PHOTOSYNTHETICA 45 (1): 110-119, 2007

Foliar $\delta^{13}C$ values of nine dominant species in the Loess Plateau of China

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Abstract

The foliar stable carbon isotope compositions ($\delta^{13}C$) of nine dominant species in seven sites, Yangling, Yongshou, Tongchuan, Fuxian, Ansai, Mizhi, and Shenmu, standing from the south to the north in the Loess Plateau of China were studied. The results showed that foliar $\delta^{13}C$ values ranged from –22.61 to –30.73 ‰ with an average of –27.23 ‰ in 141 C_3 plant samples collected from the Loess Plateau. Foliar $\delta^{13}C$ values varied significantly ($p<0.001$) among the nine C_3 species, which were *Pinus tabulaeformis* Carr., *Robinia pseudoacacia* L., *Zizyphus jujuba* Mill. var. *spinosus* Hu., *Rubus parvifolius* L., *Hippophae rhamnoides* L., *Caragana korshinskii* Kom., *Lespedeza davurica* (Laxm.) Schindl., *Artemisia sacrorum* Ledeb. var. *incana* Mattf., and *Agropyron cristatum* Gaertn. Comparatively, *R. pseudoacacia*, *H. rhamnoides*, and *C. korshinskii* had much higher $\delta^{13}C$ values than the other six species, while *A. sacrorum* had the lowest $\delta^{13}C$ value. There was no significant difference in foliar $\delta^{13}C$ value among five species, *P. tabulaeformis*, *Z. jujuba*, *R. parvifolius*, *L. davurica*, and *A. cristatum*. Considering the life forms categorized from nine C_3 species, trees and shrubs had significantly higher $\delta^{13}C$ values than herbs ($p<0.001$). The deciduous tree *R. pseudoacacia* had much higher $\delta^{13}C$ value than the evergreen tree *P. tabulaeformis* ($p<0.01$). Among the four shrubs, foliar $\delta^{13}C$ values in *H. rhamnoides* and *C. korshinskii* were markedly higher ($p<0.01$) than those in *Z. jujuba* and *R. parvifolius*. Among the three herbs, *L. davurica* and *A. cristatum* had significantly higher $\delta^{13}C$ values than *A. sacrorum* ($p<0.01$). Leguminous species such as *R. pseudoacacia*, *C. korshinskii*, and *L. davurica* as well as a non-leguminous species with nitrogen-fixation capacity, *H. rhamnoides*, had higher $\delta^{13}C$ values than other non-leguminous species with same life-form. The mean $\delta^{13}C$ value increased by about 7 % from Yangling in the south to Shenmu in the north as climatic drought increased, and foliar $\delta^{13}C$ values differed much ($p<0.001$) among the seven sites. For nine species in the Loess Plateau, foliar $\delta^{13}C$ values were significantly and negatively ($p<0.001$) correlated with the mean annual precipitation, moreover, an increase of 100 mm in annual precipitation would result in a decrease of 1.2 ‰ in $\delta^{13}C$ value.

Additional key words: C_3 species; mean annual precipitation; species differences; $\delta^{13}C$.

Introduction

The stable carbon isotope technique has been widely used in the field of plant physiological ecology, for distinguishing photosynthetic pathways (O'Leary 1981, Farquhar *et al.* 1989, Cerling *et al.* 1993), studying physiological response to water stress (Parolin 2001, Chen *et al.* 2005), and evaluating nutrient absorption (Evans 2001), water sources (Máguas and Griffiths 2003), water balance, and water use efficiency (WUE) for different species (Schwinning and Ehleringer 2001, Sun *et al.* 2005). Currently, using stable carbon isotopes to analyze intra-specific or inter-specific differences in photosynthetic and physiological characteristics, and understanding long-term WUE under different environmental conditions, the $\delta^{13}C$ responses in plants to climatic variables have been hot topics in eco-physiological research (Ziegler 1995, Van de Water *et al.* 2002, Máguas and Griffiths 2003, Foster and Brooks 2005).

WUE (the ratio of net photosynthetic rate to transpiration rate), as one of the most important physiological characteristics in process of plant growth, is an objective

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Received 22 March 2006, *accepted* 22 June 2006.

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Abbreviations: MAP – mean annual precipitation; WUE – water use efficiency; $\delta^{13}C$ – stable carbon isotope composition.

Acknowledgements: This research was supported by the Important Direction Project of Innovation of CAS (KSCX2-YW-N-003), West-action Program of Chinese Academy of Sciences, and the United Scholar's Item of Talent Training Program in West China of the Chinese Academy of Sciences (grant No. 2005LH01).

index to evaluate water use and drought-tolerant characteristics and is thus capable of providing the theoretical basis for selecting proper plant species for specific environments (Yan *et al.* 2001). Generally, WUE is determined in a photosynthetic apparatus, it only shows instantaneous behaviour of partial leaf at a certain time and varies remarkably as growing season and environmental conditions change (Chen *et al.* 2002). The carbon isotope fractionation exists in photosynthetic process from the absorption of atmospheric CO_2 to the production of organic matter. Stable carbon isotope composition (δ^{13}C) can indicate water use status by analyzing the production of carbon metabolism accumulated in leaves of whole plant, and thus evaluate the long-term WUE (Farquhar *et al.* 1989). In general, there is a positive relationship between δ^{13}C and WUE, and the plant with higher δ^{13}C value has higher WUE (Duquesnay *et al.* 1998, Shangguan *et al.* 2000, Qu *et al.* 2001, Zheng and Shangguan 2005).

The Loess Plateau is a region with the severest soil erosion in the world and one of the most fragile ecosystems in China. Presently, it is a critical question how to select proper plant species with strong drought-tolerance according to the specific environments in the process of vegetation recovery in the Loess Plateau (Shangguan *et al.* 2002). Research on relations between plant WUE and climatic variables is especially important in this region. In this study, we investigated the δ^{13}C changes of nine dominant species distributed widely in the Loess Plateau, and their relationships with the precipitation in order to provide the scientific basis for vegetation reconstruction in this region.

Materials and methods

Study sites: The study was conducted in seven sites, Yangling, Yongshou, Tongchuan, Fuxian, Ansai, Mizhi, and Shenmu, distributed from south to north in the Loess Plateau of China (Fig. 1). The study area is located at 34°16′–38°47′ N and 108°02′–110°21′ E, and belongs to a temperate zone. Their vegetation types span from warm temperate humid and semi-humid forest zone to moderate temperate arid and semi-arid desert grassland. The climatic and geographical conditions of seven sites were listed in Table 1. The latitudes and longitudes of the sampling sites were measured with a Global Position System (GPS). In order to minimize the influence of human disturbances, the sampling areas were chosen to be far from human habitats.

