# $\delta^{13}$ C Values of C<sub>3</sub> herbaceous plants and their relationships with humidity indexes in arid and humid climatic regions in northern China Liu X. Z.<sup>1, 2, 3\*</sup> and Wang C. Z.<sup>3</sup>

1. State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Water and Soil Conservation, Chinese Academy of Sciences, Yangling 712100, CHINA

2. College of Architecture and Urban Planning, Hunan University of Science and Technology, Xiangtan 411201, CHINA

3. College of Geography and Planning, Ludong University, Yantai 264025, CHINA

\*xianzhaoliu@sina.com

## Abstract

By measuring the stable carbon isotopes of  $C_3$ herbaceous plants and collecting the carbon isotope data of vegetation in northern China, such data as the geographic locations and climate factors of 47 sampling sites (33 of which were measured in this study) and carbon isotope of 325 plant samples (217 of which were measured in this study) were obtained. In addition, the humidity indexes in different climatic zones in northern China were calculated and moreover the spatial features of  $\delta^{13}C$  values of  $C_3$ herbaceous plants and their relationships with environmental factors such as humidity indexes were analyzed. Within the research scope, 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 semi-hunid zone to the semi-arid zone to the arid zone: the variation ranges of  $\delta^{13}C$  values of  $C_3$  plants in the above three climatic zones were -29.9% to -26.7% (semi-humid area), -28.4 % to -25.6% (semiarid a ea) and -28.0 % to -25.4 % (arid area), respectively.

Simple regression analysis showed that differences existed in the relationship between  $\delta^{13}C$  values of  $C_3$ herbaceous plants and humidity indexes. In the semiarid zone, semi-humid zone and overall northern area,  $\delta^{13}C$  values of  $C_3$  herbaceous plants 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, linear positive correlation existed between the  $\delta^{13}C$  values of  $C_3$  herbaceous plants and the humidity indexes (P < 0.05). With every 0.1 increase in the humidity index, the average  $\delta^{13}C$  value increased significantly by 1.3 %. Annual average temperature may be the main reason for the differences in humidity indexes of the sampling sites and for the  ${}^{13}C$  fractionation abilities of  $C_3$ herbaceous plants in arid area.

Keywords: Arid and humid climate zones, C<sub>3</sub> herbaceous plants, carbon isotope, humidity index, northern China.

## Introduction

Global warming and CO<sub>2</sub> concentration enrichment exerted a deep influence to the physiology and ecological process of plants<sup>1-2</sup>. The complicated relationships between plants and climatic environmental factors can be reflected by the stable carbon sotope  $(\delta^{13}C)$  in plant tissues, as the carbon isotope carried a lot of information reflecting the plants' environmental changes in the past, which was consequently used to extract the climatic change information such as temperature, humidity and precipitation or to reconstruct the paleoclimate and paleoenvironment<sup>3-6</sup>. Currently, relationships between  $\delta^{13}$ C compositions of vegetations and environmental factors in northern China have been studied by researchers at home and abroad<sup>7-15</sup>; however, most studies are limited to a certain single climatic or environmental factor, such as air temperature, precipitation (soil moisture), or altitude and few studies have been conducted on the relationships between  $\delta^{13}C$  values of plants and humidity indexes in northern China which can comprehensively reflect the water heat balance.

Temperature and precipitation are two 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 C3 plants were negatively correlated to temperature rise<sup>16-20</sup> while there are even more studies indicating that positive correlation existed between carbon isotope and temperature<sup>21-24</sup>. Still, there are some other studies suggesting that the above two factors were not remarkably correlated<sup>25</sup>.

In addition to differences in ecological and physiological processes of different plant species and genetic characteristics, the uncertainty regarding the relationship between  $\delta^{13}C$  values of  $C_3$  plants and temperature may relate to the difficulty in distinguishing the influence and degree of influence of environmental factors such as precipitation and lighting to the  $\delta^{13}$ C values, as well as the interaction of various factors. Besides being controlled by

\*Author for Correspondence

the carbon physiological metabolism process by itself,  $\delta^{13}C$  values of plants are also greatly affected due to the combined effects of various environmental factors.

