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Responses of carbon isotope ratios of C_3 herbs to humidity index in northern China*

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Abstract: Uncertainties would exist in the relationship between $\delta^{13}C$ values and environmental factors such as temperature, resulting in unreliable reconstruction of paleoclimates. It is therefore important to establish a rational relationship between plant $\delta^{13}C$ and a proxy for paleoclimate reconstruction that can comprehensively reflect temperature and precipitation. By measuring the $\delta^{13}C$ of a large number of C_3 herbaceous plants growing in different climate zones in northern China and collecting early reported $\delta^{13}C$ values of C_3 herbs in this study area, the spatial features of $\delta^{13}C$ values of C_3 herbs and their relationships with humidity index were analyzed. The $\delta^{13}C$ values of C_3 herbaceous plants in northern China ranged from −29.9‰ to −25.4‰, with the average value of −27.3‰. The average $\delta^{13}C$ value of C_3 herbaceous plants increased notably from the semihumid zone to the semiarid zone to the arid zone; the variation ranges of $\delta^{13}C$ values of C_3 plants in those 3 climatic zones were −29.9‰ to −26.7‰ (semihumid area), −28.4‰ to −25.6‰ (semiarid area), and −28.0‰ to −25.4‰ (arid area). In the semiarid zone, the semihumid zone, and the whole northern area, $\delta^{13}C$ values of C_3 herbs showed obvious linear negative correlation to humidity indexes ($P < 0.05$). With the increase of humidity indexes, the average $\delta^{13}C$ value of C_3 herbaceous plants tended to decrease to different extents. In the arid zone, however, a linear positive correlation was found between them ($P < 0.05$). With every 0.1 increase in humidity index, the average $\delta^{13}C$ value increased significantly by 1.3‰. Temperature is the main reason for different ^{13}C fractionation abilities of C_3 herbs occurring in different sampling sites. The highly varying response of $\delta^{13}C$ of C_3 herbaceous plants to humid index reminds us that $\delta^{13}C_{plant}$-based paleoclimate reconstruction in northern China should be carried out according to the different climatic zones.

Key words: Arid and humid climate zones, C_3 herbaceous plants, carbon isotope, humidity index, northern China

1. Introduction

Over recent decades, stable carbon isotopes ($\delta^{13}C$) from terrestrial archives have been used to trace the course of past climatic and environmental changes (Dawson and Siegwolf, 2007; Werner et al., 2012). This is because variations in plant carbon isotope values record a lot of information reflecting past climatic and environmental changes such as temperature, humidity, and precipitation (Saurer et al., 1995; Loader et al., 2007; Dodd et al., 2008; Diefendorf et al., 2010). Consequently, an understanding of factors controlling plant carbon isotope value enhances reconstructions of past climate and ecology using carbon isotope records of ancient terrestrial sediment (Kohn, 2010). The relationships between $\delta^{13}C$ compositions of vegetations and environmental factors in northern China have been studied by researchers at home and abroad (Su et al., 2003; Wang et al., 2005, 2010; Zhao et al., 2005; Chen et al., 2007; Sun et al., 2007, 2009; Ma et al., 2007, 2012); however, most studies are limited to a certain single climatic or environmental factor, such as air temperature, precipitation (soil moisture), or altitude.

Temperature and precipitation are 2 decisive factors affecting plant growth and vegetation distribution and hence affect the stable carbon isotope compositions of plants. As for plants, temperature can affect their carbon isotope fractionation via the change in biochemical reaction speed during the photosynthesis process (such as the activity of enzymes participating in photosynthesis) and the stomatal conductance of leaves. There are some studies showing that carbon isotope values of C_3 plants were negatively correlated to temperature (Körner et al., 1991; Ning et al., 2002; Li et al., 2009), while there are even more studies indicating that positive correlation existed between carbon isotopes and temperature (Li et al., 1999; Wang et al., 2002; Liu et al., 2007; Lin, 2008). Still, some other studies suggested that there were no links between

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δ13C and temperature (Zhang et al., 2003; Gebrekirstos et al., 2009; Diefendorf et al., 2010). In addition to differences in carbon physiological metabolism processes of different plant species and genetic characteristics, the uncertainty regarding the relationship between δ13C values of C_3 plants and temperature may relate to the difficulty in separating the influence of other environmental factors, such as precipitation (soil moisture), evaporation, and lighting on the δ13C values, as well as the interaction of various factors. This is because the temperature factor influencing δ13C values of plants is often cross-correlated with other environmental factors.

Farquhar et al. (1982) stated that precipitation, as an important environmental factor, cannot be ignored regarding its influence on the δ13C values of plants. For example, carbon isotope values often decrease with the increase of precipitation (Wang et al., 2003, 2008; Kohn, 2010), although there are also some studies obtaining opposite results (Su et al., 2000). Therefore, if the interference of precipitation cannot be eliminated, uncertainties will exist in the relationship between δ13C values and environmental factors such as temperature, resulting in unreliable reconstruction of the paleoclimate, extraction of paleoecology information, and explanation of stable carbon isotopic composition (Edwards et al., 2000; Valery et al., 2008). Thus, it is important to establish a rational relationship between δ13C values of plants and a proxy for climatic reconstruction that can comprehensively reflect air temperature and precipitation.

The humidity index (HI), a parameter suggested by Hulme et al. (1992), can comprehensively reflect the dry/wet state in that it considers 2 major factors affecting the water and heat balance of land surface, precipitation and potential evaporation, simultaneously. It is thus a rational parameter to be used for analyzing the relationship between climate variable and plant δ13C values. So far, however, there are few reports that combined climatic HIs and δ13C values.

Northern China is a region with a fragile ecological environment and serious land desertification. The vegetative ecosystem is an obvious indicator of climatic changes. In the present study, in order to provide a reference for climatic reconstruction using the carbon isotope of plants, we calculated the HIs of all sampling sites in different climatic areas in northern China and investigated the spatial features of δ13C compositions of C_3 herbaceous plants and their relationships with HIs based on the measured carbon isotopes of plants and results reported at home and abroad.

