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# EFFECTS OF SIMULATED $SO_2$ POLLUTION ON SUBTROPICAL FOREST SUCCESSION: TOWARD CHLOROPHYLL FLUORESCENCE CONCEPT

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**Abstract**

The effects of simulated $SO_2$ treatment ($NaHSO_3$ with different concentrations) on chlorophyll fluorescence in five subtropical forest plants, namely *Pinus massoniana* Lamb, *Schima superba* Gardn., and Champ, *Castanopsis fissa* R and W, *Acmena acuminatissima* Bi.merr., and Perry, and *Cryptocarya concinna* Hance, were investigated. After the leaf sections were immersed in 0, 20, 50, 100 mM $NaHSO_3$ for 10 h, the low temperature (77 K) chlorophyll fluorescence emission, $F_{PSI}/F_{PSII}$ ratio and chlorophyll fluorescence induction curves of all five woody plants reduced to different extents, while an increase in low temperature fluorescence polarization was found. Short-term treatment of $SO_2$ damaged PSII, decreased the fluidity of thylakoid membrane, and affected the process of electron transport. Sulfite oxidase activities of five plants grown in three light environments (100, 32, and 12% of natural light intensities) were varied, which showed different resistance to $SO_2$ pollution. *A. acuminatissima* and *C. concinna*, the dominant species in the late succession stage of a subtropical forest in South China, was less sensitive to $SO_2$ pollution. And *P. massoniana*, the pioneer heliophyte species was less able to resist $NaHSO_3$. It is suggested that $SO_2$ pollution may accelerate the succession of subtropical forest.

## Introduction

Sulphur dioxide ($SO_2$) is a widespread air pollutant, and its environmental effects include acidification of soils, lakes and rivers and damage to plants and crops. The introduction of legislation in recent years, at least in developed world, has led to very substantial reductions in emissions (Mansfield, 1999). However, Emissions of $SO_2$ in the area with rapidly developing economies of south and East Asia, as well as Africa, South and Central America have been increasing quickly, the southeast Asia now emits more S into the atmosphere than either Europe or North America (Percy & Ferretti, 2004). Acid deposition derived from combustion of fossil fuels may occur close to the point or area source as well as over long distances of 1000 km or more. Therefore, acid pollution becomes the world wide subject. As the world's largest consumer of primary commercial energy and emitter of $SO_2$, and the second largest producer of hard coal, China faces greater risks of acid pollution than any other Third World country. The emission of $SO_2$ in China was 22.549 million tons during the year of 2004 (State Environmental Protection Administration of China, 2005).

Chronic $SO_2$ exposure affects photosynthesis in forest plants, depending on exposure dose and species analyzed. Alterations were found mainly at PSII level in young spruce trees, following $SO_2$ exposure, particularly affecting the structure of D1 protein (Lütz *et al*., 1992). Chloroplast is one of the main targets of $SO_2$ or its degradation products generated in aqueous solution, resulting in an impairment of chloroplast functionality through a loss of net $CO_2$ assimilation, decline in photosynthetic electron transport rate

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and inhibition of dark reactions of photosynthesis (Veljovic-Jovanovic *et al*., 1991). $SO_2$ can react with water to yield bisulfite. Within the chloroplasts, bisulfite will be further converted to sulfite because of the pH value in stroma is at 8.8 (Kurkdjian & Guern, 1989). $SO_2/HSO_3^-$ may cause injury in a certain position, restrain Calvin cycle and affect electron transport rate (Veljovic-Jovanovic *et al*., 1991). Sulfite is known to present some cytotoxic, mutagenic and antinutritional effects (Stammati *et al*., 1992). Sulfite oxidase (E.C.1.8.3.1) catalyses the final reaction in oxidative degradation of sulfite, and its activities can be used to evaluate the resistance of plants to $SO_2$ pollution.

Measuring chlorophyll fluorescence is an important technique in investigating energy transportation of photosynthesis. In vivo, chlorophyll fluorescence is almost from chlorophyll a in PSII (including antenna chlorophyll a), which supplies a quick information of utilizing and dissipating excitation energy in PSII. Each step in photosynthesis is connected and can be reflected by PSII fluorescence. Fluorescence emission at 77 K demonstrated the alterations in the overall distribution of excitation energy between PSII and PSI. The balance of absorbed light energy (or excitation energy) between the two photosystems is beneficial to achieve the maximum photochemical energy conversion efficiency. Fluorescence polarization can be used to detect the alterations of biology membrane, thus to evaluate the fluidity of thylakoid membrane responding to environmental signal. Since the early 1970s, the use of fluorescence polarization to investigate membrane fluidity has gained popularity due to its many advantages. Fluorescence polarization is a very sensitive technique, being more informative than differential scanning calorimetry (DSC) and easier to use than electron spin resonance (ESR) (Borenstain & Barenholz, 1993). Alteration of membrane fluidity in vitro modified the response of isolated thylakoid membranes to photoinactivation of PSI and PSII activities at room and low temperature (Velitchkova *et al*., 2001). The kinetics of fluorescence induction (Wang & Shen, 1989) measured under very strong actinic light is of special interest because the rate of the photochemical reactions is very high under these conditions and the photochemical and non-photochemical events are well resolved (Schreiber, 2002). Therefore, chlorophyll fluorescence is measured in our studies to detect the influences of $SO_2$ pollution on photosynthetic machinery.

Most data reported in literature concerned fumigations with various concentrations of $SO_2$, alone or in combination with other pollutants, either for short (Sandhu *et al*., 1992; Veljovic-Jovanovic *et al*. 1991) or long periods (Garcia *et al*., 1998; Ranieri *et al*., 1999). Whereas, little information is available on the effects of $NaHSO_3$ permeation on biochemical and physiological responses of forest plants for understanding the influence of the intermediate of $SO_2$ metabolism on forest ecosystem. And to our knowledge, no report in measuring the activities of sulfite oxidase has been found in analyzing the damage of $SO_2$ to plants.

