

# Responses of chlorophyll fluorescence and xanthophyll cycle in leaves of *Schima superba* Gardn. & Champ. and *Pinus massoniana* Lamb. to simulated acid rain at Dinghushan Biosphere Reserve, China

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Received: 6 June 2004 / Accepted: 24 April 2006 / Published online: 14 December 2006  
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**Abstract** *Schima superba* and *Pinus massoniana* distributed over large areas in southern China both are dominant species at Dinghushan Biosphere Reserve. In the present study, the changes of chlorophyll fluorescence and xanthophyll cycle in the leaves of *S. superba* and *P. massoniana* exposed to simulated acid rain (SAR) were measured. When exposed to high light, the PSII photochemistry efficiency ( $F_v/F_m$ ), efficiency of energy conversion in PSII ( $\Phi$ PSII) and photochemical quenching (qP) of both *S. superba* and *P. massoniana* all decreased when acidity of SAR increased. Regarding non-photochemical quenching (qN), *S. superba* exposed to SAR had higher value than control plants, but there was no significant difference between the respective seedlings of *P. massoniana*. As for xanthophyll cycle of the two plant species, the leaves of *S. superba* exposed to SAR showed a higher content of carotenoids and a higher ability to convert violaxanthin to zeaxanthin than leaves of *P. massoniana*, which was consistent with *S. superba* exhibiting a stronger resistance to high light than *P. massoniana*. Although both species were susceptible to acid rain as shown by our results, *P. massoniana* was more susceptible compared to *S. superba*. These results provide an insight into how to protect the forest ecosystem at Dinghushan Biosphere Reserve.

**Keywords** Dominant species · Carotenoids ·  $F_v/F_m$  ·  $\Phi$ PSII · qP · qN · Violaxanthin · Zeaxanthin · Photosynthesis

## Abbreviations

SAR	Simulated acid rain
A	Antheraxanthin
Car	Carotenoids
DES	Deepoxidation state
V	Violaxanthin
Z	Zeaxanthin
FW	Fresh weight
$F_v/F_m$	PS II photochemistry efficiency
$\Phi$ PSII	Efficiency of energy conversion in PSII
qP	Photochemical quenching
NPQ	Non-radiative energy dissipation
qN	Non-photochemical quenching

## Introduction

About 80 km west of Guangzhou nearby the city of Zhaoqing, Dinghushan Biosphere Reserve is the first natural reserve in China, and also is a part of the Network of World's Biosphere Reserve organized by MAB, UNESCO. Dinghushan Biosphere Reserve is near the Tropic of Cancer. While 2/3 of the area of the world near the Tropic of Cancer consists of deserts and semideserts, the Dinghushan Biosphere Reserve area is covered by tropical–subtropical forests. The monsoon evergreen broad-leaved forest at Dinghushan Biosphere Reserve is the climax in this zone and is more than 400 years old. Thus Dinghushan Biosphere Reserve has become one of the most valuable areas for

Communicated by H. Gabrys.

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scientific research. Some recent studies have shown that human activities have a negative effect on the forest ecosystems at Dinghushan Biosphere Reserve (Seip et al. 1999; Kong et al. 1993, 1997). Due to a well-developed road, the prosperous tourism is affecting the environment. Kong et al. (1993) indicated that almost 700,000 tourists visited the reserve and about 120,000 vehicles entered there each year. Seip et al. (1999) also reported that acid deposition has become a serious problem at Dinghushan Biosphere Reserve.

Both *Schima superba* and *Pinus massoniana* are heliophilous and dominant species in forest ecosystems at Dinghushan Biosphere Reserve. These two species distributed over large areas in southern China are used for firewood. Previous studies showed that the population size of *S. superba* and *P. massoniana* at Dinghushan Biosphere Reserve decreased during the past 10 years. The serious damage symptoms of *P. massoniana* affected by acid rain have been found in some other forests in China, like the forests in the mountain of Chongqing, Baiyunshan mountain at Guangzhou, and in the Liuzhou area located in the Guangxi province. Is acid rain one of the reasons for the decrease of the population size of *S. superba* and of *P. massoniana* at Dinghushan Biosphere Reserve? No studies yet dealt with the effects of acid rain on *S. superba*, however many papers have focused on the mechanisms by which acid rain affects pine trees and provide information about what the mechanism could be for *P. massoniana*. Acid rain affects pine's normal growth directly, altering the contents of free amino acids in needles (Huhn and Schulz 1996), decreasing photosynthesis rate (Reich and Schoettle 1987; Shan and Feng 1988) and inflecting leaf nutrient balance (Thelin et al. 1998). It can also affect it indirectly through aluminium toxicity (Tepper 1989; Nowak and Friend 1995) and changes in the rates of insect herbivory (Asai and Futai 2001). In southern China, sun rays are always fierce and acid rain becomes a serious environmental problem, however, we still have little knowledge concerning the way they interact and the ability of light protection mechanisms of pines to sustain high irradiance when exposed to acid rain.