2. Materials and methods

2.1. Study area and data sources

The study area is located in the arid, semiarid, and semihumid regions of northern China (Figure 1). The

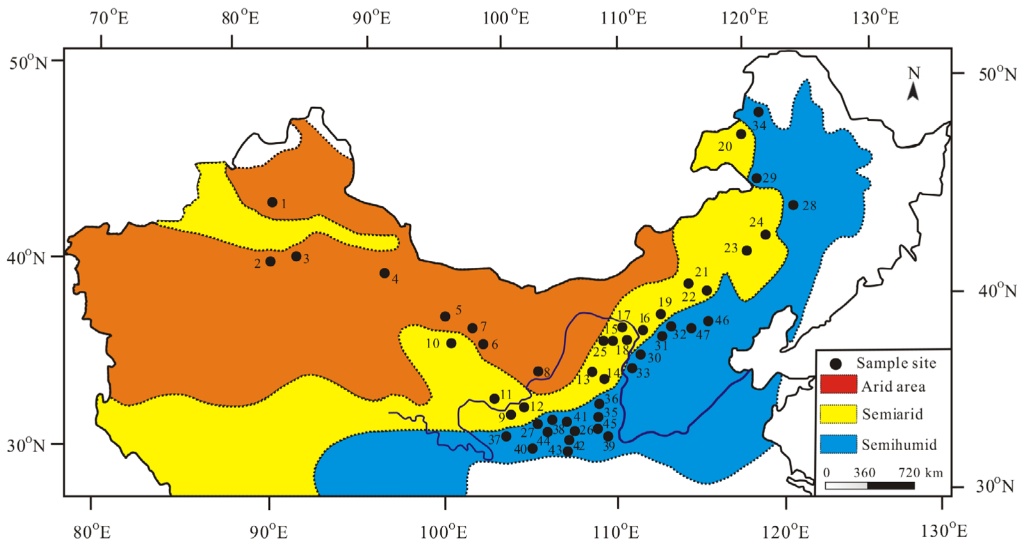

Figure 1. Distribution of sampling sites in the different climatic areas in northern China. Sample sites are indicated with numbers. 1, Junggar Basin; 2, Urumqi; 3, Fukang; 4, Kami; 5, Jinta; 6, Shandan; 7, Pingchuan; 8, Shapotou; 9, Lanzhou; 10, Su'nan; 11, Huangzhong; 12, Yuzhong; 13, Jinbian; 14, Hengshan; 15, Dongsheng; 16, Ejin Horo Banner; 17, Ordos; 18, Jungar Banner; 19, Feng Zhen; 20, Yakeshi; 21, Zhengxiangbai Banner; 22, Duolun; 23, Bairin Left Banner; 24, Jarud Banner; 25, Yulin: 26, Changwu; 27, Xiji; 28, Ulan hot; 29, Arxan: 30, Shenmu; 31, Hequ; 32, Youyu; 33, Mizhi; 34, Genhe: 35, Lochuan: 36, Ansai; 37, Xifeng; 38, Pingliang; 39, Linxia; 40, Guyuan; 41, Fuxian; 42, Chengxian; 43, Yangling; 44, Yongshou; 45, Tongchaun; 46, Beijing; 47, Hezuo.

semiarid area in northern China is a transition zone, which is a marginal region where the East Asian summer monsoons are getting so weak that the monsoonal rainfall may be extremely low in some years. To the south of this region is the semihumid North Plain, where 50%–60% of annual precipitation falls in July and August and the annual temperature is around 6–8 °C. To the north is the nomadic region, and the annual precipitation decreases from the southeast to the northwest. Due to insufficient rainfall, frequent droughts and overgrazing, the environment in the study area has been recognized as one of the most ecologically fragile zones in China.

A part of the data used in our study was derived from international and domestic literature regarding carbon isotopes of plants in northern China, including carbon isotope data of C_3 herbaceous plants and corresponding geographic data (longitude, latitude, and altitude) of 13 sampling sites (Table 1; Figure 1); the other part of the $\delta^{13}C$ data originated from 217 plant samples collected from 34 sampling sites in the farming-pastoral ecotone of northern China (Table 1; Figure 1). The climatic data, including the mean annual temperature (MAT), mean annual precipitation (MAP), monthly precipitation, and monthly mean temperature of each sampling site in the sampling year, were provided by the local weather bureau or from the Chinese Climate Center and China Meteorological Science Data Sharing Service System (http://cdc.cma.gov.cn). In addition, the corresponding longitude, latitude, and altitude of each site were measured by portable GPS (Magellan GPS Field PROV™, USA). The dominant vegetation types of all sites spanned from cold temperate semihumid forest zone to temperate arid and semiarid desert grassland. Detailed information of the sites is given in Table 1.

2.2. Plant sampling and measurement of leaf $\delta^{13}C$

In our investigation of 34 sampling sites, plants were sampled in the summer of 2008 between 25 July and 30 August. All plants collected were either the dominant species in the local area or occurred widely in the 3 climatic zones to obtain spatial variations of carbon isotope compositions of the same plant species. In order to minimize the influence of human activity, sunshine regime, and location within the canopy, sampling was restricted to flat, broad, and bright sites far from human habitats. Mature sunny leaves of 118 species of C_3 herbaceous plants were collected. Upon sampling, the number of the same plant species collected in a sampling site could not be less than 5 to 7 individual plants. Depending on the number of leaves of each species, the same number of leaves were collected from each plant and then mixed together as a sample for this species. A total of 217 samples were collected (Table 1).

The plant samples were oven-dried at 70 °C for 48 h and ground to 40 mesh-size. Leaf carbon isotope ratios were determined using a Delta-^{Plus}XP mass spectrometer (Thermo Scientific, Germany) coupled with an elemental analyzer (Flash EA 1112; CE Instruments, UK) in continuous flow mode at the College of Resources and Environment, China Agricultural University. The combustion temperature of the elemental analyzer was 1020 °C. The measurement error was ±0.15‰ and the $\delta^{13}C$ data were expressed relative to the V-PDB standard.

