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Differences in photosynthetic capacity, chlorophyll fluorescence, and antioxidant system between invasive *Alnus formosana* and its native congener in response to different irradiance levels

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Abstract

We compared the invasive *Alnus formosana* with its native congener (*A. cremastogyne*) at three irradiances in terms of photosynthesis, chlorophyll fluorescence and antioxidant system. The increased light-saturated photosynthetic rate ($A_{\max}$) and light saturation point (LSP) contributed directly to the increased performance of the invasive. The invasive species had also higher plasticity in carotenoid and total chlorophyll than the native species at 100% irradiance, potentially contributing to invasion success in high-irradiance locations via photoprotection. Moreover, the diurnal photoinhibition of photosynthesis, as judged by the maximum photochemical efficiency of PSII (F_v/F_m) of dark-adapted leaves, was more severe in the natives than in the invasive. With increasing irradiance, the invasive exhibited increased antioxidant activities and higher antioxidant levels to support the adverse conditions of both low- and high-irradiance acclimation. In contrast, the intercellular CO_2 concentration (C_i) and stomatal limitation (L_s) decreased with increases in the net photosynthetic rate (A_n), stomatal conductance (G_s), and transpiration rate (T_r). We speculated that L_s was the main factor inhibiting the A_n for both studied species. These results first indicated that the invasive may occupy new habitats successfully through tolerating shading at low irradiance and out-compete native species through higher $A_{\max}$ and antioxidant levels when irradiance is increased.

Keywords: Invasiveness; Irradiance acclimation; Photoinhibition; Photosynthesis; Plasticity

Abbreviations: $A_{\max}$, light-saturated photosynthetic rate; A_n , net photosynthetic rate; APX, ascorbate peroxidase; AQY, apparent quantum yield; CAT, catalase; Car, carotenoid; Car/Chl, carotenoid/total chlorophyll; Chl, total chlorophyll; Chla, chlorophyll *a*; Chlb, chlorophyll *b*; Chla/*b*, chlorophyll *a*/chlorophyll *b*; C_i , intercellular CO_2 concentration; EEE, excessive excitation energy; F_m , maximum fluorescence of dark-adapted state; F'_m , maximum fluorescence of light-adapted state; F_o , minimal fluorescence of dark-adapted state; F'_o , minimal fluorescence of light-adapted state; F_s , steady-state fluorescence yield; F_v/F_m , maximum photochemical efficiency of PSII; FW, fresh weight; G_s , stomatal conductance; H_2O_2 , hydrogen peroxide; LCP, light compensation point; LHC, light-harvesting complex; L_s , stomatal limitation; LPO, lipid peroxidation; LSP, light saturation point; LUE, light use efficiency; MDA, malondialdehyde; NPQ, non-photochemical quenching; $\text{O}_2^{\cdot-}$, superoxide radicals; PPFD, photosynthetic photon flux density; PSII, photosystem II; ROS, reactive oxygen species; SOD, superoxide dismutase; T_r , transpiration rate.

Introduction

Sunlight is a key limiting resource for photoautotrophic plants. In nature, plants are often exposed to continuous changes in the intensity of light (e.g., sunflecks) (Chazdon and Pearcy, 1991; Rodríguez-López et al. 2014), and their ability to acclimate to external conditions that fluctuate temporally and spatially is of considerable importance for establishing plants in forest ecosystems (Favaretto et al. 2011). Through the process of photosynthesis, light energy is used to produce ATP and NADPH in the light reaction. In the light-independent reaction, carbon is subsequently fixed into carbohydrates, and oxygen is produced (Dai et al. 2009). At high irradiance, the photosynthetic apparatus absorbs excessive excitation energy (EEE), resulting in the inactivation or impairment of the chlorophyll-containing reaction centers (Huang et al. 2014). Consequently, photosynthetic activity is depressed by photoinhibition. Conversely, plants grown at low irradiance generally have a lower light-compensation point (LCP) and light saturation point (LSP) as well as lower synthesis of some components involved in electron transport (generation of ATP and CO₂ fixation) (Huang et al. 2011). Nonetheless, the photosystem (especially photosystem II, PSII) is the primary target of photodamage in high- and low-light-grown seedlings, which increases the production of reactive oxygen species (ROS) that aggravate PSII photoinhibition (Nishiyama et al. 2011).