The objectives of our study were (1) to investigate the changes in PSII photochemistry and to find out how simulated acid rain (SAR) affected PSII function in leaves of both *S. superba* and *P. massoniana*; (2) to determine whether the xanthophyll cycle played a role in dissipating excess light energy in the leaves of these two species treated by SAR. The results will provide an insight into the possible mechanisms of the action of acid rain on these two important species. The results will further be used to establish a risk management

strategy for anthropogenic stress on forest ecosystems at Dinghushan Biosphere Reserve.

#### Study site and experimental design

Dinghushan Biosphere Reserve is located between 23°09'21" and 23°11'30"N and 112°30'39" and 112°33'41"E. The whole area of Dinghushan Biosphere Reserve occupies 1155 hm<sup>2</sup>. The climate here is humid subtropical monsoon weather. The total solar radiation is  $4.67 \times 10^5$  J/cm<sup>2</sup> annually, and the effective radiation utilized by plants is 229 J/cm<sup>2</sup> a year. The mean annual temperature is 21°C with a maximum of 28° in July and a minimum of 12.6° in January. The annual precipitation varies generally among 1,560 and 2,278 mm with an average value of 1,900 mm. The mean relative humidity is 80%.

In August 2000, 2-year-old seedlings of *S. superba* and *P. massoniana* were collected from broad-leaf and coniferous forests at Dinghushan Biosphere Reserve and then transplanted into pots. The pots were filled with soil collected in the same forests as the plants and placed in an open area. Young trees were sprayed with a SAR with a pH 3.05, 4.00 or with tap water once a week. The responses of chlorophyll fluorescence and xanthophyll cycle to SAR were studied in leaves of *Schima superba* Gardn. & Champ. and *Pinus massoniana* Lamb. in September 2002.

Simulated acid rain was prepared by adding both H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> to a base solution, with a 1:1 mol ratio of H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub>. Tap water was used as the base solution.

#### Materials and methods

Samples of tap water were collected immediately after tap water was used to make SAR. The concentrations of major elements in tap water were shown in Table 1. The pH of water samples was determined electrometrically. Ca<sup>2+</sup> and Mg<sup>2+</sup> in water samples were measured by flame atomic-absorption photometry, and Na<sup>+</sup> and K<sup>+</sup> were determined by flame emission photometry. Pb, Mn, Al, and Sr in the water were analyzed by ICP (Optima2000). For each treatment, photosynthetic rate (oxygen production) was measured in the field on sunny days with a LI-6400 (Li-Cor, USA). The temperature control of the LI-6400 was set to track the ambient air temperature.

The chlorophyll content was quantified using the procedure described by Arnon (1949). Pigments were extracted from leaf discs frozen in liquid nitrogen immediately after detachment and grounded in a

**Table 1** The pH value and the concentrations of major elements in tap water. Each value in this table is averages  $\pm$  SD,  $n = 50$ . (Unit  $\mu\text{g/L}$ )

Parameters	pH	Pb	Mn	Al	Mg	Sr	K	Ca	Na
Averages $\pm$ SD	4.27 $\pm$ 0.01	45.00 $\pm$ 0.03	21.14 $\pm$ 0.03	161.55 $\pm$ 0.23	79.00 $\pm$ 0.06	4.55 $\pm$ 0.03	414.50 $\pm$ 0.76	741.95 $\pm$ 0.71	537.35 $\pm$ 0.34

mortar with ice-cold 100% acetone. The extract was kept in darkness at  $-80^\circ\text{C}$  prior to HPLC analysis for carotenoid composition following the method of Gilmore and Yamamoto (1991). The pigment content was calculated using their conversion factors. Chlorophyll fluorescence was measured at room temperature with a pulse-modulated fluorometer (PAM 101/102/103, Walz, Effeltrich, Germany). The minimal (dark,  $F_0$ ) fluorescence yield were obtained with weak modulated light ( $0.04 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the maximal ( $F_m$ ) with a 1 s pulse of saturated light ( $6120 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The actinic light intensity was  $280 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The ratio of  $F_v/F_m$  was used as a measure of the maximum photochemical efficiency of PSII. Photochemical quenching (qP) and non-photochemical quenching (qN) were calculated according to Schreiber et al. (1986). The efficiency of energy conversion in PSII ( $\Phi\text{PSII}$ , Genty et al. 1989) was calculated as  $(F_{m'} - F_s)/F_{m'}$  ( $F_s$  = stationary level of fluorescence emission,  $F_{m'}$  = maximum fluorescence during illumination). All analyses on leaves were carried out at midday on sunny days. The collected data were analyzed using ANOVA and we chose a significance level of  $p < 0.01$ .