Farquhar et al<sup>26</sup> stated that precipitation, as an important environmental factor, cannot be ignored regarding its influence on the  $\delta^{13}$ C values of plants. For example, carbon isotope values often decrease with the increase of precipitation $^{26-28}$ ; no doubt that there are also some studies obtaining results opposite to such changing law<sup>29</sup>. Therefore, if the interference of precipitation cannot be eliminated, uncertainties would exist in the relationship between  $\delta^{13}$ C values and environmental factors such as precipitation and temperature, resulting in unreliable reconstruction of paleoclimate, extraction of paleoecology information and explanation of stable carbon isotopic composition<sup>30</sup>. From the above, it is important to establish rational relationships between the  $\delta^{13}$ C values of plants and compound humidity and temperature indexes which can comprehensively reflect air temperature and precipitation and apply the results to the inversion of paleoclimate, paleoenvironment and prediction of future trends.

The humidity index comprehensively describes the water heat balance conditions and quantitatively reflects the influences of meteorological factors such as air temperature and precipitation to the dry and wet features in a certain area which not only includes water balance, but also the variation of ground energy based on temperature and hence reflects the interactive action of water heat balance. So far, however, there are rare reports which combined climatic humidity indexes and  $\delta^{13}$ C values.

Northern China is a region with a fragile ecological environment and serious land descriptication. The vegetative ecosystem is an obvious indicator of climatic changes. Based on the stable carbon isotope results of plants in northern China reported at home and abroad, through field survey, sampling and laboratory test, the humidity indexes of different sampling sites were calculated and the spatial features of  $\delta^{13}$ C compositions of C<sub>3</sub> herbaceous plants and their relationships with humidity indexes in different climatic regions in northern China were probed into, which provided important reference for quantitative studies on climatic environmental changes while using the stable carbon isotope of plants as a substitute index.

## **Data and Methods**

**Data sources:** A part of the experimental data was obtained from international and domestic literature regarding carbon isotopes of plants in northern China, including carbon isotope data of C<sub>3</sub> herbaceous plants and corresponding geographic data (longitude, latitude and altitude) of 13 sampling sites; the other part of the experimental data (i.e.  $\delta^{13}$ C data) sourced from 217 plant samples collected from 33 sampling sites in a farming-pastoral zone in northern China in July and August of 2008

and 2009. All the sampling sites chosen were flat, broad, bright and distant from villages to avoid human activity and micro relief influence on plant isotopes. The collected samples were all from growing plants. They were either the dominant species in the local area or plants collected in the three climatic zones to obtain spatial variations of carbon isotope compositions of the same plant species. Upon sampling, the number of the same plant species collected in a sampling site could not be less than 5 to 7 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.

After the samples were washed with water and air dried naturally, they were oven-dried at 70°C for 48 hours and were then ground and screened through the 80 mesh sieve into samples. Finally, 3-5 mg of the samples was put into a vacuum combustion ube with catalysts and oxidants added and were then fired a a emperature of 1020°C while CO<sub>2</sub> was produced. After being transformed by an element analyzer (Flash EA1112), the samples were placed on a Delta <sup>Plus</sup> AP in College of Resources and Environment, China Agr cultural University to measure the plant carbon isotope value. Each sample was measured 3-5 times as above. The measurement error was  $\pm 0.15\%$  and the analysis results were expressed by  $\delta^{13}C_{PDB}$ .

