

Lvliu ZOU, Guchou SUN, Ping ZHAO, Xi'an CAI, Xiaoping ZENG, Xiaojing LIU

Carbon isotope discrimination in leaf juice of *Acacia mangium* and its relationship to water-use efficiency

© Higher Education Press and Springer-Verlag 2009

Abstract Using the PMS pressure chamber and isotope mass spectrometer (MAT-252), the leaf juice of *Acacia mangium* was obtained, and the carbon isotope discrimination (Δ) representing the most recently fixed carbon in the juice was determined. At the same time, the water-use efficiency of *A. mangium* was estimated. The results indicated that the carbon isotope ratio in the air of forest canopy (δ_a), 10 m high above ground averaged -7.57 ± 1.41 ‰ in cloudy days, and -8.54 ± 0.67 ‰ in sunny days, respectively. The diurnal change of the carbon isotope ratio in the photosynthetic products of the leaf juice (δ_p) was of saddle type in cloudy days, but dropped down from morning to later afternoon in sunny days. A strong negative correlation between δ_p and leaf-to-air vapor pressure deficit (D) was observed in sunny days, but a slight change in δ_p was found in cloudy days. The δ_p also decreased with decreasing leaf water potential (Ψ), reflecting that water stress could cause the decrease of δ_p . The carbon isotope discrimination of the leaf juice was positively correlated with the ratio between intercellular (P_i) and atmospheric (P_a) partial pressure of CO_2 . For *A. mangium*, the isotope effect on diffusion of atmospheric CO_2 via stomata was denoted by $a = 4.6$ ‰, and that in net C_3 diffusion with respect to P_i was indicated by $b = 28.2$ ‰. The results were in reasonable accord with the theoretically diffusive and biochemical fractionation of carbon isotope. It was defined that carbon isotope discrimination of photosynthetic products in *A. mangium* leaf juice was in proportion to that from photosynthetic products in dry material. The water-use efficiency estimated by the carbon isotope discrimination in leaf juice, fit well with that measured by gas exchange system

($R^2 = 0.86$, $p < 0.0001$). The application of leaf juice in measuring the stable carbon isotope discrimination would reduce the effects of fluctuating environmental factors during the synthesis of dry matter, and improve the eco-physiological studies on carbon and water balance when scaling from the plant to canopy in the fields.

Keywords carbon isotope discrimination, water-use efficiency, photosynthetic products in leaf juice

1 Introduction

The fourth assessment report of the IPCC (Intergovernmental Panel on Climate Change, 2007) pointed out that the global climate warming was an undisputed fact, and the temperature of the earth's surface would increase by 1.7 – 1.4°C on average to the end of 21st century (IPCC, 2007). Chinese researchers forecasted that the temperature from 2020 to 2030 in China would increase 1.3°C , and 2.2°C to 2050, in comparison with that in 2000 (Qin, 2003). The climate warming would enhance the evaporation of the earth's surface and transpiration in plant leaves and then increase water losses from plant and water uptake from soil by roots, making the soil dry. Usually, plants take up water from soil by soil-plant hydraulic system and balance the vapor pressure deficit by transpiration via leaf stomata and cuticle. The stomata are diffusion pathways for CO_2 and H_2O in plant leaves. Atmospheric CO_2 enters mesophyll and transfers to the site where carboxylation takes place. Photosynthesis (A) goes on at the same time the water evaporates through stomata and cuticle (E). The A/E ratio reflects the ratio of CO_2 assimilation rate to transpiration rate, namely water-use efficiency (WUE). The WUE of plant species differs markedly due to their different genetic and physiological characteristics, as well as different habitats. A gas exchange system is often employed to measure leaf WUE, but it fails when it is measured on a larger scale in the fields (Farquhar et al., 1989).

CO_2 presents in the forms of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in the

Translated from *Chinese Journal of Ecology*, 2008, 27(4): 497–503 [译自: 生态学杂志]

Lvliu ZOU, Guchou SUN, Ping ZHAO (✉), Xi'an CAI, Xiaoping ZENG, Xiaojing LIU
South China Institute of Botany, Chinese Academy of Sciences, Guangzhou 510650, China
E-mail: zhaoping@scib.ac.cn

