precipitation. This sensitivity is much greater than that reported by Steward et al. (1995) ( $-0.34\text{‰}/100\text{ mm}$ ) and Wang et al. (2003) ( $-0.49\text{‰}/100\text{ mm}$ ). Relative to  $\text{C}_3$  plants, the  $\delta^{13}\text{C}$  of  $\text{C}_4$  plants is not sensitive to water availability (Farquhar et al., 1982; Henderson et al., 1992; Wang et al., 2005, 2006). Studies on  $\text{C}_4$  species mostly showed slight increases in  $\delta^{13}\text{C}$  with increasing water availability (Buchmann et al., 1996; Schulze et al., 1996; Wang et al., 2005, 2006). However, Liu et al. (2005) observed an opposite pattern in *Bothriochloa ischaemum* ( $\text{C}_4$ ), i.e.,  $\delta^{13}\text{C}$  decreasing significantly with increasing precipitation ( $-0.61\text{‰}/100\text{ mm}$ ). Our study includes an intensive investigation on the plants'  $\delta^{13}\text{C}$  response to precipitation in an arid and semiarid region by averaging a large number of species in 33 sites in North China with annual precipitation ranging from 15 to 650 mm.

The  $^{13}\text{C}$  enrichment in soil organic matter has been observed in both field and laboratory investigations. Some observations have come from field or laboratory incubation experiments (e.g. Natelhoffer and Fry, 1988; Wedin et al., 1995; Fernandez et al., 2003; Connin et al., 2001). Time spans used in these experiments are relatively short, typically less than 5 years, while the time for decomposition of organic matters in natural systems is much longer, on timescales from decades to millennia. Therefore, these short-term experimental results represented  $^{13}\text{C}$  enrichments only during

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the early stage of decomposition. In fact, progressive  $^{13}\text{C}$  enrichment continues even during decomposition of resistant soil organic matter having slow turnover rates (Chen et al., 2002; Feng et al., 1999; Torn et al., 2002). Other observations have come from isotopic measurements of soil profiles (e.g. Stout et al., 1978; Dzurec et al., 1985; Gregorich et al., 1995; Balesdent et al., 1993; Boutton, 1996; Bowman et al., 2002). Most of such studies aimed to understand vegetation dynamics, and thus study sites were chosen in areas where  $\text{C}_3/\text{C}_4$  ratios in local vegetation have not been constant in the past (e.g. Dzurec et al., 1985; Schwartz et al., 1986; Boutton et al., 1998; Guillaume et al., 2001; Krull et al., 2005). As a result, the observed  $\delta^{13}\text{C}$  differences between soil organic matter and surface standing vegetation included not only the isotopic enrichment during organic matter decomposition, but also changes of the  $\text{C}_3/\text{C}_4$  ratio in the local vegetation. To quantify isotopic enrichment in soil organic matter, we studied isotopic variations in 14 soil profiles at three sites. All three sites are undisturbed, and have  $\text{C}_3$  only vegetations; they are chosen especially because we intended to avoid isotopic variations in soil organic matter due to changing abundance of  $\text{C}_3$  and  $\text{C}_4$  plant types. We then evaluate how various corrections affect the paleovegetation reconstruction for Chinese Loess Plateau.

## 2 Study sites and methods

### 2.1 Study sites of plant sampling and plant collection

A total of 33 sites in North China were studied, among which 27 sites are in Northwest China (Fig. 1). From west to east, North China is characterized by temperate arid, semiarid and semi-humid climate. The dominant control over the precipitation amount is the strength of the East Asian summer monsoon system. The precipitation occurs mostly in the summer season (from May to September), which accounts for approximately 68% to 87% of the total annual precipitation. From southeast to northwest in the study region, the annual rainfall amount decreases from 650 to 15 mm. The vegetation

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is dominated by shrubs and herbs.

Plants were sampled in August of 1998 to 2005. In order to minimize the influences attributed to human disturbance, local water supply, light regime or location within the canopy, sampling was restricted to unshaded sites far from human habitats. All the species collected were those having high abundance in the community or those widely occurring in North China. At each site, 5–7 plants of each species of interest were identified, and the uppermost and the second fully opened leaves of each individual were obtained. The leaves from each species at each site were mixed together into one sample. A total of 557 C<sub>3</sub> samples (including 255 species) and 136 C<sub>4</sub> samples (including 43 species) were collected.

## 2.2 Study sites of soil sampling and soil collection

One of the three soil sampling sites (Site 1) is located at the northeastern part of the Qinghai-Tibetan Plateau in which the Chinese Academy of Sciences has a research station, Haibei Highland Frigid Meadow Ecosystem Experimental Station (101°12'E, 37°45'N; 3200 m a.s.l.). The other two sites (Sites 2 and 3) are in the Donglingshan Mountain situated on the boundary between Beijing and Hebei Province in North China, approximately 130 km west of Beijing city (Fig. 1). These two sites are within Beijing Forest Ecosystem Research Station, which covers 16 000 ha of land, and was established on Donglingshan Mountain by the Chinese Academy of Sciences in 1990.