To protect against these phytotoxic symptoms, plants have many photoprotection mechanisms. One mechanism is known as non-photochemical quenching (NPQ) of chlorophyll fluorescence because it competes with photochemistry to quench the little energy that the excited chlorophyll *a* (Chl*a*) molecules can re-emit as fluorescence (Dai et al. 2009; Giorio et al. 2012). Chl*b* is mainly contained in the light-harvesting Chl protein complex II (LHCII), and shaded leaves are relatively rich in Chl*b* (Walters, 2005; Huang et al. 2011). In another mechanism, plants use an antioxidant defense system that consists of oxygen free-radical detoxifying enzymes and other antioxidant compounds to avoid ROS (Huylensbroeck et al. 2000; Shi et al. 2006; Jaleel et al. 2009; Foyer and Shigeoka, 2011). Nonetheless, carotenoids (Car) harvest the EEE, quench the excited state of Chl, and play an effective role in photoprotection (Gill and Tuteja, 2010; Rodríguez-López et al. 2014). Nevertheless, data related to the susceptibility and tolerance of invasive trees to photoinhibition and oxidative stress are scarce.

Accumulating evidence suggests that biological invasions are a major threat to natural

ecosystems (Daehler, 2003; Allred et al. 2010; Fan et al. 2013; Liao et al. 2013). Thus, the identification of the factors that contribute to the success of invasive plants is important for predicting/controlling potentially invasive plants. The ability to compete with native species for resources (limited or not) may greatly improve the probability of establishment and the overall fitness of the invasive plants (Li et al. 2012). However, the mechanisms by which introduced species grow more aggressively in their nonnative ranges than in their native ranges are still not well elucidated. Some studies have reported that some invasive species have a higher light-saturated photosynthetic rate (A_{max}), photosynthetic nitrogen use efficiency (PNUE), water use efficiency (WUE), and specific leaf area (SLA) than native species (e.g., Yamashita et al. 2000; Feng et al. 2007; Zheng et al. 2012). Other studies demonstrated that higher carbon gain and growth may enable invasive species to easily out-compete more slowly growing species by facilitating colonization or resource acquisition (Allred et al. 2010 and references therein). However, Palacio-López and Gianoli (2011) used a meta-analysis to reveal that invasive plant species do not display greater phenotypic plasticity (at least for some functional traits) than their native counterparts' responses to light, nutrients, water, carbon dioxide, and support availability. Furthermore, invasive species did not always exhibit increases in A_{max} , PNUE, WUE and SLA. From 24 photosynthetic comparisons between invasive and native species, Daehler (2003) demonstrated that 11 comparisons revealed similar photosynthetic rates for invasive and native species; 7 and 6 comparisons reported higher and lower photosynthetic rates for invasive species, respectively. One explanation is that most of the studies compared phylogenetically unrelated invasive and native species. Phylogenetic relatedness may constrain morphological/physiological variations; closely related plants may share more common traits and more overlapping resource requirements than unrelated species (Feng, 2008; Morais et al. 2012). In contrast, when invasive species are compared with distantly related and hence markedly different counterparts, hypothetical differences in trait plasticity may be equally explained by several traits other than the invasive feature (Palacio-López and Gianoli, 2011). An alternative explanation for the inconsistent results is the intrinsic weakness of the approach of invasive and native comparisons (Grotkopp and Rejmánek, 2007). Consequently, comparisons between related invasive and native species are more meaningful in explaining the invasiveness of invasive species (Zheng et al. 2012; Fan et al. 2013).

Alnus formosana (Burk.) Makino (Betulaceae), an excellent producer of pulpwood and a species

