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Diurnal gas exchange and superior resources use efficiency of typical C_4 species in Hunshandak Sandland, China

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Abstract

Net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), leaf water potential (Ψ_{leaf}), leaf nitrogen content, and photosynthetic nitrogen use efficiency (PNUE) were compared between a typical C_4 plant, *Agriophyllum squarrosum* and a C_3 plant, *Leymus chinensis*, in Hunshandak Sandland, China. The plant species showed different diurnal gas exchange patterns on June 12-14 when photosynthetic photon flux density (PPFD), air temperature (T_{air}), and water potential were moderate. P_N, E, and g_s of *A. squarrosum* showed distinct single peak while those of *L. chinensis* were depressed at noon and had two peaks in their diurnal courses. Gas exchange traits of both species showed midday depression under higher photosynthetic photon flux density (PPFD) and T_{air} when Ψ_{leaf} was significantly low down on August 6-8. However, those of *A. squarrosum* were depressed less seriously. Moreover, *A. squarrosum* had higher P_N, Ψ_{leaf}, water use efficiency (WUE), and PNUE than *L. chinensis.* Thus *A. squarrosum* was much more tolerant to heat and high irradiance and could utilise the resources on sand area more efficiently than *L. chinensis*. Hence species like *A. squarrosum* may be introduced and protected to reconstruct the degraded sand dunes because of their higher tolerance to stress and higher resource use efficiency.

Additional key words: adaptation to arid environment; *Agriophyllum*; C_3 species; *Leymus*; photosynthetic nitrogen use efficiency; stomatal conductance; transpiration rate; water use efficiency.

Introduction

Since the initial discovery of C_4 photosynthesis in the late 1960s, a significant progress has been made in elucidating the biochemical and physiological characteristic of this pathway (Pearcy and Ehleringer 1984). The higher photosynthetic efficiency of C_4 plants under tropical conditions and their consequently higher growth rate and daily organic production is widely recognised (Hatch 1999, Smith 1997). Based on the higher carboxylation ability, C_4 species have higher water use efficiency (WUE) and nitrogen use efficiency (PNUE) (Makino *et al.* 1994, Schulze *et al.* 1996, Orsenigo *et al.* 1997, Rudmann *et al.* 2001). However, Pearcy *et al.* (1981) found that water limited conditions did not enhance the competitive ability of C_4 relative to C_3 that was observed under well-watered conditions. The diurnal changes of gas exchange of C_3 and C_4 species has also been introduced (Read *et al.* 1997, Matos 1998, Maragni *et al.* 2000). Nevertheless, the midday depression induced by high irradiance of these two typical photosynthetic pathways has not been compared in detail, and their corresponding physiological mechanisms have not been interpreted clearly. Although many experiments comparing C_3 and C_4 plants have been done, most of them were conducted in the controlled ideal environments. How do they respond to the stressful habitats, such as high irradiance, heat, drought, and N limitation that are common in the arid sandy areas? Does the superiority of C_4 plants still be expressed in the resource limited conditions? In this study the differences between *A. squarrosum* and *L. chinensis* were analysed in diurnal courses of photosynthesis, WUE, and PNUE under field conditions. Our aim was to explore the different adaptive strategies of C_3 and C_4 species to arid environment.

A. squarrosum is a pioneer species in sandland, which widely distributes in shifting sand dunes (Chen 1986). It is crucial in fixing the shifting sand dunes. *L. chinensis*,

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which is mainly distributed in fixed sand dunes and lowland, is a dominant species and occurs at the later-succession stage when environment is stabilised. Both of them are annual grasses while the former is a typical C_4 plant and the latter is a typical C_3 plant (Yin and Li 1997). In spite of their ecological importance, the information on the diurnal variations of gas exchange and water potential of these two species has not been documented yet. In this paper, the diurnal CO_2 and water vapour exchange of two species and their difference in WUE and PNUE were compared in order to understand the eco-physiological mechanisms, the successful resource use, and fast growth of *A. squarrosum* in the temperate sandy areas.