Site 1 has the characteristics of a highland continental climate, and is cold and humid with the mean annual temperature of -2.0°C and the mean annual precipitation of around 600 mm. Vegetation there is highland frigid meadow with most dominant species of *Potentilla fruticosa*, *Kobresia pygaea* and *Kobresia humilis*. There are no C<sub>4</sub> species. Soil type is alpine meadow soil. The area of Sites 2 and 3 is characterized by temperate semimoist climate. The amount of annual precipitation is 611.9 mm and the mean annual temperature is from 2°C (2303 m a.s.l. at the peak of Donglingshan Mountain) to 8°C (400 m a.s.l., Zaitang). The vegetation is characterized by forest, shrub and subalpine meadow. Site 2 is situated on the north-facing slope of Dongling-

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shan Mountain at an elevation of 1600 m. The vegetation is a deciduous broad-leaved forest with only one birch tree species, *Betula platyphylla*, having an average height of 5 m and an average canopy area of 10 m<sup>2</sup>. In addition, two C<sub>3</sub> grass species, *Trullius chinensis* and *Galium verum*, with about 0.15 m and 0.10 m high, respectively, occur within birch stands. No C<sub>4</sub> species is present at this site. The site has a brown forest soil. Site 3 is located on a ridge with an elevation of 1700 m, approximately 300 m southeast of Site 2. The vegetation at Site 3 is a subalpine meadow with *Roegneria kamoji* and *Artemisia lavandulaefolia* grasses as dominant species. Except for few *Setaria viridis* (C<sub>4</sub>) occurring on roadsides and neighbor of the climatic station, which is also located at the ridge (1700 m a.s.l.), no C<sub>4</sub> species exists elsewhere, particularly no C<sub>4</sub> species at the sampling plots. Soil type is subalpine meadow soil. There is no human habitat within 20 km<sup>2</sup> of Site 2 and Site 3, mainly because of strong winds and low temperature for many days of each year. Grazing and cutting have been strictly prohibited in the area since 1980's.

At Site 1, six 0.5 m×0.5 m plots were laid out within one of the enclosures in Haibei Highland Frigid Meadow Ecosystem Experimental Station with *Kobresia pygaea* and *Kobresia humilis* as dominant species. For each plot, all aboveground plants were collected (and will be used as litter input to the soil), and then the soil profile was dug to the weathered rock (saprolite). Soil profiles were sampled at 5 cm intervals. The depths of the six soil profiles are 60 cm, 120 cm, 110 cm, 60 cm, 70 cm and 90 cm, respectively.

At Site 2, five plots of 0.5 m×0.5 m were sampled in *Betula platyphylla* stands. In order to avoid birch roots, all plots were chosen at 2–3 m away from tree trunks. At each plot, we first sampled the litter layer, and then dug a soil profile to the weathered rock. The depths of the five soil profiles are 75 cm, 105 cm, 100 cm, 100 cm and 120 cm, respectively. All soil profiles were sampled at 5 cm intervals except for one that were sampled at 10 cm intervals.

At Site 3, three plots of 0.5 m×0.5 m were sampled in the same way as described for Site 1. The depths of the soil profiles are 60 cm, 70 cm and 95 cm, respectively, and

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soils were sampled at 5 cm intervals.

## 2.3 Measurements

The plant and litter samples were oven-dried, and ground to 40 mesh. Soil samples were air-dried and sieved with a 2-mm sieve. Plant fragments and the soil fraction coarser than 2 mm were removed. The <2 mm soil fraction was treated with 1N HCl at room temperature overnight to remove carbonates, after which it was washed and oven-dried at 70°C. Carbon contents and carbon isotopic ratios of samples were determined on an elemental analyzer coupled with a Delta<sup>Plus</sup> XP mass spectrometer in a continuous flow mode. The <sup>13</sup>C to <sup>12</sup>C ratio of the sample is reported in the  $\delta$ -notation as the relative difference in parts per thousand (per mil) from the PDB standard. The repeatability of C contents is better than 0.1% (1 $\sigma$ ) and that of  $\delta^{13}\text{C}$  is better than 0.15‰ (1 $\sigma$ ).

## 3 Results

### 3.1 $\delta^{13}\text{C}$ variations of C<sub>3</sub> and C<sub>4</sub> plants with annual precipitation

Values of  $\delta^{13}\text{C}$  in C<sub>3</sub> plants we measured range from -21.7‰ to -30‰ with a mean value of -26.7‰ ( $n=557$ , s.d.=1.56), and those of C<sub>4</sub> plants vary from -10.0‰ to -15.8‰ with a mean of -12.8‰ ( $n=136$ , s.d.=1.06).

Fig. 2a plots the site-averaged  $\delta^{13}\text{C}$  values for C<sub>3</sub> plants as a function of precipitation, showing that  $\delta^{13}\text{C}$  increases significantly with decreasing precipitation with a slope of -0.004‰/mm (0.001 s.e.,  $t$ -test,  $P<0.0001$ ). C<sub>4</sub> plant  $\delta^{13}\text{C}$  values display a slight increase trend with increasing annual precipitation with a slope of 0.001‰/mm (0.001 s.e.,  $t$ -test,  $P=0.220$ ), although the correlation is not significant (Fig. 2b). *Setaria vidis* (an annual grass with a stem length of 10–110 cm) is one of the most common C<sub>4</sub> species in China (Institute of Botany, CAS, 1987). Using this species alone, the  $\delta^{13}\text{C}$

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also slightly increases with precipitation, with a slope of 0.001‰/mm (0.001 s.e.*t*-test,  $P=0.321$ ), but again the correlation is not significant (Fig. 2c).

### 3.2 Variations in $\delta^{13}\text{C}$ in soil profiles

In soil, the carbon contents drop rapidly with depths near the surface, and then level off and slowly approach zero in deeper layers. All three sites share this characteristic carbon distribution with depth. One example is given for Site 1 in Fig. 3a.