atmosphere, and they are discriminated by plants when photosynthesis fixes CO₂. ¹³C can be discriminated by enzyme Rubisco which assimilates CO₂ in C₃ plants (Park and Epstein, 1960). The mass of C atoms influences CO₂ diffusion via the stomata, making dissimilar allocations of ¹³C and ¹²C in photosynthetic products (O’Leary, 1981). Farquhar et al. (1982) pointed out that there was a linear relationship between carbon isotope discrimination (Δ) of C₃ plants and the ratio of intercellular CO₂ partial pressure to atmospheric CO₂ partial pressure. Furthermore, Farquhar (1984) proved that the WUE of C₃ plants correlated with the Δ . Potted plants e.g. groundnut (Hubick et al., 1988), cotton (Martin and Thorstenson, 1988), barley (Hubick and Farquhar, 1989) took on such similar relationships. Wright et al. (1988) proved that there was a relationship between Δ and WUE of products in aboveground organs from eight genotypes of groundnut, whereas it needed more research to find out whether such a relationship is applicable at larger spatial scale. The Δ of dry matter in aboveground organs was applied to estimate WUE in early studies in that the stable isotope ratio of dry matters was taken as an aggregate of a series of complicated physiological and biochemical reactions for longer time period, and in which the change of stomatal conductance and water loss by transpiration, caused by the fluctuation of vapor pressure deficit, was difficult to estimate. On the assumption that soluble sugar was the main photosynthetic product in leaf and accumulated in mesophyll cells or transferred to outside, the carbon in leaf juice collected by pressuring could represent the carbon that was newly assimilated, and the fluctuation of microclimate which caused changes of photosynthesis and stomatal conductance could be supposed to be relatively stable at this period of time.

Since mid 1980s *A. mangium* was introduced to the Guangdong hilly land region located in the low subtropics. *A. mangium* plantation played an important role in the utilization of spatial energy resource, regulation of microclimate and improvement of ecological environment of hilly land. The soil of the degraded ecosystem in south China is very infertile. Soil erosion is serious as a result of vegetation loss on the land surface. Its physical and chemical characters were deteriorated. During the rainy season, water supply is discontinuous because of the lower water-holding capability of soil, and it is even worse in dry season (from September to December). These conditions above are disadvantageous for the growth of many tree species, and climatic warming would make it worse. The water deficit in the soil caused by a prolonged dry season is an adverse factor for the introduced tree species. In this study, carbon isotope discrimination (Δ) is employed to estimate the WUE of *A. mangium*, inspect the effect of environmental change on photosynthesis and water-use, and is suitable and effective experimental method for the study on forest ecosystem carbon and water balance.

2 Study area and methods

2.1 Study site

We conducted this study in Heshan Hilly Land Interdisciplinary Experimental Station, Chinese Academy of Sciences, which is located at 22°41’N and 112°54’E, in Heshan city, Guangdong Province. The station is characterized with southern subtropical monsoon climate and crimson soil. The mean annual temperature is 21.7° C, precipitation 1700 mm and evaporation amounts to 1600 mm. Dry season is from September to December. The *A. mangium* plantation in which the field measurement was conducted is 23 years old and 10 m to 17 m high. The fieldwork was carried out on sunny days and cloudy days in December, 2006. The weather during the consecutive 3 days before experimental measurement was stable.

2.2 Carbon isotope ratio measurement

We collected air samples from canopy of the *A. mangium* plantation (about 10 to 15 m above ground) with an electromagnetic pump and a plastic tube. The samples were then injected right away into sampling bags which were vacuumed beforehand. Air samples were collected once every 1 h and repeated twice. At the same time, an infrared CO₂ analytical instrument (Li-Cor 6400, Inc., Lincoln, NE, USA) was employed to record the atmospheric CO₂ concentration. CO₂ isotope ratio of air samples which were pretreated was determined on the MAT-252 stable isotope mass spectrograph (δ_a):

$$\delta_a = \left(\frac{R_a}{R_{std}} - 1 \right) \times 1000 \quad (1)$$

where R_a is the atmospheric ¹³C/¹²C ratio, and R_{std} is the ¹³C/¹²C ratio of Pee Dee Belemnite (PDB) standard sample.

2.3 The collection of plant leaf photosynthetic products samples and the measurement of carbon isotope ratio

We collected mature leaves from the canopy of *A. mangium* every 2 h at the same time with the air sample collection which were then pressured with PMS pressure chamber (PMS Instruments, Cregan, USA), and then sucked the leaf juice with the mini-sucker. These were then injected into a 10-mL vial at once, frozen in the refrigerator and afterwards, we measured the carbon isotope ratio (δ_p) of leaf juice with MAT-252 stable isotope mass spectrograph:

$$\delta_p = \left(\frac{R_p}{R_{std}} - 1 \right) \times 1000 \quad (2)$$

where R_p is the ¹³C/¹²C ratio of plant samples. According to the method presented by Farquhar and Richards (1984)

carbon isotope discrimination (Δ) of plant photosynthetic products was calculated:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad (3)$$

2.4 Determination of leaf photosynthetic and transpiration rate

With respect to the sample site slope, we set up a bracket on higher land and employed other Li-Cor 6400 photosynthesis measurement system to measure photosynthetic and transpiration rate of canopy leaf on the lower land, collecting 20 pieces of leaves randomly every 2 h, detecting continuously and recording the temperature, relative humidity and photosynthetic active radiation.