used in vegetation restoration, is native to Taiwan Province (an island) in China and was introduced to South Africa and other parts of Asia (Zwolinski, 1992; Lai et al. 2012). According to field observations, *A. formosana* can spread over larger spatial scales than some native species (e.g., *A. cremastogyne* Burk.) in the introduced range, and it has successfully colonized a variety of habitats, including woodlands, riparian forests, and the borders of agricultural fields. Our previous study also demonstrated that *A. formosana* exhibits higher phenotypic plasticity than *A. cremastogyne* in three irradiance levels; the increased plasticity is mainly noted in its higher relative growth rate (RGR) but also in its lower leaf area ratio (LAR)(data not shown). The relative performance of invasive plants is environment-dependent, and comparisons between related invasive and native plants in diverse environments are more informative in elucidating plant traits contributing to invasiveness (Allred et al. 2010; Hyldgaard and Brix, 2012; Pintó-Marijuan and Munné-Bosch, 2013). The ability of plants to capture and utilize light is an important determinant of growth potential and fitness (Zheng et al. 2012). Competition for sunlight is an important factor influencing some plant invasions. Yamashita et al. (2000) reported that invasive *Bischofia javanica* achieves a rapid photosynthetic acclimation when transferred from shade to a sunny environment by minimizing photoinhibition in existing shade leaves and rapidly deploying new sun leaves with high photosynthetic capacity. Thus, it is reasonable to hypothesize that *A. formosana* might have an increased $A_{\max}$ and/or antioxidant functions to enhance photosynthesis compared with its native congener. However, the physiological mechanisms associated with the higher performance of *A. formosana* were unclear, and the relationship between the plasticity of physiological traits and invasiveness was also not assessed.

Against this background, we examine possible strategies of competition and tolerance that may be responsible for or aid in the successful invasion of *A. formosana* at three irradiances. The main objectives of this study were to (i) explore which physiological traits contributed to the higher performance of the invasive and (ii) analyze the relationships between invasiveness and plasticity of these physiological traits and performance. The results from this work will contribute to the scientific discussion of the differences between the invasive and native species in photosynthetic acclimation and photoprotection strategies.

Materials and methods

Site description

The experiment was performed in Lingyan Mountain Botanical Garden (31°01' N, 103°35' E, and 800 m.a.s.l.) of Sichuan Agricultural University (SICAU) located in Dujiangyan County, Sichuan Province in southwest China. The mean annual temperature is 15.2°C, and the annual accumulated temperature ($\geq 10^{\circ}\text{C}$) is 4677.1°C, with a mean of 26.5°C in the hottest month (July) and 5.6°C in the coolest month (January). The annual precipitation of the base ranges from 1300 to 1800 mm, with a dry period lasting from November to February. A heavy fog, which usually starts at ca. 23:00 and lasts to ca. 10:00, is present almost daily from October to February.

We conducted this study in a 5-year-old *Alnus*-dominated heath originating from an illegal deforestation in a *Pinaceae-Alnus* forest in Dujingyan-Longchi National Park (DLNP), Sichuan Province. *A. cremastogyne* (including adult trees and young saplings) are native species with a stature similar to that of *A. formosana* in this region. This species has sympatric distributions with *A. formosana* and also occurs in various habitats, such as cut-over areas, forest gaps, and forest canopies. Based on our field observations, the native congener is typically distributed in slightly shaded environments and rarely in low- or high-irradiance environments. However, *A. formosana* has high density at ca. 50–100% irradiances, whereas it is still common at ca. 20–50% irradiances. *A. formosana* is also found at ca. 10% irradiance. The seeds of the studied species were collected from natural populations from mid-to-late December 2008 to mid-January 2009 at two sites 20 km apart from each other around Dujingyan city, Sichuan Province, China.

Experimental treatments

In February 2009, the seeds of the studied species were sown at a seedbed in a greenhouse. In April, when the seedlings of the species were ca. 15 cm tall (with three leaf pairs), similarly sized vigorous seedlings were transplanted singly into 15 dm³ plastic pots (30 cm in diameter and 23 cm in height) located under shade netting that provided 50% irradiance. The pots were filled with equal proportions of river sand and forest topsoil. Topsoil was used to provide a natural supply of macro- and micro-nutrients, and the river sand provided a texture with adequate drainage and facilitated the harvest of fine roots. After 20 days of growth in 50% irradiance, all seedlings were transitioned to full sunshine by removing the shade net. The seedlings were divided randomly into three groups, and each group (20 seedlings) was grown at one of the three irradiances (12.5%, 56.2%, and 100%). The seedlings were watered with tap water (ca. 3 dm³) every three day and fertilized with complex

compound fertilizer (15% N, 15% P, and 15% K) at 2 g per pot. On June 8th, the transplanting work was completed.

The soil in the area is a nutrient-rich, poor-to-rapidly drained, powdery, yellow-brown loam with a pH value between 5.5 and 6.5. Before fertilization, the organic matter content, including total N, P and K and available N, P and K, in the growing substrate were 19.32 g kg⁻¹, 813.18 mg kg⁻¹, 235.26 mg kg⁻¹, 39.32 mg kg⁻¹, 10.36 mg kg⁻¹, and 58.54 mg kg⁻¹, respectively. After fertilization, the contents of available N, P, and K were 45.65, 26.46, and 76.23 mg kg⁻¹, respectively.