Materials and methods

Study area: The experiment was carried out in Hunshandak Sand Area (42°30'–43°54'N; 112°45'–116°36'E), Inner Mongolia Autonomous Region of China, warm desert area of China. The prevailing climate is of the temperate semiarid type with annual mean temperature of 1.7 °C, annual precipitation of 250-350 mm, and annual potential transpiration of 2 000–2 700 mm. Total nitrogen content in rhizosphere soil is very low (only 96.5 mg kg^{-1}). The main habitats included shifting sand dunes, semi-fixed sand dunes, fixed sand dunes, and lowlands. The coverage of vegetation in these habitats is < 10 %, 10—40 %, > 40 %, and 60 %, respectively. In recent years, some fixed sand dunes have been reactivated and grassland degeneration has been accelerated with increasing human activities. Therefore, the shifting sand dune account or will account for higher proportions in the sandy area.

Photosynthesis was measured on clear days at two stages (on June 12-14 and August 6-8, 2002) throughout daytime at two-hour intervals. Photosynthetic photon flux density (PPFD), air temperature (T_{air}), net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were measured using a portable gas exchange system (*LCA*-4, *ADC*, Hoddesdon, England). The leaf area was measured using an area meter (*AM100*, *ADC*, UK). WUE was calculated as P_N/E. Before measuring, the CO_2 and H_2O analysers were calibrated using CO_2 standards (460 µmol mol^{-1}) and *WG-602* Water Vapour Generator (*ADC*, Hoddesdon, England), respectively. During operation, air was collected from 6 m above the floor and dried (by passing through "drier") to 20 % relative humidity before being pumped into the analyser. Relative air flow through the leaf chamber was 6 250 mm^3 s^{-1}. The central portion of most leaves was approximately horizontal, and the leaf cuvette was clamped on this portion of the leaf and kept in the horizontal so that the effect of leaf angle on incident photon flux was minimised during the measurements. Full-expanded functional leaves in upper shoots were selected. Gas exchange data were recorded as soon as readings became stable, usually 30-90 s after leaf insertion into the cuvette. Three replications were carried out for each species at each time.

Leaf water potential (Ψ_{leaf}): After gas exchange measurements, Ψ_{leaf} was measured with a *WP4* Dewpoint Potential Meter (*Decagon Devices*, Pullman, Washington, USA). Fully expanded leaves were taken near to the tops of plants, and then kept in a sealed plastic bag and stored in an ice box after being collected. Measurements were done immediately after being brought into laboratory. Calibration was checked hourly. Five to eight maturing leaves were sampled for each determination. When measuring, small linear leaves or leaf section was put into chamber for measurement. Mature leaf tissue was used to minimise growth effects on leaf water potential. Three replications were made for each species.

Nitrogen contents in leaves: Leaves were collected from upper canopy on June 12-14 and August 6-8, respectively. Three replicates were taken. Leaves were dried at 70 °C to constant mass. N content was determined with an automatic N analyser (*KJELTEC system, 1026* distilling unit, U.S.A.). The method and calculation were introduced by Miller and Keoney (1982).

Data analysis: The large data sets of photosynthesis were entered into an *Excell* spread sheet which included physiological measurements, leaf areas, times of measurement, and species. Analysis of variance of leaf traits was carried out on each measurement and the significance of plant mean square determined by testing against the standard error (species×replicate). The least significances of the differences (LSD) between the means were estimated at 95 % confidence level. Calculations and linear regressions were performed by *Sigma-Plot 8.0* program. Significant differences between species are reported at $p< 0.05$ if not otherwise indicated. The final data of each time in June were the means of the same time on June 12-14, while that in August was the mean on August 6-8.

Results

The diurnal courses of gas exchange and weather parameters: The maximal PPFD and T_{air} on June 12-14 and August 6-8 at 14:00 were 1 980 and 2 270 μmol $m^{-2} s^{-1}$, and 33.3 and 37.7 °C, respectively (Fig. 1*AB*). Mean diurnal air temperature on August 6-8 was 3.5 °C higher than that on June 12-14 (Fig. 1*B*).