2.5 Data processing

2.5.1 WUE calculation

According to Farquhar et al. (1985), the ratio of leaf CO₂ assimilation rate (A) to transpiration rate (E) (i.e., WUE):

$$\frac{A}{E} = WUE = \frac{P_a - P_i}{1.6D} \quad (4)$$

$$\Delta = a + (b - a) \times \frac{P_i}{P_a} \quad (5)$$

$$WUE = \frac{P_a \times \frac{b - d - \Delta}{b - a} \times (1 - \phi_c)}{1.6 \times D \times (1 + \phi_w)} \quad (6)$$

where P_a and P_i are atmospheric and intercellular CO₂ partial pressure, respectively, and D is the leaf-to-air vapor pressure deficit (kPa). When C₃ plants photosynthesize, the carbon isotope discrimination of atmospheric ¹³CO₂ and ¹²CO₂ that diffuse via stomata was denoted by $a = 4.4$ ‰ (Craig, 1954), and the carbon isotope discrimination related to intercellular CO₂ partial pressure P_i and carbon fixation is indicated by $b = 27$ ‰ (Farquhar et al., 1989), d is the emendation value related to CO₂ assimilation rate and negligible under daytime light radiation conditions (Evans et al., 1986), whereas the effect of respiration in the dark or without illumination on the discrimination is supposed to be 7 ‰ (Werner et al., 2006). ϕ_c is a parameter related to soil humidity, $(1 - \phi_c) = 0.7$ in dry season, and $(1 - \phi_c) = 1$ under irrigative conditions (Hubick and Farquhar, 1989), $\phi_w = 0$ among the same plant species.

2.5.2 Leaf-to-air vapor pressure deficit

According to Cambell and Norman (1998), it is obtained based on the calculation of measured leaf temperature and air relative humidity.

2.6 Statistical analysis

The data were mean values from three or four measurements. They were analyzed using variable analysis (ANOVA) and least significance detection (LSD) with SPSS (SPSS, INC, Chicago IL), and the least significance level is 0.05.

3 Results and analysis

3.1 The carbon isotope ratio of canopy atmospheric CO₂ in *A. mangium* plantation

The fluctuation of atmospheric δ_a in *A. mangium* plantation canopy in the experimental site was significant ($p < 0.05$). Figure 1 shows that atmospheric CO₂ concentration of canopy was higher in the morning and δ_a was lower. Keeling et al. (1979) pointed out that atmospheric ¹³CO₂ consumption was slow as ¹³C was discriminated when photosynthesis assimilated CO₂ during daytime. The CO₂ assimilation decreases the concentration of ambient CO₂, then remaining CO₂ containing ¹³C in atmosphere was denser than before, and δ_a was higher. The atmospheric CO₂ concentration of *A. mangium* plantation canopy was less fluctuant, so was δ_p which changed along with CO₂ concentration (Fig. 1).

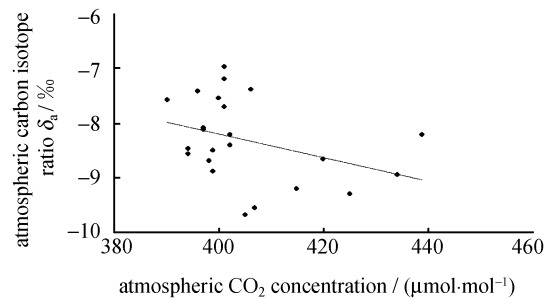


Fig. 1 Relationship between carbon isotope ratio (δ_a) and CO₂ concentration of air in the canopy of *A. mangium* plantation
Note: Leaf temperature is 21.9°C, leaf-to-air vapor pressure deficit is 1.45 kPa.

On December 7, 2006, which was a cloudy day, the atmospheric CO₂ concentration in the *A. mangium* plantation canopy (10 to 15 m above ground) was 445 $\mu\text{mol/mol}$ in the morning, declined with the time and dropped down to 401 ± 18.3 $\mu\text{mol/mol}$ at noon. The minimum was 390 $\mu\text{mol/mol}$ in the afternoon (Fig. 2(a)). During daytime the mean value of atmospheric carbon isotope ratio δ_a was -7.57 ± 1.41 ‰ ($n = 14$) and lower at noon, the diurnal change was of saddle type (Fig. 2(b)). On December 17, 2006, which was a sunny day, the atmospheric CO₂ concentration of *A. mangium* plantation canopy was 400.2 ± 4.3 $\mu\text{mol/mol}$ on the average and the atmospheric CO₂ carbon isotope ratio δ_a was -8.5 ± 0.67 ‰, which was