The carbon isotopic ratio in the soil organic matter at Site 1 increases with depth (Fig. 3b). Compared to the carbon contents (Fig. 3a), changes of  $\delta^{13}\text{C}$  with depth are more gradual. The isotopic enrichments with depth do not seem to approach a constant value within the depth of observation. Carbon isotope profiles in Site 2 and Site 3 (Figs. 4a and 4b) also show the typical pattern of  $^{13}\text{C}$  enrichment with depth.

We are mostly interested in the isotopic difference between vegetation and soil organic matter. Obviously, this difference depends upon which depth of soil is under discussion. When dealing with a paleosol, the specific paleo-soil horizon it is not always clear. We, therefore, tabulated in Table 1 observed  $\delta^{13}\text{C}$  differences between vegetation (Sites 1 and 3) or litter (Site 2) and the soil organic matter either near the surface (0~5 cm of mineral soil) or at the bottom of soil profiles. Between the surface soil and vegetation, the  $\delta^{13}\text{C}$  difference ranges from 0.6 to 2.5‰ (Sites 1 and 3), and between the bottom soil and vegetation, the  $\delta^{13}\text{C}$  difference ranges from 2.6 to 5.9‰. At the forested Site 2, the  $\delta^{13}\text{C}$  difference is 1.5–3.0‰ between the surface soil and litter, and 3.7–5.0‰ between bottom soil and litter (Table 1). Typically, the  $\delta^{13}\text{C}$  of litter on the forest floor is about 0.5‰ higher than the foliage input (e.g. Stout et al., 1978; Dzurec et al., 1985; Gregorich et al., 1995; Balesdent et al., 1993; Wang, 2003). If this enrichment were taken in to account, the  $\delta^{13}\text{C}$  difference between soil and vegetation would be 2.0–3.5‰ for the surface soil and 4.2–5.5‰ for the bottom soil.

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## 4 Discussion

### 4.1 $\delta^{13}\text{C}$ variations of plants

For both  $\text{C}_3$  and  $\text{C}_4$  plants, water deficit usually causes plants to close stomatal pores to reduce water loss by transpiration, and thus the stomatal conductance ( $g$ ) decreases.

5 As a result, the ratio of intercellular to ambient  $\text{CO}_2$  concentration ( $c_i/c_a$ ) decreases. For  $\text{C}_3$  plants, a decrease in  $c_i/c_a$  directly results in an increase in its carbon isotopic ratio (Farquhar et al., 1982). Observations of  $\text{C}_3$  plant  $\delta^{13}\text{C}$  values decreasing along a precipitation gradient reported by this work and others (e.g. Steward et al., 1995; Wang et al., 2005; Liu et al., 2005; Schulze et al., 2006) are consistent with the expectation. For  $\text{C}_4$  plants, a decrease in the  $c_i/c_a$  ratio may lead to two opposite isotopic responses; the  $\delta^{13}\text{C}$  may increase or decrease depending on the degree of leakiness,  $\phi$  (Farquhar, 1983), the proportion of carbon dioxide produced within bundle sheath cells from  $\text{C}_4$  acids that is not fixed by Rubisco but leaked back to mesophyll cells. According to the model developed by Farquhar (1983) (modified by Henderson et al., 1992), if the  $\phi$  value is less than 0.35, the  $\delta^{13}\text{C}$  of  $\text{C}_4$  species decreases with decreasing  $c_i/c_a$  ratio; otherwise,  $\delta^{13}\text{C}$  increases. The  $\phi$  parameter seems to remain relatively constant for a given species under a wide range of environmental conditions (e.g., temperature, moisture and light conditions). Henderson et al. (1992) found that the  $\phi$  values measured in 10  $\text{C}_4$  species, using a gas exchange method, remained around 0.21 over a range of irradiance and leaf temperature. In this and other studies (Schulze et al., 1996; Buchmann et al., 1996; Ghannoum et al., 2002; Wang et al., 2005),  $\delta^{13}\text{C}$  values of  $\text{C}_4$  species were observed to decrease with increasing water stress, corresponding to  $\phi$  values less than 0.35. Therefore for the observation on *Bothriochloa ischaemum* by Liu et al. (2005) where the plant  $\delta^{13}\text{C}$  significantly decreased with increasing annual precipitation amount, it is possible that the  $\phi$  value of this species is greater than 0.35. *Bothriochloa ischaemum* seems to be a unique case, since it is the only  $\text{C}_4$  species reported to have a negative  $\delta^{13}\text{C}$ -precipitation correlation.

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## 4.2 Variations in $\delta^{13}\text{C}$ and carbon contents of soil organic matter

The soil profiles of carbon contents and  $\delta^{13}\text{C}$  show very different characteristics. The variations of carbon contents with depths are very similar within all profiles at Sites 1 to 3 (Figs. 3a), which are similar to thousands of reported carbon distributions in soil profiles (Zinke et al., 1986; 1998). Typically, carbon contents decreases rapidly near the surface, and then slowly at deeper soil. Judging from the carbon content profile alone it seems that little soil processes is occurring at depth. Compared to the carbon contents, the profile of soil  $\delta^{13}\text{C}$  also shows relatively rapid change near surface (although less than that of carbon contents), but the  $^{13}\text{C}$  enrichment continues in deeper layers. This suggests that even slow decomposition of resistant organic matter is associated with isotopic enrichment.

