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Response of chlorophyll fluorescence to dynamic light in three alpine species differing in plant architecture

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Abstract

A study was carried out to examine the effect of dynamic photosynthetically active photon flux density (PPFD) on photoinhibition and energy use in three herbaceous species, prostrate *Saussurea superba*, erect-leaved *S. katochaete*, and half-erect-leaved *Gentiana straminea*, from the Qinghai—Tibet Plateau. Chlorophyll fluorescence response was measured under each of three sets of high–low PPFD combinations: 1700–0, 1400–300, and 1200–500 $\mu mol\, m^{-2}\, s^{-1}$, illuminating in four dynamic frequencies: 1, 5, 15, and 60 cycles per 2 h. The total light exposure time was 2 h and the integrated PPFD was the same in all treatments. The highest frequency of PPFD fluctuation resulted in the lowest photochemical activity, the highest level of non-photochemical quenching, and the greatest decrease of F_v/F_m (maximal photochemical efficiency of PSII). The 5 and 15 cycles per 2 h treatments resulted in higher photochemical activity than the 1 cycle per 2 h treatment. The 1700–0 PPFD combination led to the lowest photochemical activity and more serious photoinhibition in all species. *S. superba* usually exhibited the highest photochemical activity and CO_2 uptake rate, the lowest reduction of F_v/F_m, and the smallest fraction of energy in thermal dissipation. With similar fractions of thermal dissipation, *S. katochaete* had relatively less photoinhibition than *G. straminea* owing to effective F_0 quenching. The results suggest that high frequency of fluctuating PPFD generally results in photoinhibition, which is more serious under periods of irradiation with high light intensity.

Keywords: Alpine herbs; CO_2 uptake; Energy utilization; Photoinhibition; Thermal dissipation

1. Introduction

Plants in natural environments often experience considerable temporal variation in photosynthetically active photon flux density (PPFD) caused by canopy structure, wind and cloud conditions, and solar elevation (Smith et al., 1989; Knapp and Smith, 1990; Pearcy et al., 1996; Kirschbaum et al., 1998). The response of understory plants to the dynamic light environment within forests has attracted much attention, and many understory plant species can use sunflecks efficiently for photosynthetic CO_2 uptake (e.g. Pearcy, 1990; Hamerlynck and Knapp, 1994; Whitehead and Teskey, 1995; Valladares et al., 1997; Allen and Pearcy, 2000; Leakey et al., 2003; Tang et al., 2003). However, little evidence is available to explain how plants in grassland respond to a dynamic light environment, or the underlying mechanism (Pearcy et al., 1996).

Grassland plants live in a more stable light environment than forest understory species, but a more variable one than species in drier habitats. The light fluctuation pattern is very different in grassland than in forest understory (Pearcy et al., 1996). For example, in grassland, sunflecks or sunpatches have a much higher maximum PPFD, and short-term sunflecks with a high frequency contribute much more to total PPFD than in forest understory. Cumulus shadow is another factor causing light fluctuations in open habitats; such fluctuations lasted for 4–10 min in an alpine area in the Rocky

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Mountains, USA (Knapp and Smith, 1989). Species from an environment with a higher temporal heterogeneity of light responded to dynamic PPFD more quickly in terms of stomatal conductance (g_s, Knapp and Smith, 1990). The CO_2 uptake rate responds to light fluctuations faster than g_s does (Whitehead and Teskey, 1995), and photosystems change even more quickly upon PPFD variation (Roháček and Barták, 1999). Therefore, grassland plants could be expected to respond quickly to dynamic PPFD, especially in CO_2 fixation and chlorophyll fluorescence.

Frequency (and the converse, duration) of light fluctuation varies greatly in nature, from a few times a second to once every several minutes (Knapp and Smith, 1989; Smith et al., 1989; Pearcy et al., 1996). The relative contribution to carbon fixation of sunflecks depends on their duration and light intensity (Pearcy et al., 1996). Different frequencies of dynamic PPFD influenced photosynthetic light use efficiency (Valladares et al., 1997) and plant growth (Sims and Pearcy, 1993; Watling et al., 1997).

Although brief sunflecks contribute mostly to carbon gain in understory plants, photosynthesis may be inhibited in shade-adapted species under prolonged exposure to high light intensity by photoinhibition (Valladares and Pearcy, 1997). In fact, photoinactivation is inevitable for individual photosystem II (PSII) reaction centers, even under weak irradiation (Lee et al., 1999). The loss of function in PSII reaction centers depends on photon dose, that is, the product of light intensity and duration of illumination (Park et al., 1995). The net rate of decline in PSII function is determined by damage to and repair of PSII reaction centers. The repair process is saturated under weak light, while damage linearly increases with light intensity up to saturation (Park et al., 1996; Anderson et al., 1997). Therefore, a following period of low light may benefit the release of photoinhibition pressure induced by high light (Pearcy, 1990). For a certain photon dose, the effect on relaxation of photoinhibition pressure should be related to the intensities of low and high light. The frequency of light fluctuation, which determines the duration of periodical high and low light, should also affect the degree of photoinactivation, owing to the limited speed of the repair process in low light (Anderson et al., 1997). However, there are few experimental studies on the effect of frequency of dynamic PPFD on photosynthetic photoinhibition (Ögren, 1991).