South China is located in south subtropical area with rapid industrial development, and it is one of the main acid rain polluted areas. The current paper focuses on the responses of several dominant plant species in forest succession grown under different light intensities to simulated $SO_2$ treatment, in order to deduce the effects of acid deposition on subtropical forest structures. The differences of physiological responses and protection strategies toward the change in environmental conditions may be of major importance for the overall physiological performance and the contribution to competitive differences among species.

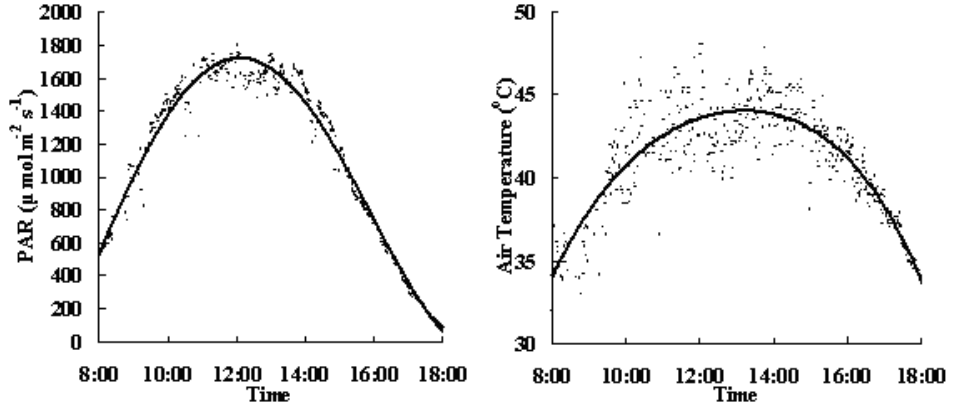


Fig. 1. Diurnal changes of photosynthetically active radiation (PAR) and air temperature in August at experimental site of South China Botanical Garden, Guangzhou, China

## Materials and Methods

**Field site and plant materials:** The experimental site is at South China Botanical Garden, Guangzhou, Guangdong Province, China (23°35' N and 122°57' E), belonging to south subtropical monsoon climate. The annual average temperature is 21.4-21.9°C. The average temperature of July and August, the hottest months of the year, is 28.0-28.7 °C and January or February, the coldest month, with the average temperature of 12.4-13.5 °C. Average precipitation is 1623.6-1899.8 mm and average radiation is 4367.2-4597.3 MJ $m^{-2}$ during the whole year. Five typical woody plants, *Pinus massoniana* Lamb, *Schima superba* Gardn. and Champ, *Castanopsis fissa* R and W, *Acmena acuminatissima* Bi.merr. and Perry, and *Cryptocarya concinna* Hance, represent three succession periods, were chosen for studies. *P. massoniana* is the pioneer heliophyte species; *S. superba* and *C. fissa* are medium-succession species, while *A. acuminatissima* and *C. concinna* are climax species in a subtropical wildwood at Dinghu Mountain nature reserve. The potted seedlings were grown under different light intensities (100%, 32% and 12% of natural light), respectively with regular management of water and fertilizer for 21 months. Havelocks were used in setting light gradients and 12 potted plants were put under each light intensity treatment. Diurnal change of natural light intensity and air temperature in August (Fig. 1) were measured using PAR and temperature sensors (PAM-2100, Germany). 100% natural light intensities reached 1801 μ mol $m^{-2}$ $s^{-1}$ at midday.

**Sampling and simulated $SO_2$ treatment:** Leaves from different individuals (n>3) with similar position on plants were taken for in vitro treatments and measurements. According to the rain acidity of Guangzhou city (Huang *et al*., 2004), leaf disks (2.0 cm length for *P. massoniana* needles) were immerged, adaxial side up, in 20, 50, 100 mM $NaHSO_3$ (pH 5.3, 5.0, 4.8, respectively), and 0 mM $NaHSO_3$ (distilled water, pH 6.0) treatment as control under 20 μ mol $m^{-2}$ $s^{-1}$ illumination for 10 h (25 °C).

**Measurements of sulfite oxidase activities:** According to the method described by Sezgintürk & Dinçkaya (2005) with alterations, leaf samples with known weight were

ground in a mortar with addition of phosphate buffer (pH 7.5, 50 mM). The mixture was centrifuged for 15 min (10000×g, 0-4°C). Supernatant with 30 µl (equal to 1.5 mg leaf samples) was added to $Na_2SO_3$ solution with different concentrations (0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, 1 M). Absorptions were measured at 240 nm due to the formation of $H_2O_2$ with an Ultraviolet-Visible spectrophotometer (Lambda 25, USA). Enzyme activities were expressed as $\Delta A_{240\ nm}$ $min^{-1}$ $mg^{-1}$ FW.

**Measurements of 77K Chlorophyll fluorescence emission spectra and polarization:** Leaf samples treated after $NaHSO_3$ or water were washed by distilled water, cut into strips and inserted into quartzose tubes. 77K Chlorophyll fluorescence emission spectra were recorded by a fluorescence spectrophotometer with low temperature affiliation (LS55, Perkin-Elmer Inst. USA). The excitation wavelength was at 436 nm (slit 10 nm) and the emission was between 600 to 800 nm (slit 10 nm). Chlorophyll fluorescence polarization was measured and calculated with the polarizing function of the instrument at 742 nm spontaneously. The fluorescence polarization (*P*) was calculated as $P= (I_w- GF \cdot I_{vh})/(I_w+ GF \cdot I_{vh})$, where $I_w$ was the intensity with the polarizers vertical and vertical (excitation and emission), $I_{vh}$ was the intensity with the polarizers vertical and horizontal (excitation and emission) and *GF* is the Grating Factor. The Grating Factor (*GF*) corrects for instrumental polarization which can be calculated by the ratio of vertically over horizontally polarized light ($GF= I_{hv}/I_{hh}$) when the exciting beam of light was polarized horizontally (Litman & Barenholz, 1982).

**Measurements of chlorophyll a fluorescence induction curves:** Leaf disks or needles treated after $NaHSO_3$ or water were washed by distilled water and dark adapted for 30 min. The fast kinetics curves (within 3.5s) were measured by a portable pulse-modulated fluorimeter PAM-2100 (Walz, Germany) with the software of PamWin. Red excitation light peaking at 650 nm (intensity of 600 µmol photons $m^{-2}$ $s^{-1}$) was used for fluorescence induction.