Several mechanisms have been proposed to account for  $^{13}\text{C}$ -enrichment of soil organic matter relative to original plants. The first mechanism is the decrease in the  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  since the industrial revolution because of combustion of  $^{13}\text{C}$ -depleted fossil fuels, and from the pre-industrial era to  $\sim 11\,000$   $^{14}\text{C}$  yr BP, the  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  was relatively constant, with a mean of  $-6.40\text{‰}$  (Marino et al., 1992). This mechanism is responsible for an isotopic difference of about  $1.3\text{‰}$  between the pre-industrial organic carbon and the present vegetation (Marino et al., 1992; Leuenberger et al., 1992). The second mechanism involves different decay rates of various components in organic matter having different  $\delta^{13}\text{C}$ . These effects also significantly alter the total  $\delta^{13}\text{C}$ , but the expected magnitude and direction depends on the relative proportions of components, and are not completely known (Feng, 2002). The third mechanism is isotopic fractionation during decomposition. The most important processes are microbial respiration and fermentation, leading to  $^{13}\text{C}$ -enrichments in microbial products compared to the organic substrate (Macko and Estep, 1984; Poage and Feng 2004). This mechanism is considered to be the main reason for observed  $^{13}\text{C}$  enrichment between litter and vegetation and with increasing soil depth.

The soil organic matter at the deepest depth of our soil profiles are likely derived

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from plant materials not affected by fossil fuel combustion, because the age of soil organic matter in deep soil can be thousands of years old (Shen, 2000). We, therefore, subtract 1.3 from the observed mean  $\delta^{13}\text{C}$  difference between deep soil and vegetation to account for the effect of the  $\delta^{13}\text{C}$  decrease in atmospheric  $\text{CO}_2$  since the industrial revolution. The  $\delta^{13}\text{C}$  values of soil organic matter at 5 cm depth, may also have been affected by the  $\delta^{13}\text{C}$  decrease in atmospheric  $\text{CO}_2$  in recent decades. However, we did not measure the age of the soil organic matter, and thus cannot determine how much correction would be needed for the surface soil. We will use the observed difference without a correction for later discussions, but note here that this difference may be slightly overestimated.

Summarizing the above discussions, we report that the  $\delta^{13}\text{C}$  difference is  $1.8 \pm 0.3\%$  (1 s.e.,  $n=14$ ) between the surface soil and vegetation, and  $2.8 \pm 0.3\%$  (1 s.e.,  $n=14$ ) between the bottom soil and vegetation. If all values in isotopic differences between soil and vegetation are pooled, the difference is normally distributed with a mean of  $2.3\%$  and standard deviation of  $1.1\%$  ( $n=28$ ).

Previous investigations (e.g. Stout et al., 1978; Dzurec et al., 1985; Gregorich et al., 1995; Balesdent et al. 1993) showed that the  $\delta^{13}\text{C}$  difference between vegetation and the topsoil (down to 20 cm depth) varied between  $0.5\%$  and  $2.5\%$ . Wang (2001) obtained similar results from 36 soil profiles in Northwest China, and the mean  $\delta^{13}\text{C}$  difference between vegetation and the topsoil layer is  $2.2\%$ . These  $\delta^{13}\text{C}$  differences obtained by previous studies were not corrected for the effect of the  $\delta^{13}\text{C}$  decrease in atmospheric  $\text{CO}_2$ . Therefore, the  $2.8\%$  carbon isotopic difference we obtained between the bottom organic matters and vegetation is probably the maximum  $^{13}\text{C}$  enrichment due to organic matter decomposition, because most paleosols may not represent the soil at deepest horizons. If the  $\delta^{13}\text{C}$  difference of  $1.8\%$  between the surface soil and vegetation are considered to be the most likely value for isotopic correction due to organic matter decay, Eq. (1) should be modified to the following:

$$C_3(\%) = [(\delta^{13}\text{C}_{\text{SOM}} - 1.8 - \delta^{13}\text{C}_{\text{C}_4}) / (\delta^{13}\text{C}_{\text{C}_3} - \delta^{13}\text{C}_{\text{C}_4})] \times 100 \quad (3)$$

### 4.3 Reconstructions of paleovegetation

Equations (1) and (3) are mass balance equations for estimating  $C_3$  plants' abundance in the vegetation using the measured  $\delta^{13}C$  of paleosol organic matter without or with a correction for isotopic enrichment due to soil organic matter decomposition. Before using these equations, however, we also need to obtain the end member  $\delta^{13}C$  values of  $C_3$  and  $C_4$  plants at the time when the plants grew. If we start with modern isotopic compositions of  $C_3$  and  $C_4$  plants, we have to make several corrections to obtain the  $\delta^{13}C_{C_4}$  and  $\delta^{13}C_{C_3}$  values for the time in the geological history, e.g., Holocene or the Last Glaciation. These corrections account for the effects on plant  $\delta^{13}C$  of 1) changes in precipitation, 2) changes in the  $\delta^{13}C$  of atmospheric  $CO_2$ , and 3) changes in the  $CO_2$  concentration in the atmosphere. Here we use the published loess  $\delta^{13}C$  data of Holocene and the Last Glaciation (LG) in Chinese Loess Plateau reported by Vidic and Montañez (2004) to show how different types of corrections affect the qualitative reconstruction of paleovegetation.

First, we obtain plant  $\delta^{13}C$  values in Holocene. The study site in Vidic and Montañez (2004) is located at Jiaodao close to Yanan city on the central Chinese Loess Plateau, which presently has a temperate semimoist climate with an annual precipitation amount of about 600 mm. Our study shows the mean  $\delta^{13}C$  values of standing  $C_3$  and  $C_4$  plants in this area are  $-27.5\%$  and  $-12.5\%$  respectively. For Holocene, we could assume that precipitation was similar to the current level, and therefore no need to correct for the climate effect on plant  $\delta^{13}C$  value. The  $\delta^{13}C$  value of the atmospheric  $CO_2$  in most part of Holocene was  $1.3\%$  higher than it is today (Marino et al., 1992; Leuenberger et al., 1992). Thus,  $\delta^{13}C$  values of  $C_3$ ,  $C_4$  plants during Holocene would be  $-26.2\%$  and  $-11.2\%$  respectively (Table 2).