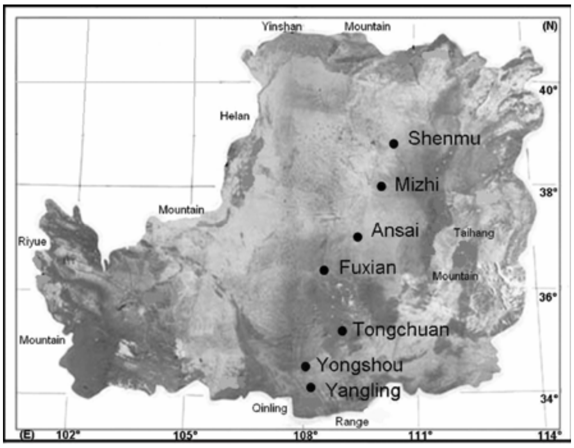

Fig. 1. Seven sampling sites, Yangling, Yongshou, Tongchuan, Fuxian, Ansai, Mizhi, and Shenmu, standing from the south to the north in the Loess Plateau of China.

Plant sampling: Nine dominant species with C_3 photosynthetic pathway, including two trees, four shrubs, and three herbaceous plants, distributed widely in the Loess Plateau, were taken in June 2005 when plants were actively growing. The two tree species were *Pinus tabulaeformis* Carr., an evergreen species, and *Robinia pseudoacacia* L., a deciduous species. The four shrubs were *Zizyphus jujuba* Mill. var. *spinosus* Hu., *Rubus parvifolius* L., *Hippophae rhamnoides* L., and *Caragana korshinskii* Kom. The three herbaceous plants were *Lespedeza davurica* (Laxm.) Schindl., *Artemisia sacrorum* Ledeb. var. *incana* Mattf., and *Agropyron cristatum* Gaertn. Among nine species, *P. tabulaeformis*, *R. pseudoacacia*, *Z. jujuba*, *L. davurica*, and *A. cristatum* have stronger adaptabilities and are distributed widely in the humid and semi-humid climatic zones as well as in the arid and semi-arid zones in the Loess Plateau. *R. parvifolius* mainly grows in the humid and semi-humid zones as well as the arid zone, and *H. rhamnoides*, *C. korshinskii*, and *A. cristatum* mainly grow in the arid and semi-arid climatic zones.

The leaf samples of the trees were mostly taken from their lower canopies (2–3 m aboveground) on semi-adret slopes of the Loess Plateau, while those of the shrubs and herbaceous plants were mostly taken from their upper canopies on the adret (southward-facing) slopes. Three to five healthy and fully expanded leaves from individual plants were randomly selected, and each sample involved 2–3 individual plants of the same species. Three samples of each species and totally 141 plant samples from nine species were collected from seven sites of the Loess Plateau.

Analysis of stable carbon isotope composition (δ^{13}C): Plant samples were first ultrasonically washed with distilled water and air-dried, then oven-dried at 70 °C for at least 48 h to a constant mass. All the leaves from the same species were put together, ground with a plant-sample mill (*1093* Sample Mill, Sweden) into uniformly fine powder, and finally sieved with a 1-mm-mesh screen. δ^{13}C, a carbon isotope, was measured with a *MAT-251* mass spectrometer (*Finnigan*, San Jose, USA) in the State

Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences. Treated samples of 3–5 mg were put into a vacuum quartz tube in which they were mixed with some activator and desiccant, and then oxidized in an oxygen flux of 850 ºC, and later on the CO_2 thus produced was cryogenically purified with both a dry ice-ethanol trap and a liquid nitrogen trap. Then, according to the PDB (belemnite from the Pee Dee Formation) standard, carbon isotope CO_2 was measured with a *MAT-251* mass spectrometer at the precision of <0.02 %. The results were expressed as $\delta^{13}C$ [‰] = $\{[(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}]/(^{13}C/^{12}C)_{standard}\} \times 1\,000$, in which, $(^{13}C/^{12}C)_{sample}$ and $(^{13}C/^{12}C)_{standard}$ were the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively (Farquhar *et al.* 1989).

Mean annual precipitation (MAP) for Yangling, Yongshou, Tongchuan, Fuxian, Ansai, Mizhi, and Shenmu in the Loess Plateau was provided by the Meteorological Bureau of Shaanxi Province.

Table 1. Geographical and climatic conditions of seven sampling sites in the Loess Plateaua of China. Mean annual precipitation (MAP), mean annual temperature (MAT).

Sampling site	Geographical location	Altitude [m]	Climate and vegetation	Soil type	MAP [mm]	MAT [ºC]	Aridity index
Yangling	34º16´N, 108º04´E	468	Warm temperate humid or semi-humid climate and forest zone	Silty clay loam	635	12.9	1.33
Yongshou	34º49´N, 108º02´E	1454	Warm temperate semi-humid climate and forest zone	Calcareous loam	602	10.8	1.22
Tongchuan	35º03´N, 109º08´E	1324			555	12.0	1.16
Fuxian	36º04´N, 108º32´E	1353			570	9.1	1.09
Ansai	36º46´N, 109º15´E	1125	Warm temperate semi-arid climate and forest-grassland zone	Loessial silty soil	505	8.8	1.20
Mizhi	37º51´N, 110º10´E	1103	Moderate temperate semi-arid climate and grassland zone	Loessial silty soil	451	8.8	1.70
Shenmu	38º47´N, 110º21´E	1255	Moderate temperate arid or semi-arid climate and deserted grassland zone	Sandy loess	441	8.5	1.80

Data analysis: The correlation analysis, regression analysis, and one-way analysis of variance (ANOVA) were conducted with *SPSS* software (2004, version *13.0*; *SPSS*, USA). The site or species groupings for means of foliar $\delta^{13}C$ values were compared by Independent-Sample *t*-test for two-group tests, or by ANOVA followed by multiple comparison tests where more than two groups were compared. The homogeneity of the variance among the groups was assessed by Levene's test. LSD test was used for multiple comparison where the variances were deemed homogeneous, otherwise Tamhane's T2 test was used. The normal distribution test of the values of foliar $\delta^{13}C$ was conducted by One-Sample Kolmogorov-Smirnov Test (K-S test in short).

Results

Patterns of foliar $\delta^{13}C$ values for nine C_3 species in the Loess Plateau: In all the 141 plant samples from nine species taken in the Loess Plateau, foliar $\delta^{13}C$ values ranged from –22.61 to –30.73 ‰, with 91 % of them falling in the range from –24.0 to –29.0 ‰. Moreover, the $\delta^{13}C$ values below –29.0 ‰ and above –24.0 ‰ were both accounted for 4 % of total data, while $\delta^{13}C$ values varying between –25 ‰ and –29 ‰ were accounted for 86 %. The mean $\delta^{13}C$ value was –27.23 ‰ (Fig. 2). The $\delta^{13}C$ values of nine species in the Loess Plateau did not follow the normal distribution by K-S test ($p<0.05$), and the skewness coefficient for $\delta^{13}C$ value distribution was less than 1, but the kurtosis coefficient was more than 1.

Foliar $\delta^{13}C$ values of nine species in seven sampling sites: Foliar $\delta^{13}C$ value in *P. tabulaeformis* varied significantly ($p<0.001$) across seven sites, Yangling, Yongshou, Tongchuan, Fuxian, Ansai, Mizhi, and Shenmu, standing from south to north in the Loess Plateau (Table 2). Comparatively, the $\delta^{13}C$ values for *P. tabulaeformis* growing in Shenmu and Mizhi were significantly higher than those in other five sites, while the $\delta^{13}C$ values were relatively lower and differed little ($p>0.05$) between Fuxian and Ansai. There was significant difference ($p<0.001$) in foliar $\delta^{13}C$ value for *R. pseudoacacia* growing in six sites except Shenmu. *R. pseudoacacia* in Mizhi and Ansai had much higher mean $\delta^{13}C$ values that were –24.59 and –24.78 ‰, respectively, while *R. pseudoacacia* in Fuxian had the lowest $\delta^{13}C$ value (–28.73 ‰) (Table 2).