## Results

**Sulfite oxidase activity:** Sulfite oxidase is physiologically important and its absence may even lead to death (Ganai *et al*., 1997). The enzyme activities ($\Delta A_{240\ nm}$ $min^{-1}$ $mg^{-1}$ FW) of the five forest species were increased with the elevation of $Na_2SO_3$ concentration gradually but became stable after 0.5 M (Fig. 2). The sulfite oxidase activity in the extract of *A. acuminatissima*, a late succession stage species, increased by 1.17-5.79 times over the control ($Na_2SO_3$- free) after reacting with 0.5 M $Na_2SO_3$, showing its better capacity in transforming and detoxifying sulfite. However, compared with the broad-leaved species, the pioneer species *P. massoniana* changed least, with 99.95, 85.49 and 87.12% increments at three natural light treatments (100, 32, 12%), respectively. The increments in sulfite oxidase acitivities of *S. superba*, *C. fissa* and *C. concinna* were in between, with those under 100% natural light changed little. 32% natural light treatment made these three species higher enzyme activities and alterations. Moreover, the increasing rate of sulfite oxidase activity ($Na_2SO_3$ concentration in the linear part of curve in Fig. 2) revealed that *P. massoniana* under three light intensities had less activity rates than the other four species, while *C. fissa* showed the highest rates. In most cases, the activity rate at the lower $Na_2SO_3$ concentration was repressed significantly by natural light (Table 1).

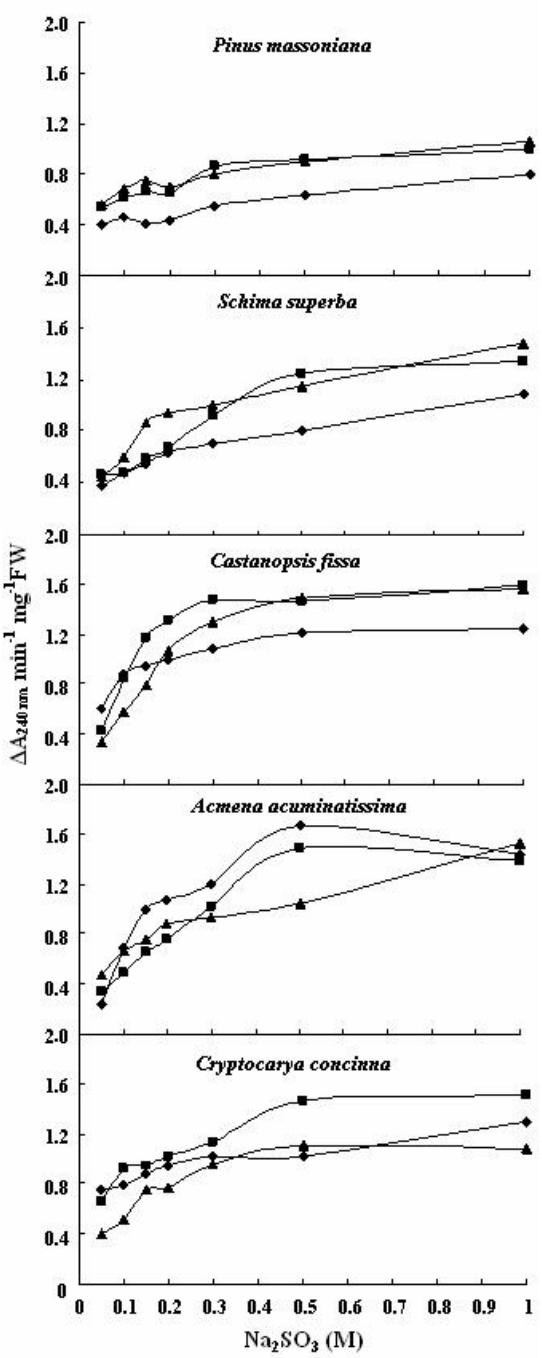


Fig. 2. Changes in sulfite oxidase activities along with $Na_2SO_3$ concentration in five forest plants *P. massoniana*, *S. superba*, *C. fissa*, *A. acuminatissima* and *C. concinna* by long-term light gradient treatments. (-♦- 100% light intensity; -■-32% light intensity; -▲-12% light intensity=4)

**Table 1. Increasing rate of sulfite oxidase activities ($\Delta A_{240\ nm}$ $min^{-1}$ $mg^{-1}$ FW $M^{-1}$ $Na_2SO_3$, the slopes in the linear part of curves in Fig. 2 between 0.05 and 0.2 M $Na_2SO_3$, n=4) in five plants *P. massoniana*, *S. superba*, *C. fissa*, *A. acuminatissima* and *C. concinna* by long term light gradient treatments.**

| Species | Relative light intensity (%) | $Na_2SO_3$ Concentration (M) | | | | |
|---|---|---|---|---|---|---|
| | | 0.05 | 0.10 | 0.15 | 0.20 | Increasing rate |
| *P. massoniana* | 100 | 0.40 | 0.46 | 0.42 | 0.44 | 0.17a |
| | 32 | 0.54 | 0.61 | 0.66 | 0.64 | 0.73b |
| | 12 | 0.57 | 0.69 | 0.75 | 0.70 | 0.91c |
| *S. superba* | 100 | 0.38 | 0.48 | 0.55 | 0.63 | 1.64a |
| | 32 | 0.47 | 0.47 | 0.58 | 0.67 | 1.44b |
| | 12 | 0.45 | 0.60 | 0.87 | 0.94 | 3.52b |
| *C. fissa* | 100 | 0.60 | 0.88 | 0.95 | 0.99 | 2.49a |
| | 32 | 0.42 | 0.85 | 1.16 | 1.31 | 5.96b |
| | 12 | 0.34 | 0.58 | 0.79 | 1.07 | 4.79c |
| *A. acuminatissima* | 100 | 0.24 | 0.69 | 0.10 | 1.06 | 5.54a |
| | 32 | 0.34 | 0.49 | 0.66 | 0.75 | 2.75b |
| | 12 | 0.48 | 0.67 | 0.75 | 0.89 | 2.60b |
| *C. concinna* | 100 | 0.76 | 0.80 | 0.88 | 0.95 | 1.28a |
| | 32 | 0.66 | 0.92 | 0.94 | 1.02 | 2.20b |
| | 12 | 0.40 | 0.51 | 0.76 | 0.77 | 2.71b |