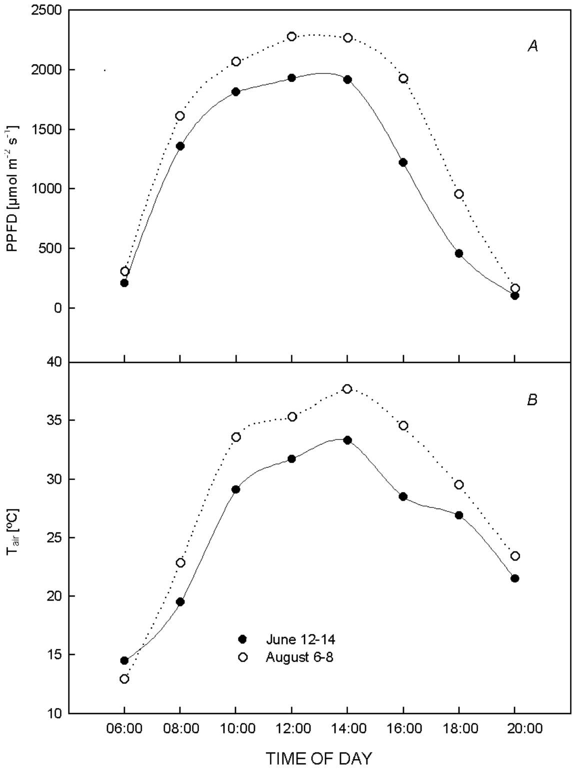

Fig. 1. Diurnal changes in photosynthetic photon flux density (PPFD) and air temperature (T_{air}) on June and August.

A. squarrosum showed one-peak and *L. chinensis* showed two-peaks pattern in the diurnal courses of P_N, *E,* and g_s in June. The peaks of these three parameters for *A. squarrosum* all appeared at 10:00. *L. chinensis* showed two peaks at 08:00 and 16:00 for both P_N and $g_{s,}$ while that of *E* occurred at 10:00 and 14:00 (Fig. 2*ACE*).

With the higher irradiance and air temperature in August, both *A. squarrosum* and *L. chinensis* showed two peaks of P_N, *E*, and g_s. The first one appeared at 08:00, except that *A. squarrosum* showed its first peak of *E* at about 10:00, and the second one appeared at 16:00 for both species (Fig. 2*BDF*). However, the degree of the midday depression was different: at noon, P_N of *A. squarrosum* and *L. chinensis* decreased by 43 and 55 %, respectively, whereas g_s was 55 and 84 % lower than the maximal one in the morning. With few exceptions, *A. squarrosum* showed higher P_N, *E*, and g_s than *L. chinensis* on both stages, of which the former showed 80 and 41 % greater P_{max} than the latter in June and August, respectively.

Differences in WUE and Ψ_{leaf} between two species: *A. squarrosum* had higher WUE on June 12-14 and August 6-8, even though of different degree (Fig. 3*A,B*). An obviously inversed single peaked curve was observed in the diurnal variation of Ψ_{leaf} in leaves, which was similar in both species with the range from –0.14 to –0.63 MPa on June 12-14 (Fig. 3*C*). However, the difference between two species was greater and on average for *A. squarrosum* was about 18 % more than for *L. chinensis* during the second measuring period (Fig. 3*D*).

Leaf N content and PUE: Leaf N content on area basis in *A. squarrosum* was 15 and 16 % lower than that in *L. chinensis* for June and August, respectively ($p<0.05$) (Fig. 4*A*). However, PNUE of *A. squarrosum* was 2.1 and 1.7 times that of *L. chinensis* in June and August, respectively.

Discussion

Differences in gas exchange pattern and their possible reasons: Under the conditions shown in Fig. 1, *A. squarrosum* exerted a distinct single peak response of P_N and g_s on June 12-14, whereas *L. chinensis* had two peaks because there were pronounced midday depressions. This illustrated that *A. squarrosum* was much more tolerant to heat and high irradiance than *L. chinensis*. The maximal PPFD and T_{air} on August 6-8 were higher than those on June 12-14. Such conditions, together with the low Ψ_{leaf}, caused midday depression in both *A. squarrosum* and *L. chinensis* (Fig. 2*B*). However, the extent of depression in the former was lower than that in the latter, *i.e.* P_N of *L. chinensis* was inhibited more seriously than that of *A. squarrosum* which can be seen from the curves of P_N and g_s (Fig. 2*B,F*).