Foliar $\delta^{13}C$ value in *Z. jujuba* varied significantly ($p<0.001$) across the seven sites, moreover, plants in

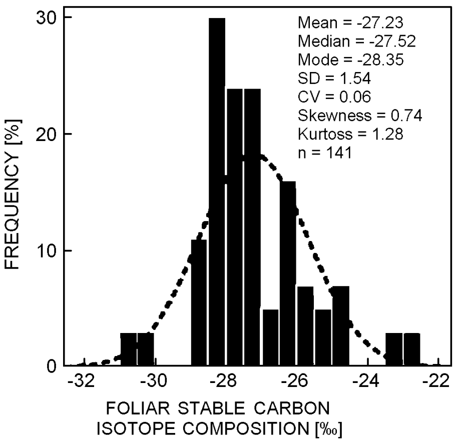

Fig. 2. Frequency histograms of foliar stable carbon isotope compositions for 141 plant samples from nine C_3 species in the Loess Plateau of China. *Dashed curve* indicates fitted log-normal curves.

Mizhi and Shenmu had higher δ^{13}C values, but plants in Yangling had the lowest δ^{13}C value (–30.39 ‰) (Table 2). There was significant difference (p<0.001) in foliar δ^{13}C value for *R. parvifolius* growing in four sites, Yangling, Yongshou, Fuxian, and Ansai, and plants in Ansai had the highest δ^{13}C value (–27.07 ‰). Foliar δ^{13}C value in *H. rhamnoides* varied significantly (p<0.001) among Tongchuan, Fuxian, Ansai, and Shenmu, and plants in Shenmu had the highest δ^{13}C value (–22.66 ‰). There was significant difference (p<0.001) in foliar δ^{13}C value for *C. korshinskii* growing in Ansai, Mizhi, and Shenmu. Moreover, plants in Mizhi had the highest δ^{13}C value (–23.27 ‰), while plants in Ansai and Shenmu had similar δ^{13}C values (Table 2).

Foliar δ^{13}C value in *L. davurica* varied significantly (p<0.001) across the six sites except Tongchuan, moreover, plants in Mizhi and Shenmu had higher δ^{13}C values, but plants in Yongshou had the lowest δ^{13}C value (–28.35 ‰) (Table 2). There was significant difference (p<0.001) in foliar δ^{13}C value for *A. sacrorum* growing in six sites except Shenmu, and plants in Fuxian and Mizhi had higher δ^{13}C values, while plants in Yangling had the lowest δ^{13}C value (–30.69 ‰). Foliar δ^{13}C value in *A. cristatum* varied significantly (p<0.001) among four sites, Fuxian, Ansai, Mizhi, and Shenmu, and plants in Mizhi had the highest δ^{13}C value (–26.18 ‰) (Table 2).

Table 2. Foliar stable carbon isotope compositions [δ^{13}C, ‰] of nine species in seven sites of the Loess Plateau. Species: 1. *Pinus tabulaeformis* Carr. (Pinaceae), 2. *Robinia pseudoacacia* L. (Leguminosae), 3. *Zizyphus jujuba* Mill. var. *spinosus* Hu. (Rhamnaceae), 4. *Rubus parvifolius* L. (Rosaceae), 5. *Hippophae rhamnoides* L. (Eleagnaceae), 6. *Caragana korshinskii* Kom. (Leguminosae), 7. *Lespedeza davurica* (Laxm.) Schindl. (Leguminosae), 8. *Artemisia sacrorum* Ledeb. var. *incana* Mattf. (Compositae), 9. *Agropyron cristatum* Gaertn. (Gramineae). In each column, values followed by the same letter are not significantly different at p<0.05 according to LSD tests; ***significantly different at p<0.001.

Sampling site	Species number 1	2	3	4	5	6	7	8	9	Significance
Yangling	–27.15 ±0.07 c	–25.06 ±0.09 c	–30.39 ±0.08 f	–27.96 ±0.10 bc	/	/	–27.57 ±0.08 c	–30.69 ±0.05 c	/	***
Yongshou	–27.45 ±0.08 d	–26.28 ±0.10 e	–27.36 ±0.08 c	–27.77 ±0.08 c	/	/	–28.35 ±0.09 e	–28.45 ±0.10 b	/	***
Tongchuan	–28.09 ±0.06 e	–25.90 ±0.05 d	–28.36 ±0.09 e	/	–27.55 ±0.07 c	/	/	–28.35 ±0.07 b	/	***
Fuxian	–28.47 ±0.10 f	–28.73 ±0.08 f	–28.06 ±0.11 d	–28.06 ±0.10 c	–27.53 ±0.06 c	/	–27.68 ±0.07 cd	–27.46 ±0.08 a	–27.43 ±0.09 b	***
Ansai	–28.57 ±0.06 f	–24.78 ±0.06 b	–28.24 ±0.10 e	–27.07 ±0.13 a	–26.49 ±0.04 b	–27.04 ±0.07 b	–27.68 ±0.05 d	–28.35 ±0.10 b	–28.55 ±0.09 c	***
Mizhi	–26.03 ±0.15 b	–24.59 ±0.05 a	–25.66 ±0.11 a	/	/	–23.27 ±0.09 a	–26.44 ±0.05 a	–27.56 ±0.08 a	–26.18 ±0.06 a	***
Shenmu	–25.35 ±0.08 a	/	–26.16 ±0.09 b	/	–22.66 ±0.06 a	–27.01 ±0.07 b	–27.34 ±0.07 b	/	–28.45 ±0.09 c	***
Significance	***	***	***	***	***	***	***	***	***	/

Comparison of foliar δ^{13}C among nine species and seven sites: Foliar δ^{13}C values of plants varied significantly among seven sampling sites and nine species (p<0.001), and both site and species had significant effects on δ^{13}C values (Table 3). Foliar δ^{13}C values of the same species in different sampling sites and different species in the same site varied greatly (p<0.001, Table 2). Comparatively, plants growing in Mizhi and Shenmu had significantly higher δ^{13}C values than those in other five sites, while the δ^{13}C value differed little for plants in Yangling, Yongshou, Tongchuan, Fuxian, and Ansai. The mean δ^{13}C values of plants in seven sites ranked in the order: Mizhi (–25.68 ‰, n = 21) > Shenmu (–26.16 ‰, n = 18) > Ansai (–27.42 ‰, n = 27) > Yongshou (–27.61 ‰, n = 18) > Tongchuan (–27.65 ‰, n = 15) > Fuxian (–27.93 ‰, n = 24) > Yangling (–28.13 ‰, n = 18) (Fig. 3).

Table 3. Two-way ANOVA for the effects of sampling sites and species on foliar stable carbon isotope compositions.