Without the anthropogenic input of fossil fuel  $CO_2$ , the atmospheric  $CO_2$  concentration before the industrial revolution was 80 ppm lower than it is today. It is possible that the  $CO_2$  concentration level also affect the  $\delta^{13}C$  values of  $C_3$  plants. However, the magnitude of this effect is not entirely clear. Polley et al. (1993) showed that  $C_3$

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plants, growing over a range of  $\text{CO}_2$  concentrations characteristic of the Last Glacial Maximum to the present atmosphere, tended to have constant  $c_i/c_a$  ratios, suggesting a constant carbon isotope discrimination ( $\Delta$ ). Feng and Epstein (1995), on the other hand, reported that the mean  $\Delta$  value of tree rings in four trees is positively correlated to the  $\text{CO}_2$  concentration with a mean slope of  $0.02\text{‰}/\text{ppm}$ . We have recently conducted a detailed study on the  $\Delta$  response of  $\sim 50$  tree-ring series from widely distributed locations to atmospheric  $\text{CO}_2$  concentration in the past 100–200 years. We found that the pattern of the  $\delta^{13}\text{C}$  response to the  $\text{CO}_2$  concentration is complex. Before 1950, the mean  $\Delta$  value tended to increase with the  $\text{CO}_2$  concentration increase, while since 1950  $\Delta$  have decreased. The instantaneous rate of  $\Delta$  change with the  $\text{CO}_2$  concentration varied systematically, ranging from  $0.025\text{‰}/\text{ppm}$  in 1850 to  $-0.013\text{‰}/\text{ppm}$  in 1995 (Wang and Feng, 2008<sup>1</sup>). Apparently, the  $\delta^{13}\text{C}$  response to the  $\text{CO}_2$  concentration is not linear during the past 100 years, and it is likely that this response not only depends upon the absolute level of  $\text{CO}_2$  but also upon the rate of  $\text{CO}_2$  concentration change. Thus, it is difficult to determine whether corrections for a  $\text{CO}_2$  concentration change is necessary, and if so how much correction should be made. Here we will make paleoecological reconstruction considering the two situations, with and without a  $\text{CO}_2$  concentration correction. Under the situation with the correction, we use the coefficient of  $\delta^{13}\text{C}$ - $\text{CO}_2$  concentration of Feng and Epstein (1995) ( $-0.02\text{‰}/\text{ppm}$ ), which has already been used by several previous authors.  $\delta^{13}\text{C}$  value of  $\text{C}_3$  plants would then be  $-24.6\text{‰}$  in the Holocene after a  $1.6\text{‰}$  correction for 80 ppm change in the  $\text{CO}_2$  concentration of the atmosphere. For  $\text{C}_4$  plants,  $\delta^{13}\text{C}$  has a lower sensitivity to  $\text{CO}_2$  concentration change (Henderson et al., 1992), so it remains  $-11.2\text{‰}$  (Table 2).

We now obtain  $\delta^{13}\text{C}_{\text{C}_4}$  and  $\delta^{13}\text{C}_{\text{C}_3}$  values for the LG. Atmospheric  $\text{CO}_2$  concentration was 80 ppm lower and  $\delta^{13}\text{C}$  value of  $\text{CO}_2$  was  $0.3\text{‰}$  lower than that of Holocene (Marino et al., 1992; Leuenberger et al., 1992). With no correction for the effect of  $\text{CO}_2$

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<sup>1</sup> Wang, G. A. and Feng, X.: Long-term response in carbon isotope discrimination of tree rings to the atmospheric  $\text{CO}_2$  concentration, in preparation, 2008.

concentration, the  $\delta^{13}\text{C}$  values of  $\text{C}_3$  and  $\text{C}_4$  plants in the last ice age become  $-26.5\text{‰}$  and  $-11.5\text{‰}$ , respectively. With corrections for the effect of  $\text{CO}_2$  concentration,  $\delta^{13}\text{C}$  values of  $\text{C}_3$  plants in the last ice age become  $-23.3\text{‰}$ .  $\delta^{13}\text{C}$  value of  $\text{C}_4$  plants is still  $-11.5\text{‰}$ .

In addition, the precipitation at the location during the Last Glaciation was about 200 mm lower than that in Holocene (Wu et al., 1995). We correct the precipitation effect on the  $\delta^{13}\text{C}$  of  $\text{C}_3$  plants using the  $\delta^{13}\text{C}$ -precipitation coefficient of  $-0.4\text{‰}/100$  mm obtained in this study, which yields a  $0.8\text{‰}$  correction for  $\text{C}_3$  plants. Therefore, the  $\delta^{13}\text{C}_{\text{C}_3}$  of vegetation becomes  $-25.7\text{‰}$  without the correction for the  $\text{CO}_2$  concentration, and  $-22.5\text{‰}$  with the  $\text{CO}_2$  correction. The precipitation change does not significantly affect the  $\delta^{13}\text{C}_{\text{C}_4}$  value, and thus no further corrections are made for the  $\delta^{13}\text{C}$  of  $\text{C}_4$  plants. Values for various corrections are listed in Table 2.