In addition, the corresponding latitude, longitude and altitude of the sampling sites were all measured by the global positioning system (Magellan GPS Field PRO V<sup>TM</sup>, California). The meteorological data were obtained from meteorological stations near the sampling sites or from the Natural Resource Database of the Institute of Geographical Sciences and Resource (http://www.natural resources csdb.cn/index.asp), the Meteorological Science Data Sharing Service Network (http://cdc.cma. gov.cn/ shishi/ climate.jsp) and the Meteorological Reference Office of National Meteorological Information Centre. The meteorological data obtained from the above sources included the annual average temperature and annual average precipitation from 1971 to 2008 and the average air temperature and average precipitation of each month over the years (1971-2008). Related information on distribution of the researched sampling sites, average carbon isotope values and sampling sites are shown in figure 1 and table 1.

## Calculation of humidity index

Humidity index is used to represent the water budget, thermal balance and dry/wet degree of a certain area. Its expression is as follows:

$$HI = \frac{R}{PE} \tag{1}$$

where R means the annual precipitation (mm) and PE means the potential evapotranspiration (mm), which is calculated as per Holdridge<sup>31</sup>, i.e. considering the potential evapotranspiration as a function of temperature, it can be expressed by the formula as follows:

$$PE = 58.93 \times ABT \tag{2}$$

where ABT indicates the annual biology temperature (°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 calculation formula of ABT is as follows:

$$ABT = 1/12 \sum_{1}^{12} T$$
 (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 formulas (1) to (3), we obtain the calculation formula for humidity index as follows:

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

Statistical analysis: The SPSS statistical analysis software (SPSS12.10 for Windows, Chicago, USA) was used for data correlation analysis, regression analysis and One-Way ANOVA variance analysis. 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, micro relief form and altitude, the carbon isotope data of plants collected from sampling sites on high mountains were avoided as much as possible.

#### **Results and Discussion**

Characteristics of  $\delta^{13}$ C compositions of C<sub>3</sub> herbaceous plants in different climatic zones: Figure 2A showed the average values and distribution ranges of carbon isotopes of  $C_3$  herbaceous plants in northern China as well as in various climatic zones (arid zone, semi-arid zone and semi-humid zone). Overall, the  $\delta^{13}$ C values of the 325 C<sub>3</sub> plant samples ranged from -29.9% to -25.4%, with the average value of -27.3‰. These results were within the range (-22% to -33%)of  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants from around the world. With regards to climatic zone, the variation range of  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in the arid zone of the northern China was narrow, mainly between -28.0‰ and -25.4‰, with an average value of -26.84% (n = 81) which was slightly more than the average value (-27.0%) of continental  $C_3$ herbaceous plants worldwide<sup>35</sup> and the average value (– 27.1%) was obtained via the isotope analysis for the 461 C<sub>3</sub> plant samples collected from northern China<sup>37</sup>.

The main reason for this may be that the plant samples were collected from the arid zone and  $\delta^{13}$ C values usually increased with the decrease in moisture<sup>25,28, 38-39</sup>, so plant carbon isotopes of these zones were slightly more positive compared with that of the humid and semi-humid zones.

The reason why the distribution of  $\delta^{13}$ C values of plants in the arid zone was relatively more concentrated might be due to the climatic environmental conditions of the sampling sites which were very similar. Statistical analysis was made for the annual average temperature and annual average precipitation and the results showed that the annual average precipitation of the sampling sites was  $158.0 \pm 40.11$  mm, the variation of average annual temperature was  $5.7-9.6^{\circ}$ C and the average temperature was  $7.53 \pm 1.24^{\circ}$ C. These results indicated that the degree of variation in the arid zone was significantly less than that of the semi-arid zone and semi-humid zone in northern China (Table 2).

The C<sub>3</sub> herbaceous plants in the semi-arid climatic zone were collected from Lanzhou, Su'nan and Yuzhong of Gansu Province, Jingbian, Hengshan and Yulin of Shaanxi Province, Ejin Horo Banner, Jungar Banner, Jarud Banner Doran, Dongsheng, Ordos, rakeshi, Fengzhen and Baarin Left Bannerm of Inner Mongolia and Huangzhong of Ningxia Province. Altogether 17 regions and a total of 124 samples were collected. An ual average precipitation of this climatic zone was 200–400 mm. The variation range of the  $\delta^{13}$ C values of C<sub>3</sub> herba eous plants was from –28.4‰ to –25.6‰, with an average value of –27.2‰ (Fig. 2A), which was slightly more negative than the average  $\delta^{13}$ C value (–27.0‰) of C<sub>3</sub> herba eous plants worldwide.