Source of variation	Sum of squares	*df*	Mean square	*F*	*p*
Sites	98.08	6	16.35	2475.34	<0.001
Species	101.99	8	12.75	1930.59	<0.001
Sites×species	124.15	32	3.88	587.52	<0.001
Error	0.621	94	0.007		

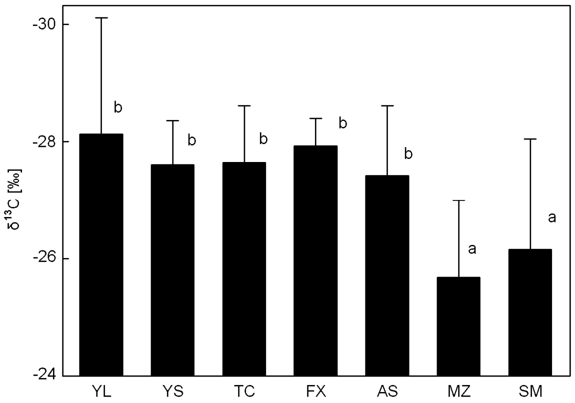

Fig. 3. Foliar stable carbon isotope compositions of plant samples in seven sites of the Loess Plateau in China, Yangling (YL), Yongshou (YS), Tongchuan (TC), Fuxian (FX), Ansai (AS), Mizhi (MZ), and Shenmu (SM). *The same lowercase letters* near vertical bars indicate non-significant difference at $p<0.05$ according to LSD's tests. Error bars are +SD, $n = 15–27$.

Among the nine C_3 species, *R. pseudoacacia*, *H. rhamnoides*, and *C. korshinskii* had significantly higher δ^{13}C values than other six species, and *A. sacrorum* had the lowest δ^{13}C value, whereas the δ^{13}C value differed little among *P. tabulaeformis*, *Z. jujuba*, *R. parvifolius*, *L. davurica*, and *A. cristatum* (Fig. 4). Considering the life forms categorized from C_3 species, there was significant difference ($p<0.001$) in foliar δ^{13}C values among trees, shrubs, and herbaceous plants. Trees and shrubs had larger variation extents and higher δ^{13}C values than herbs, and the mean δ^{13}C values for three life-forms ranked in the order: trees (–26.65 ‰, $n = 39$) > shrubs (–27.01 ‰, $n = 53$) > herbs (–27.91 ‰, $n = 49$) (Fig. 5). The deciduous tree, *R. pseudoacacia* had significantly higher δ^{13}C value (–25.89 ‰, $n = 18$) than the evergreen tree, *P. tabulaeformis* (–27.30 ‰, $n = 21$). Among the four shrubs, *C. korshinskii* and *H. rhamnoides* had much higher δ^{13}C values than *Z. jujuba* and *R. parvifolius*, and mean δ^{13}C values were –25.77 ‰ ($n = 9$) and –26.06 ‰ ($n = 12$), respectively, in both former species and –27.74 ‰ ($n = 21$) and –27.72 ‰ ($n = 12$), respectively, in both latter species. Among the three herbaceous plants, *L. davurica* and *A. cristatum* had very high δ^{13}C values, which were –27.51 ‰ ($n = 18$) and –27.65 ‰ ($n = 12$), respectively, and *A. sacrorum* had the lowest δ^{13}C value (–28.48 ‰, $n = 18$) (Fig. 4). *R. pseudoacacia*, *C. korshinskii*, and *L. davurica* are all leguminous plants and their δ^{13}C values are the highest in their each life-form, trees, shrubs, and herbs, respectively. Like leguminous plants, *H. rhamnoides* also had higher δ^{13}C value because its roots are in symbiosis with abundant rhizobia and thus have higher N-fixation capacities.

Variation trends in foliar δ^{13}C values for nine species in the Loess Plateau: Foliar δ^{13}C values of nine species exhibited an increasing trend in seven sampling sites standing from the south to the north in the Loess Plateau (Fig. 6*A*), and there existed a general increasing tendency for the mean δ^{13}C values (Fig. 6*B*), and the mean δ^{13}C value increased by about 7 % from Yangling to Shenmu.

The variations of δ^{13}C values in five species *P. tabulaeformis*, *R. pseudoacacia*, *Z. jujuba*, *L. davurica*, and *A. sacrorum* distributing widely in seven sites of the Loess Plateau were also studied. Foliar δ^{13}C values of *P. tabulaeformis* and *R. pseudoacacia* sharply decreased first and then increased steeply (Fig. 7*A*), but plants in Mizhi and Shenmu still had higher δ^{13}C values.

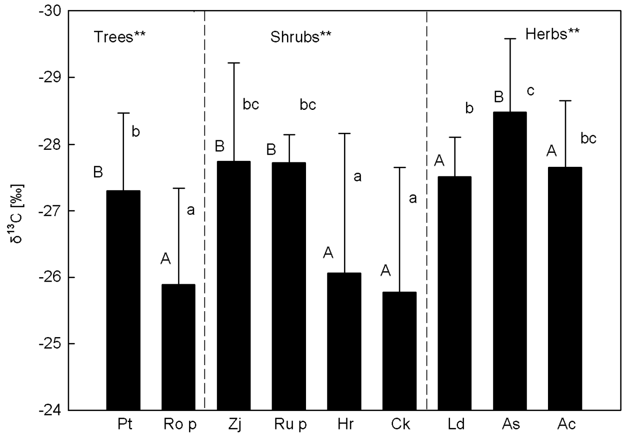

Fig. 4. Foliar stable carbon isotope compositions of nine C_3 species in the Loess Plateau of China. *Lowercase letters* indicate the differences in foliar δ^{13}C values among nine species, while *capital letters* indicate the differences among different species from the same life-form groups, *i.e.* trees, shrubs, and herbs. Significant differences among variables were determined by *t*-test or ANOVA (LSD test, $p<0.05$). Values with any letter in common are not significantly different. **significant difference at $p<0.01$. Error bars are +SD, $n = 9–21$. Abbreviations: Pt: *Pinus tabulaeformis*; Ro p: *Robinia pseudoacacia*; Zj: *Zizyphus jujuba*; Ru p: *Rubus parvifolius*; Hr: *Hippophae rhamnoides*; Ck: *Caragana korshinskii*; Ld: *Lespedeza davurica*; As: *Artemisia sacrorum*; Ac: *Agropyron cristatum.*

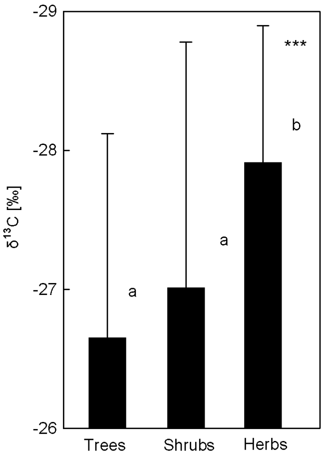

Fig. 5. Foliar stable carbon isotope compositions of the trees, shrubs, and herbs categorised from the life-forms of nine C_3 species in the Loess Plateau of China. *The same lowercase letters* near vertical bars indicate non-significant difference at $p<0.05$ according to LSD's tests. Error bars are +SD. Sampling sizes for the trees, shrubs, and herbs are 39, 53, and 49, respectively. ***significant difference at $p<0.001$ among the three life-forms.