The average  $\delta^{13}\text{C}_{\text{SOM}}$  value of  $\text{S}_0$  (Holocene paleosol) is reported to be  $-19.5\text{‰}$  and that of  $\text{L}_1$  (loess from the last glacial period)  $-22.4\text{‰}$  (Vidic and Montañez, 2004). Table 2 shows the effect of each type of corrections on the estimate of  $\text{C}_4$  plant abundance in the vegetation of a given age. One important comparison is shown by the difference using Eq. (1) versus Eq. (3) (column 4 and 5). Other things being equal, a correction for isotopic enrichment due to organic matter decomposition yields a  $\text{C}_4$  plant abundance in the vegetation about 12% lower than that obtained without such a correction.

The correction for  $\text{CO}_2$  concentration causes additional decrease in the estimated  $\text{C}_4$  plant abundances. In Holocene, the  $\text{CO}_2$  concentration correction reduces the estimated  $\text{C}_4$  abundances by about 6–8%. The effect is more substantial for the LG; the estimated  $\text{C}_4$  abundances become 0.9% using (1) but negative using Eq. (3). The negative value indicates that we may have overcorrected either for the enrichment of soil organic matter, or the  $\delta^{13}\text{C}$  end member of  $\text{C}_3$  plants, or both. Since our correction for organic matter decomposition is rather conservative (we used the difference between vegetation and surface soil), an overcorrection for the  $\text{CO}_2$  concentration effect is likely

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the cause. As discussed earlier, our recent compilation on tree-ring  $\delta^{13}\text{C}$  series does not yield a mean sensitivity of  $-0.02\text{‰}/\text{ppm}$ . The highest sensitivity,  $-1.3\text{‰}/\text{ppm}$ , was obtained for the most recent year of 1995 in the series, and before 1995 it was consistently lower. Nevertheless, our calculations indicate that the  $\text{C}_4$  abundance was probably very low and close to zero during the LG.

Compared to previously published paleovegetation reconstructions the estimated  $\text{C}_4$  abundances from this study are consistently lower. Vidic and Montañez (2004) reported 53% for  $\text{C}_4\%$  in Holocene, and 34% in the LG. Liu et al. (2005) also reported 53% of  $\text{C}_4$  vegetation in Holocene, but 11% in the LG.

The differences in the percentage of  $\text{C}_4$  plants between this study and those by Vidic and Montañez (2004) and Liu et al. (2005) come from the two sources. First, both Vidic and Montañez (2004) and Liu et al. (2005) did not take into account the  $^{13}\text{C}$  enrichment during decomposition of organic matters (1.8‰ used in this study). Second, their corrections to obtain end members of  $\delta^{13}\text{C}_{\text{C}_3}$  and  $\delta^{13}\text{C}_{\text{C}_4}$  are difference from ours. Vidic and Montañez (2004) did not make any correction, and therefore they obtained 53% and 34%  $\text{C}_4$  contribution in Holocene and the LG, respectively (see Table 2). When estimating the  $\text{C}_4\%$  in the Last Glaciation, Liu et al. (2005), did corrections on the precipitation effect on plant  $\delta^{13}\text{C}$  for both  $\text{C}_3$  and  $\text{C}_4$  plants ( $-1.1\text{‰}/100\text{ mm}$ ,  $-0.61\text{‰}/100\text{ mm}$  for  $\text{C}_3$ ,  $\text{C}_4$  plants, respectively). We think that the sensitivity they used for  $\text{C}_3$  plants might have been too high considering that our study is more comprehensive. In addition, their observation on the  $\text{C}_4$  plant response to precipitation based on one  $\text{C}_4$  species (*Bothriochloa ischaemum*) may have been an exception rather than the rule. While Liu et al. (2005) did correct for the effect of  $\text{CO}_2$  concentration, they overlooked the fact that the  $\delta^{13}\text{C}$  value of  $\text{CO}_2$  was 1.3‰ higher in Holocene than it is of today.

It is possible that Vidic and Montañez (2004) and perhaps also Liu et al. (2005) over-estimated the percentages of  $\text{C}_4$  plants. For example, the percentages of  $\text{C}_4$  plants in the last ice age obtained by Vidic and Montañez (2004) reaches 34%, while we consider it unlikely that so much  $\text{C}_4$  plants occurred during the LG when the annual

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temperature was expected to have dropped by 8–10°C compared to Holocene in the China Loess Plateau (Wu et al., 1995; Ganopolski et al., 1998). The annual temperature today in Yanan is around 9°C. If the annual temperature in Holocene is close to this value, the annual temperature during the last glacial time might have approached 0°C, close to the mean annual temperature at the peak of Donglingshan Mountain in North China (2303 m a.s.l. the annual temperature is around 0°C) (Fig. 1), where no C<sub>4</sub> species are present above 1750 m (Liu, 2003). In addition, an investigation in Northwest China (including the Loess Plateau) showed that C<sub>4</sub> species in natural vegetation are rarely found at sites with annual temperature less than 3°C (Wang, 2001). Thus, we argue that the C<sub>4</sub> plants abundance during the Last Glaciation were likely to be close to zero.

## 5 Conclusions

The <sup>13</sup>C enrichment by decomposition of organic matters can result in overestimation of the percentage of C<sub>4</sub> plants in paleovegetation using  $\delta^{13}\text{C}$  values of soil organic matter. This effect has been overlooked by previous investigators. This work provides data for quantitative corrections of the decomposition effect by measuring the  $\delta^{13}\text{C}$  variation in soil organic matter with depth in 14 soil profiles from three sites. These sites all have C<sub>3</sub> only vegetation, and include both forest and grassland vegetation type. We report that the average  $\delta^{13}\text{C}$  difference between soil organic matter and vegetation is 1.8 for the surface soil and 2.8 for the bottom (of the profiles) soil.

