Relationship between $\delta^{13}C$ and photosynthetic parameters and their responses to leaf nitrogen content in six broadleaf tree species

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Abstract

Stable carbon isotope composition ($\delta^{13}C$), net photosynthetic rate (P_N), actual quantum yield of photosystem 2 (PS2) electron transport (Φ_{PS2}), nitrogen content (N_c), and photosynthetic nitrogen use efficiency (PNUE) in the leaves of six broadleaf tree species were determined under field environmental conditions. The six tree species were *Magnolia liliflora* Desr., *M. grandiflora* Linn., *M. denudata* Desr., *Prunus mume* (Sieb.) Sieb. *et* Zucc. cv. Meiren Men, *P. mume* (Sieb.) Sieb. *et* Zucc. f. *alphandii* (Carr.) Rehd., and *P. persica* (L.) Batsch. var. *rubro-plena*. The relationships among $\delta^{13}C$, Φ_{PS2}, P_N, and PNUE, as well as their responses to N_c in the six species were also studied. Both P_N and $\delta^{13}C$ negatively correlated with N_c, but Φ_{PS2} positively correlated with N_c. This indicated that with N_c increase, P_N and $\delta^{13}C$ decreased, while Φ_{PS2} increased. There were weak negative correlations between $\delta^{13}C$ and PNUE, and strong negative correlations ($p<0.01$) between Φ_{PS2} and PNUE. According to the variance analysis of parameters, there existed significant interspecific differences ($p<0.001$) of $\delta^{13}C$, P_N, Φ_{PS2}, PNUE, and N_c among the tree seedlings of the six tree species, which suggests that the potential photosynthetic capacities depend on plant species, irradiance, and water use capacity under field conditions.

Additional key words: actual quantum yield of PS2 electron transport; broadleaved tree species; leaf nitrogen content; net photosynthetic rate; photosynthetic nitrogen use efficiency; stable carbon isotope composition.

Introduction

The absorption, transfer, and utilization of radiation energy in plant leaves depend on plant species and environmental variables. 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 ($\delta^{13}C$), which can reflect physio-ecological phyto-adaptability in whole growth, has been widely adopted in studies of plant photosynthetic metabolism and water use (Farquhar *et al.* 1982, Shangguan *et al.* 2000b, Duranceau *et al.* 2001, Zheng and Shangguan 2005). Leaf nitrogen content (N_c) and PNUE significantly correlate with leaf photosynthetic capacity (Poorter and Evans 1998, Shangguan *et al.* 2000a,b, 2004, Frak *et al.* 2002, Hikosaka 2004, Takashima *et al.* 2004). Many studies have shown that N_c positively correlates with P_N and Φ_{PS2} (Poorter and Evans 1998, Taub and Lerdau 2000, Takashima *et al.* 2004, Shangguan *et al.* 2005). However, some researchers have argued that N_c negatively correlates with P_N (Zhao *et al.* 1999), but there exists no correlation between P_N and N_c in diverse species (Evans 1989). Moreover, P_N–N_c relationship in the species with different life-forms and metabolisms significantly differ and the ratio between P_N and organic nitrogen, termed as PNUE, is not constant (Field and Mooney 1986, Anten *et al.* 1995, Hikosaka *et al.* 1998, Hanba *et al.* 1999, Hikosaka 2004). Therefore, there have been controversies on the relationships between leaf photosynthetic parameters, $\delta^{13}C$, and N_c (Evans 1989, Poorter and Evans 1998, Shangguan *et al.* 2000b, 2005, Hikosaka 2004, Takashima *et al.* 2004).

We studied the relationships among $\delta^{13}C$, Φ_{PS2}, P_N, and PNUE, as well as their responses to N_c in six broadleaf tree species. The objective was to better understand the physio-ecological adaptability of plant photosynthesis by combining the techniques of leaf gas exchange, chlorophyll (Chl) fluorescence, and stable carbon isotope.

Materials and methods

Location and climatic conditions: The experimental site is a field of the Rougu Experiment Farm, the Institute of Soil and Water Conservation, the Chinese Academy of Sciences, in Yangling District, Shaanxi Province, China (34°18′N, 108°15′E, and elevation of 450 m above sea level). The climate belongs to warm temperate humid and semi-humid. The mean annual temperature and growing degree days (>10 °C) are 12.9 and 4 143 °C. The total annual sunshine, rainfall, and frost-free period are 2 163 h, 635 mm, and 228 d, respectively. The 51 % of the rainfall distributes between July and September.