The experiment, which involved simulating the light regimes of forest openings (cut-over areas), forest gaps, and forest canopies in DLNP of China, was conducted in three large shade houses (40 m × 4.0 m × 1.5 m) that were constructed of steel tubes in an open site. The lower 30 cm of each shade house remained open to facilitate airflow. The light intensity in each shade house was controlled by different layers of black nylon shade netting, including no netting to create 100% irradiance (full sunlight). Two plots, a forest gap and a forest canopy, were selected in the experimental area. These plots were 200 m apart at 750 m.a.s.l. The two plots boast the same or similar exposure, inclination, and edaphic conditions. Quantum sensors and a Li-1400 Datalogger (LI-Cor Inc., Lincoln, NE, USA) were used to measure photosynthetic photon flux density (PPFD) in the two plots and two shade houses ([Supplementary figure S1](#)) during a clear day. The relative irradiance above each sample individual was calculated as the ratio of the light intensity above it to that in the open site. The PPFDs of the forest gaps and forest canopies were $54.6 \pm 0.04\%$ and $10.3 \pm 0.90\%$, respectively, during full sunlight exposure, whereas the PPFDs of the two shade houses reached $56.2 \pm 1.03\%$ irradiance (medium shade) and $12.5 \pm 0.21\%$ irradiance (excessive shade). These results suggest that the simulated sunlight environment resembled that of the sample lands. To avoid position effects, the pots were arranged in the central part of each house and randomized fortnightly within each shade house throughout the experiment. As in most experiments that control irradiance ([Feng, 2008](#); [Zheng et al. 2012](#)), we had no replication of the shade house with similar irradiance, and the seedlings in each shade house were used as replicates for statistical analyses.

Light-response curves

From August 18th to 25th, on clear-sky days, randomly selected, new fully expanded leaves (the fifth or sixth leaf from the top) of three individuals per species and treatment were analyzed. The photosynthetic light-response curves ($A/PPFD$) were obtained by decreasing the PPFD in fifteen

sequential steps from strong to weak (2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, 50, 20, 10 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at 25°C. The leaf tissues were initially exposed to a low ambient CO_2 concentration (50 $\mu\text{mol mol}^{-1}$ air for 5 min to allow for stomatal opening); the A/PPFD curves were then produced at a CO_2 concentration of 400 $\mu\text{mol mol}^{-1}$ air. The A_{max} , apparent quantum yield (AQY), LCP, and LSP were determined from these curves by fitting the data to a modified rectangular hyperbole model proposed by Ye (2007). The model was fitted to the data using nonlinear regression analysis by applying iterative curve fitting (minimum least square difference) using the Microsoft Excel Solver tool (Microsoft Corporation, Redmond, WA, USA). It was assumed that the LSP corresponded to the PPFD required to achieve 90% of the A_{max} . The relative humidity of the air in the leaf chamber was controlled at ca. 70%, and leaf temperature was maintained at $25 \pm 0.4^\circ\text{C}$ during all measurements.

Gas exchange and chlorophyll fluorescence measurements

The A_n , stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) were measured between 9:00 and 10:30 am from the same leaves (and time periods) using a Li-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA) with constant irradiation (600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The stomatal limitation (L_s) of A is an estimate of the proportion by which actual G_s decreases A relative to what A would be assuming infinite conductance between the atmosphere and the leaf sub-stomatal airspace (A^0) and is typically estimated from A vs. C_i curves using the following relationship (Farquar and Sharkey, 1982): $L_s = 1 - A/A^0$. A is the rate of carbon assimilation at the measurement [CO_2] and based on the actual G_s , and A^0 is photosynthesis assuming infinite G_s (i.e., when $C_i = \text{atmospheric } [\text{CO}_2]$). The light use efficiency (LUE) was calculated by $LUE = A_n/\text{PPFD}$.