The main reason for this difference may be that the samples of this climatic zone were all C<sub>3</sub> herbaceous plants, while previous research included woody plants and shrub plants and the  $\delta^{13}$ C values of different life-form plants in the same area usually decreased in the sequence of arbor > shrub > herb<sup>40-43</sup>. In addition, the distribution range of  $\delta^{13}$ C values of plants in this zone was slightly wider than that of the arid zone (Fig. 2A) and the reason might be that the climatic environmental conditions of the sampling sites in this region were largely different from each other (Table 2).

The plant samples from the semi-humid climatic zone were mainly collected from the middle part of Shaanxi province in Loess Plateau, eastern Gansu and southeastern edge of the Inner Mongolia Plateau, altogether 22 regions, with a total of 122 samples. The annual average precipitation of each sampling site was greater than 400 mm which ranged basically from 420 mm to 660 mm, indicating that this region was a typical semi-humid climatic zone. The  $\delta^{13}$ C values of the C<sub>3</sub> herbaceous plants in this climatic zone varied from –29.9‰ to –26.7 ‰, with an average value of –27.8 ‰ (Fig. 2A). In addition, the distribution range was wider than that of the semi-arid and arid zones. This may relate to the large differences in annual average temperature and annual average precipitation at the sampling sites in this climatic zone.

In addition to the variation characteristics of the  $\delta^{13}$ C values of all plants in the arid and humid climatic zones, we also analyzed the carbon isotope values of five kinds of eurytropic C<sub>3</sub> herbaceous plants, which were collected from all three climatic zones in northern China. Figure 2B

showed that the obvious differences (P<0.05) existed in the average  $\delta^{13}$ C 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 semi-humid zone slightly less than that in the semi-arid and arid zones.

This indicated the carbon isotope compositions of  $C_3$ herbaceous plants had consistent variation patterns for both an individual plant and plants as a whole in different climatic zones, suggesting that changes of precipitation were important for the variation of  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in different climatic zones over the whole northern area. Additionally, such significant variance in the carbon isotope compositions of C<sub>3</sub> herbaceous plants in different climatic zones also reminded us that when using  $\delta^{13}$ C values of soil organic matter and soil carbonate to estimate the proportion of C<sub>3</sub> herbaceous plants in past vegetation and the relative biomass contribution in research on paleoclimate and the paleoecological environment, the sediment surroundings, especially the climatic environment, must be considered so as to choose the proper end member value of  $\delta^{13}$ C for C3 plants.

Relationships between  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants and humidity indexes in different climatic zones: The influence of the humidity index on  $\delta^{13}$ C of C<sub>3</sub> herbaceous plants results from the interactions of multiple meteorological factors such as temperature, precipitation, evaporation, soil humidity and pressure of water vapor. Figure 3 shows the relationships between  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants as a whole and humidity indexes in different climatic zones. As seen from figure 3A, with the increase in the humidity index, the  $\delta^{13}$ C value (water use efficiency) of all plants as a whole gradually increased (P < 0.05) in the arid zone indicating that these plants can make full use of rainwater resources and absorb as much water as possible in seasons with large precipitation. Schulze et al<sup>44</sup>, Su et al<sup>19</sup> and Skrzypek et al<sup>45</sup> observed that  $\delta^{13}$ C values of a small number of C<sub>3</sub> herbaceous plants increased with the increase in relative humidity or annual precipitation.