Plants: Six broadleaf tree species were selected for this study, including three species of Magnoliaceae (*M. liliflora* Desr., *M. grandiflora* Linn., and *M. denudata* Desr.) and three species of Rosaceae [*P. mume* (Sieb.) Sieb. *et* Zucc. cv. Meiren Men, *P. mume* (Sieb.) Sieb. *et* Zucc. f. *alphandii* (Carr.) Rehd, and *P. persica* (L.) Batsch. var. *rubro-plena*]. The young seedlings (1–2 years old) were grown in pots (60 cm tall and 37 cm in diameter). The growth media was a mixture of compound fertilizer, woody scraps, plant ash, pine bark, and sands. All the pots were well watered and fertilized.

Three pot-planted seedlings of each species that had the same age and floor caudex, height, and canopy breadth were chosen for measurements. Healthy and mature functional leaves at sunlit canopy positions were marked with tags and then the leaves were fixed with a line to keep the leaf clamps unchanged in position. During measurements, the leaves were kept at their natural growing angles. The field measurements were taken in October, 2004 under clear sky conditions, and each measurement had three replications.

P_N of the fully expanded leaves of the six species was measured in field between 10:00~11:30 using a *CIRAS-2* portable photosynthesis system (*PPS*, Hertfordshire, UK). The gas entry was connected to a gas pole, 3 m above ground. The open pathway was turned on to adjust the air flow rate to 2×10^5 mm^3 s^{-1}. The P_N values were measured 2 min after their photosynthesis became stable.

Field measurement of the actual quantum yield of photosystem 2 (PS2) electron transport (Φ_{PS2}): The Φ_{PS2} in the fully expanded leaves of the six species was measured between 10:00~11:30 using a *FMS 2.02* pulse modulation fluorescence meter (*Hansatech*, King's Lynn, UK). Following the measurement of steady-state fluorescence under natural irradiance (F_s), maximum fluorescence at light adaptation (F_m') was measured after a strong flash (5 000 μmol m^{-2} s^{-1}, pulse duration 0.7 s) was exposed to the leaves. The Φ_{PS2} was finally calculated according to formula $\Phi_{PS2} = (F_m' - F_s)/F_m'$ (Roháček 2002).

Determination of leaf nitrogen content (N_c): One or two healthy and mature leaves in sunlit canopy positions were collected from each seedling, then oven-dried at 105 °C for 10 min and at 70 °C for at least 48 h to a constant mass. The leaves were ground to a uniformly fine powder with a thin agate mortar and then passed an 80-mesh sieve. 200 mg of ground material was used to determine N_c by the modified Kjeldahl analysis for which concentrated sulphuric and salicylic acid and Na$_2$SO$_4$, K$_2$SO$_4$, and Se in a ratio of 62 : 1 : 1 (m/m/m) as the catalyst (Page *et al.* 1982). The digests were used to determine nitrogen concentration with a continuous flow analyser (*Skalar*, York, UK). The PNUE was determined as the ratio of P_N to N_c (Poorter and Evans 1998).

Stable carbon isotope composition (δ^{13}C): One or two healthy mature leaves in sunlit canopy positions were collected from each seedling, then ultrasonically washed with distilled water, and air-dried. The leaves were oven-dried at 70 °C for at least 48 h to a constant mass, then ground to a uniformly fine powder with a thin agate mortar, and finally seized with an 80-mesh sieve. δ^{13}C, a carbon isotope, was measured with a *MAT-251* mass spectrometry (*Finnigan*, San Jose, USA). 3~5 mg treated samples 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 °C, and later 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 δ^{13}C [‰] = {[(^{13}C/^{12}C)$_{sample}$ − (^{13}C/^{12}C)$_{standard}$]/(^{13}C/^{12}C)$_{standard}$}×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 (Farquhar *et al.* 1989).

A theoretical analysis was made based on a model developed to describe the fractionation of carbon isotopes in C$_3$ photosynthesis (Farquhar *et al.* 1982):

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \times C_i/C_a$$
$$WUE = P_N/E_r = (C_a - C_i)/1.6 \, \Delta W$$
$$WUE = C_a \, [1 - (\delta^{13}C_a - \delta^{13}C_p)/a \, (b - a)]/1.6 \, \Delta W$$

where a was the fractionation occurring due to diffusion in air (4.4 ‰), b was the net fractionation resulting from carboxylation, and C_a and C_i were ambient and intercellular CO$_2$ concentrations, respectively. $\delta^{13}C_a$ and $\delta^{13}C_p$ were referred to as the carbon isotopic compositions of air (–8.0 ‰) and the plant material, respectively. E_r was transpiration rates and ΔW was the difference between vapour pressures inside and outside the leaves.