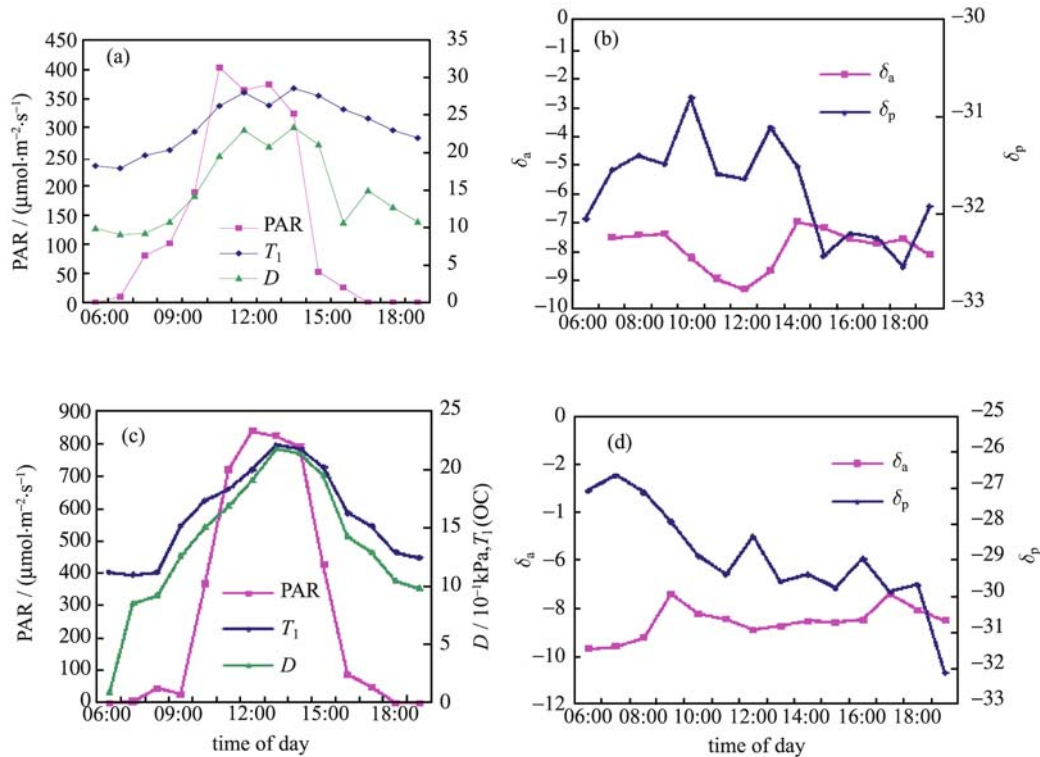


Fig. 2 Daily changes of light intensity, leaf temperature and leaf-to-air vapor pressure difference (a, b), carbon isotope ratio (c, d) of photosynthetic product in leaves of *A. mangium*
Note: (a), (c): cloudy day; (b), (d): sunny day.

significantly lower than in the cloudy day ($p < 0.05$). During sunny days, the diurnal change of δ_a was relatively more stable but lower in the morning (Fig. 2(d)). Farquhar et al. (1989) suggested that the atmospheric δ_a was supposed to 8.0‰ when estimating carbon isotope discrimination of plants in the field.

3.2 The carbon isotope ratio of photosynthetic products from *A. mangium* leaves

Figure 2 shows that solar radiation was lower in the morning on December 7 (cloudy day). Mean photosynthetically active radiation was $324 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$ from 9:00 to 14:00 and then declined gradually. The mean diurnal temperature was $23.5 \pm 3.6^\circ\text{C}$, and leaf-to-air vapor pressure deficit (D) was 0.94 kPa before 8:00, which then increased from 1.89 kPa at noon to 2.17 kPa in the afternoon and declined to 1.22 kPa after 15:00 (Fig. 2(a)). During cloudy days, the carbon isotope ratio of plant photosynthetic products was lower in the morning and afternoon, but took on two peak values at 11:00 and 14:00, and lower at 12:00, the diurnal change was of saddle type. The diurnal mean δ_a was $-31.75 \pm 0.59\text{‰}$ (Fig. 2(b)). On December 17 (sunny day), the mean photosynthetically active radiation was $650 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$ from 9:00 to 15:00, and the diurnal mean temperature was

$16.12 \pm 4.0^\circ\text{C}$; leaf-to-air vapor pressure deficit (D) was 2.18 kPa at noon, which declined in the morning and afternoon, and diurnal mean D was $1.43 \pm 0.47 \text{ kPa}$ (Fig. 2(d)). The results show that the carbon isotope ratio δ_a of photosynthetic products in *A. mangium* leaf was $-28.89 \pm 1.42\text{‰}$ in sunny days, which was higher than that in cloudy days ($p < 0.05$).