## Results

### Changes in net photosynthetic rate and chlorophyll contents

The changes in net photosynthetic rate of *S. superba* and *P. massoniana* leaves exposed to SAR are shown

in Fig. 1 a. In both plants, SAR with a pH of 3.05 caused a larger decrease of net photosynthetic capacity than SAR with a pH of 4.0. The content of total chlorophyll increased with the increasing acidity of SAR (Fig. 1b). The increase of the chlorophyll content in the leaves of *S. superba* was higher than that in the leaves of *P. massoniana*.

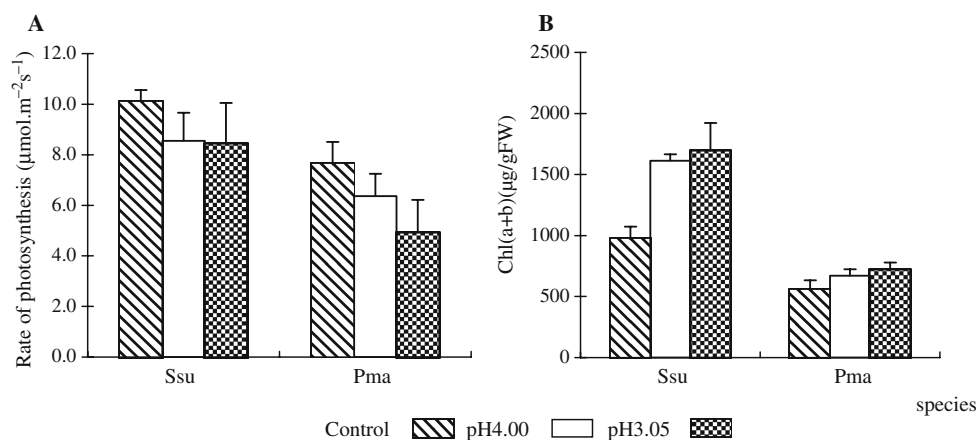
### Changes in chlorophyll fluorescence parameters

The parameters of chlorophyll fluorescence were sensitive to SAR (pH 3.05 and 4.0) applied to both *S. superba* and *P. massoniana*. The  $F_v/F_m$  ratio, which characterizes the maximal quantum yield of the primary photochemical reaction in dark-adapted leaves, decreased with the increasing acidity of SAR (Fig. 2a). It decreased to 85 and 81% of the control in leaves of *S. superba* and *P. massoniana*, respectively.  $\Phi\text{PSII}$  and qP showed a similar pattern as  $F_v/F_m$  (Fig. 2b and c). qN is considered to be a good estimate of the amount of energy dissipated by non-photochemical quenching by plants. The qN increased by 32% at pH 4.00 and 36% at pH 3.05 in *S. superba* but no difference appeared in *P. massoniana* exposed to SAR (Fig. 2d).

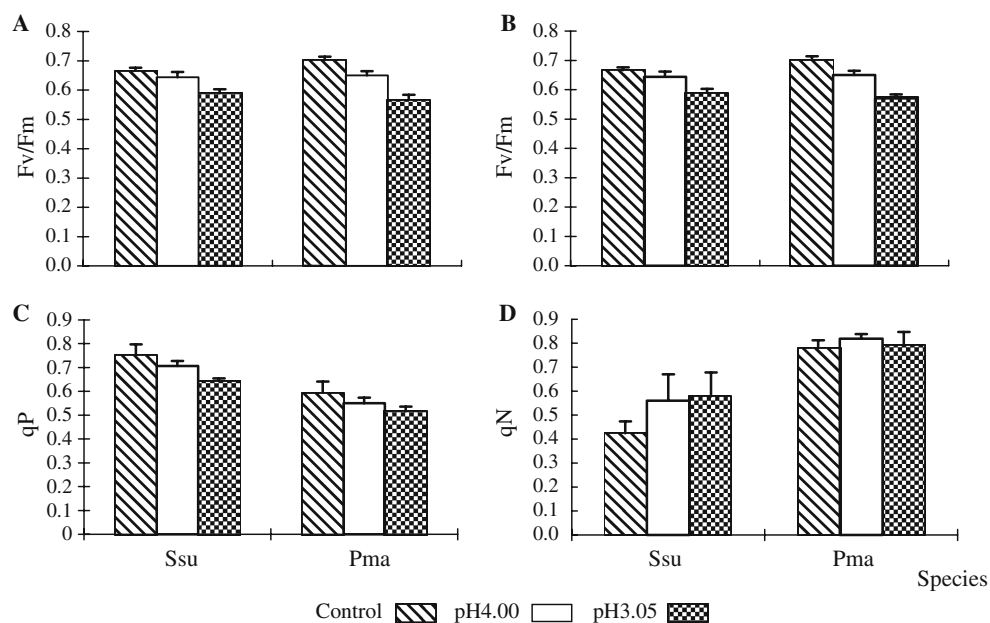
### Changes in leaf carotenoid composition with increasing acidity of SAR