Data analysis: The correlation analysis and one-way analysis of variance (ANOVA) were conducted with the *SPSS 12.0* statistical package.

Results and discussion

Responses of leaf $\delta^{13}C$, P_N, and Φ_{PS2} to N_c: In this experiment, both P_N and $\delta^{13}C$ negatively correlated with N_c in the leaves of the six species, while Φ_{PS2} positively correlated with N_c (Fig. 1). P_N and $\delta^{13}C$ decreased, but Φ_{PS2} increased as N_c increased. In general, a strong positive correlation exists between N_c and P_N in many species primarily because the proteins of the Calvin cycle and thylakoids cover the majority of leaf N, and thus P_N increases with increased nitrogen content per unit leaf area. These conclusions have been widely confirmed (Poorter and Evans 1998, Taub and Lerdau 2000, Takashima *et al*. 2004, Zhang and Feng 2004). Growth irradiance has an important influence on the relationship between P_N and N_c, and thus considerable variation of photosynthetic capacity is found at a given N_c among species, which reflects different strategies of N partition in various major components of the photosynthetic apparatus, the electron transport capacity per unit of chlorophyll (Chl), and the specific activity of the enzyme ribulose-1,5-bisphosphate carboxylase (RuBPC) (Zhao *et al*. 1999, Frak *et al*. 2002, Zhang and Feng 2004).

Evans (1989) argued that plants in particular environments did not always have high photosynthetic capacity even if they ha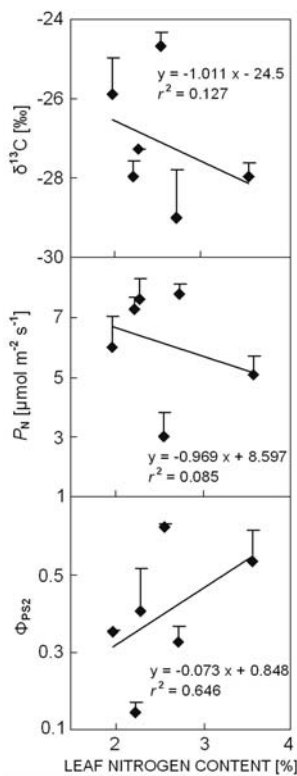d higher N_c; in particular, species that flourished under shade always allocate relatively more N to the thylakoids and show a decline in electron transport capacity per unit of Chl which limits the increases in P_N. Therefore, there was not always a general positive relationship between N_c and P_N. Different species had different correlations between P_N and N_c. Leaf P_N strongly and positively correlated with N_c in wheat, rice, *Panicum laxum*, *Raphanus raphanistrum*, *Chenopodium album*, *Spinacia oleracea*, and *Helianthus annuus* while the correlations in Californian evergreen trees, rainforest trees, and Australian sclerophylls were weak (Evans 1989). Zhao *et al*. (1999) found that in shaded habitat, the leaf P_N of three European trees was low, but N_c was relatively higher. The reason for this mainly contained two points: on one hand the decrease of P_N resulted in the reductions of saccharide accumulation and leaf biomass under low irradiance which led to increased N_c per unit leaf dry matter; on the other hand, low irradiance would cause the changes of physiological and biochemical mechanisms of plants. With well-balanced N application, such deoxidizing agents as NADPH and NADH for the deoxidation of CO_2 and nitrate absorbed by leaves would compete with energy supplier (ATP). The P_N decrease of plants grown under low irradiance was beneficial to the deoxidation of nitrate, thereby resulting in increased N_c. In our experiment, the P_N of the leaves of the six seedlings negatively correlated with their N_c, which was probably because the irradiance was relatively lower in October only amounting to 1 080 µmol m^{-2} s^{-1}, the temperature was about 23.9 °C, and the relative humidity was about 52.8 %.