Plant form and leaf position are directly linked with in situ leaf light interception (Germino and Smith, 2001; Cui et al., 2003; Pearcy et al., 2004). Leaves seem adapted to the light environment of their original position: artificial change of a vertical leaf to a horizontal position leads to serious photoinhibition (Valladares and Pearcy, 1999).

In alpine regions, cumulative radiation is no higher than in lowlands at a similar latitude (Körner, 1999). However, global radiation above the canopy usually approaches, or even surpasses, the solar constant during the plant growth season on the Tibetan Plateau (Chen and Xu, 2000). In this study we selected three herbaceous species, *Saussurea superba*, *S. katochaete*, and *Gentiana straminea*. They varied in plant architecture and in experience of dynamic PPFD regime in their local habitats due to broken cumulus, common in the afternoon, and neighbor shading. Species *S. katochaete* and *G. straminea* have lower and *S. superba* has much higher maximal CO_2 uptake rates and photosynthetic light saturation point (approximately 800 $\mu mol\ m^{-2}\ s^{-1}$ in the former two species and 1500 $\mu mol\ m^{-2}\ s^{-1}$ in the latter, see Cui et al., 2003, 2004). Photoinhibition is induced by high light and high temperature in the former two species but not in the latter. *S. superba* also has higher stomatal conductance even at high temperature (Cui et al., 2003). Thus, the objectives of this study were: (1) to determine whether intermittent low light mitigates photoinhibition caused by high light and (2) to clarify the effects of dynamic PPFD frequency, PPFD combination, and species on photoinhibition. We hypothesize that low light facilitates the recovery from previous high-light-induced photoinhibition and that the effect depends on dynamic light frequency and light intensity. The prostrate species, living in a naturally higher light environment, is less sensitive to dynamic PPFD than the erect-leaved ones were.

2. Material and methods

2.1. Plant materials

Three herbaceous species – *S. superba* Anth., *S. katochaete* Maxim. (Asteraceae), and *G. straminea* Maxim. (Gentianaceae) – which differ in plant stature and leaf inclination were selected for the study. *S. superba* is a dwarf rosette plant with two or three rounds of leaves that expand horizontally on the soil surface. *S. katochaete* usually has two to four small vertical leaves that are extended to the upper canopy of the community on long petioles. Fully expanded leaves of *S. superba* are three to four times larger than those of *S. katochaete*. *G. straminea* grows linear leaves slantwise from the soil surface to the canopy top. Its mature leaves are 20–30 cm long. Leaves of *S. superba* generally intercept full sunlight except in the early morning or late afternoon, when they are shaded by neighboring plants. The leaves of *S. katochaete* intercept highly variable light as they swing in the wind. *G. straminea* leaves are partly shaded by other species. They often curl a bit, especially in full sunlight at midday.

The plants were taken from a *Kobresia humilis* meadow around Haibei Alpine Meadow Ecosystem Research Station (latitude 37°29′N, longitude 101°12′E), at the northeast edge of the Qinghai—Tibet Plateau, at an altitude of approximately 3250 m. The annual mean air temperature is −2 °C and the annual precipitation is 500 mm (Klein et al., 2001). The height of vegetation layer was about 10–20 cm and maximal leaf area index was about 3.0. The grassland was moderately degraded with large spatial heterogeneity in aboveground biomass. The mean ratio of PPFD at soil surface to that above canopy was 0.12 in 1 h at noon in a clear day. We measured PPFD at leaf upper surface in species *S. superba* and *S. katochaete* for 4 days with the method described in

earlier paper (Cui et al., 2003). Low light intervals (indicated by a decrease of PPFD from preceding high light by more than 700 μmol m^{-2} s^{-1}) lasting for 1–2, 3–5, 6–12, and 13–40 min accounted for 35%, 22%, 25%, and 18% of total time of low light intervals shorter than 40 min in *S. superba*. The values were 45%, 32%, 15%, and 8% in *S. katochaete*.

The experimental plants were transplanted from the study site to 8-L plastic pots with their original soil columns. The pots were then transported to the garden at the station. Vegetation was similar in the garden to that in the field where plants lived. Plants were well watered so that the leaves did not wilt even under full sunlight at midday. Plants were used for experiment, 1 week after transplanted in pots. During the experimental period, frost frequently occurred at night. The pots were temporarily moved into a laboratory to avoid frost damage in late afternoon every day.