Table 2 shows the changes of relative xanthophyll pigment composition in leaves of both species following application of SAR. The lutein, neoxanthin and total  $V + A + Z$  per chlorophyll were higher than those

**Fig. 1** Effects of simulated acid rain on rate of photosynthesis (a) and chl(a + b) (b) in leaves of *S. superba* (Ssu) and *P. massoniana* (Pma). Bars represent SD of four replicates



**Fig. 2** Parameters of chlorophyll fluorescence induction kinetics and its quenching in control and simulated acid rain-treated (pH 4.00 or 3.05) leaves of *S. superba* and *P. massoniana*. Bars represent SD of four replicates



**Table 2** Changes in the level of total and individual carotenoids and pigment ratios in leaves of *S. superba* and *P. massoniana* exposed to SAR with different pH values. Pigment content in mmol/mol chl(*a* + *b*), all values in this table are averages  $\pm$  SD,  $n = 4$

Parameters	<i>S. superba</i>			<i>P. massoniana</i>		
	Control	pH 4.00	pH 3.05	Control	pH 4.00	pH 3.05
Lutein	199.71 $\pm$ 11.32	247.64 $\pm$ 17.13	231.13 $\pm$ 2.02	162.83 $\pm$ 5.16	215.92 $\pm$ 21.29	210.26 $\pm$ 12.31
Neoxanthin	43.11 $\pm$ 5.26	46.58 $\pm$ 1.29	46.36 $\pm$ 3.24	30.95 $\pm$ 0.60	49.81 $\pm$ 6.11	46.80 $\pm$ 9.19
$\beta$ -carotene	16.97 $\pm$ 2.11	17.50 $\pm$ 1.43	17.44 $\pm$ 1.86	27.54 $\pm$ 0.12	20.12 $\pm$ 1.33	22.62 $\pm$ 1.90
( <i>V</i> + <i>A</i> + <i>Z</i> )/ chl( <i>a</i> + <i>b</i> )	145.5	163.18	160.43	87.31	125.59	130.91
Deep oxidation state	0.65 $\pm$ 0.01	0.76 $\pm$ 0.02	0.82 $\pm$ 0.02	0.56 $\pm$ 0.01	0.53 $\pm$ 0.02	0.48 $\pm$ 0.01

found in the control leaves of *P. massoniana*, but no obvious changes were observed in leaves of *S. superba*. However, different changes in  $Z/(V + A + Z)$  ratio were found between *S. superba* and *P. massoniana* leaves.  $Z/(V + A + Z)$  increased from 54% in control to 75% in the leaves of *S. superba* treated with SAR at pH 3.05, while  $Z/(V + A + Z)$  decreased from 43% in control to 33% in the leaves of *P. massoniana* treated with SAR at pH 3.05 (Fig. 3).