According to the photosynthetic model developed by Farquhar and Caemmerer (1982), nitrogen involved in photosynthesis is divided into two parts. The first part is in the form of soluble protein dominated by RuBPC, and the second is in the form of protein in the thylakoid membranes in chloroplast. Most evidences are concerned with the relationship between P_N and intercellular CO_2 concentration (C_i). At low C_i and moderate irradiance, P_N is proportional to maximum RuBPC activity per unit leaf area, while both P_N and RuBPC correlate with soluble protein per unit leaf area. At high C_i, P_N depends on irradiance and RuBPC regeneration rate that reflects the rate of electron transport, and thus photosynthesis correlates with thylakoid nitrogen. If there is considerable variation in growth irradiance, the strong correlation between Chl and leaf N may not directly point to a strong correlation between electron transport capacity and leaf N_c (Evans 1989). The decline in electron transport capacity associates with a proportional decrease in cytochrome *f* content per unit of Chl so that no effect of growth irradiance occurs when the electron transport capacity is expressed per unit of cytochrome *f*. Although growth under low irradiance is generally associated with low rates of electron transport per unit of Chl, the electron transport per unit of

Fig. 1. Relationships of leaf stable carbon isotope composition ($\delta^{13}C$), net photosynthetic rate (P_N), and actual quantum yield of PS2 electron transport (Φ_{PS2}) with leaf nitrogen content in the six tree seedlings. Error bars are +SE, $n = 3$.

N is less affected by irradiance because plants tend to increase relative amounts of Chl per unit of N (Evans 1989). The dependence on irradiance is particularly significant in different plants. For example, *Spinacia oleracea* retains strong dependence on irradiance during growth on either a Chl or N basis, but both *Flindersia brayleyana* and *Solanum dulcamara* show a reversal that the electron transport per unit of N increases when they are grown at low irradiance (Evans 1989). In this experiment, the Φ_{PS2} positively correlated with N_c in the leaves of the six species, which indicated that the electron transport per unit of N was also increasing.

According to a theoretical model developed to describe the fractionation of carbon isotopes during C_3 photosynthesis (Farquhar *et al.* 1982), there exists close correlation between $\delta^{13}C$ and C_i, and $\delta^{13}C$ significantly decreases with increased C_i while C_i/C_a, as a driving factor for leaf gas exchange, closely relates to RuBPC, stomatal regulation, and environmental variables. Shangguan *et al.* (2000b) suggested that in the leaves of winter wheat $\delta^{13}C$ increased with increased drought. At a given C_i/C_a, $\delta^{13}C$ was reduced by N deficiency. $\delta^{13}C$ was weakly and negatively correlated with C_i/C_a, and leaf $\delta^{13}C$ in the low-N treatment was significantly higher than that in the high-N treatment. The Φ_{PS2} significantly decreased as leaf water stress gradually became intensified. However, Φ_{PS2} is increased by N application (Shangguan *et al.* 2005). In our experiment, with increased N_c, $\delta^{13}C$ decreased while Φ_{PS2} increased, which was consistent with the above conclusions.

Relationships among $\delta^{13}C$, Φ_{PS2}, and PNUE: In our experiment, $\delta^{13}C$ weakly and negatively correlated with the PNUE in the leaves of the six species while Φ_{PS2} strongly and negatively correlated with PNUE ($p<0.01$) (Fig. 2). The photosynthesis-nitrogen relationship significantly differed among species. PNUE is an important leaf trait to depict species in terms of their leaf economy, physiology, and strategy. Generally, high PNUE species tended to have high growth rates and appeared in undisturbed or high productivity habitats, while the low PNUE ones appeared in unfavourable or low productivity habits. PNUE is an important leaf trait that correlates with other traits, such as leaf mass per area (LMA) and leaf life-span regardless of life-forms, phylogeny, and biome. Various factors are involved in inter-specific difference. In particular, N allocation inside leaves and mesophyll conductance for CO_2 diffusion are important. To produce tough leaves, plants need to allocate more biomass and N to form thick cell walls that result in a reduction in mesophyll conductance and N allocation to the photosynthetic apparatus. Allocation of biomass and N to cell walls may lead to the negative relationship between PNUE and LMA (Hikosaka 2004). However, despite a strong connection between P_N and N_c, PNUE is not constant (Poorter and Evans 1998). Field and Mooney (1986) reported a three-fold difference in PNUE between annual