2.2. Chlorophyll fluorescence measurement

An LI-6400 portable fluorescence and gas exchange system (LI-COR Inc., Lincoln, NE, USA) was used, which provided artificial light composed of 10% blue light (center wavelength: 470 nm) and 90% red light (center wavelength: 630 nm). Three high–low PPFD combinations were used: 1700–0, 1400–300, and 1200–500 μmol m^{-2} s^{-1}. Previous measurement showed that the maximum PPFD reaching vertical *S. katochaete* leaves in the field is around 1700 μmol m^{-2} s^{-1} during daytime (Cui et al., 2003). Under heavy cloud cover or direct shading, radiation can decline to below 100 μmol m^{-2} s^{-1}. For each PPFD combination there were four PPFD alternation frequencies: 1, 5, 15, and 60 cycles per 2 h (named as F-1, F-5, F-15, F-60, and thereafter). The total exposure time to either high or low light was 1 h in each treatment. During the measurement, the block temperature of the leaf chamber was set to 15 °C. The air temperature inside the chamber varied between 15.3 and 16.2 °C. Leaf temperature fluctuated between 15.3 and 20.5 °C, depending on light intensity and species. Air RH was above 45% and leaf vapor pressure deficit was lower than 1.5 kPa.

2.3. Experimental schedule

All the treatments were measured totally randomly. Before each measurement, pots with plants were brought to laboratory for about 4 h in weak light less than 20 μmol m^{-2} s^{-1}. Test before formal experiment indicated that treatment effects were not significantly affected by the time of measurements. The LI-6400 was warmed up till the block temperature stabilized at 15 °C. A healthy, fully expanded leaf was then sealed in the leaf chamber and kept in darkness for 30 min. During the dark period, maximal fluorescence (F_m) and minimal fluorescence (F_0) were recorded every 2 min. Actinic light was turned on and high–low light cycles began immediately after the dark period. During the period of light exposure, maximal fluorescence under light (F'_m), steady-state fluorescence under light (F_t), and minimal fluorescence under light (F'_0) were measured every 1 min for the 60 cycles per 2 h treatment and 4 min for other ones. After the dynamic PPFD cycles were completed, the light was switched off and the leaf was kept in darkness for another 30 min, during which F_m and F_0 were recorded. Fluorescence values were calculated as described by Schreiber et al. (1994). The quantum efficiency of PSII (F'_v/F'_m) and its maximum value (F_v/F_m) were estimated from $(F'_m - F'_0)/F'_m$ and $(F_m - F_0)/F_m$, respectively. The photochemical efficiency of the whole PSII photosystem ($\Delta F/F'_m$) was derived from $(F'_m - F_t)/F'_m$. Photochemical quenching (qP) equaled $(F'_m - F_t)/(F'_m - F'_0)$. Non-photochemical quenching (qN) was calculated by $(F_m - F'_m)/(F_m - F'_0)$. Apparent photochemical electron transport rate (ETR) was derived from $0.5 \times 0.84 \times \Delta F/F'_m \times$ PPFD. According to Demmig-Adams et al. (1995), the fraction of absorbed light used in photochemistry was given by $\Delta F/F'_m$. The fraction of absorbed light dissipated via thermal energy dissipation was estimated by $(F_v/F_m - F'_v/F'_m)$. The fraction of absorbed light used in other ways was the unaccounted part from the total of 0.84, the maximal intrinsic PSII efficiency. In calculation, 0.84 was normalized to be 1.0. During the experiment, CO_2 uptake was measured continuously. The quantum efficiency of CO_2 uptake (ΦCO_2) was determined as integrated CO_2 uptake per integrated incident PPFD. For each treatment, three to four leaves from different plants were measured.

2.4. Statistical analysis

The SAS® Software was used to perform factorial analysis of variance (two-way ANOVA analysis) with three experimental factors: species, PPFD combination, and dynamic frequency. Species had three, PPFD combination had three, and dynamic frequency had four levels. Interaction effects between any two of these factors or among the three factors were not significant at $P = 0.05$ level. Therefore, only main effects of the factors were reported. Duncan's multiple-range test was used for comparison of means among different levels within a factor.

3. Results

3.1. Effects of dynamic PPFD regimes on changes in F_v/F_m

Before exposure to dynamic PPFD, the F_v/F_m of the leaves of each species was around 0.8 after 30-min dark adaptation, indicating that the leaves were healthy and had similar initial status with respect to PSII reaction centers (Fig. 1).

F_v/F_m decreased after dynamic PPFD treatment in all the leaves. After the light was switched off, F_v/F_m recovered quickly in the first 10 min and leveled off to near stability after 30 min of darkness (Fig. 1).