Chlorophyll fluorescence was measured simultaneously with the gas exchange measurements for leaves using a fluorometer leaf chamber (6400-40, Li-Cor Inc. Lincoln, NE, USA). The initial fluorescence (F_0) was measured by switching on the modulated irradiation of 0.6 kHz. PPFD was less than 0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on the leaf surface, which was adapted to darkness for 30 min. The maximal fluorescence (F_m) was measured at 20 kHz with a 1-s pulse of 6000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of white light. The maximum photochemical efficiency of PSII (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$. During illumination, the steady-state fluorescence (F') was obtained. The maximal fluorescence (F'_m) was also determined by applying a saturating pulse. The non-photochemical

quenching coefficient (NPQ) was calculated as $NPQ = (F_m - F'_m) / F'_m$.

All of the above photosynthetic measurements were repeated five times, so the photosynthetic parameters for each replicate were computed as the average values for the measured days. Within each time period, measurements were carried out in blocks in an attempt to minimize the effects of potential variations of the environmental conditions.

Determination of photosynthetic pigment concentrations

Leaf discs were collected on clear-sky days, flash frozen in liquid nitrogen, and subsequently stored at -80°C until analysis. Subsequently, the plant material was ground. To calculate the Car/Chl ratio, total Chl, Chl*a*, Chl*b*, Chl*a/b* and Car concentrations were determined spectrophotometrically after extraction overnight in the darkness in 80% ethanol according to [Lichtenthaler \(1987\)](#).

Hydrogen peroxide content and lipid peroxidation assay

Fully expanded and exposed leaves were randomly selected from each replication and treatment to measure the H_2O_2 and MDA levels. The H_2O_2 concentration was estimated following the method of [Patterson et al. \(1984\)](#). The absorbance of the solution was measured at 410 nm against blanks that had been similarly prepared but did not contain plant tissue. LPO in plant tissue was determined by measuring MDA, a major thiobarbituric acid-reactive species ([Heath and Packer, 1968](#)). The absorbance of the supernatant was read at 532 nm and corrected for non-specific absorbance at 600 nm.

Antioxidant activity assays

Plant samples (0.5 g) were homogenized in 10 ml of chilled 0.1 M potassium phosphate buffer (pH 7.0) under ice-cold conditions. The homogenate was filtered through four layers of cheesecloth and centrifuged at $15,000\times g$ at 4°C for 30 min. The supernatant was stored at 4°C before use in protein determination and enzyme activity assays. The protein content in the homogenate was determined using bovine serum albumin as a standard. All spectrophotometric analyses were performed at 25°C using a UV-VIS spectrophotometer (Model UV-190, Shimadzu, Japan).

Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium ([Beauchamp and Fridovich, 1971](#)). One SOD unit was calculated as the amount of enzyme required to inhibit the photoreduction of NBT by 50% at 25°C . Tubes containing the reaction mixture were incubated under two 15 W fluorescent lamps for 15 min and then transferred to the dark for 15 min. The absorbance was read at

560 nm. The SOD activity is expressed as U mg^{-1} protein. Catalase (CAT, EC 1.11.1.6) activity was determined by directly measuring the rate of H_2O_2 conversion to O_2 at 240 nm according to the method of Cakmak and Marschner (1992). Enzyme activity was calculated using an extinction coefficient of $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured as the decrease in absorbance at 290 nm, which results from ascorbate oxidation (Nakano and Asada 1981). Enzyme activity was calculated using an extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$. The CAT and APX activities are expressed as U mg^{-1} protein.

Statistical analyses

The data were statistically analyzed following either a randomized block design (*A*, gas exchange parameters and Chl fluorescence parameters) or a completely randomized design (the remaining parameters) with four to six replicates. Statistical analyses were performed with the software Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., IL, USA). A two-way analysis of variance (ANOVA) was used to assess the effects of species (S), irradiance (I), and their interaction ($S \times I$) on each variable evaluated in this study. One-way ANOVAs (Duncan test) were used to assess the differences among species at the same irradiance and the differences among irradiances for the same species in each variable. Data are presented as the means $\pm$ one standard error (SE).

Results

Photosynthesis

To explore the photosynthetic characteristics of invasive *A. formosana* and its native congener, light-response curves of *A*/PPFD were measured. The patterns of the light-response curves were similar for all three irradiances (Fig. 1). A_n variations for both studied species exhibited similar trends, increasing rapidly up to $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD then rising slowly until reaching their maximum values (8.56 , 7.30 , and $5.40 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for *A. formosana* and 7.24 , 6.70 , and $4.80 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for *A. cremastogyne* at 100%, 56.2%, and 12.5% irradiances, respectively) of $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Plants grown at higher irradiances had higher A_n than those grown at low irradiances.