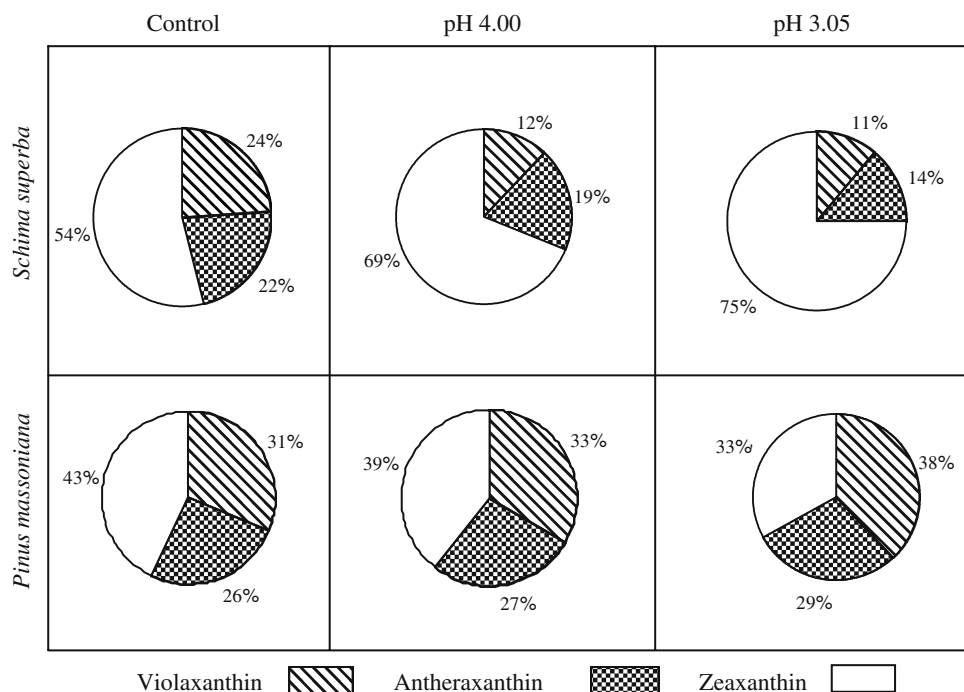
## Discussion

Figure 1 shows that the application of SAR on the seedlings of *S. superba* and *P. massoniana* lead to a decrease of photosynthetic rate, indicating that both species are susceptible to acid rain. Shan (1998) showed that photosynthetic rate of *Pinus densiflora* treated by SAR decreased too. Compared to control trees, photosynthetic rate of *S. superba* exposed to SAR decreased by 16 and 17%, respectively.

Photosynthetic rate of *P. massoniana* treated by SAR decreased by 16 and 35%, which suggests that *P. massoniana* is more susceptible than *S. superba* when exposed to acid rain.

Environmental stresses are supposed to affect photosynthetic processes in the long term. The effects are a decrease in the photosynthetic quantum energy conversion and a concomitant increase in fluorescence and energy dissipation as heat (Lichtenthaler 1996). One of the protection mechanisms against overheating is the interconversion between zeaxanthin, antheraxanthin and violaxanthin in the xanthophyll cycle. In this paper, we have studied the effects of SAR on the photosynthetic processes by means of chlorophyll fluorescence techniques and photosynthetic pigment analysis. At midday, we observed a significant decrease in  $F_v/F_m$  ratios and the actual PSII efficiency ( $\Phi_{PSII}$ ) (Fig. 3), measured as an indicator of the extent of photoinhibition, which suggests that some important protective or regulatory mechanisms to avoid photodamage to the photosynthetic

**Fig. 3** Fluctuation of xanthophyll cycle components of *S. superba* and *P. massoniana* expressed on a chl(*a* + *b*) basis. For both species, data are means from four independent determinations



apparatus under excess light energy were activated (Demmig-Adams and Adams 1992).

It has been shown that qP can be used as a measure of the proportion of closed PSII centers and qN reflects the capacity of plants to carry out non-radiative dissipation of excess energy (Demmig-Adams et al. 1990; Genty et al. 1989). Our results showed a decrease in qP and an increase in qN in the SAR-treated leaves of *S. superba* when compared to control. This suggests that SAR-treated leaves have a higher capacity for dissipating excess energy non-radiatively. The pigment analysis yielded similar DES values in the control and SAR-treated leaves. Thus, we suggest that the increase in DES in SAR-treated seedlings may be a form of acclimation that dissipates excess excitation energy and thus protects PSII from photodamage. Whereas there was no significant changes in qN, and only a little decrease in DES in SAR-treated *P. massoniana* seedling. This result indicates that the seedling of *P. massoniana* have a low ability for dissipating excess energy non-radiatively. From the results as we mentioned, we may conclude that *P. massoniana* is more susceptible to acid rain than *S. superba* in south China. The susceptibility of *P. massoniana* to acid rain may be one of the reasons of the dieback of *P. massoniana* in China. Our results also support the idea that conifers are more susceptible to acid rain than broadleaf trees (Christopher 1993; Cappellato et al. 1993). Amezaga (1997) showed that conifers had less ability of acid neutralization than broad-leaf trees.

Our results showed the effects of SAR on two major plant species at Dinghushan Biosphere Reserve. Both *S. superba* and *P. massoniana* were susceptible to acid rain.

As acid deposition increases in the Reserve, we conclude that this is one of the stresses that are currently leading to the decrease of the population size of *S. superba* population and the decline of *P. massoniana* in the Reserve.

**Acknowledgments** This work was supported by Guangdong Provincial Natural Science Foundation of China (04002320), Director Foundation of South China botanical Garden (2004–2139) and National Natural Science Foundation of China (30470306).

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