2.3. Calculation of HI

According to the suggestion of Hulme et al. (1992), the HI can be written as follows:

$$HI = \frac{R}{Pe} \quad (1)$$

where R is the annual precipitation (mm) and Pe is the annual potential evapotranspiration (mm). As Holdridge's scheme has clear applicability and ecological significance (Meng et al., 2004), it is suitable for calculating the potential evapotranspiration and can be expressed as follows:

$$Pe = 58.93 \times ABT \quad (2)$$

where ABT indicates the annual biotemperature (°C). It refers to the average temperature for the vegetative growth of the plants, ranging from 0 °C to 30 °C in general, excluding daily average temperatures below 0 °C and above 30 °C, and hence the formula for calculation of ABT is as follows:

$$ABT = 1/12 \sum_1^{12} T \quad (3)$$

where T represents the monthly average temperature higher than 0 °C; however, the monthly average temperature higher than 30 °C shall be regarded as 30 °C and the monthly average temperature lower than 0 °C shall be regarded as 0 °C. Combining Eqs. (1) through (3), we obtain the calculation formula for HI as follows:

$$HI = R / \left(58.93 \times \frac{1}{12} \sum_1^{12} T \right) \quad (4)$$

2.4. Statistical analysis

SPSS 12.10 for Windows (SPSS Inc., USA) was used for data correlation analysis, regression analysis, and one-way analysis of variance (ANOVA). If the variance analysis results for the $\delta^{13}C$ values of all plants in the various climatic zones were significant ($P < 0.05$), then the least significant range method (Duncan's new multiple range method) was used for multiple comparison. As the $\delta^{13}C$ values of plants were affected by mountain trend, microrelief form, and altitude, the carbon isotope data of plants collected from sampling sites on high mountains were avoided as much as possible.

Table 1. Information of the sampling sites.

Site no.	Longitude (°E)	Latitude (°N)	MAT (°C)	MAP (mm)	Altitude (m)	Averaged $\delta^{13}C$ (‰)	Vegetation type	Sampling date	n	Data source	Climatic region
1	85.92	44.65	8.0	150	477	−26.9 ± 0.85	Herbage	18.05.2006	23	Sun et al., 2009	
2	86.62	42.75	6.4	184	690	−27.1 ± 0.00	Herbage	26.07.1995	1	Feng et al., 2003	
3	87.83	43.17	6.1	164	650	−26.5 ± 1.25	Shrubs	31.07.1997	24	Chen et al., 2002	
4	93.67	42.82	9.8	65	800	−27.6 ± 0.65	Herbage	26.07.2008	8	Observed	Arid area
5	98.90	40.00	8.0	154	1250	−26.9 ± 2.34	Shrubs	31.07.1997	8	Chen et al., 2002	
6	101.00	38.17	5.7	177	1764	−25.2 ± 0.49	Shrubs	31.07.2008	3	Observed	
7	100.01	39.33	7.6	186	1547	−25.4 ± 0.49	Shrubs	30.08.2002	2	Su and Yan, 2008	
8	104.95	37.45	9.6	184	1250	−28.0 ± 1.42	Shrubs	11.08.2008	12	Observed	
9	103.83	36.00	6.6	327	1517	−28.0 ± 0.00	Herbage	28.07.2008	1	Observed	
10	99.63	38.82	3.6	214	2204	−25.9 ± 0.65	Herbage	05.08.2008	6	Observed	
11	101.52	36.65	2.9	380	2260	−25.6 ± 0.50	Herbage	07.08.2008	2	Observed	
12	104.02	36.55	6.6	350	1896	−27.0 ± 0.56	Herbage	30.08.2008	8	Observed	
13	108.50	37.37	7.8	395	1333	−27.3 ± 0.37	Herbage	29.08.2008	6	Observed	
14	109.17	37.28	8.5	390	1019	−26.9 ± 0.45	Herbage	27.08.2008	7	Observed	
15	109.98	39.03	5.4	363	1461	−27.1 ± 0.54	Herbage	26.08.2008	10	Observed	
16	110.05	39.17	6.2	380	1276	−27.2 ± 0.65	Herbage	25.08.2008	9	Observed	
17	110.47	39.35	6.4	350	1108	−27.3 ± 0.68	Herbage	24.08.2008	8	Observed	Semiarid area
18	110.27	39.03	7.5	392	1249	−26.7 ± 1.23	Herbage	23.08.2008	9	Observed	
19	113.45	40.27	4.7	347	1195	−27.9 ± 0.74	Herbage	22.08.2008	10	Observed	
20	120.43	49.17	−2.9	289	676	−28.2 ± 0.84	Forest	01.08.2008	11	Observed	
21	115.12	42.23	1.5	314	1405	−27.5 ± 0.36	Herbage	03.08.2008	8	Observed	
22	116.47	42.18	2.4	386	1245	−28.4 ± 0.65	Herbage	04.08.2008	8	Observed	
23	119.40	43.98	5.3	390	486	−27.5 ± 0.58	Herbage	06.08.2008	10	Observed	
24	120.90	44.57	2.8	383	491	−28.8 ± 0.73	Herbage	08.08.2008	8	Observed	
25	109.50	38.20	10.0	400	1025	−27.3 ± 1.23	Herbage	16.08.2008	3	Observed	
26	107.73	35.20	9.1	584	847	−27.8 ± 0.42	Forest	09.08.2008	3	Observed	
27	105.72	35.97	5.3	425	1931	−26.4 ± 0.50	Herbage	15.08.2008	7	Observed	
28	122.03	46.05	4.1	443	433	−27.8 ± 0.34	Herbage	10.08.2008	10	Observed	
29	119.93	47.17	−2.7	453	997	−28.3 ± 0.45	Herbage	13.08.2008	10	Observed	
30	110.48	38.23	8.9	441	1226	−27.1 ± 1.39	Herbage	20.08.2008	10	Observed	
31	111.13	39.38	8.8	460	875	−27.4 ± 0.57	Herbage	12.08.2008	8	Observed	
32	112.27	40.00	8.6	450	1358	−27.4 ± 0.47	Herbage	19.08.2008	8	Observed	
33	110.23	37.67	8.3	441	2104	−27.2 ± 0.96	Herbage	30.06.2005	7	Zheng & Shang, 2007	
34	121.32	50.47	−5.3	437	718	−28.6 ± 0.63	Forest	28.08.2008	9	Observed	
35	109.40	35.70	9.2	621	1100	−28.0 ± 1.62	Forest	12.08.2008	4	Observed	
36	109.33	36.75	8.8	531	1372	−27.6 ± 1.03	Herbage	12.06.2005	9	Zheng & Shang, 2007	Semihumid area
37	107.67	35.70	8.9	594	1421	−26.9 ± 0.07	Herbage	21.08.2008	2	Observed	
38	106.67	35.55	8.6	511	1560	−26.8 ± 0.00	Herbage	14.08.2008	1	Observed	
39	103.20	35.60	6.3	501	2000	−26.7 ± 0.46	Herbage	17.08.2008	4	Observed	
40	106.27	36.00	6.2	478	1753	−26.4 ± 0.64	Herbage	18.08.2008	2	Observed	
41	109.42	35.50	9.0	600	1085	−28.1 ± 0.97	Herbage	18.06.2005	9	Zheng & Shang, 2007	
42	105.38	34.35	11.9	650	997	−30.9 ± 0.00	Forest	26.07.1995	1	Feng et al., 2003	
43	107.93	34.23	12.9	637	447	−29.2 ± 2.20	Herbage	20.06.2005	2	Zheng & Shang, 2007	
44	107.93	34.48	10.8	609	421	−27.5 ± 1.69	Herbage	22.06.2005	5	Zheng & Shang, 2007	
45	108.57	35.57	10.2	650	970	−27.5 ± 0.66	Herbage	25.06.2005	6	Zheng & Shang, 2007	
46	116.38	40.00	11.5	595	1100	−27.7 ± 0.42	Forest	02.08.2008	2	Observed	
47	112.90	39.98	3.2	530	2400	−28.9 ± 0.98	Forest	26.07.1995	3	Feng et al., 2003	