Therefore, their δ^{13}C values still exhibited the increasing trends and increased by about 14.6 % from Yangling to Shenmu for *P. tabulaeformis* and by about 1.9 % from Yangling to Mizhi for *R. pseudoacacia*. Comparatively, *Z. jujuba* and *A. sacrorum* exhibited more significant increasing trends in δ^{13}C values (Fig. 7*B*), which increased by about 13.9 % from Yangling to Shenmu for *Z. jujuba*, and by about 10.2 % from Yangling to Mizhi for *A. sacrorum*. Whereas *L. davurica* showed a slightly increasing trend in δ^{13}C and it increased only by about 0.01 % from Yangling to Shenmu, but by about 4.1 % from Yangling to Mizhi.

Relationships between foliar δ^{13}C values and MAP: For nine species in the Loess Plateau, foliar δ^{13}C values in 141 C_3 plant samples were significantly and negatively (r = –0.364, $p<0.001$) correlated with MAP, generally, foliar δ^{13}C values decreased significantly as MAP increased (Fig. 8). MAP as the main influencing factor could explain 25.5 % of the spatial variations in foliar δ^{13}C values. An increase of 100 mm in MAP would result in a decrease of 1.2 ‰ in foliar δ^{13}C value.

Discussion

Patterns of foliar δ^{13}C values in the Loess Plateau: The carbon isotope fractionation exists in photosynthetic process from the absorption of atmospheric CO_2 to the production of organic matter (Farquhar *et al.* 1989). Plants with different photosynthetic pathways (C_3, C_4, and CAM) have different foliar δ^{13}C values due to the difference in their primary carboxylating enzymes (ribulose-1,5-bisphosphate carboxylase/oxygenase and phospho*enol*pyruvate carboxylase for C_3 and C_4 species, respectively) (Farquhar *et al.* 1989). Generally, C_3 plant's δ^{13}C values vary between –20 and –35 ‰ (average: –27 ‰), C_4 plant's δ^{13}C values range from –7 to –15 ‰ (average: –12 ‰), and CAM-plant's δ^{13}C values lie between C_3 and C_4 plants, about –10 to –22 ‰ (average: –16 ‰) (O'Leary 1981). Foliar δ^{13}C values of 141 plant samples from nine C_3 species taken in the Loess Plateau ranged from –22.66 to –30.70 ‰, with an average of –27.23 ‰. Therefore, these plants belong to C_3 species.

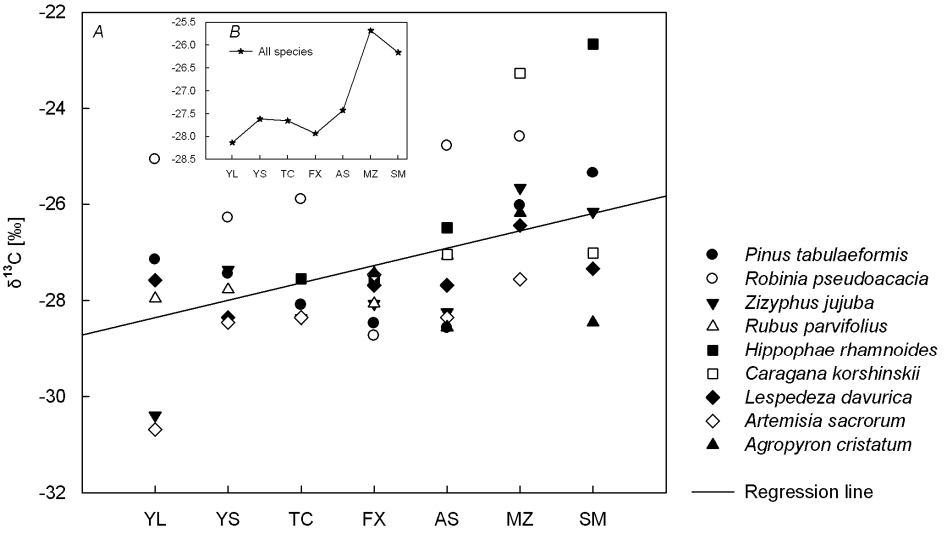

Fig. 6. Scatter plot (*A*) of foliar stable carbon isotope compositions of nine plant species and line plot (*B*) of mean foliar stable carbon isotope compositions of nine species in seven sites of the Loess Plateau in China, Yangling (YL), Yongshou (YS), Tongchuan (TC), Fuxian (FX), Ansai (AS), Mizhi (MZ), and Shenmu (SM).

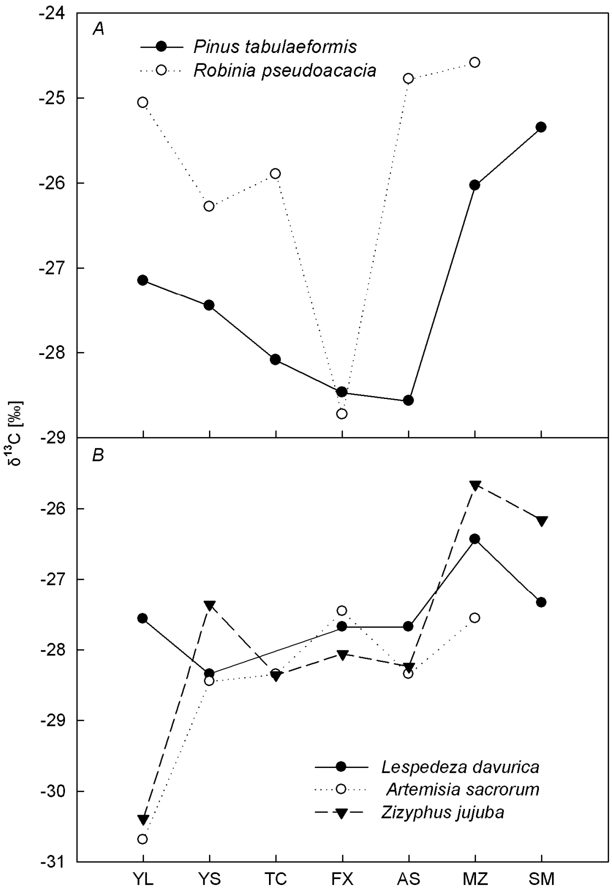

Fig. 7. Line plots of mean foliar stable carbon isotope compositions of *Pinus tabulaeformis* and *Robinia pseudoacacia* (*A*) as well as *Zizyphus jujuba*, *Lespedeza davurica*, and *Artemisia sacrorum* (*B*) in seven sites of the Loess Plateau in China, Yangling (YL), Yongshou (YS), Tongchuan (TC), Fuxian (FX), Ansai (AS), Mizhi (MZ), and Shenmu (SM). *R. pseudoacacia* and *A. sacrorum* were collected from six sites excluding Shenmu, and *L. davurica* was collected from six sites excluding Tongchuan.