All three experimental factors – species, dynamic PPFD frequency, and PPFD combination – had significant effects on

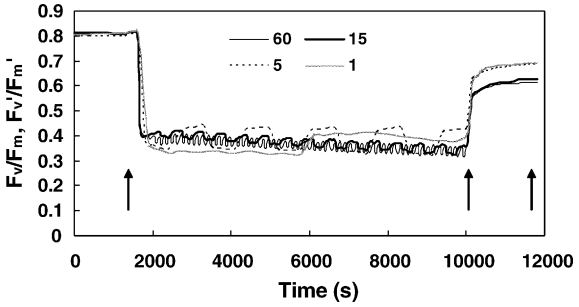

Fig. 1. Typical response of maximal quantum efficiency in darkness (F_v/F_m) and light (F'_v/F'_m) to various frequencies of dynamic irradiation (60, 15, 5, and 1 cycles per 2 h). Only *S. katochaete* under the high–low PPFD combination of 1200–500 μmol m^{-2} s^{-1} is shown as an example. Other light combinations or species showed a similar pattern. The first arrow in the diagram indicates the end of the first 30 min darkness and the time of measuring F_v/F_m before dynamic light treatment. Dynamic PPFD treatment begins then, and F'_v/F'_m is monitored till the end of the light period, as indicated by the second arrow. The third shows the end of the second 30-min darkness and time of measuring F_v/F_m again. Higher F'_v/F'_m corresponds to the low-light period of the PPFD combination.

F_v/F_m response (Table 1). *S. superba* usually had the highest F_v/F_m values and the lowest relative reduction of F_v/F_m by dynamic PPFD (Table 1), but *G. straminea* was at the other extreme in most cases. F_v/F_m declined more in the 1700–0 PPFD combination than in the other two. It decreased more as the dynamic PPFD frequency increased (Table 1).

3.2. Effects of dynamic PPFD on PSII photochemical activity and non-photochemical activity in light

Under continuous irradiation, PSII efficiency decreased linearly at PPFD of ≥1200 μmol m^{-2} s^{-1} in both *S. katochaete* and *G. straminea* (Fig. 2b and c). It largely recovered and kept almost constant in *S. superba* (Fig. 2a). $\Delta F/F'_m$ and qP showed the same tendency as F'_v/F'_m in Fig. 2.

Table 1
Effects of dynamic PPFD on maximal quantum efficiency of PSII in *S. superba*, *S. katochaete*, and *G. straminea*

Factor	Level	Relative decrease of F_v/F_m[a]
Species	*S. superba*	0.897a
	S. katochaete	0.847b
	G. straminea	0.829b
	Total	0.865 ($P<0.001$)
PPFD combination	1200–500	0.889a
	1400–300	0.878a
	1700–0	0.815b
	Total	0.865 ($P<0.001$)
Frequency (cycle/2 h)	1	0.898a
	5	0.884a
	15	0.873ab
	60	0.830b
	Total	0.865 ($P=0.029$)

Note: Values with the same letter within one factor are not significantly different.

[a] Relative decrease of F_v/F_m was quantified from dividing the initial F_v/F_m value by the final value two-way ANOVA was used for statistical analysis. Interaction effects among the factors were not significant at $P=0.05$ level. Duncan's test was used to compare the mean effects of levels in each factor.

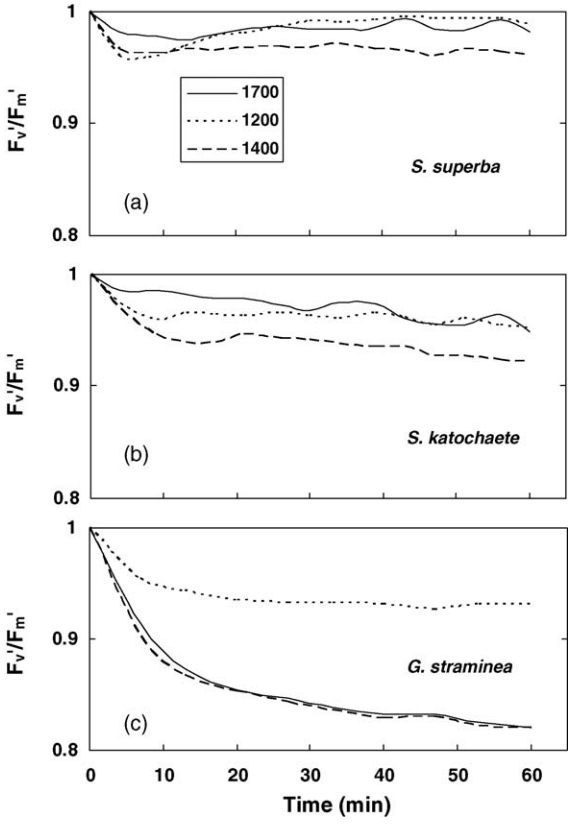

Fig. 2. Change in normalized F'_v/F'_m under continuous high light (dotted, continuous and broken lines for 1200, 1400, and 1700 μmol m^{-2} s^{-1}, respectively) in *S. superba* (a), *S. katochaete* (b), and *G. straminea* (c). All treatments followed a 30-min dark period. The beginning values of F'_v/F'_m were set to 1.0 to facilitate comparison among species, respectively.

Under dynamic irradiation, PSII efficiency at low light intensity was generally the lowest in F-60 treatment in all species (Fig. 1; Table 2). F_v/F_m after 30 min of recovery in darkness was highly correlated with F'_v/F'_m at the end of the last preceding low-light period (Fig. 3).