Difference in WUE between two species: P_N/E reflects the instantaneous WUE of the plant at leaf level. Larger g_s could result in higher *E* and P_N (Fig. 2). However, larger increase of P_N than *E* made a greater WUE in *A. squarrosum* than in *L. chinensis* though at the expense of higher *E*. The superiority of *A. squarrosum* in WUE is expressed more clearly under drought conditions. For instance, when water content in plant was sufficient in June (Ψ_{leaf} was from –0.14 to –0.63 MPa, Fig. 3*A*), *A. squarrosum* and *L. chinensis* had similar Ψ_{leaf}. But the difference between the two species was enlarged in August because leaf water deficits occurred (the lowest Ψ_{leaf} was

–3.34 MPa in *L. chinensis*, Fig. 3*B*), and then *A. squarrosum* maintained greater Ψ_{leaf} than *L. chinensis*. The ability to maintain higher Ψ_{leaf} at moderate water deficits may be an adaptation mechanism of plant to water-limited conditions (Ehsaie *et al.* 1991, Zhu *et al.* 2002). When leaves of plants were subjected to water stress at noon, *A. squarrosum* showed higher WUE and Ψ_{leaf} than *L. chinensis*. So the risk of photoinhibition was reduced.

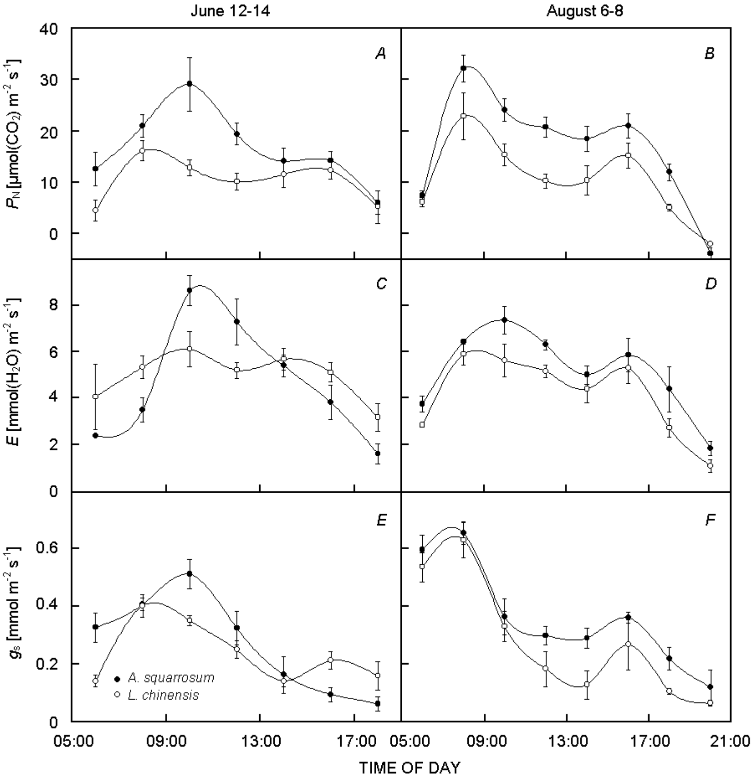

Fig. 2. Diurnal courses of net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) of *A. squarrosum* and *L. chinensis* on June 12-14 (*A, C, E*) and August 6-8 (*B, D, F*).