3. Results

3.1. δ13C compositions of C_3 herbaceous plants in different climatic zones

Figure 2 shows the average values and ranges of δ13C values of C_3 herbaceous plants in northern China as well as in various climatic zones. Overall, the δ13C values in all C_3 plant samples in northern China ranged from –29.9‰ to –25.4‰ with an average value of –27.3‰ (n = 327, SD = 1.47), while those in the arid zone of northern China were narrow, mainly between –28.0‰ and –25.4‰ with a mean value of –26.84‰ (n = 81, SD = 1.25), which was slightly more than the average value of –27.2‰ (n = 124, SD = 1.31) obtained via the isotope analysis for the 124 C_3 plant samples collected from the semiarid climatic zone and significantly more positive compared with that of the semihumid zones (a mean value of –27.8‰, n = 122, SD = 1.35) in northern China.

3.2. Relationships between δ13C values of C_3 herbaceous plants and HIs in different climatic zones

Figure 3 plots the relationships between δ13C values of C_3 herbaceous plants as a whole and HIs in different climatic zones. In the arid zone, δ13C of plants as a whole increased significantly with rising HI, with a coefficient of 1.3‰ for every 0.1 increase in HI ($P < 0.05$; Figure 3a). In contrast to the arid zone, all C_3 herbaceous plants in the semiarid and semihumid zones displayed decreasing δ13C with the increase in HI, and for every 0.1 increase in HI, the δ13C value decreased by 1.1‰ for the semiarid zone (Figure 3b) and by 0.4‰ for the semihumid zone (Figure 3c). Remarkable negative relations between plant δ13C values and HI were observed in northern China (Figure 3d).

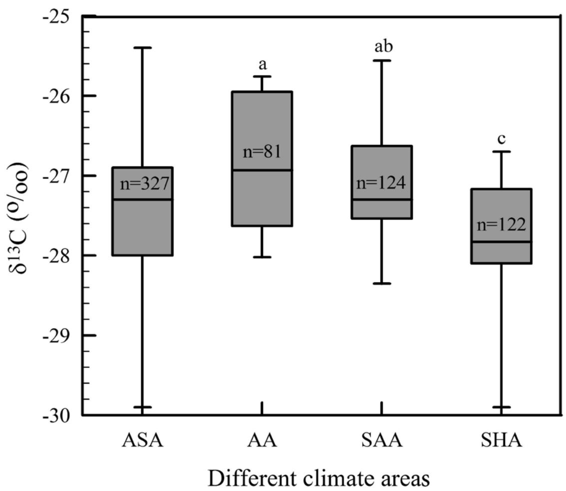

Figure 2. The spatial characteristics of δ13C values for whole C_3 herbaceous plants in the arid and humid climate areas of northern China. Different letters represent significant differences among different climate areas at α = 0.05 level. ASA, all the sampling areas; AA, arid area; SAA, semiarid area; SHA, semihumid area.

4. Discussion

4.1. δ13C variation of C_3 herbaceous plants

Among many environmental factors, precipitation and temperature are 2 of the most important factors exerting effects on plant δ13C. Except in extremely wet environments, δ13C of C_3 plants generally increases with decreasing rainfall (Korol et al., 1999; Wang et al., 2003; Zhang et al., 2003; Wang et al., 2010), although patterns of variation of δ13C in living plants with temperature remain unresolved (Wang et al., 2008; Kohn, 2010). In our study, the 124 C_3 plant samples in the semiarid climatic zone were collected from 17 sites, and the MAP of this climatic zone was 200–400 mm. Furthermore, the 122 plant samples from the semihumid climatic zone were mainly collected from the middle part of Shaanxi Province on the Loess Plateau, eastern Gansu, and the southeastern edge of the Inner Mongolia Plateau. The MAP of each sampling site was greater than 400 mm, which ranged basically from 420 mm to 660 mm. However, the 81 sample plants from the arid zone grew in an environment where precipitation is less than 200 mm. Thus, δ13C values of plants in the arid zone were slightly more positive than those in the semiarid and semihumid zones.