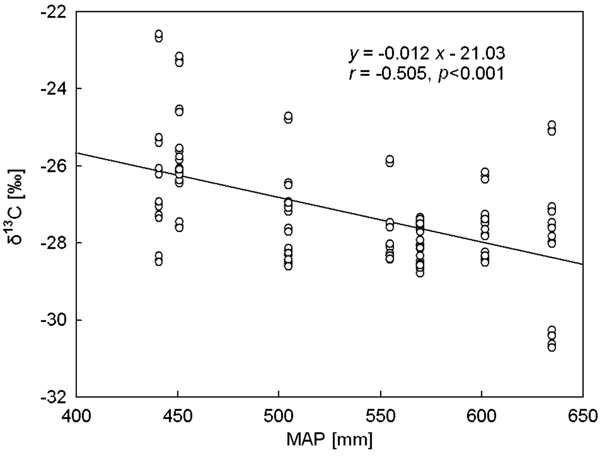

Fig. 8. Relationships between foliar stable carbon isotope compositions of nine species and the mean annual precipitation (MAP) in the Loess Plateau.

Wang *et al.* (2003) reported that foliar $\delta^{13}C$ values of 367 C_3 herbaceous plants in the loess area of northern China varied between –21.7 and –30.0 ‰ with an average of –26.7 ‰, which was consistent with the results in our study.

Foliar $\delta^{13}C$ variations among nine species: The $\delta^{13}C$ values of organic matters in leaves are mainly influenced by the relative variation between diffusion fractionation and enzyme fractionation, further dependent on the intercellular (C_i) and ambient CO_2 (C_a) concentration. While the ratio of C_i/C_a, as a driving factor for leaf gas exchange, is closely related to net photosynthetic rate and stomatal conductance, it also highly correlates with WUE. Because the carbons in plant tissues are accumulated for a period of time, the $\delta^{13}C$ values can indicate the mean value of C_i/C_a for the period. Therefore, foliar $\delta^{13}C$ value is positively correlated with WUE, and can indirectly estimate the long-term plant WUE (Farquhar *et al.* 1989).

Large differences in foliar $\delta^{13}C$ values among the nine species showed that different species have different adaptabilities to climatic environment and thus exhibit considerable differences in plant WUEs. Considering the life-forms, trees and shrubs had significantly higher $\delta^{13}C$ values than herbs, and the deciduous tree *R. pseudoacacia* had higher $\delta^{13}C$ value than the evergreen tree *P. tabulaeformis*. Hence trees and shrubs may have higher WUE than herbs, and deciduous trees have higher WUEs than evergreen trees, which is in agreement with the results of Qu *et al.* (2001) and Chen *et al.* (2002). We hypothesized that significant differences in $\delta^{13}C$ value and WUE among life-forms were likely caused by different root systems for specific water availabilities. Trees and shrubs generally have deeper root systems than herbs in water-limited ecosystems. Therefore, herbs use resources mostly from the upper soil layers, while trees and shrubs take up water mainly from the deeper soil layer (Dodd *et al.* 1998, Schenk and Jackson 2002). Because water resources in deeper layers can be kept more constant and separation in resource utilization reduced the competition between three life-form species, trees and shrubs could employ a relatively prodigal water-use pattern (Gebauer *et al.* 2002). When the precipitation is relatively low in summer, deciduous trees tend to utilize more stable water resources such as groundwater than evergreen trees (Stratton *et al.* 2000).

P. tabulaeformis and *R. pseudoacacia* have better physio-ecological adaptabilities and anti-adversities to dry and barren habitats, and have great functions in conserving soil and water, enriching headwaters and improving soil fertility. Thus, both tree species are dominant in the project of transforming farming land to forestland in the Loess Plateau in China (Cheng and Wan 2002). Recently, numerous studies have been conducted on the community structures (Wang and Wang 2002, Zhang and Shangguan 2005a), biological productivities (Zhang and Shangguan 2005b), and maintenance of nutrients and headwaters (Wang and Shao 2004) for both species in the loess regions, China. In this study, *R. pseudoacacia* had higher foliar δ^{13}C value than other seven species except *C. korshinskii*, while *P. tabulaeformis* had higher δ^{13}C value than other five species except *R. pseudoacacia*, *C. korshinskii*, and *H. rhamnoides*, showing that both species have higher WUEs and more efficient water-use strategies.

H. rhamnoides and *C. korshinskii* grow quickly and possess strong abilities to burgeon and develop large root systems, thus they have great capacities for soil and water conservation, wind and sand prevention, and N-fixation for improving soil fertility. Therefore, both shrub species are considered pioneer species in the project of dryland forestation (Cheng and Wan 2002). Up to now, their biological features (Ma *et al.* 2003b, Li *et al.* 2004), physiological and biochemical characteristics (Ma *et al.* 2003a, Xu and Shan 2004), and drought-tolerant adaptabilities (Xu and Shan 2004, Cheng *et al.* 2005) have been widely studied in China. In this study, foliar δ^{13}C values of *H. rhamnoides* and *C. korshinskii* were significantly higher than those of other six species except *R. pseudoacacia*, suggesting they have higher WUE and better adaptability to dry and barren habitats.

Three leguminous species, *R. pseudoacacia*, *C. korshinskii*, and *L. davurica*, and one non-leguminous species, *H. rhamnoides* with N-fixation capacity, had significantly higher δ^{13}C values than other non-leguminous species with the same life-forms, showing that leguminous species have higher WUEs than non-leguminous species, which is in agreement with the results of Schulze *et al.* (1998). It is probably because leguminous species have higher leaf N contents due to N-fixation, thus they have greater photosynthetic capacities and higher WUE. Quinos (1998) considered that leguminous species living in symbiosis with rhizobia to fix N can not only supply enough N for their growth, but also accelerate the mineralization of litterfalls and root-remnants, which is favourable for increasing soil available N and maintaining sustainable soil productivity. Additionally, leguminous species have great adaptability to poor sandy habitats and play important roles in improving soil fertility and conserving soil and water resources, so they are pioneer species in the recovery and reconstruction of degraded ecosystem in arid areas (Glasener *et al.* 2002).

Relations between foliar δ^{13}C values and environmental factors: Species with different photosynthetic pathways have much different δ^{13}C values (Farquhar *et al.* 1989). Furthermore, foliar δ^{13}C is also influenced by many environmental factors such as precipitation, temperature, humidity, irradiance, air CO_2 concentration, *etc.* (Dawson *et al.* 2002). Thus plants with different geographic distributions have different δ^{13}C values. Austin and Vitousek (1998) evaluated foliar δ^{13}C values in five native forests in Hawaii with the MAP ranging from 500 to 5 500 mm: the δ^{13}C values varied from –25.6 to –29.9 ‰ and decreased by about 16.8 % with increasing MAP. Many studies have shown that foliar δ^{13}C values are negatively correlated with precipitation, and the negative correlation is more distinct in arid areas than in moist areas (Austin and Vitousek 1998, Chen *et al.* 2002, Van de Water *et al.* 2002, Wang *et al.* 2003, 2005). In our study, foliar δ^{13}C values of 141 plant samples in the Loess Plateau were significantly and negatively correlated with the MAP ($p<0.001$), which was in agreement with the above viewpoints. The reason is that low precipitation, low air humidity, and low soil water availability in arid areas would lead to stomata closure and decreases in stomatal conductance and C_i, and then the δ^{13}C value would increase.