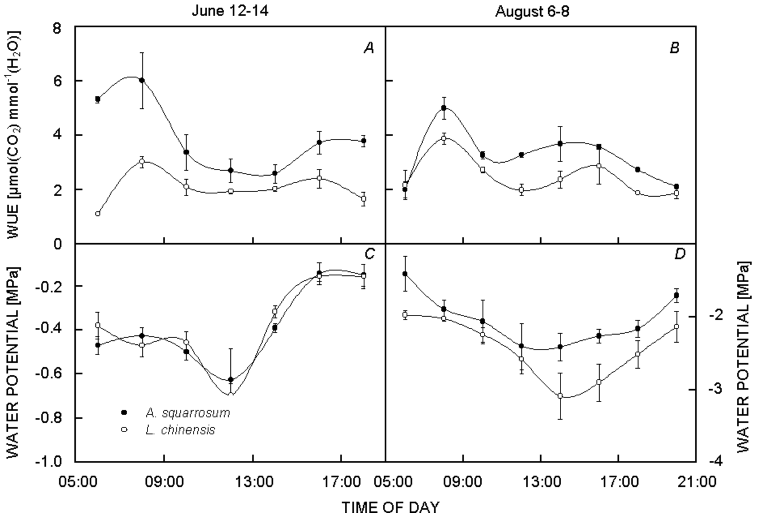

Fig. 3. The daily changes in water use efficiency (WUE) and water potential of *A. squarrosum* and *L. chinensis* on June 12-14 (*A, C*) and August 6-8 (*B, D*).

Leaf N content and utilisation: Positive correlation has been observed between the rate of irradiance-saturated photosynthesis of a leaf and its N content (Reich *et al.* 1994, 1995, Niinemets *et al.* 2002). This is because a large percentage of leaf organic N (up to 75 %) is present in chloroplasts (Makino *et al.* 1994, Nakano *et al.* 1997). However, their extents differ according to plant species, which reflects diversity in PNUE (Reich *et al.* 1998). PNUE, the ratio of CO_2 assimilation rate to leaf organic N content, is important in determining the efficiency with which species utilise N to achieve growth (Garnier *et al.* 1995). The inter-specific difference in PNUE has been described for many herbaceous species (Reich *et al.* 1995, Milka *et al.* 1999). In our study, the higher PNUE of *A. squarrosum* probably resulted from the C_4 concentrating mechanism that leads to CO_2 saturation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). Because far less RuBPCO is required to achieve high photosynthetic rates in C_4 than C_3 plants (Sage *et al.* 1987), and RuBPCO is a large, N expensive enzyme accounting for typically 20-30 % of total leaf N in C_3 plant (Evans 1989), the N saving in C_4 plants is obvious. So the high PNUE was maintained in the C_4 plant. Another explanation may be the N localisation. In our study, *A. squarrosum* showed slightly lower leaf N content and Chl content (values not shown) but significantly greater PNUE than *L. chinensis* (Fig. 4). This shows that much more N was located in carboxylation components (such as RuBP carboxylation and regeneration enzymes) rather than in light-harvesting (such as Chl and carotenoids) ones (Field 1983, Ishida *et al.* 1999). We therefore infer that *A. squarrosum* is more effective in N distribution and utilisation.

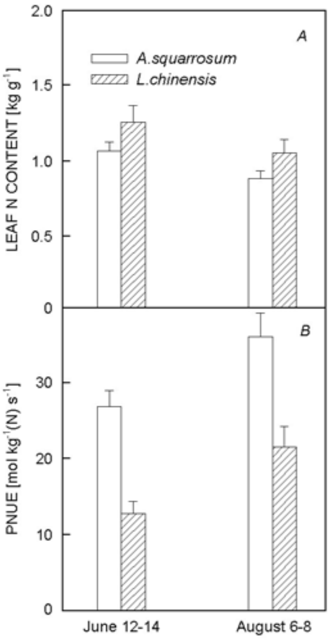

Fig. 4. Leaf nitrogen content and photosynthetic nitrogen use efficiency (PNUE) of *A. squarrosum* and *L. chinensis* on June 12-14 (*A*) and August 6-8 (*B*).

In conclusion, the C_4 species *A. squarrosum* had higher photon, water, and nitrogen use efficiency than the C_3 species *L. chinensis* in the semi-arid sandy area, which can be attributed mainly to its C_4 photosynthetic mechanism. This may be the reason for its survival in shifting sand dunes. The results also suggested that *A. squarrosum* should be first introduced and protected at recovering the degraded sandy land in Hunshandak.

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