There was a significant difference among species in PSII photochemical activity and non-photochemical activity at the end of dynamic PPFD treatment (Table 2). The PPFD combination also significantly affected these values except for F'_v/F'_m ($P=0.215$, Table 2). Dynamic frequency had a significant effect on non-photochemical fluorescence quenching and F'_v/F'_m, but not in $\Delta F/F'_m$ or qP ($P=0.239$ and 0.592, Table 2). Generally, *S. superba* and the 1200–500 PPFD combination resulted in the highest photochemical activity (F'_v/F'_m, $\Delta F/F'_m$, and qP) and the lowest non-photochemical activity (qN). The highest frequency (F-60) and the lowest frequency (F-1), with similar values, gave the lowest photochemical activity and highest non-photochemical activity.

With an increase in light intensity, the fraction of absorbed energy used by photochemical pathways was remarkably reduced. Energy dissipated by heat or used in other ways

Table 2
Effects of species, PPFD combination, and dynamic PPFD frequency on fluorescence measures at the end of dynamic PPFD exposure

Factor	Level	F'_v/F'_m	$\Delta F/F'_m$	qP	qN
Species	*S. superba*	0.603b	0.512b	0.784c	0.600a
	S. katochaete	0.464a	0.352a	0.659b	0.834b
	G. straminea	0.446a	0.303a	0.591a	0.844b
		$P<0.001$	$P\leq0.001$	$P=0.005$	$P<0.001$
PPFD combination	1200–500	0.490a	0.339a	0.558a	0.812b
	1400–300	0.555b	0.417b	0.726b	0.691a
	1700–0	0.553b	0.570c	0.981c	0.594a
		$P=0.215$	$P\leq0.001$	$P<0.001$	$P=0.003$
Frequency (cycle per 2h)	1	0.526b	0.400a	0.647a	0.789c
	5	0.566b	0.452a	0.748b	0.676a
	15	0.534b	0.400a	0.696ab	0.696ab
	60	0.465a	0.381a	0.686ab	0.773bc
		$P=0.003$	$P=0.239$	$P=0.592$	$P=0.086$

Two-way ANOVA was used for statistical analysis. Interaction effects among the factors were not significant at $P=0.05$ level. Duncan's test was used to test the significance of difference between levels in each factor. The degree of significance for factor effect was also shown as P-values at the end of each parameter.
Note: Values with the same letter in a column within one factor are not significantly different ($P>0.05$).

decreased linearly with an increase in photochemical activity in all species (Fig. 4a). The slopes were −0.514 and −0.486 in *S. katochaete*, −0.494 and −0.506 in *G. straminea*, and −0.579 and −0.421 in *S. superba*, which used a higher proportion of energy in photochemical pathways and a lower proportion in heat dissipation or other ways, especially at high light intensities (Fig. 4b and c). The highest frequency led to the lowest proportion of photochemical energy use in low light (PPFD 500, 300, and 0 μmol m^{-2} s^{-1}, Fig. 4a).

At the end of light exposure, qN was highest in the 1200–500 and the lowest in the 1700–0 PPFD combination (Table 2). The fast recovery component of qN (qN-fast) was significantly higher for the 1200–500 PPFD combination than for the other two, while the slow-recovery component (qN-slow) was significantly higher for the 1700–0 PPFD combination (Table 3). *S. superba* had a lower qN-slow than

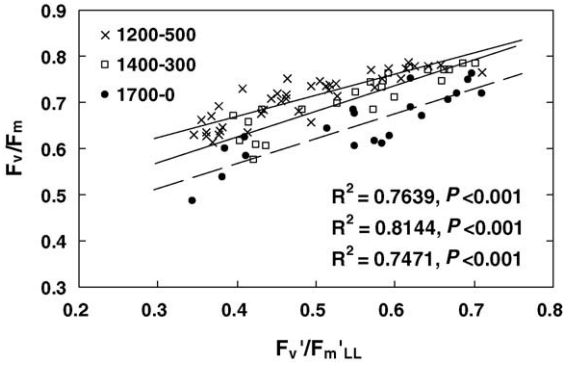

Fig. 3. Relationship between PSII efficiency in the low-light period of the last cycle of dynamic PPFD treatment (F'_v/F'_{mLL}) and F_v/F_m after recovery in 30 min of darkness at the end of each treatment. The diagram includes data from the three species under different dynamic PPFD frequencies. The curves for 1200–500, 1400–300, and 1700–0 PPFD combinations were $Y=0.4611X+0.4855$ (dotted line), $Y=0.5605X+0.4006$ (continuous line), and $Y=0.5428X+0.3501$ (broken line).