Species [except for AQY ($F = 3.341$, $P = 0.632$)] and irradiance significantly ($P < 0.05$ for AQY, $P < 0.01$ for others) affected photosynthesis variables evaluated in this study according to a two-way ANOVA (Fig. 2). The interactions ($S \times I$) between species and irradiance were also significant for

the variables ($F = 8.409$, $P = 0.005$ for $A_{\max}$; $F = 6.278$, $P = 0.014$ for LCP) except LSP ($F = 0.989$, $P = 0.400$) and AQY ($F = 0.477$, $P = 0.632$), indicating that the patterns of the differences in $A_{\max}$, LSP, and LCP between species were different at three irradiances. For example, *A. formosana* compared with its native congener exhibited significantly higher $A_{\max}$ and LSP at all studied irradiances (Fig. 2B and C). However, the invasive had lower LCP than the natives only at 12.5% irradiance ($F = 36.24$, $P = 0.004$) but not at 100% and 56.2% irradiances (Fig. 2D). For both studied species, $A_{\max}$, LSP, and LCP increased significantly with increasing irradiance and peaked at 100% irradiance, whereas AQY was not significantly ($F = 1.500$, $P = 0.296$ for *A. formosana*; $F = 2.700$, $P = 0.146$ for *A. cremastogyne*) influenced by three irradiances (Fig. 2A).

Maximum photochemical efficiency of PSII (F_v/F_m) and diurnal variation of NPQ

Photoinhibition of PSII can be easily detected *in vivo* by a decrease in the 'dark-adapted' ratio of variable to F_v/F_m (Favaretto et al. 2011). Thus, decrease in the F_v/F_m ratio, which represents the maximum photochemical efficiency of PSII, is often used to estimate the degree of photoinhibition. In this study, the patterns of the F_v/F_m ratios were similar for all three irradiances (Fig. 3). As time progressed, the F_v/F_m ratio was significantly ($F = 11.545$, $P = 0.009$ for *A. formosana*; $F = 9.348$, $P = 0.014$ for *A. cremastogyne*) influenced by irradiances (Fig. 3A and B). For both studied plants, the F_v/F_m ratio decreased significantly with increased irradiance, reached the lowest points at ca. 13:00 h (0.77, 0.79, and 0.73 for *A. formosana* and 0.75, 0.79, and 0.71 for *A. cremastogyne* at 100%, 56.2% and 12.5% irradiances, respectively), and then increased until rebounding to the level of the morning at ca. 19:00 h. For *A. formosana*, the F_v/F_m ratio ranged from 0.77 to 0.83 at 100% and 56.2% irradiances and was significantly higher than the ratio at 12.5% irradiance (0.73–0.81). For *A. cremastogyne*, the values (0.71–0.79) at 12.5% irradiance were slightly lower than those for the invasive.

For both studied species, NPQ increased continuously with increasing irradiance, whereas these variables peaked at ca. 13:00 h (4.53, 2.79, and 1.45 for native species and 3.58, 2.14, and 0.61 for invasive species at 100%, 56.2%, and 12.5% irradiances, respectively) and then rapid decreased to morning levels (Fig. 3C and D). Similarly to F_v/F_m , the levels of NPQ at 100% irradiance were markedly ($P < 0.05$) higher than the levels at 56.2% and 12.5% irradiances for both plants. Further, the natives exhibited a broader NPQ ascendant range and a larger max NPQ than the invasive at all studied irradiances.

Gas exchange parameters and light use efficiency

Irradiance ($P < 0.01$) and species [except for C_i ($F = 0.301$, $P = 0.593$) and L_s ($F = 1.898$, $P = 0.193$)] significantly affected all gas exchange parameters in this study based on two-way ANOVA (Fig. 4A–F). However, the interactions between species and irradiance were not significant for most of the variables (e.g., $F = 0.729$, $P = 0.503$ for T_r ; $F = 0.174$, $P = 0.842$ for G_s ; $F = 0.032$, $P = 0.968$ for C_i ; $F = 0.844$, $P = 0.454$ for L_s) except A_n ($F = 8.200$, $P = 0.006$), indicating species differences in plasticity for these variables. With increasing irradiance, the values of A_n , T_r , and G_s increased for *A. formosana* and its native congener and was significantly increased for the invasive compared with the natives at three irradiances (except A_n at 100% and 12.5% irradiances and G_s at 26.5% irradiance) (Fig. 4A, C, and E). For both studied species, however, the values of C_i , L_s , and LUE decreased with increasing irradiance, although the differences were not significant ($P > 0.05$) at 100% and 56.2% irradiances [except LUE ($P = 0.023$) for *A. cremastogyne*]. Further, the differences in C_i , L_s , and LUE values between invasive and native species were not significant at three irradiances (Fig. 4B, D, and F).