In sunny days, *A. mangium* leaf took on higher photosynthetic rate and lower intercellular CO_2 partial pressure (P_i), e.g. P_i was 25.78 Pa in sunny days and 31.06 Pa in cloudy days; the ratio of intercellular CO_2 partial pressure to atmospheric CO_2 partial pressure (P_i/P_a) was 0.648 at noon in sunny days but 0.772 in cloudy days. Therefore δ_a of leaves was higher in sunny days than that in cloudy days.

From Fig. 3 it can be seen that the carbon isotope ratio (δ_a) of *A. mangium* leaf presented small change along with the increase of leaf-to-air vapor pressure deficit in cloudy days, whereas in sunny days the δ_p of *A. mangium* leaf was higher, but dropped down markedly with the increase of D . This study indicated that δ_p of *A. mangium* leaf fluctuated more obviously in sunny days under similar D conditions.

When D was 1.4 kPa, the carbon isotope ratio (δ_p) of photosynthetic products in *A. mangium* leaf declined with the decrease of leaf water potential (Ψ) (Fig. 4). The

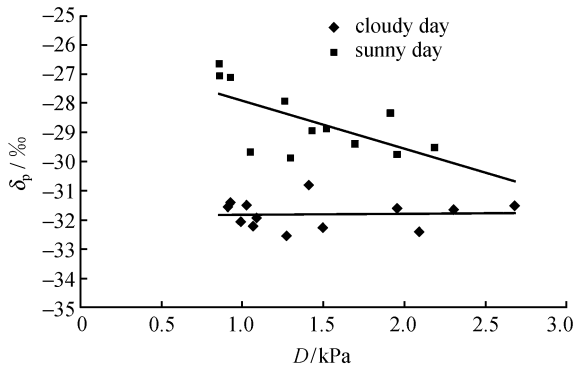


Fig. 3 The relationship between leaf-to-air vapor pressure deficit (D) and carbon isotope ratio (δ_p) of photosynthetic products in leaves of *A. mangium*

Note: Cloudy day with average irradiance of $290 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$ and mean leaf temperature of $19.9 \pm 2.1^\circ\text{C}$; Sunny day with average irradiance of $536 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$ and mean leaf temperature of $23.5 \pm 3.6^\circ\text{C}$.

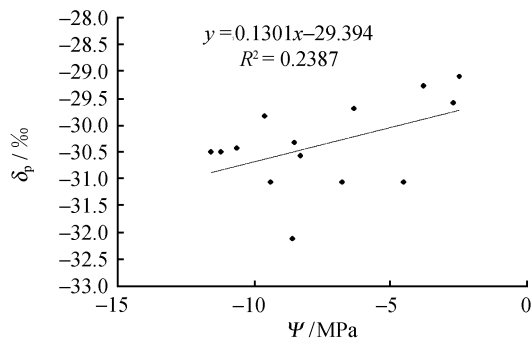


Fig. 4 The relationship between leaf water potential (Ψ) and carbon isotope ratio (δ_p) of photosynthetic products in leaves of *A. mangium*

Note: Ambient CO_2 partial pressure was 38.6 Pa ; leaf-to-air vapor pressure deficit was 1.46 kPa .

decrease of leaf water potential reflected an increase of water stress, and caused the leaf stomata to close and intercellular CO_2 partial pressure to increase, which then resulted in a decline of δ_p (Ehleringer and Cooper, 1986).

3.3 The carbon isotope discrimination (Δ) of photosynthetic products in leaf in relation to water-use efficiency (WUE)

With the increase of intercellular and atmospheric CO_2 partial pressure, Δ of *A. mangium* leaf juice increased, and there was significant correlation between Δ and the P_i/P_a ratio which was measured by gas change system (Fig. 5). It was identical to the result proved by Farquhar et al. (1982) that dry matters of different plant species were significantly correlative with the P_i/P_a ratio.

When the atmospheric CO_2 partial pressure was 38.6 Pa and D was 1.4 kPa , there was negative relationship between Δ obtained by measurement and calculation with

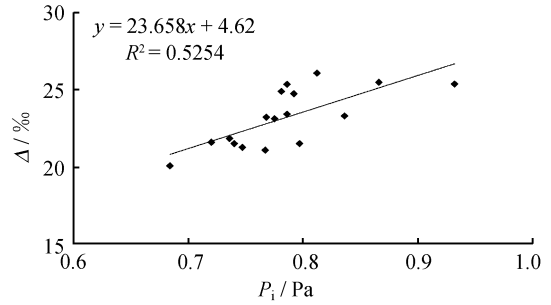


Fig. 5 Carbon isotope discrimination in relationship to the ratio between intercellular and ambient CO_2 partial pressure (P_i/P_a)

Note: P_i and P_a were measured with gas exchange system, Δ was calculated from Eq. (3).