*Within the last line, means with the same letter are not significantly different at $P<0.05$

**Low-temperature (77 K) chlorophyll fluorescence:** Fluorescence emission at 77 K showed the excitation densities within LHCII, PSII and PSI determine the amplitude of emitted fluorescence at 685 nm, 695 nm (emitted by LHCII-PSII complexes) and 735 nm (emitted by PSI-LHCI) (Krause & Weis, 1991). As shown in Fig. 3, the corresponding fluorescence peaks of PSII and PSI among five species were somewhat different. Two peaks in *P. massoniana* were at 697 and 736 nm, while that in broad-leaved plant *C. fissa* were at 697 and 740 nm, also at 687 and 740 nm in other three species. With the treatment of grown light gradients and consequent $NaHSO_3$, the features of low temperature fluorescence emission spectra were altered at different extents. In the control leaves (non $NaHSO_3$ treatment) of all tested plants, fluorescence emission, especially that from PSI reduced with the decrease in light intensity (Fig. 3 A. B. C, curve 1). $NaHSO_3$ treatment led to the changes in height and site of fluorescence peak. As the concentration of $NaHSO_3$ increased, PSI related peak was lowered down and PSII peak went up in the leaves grown at low light (12%) condition. The blue shift and reduction of PSI emission peak were observed in leaves of five species grown under 100% and 32% of natural light and treated with $NaHSO_3$. It was exacerbated by the higher concentration of $NaHSO_3$ (50- 100 mM, Fig. 3, curve3 and 4). However, the change in peaks relative to PSII differed from plant species, which showed decrease, increase or even unchanged. A significant increase of PSII fluorescence emission was found in *P. massoniana* leaves at 100% and 32% light and *A. acuminatissima* at 32% light when treated with 100 mM of $NaHSO_3$.

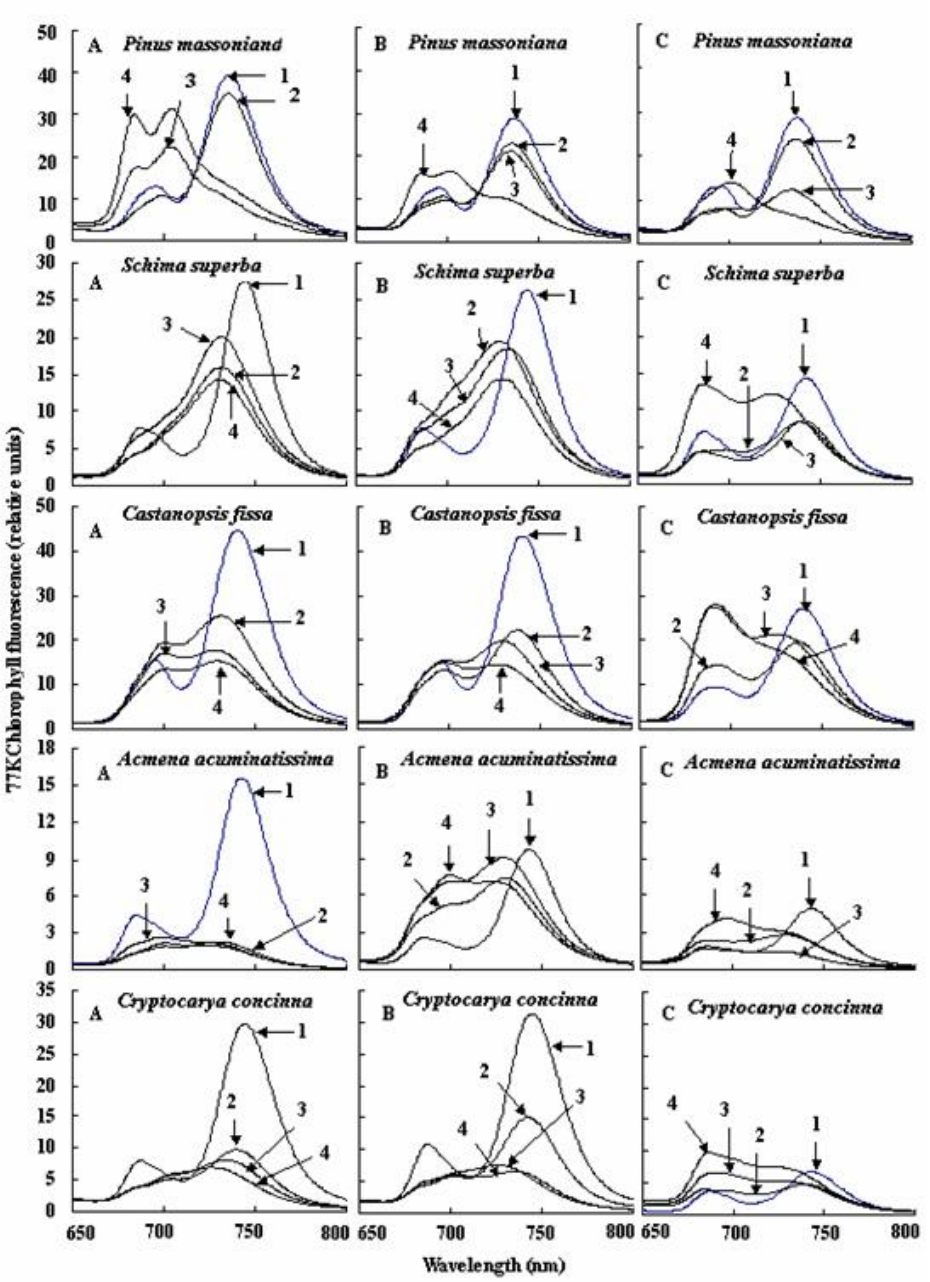


Fig. 3. 77 K fluorescence emission in five plants *P. massoniana*, *S. superba*, *C. fissa*, *A. acuminatissima* and *C. concinna* by long term light gradient and short term $NaHSO_3$ solution treatments. A-100% light intensity; B-32% light intensity; C-12% light intensity

The number of 1, 2, 3, 4 represents the treatment with 0, 20, 50, 100 mM $NaHSO_3$, respectively. Each curve was the mean of 3 or 4 individual experiments.