As for why the variation range of plant δ13C values in the arid zone was relatively more concentrated, it can be attributed to the climatic environmental conditions of the sampling sites, which were very similar. Statistical analysis was done for the MAT and MAP, and the results showed that the MAP of the sampling sites was 158.0 ± 40.11 mm, the variation of MAT was 5.7–9.6 °C, and the average temperature was 7.53 ± 1.24 °C. These results indicated that the degree of variation in the arid zone was significantly less than that of the semiarid and semihumid zones in northern China (Table 2).

In addition to the variation characteristics of δ13C values of all plants in the study area, we also analyzed the δ13C values of 5 eurytropic C_3 species, which were collected from 3 climatic zones in northern China. Figure 4 shows that obvious differences ($P < 0.05$) existed in the average δ13C values of *Chenopodium glaucum*, *Artemisia lavandulaefolia*, *Plantago depressa*, *Artemisia capillaris*, and *Lepidium apetalum* in different climatic zones, which caused the average values of the above plants in the semihumid zone to be slightly less than those in the semiarid and arid zones. This indicated that the carbon isotope compositions of C_3 herbaceous plants had consistent variation patterns for both individual plants and plants as a group in different climatic zones, suggesting that changes of precipitation were important for the variation of δ13C values of C_3 herbaceous plants in different climatic zones over the whole northern area. Additionally, such significant variance in the carbon isotope compositions of C_3 herbaceous plants in different climatic zones also

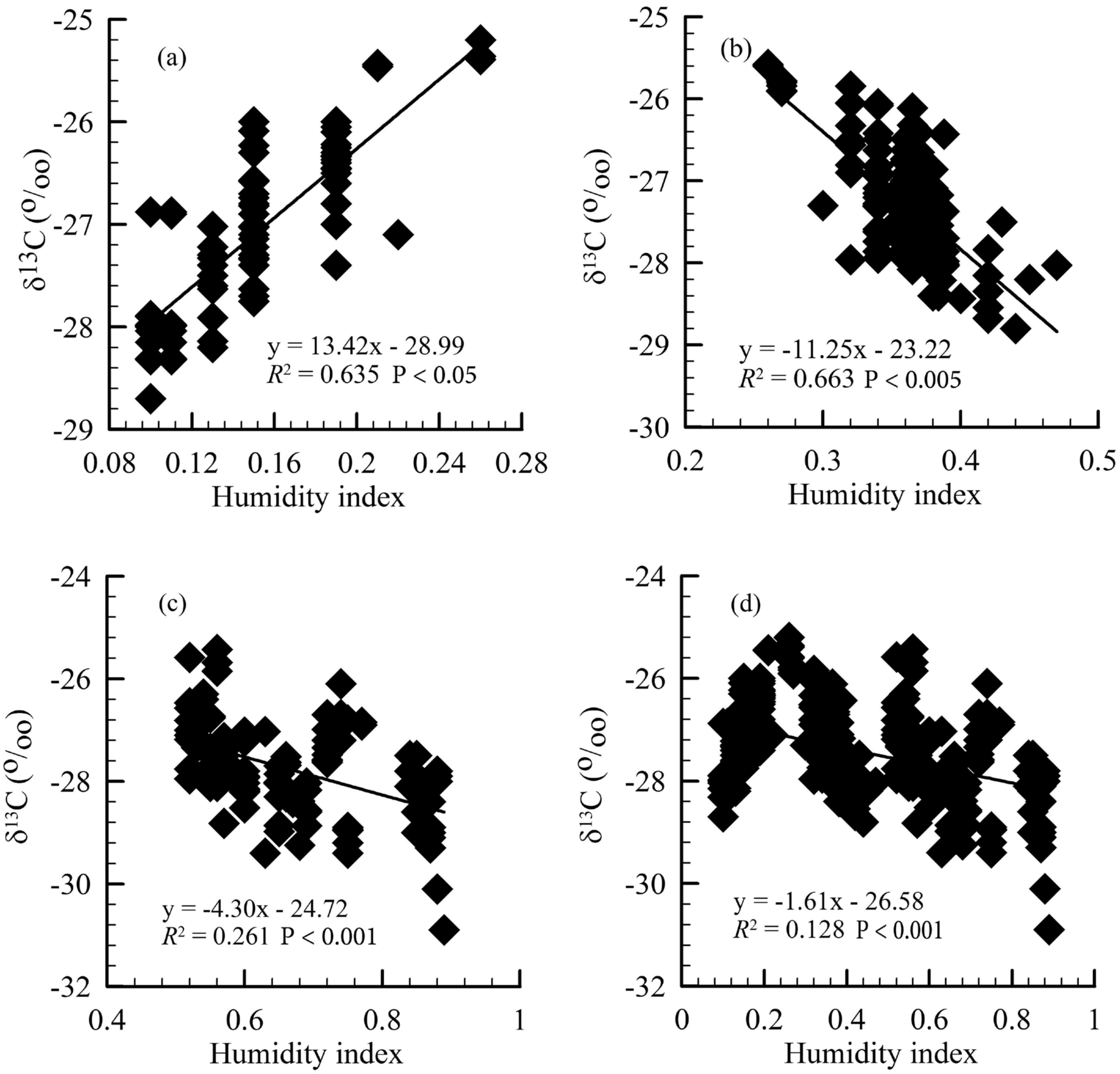

Figure 3. Relationships between $\delta^{13}C$ values of C_3 herbaceous plants and HI in different climate areas of northern China: a) arid area; b) semiarid area; c) semihumid area; d), all the sampling areas.

Table 2. The average value and variation coefficients of MAT and MAP for all sampling sites in different climatic areas of northern China.