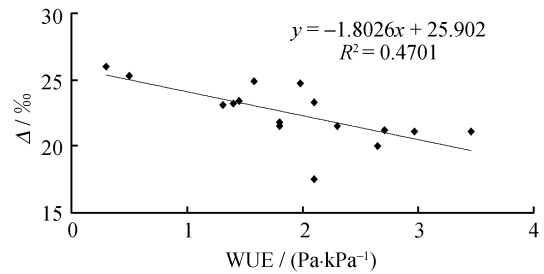


Fig. 6 Relationship between water-use efficiency (WUE) and leaf carbon isotope discrimination (Δ) of *A. mangium*

Note: WUE data were obtained from Fig. 2, ambient CO_2 partial pressure was 38.6 Pa , leaf-to-air vapor pressure deficit was 1.46 kPa .

Eq. (3) and leaf water-use efficiency (WUE, P_a/kPa). If the WUE was higher, the Δ was lower (Fig. 6). Scatter dots figure (Fig. 7) based on two types of WUE obtained from Δ and gas exchange system show that two results were similar. Using the relationship between Δ of leaf juice and the P_i/P_a ratio, we calculated that $a = 4.6 \text{ ‰}$ if $P_i/P_a = 0$, $b = 28.2 \text{ ‰}$ if $P_i/P_a = 1$. Two types of WUE were close to each other ($R^2 = 0.86$, $p < 0.0001$) (Fig. 7(b)).

4 Discussion

^{12}C and ^{13}C are two types of stable carbon isotope existing in nature, and their allocations are uneven in different compounds, which occur in the physico-chemical and metabolism processes when plants take up and assimilate CO_2 . The abundance of ^{13}C relative to ^{12}C in plants tissues is smaller than that in atmospheric CO_2 , which reflects that the carbon isotope is discriminated during CO_2 assimilation. Starting with O'Leary's study (1981), this research field made great progress and researchers uncovered the effect of the photosynthetic metabolism to the environment making use of the carbon isotope discrimination characteristics in plant photosynthesis. In recent years, stable

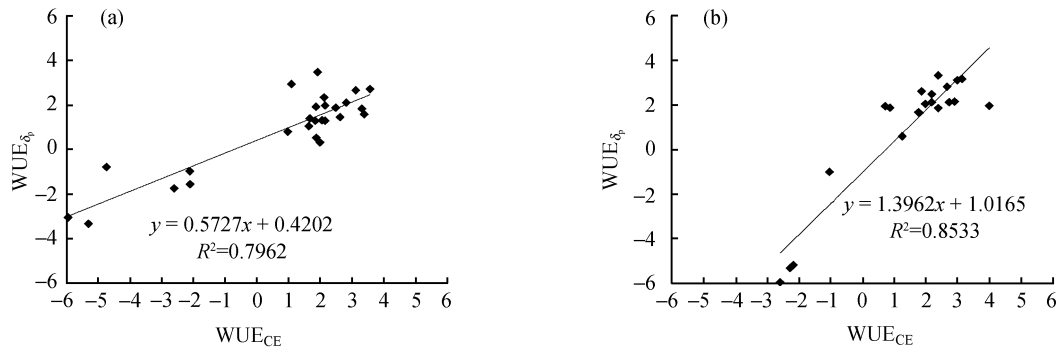


Fig. 7 Leaf water-use efficiency (WUE_{CE}) value calculated from the measurement using CO_2 exchange method in comparison with that based on carbon stable isotope measurement of photosynthetic product of *A. mangium* ($WUE_{\delta p}$)
 Note: WUE values on Y-axis were calculated from Δ using equation 6, values of a and b are 4.4‰ and 27‰ respectively in Fig. 7(a), while they are 4.6‰ and 28.2‰ respectively obtained from this issue Fig. 7(b).

carbon isotope detecting techniques have been applied to studies on the forest ecosystem carbon cycling (Gessler et al., 2001; Ponton et al., 2006; Werner et al., 2006), which also received attention in China (Yi and Zhuang, 2005; Zheng et al., 2005).