The effect of precipitation on  $\delta^{13}\text{C}$  of C<sub>3</sub> and C<sub>4</sub> plants has been assessed by a comprehensive investigation of a large number of species (255 of C<sub>3</sub> and 43 C<sub>4</sub> species) from 33 sites in North China, adding additional data to the exiting database for semi-arid to semi-moist ecosystems. A strong negative relationship is found between the site-averaged  $\delta^{13}\text{C}$  of C<sub>3</sub> plants and the annual rainfall amount with a coefficient of –0.40%/100 mm, while no significant correlations are observed for both C<sub>4</sub> plants as a group and *Setaria viridis* as a single C<sub>4</sub> species, although  $\delta^{13}\text{C}$  trends toward slight

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increase with increasing precipitation.

This study demonstrates that the paleovegetation reconstruction can be significantly affected by various corrections to the  $\delta^{13}\text{C}_{\text{SOM}}$  value, and to the end member  $\delta^{13}\text{C}$  values of  $\text{C}_3$  and  $\text{C}_4$  plants before using mass balance calculations. We recommend that corrections for the changes in the atmospheric  $\delta^{13}\text{C}$ ,  $^{13}\text{C}$  enrichment by decomposition, and changes in precipitation at the given location are necessary. Corrections for changes in the atmospheric  $\text{CO}_2$  concentration may also be needed, but we currently have relatively poor knowledge on how much that correction should be. Using these corrections, we show that  $\text{C}_4$  plants were likely to be absent during the Last Glaciation on the central Chinese Loess Plateau, which, considering the paleo-temperature and precipitation condition, is consistent with distributions of  $\text{C}_4$  plants in the modern environment.

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**Table 1.** Observed carbon isotopic differences between vegetation or plant litter and soil organic matter.

	Site 1		Site 2		Site 3	
	$\delta^{13}\text{C}_{\text{sur-veg}}$	$\delta^{13}\text{C}_{\text{botm-veg}}$	$\delta^{13}\text{C}_{\text{sur-litter}}$	$\delta^{13}\text{C}_{\text{botm-litter}}$	$\delta^{13}\text{C}_{\text{sur-veg}}$	$\delta^{13}\text{C}_{\text{botm-veg}}$
Profile 1	0.7	5.2	1.5	3.7	2.5	3.9
Profile 2	1.0	3.7	2.1	3.8	1.4	3.4
Profile 3	0.6	5.9	2.4	3.7	2.2	3.3
Profile 4	1.0	5.2	3.0	5.0		
Profile 5	1.1	2.6	2.5	4.5		
Profile 6	1.0	3.2				
Mean	0.9	4.3	2.3	4.1	2.0	3.5
Stand. Error	0.1	0.5	0.2	0.2	0.3	0.2

The subscripts: sur=surface soil; botm=bottom soil; veg=vegetation; litter=plant litter at the soil surface.

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**Table 2.** Comparison of  $\text{C}_4\%$  contribution to paleovegetation with various corrections.

<b>Holocene</b>			Using Eq. (1)*	Using Eq. (3)*
	$\delta^{13}\text{C}_{\text{C}_3}$	$\delta^{13}\text{C}_{\text{C}_4}$	$\text{C}_4\%$	$\text{C}_4\%$
Today's vegetation	-27.5	-12.5	53.3	41
$\delta^{13}\text{C}_{\text{atm}}$ correction (+1.3)	-26.2	-11.2	44.7	32.7
$\text{CO}_2$ concentration correction ( $\text{C}_3$ : +1.6‰)	-24.6	-11.2	38.1	24.6
<b>Last Glaciation</b>			Using Eq. (1)*	Using Eq. (3)*
	$\delta^{13}\text{C}_{\text{C}_3}$	$\delta^{13}\text{C}_{\text{C}_3}$	$\text{C}_4\%$	$\text{C}_4\%$
Today's vegetation	-27.5	-12.5	34	22
$\delta^{13}\text{C}_{\text{atm}}$ correction (+1.3‰-0.3‰)	-26.5	-11.5	27.3	15.3
Precipitation correction ( $\text{C}_3$ : +0.8‰)	-25.7	-11.5	23.2	10.6
$\text{CO}_2$ concentration correction ( $\text{C}_3$ : +1.6‰+1.6‰)	-22.5	-11.5	0.9	-15.5

\* Calculations assume that  $\delta^{13}\text{C}_{\text{SOM}}$  is -19.5‰ for Holocene and -22.4‰ for LG.

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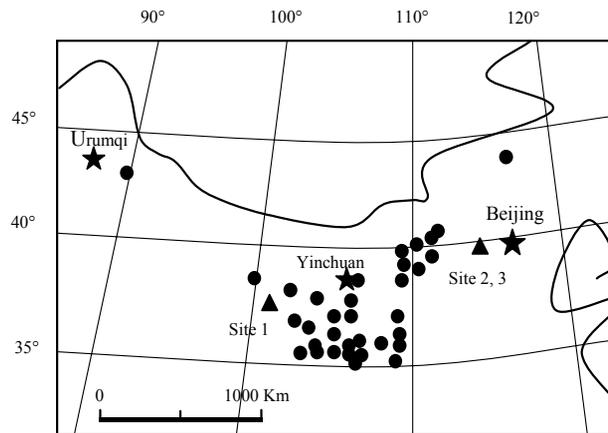
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**Fig. 1.** Sample sites of this study. Solid circles and triangles represent plant and soil profile sites, respectively.