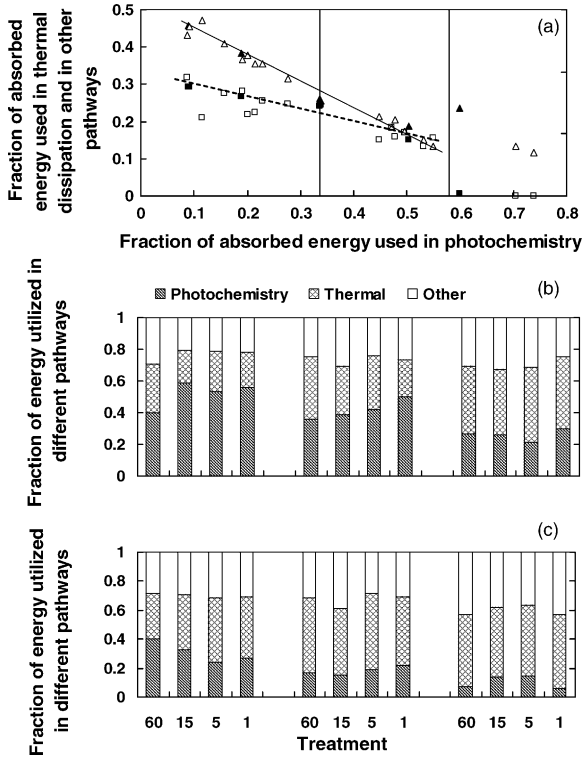

Fig. 4. (a) Distribution of absorbed energy to thermal dissipation (triangles) and by other pathways (squares) relative to that used in PSII photochemical pathways. The vertical lines divided the PPFD into three zones: high light (1200, 1400, and 1700 μmol m^{-2} s^{-1}), low light (300 and 500 μmol m^{-2} s^{-1}), and darkness along x-axis. The 60 cycles per 2 h treatment is indicated by solid symbols. The slopes for thermal dissipation verse photochemistry (the continuous line) and for other pathways verse photochemistry (the broken line) in light were −0.680 and −0.318. (b and c) Average fraction of absorbed energy used in the three components in low (b, 500 μmol m^{-2} s^{-1}) and high (c, 1200 μmol m^{-2} s^{-1}) light under dynamic PPFD.

Table 3
Effects of species, PPFD combination, and dynamic frequency on fast and slow recovery components of qN at the end of the light period

Factor	Level	qN-fast	qN-slow
Species	*S. superba*	0.381a	0.460a
	S. katochaete	0.581b	0.614b
	G. straminea	0.584b	0.682c
		$P = 0.008$	$P < 0.001$
PPFD combination	1200–500	0.553b	0.527a
	1400–300	0.390a	0.517a
	1700–0	0.441a	0.648b
		$P < 0.001$	$P < 0.001$
Frequency	F-1	0.540b	0.530b
	F-5	0.372a	0.437a
	F-15	0.486b	0.554b
	F-60	0.482b	0.644c
		$P = 0.001$	$P = 0.001$

Two-way ANOVA was used for statistical analysis. Interaction effects among the factors were not significant at $P = 0.05$ level. Duncan's test was adopted to test the significance of difference between levels in each factor. qN components were calculated as qN-fast $= 1 - (F''_m - F''_0)/(F'_m - F'_0)$ and qN-slow $= 1 - (F''_m - F''_0)/(F'_m - F'_0)$, where F'_m and F'_0 are the maximum and minimum fluorescence intensity measured at the end of light exposure; F_m, and F_0 are the values measured at the end of the first 30-min dark period, F''_m and F''_0 are the values measured after 2 min of darkness immediately following dynamic PPFD exposure. *Note:* Values with the same letter in a column within one factor are not significantly different ($P > 0.05$).

the other two species. Among the four dynamic frequencies, the highest one gave an obviously higher qN-slow and the 5 cycles per 2 h treatment gave the lowest qN-fast and qN-slow components.

The quantum efficiency of CO_2 uptake (ΦCO_2) was linearly correlated with $\Delta F/F'_m$ in all three species. The slope of the linear correlation was highest in *S. superba* and lowest in *G. straminea* (Fig. 5). Neither light intensity nor dynamic frequency had a significant effect on the slope.

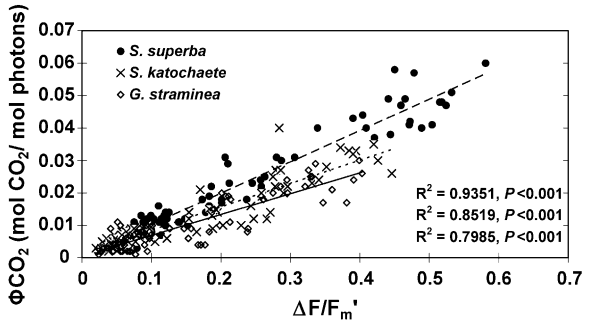

Fig. 5. Relationship between PSII photochemical efficiency ($\Delta F/F'_m$) and quantum efficiency of CO_2 uptake (ΦCO_2) in *S. superba*, *S. katochaete*, and *G. straminea* during dynamic PPFD. Data from all treatments in one species were mixed, and only part of them was drawn to make the diagram clear. Data from one replicate in each treatment of a species were sorted and points were peaked in sequence at interval of five data. Slope of the fitted line is 0.0965 for *S. superba* (broken line), 0.0722 for *S. katochaete* (dotted line), and 0.0641 for *G. straminea* (continuous line). R^2 and significance level was shown in the same sequence.