Researchers explained the allocations of stable carbon isotope in C_3 plants photosynthesis with multi-modes which were in common with the assumption that the different allocations of carbon isotope were attributed to the difference of $^{12}CO_2$ and $^{13}CO_2$ diffusion via the stomatal pathway and the difference of enzyme Rubisco carboxylation capability (Keeling et al., 1979; Vogel, 1980; Farquhar et al., 1989). The C_3 plants leaf carbon isotope discrimination proposed by Farquhar et al. (1982) was simple, which used in the relationship between Δ of dry matter from different herbaceous species and the P_i/P_a ratio to determine a and b directly. When stomatal conductance relative to the capability of CO_2 fixation was lower, intercellular CO_2 partial pressure (P_i) was lower and carbon isotope discrimination (Δ) increased to $a = 4.4\%$. However, when the stomatal conductance was higher and P_i was close to atmospheric CO_2 partial pressure (P_a), Δ inclined to $b = 27\%$, i.e. net allocation or Rubisco discrimination resulted from carboxylation. Farquhar et al. (1982) proved that Δ of dry matters in plant photosynthetic products significantly correlated with the P_i/P_a ratio, and our study also proved that Δ of *A. mangium* leaf photosynthetic products positively correlated with the P_i/P_a ratio measured with gas exchange system (Fig. 5). Thus a and b of woody plants were 4.6‰ and 28.2, respectively, which fitted with the WUE calculated from Eq. (6), and accorded with the WUE measured with the gas exchange system. Although the sugar content in the leaf juice of *A. mangium* was not measured in this study, most fixed carbon came into being soluble sugar in the first instance. This study indicated that the increase of atmospheric CO_2 concentration for a short period of time caused the stomata to close, and then the concentration of cellular CO_2 increased and made the C_i/C_a ratio or P_i/P_a ratio

change, where the Δ of the leaf juice increased and the WUE declined, i.e., the increase of atmospheric CO_2 concentration would change the WUE of plants. Comstock and Ehleringer (1993) also proved that the increase of atmospheric CO_2 concentration for longer periods of time and the fluctuation of leaf temperature would influence δ_p and WUE. Brugnoli et al. (1988) proved that the Δ of soluble sugar in the cotton leaf correlated intimately with the P_i/P_a ratio and calculated Δ of sugar reflected the allocation of carbon isotope in middle temporal scale. In this study we extracted leaf juice within 1 hour after photosynthesis, which could reflect photosynthetic products in the short term. From Fig. 5, the P_i/P_a ratio was 0.7–0.9, which was in accordance with the results of Eqs. (4) and (5) related to stomatal diffusion and bio-chemical discrimination.

Francey et al. (1985) found that atmospheric carbon isotope ratio (δ_a) presented an obvious gradient change in the *Lagastrobos franklinii* plantation, and in the *A. mangium* plantation we found δ_a fluctuated with time in the diurnal course. Due to the ^{13}C discrimination in leaf photosynthesis, the difference of forest canopy photosynthetic activity, soil respiration and litter decomposition caused a slow change of the atmospheric CO_2 isotope ratio. On account of the fluctuation of δ_a in *A. mangium* plantation, and especially the significant difference of δ_a during cloudy days and sunny days, relevant δ_a needed to be measured when estimating carbon isotope discrimination (Δ) of plant grown on farm. The modified atmospheric CO_2 carbon isotope in field could reflect atmospheric CO_2 carbon isotope ratio of *A. mangium* plantation stand in a larger scale. The carbon isotope discrimination measurement system has become an effective method in C_3 plants eco-physiological photosynthesis and relevant water-use research. However, there is much work to be done on how to scale up from individual plant to canopy, e.g. the regulation of water dissipation is not an independent variable and soil evaporation negatively correlates with the relevant leaf area. Vapor pressure deficit fluctuated

frequently and is not an independent variable. Vapor pressure deficit changes with the fluctuation of stomatal conductance and canopy aerodynamic data are also needed to ascertain the difference of Δ at the canopy level. The experimental site of this study is located in the Zhujiang Delta Region where the cloudy days are quite frequent, and we measured the mean carbon isotope ratio of leaf juice in sunny days and cloudy days, respectively, which are in accordance with the local climate. The short-term change of the C_i/C_a ratio can be estimated with the use of leaf juice carbon isotope discrimination measurement system, whereby the water-use efficiency of C_3 plants in a short time (e.g. 1 h) can be calculated. This method can reduce the effect of fluctuating environmental factors on water-use and offers a new approach to measure the C_i/C_a ratio during the growth season, and it is good for the measurement of water-use efficiency in different season. It is also helpful for further research on the carbon and water balance when scaling from an individual plant to canopy.

Acknowledgements This study was supported by the National Natural Science Foundation of China (Grant No. 30428022, 30770328, 30871998) and Provincial Nature Science Foundation of Guangdong (No. 07006917).