A concomitant redistribution of excitation energy in favor of PSI relative to PSII can be detected as an increase in 77 K fluorescence emission associated with PSI relative to PSII (Bruce *et al*., 1989). From the ratios of the two peak values (Table. 2), gradient changes of $F_{PSI}/F_{PSII}$ were found according to different natural light treatments except *A. acuminatissima*, which demonstrated that weak light is beneficial to distribute more excitation energy from PSII to PSI, whereas strong light may increase the fraction of excitation energy at PSII but PSI. It is apparent from most of the data in Table 2 that $NaHSO_3$ treatment resulted in the decrease in $F_{PSI}/F_{PSII}$ ratios regardless of the difference between either species or light intensity. Compared with the other four species, $F_{PSI}/F_{PSII}$ of *P. massoniana* under 12% natural light decreased most (by 84.4%) after immerging in 100 mM $NaHSO_3$. On the contrary, changes of *C. concinna* were relative small, with the alteration of 53.6%, 39.4% and 48.2%, under three light intensities, respectively, which showed higher tolerance ability to simulated $SO_2$ pollution.

**Low-temperature (77 K) Chl fluorescence polarization:** Membrane fluidity is inversely related to the probe polarization ratio. As membrane fluidity decreases, the polarization ratio increases and vice versa (Litman & Barenholz, 1982). In general, membrane fluidity is detected by the polarization of membrane lipids with an exogenous fluorescence probe such as DPH (1,6-diphenyl-1,3,5-hexatriene). In the present study, the polarization of chlorophyll fluorescence was monitored directly for understanding the perturbation of membrane properties because chlorophylls are bounded with proteins as complexes which locate in thylakoid membrane. Table 3 showed that fluorescence polarization was elevated obviously by the increasing $NaHSO_3$ concentration, reflecting that thylakoid membrane of these five forest plants were impacted with their fluidity decreasing to different extents. Among the examined plants, the levels of polarization in *P. massoniana* and *S. superba* treated with 100 mM $NaHSO_3$ increased gradually with decreasing light intensity. A 4.64 and 6.16 times increment of polarization under 12% natural light was found compared with the corresponding controls. However, less change was presented in *C. concinna*, with the polarization increasing of 226.8, 82.5 and 105.6%, respectively. The susceptivities of this parameter in *C. fissa* and *A. acuminatissima* were in between.

**Chlorophyll a fluorescence induction curves:** The fluorescence induction curves in Fig. 4 were similar to former studies, with O, I, D, P and S states appeared (Widell *et al*., 1983). After dark adaptation, fluorescence came out immediately with Fo level (O on the curve) when illuminated by excitation light. Thereafter, increases (or decreases at S state) in most curves were found including I, D, P and S points. Five forest species had similar induction curves in shapes but were different in fluorescence emissions after treatments of light gradients and $NaHSO_3$, with those grown under 12% natural light higher than 100% natural light. Fluorescence emission (especially Fo and Fp) of the other four species declined with the elevated $NaHSO_3$ concentration gradually except *C. concinna*, which showed less sensitive to $SO_2$ pollution with crossed curves appeared. On the contrary, the induction curves of *P. massoniana* became smooth with P peaks disappeared after immerged in 100 mM $NaHSO_3$. *S. superba* and *A. acuminatissima* grown under 100% natural light shifted similar as *P. massoniana*. Meanwhile, 1- (Fo/Fp) values were calculated in Table 4, which expressed variable fluorescence of plant leaves. Similar with other fluorescence parameters, the lower values of 1- (Fo/Fp) in *P. massoniana* again demonstrated that it responded sharply to elevated concentrations of $NaHSO_3$.

**Table 2. Changes in $F_{PSI}/F_{PSII}$ at 77 K in five plants *P. massoniana*, *S. superba*, *C. fissa*, *A. acuminatissima* and *C. concinna* by long term light gradient and short term $NaHSO_3$ solution treatments.**

| Species | Relative light intensity (%) | $NaHSO_3$ concentration (mM) | | | |
|---|---|---|---|---|---|
| | | 0 | 20 | 50 | 100 |
| *P. massoniana* | 100 | 2.20a±0.09 | 3.00a±0.21 | 2.49a±0.30 | 0.53b±0.13 |
| | 32 | 2.09a±0.27 | 1.80a±0.60 | 2.51a±0.41 | 0.64b±0.22 |
| | 12 | 3.15a±0.19 | 3.13a±0.05 | 0.50b±0.05 | 0.49b±0.07 |
| *S. superba* | 100 | 2.06a±0.24 | 1.95a±0.07 | 1.83a±0.14 | 0.90b±0.07 |
| | 32 | 3.40a±0.55 | 2.54b±0.09 | 2.12b±0.22 | 1.70c±0.39 |
| | 12 | 3.75a±0.88 | 3.24a±0.40 | 2.08b±0.05 | 1.45c±0.75 |
| *C. fissa* | 100 | 2.92a±0.16 | 1.37b±0.04 | 0.79c±0.03 | 0.60c±0.14 |
| | 32 | 2.88a±0.12 | 1.70b±0.91 | 1.37b±0.06 | 1.05b±0.01 |
| | 12 | 2.93a±0.14 | 1.32b±0.07 | 1.04b±0.03 | 1.06b±0.01 |
| *A. acuminatissima* | 100 | 2.59a±0.20 | 1.17b±0.19 | 0.86b±0.07 | 0.82b±0.08 |
| | 32 | 3.66a±0.20 | 1.74b±0.15 | 1.29c±0.13 | 0.92c±0.07 |
| | 12 | 2.35a±0.17 | 1.50b±0.01 | 0.88c±0.03 | 0.85c±0.02 |
| *C. concinna* | 100 | 1.83a±0.21 | 1.23a±0.17 | 0.92b±0.08 | 0.85b±0.06 |
| | 32 | 2.82a±0.72 | 3.01a±0.78 | 1.74b±0.05 | 1.71b±0.12 |
| | 12 | 3.40a±0.17 | 2.44b±0.85 | 2.25b±0.57 | 1.76c±0.21 |