4. Discussion

4.1. Photoinhibition in alpine herbs and effects of dynamic PPFD

Excess energy absorbed by chlorophyll that cannot be used in photochemical activity may lead to photoinhibition, as indicated by a decrease in primary photochemical activity and in the chlorophyll fluorescence parameter F_v/F_m (Osmond et al., 1999). Alpine species are well adapted to high irradiation, even though photoinhibition is induced after low-temperature stress (Lütz, 1996; Fetene et al., 1997; Germino and Smith, 2000a,b). In this study, F_v/F_m decreased in all treatments in all three species (Fig. 1; Table 1). F_v/F_m was reduced by more than 35% in some treatments in *G. straminea* and *S. katochaete* (data not shown). The significantly higher reduction of F_v/F_m in these two species was consistent with their much lower photosynthetic light saturation points than in *S. superba* (Cui et al., 2003, 2004).

The rate of decline of PSII function depends on both light intensity and duration (Park et al., 1995). The reduction in F_v/F_m is well correlated with the photon dose (Ögren, 1991; Park et al., 1995). The photon dose, in the present study, was uniformly 6.12 mol m^{-2}, which is low compared with the integrated daytime solar radiation in the study area. Nevertheless, F_v/F_m was declined and qN-slow was high after dynamic PPFD exposure in all treatments (Fig. 1; Tables 1 and 3). Excessive light is almost unavoidable for most plants on a daily basis (Ort, 2001). It is very common at the study site according to in situ measurements (Cui et al., 2003). Since photoinhibition may occur even when PPFD was below the photosynthetic light saturation point (Demmig-Adams and Winter, 1988; Lichtenthaler and Burkart, 1999), we suggest that photoinhibition is common under natural local conditions on the Qinghai—Tibetan Plateau. Midday depression of CO_2 uptake, which generally occurs in these alpine herbs (Shi et al., 2001), may be partly caused by photoinhibition.

Compared with constant light (1 cycle per 2 h treatment), high-frequency dynamic PPFD resulted in much more reduction of F_v/F_m (Table 1). The slower dynamic PPFD regimes had no significant effect on F_v/F_m change. The rate of photoinactivation is determined by the balance between damage to and repair of PSII centers (Park et al., 1995). In low light, F_v/F_m of a high-light-adapted leaf does not decrease (Russell et al., 1995). Therefore, a switch to weak light should facilitate the repair of PSII centers damaged by high-intensity light (Pearcy et al., 1996). We suggest two reasons for the small effect of low dynamic frequencies on F_v/F_m: (1) plants suffered from other stresses. Under such conditions, even the low light was too strong for photochemical activity (Ort, 2001). This was not likely true in this study because the initial F_v/F_m values were normal (Fig. 1). (2) The high light intensity was too strong (Demmig-Adams and Winter, 1988; Pearcy, 1990). Non-functional PSII centers accumulate under high light intensity to such an extent that they cannot be repaired quickly enough in the following low light period,

or the remaining functional PSII centers cannot cope with the following low light. Deeply shaded leaves store a large number of non-functional PSII centers in huge granal stacks in chloroplasts awaiting the slow repair processes after high light exposure (Anderson and Aro, 1994). Species that experience periodic extremely strong light may also have such a response.

To avoid photoinactivation, either photochemical activity or thermal dissipation or both should be promoted under high light (Ort, 2001). Both photochemical and non-photochemical fluorescence values fluctuated synchronistically at all dynamic frequencies (e.g. F'_v/F'_m, Fig. 1), suggesting that alpine plants can quickly redistribute absorbed energy among different pathways. However, in F-60 treatment, the difference in fluorescence values between high and low light was obviously lower than at other frequencies (Fig. 1). Photochemical activity in low light decreased more than in high light. Low photochemical activity under low-intensity light showed that the high pressure of photoinhibition in the preceding high-light period could not be relaxed at the end of the following low light period. We found that the final F_v/F_m was linearly correlated with F'_v/F'_m in low light (Fig. 3), indicating that recovery in low light was important in determining the degree of photoinhibition under dynamic PPFD. The significantly high value of qN-slow also implied photoinhibition after dynamic PPFD (Demmig-Adams and Winter, 1988; Lichtenthaler and Burkart, 1999). Non-functional PSII centers serve in thermal dissipation and photoprotection (Ottander et al., 1993). qN-fast needs 2–4 min to relax (Lichtenthaler and Burkart, 1999). The 1-min fluctuation was too fast for PSII to fully recover in low light. A slower frequency of high–low light benefited PSII photochemical activity (Tables 1 and 2).