Therefore, it is possible that the  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in arid zones of northern China increased with the increase in humidity indexes and the fact that the  $\delta^{13}$ C values of single *Chenopodium glaucum* increased significantly with the increase in humidity index can be taken as strong evidence (Fig. 4B). This was, however, exactly opposite to the influence of the humidity index on  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in the semi-arid and semi-humid zones. Figures 3B to 3C show that in the northern semi-arid and semi-humid zones,  $\delta^{13}$ C values of all C<sub>3</sub> herbaceous plants significantly decreased with the increase in the humidity index and for every 0.1 increase in the humidity index, the  $\delta^{13}$ C value of C<sub>3</sub> herbaceous plants decreased by 1.1% (for semi-arid zone) and by 0.4% (for semi-humid zone), which is consistent with the results of previous research<sup>46-49</sup>.

Winter et al<sup>50</sup> reported that  $\delta^{13}C$  values of C<sub>3</sub> herbaceous plants such as wheat (Triticum aestivum) and Poa annua were slightly more positive in a low-humidity than in a high-humidity ecological environment; Stuiver and Braziunas<sup>3</sup> analyzed the relationship between relative humidity and the  $\delta^{13}$ C value of coniferous forest, which showed high negative correlation; Wang and Han<sup>51</sup> also found that the  $\delta^{13}$ C values of several C3 herbaceous plants were obviously more positive in dry seasons than those in rainy seasons in their researches The trend that  $\delta^{13}$ C value significantly increased with the decrease of humidity index might be related to the dry air or insufficient water content in the soil causing the increase of the stomatal conductance of plants which meanwhile indicated that these plant species under semi-and and semi-humid 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 of all C<sub>3</sub> he baceous plants and the humidity indexes 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 humidity indexes because annual precipitation gradually decreased from east to west. In this study, the overall trend that  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants significantly decreased with the increase in humidity index (Fig. 3D) was consistent with the results concluded by Wang et al<sup>28</sup> where values of carbon isotopes of the 367 C<sub>3</sub> herbaceous plant samples in northern China were obviously negative with the increase in the annual average precipitation. Therefore, when the carbon isotopic composition was used as a substitute index to study the paleoclimate or paleoenvironment of northern China, it was rational to use the  $\delta^{13}$ C value as the substitute index of the climatic humidity index. Of course, this result was also related to the quantity of samples analyzed and the climatic zones researched and hence further systematic studies were very necessary.

For the single C<sub>3</sub> plant species, statistical analysis could not be made for the vast majority of plants due to the limitation of sample amount. Therefore, in this study, only three kinds of C<sub>3</sub> herbaceous plants (*Plantago depressa*, *Chenopodium glaucum* and *Lepidium apetalum*), which were widely distributed in the same climatic zone with multiple data points, were analyzed. Figure 4 shows the change in  $\delta^{13}$ C values for the three plants with the change in the humidity index. All showed a decrease, except for *Chenopodium glaucum* in the arid zone, but the magnitude of descent and degree of relevance between  $\delta^{13}$ C values of plants and the humidity indexes varied, even for the same species and varied from zone to zone. This indicated their sensitivities were different against the humidity indexes, with the reason that  $\delta 13C$  values of the plants were the result of the joint action of the plant species and environmental factors<sup>52</sup>.

Such variances reflected by the change in  $\delta^{13}$ C values due to the change in humidity index might be related to the variances produced in the carbon isotope fractionation caused by the change of the physiological characteristics made by the plants to adapt to the environmental changes. In addition, these variances may also relate to the small sample size of individual plant species. The above different plants and the same species having different  $\delta^{13}$ C variation rates in different climatic zones reminded us that when we used  $\delta^{13}$ C values of plants to study the paleoclimate and paleoenvironment, choosing the plant species which were the most sensitive to the changes in environmental indicators might gain the most valuable results.