Climatic area	MAT		Climatic area	MAP	
	Mean value of sites (°C)	CV (%)		Mean value of sites (mm)	CV (%)
Arid area	7.53 ± 1.24a	0.165b	Arid area	158.0 ± 40.11c	0.085c
Semiarid area	5.02 ± 3.09a	0.614a	Semiarid area	360.9 ± 48.13b	0.133ab
Semihumid area	7.40 ± 4.26a	0.575a	Semihumid area	530.6 ± 80.16a	0.151a

Note: MAT and MAP as in Table 1; CV, coefficient of variation. Different letters indicate significant difference ($P < 0.05$).

Note: The numbers of sampling sites are the same as in Figure 1; MAT and MAP are the abbreviations of mean annual temperature and mean annual precipitation, respectively. MAT and MAP represent the average values of more than 30 years; dominant vegetation types are from "The Vegetation Atlas of China".

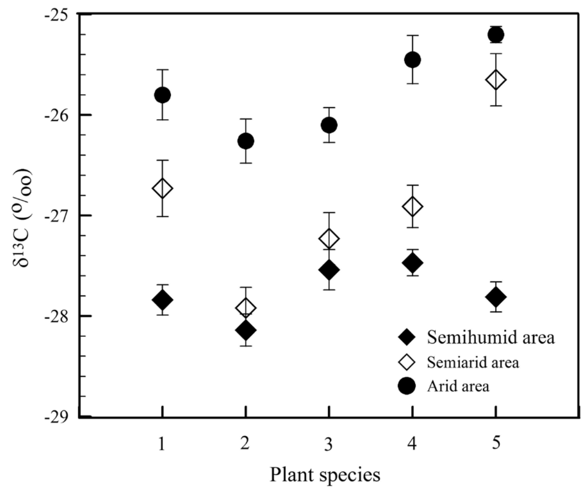

Figure 4. The spatial characteristics of $\delta^{13}C$ values for individual C_3 herbaceous plant in the arid and humid climate areas of northern China. The numbers 1–5 on horizontal axis denote *Chenopodium glaucum*, *Artemisia lavandulaefolia*, *Plantago depressa*, *Artemisia capillaris*, and *Lepidium apetalum*, respectively.

reminds us that when using $\delta^{13}C$ values of soil organic matter and soil carbonate to estimate the proportion of C_3 herbaceous plants in past vegetation, the sediment surroundings, especially the climatic environment, must be considered so as to choose the proper end member value of $\delta^{13}C$ for C_3 plants.

4.2. Response of plant $\delta^{13}C$ values to HIs

The influence of the HI on $\delta^{13}C$ composition of C_3 herbaceous plants results from the interactions of multiple meteorological factors such as temperature, precipitation, evaporation, soil humidity, and pressure of water vapor. In our study, a strong positive correlation was found between plant $\delta^{13}C$-values and HI for the arid zone ($R^2 = 0.6351$, $P < 0.05$); in other words, with the increase in HI, the $\delta^{13}C$-value of all plants as a whole gradually increased. Schulze et al. (1996), Su et al. (2000), and Skrzypek et al. (2007) also observed that $\delta^{13}C$ values of C_3 herbaceous plants increased with the increase in relative humidity or annual precipitation. Therefore, it is possible that the $\delta^{13}C$ values of C_3 herbaceous plants in arid zones of northern China increased with the rising HI, and the fact that the $\delta^{13}C$ values of single *Chenopodium glaucum* increased significantly with the increase in HI can be taken as strong evidence (Figure 5a). However, the HI–$\delta^{13}C$ correlation for the semiarid and semihumid zones was the opposite, which is consistent with the results of previous research (Li et al., 1995; Sparks and Ehleringer, 1997; Anderson et al., 2000; Wang et al., 2006).

For example, Stuiver and Braziunas (1987) analyzed the relationship between relative humidity and the $\delta^{13}C$ value of coniferous forest, which showed high negative correlation; Wang and Han (2001) also found that the $\delta^{13}C$ values of several C_3 herbaceous plants were obviously more positive in dry seasons than those in rainy seasons. The trend by which $\delta^{13}C$ values significantly increased with the decrease of HI might be related to the dry air or insufficient water content in the soil causing the decrease of the stomatal conductance of plants, which meanwhile indicated that these plant species under semiarid and semihumid conditions adapted to the ecological environment of different water contents by adjusting the stomatal conductance to change the water use efficiency.

Although the relationship between $\delta^{13}C$ values and HIs varied from zone to zone, it generally represented the variation of carbon isotope compositions of C_3 herbaceous plants in northern China according to the change in HIs because annual precipitation gradually decreased from east to west. In this study, the overall trend that $\delta^{13}C$ values of C_3 herbaceous plants significantly decreased with the increase in HI ($R^2 = 0.1281$, $P < 0.001$) was consistent with the results reported by Wang et al. (2003) that the values of carbon isotopes of 367 C_3 herbs samples in northern China were obviously negative with the increase in MAP. Therefore, it was rational to use the $\delta^{13}C$ value as a proxy of the climatic HI to study the paleoclimate or paleoenvironment of northern China.

For the single C_3 species, Winter et al. (1982) reported that $\delta^{13}C$ values of C_3 herbaceous plants such as *Triticum aestivum* and *Poa annua* were slightly more positive in a low-humidity than in a high-humidity environment. In this study, as statistical analysis could not be done for the vast majority of plants due to the limitation of sample size, only 3 C_3 species (*Plantago depressa*, *Chenopodium glaucum*, and *Lepidium apetalum*), which were widely distributed in the same climatic zone with multiple data points, were analyzed. The $\delta^{13}C$ values for the 3 plants showed a decrease with the increase in HI, except for *Chenopodium glaucum* in the arid zone, but the magnitude of descent and degree of relevance between plant $\delta^{13}C$ values and the HIs varied, even for the same species, and varied from zone to zone (Figures 5a–5c). This indicated that their sensitivities were different against the HIs, with the reason that $\delta^{13}C$ values of the plants were the result of the joint action of the plant species and environmental factors (Yan et al., 1998).