Photosynthetic pigments

The responses of Chl a , Chl b , Chl, and Car to irradiances significantly ($P < 0.01$) differ between species, whereas the responses of Chl a/b and Car/Chl did not differ (Fig. 5A–F). The interactions between species and irradiance were significant for the variables ($F = 14.450$, $P = 0.001$ for Chl a/b ; $F = 24.551$, $P = 0.000$ for Chl b ; $F = 8.294$, $P = 0.005$ for Chl) except Chl a ($F = 2.832$, $P = 0.098$), Car ($F = 3.040$, $P = 0.085$) and Car/Chl ($F = 4.570$, $P = 0.033$). For both studied species, Chl a , Chl b , and total Chl decreased, whereas Car and Car/Chl increased with increasing irradiance, although the differences in these variables between invasive and native congeners were not significant at 100% and 56.2% irradiances (except Car and Chl in natives). Similarly, the difference in Chl a/b between *A. formosana* and its native species was not significant at 100% and 56.2% irradiances, whereas Chl a/b increased in invasive with increasing irradiance (Fig. 5B). Furthermore, compared with its native congener, *A. formosana* exhibited significantly increased Chl b ($F = 135.779$, $P = 0.000$), Chl ($F = 12.436$, $P = 0.024$) and Car ($F = 27.923$, $P = 0.006$) but markedly lower Chl a/b ($F = 42.135$, $P = 0.003$) and Car/Chl ($F = 46.154$, $P = 0.002$) at 12.5% irradiance as well as slightly higher Chl a ($F = 9.076$, $P = 0.039$) and Chl ($F = 8.770$, $P = 0.041$) at 100% irradiance.

Lipid peroxidation and antioxidative enzyme activity

Both irradiance ($P < 0.01$) and species ($P < 0.05$ for H_2O_2 , $P < 0.01$ for others) had significant effects on all antioxidant variables measured in this study based on a two-way ANOVA (Fig. 5A–F). The interactions between irradiance and species were not significant for the variables ($F = 2.870$, $P = 0.096$ for MDA; $F = 1.802$, $P = 0.207$ for SOD; $F = 0.322$, $P = 0.731$ for CAT) except APX ($F = 0.368$; $P = 0.038$). With increasing irradiance, H_2O_2 , SOD, CAT, and APX markedly ($P \leq 0.01$) increased for all studied species, although the differences were not significant at 56.2% and 12.5% irradiances [except H_2O_2 ($P = 0.026$) and APX ($P = 0.007$) in *A. formosana* and CAT ($P = 0.007$) and APX ($P = 0.001$) in *A. cremastogyne*], whereas MDA in *A. formosana* revealed no self-evident variation ($F = 1.571$, $P = 0.283$; Fig. 6B). Moreover, compared with its native congener, *A. formosana* revealed significantly ($P < 0.01$) higher MDA, SOD, and APX at all studied irradiances and CAT only at 100% and 12.5% irradiances.

Discussion

Why some introduced species become invasive and others do not is the central open question of aggression biology, and general trends indicate that increased reproductive success makes a species more invasive? When performing aggression studies, it is important to include an in-depth physiological characterization of the plant species under investigation (Li et al. 2012; Fan et al. 2013). Two characteristics of functional traits are believed to support the process of invasive species becoming invasive: higher prevalence of competitively advantageous traits and greater phenotypic plasticity that permits the species to survive the colonization period and to spread within a broad range of environments (Allred et al. 2010; Hyldgaard and Brix, 2012; Pintó-Marijuan and Munné-Bosch, 2013). Thus, we discuss photosynthesis adjustment and the production of antioxidants as the two mechanisms that help plants to cope with suboptimal conditions found in various irradiance conditions.