*Within each row, means with the same letter (a for 0 mM $NaHSO_3$) are not significantly different at $P<0.05$, n=3

**Table 3 Changes in chlorophyll fluorescence polarization ($\times 10^{-2}$) at 77 K in five plants *P. massoniana*, *S. superba*, *C. fissa*, *A. acuminatissima* and *C. concinna* by long term light gradient and short term $NaHSO_3$ solution treatments.**

| Species | Relative light intensity (%) | $NaHSO_3$ concentration (mM) | | | |
|---|---|---|---|---|---|
| | | 0 | 20 | 50 | 100 |
| *P. massoniana* | 100 | 2.69a±0.70 | 4.76b±0.35 | 5.47b±0.28 | 2.36a±0.19 |
| | 32 | 2.18a±0.11 | 2.06a±0.08 | 3.34b±0.14 | 7.11c±0.51 |
| | 12 | 1.92a±0.83 | 1.88a±0.35 | 4.94b±0.35 | 10.82c±0.41 |
| *S. superba* | 100 | 4.41a±0.74 | 5.76a±0.11 | 8.87b±0.12 | 7.42b±0.02 |
| | 32 | 2.09a±0.93 | 4.62b±0.18 | 5.92c±0.34 | 5.98c±0.16 |
| | 12 | 1.12a±0.39 | 3.61b±0.07 | 8.77c±0.66 | 8.02c±0.64 |
| *C. fissa* | 100 | 1.19a±0.34 | 2.36a±0.83 | 5.35b±0.63 | 6.22b±0.37 |
| | 32 | 1.59a±0.46 | 3.37b±0.92 | 4.14b±0.20 | 4.40b±0.04 |
| | 12 | 1.54a±0.19 | 2.29b±0.13 | 3.21c±0.20 | 3.95c±0.47 |
| *A. acuminatissima* | 100 | 5.92a±0.13 | 6.33a±0.29 | 9.11b±0.10 | 11.58c±0.21 |
| | 32 | 4.69a±0.69 | 6.28b±0.55 | 7.80b±0.89 | 8.67c±0.29 |
| | 12 | 1.61a±0.55 | 4.42b±0.53 | 4.32b±0.11 | 4.91b±0.73 |
| *C. concinna* | 100 | 2.84a±0.78 | 7.81b±0.47 | 8.25b±0.82 | 9.28c±0.22 |
| | 32 | 2.51a±0.83 | 4.75b±0.96 | 4.38b±0.71 | 4.58b±0.25 |
| | 12 | 2.49a±0.10 | 3.32b±0.83 | 5.05c±0.75 | 5.12c±0.15 |

*Within each row, means with the same letter (a for 0 mM $NaHSO_3$) are not significantly different at $P<0.05$, n=4