Such differences, expressed by the change in $\delta^{13}C$ values due to the HI, might be related to the variances in the carbon isotope fractionation caused by the change of plant physiological characteristics to adapt to the environmental conditions. Additionally, these variances may also relate to the small sample size of individual species. The above different plants and the same species having different $\delta^{13}C$ variation rates in different climatic

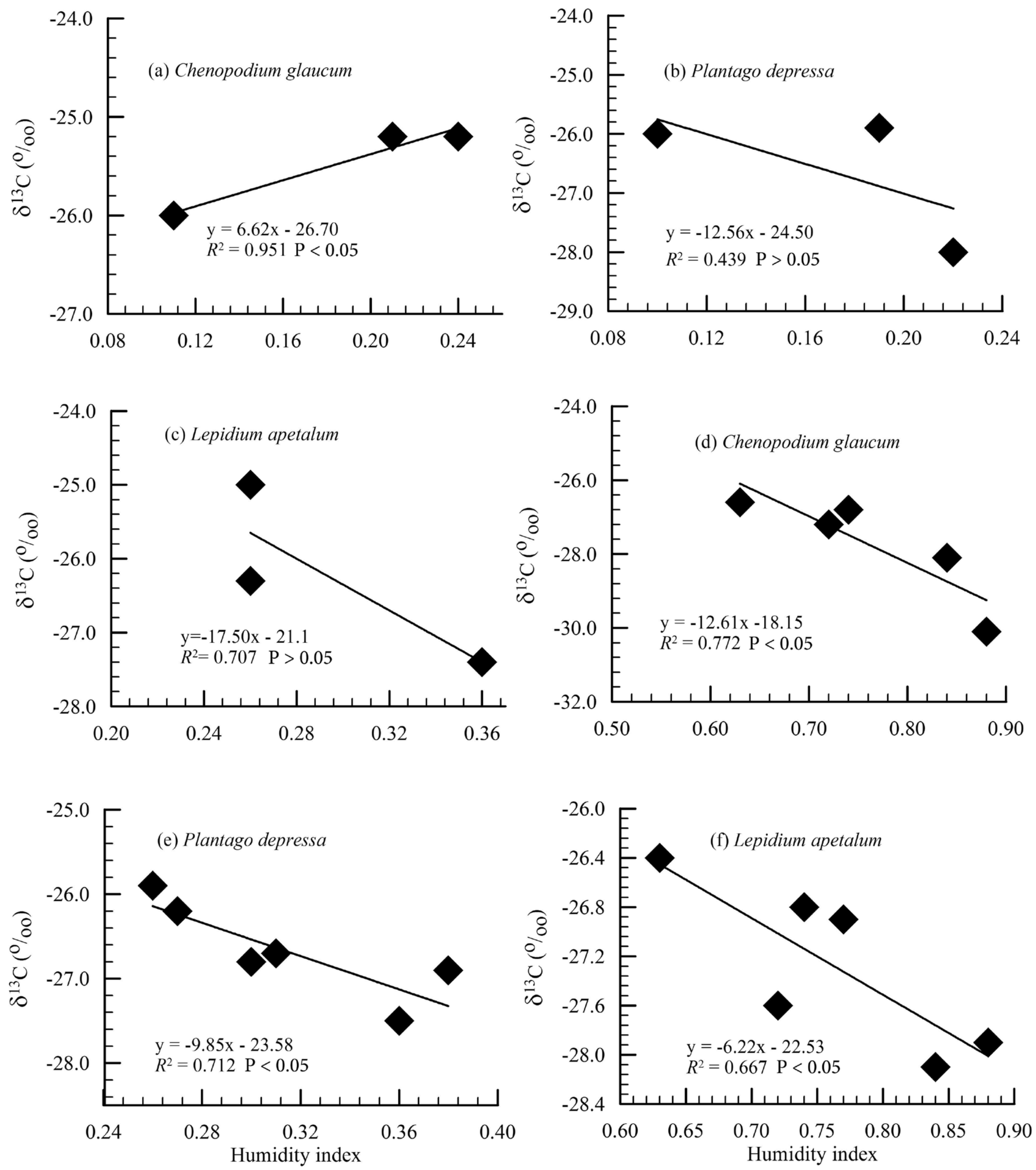

Figure 5. The correlation between $\delta^{13}C$ values of single plant C_3 and humidity index in different climate areas of northern China: a) and b), arid area; c) and d), semiarid area; e) and f), semihumid area.

zones reminded us that when using $\delta^{13}C$ values of plants to reconstruct the paleoclimate and paleoenvironment, choosing the plant species that are the most sensitive to the changes in environmental indicators might gain the most valuable results.

Based on the theories of Farquhar et al. (1982, 1989), when precipitation is insufficient or air humidity is reduced, the stoma of plants close and stomatal conductance is reduced, which can lead to CO_2 concentration decrease in plant leaves and the increase of the carbon isotope ratio of photosynthetic products. The fact that the $\delta^{13}C$ values of C_3 herbs in the semiarid zone, semihumid zone, and the whole northern area were negatively correlated to HIs provides strong evidence for the above point. However, in the northern arid zone, the influence of HIs on the $\delta^{13}C$ values was not that simple. Viewed from the whole northern area, as precipitation gradually decreased from east to west the plant $\delta^{13}C$ values significantly decreased with the increase in HI, but the $\delta^{13}C$ values in the arid zone showed an ascending trend. A very possible reason why this happens is that the influence of annual temperature on C_3 plant $\delta^{13}C$ outweighs that of precipitation.

According to Eqs. (1) through (4), the HI is a ratio between annual precipitation and annual maximum evapotranspiration, while in the arid zone of northern China the annual precipitation was the main factor limiting plant growth. The MAP of each sampling site in this zone varied slightly (Table 2), which to a certain degree eliminated the interference of precipitation. Thus, the change of HI mainly depended on the evapotranspiration, which was closely related to temperature. That is, when the temperature increased, the soil evaporation and transpiration would be intensified, the evapotranspiration would increase, and, hence, the HI would decrease. Simple regression analysis showed that in the northern arid zone, linear negative correlation existed among the HIs, the $\delta^{13}C$ values of C_3 herbaceous plants, and the MAT. With the increase in MAT, both HIs and $\delta^{13}C$ values of C_3 herbaceous plants significantly decreased (Figures 6a and 6b), while the relationships among HIs, $\delta^{13}C$ values of plants, and MAP were not obvious (Figures 6c and 6d). Thus, the $\delta^{13}C$ values of C_3 herbaceous plants and HIs were significantly and positively correlated.