herbs and evergreen woody species grown in the field, and a considerable inter-specific PNUE variation that was observed in a wide range of herbaceous species. Poorter and Evans (1998) showed that nitrogen partition to RuBPC was slightly smaller in low PNUE species. However, in other researches, N partition in the photosynthetic apparatus seemed to be independent of PNUE (Hikosaka *et al.* 1998, Takashima *et al.* 2004). Significant amounts of N were allocated to electron carriers, ATP synthase, Calvin cycle enzymes other than RuBPC, and enzymes of sugar metabolisms. The amount of these proteins may determine P_N to a certain degree (Evans and Seemann 1989, Hikosaka and Terashima 1996). In our experiment, Φ_{PS2} significantly and negatively correlated with PNUE, which indicated that the capacity of electron transport of PS2 decreased with increased PNUE.
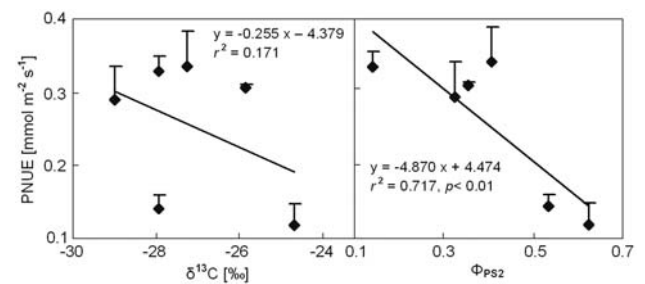

Fig. 2. Relationships of leaf stable carbon isotope composition ($\delta^{13}C$) and actual quantum yield of PS2 electron transport (Φ_{PS2}) with photosynthetic nitrogen use efficiency (PNUE) in the six tree seedlings. Error bars are +SE, $n = 3$.

Variance analysis of typical photosynthetic parameters, N_c, and $\delta^{13}C$ of leaves in six species: The variance analysis of the parameters showed significant interspecies differences ($p<0.001$) of $\delta^{13}C$, P_N, Φ_{PS2}, PNUE, and N_c among six tree seedlings (Table 1), which indicated that the potential photosynthetic capacity depended on plant species. Irradiance and water use capacity in the field differed among the six species and further implied that the adaptabilities of different species to the same environment differed. The mean values of $\delta^{13}C$, N_c, and Φ_{PS2} were -27.59 ± 0.83, 2.30 ± 0.26, and 0.27 ± 0.03, respectively, which were higher in the three species of Rosaceae, *P. mume* (Sieb.) Sieb. *et* Zucc. cv. Meiren Men, *P. mume* (Sieb.) Sieb. *et* Zucc. f. *alphandii* (Carr.) Rehd., *P. persica* (L.) Batsch. var. *rubro-plena*, than in the three species of Magnoliaceae, *M. liliflora*, *M. grandiflora*, and *M. denudata*, which were -26.83 ± 0.25, 2.80 ± 0.14, and 0.52 ± 0.07, respectively. However, the mean values of P_N and PNUE were 7.03 ± 0.58 and $3.09\pm0.24\times10^2$ $\mu mol\ m^{-2}\ s^{-1}$ in the three species of Rosaceae, lower than those in the three species of Magnoliaceae, which were 5.22 ± 0.73 and $1.99\pm0.31\times10^2$ $\mu mol\ m^{-2}\ s^{-1}$.

There are many studies showing significant PNUE differences among plant species. The differences relate to ecological traits of plant species: higher PNUE tends to

Table 1. Variance analysis of δ^{13}C, N_c, and photosynthetic parameters in the six tree seedlings. [***]Significant difference ($p<0.001$).

Variable		ANOVA			
		Sum of squares	df	Mean square	F
δ^{13}C	Between groups	37.522	5	7.504	16.588[***]
	Within groups	5.429	12	0.452	
	Total	42.951	17		
N_c	Between groups	4.673	5	0.935	15.433[***]
	Within groups	0.727	12	6.056E-02	
	Total	5.399	17		
P_N	Between groups	51.436	5	10.287	21.506[***]
	Within groups	5.740	12	0.478	
	Total	57.176	17		
Φ_{PS2}	Between groups	0.430	5	8.604E-02	23.710[***]
	Within groups	4.355E-02	12	3.629E-03	
	Total	0.474	17		
PNUE	Between groups	14.231	5	2.846	28.389[***]
	Within groups	1.203	12	0.100	
	Total	15.434	17		