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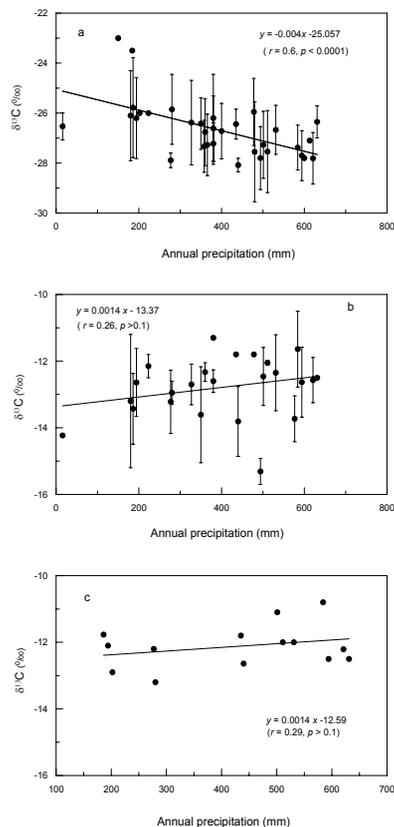
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**Fig. 2.**  $\delta^{13}\text{C}$  variations of plant leaves with the annual precipitation amount along a rainfall gradient in North China. **(a)** Variations of site-averaged  $\delta^{13}\text{C}$  in  $\text{C}_3$  plants. **(b)** Variations of site-averaged  $\delta^{13}\text{C}$  in  $\text{C}_4$  plants. **(c)**  $\delta^{13}\text{C}$  variations of *Setaria viridis* (a  $\text{C}_4$  species). Each bar represents the mean  $\delta^{13}\text{C}$  value averaged for a given site and the standard deviation of that site.

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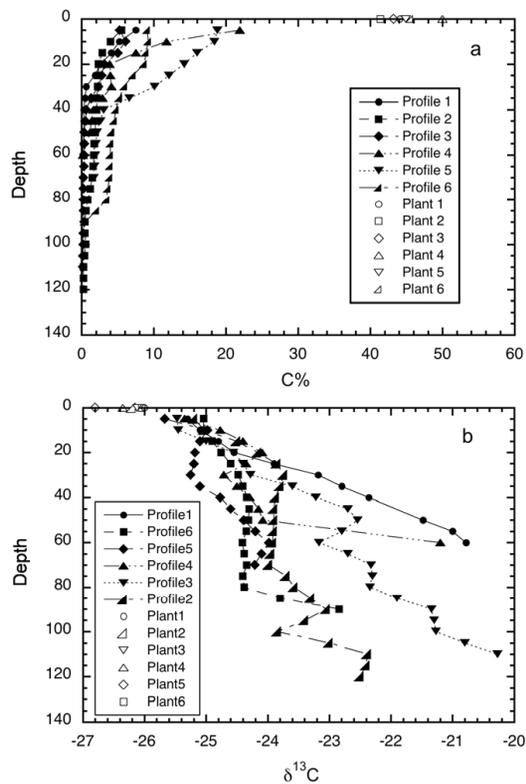
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**Fig. 3.** Carbon contents of soil and aboveground plants (a) and  $\delta^{13}\text{C}$  values of soil organic matter and aboveground plants (b) at Site 1. The organic carbon contents and  $\delta^{13}\text{C}$  values at 0 cm depth of each profiles are those for aboveground plants within each plots.

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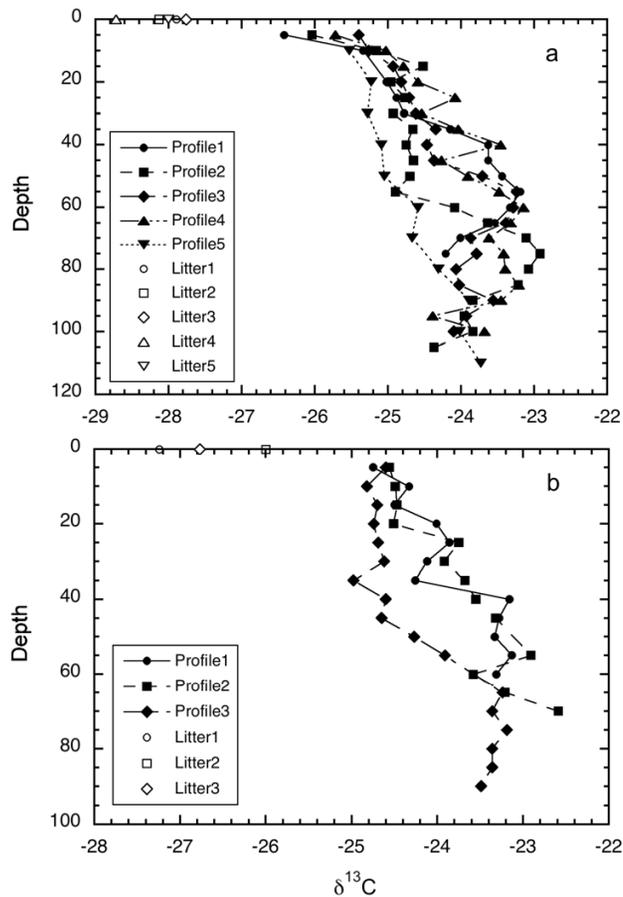
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**Fig. 4.** Variations of  $\delta^{13}\text{C}$  values of organic matter with depths at **(a)** Site 2, and **(b)** Site 3. The  $\delta^{13}\text{C}$  value at the 0 cm depth of each profile is that of litter within each plot in (a), and aboveground plants in (b).

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