According to theories of Farquhar et al<sup>26,53</sup>, precipitation reflects the moisture condition for plant growth to a certain extent. When precipitation is insufficient or air humidity is reduced, the stoma of plants close and stomatal conductance is reduced, which can lead to CO<sub>2</sub> concentration loss in plant leaves and carbon isotope ratio increase of photosynthetic products. The fact that the  $\delta^{13}C$  values of the  $C_3$  herbaceous plants in the semi-arid zone, semi-humid zone and the whole northern area were negatively correlated to humidity indexes provides strong evidence for the above point. However, in the northern arid climatic zone, the influence of humidity indexes on the  $\delta^{13}$ C values was not that simple. Viewed from the whole northern area, as precipitation gradually decreased from east to west and the  $\delta^{13}C$  values of plants significantly decreased with the increase in humidity indexes (Fig. 3D) the  $\delta^{13}$ C values of plants in the arid zone showed an ascending trend (Fig. 3A), which we thought might be the influence of annual maximum temperature outweighing that of the precipitation.

According to formulas (1) - (4), the humidity index is a ratio between annual maximum precipitation and annual maximum evapotranspiration, while in the arid zone of northern China the annual precipitation was the biggest factor limiting plant growth. The annual average precipitation of each sampling site in this zone varied slightly (Table 2) which to a certain degree eliminated the interference of precipitation. Therefore, the change of humidity index 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 humidity index would decrease. Simple regression analysis showed that in the northern arid zone, linear negative correlation existed among the humidity indexes, the  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants and the annual average temperature. With the increase in annual average temperature, both humidity indexes and  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants significantly decreased (Fig.5A and 5C) while the

relationships among humidity indexes,  $\delta^{13}$ C values of plants and annual average precipitation were not obvious (Fig.5B and 5D).

Thus, the  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants and humidity indexes were significantly and positively correlated, indicating that the variation pattern of  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in the northern arid zone affected by humidity indexes was actually due to temperature change. This result was consistent with the temperature restricted the  $\delta^{13}$ C values of C3 herbaceous plants to a certain extent obtained by scholars in North America<sup>54-55</sup> and Australia<sup>56</sup>. From the above, we speculated that the average annual precipitation in the northern arid zone was not the decisive factor causing the variances in relationships between  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants and humidity indexes in different sampling sites and the influence of the humidity index in local areas may show a different pattern entirely from that in a larger scale range.

It should be pointed out that the humidity indexes used in this study were calculated via the Holdridge model, which only considered the influence of precipitation and temperature, while in fact the humidity index referred to the joint action of temperature, precipitation, evaporation, wind speed, solar radiation, pressure of water vapor and other meteorological factors<sup>57</sup>. Hence, the humidity indexes calculated here may have some limitations. Therefore, establishing a scientific and rational method to calculate the humidity indexes, as well as determining the mechanism of how humidity indexes affect plant carbon isotopic compositions and their relationship requires further study.

## Conclusion

Through analysis on spatial variances of carbon isotopic compositions of  $C_3$  herbaceous plants in northern China and their correlation with humidity indexes, the following conclusions were drawn:

(1) The variation range of  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in northern China was from -29.9‰ to -25.4‰, with an average value for all samples of -27.3‰, which was close to the global average. The average value of the climatic zones significantly increased with the increase in precipitation by sequence of semi-humid zone, semi-arid zone and arid zone. The distribution intervals of the  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in the semi-humid, semiarid and arid zones were -29.9‰ to -26.7‰, -28.4‰ to -25.6‰ and -28.0‰ to -25.4‰, respectively.

(2) Within the research scope, the  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants significantly decreased with the increase in humidity indexes, with every 0.1 increase in the humidity index, the average  $\delta^{13}$ C value of C<sub>3</sub> herbaceous plants decreased by -0.2% or so. This variation trend was more obvious in the semi-humid and semi-arid zones. However, the  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants in the arid climatic zone and humidity indexes were significantly and positively correlated, with every 0.1 increase in the humidity index, the average  $\delta^{13}$ C value increased by 1.3%.

Average annual temperature may be the main reason for differences in the humidity indexes and  $^{13}\mathrm{C}$  fractionation

abilities of  $C_3$  herbaceous plants in the sampling sites of northern China.