be more possibly found in C_4 species than in C_3 species (Anten *et al.* 1995), in herbaceous than in evergreen woody species (Evans 1989, Hikosaka *et al.* 1998), in deciduous than in evergreen species (Takashima *et al.* 2004), in gap species than understory species (Chazdon and Field 1987), in species that grow with higher nutrient availabilities (Poorter *et al.* 1990), in species whose leaves have a shorter leaf life-span (Reich *et al.* 1992, 1994), in species that live at earlier succession stages (Ellsworth and Reich 1996), and in species that grow at lower altitudes (Hikosaka *et al.* 2002). Taub and Lerdau (2000) suggested that there were significant PNUE differences among the six grass species, including three of C_4 subtype NAD-ME and three of C_4 subtype NADP-ME, with either N_c at a given N supply or leaf nitrogen-photosynthesis relationship. The PNUE difference between C_3 and C_4 species can be explained by the fact that C_4 metabolism involves efficient CO_2 concentrating mechanisms (Sage and Pearcy 1987). However, there is significant variation among C_3 species though they have the same metabolisms. That probably relates to CO_2 diffusion, N allocation between photosynthetic and non-photosynthetic nitrogenous compounds, N partition within the photosynthetic apparatus, and photosynthetic enzyme kinetics (Field and Mooney 1986). Poorter and Evans (1998) suggested that the respiration under light and leaf photon absorption were two other possible causes. In the nearly 100 years, δ^{13}C varied from –29.75 to –25.05 ‰ in *Quercus liaotungensis* Koidz., *Ostryopsis davidiana* Decne., *Zizyphus jujuba* Mill. var. *spinosa* (Bunge) Hu *ex* H. F. Chow, and *Sophora viccifolin* Hence, four C_3 species growing in the Loess Plateau, and differed much among the four species (Zheng and Shangguan 2005). In our experiment, there existed significant inter-specific differences ($p<0.001$) of δ^{13}C, P_N, Φ_{PS2}, PNUE, and N_c among the six tree seedlings and this is consistent with

the results of Anten *et al.* (1995) and Zheng and Shangguan (2005).

Hikosaka *et al.* (1998) suggested that 50–60 % of leaf N was involved in photosynthetic proteins of herbaceous species. They determined the contents of RuBPC in an annual (*Chenopodium album*) and an evergreen tree (*Quercus myrsinaefolia*) and showed that at the same N content, the former had 23 % higher amount of RuBPC than the latter, and thus higher PNUE was found in the former than in the latter. Hanba *et al.* (1999) suggested that leaves of the evergreens had thicker cell walls, weaker mesophyll conductances, and stronger resistance to CO_2 diffusion than those of herbaceous and deciduous plants, and thus lower PNUE occurred in the latter two species. Takashima *et al.* (2004) found that evergreen *Quercus* species allocated more N to detergent-insoluble proteins, which were presumed to be structural proteins closely associated with cell walls, and less N to the photosynthetic proteins than deciduous species did. As a result lower PNUE appeared in evergreen *Quercus* species than in deciduous species. Therefore, N allocation to the photosynthetic apparatus may be a major factor for the inter-specific PNUE variation. Recently, Warren and Adams (2004) suggested that evergreen species have a low PNUE partly due to an accumulation of inactive RuBPC for N reserve. Excessive RuBPC may not contribute to photosynthesis but lead to a PNUE reduction. However, the activation state is an important but not always a major factor for the low PNUE in evergreen species.

In our experiment, both the means of P_N and PNUE in *M. liliflora*, *M. grandiflora*. and *M. denudata*, the three evergreen species, were lower than those in *Prunus mume* (Sieb.) Sieb. *et* Zucc. cv. Meiren Men, *P. mume* (Sieb.) Sieb. *et* Zucc. f. *alphandii* (Carr.) Rehd., and *P. persica* (L.) Batsch. var. *rubro-plena*, the three deciduous

species. This conclusion was inconsistent with the results of Hanba *et al.* (1999), Takashima *et al.* (2004), and Warren and Adams (2004). It was probably related to plant species and environmental conditions. Hikosaka (2004) also suggested that PNUE was related to physiological characteristics of plants and was influenced by environmental conditions, such as atmospheric CO_2 concentration, growth irradiance, nutrient availabilities, canopy gradients, *etc.* Furthermore, similar PNUEs do not imply similar adaptability mechanism to environmental conditions. For example, about 27 % of the total N of rice leaves exists in RuBPC while only 20 % of the total N of wheat leaves is in RuBPC. In rice leaves, higher proportion of RuBPC nitrogen partially offset

lower specific activity of RuBPC. Rice also has a relatively high leaf conductance. Accordingly, wheat and rice have similar P_N at given N content, which results in their similar PNUEs (Makino *et al.* 1988). Compared with *Glycine*, *S. oleracea* has a low proportion of total leaf N in RuBPC, but its superior specific activity of RuBPC still results in higher PNUE than that of *Glycine* does (Evans 1989).

The relationships among photosynthetic characteristics, N_c, and PNUE are fairly complicated, and various controversies revealed by current studies demand that the relationships involve more physio-ecological mechanisms.

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