Chen *et al.* (2002) studied foliar δ^{13}C values of species occurring within two desert communities of Fukang, Xinjiang, Jinta, and Gansu, and showed that dry conditions led to higher foliar δ^{13}C values. An increase of 100 mm in MAP would result in a decrease of 1.0–1.5 ‰ in δ^{13}C value. Wang *et al.* (2003) reported that foliar δ^{13}C values of 367 C_3 herbaceous species in loess area of northern China decreased steeply with increasing MAP, and the mean change was –0.49 ‰ per 100 mm. In the Loess Plateau, annual precipitation is the main factor that makes the δ^{13}C value in the west part greater than that in the central part. In this study, an increase of 100 mm in the MAP in the Loess Plateau would result in a decrease of 0.88 ‰ in foliar δ^{13}C. This change was much higher than that in the study of Wang *et al.* (2003), but slightly lower than that in the study of Chen *et al.* (2002), which was probably related to plant species and sampling areas.

Water availability is the most limiting factor for plant growth in the Loess Plateau, thus foliar δ^{13}C was mainly influenced by MAP, which could explain 13.3 % of the variation in δ^{13}C value. In the seven sites standing from south to north in the Loess Plateau, the δ^{13}C value increased remarkably when MAP was gradually decreased (Fig. 6*A*), showing that plant WUE increased as climatic drought increased. Generally, plants growing in droughty habits tend to improve their WUEs and employ more conservative water-use patterns to survive in unfavourable environments. Plants need a long period to adapt to climatic change and complex environment. Using carbon isotope analysis to study the responses of WUE in different plants to environment, especially to the precipitation, has important significance on revealing the drought-tolerant mechanisms and adaptive strategies of dominant species in the Loess Plateau to climatic change.

References

Austin, A.T, Vitousek, P.M.: Nutrient dynamics on a precipitation gradient in Hawaii. – Oecologia **113**: 519-529, 1998.

Cerling, T.E., Wang, Y., Quade, J.: Expansion of C4 ecosystems as an indicator of global ecological change in the late miocene. – Nature **361**: 344-345, 1993.

Chen, S.P., Bai, Y.F., Lin, G.H., Han, X.G.: Variations in life-form composition and foliar carbon isotope discrimination among eight plant communities under different soil moisture conditions in the Xilin River Basin, Inner Mongolia, China. – Ecol. Res. **20**: 167-176, 2005.

Chen, T., Feng, H.Y., Xu, S.J., Qiang, W.Y., An, L.Z.: Stable carbon isotope composition of desert plant leaves and water-use efficiency. – J. Desert Res. **22**: 288-291, 2002.

Cheng, J.M., Wan, H.E.: Vegetation Construction and Soil and Water Conservation in the Loess Plateau of China. – Chinese Forestry Press, Beijing 2002.

Cheng, J.M., Wan, H.E., Wang, J., Yong, S.P.: Growth of *Caragana korshinskii* and depletion process of soil water in semi-arid region. – Sci. Silv. sin. **41**(2): 37-41, 2005.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P.: Stable isotopes in plant ecology. – Annu. Rev. Ecol. System. **33**: 507-559, 2002.

Dodd, M.B., Lauenroth, W.K., Welker, J.M.: Differential water resource use by herbaceous and woody plant life-forms in a shortgrass steppe community. – Oecologia **117**: 504-512, 1998.

Duquesnay, A., Bréda, N., Stievenard, M., Dupouey, J.L.: Changes of tree-ring δ^{13}C and water use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. – Plant Cell Environ. **21**: 565-572, 1998.

Evans, R.D.: Physiological mechanisms influencing plant nitrogen isotope composition. – Trends Plant Sci. **6**: 121-126, 2001.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T.: Carbon isotope discrimination and photosynthesis. – Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 503-537, 1989.

Foster, T.E., Brooks, J.R.: Functional groups based on leaf physiology: are they spatially and temporally robust? – Oecologia **144**: 337-352, 2005.

Gebauer, R.L.E., Schwinning, S., Ehleringer, J.R.: Interspecific competition and resource pulse utilization in a cold desert community. – Ecology **83**: 2602-2616, 2002.

Glasener, K.M., Wagger, M.G., MacKown, C.T., Volk, R.J.: Contributions of shoot and root nitrogen-15 labeled legume nitrogen sources to a sequence of three cereal crops. – Soil Sci. Soc. Amer. J. **66**: 523-530, 2002.

Li, G.Q., Zhao, F.X., Li, X.Z., Wei, Y.: Density and biomass dynamics of *Hippophae rhamnoides* L. subsp. *sinensis* population in Mu Us Sandland. – Sci. Silv. sin. **40**(1): 180-184, 2004.

Ma, C.-C., Gao, Y.-B., Guo, Y.-H., Wang, J.-L.: Interspecific transition among *Caragana microphylla*, *C. davazamcii* and *C. korshinskii* along geographic gradient. II. Characteristics of photosynthesis and water metabolism. – Acta bot. sin. **45**: 1228-1237, 2003a.

Ma, C.-C., Gao, Y.-B., Liu, H.-F., Wang, J.-L., Guo, Y.-H.: Interspecific transition among *Caragana microphylla*, *C. davazamcii* and *C. korshinskii* along geographic gradient. I. Ecological and RAPD evidence. – Acta bot. sin. **45**: 1218-1227, 2003b.

Máguas, C., Griffiths, H.: Applications of stable isotopes in plant ecology. – Progr. Bot. **64**: 473-505, 2003.

O'Leary, M.H.: Carbon isotope fractionation in plants. – Phytochemistry **20**: 553-567, 1981.

Parolin, P.: Morphological and physiological adjustment to waterlogging and drought in seedlings of Amazonian floodplain trees. – Oecologia **128**: 326-335, 2001.

Qu, C.M., Han, X.G., Su, B., Huang, J.H., Jiang, G.M.: The characteristics of foliar δ^{13}C values of plants and plant water use efficiency indicated by δ^{13}C values in two fragmented rainforests in Xishuangbanna, Yunnan. – Acta bot. sin. **43**: 186-192, 2001.

Quinos, P.M.: Facilitative effects of *Lotus tenuis* on *Paspalum dilatatum* in a lowland grassland of Argentina. – Oecologia **114**: 427-431, 1998.

Schenk, H.J., Jackson, R.B.: Rooting depths, later root spreads and belowground/aboveground allometries of plants in water-limited ecosystems. – J. Ecol. **90**: 480-494, 2002.

Schulze, E.D., Williams, R.J., Farquhar, G.D., Schulze, W., Langridge, J., Miller, J.M., Walker, B.H.: Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. – Aust. J. Plant Physiol. **25**: 413-425, 1998.

Schwinning, S., Ehleringer, J.R.: Water use trade-offs and optimal adaptations to pulse-driven arid ecosystems. – J. Ecol. **89**: 464-480, 2001.

Shangguan, Z.P., Shao, M.A., Dyckmans, J.: Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. – Environ. exp. Bot. **44**: 141-149, 2000.

Shangguan, Z.P., Shao, M.A., Lei, T.W., Fan, T.L.: Runoff water management technologies for dryland agriculture on the Loess Plateau. – Int. J. Sustain. Develop. World Ecol. **9**: 341-350, 2002.

Stratton, L.C., Goldstein, G., Meinzer, F.C.: Temporal and spatial partitioning of water resources among eight woody species in a Hawaiian dry forest. – Oecologia **124**: 309-317, 2000.