Table 1
Information of sampling sites and carbon isotope ratios of C3 herbaceous plants in
different climate area of North China

Site No.	Longitude	Latitude	AMT (°C)	AMP (mm)	EV(m)	HI	$\delta^{13}$ C±SD (‰)	Data source	Sample (n)
1	85°55′	44°39′	8.0	150	477	0.15	$-26.9\pm0.85$	[14-15]	23
2	86°37′	42°45′	6.4	184	690	0.22	$-27.1\pm0.00$	[32]	1
3	87°50′	43°10′	6.1	164	650	0.19	$-26.5 \pm 1.25$	[33]	24
4	93°40′	42°49′	9.8	65	800	0.09	-27.6±0.65	Observed	8
5	98°54′	40°00′	8.0	154	1250	0.11	$-26.9\pm2.34$	[33]	8
6	101°00′	38°10′	7.7	177	1764	0.26	$-25.2\pm0.49$	Observed	3
7	100°06′	39°20′	7.6	186	1547	0.21	$-25.4\pm0.49$	[34]	2
8	104°57′	37°27′	9.6	184	1250	0.10	$-28.0\pm1.42$	Observed	12
9	103°50′	36°00′	6.6	327	1517	0.32	-28.0±0.00	Observed	1
10	99°38′	38°49′	3.6	214	2204	0.26	$-25.9\pm0.65$	Observed	6
11	101°31′	36°39′	2.9	380	2260	0.36	$-25.6\pm0.50$	Observed	2
12	104°01′	36°33′	6.6	350	1896	0.36	-27.0+0.56	Observed	8
13	108°30′	37°22′	7.8	395	1333	0.27	-27.3+0.37	Observed	6
14	109°10′	37°17′	8.5	390	1019	0.32	-26.9+0.45	Observed	7
15	109°59′	39°02′	5.4	363	1461	0.37	-27.1+0.54	Observed	10
16	110°03′	39°10′	6.2	380	1276	0.34	-27.2+0.65	Observed	9
17	110°28′	39°21′	64	350	1108	0.34	-27.3+0.68	Observed	8
18	110°16′	39°02′	7.5	392	249	0.37	-26.7+1.23	Observed	9
19	113°27′	40°16′	4.7	347	1195	0.38	-27.9+0.74	Observed	10
20	120°26′	49°10′	-2.9	289	676	0.38	-28.2+0.84	Observed	11
21	115°07′	42°14′	1.5	314	1405	0.37	-275+036	Observed	8
22	116°28′	42°11′	2.4	386	1245	0.38	-284+065	Observed	8
23	119°24′	43°59′	5 3	390	486	0.36	-275+058	Observed	10
24	120°54′	44°34′	2.8	383	491	0.30	-28 8+0 73	Observed	8
25	109°30′	38°12′	10.0	400	1025	0.35	-27.3+1.23	Observed	3
26	107°44′	35°12′	91	584	847	0.84	$-27.8\pm0.42$	Observed	3
27	105°43′	35°58'	5.3	425	1931	0.57	-264+050	Observed	7
28	122°02′	46°03′	4.1	443	433	0.60	$-27.8 \pm 0.34$	Observed	10
29	119°56′	47°10′	-2.7	453	997	0.65	-28.3+0.45	Observed	10
30	110°29′	38°14′	8.9	441	1226	0.52	-27.1+1.39	Observed	10
31	111°08′	39°23'	8.8	460	875	0.56	$-27.4 \pm 0.57$	Observed	8
32	112°16′	40°00′	8.6	450	1358	0.55	$-27.4\pm0.47$	Observed	8
33	110°14′	37° 40′	8.3	441	2104	0.53	$-27.2\pm0.96$	[36]	7
34	121°19′	50°28′	-3.1	437	718	0.69	$-28.6\pm0.63$	Observed	9
35	109°24′	35°42′	9.2	621	1100	0.88	$-28.0\pm1.62$	Observed	4
36	109°20′	36°45′	8.8	531	1372	0.72	$-27.6\pm1.03$	[36]	9
37	107°40′	35°42′	8.9	594	1421	0.77	$-26.9\pm0.07$	Observed	2
38	106°40′	35°33′	8.6	511	1560	0.75	$-26.8\pm0.00$	Observed	1
39	103°12′	35°36′	6.3	501	2000	0.74	-26.7+0.46	Observed	4
40	106°16′	36°00'	6.2	478	1753	0.54	-26.4+0.64	Observed	2
41	109°25′	35°30'	9.0	600	1085	0.87	-28.1+0.97	[36]	9
42	105°23′	34°21	11.9	650	997	0.89	-30.9+0.00	[32]	1
43	107°56′	34°14′	12.9	637	447	0.75	-29.2+2.20	[36]	2
44	107°56′	34°29'	10.8	609	421	0.85	-27.5+1.69	[36]	5
45	108°34′	35°34'	10.2	650	970	0.66	-27.5+0.66	[36]	6
46	116°23′	40°00′	11.5	595	1100	0.63	-27.7+0.42	Observed	2
47	102°54′	39°59′	3.2	530	2400	0.75	$-28.9\pm0.98$	[32]	3