References

- Brugnoli E, Hubick K T, von Caemmerer S, Wong S C, Farquhar G D (1988). Correlation between the carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio of intercellular and atmospheric partial pressure of carbon dioxide. *Plant Physiol*, 88: 1418–1424
- Campbell G S, Norman J M (1998). *An Introduction of Environmental Biophysics*. New York: Springer-Verlag, 36–51
- Comstock J, Ehleringer J (1993). Stomatal response to humidity in common bean (*Phaseolus vulgaris*). Implication for maximum transpiration rate, water-use efficiency and productivity. *Aust J Plant Physiol*, 20: 669–691
- Craig H (1954). Carbon-13 variations in plants and the relationship between carbon-13 and carbon-14 variations in nature. *J Geol*, 62: 115–149
- Ehleringer J R, Cooper T A (1986). Correlation between carbon isotope ratio and micro habitat in desert plants. *Oecologia*, 76: 562–566
- Evans J R, Sharkey T D, Berry J A, Farquhar G D (1986). Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plant. *Aust J Plant Physiol*, 10: 205–206
- Farquhar G D, Ball M C, von Caemmerer S, Roksadic Z (1982). Effects of salinity and humidity on $\delta^{13}C$ value of halophytes—evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different conditions. *Oecologia*, 52: 121–124
- Farquhar G D, Ehleringer J R, Hubick C T (1989). Carbon isotope discrimination and photosynthesis. *Ann Rev Plant Physiol*, 40: 503–537
- Farquhar G D, Richards R A (1984). Isotope composition of plant carbon correlates with water-use efficiency of wheat genotype. *Aust J Plant Physiol*, 11: 539–552
- Francey R T (1985). Cape Gm isotope measurements—a preliminary assessment. *J Atmos Chem*, 3: 247–260
- Gessler A, Schrempf S, Matzarakisa A, Mayer H, Rennenberg H, Adams A (2001). Radiation modified effect of water availability on the carbon isotope composition of beech (*Fagus sylvatica*). *New Phytol*, 150: 653–664
- Hubick K T, Shorter R, Farquhar G D (1988). Heritability and genotype environment interactions of carbon isotope discrimination and transpiration efficiency in peanut. *Aust J Plant Physiol*, 13: 803–816
- Hubick K T, Farquhar G D (1989). Carbon isotope discrimination and the ratio of carbon obtained to water lost in barley cultivars. *Plant, Cell Environ*, 12: 795–804
- Intergovernmental Panel on Climate Change (IPCC) (2007). Fourth assessment report of working group I. In: United Nation's Environmental Program. Geneva, Switzerland
- Keeling C D, Moor W M, Tans P P (1979). Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide. *Nature*, 277: 121–123
- Martin B, Thorstenson Y R (1988). Stable carbon isotope composition ($\delta^{13}C$), water-use efficiency, and biomass productivity of *Lycopersicon esculentum*, *Lycopersicon pennellii*, and the F1 hybrid. *Plant Physiol*, 88: 213–217
- O'Leary M N (1981). Carbon isotope fractionation in plants. *Phytochemistry*, 20: 553–567
- Park R, Epstein S (1960). Carbon isotope fractionation during photosynthesis. *Geochim et Cosmochim Acta*, 21: 110–126
- Ponton S, Flanagan L, Alstad K, Johnson B, Morgenstern K, Klugun N, Blackt A, Barr A (2006). Comparison of ecosystem water-use efficiency among Douglas-fir, aspen forest and grassland using eddy covariance and carbon isotope techniques. *Global Change Biol*, 12: 294–310
- Qin D (2003). Fact, impact, adaptation and mitigation strategy of climate change. *B Natl Nat Sci Found China*, 7: 1–3
- Vogel J C (1980). *Fractionation of Carbon Isotope During Photosynthesis*. Heidelberg: Springer-Verlag
- Werner C, Unger S, Pereir J, Maia R, David T S, Kurz-Besson C, David J S, Máguas C (2006). Importance of short-term dynamics in carbon isotope ratio of ecosystem respiration ($\delta^{13}CR$) in Mediterranean oak woodland and linkage to environmental factors. *New Phytol*, 172: 330–346
- Wright G C, Hubick K T, Farquhar G D (1988). Discrimination in carbon isotope of leaves correlated water-use efficiency of field grown peanut cultivars. *Austral J Plant Physiol*, 15: 815–825
- Yi X F, Zhuang X A (2005). Application of stable isotopic approach in ecology: a review. *Chin J Ecol*, 24(3): 306–314 (in Chinese)
- Zheng X B, Zhuang Y, Gu G H (2005). Application of carbon isotope technique in forest ecosystem carbon cycling research. *Chin J Ecol*, 24(11): 1334–1338 (in Chinese)