Many factors other than temperature and precipitation, such as altitude, longitude, and latitude, can affect plant $\delta^{13}C$ (Körner et al., 1991; Sparks and Ehleringer, 1997; Li et al., 2009; Wang et al., 2010). Here, we neglected the effects of these factors when establishing relationships between HIs and $\delta^{13}C$ because we think that these effects are unlikely to affect our results significantly. This consideration is based on the fact that the variations of altitude, longitude, and latitude tend to cause changes in temperature and/or precipitation. Therefore, their effects on plant $\delta^{13}C$ will be embodied in the effects of temperature and precipitation. Although other environmental factors may also vary with altitude, longitude, and latitude, e.g., changes in atmospheric pressure and solar radiation with elevation, it is generally thought that the altitudinal trend of plant $\delta^{13}C$ can be attributed mainly to the influence of temperature and/or precipitation rather than to changes in air pressure and solar radiation (Sparks and Ehleringer, 1997; Li et al., 2009; Wang et al., 2010, 2013). In addition, the HI calculated by the Holdridge model may have some limitations, but to a certain extent, it considers the combined influence of precipitation and evaporation related to temperature, wind speed, solar radiation, pressure of water vapor, and other meteorological factors (Wang et al., 2004). Therefore. the variation pattern of $\delta^{13}C$ values of C_3 herbaceous plants in the whole northern China affected by HIs was actually effective.

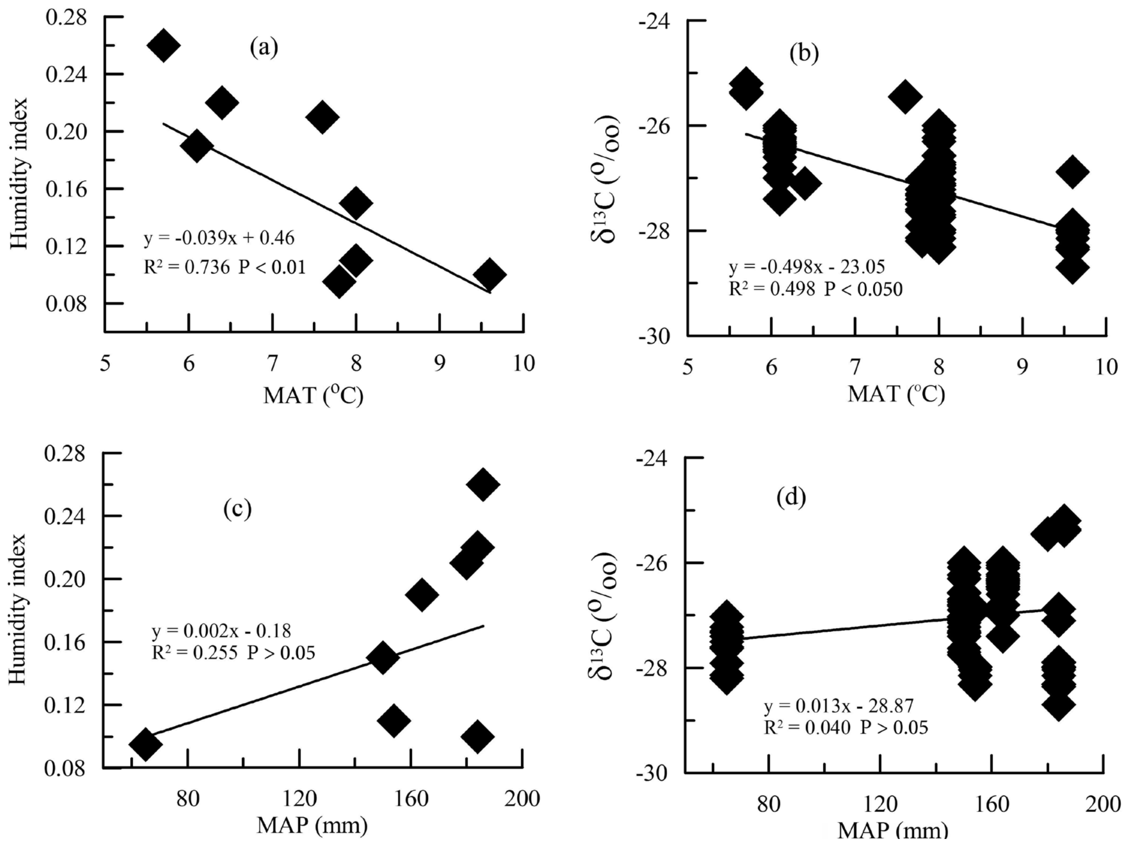

Figure 6. Relationships of humidity index and $\delta^{13}C$ for C_3 herbaceous plants with mean annual temperature (a and b) and mean annual precipitation (c and d) in arid area of northern China.

We here compared the spatial characteristics of the $\delta^{13}C$ values in different climatic areas and derived a quantitative relationship between plant $\delta^{13}C$ and HI by measuring and collecting the $\delta^{13}C$ of a large number of C_3 plants growing in northern China. The response of plant $\delta^{13}C$ to different climatic areas varies considerably. A strong negative relationship was found between $\delta^{13}C$ values of C_3 herbaceous plants and HI in the whole of northern China, with a coefficient of −0.16‰ for 0.1 increases in HI. This variation trend was more obvious in the semihumid and semiarid zones. However, the positive correlation was observed in the dry climatic zone. The highly varying response of $\delta^{13}C$ of C_3 herbaceous plants to the HI demonstrates that $\delta^{13}C_{plant}$-based paleoclimate reconstruction in northern China should be carried out according to the different climatic zones.

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