The numbers of sampling sites are the same as in figure 1. AMT: Annual mean temperature; AMP: Annual mean precipitation; EV: Elevation; HI: Humid index

Table 2
The average value and variation coefficient of annual mean temperature and annual mean precipitation for all
sampling sites in different climatic areas of north China

Climatic area	AMT		Climatic area	AMP		
Cilliatic area	Mean value of sites (°C)	CV (%)	Cillianc area	Mean value of sites (mm)	CV (%)	
Arid area	7.53±1.24a	0.165b	Arid area	158.0±40.11c	0.085c	
Semi-arid area	5.02±3.09a	0.614a	Semi-arid area	360.9±48.13b	0.133ab	
Semi-humid area	7.40±4.26a	0.575a	Semi-humid area	530.6±80.16a	0.151a	

AMT and AMP as in table 1; CV: Coefficient of variation. The different letters indicate significant difference (P<0.05).



Figure 1: Distribution of sampling sites in the different climatic areas in North part of China 1: Gurbantongut; 2: Urumqi; 3: Fukang; 4: Kani; 5: Inta; 6: Shandan; 7: Pingchuan; 8: Shapotou; 9: Lanzhou; 10: Sunan; 11: Huangzhong; 12: Yuzhong; 13: Jinbian; 4: Hengshan; 15: Dongsheng; 16: Ejin Horo Banner; 17: Ordos; 18: Jungar Banner; 19: Feng Zhen; 20: Yakeshi; 21: Zreng Liangbai 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: Feijin, 47: Hezuo



Figure 2: Spatial characteristics of δ<sup>13</sup>C values for C<sub>3</sub> herbaceous plants in the arid and humid climate area of north China. (A): The characteristics of δ<sup>13</sup>C values for whole C<sub>3</sub> herbaceous plants (Different letters represent significant differences among different climate areas at α=0.05 level). WNC: All the sampling areas; AA: Arid area; SAA: Semi-arid area; SHA: Semi-humid area; (B): The individual C<sub>3</sub> herbaceous plant, the number 1-5 of horizontal axis denote *Chenopodium glaucum*, Artemisia lavandulaefolia, Plantago depressa, Artemisia capillaris and Lepidium apetalum

respectively



Figure 3: Relationships between  $\delta^{13}$ C values of C<sub>3</sub> herbaceous plants and burnidity index in different climate areas of North China





Figure 4: The correlation between  $\delta^{13}$ C values of single C<sub>3</sub> plant and humidity index in different climate areas of North China. (A), (B) Arid area; (C), (D) Semi-arid area; (E), (F) Semi-humid area



Figure 5: Relationships of humidity index and  $\delta^{13}$ C for C<sub>3</sub> herbaceous plants with mean annual temperature (A, C) and mean annual precipitation (B, D) in arid areas of North China. AMT and AMP as in table 1

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