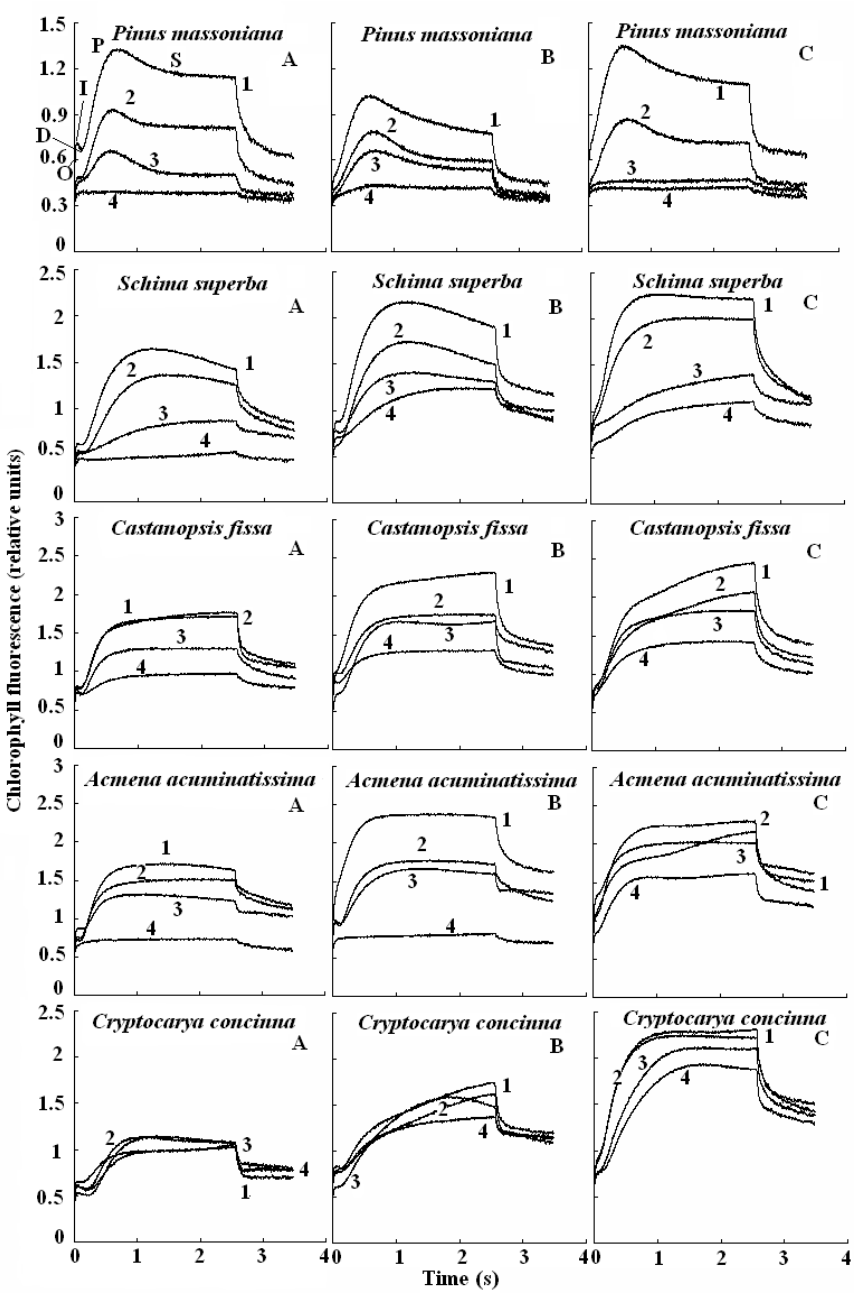


Fig. 4. Chlorophyll a fluorescence induction curves in five plants *P. massoniana*, *S. superba*, *C. fissa*, *A. acuminatissima* and *C. concinna* by long term light gradient and short term $NaHSO_3$ solution treatments. A-100% light intensity; B-32% light intensity; C-12% light intensity
The number of 1, 2, 3, 4 represents the treatment with 0, 20, 50, 100 mM $NaHSO_3$, respectively. Each curve was the mean of 3 or 4 individual experiments.

**Table 4 Changes of 1- (Fo/Fp) in chlorophyll a fluorescence in five plants *P. massoniana*, *S. superba*, *C. fissa*, *A. acuminatissima* and *C. concinna* by long term light gradient and short term $NaHSO_3$ solution treatments.**

| Species | Relative light intensity (%) | $NaHSO_3$ concentration (mM) | | | |
|---|---|---|---|---|---|
| | | 0 | 20 | 50 | 100 |
| *P. massoniana* | 100 | 0.63a | 0.61a | 0.48b | 0.18c |
| | 32 | 0.64a | 0.57b | 0.54b | 0.32c |
| | 12 | 0.59a | 0.56a | 0.22b | 0.18b |
| *S. superba* | 100 | 0.72a | 0.70a | 0.46b | 0.25c |
| | 32 | 0.72a | 0.72a | 0.56b | 0.50b |
| | 12 | 0.70a | 0.69a | 0.46b | 0.47b |
| *C. fissa* | 100 | 0.70a | 0.67a | 0.54b | 0.38c |
| | 32 | 0.70a | 0.56b | 0.71a | 0.48b |
| | 12 | 0.69a | 0.69a | 0.63a | 0.52b |
| *A. acuminatissima* | 100 | 0.68a | 0.62a | 0.51b | 0.24c |
| | 32 | 0.64a | 0.61a | 0.53b | 0.24c |
| | 12 | 0.56a | 0.68a | 0.58a | 0.60a |
| *C. concinna* | 100 | 0.46a | 0.60b | 0.64b | 0.52a |
| | 32 | 0.55a | 0.52a | 0.62b | 0.42c |
| | 12 | 0.70a | 0.71a | 0.71a | 0.68a |

*Within each row, means with the same letter (a for 0 mM $NaHSO_3$) are not significantly different at $P<0.05$, n=4

## Discussion

Acidic precipitation arises from the oxidation of sulfur dioxide and nitrogen dioxide in the atmosphere to form $H_2SO_4$ and $HNO_3$ acids deposited onto forests via rain, fog, cloud or by dry deposition of gases and vapour (Percy & Ferretti, 2004). Thus the use of buffered $H_2SO_3$ to exposed cells to different concentrations of $SO_2$ is arguably realistic (Taylor *et al*., 1981). The effects of $SO_2$ on vegetation and agriculture, and its role in the formation of acid rain, continues to be controversial. However, it is reported that, after penetration through the stomata, $SO_2$ rapidly dissolves in the aqueous phase of the cell wall and, at apoplastic pH value, it reacts with water to form toxic molecular species such as bisulfite and sulfite, which, in turn, can rapidly be converted, through a series of reactions, to a non-toxic sulfate (Rennenberg & Polle, 1994; Ranieri *et al*., 1999). Rapid metabolic conversion of sulfite to sulfate may be achieved by apoplastic sulfite oxidase activity (Pfanz *et al*., 1990). Rennenberg & Polle (1994) calculated that the capacity of apoplastic fluids for enzymatic conversion of sulfite to sulfate is three orders of magnitude higher than the influx of atmospheric $SO_2$ into the leaf via the stomata at 30ppb $SO_2$. Therefore, apoplastic sulfite oxidase activity may be able to convert the bulk of the sulfite produced from $SO_2$ influx into the leaves at the atmospheric $SO_2$ concentrations which plants experience even in polluted environments. The finding that sulfite accumulates in xylem sap of red spruce trees fumigated in the winter with low $SO_2$ concentrations is consistent with this assumption (Wolfenden *et al*., 1991; Rennenberg & Herschbach, 1996). In our experiments, extractions of different plant species reacted with $Na_2SO_3$ in creating $H_2O_2$, which demonstrated that five examined forest plants were able to catalyze sulfite into sulfate so as to avoid further damages in bioplasm (Fig. 2, Table 1). However, the sulfite oxidase activities of plant leaves along the natural light gradient were different (lowest under 100% natural light), which implies that leaves grown with long term of low light intensity possessed the better tolerant ability to $SO_2$. In this way,

activities of this enzyme were mostly consistent to chlorophyll fluorescence emission according to natural light gradient (Table 1, 2). Among the five forest plants examined, *P. massoniana* was more sensitive to high concentration of $Na_2SO_3$, with smallest alterations in absorption at 240 nm. Hence, it is deduced that $SO_2$ and $SO_3^{2-}$ might easily infiltrated into *P. massoniana* cells, yielding and accumulating more highly oxidant molecular species in bioplasm, which resulted in the damage of lipids, proteins and subcellular structures. Contrarily, *A. acuminatissima* could detoxify more sulfite by the enzyme systems to avoid further destructions of plant cells.