Both thermal dissipation and energy used in other pathways linearly increased with the decrease of energy flow through photochemical pathways when the light intensity was increased (Fig. 4a). The steeper slope of the thermal dissipation showed that this pathway generally played an important part in preventing photoinactivation in these alpine species, as in other species (Ort, 2001). The fraction of energy used in pathways other than photochemistry and thermal dissipation was very high in all species (Fig. 4). Such phenomenon was also found in plants under photoinhibition in other places (Demmig-Adams et al., 1995). Because some of the energy that is not used in photochemical pathways or dissipated thermally may cause photoinhibition (Lichtenthaler and Burkart, 1999), it is clear that photoprotection by thermal dissipation and other means was insufficient to prevent photoinhibition in these species under dynamic PPFD. Therefore, when photochemical energy use was significantly reduced by too-fast fluctuation of PPFD (Fig. 4a), photoinhibition pressure increased.

Nevertheless, dynamic PPFD at low frequency did not aggravate photoinhibition (Table 1). More energy was used in photochemical pathways under low light than in high light, especially in darkness after strong light (Fig. 4a). Therefore, the following low-light period (e.g. the 300 after 1400 and 500 after 1200 $\mu mol\,m^{-2}\,s^{-1}$ in this study) had beneficial effects on promoting photochemical activity and carbon fixation and decreasing photoinhibition pressure (as shown by qP or energy in other pathways) at low dynamic frequency (Fig. 4; Table 2).

4.2. Different responses to dynamic PPFD in species with different plant architectures

Consistent and significant differences among species were found for all fluorescence values measured after dynamic PPFD treatments (Tables 1–3). *S. superba* generally had the highest photochemical activities (F_v/F_m, F'_v/F'_m, $\Delta F/F'_m$, qP, and ETR) and the lowest non-photochemical quenching (qN). The term $(1 - qP)$ denotes photoinhibition pressure (Osmond et al., 1999). F'_v/F'_m is negatively linearly correlated with the ratio of antheraxanthin (A) plus zeaxanthin (Z) to violaxanthin (V) plus A and Z (Demmig-Adams et al., 1995), and the conversion of V to Z via A was an effective way of photoprotection. Therefore, the highest values of qP and F'_v/F'_m resulted in the lowest reduction of F_v/F_m (Tables 1 and 2).

Because the photon dose was the same, a similar degree of F_v/F_m reduction should be expected among all treatments within a species. However, greater reduction of F_v/F_m was observed in the 1700–0 PPFD combination than in the other two in *G. straminea* and *S. katochaete* (data not shown). Under illumination at an intensity of 1700 $\mu mol\,m^{-2}\,s^{-1}$, the F'_v/F'_m (Fig. 2) and qP (data not shown) steadily declined, suggesting accumulation of photoinhibition pressure. Because the 5 and 15 cycles per 2 h treatments resulted in longer recovery time in darkness after final light exposure (42 and 35 min as compared with 30 min for all the other treatments), the higher reduction of F_v/F_m in the 1700–0 PPFD combination implies that the extremely high PPFD may induce strong photoinactivation in these two species, which needs long time for full recovery.

Differences among species in energy distribution were remarkable (Fig. 4b and c). The proportion of energy invested in photochemical pathways was highest in *S. superba* and lowest in *G. straminea*. The former species had also the lowest proportion of energy loss by thermal dissipation and other routes. Although *G. straminea* had a similar fraction of thermal dissipation as did *S. katochaete*, its lower photochemical activity led to a higher fraction of energy being used in other ways.

A reduction of F_v/F_m accompanied by reduction of F_0 indicates photoprotection, but an increase of F_0 indicates photoinactivation (Osmond et al., 1999). Significant difference in change of F_0 was observed among the three species after dynamic light ($P < 0.001$, data not shown). *S. katochaete* generally decreased F_0 during the experiment, implying that photoprotection by F_0 quenching was the main cause of F_v/F_m reduction. In *G. straminea*, however, F_0 increased in most treatments. Because a slight reduction of F_0 may

mask photoinactivation (Osmond et al., 1999), we suggest that photoinactivation induced F_v/F_m reduction principally in this species. The higher degree of F_v/F_m reduction in this species also supports this suggestion (Table 1).

The large difference in response to dynamic PPFD seems to depend on the photosynthetic characteristics of these species, which are directly related to plant architecture (Cui et al., 2003, 2004). The prostrate leaves of *S. superba* intercept much stronger PPFD than the erect or half-erect leaves of the other two species (Cui et al., 2003, 2004). To adapt to high light, this species has high photochemical activity (Fig. 4; Tables 1 and 2), high CO_2 uptake capability (Fig. 5), and high capacity for photoprotection (Fig. 2a). The other two species are sensitive to high light (Fig. 2b and c). They relied more on thermal dissipation to cope with excess excitation energy (Fig. 4). And thermal dissipation was not sufficient to prevent photoinhibition under strong light or high frequency of dynamic PPFD (Table 1).

In conclusion, this study demonstrated that dynamic light affected leaf photochemistry. Although a following low light period facilitated photoinhibition induced in preceding high light, high frequency of light fluctuation led to accumulating photoinhibition pressure. Moreover, extremely high light induced photoinhibition that was difficult to be relaxed during following low light period. Plants differed in architecture also differed in photochemical activities and responses to dynamic light.

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