Sun, S.F., Huang, J.H., Lin, G.H., Zhao, W., Han, X.G.: Application of stable isotope technique in the study of plant water use. – Acta ecol. sin. **25**: 2362-2371, 2005.

Van de Water, P.K., Leavitt, S.W., Betancourt. J.L.: Leaf δ^{13}C variability with elevation, slope aspect, and precipitation in the southwest United States. – Oecologia **132**: 323-343, 2002.

Wang, G.A., Han, J.M., Liu, D.S.: The carbon isotope composition of C_3 herbaceous plants in loess area of northern China. – Sci. China D **46**: 1069-1076, 2003.

Wang, K.Q., Wang, B.R.: Study on thinning to *Robinia pseudoacacia* forest on the Loess Plateau. – Chin. J. appl. Ecol. **13**: 11-15, 2002.

Wang, L., Shao, M.A.: Study on relationship between growth of artificial *Robinia pseudoacacia* plantation and soil desiccation in the Loess Plateau of Northern Shaanxi Province. – Sci. Silv. sin. **40**: 84-91, 2004.

Wang, M., Li, Y., Huang, R.Q., Li, Y.L., Zhang, Y.X.: Responses of floral carbonate isotopic compositions of the central Qinghai-Tibet Plateau to environmental conditions. – J. Mount. Sci. **23**: 274-279, 2005.

Xu, B.C., Shan, L.: A comparative study on water use characteristics and eco-adaptability of *Hippophae rhamnoides* and *Caragana korshinskii* in semi-arid loess hilly-gully region. – Chin. J. appl. Ecol. **15**: 2025-2028, 2004.

Yan, C.R., Han, X.G., Chen, L.Z.: Water use efficiency of six woody species in relation to micro-environmental factors of different habitats. – Acta ecol. sin. **21**: 1952-1956, 2001.

Zhang, X.B., Shangguan, Z.P.: Floristic characteristics of seed plants in Ziwuling of Loess Plateau. – Chin. J. Ecol. **24**: 872-877, 2005a.

Zhang, X.B., Shangguan, Z.P.: The bio-cycle patterns of nutrient elements and stand biomass in forest communities in Hilly Loess Regions. – Acta ecol. sin. **25**: 527-537, 2005b.

Zheng, S.X., Shangguan, Z.P.: Studies on variety in the δ^{13}C value of typical plants in Loess plateau over the last 70 years. – Acta phytoecol. sin. **29**: 289-295, 2005.

Ziegler, H.: Stable isotopes in plant physiology and ecology. – Progr. Bot. **56**: 1-2, 1995.

Wise, R.R., Hoober, J.K. (ed.): **The Structure and Function of Plastids**. – Springer, Dordrecht 2006. ISBN 10 1-4020-4060-1 (hard-bound). XL + 575 pp., € 250.00, USD 329.00, GBP 192.50.

The 23rd volume of book series „Advances in Photosynthesis and Respiration" is dedicated to one of the basic topics of photosynthesis that is studied since 17th century everywhere in the world. Where the centres of recent studies on plastids are is shown by the listing of affiliation of 57 authors of this representative book. Twenty six of them work in the U.S.A., the remaining 31 authors in institutions of ten countries: Australia (5), Germany, Japan, Mexico, Sweden, and the U.K. (4 each), France and Switzerland (2 each), and Brazil and Korea (1 each).

B. Gunning, F. Koenig, and Govindjee reviewed the history of recognizing chloroplast structure. This interesting introductory text brings also five figures that are probably not known by young scientists. Twenty-seven chapters of the book are divided into five sections. Section I is entitled "Plastid Origin and Development" and its five chapters describe diversity of plastid forms, basic plastid types and specializations, their origin and evolution affected genomically, by presence of photosynthetic pigments, and by factors of environment, protein import into chloroplasts, and the mechanisms of plastid division. The reader will find here also explanation of some less known terms, such as tic- and toc-translocation (in chapter 3) or dynamin (family of eukaryote-specific proteins involved in the fission of several membrane systems; chapter 5).

Section II, "The Plastid Genome and its Interaction with the Nuclear Genome" (5 chapters), is devoted to expression, prediction, and functions of the thylakoid proteome, nucleus- and chloroplast-encoded factors in synthesis of photosynthetic apparatus (chloroplast splicing, translation, elongation, assembly, modulation by radiant energy, *etc*.), plastid transcription and functions of the respective polymerases, plastid-to-nucleus-signalling (one finds here, among others, list of mutants with morphologically aberrant plastids or regulation of the tetrapyrrole pathway), and utilization of trace metals (Fe, Cu, Mn) in chloroplasts.

Section III is entitled "Photosynthetic Metabolism in Plastids". Its four chapters deal with light/dark regulation of chloroplast metabolism (ferredoxins, thioredoxins, ferredoxin:thioredoxin reductase and target enzymes of carbon cycles), physiological significance of chlororespiratory pathway that reduces and oxidises plastoquinone pool (with special attention to studies on *Arabidopsis* and *Chlamydomonas*), CO_2 concentrating mechanism (functions of five types of carbonic anhydrases, differences in cyanobacteria, eukaryotic algae, and higher plants), and with synthesis, export, and partitioning of photosynthates (intermediates, sucrose, starch, photosynthetic carbon oxidation cycle, *etc*.).

Six chapters of Section IV ("Non-Photosynthetic Metabolism in Plastids") are devoted to biosyntheses of chlorophylls, carotenoids, lipids (glycerolipids, fatty acids; their transport is also analysed), amino acids (namely glutamine, glutamate, aspartate, branched-chain and aromatic amino acids), and sulphur metabolites. A special chapter is on the regulation of calcium fluxes in chloroplasts. Section V consists of seven chapters on plastid differentiation and responses to environment. Individual chapters deal with plastids in ripening fruits (chromoplasts, metabolism of saccharides, lipids, carotenoids, activity of polyphenol oxidase) and plastid fate during leaf senescence (gerontoplasts, degeneration of thylakoid system and basic substances, plastoglobuli, extrusion of material, catabolism of pigments, reactive oxygen species), role of plastids in gravitropism (I was pleased to find here reference to an early paper of the Czech scientist Bohumil Němec, written in 1900), with chloroplast movements as responses to environmental signals (involvement of phototropin, phytochrome, Ca ions, *etc*.), and with oxygen metabolism and stress physiology (the water-water cycle, dissipation of excess energy, and photoprotection). Special chapters are devoted to kleptoplasts (plastids acquired from photosynthetic organisms by heterotrophic organisms such as ciliates, foraminifera, and sacoglossans) and apicoplasts (chloroplast-derived organelles originating by secondary endosymbiosis).

All chapters are accompanied by explanatory figures (including electron micrographs and schemes), summarizing tables, and lists of complete references. In some chapters directions for further research are outlined. In the introductory pages prepared by the series editor Govindjee the reader will find interesting information, *e.g*. list of selected earlier books on chloroplasts or reference to a useful two-disc CD set of "Plant Biology", that contains more than 1 000 images (see www.plantcellbiologyonCD.com). Generally, it is a very useful book for students and scientists in plant biology, biochemistry, and genetics.

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