The photosynthetic apparatus in plant leaves can harmonize the relationships of different parts, including state transitions, to adapt to various light environments. This phenomenon occurs via the reversible transfer of a fraction of the light harvesting complex II (LHCII) from PSII to PSI (state I to state II transition), as the consequence of its phosphorylation (Allen, 1992; Aro & Ohad, 2003). When PSII is over excited, the photosynthetic apparatus can be converted into state II. Whereas, when PSI is over excited, the photosynthetic apparatus is converted into state I. Murata (1969) reported that when state I to state II transition happens in *Porphyridium cruentum*, 77 K fluorescence emission of PSI increases, while PSII fluorescence intensity decreases. Therefore, the gradually increasing of $F_{PSI}/F_{PSII}$ responding to reduced light intensities in Table 2 reflected the long term light environment acclimation in the forest plants by adjustments of state I to state II transition. And also in the present study, the fact that altered the site and reduced emission of PSI associated with the enhancement of PSII emission by $NaHSO_3$ treatment (Fig. 3) illuminated a state II to state I transition. Taken together with the calculated data of $F_{PSI}/F_{PSII}$ (Table. 2), clearly, the impact of $NaHSO_3$ on energy transformation between two photosystems might be from the modification of their component and structure. The degrees of state transition varied among five forest plants. $F_{PSI}/F_{PSII}$ at 77 K of *P. massoniana* changed most, indicating it was damaged more than other species. On the contrary, the photosystems of *C. concinna* had superiority for excited energy distribution in resisting $SO_2$ pollution.

Investigating membrane fluidity by using fluorescence polarization has become popular due to its many advantages including virtual real time measurements, delay between excitation and emission is $10^{-8}$ s or less and detect weak signals with high quality instruments (Denich *et al*., 2003).The fluorescent probes used in fluorescence polarization can embed into the lipid bilayer and are sensitive to alterations in membrane fluidity resulting from structural changes (Luly *et al*., 1981). The movement of membrane affects the depolarization of the exciting light. This depolarization is used to calculate fluorescence polarization values and lifetimes. These values report on the rotational rate of the probe which reflects the microviscosity of the membrane environment surrounding the probe (Adler & Tritton, 1988). Since PSI and LHCI are imbedded in the bilayer of thylakoid membrane and when excited at 436 nm, the chlorophyll fluorescence emission (at 77 K) reached maximum at 742 nm. Therefore, it is used as probe in our experiments to investigate the possible alteration of thylakoid membrane fluidity. After treating by simulated $SO_2$, the elevation of low temperature chlorophyll polarization of the five forest plants (Table. 3) was thought to elucidate the increasing thylakoid membrane mucosity and decreasing fluidity. If this is true, then the higher increment of polarization in *P. massoniana* and lower increment of polarization in *C. concinna* by the combination of short term $NaHSO_3$ (100 mM) and long term low light intensity again evidenced their different tolerance to $SO_2$.

Two phases are clearly distinguished in the kinetics of fluorescence induction: the photochemical phase is completed within 1-2 ms and the thermal phase lasts for 200 ms after the onset of light (Schreiber & Krieger, 1996). The former is attributable to the light-induced reduction of $Q_A$, the primary quinone acceptor of photosystem II, whereas the thermal phase is not directly related to the redox state of $Q_A$ (Samson *et al*., 1999). In the curve, O→I means the separation of electron from PSII (Critchley & Smillie, 1981). Hawkins (1985) believes, if the duration of O→I→D is lengthened, the water photolysis will be hindered and the electron numbers arriving at PSII will be decreased. The changes of O→P process is due to the deoxidization of PQ pool and the slowing of corresponding electron transport speed. The appearance of P→S changes may be due to the excitation of dark reaction in photosynthesis, which consumes the electrons at PSI and results in oxidation of PQ pool (Melis & Zeiger, 1982). The induction curve changes its shape according to many environmental conditions, such as light intensity, temperature, drought or chemical influences. The calculation of several phenomenological and biophysical expressions leads to the dynamic description of a photosynthetic sample at a given physiological state. The maximal measured fluorescence intensity, Fp, which can be denoted as Fm if the excitation intensity was experimentally found, in all physiological states studied, to permit the closure of all reaction centers. The fluorescence intensity at 50 μs, considered to be the intensity when all reaction centers are open (Krüger *et al*., 1997). Therefore, 1- (Fo/Fp) were used to estimate the impairments of electron transport pathway to PSII reaction centers of the five forest plant species to simulated $SO_2$ pollution (Table. 4). The results calculated from this formula in control leaves were not changed significantly by different light intensities. However, after gradient increasing of $NaHSO_3$ concentrations, the values of *P. massoniana* were lowered down most but remained unchanged in *C. concinna*. It is speculated that the impact of photosynthetic electron transport processes were aggravated by high $NaHSO_3$ concentration and the injury effect was different among species by $SO_2$ pollution.

In South China, the subtropical evergreen monsoonal broadleaf forest, stands alone in the world, develops in the succession process of conifer forest → coniferous and broad-leaved mixed forest with conifer dominated → coniferous and broad-leaved mixed forest with heliophyte broad-leaved species dominated → evergreen broad-leaved forest with heliophyte broad-leaved species dominated → evergreen broad-leaved forest with shade-tolerance species (Peng *et al*., 1996). Five woody plants selected in this experiment were the dominant species in different succession periods. Studying on the responses and adaptations of these plant species grown under different light intensities to simulated $SO_2$ and acid precipitation are useful to find out the effects of human activities on subtropical forest structures and functions. The total results demonstrated that *P. massoniana*, the dominant species in early stage of succession, was lower in sulfite oxidase activitiy and its fluorescence parameters, particularly under 12% natural light illumination were sensitive to simulated $SO_2$ treatment compared with the other broad-leaved species. Whereas the late stage dominant species *C. concinna* exhibited better tolerance to simulated $SO_2$ treatment from chlorophyll fluorescence aspects. Hence, we deduced that in subtropical coniferous and broad-leaved mixed forest, acid pollution and low light will accelerate the decline of *P. massoniana* (including other heliophyte forests) and promote succession course. On the contrary, forest climax dominant species like *C. concinna* less sensitive to simulated $SO_2$ treatment are propitious to its development in the forest.

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