On the one hand, plants can change their photosynthetic characteristics to acclimate to fluctuating irradiance (Peltzer and Polle, 2001; Rodríguez-López et al. 2014). As shown in Fig. 2, A_{max} , LSP, and LCP in both studied species were significantly increased at high irradiance (100% irradiance) than low irradiance. Similar results were obtained in studies of certain tropical rainforest tree species (e.g., Walters, 2005 and references therein; Favaretto et al. 2011), indicating that light-demanding species may protect their photosynthetic apparatus by using EEE in photosynthesis.

The excessive-shade treatments decreased the LCP (Fig. 2D). Plants that are acclimated to lower irradiance tend to have a lower photosynthetic capacity and LCP (Huang et al. 2011). Krause et al. (2001) and Zheng et al. (2012) demonstrate that the $A_{\max}$ was positively correlated with the net assimilation rate and N allocation to photosynthesis in the invasive. Thus, invasive species may increase N allocation to photosynthesis by reducing accumulation of N-based defensive chemicals due to evolutionary responses in the absence of natural enemies (Feng, 2008; Fan et al. 2013). In this work, the increased $A_{\max}$ of *A. formosana* compared with its native congener (Fig. 2C) may contribute to its higher RGR through increasing NAR, one of the determinants of RGR. In our previous work, we also reported positive correlations between RGR, total biomass, and $A_{\max}$ (data not shown). The increased RGR of *A. formosana* can facilitate the growth of ramets and branches, increasing crown area and total leaf area, which might provide a competitive advantage to the invasive by shading native plants (Grotkopp and Rejmánek, 2007; Feng et al. 2007).

Dark-adapted F_v/F_m reflects the potential quantum efficiency of the PSII and is used as a sensitive indicator of plant photosynthetic performance, with optimal values of approximately 0.83–0.85 measured for most plant species (Maxwell and Johnson, 2000). Any value of F_v/F_m that is below the unstressed values is considered representative of photoinhibition. A decrease in this number designates a stressful condition and a reduction in the maximum quantum efficiency of PSII, which thereby compromises the plant's photosynthetic potential (Favaretto et al. 2011). In early morning, the irradiance was relatively low, and the F_v/F_m value was high (Fig. 3A and B). At noon, the irradiance increases to a high level, inducing PSII photoinhibition. Because PSII photoinhibition results from an imbalance of photodamage and repair, the decrease in F_v/F_m (at ca. 13:00 h) suggests that the rate of PSII photodamage largely exceeds the rate of repair under strong irradiance. The lower F_v/F_m could be considered as a positive adaptation for down-regulation of the photosynthetic excitation pressure and might be stimulated by photoprotective mechanisms, including non-photochemical dissipation of Chl excitation or state transition (Campos et al. 2014 and references therein); the latter describes a reversible physiological mechanism that enables plants to optimize the photosynthetic electron transport for rapidly fluctuating light conditions (Papageorgiou and Govindjee, 2011), such as those in an undercover canopy. On a clear day, the relative humidity decreases, and the vapor pressure deficit increases at noon compared with the morning. As a result, leaves might close their stomata to reserve water potential. The decrease in

stomatal conductance induces a decline in CO₂ fixation (Farquhar and Sharkey, 1982), which could increase the generation of ROS that induce photooxidative damage to PSII (Huang et al. 2014). To avoid severe PSII photoinhibition under high irradiance, plants might reduce their absorption of light energy to decrease the rate of photodamage to PSII. Similarly to $A_{\max}$, *A. formosana* exhibited higher F_v/F_m and LSP at three irradiances but lower LCP only at 12.5% irradiance compared with its native congener (Fig. 2 and 3). One explanation for this result is that the invasive are capable of achieving photosynthesis at low irradiances. An alternative explanation is that the invasive can quickly attain canopy stature after canopy gap formation, which is potentially consistent with its LUE (Daehler, 2003; Fan et al. 2013).

NPQ can represent the energy that cannot be utilized to transport photosynthetic electrons being dissipated harmlessly as heat energy from PSII antennae. In this study, NPQ values in both species were increased at high irradiance compared with low irradiance (Fig. 3C and D). The lower NPQ in 56.2% and 12.5% irradiance plants indicates that these plants effectively reduced the irradiance heat and efficiently utilized the energy absorbed by antenna pigments (Giorio et al. 2012; Rodríguez-López et al. 2014). The higher NPQ in 100% irradiance plants indicates that the energy absorbed in the physiological range of irradiances was considerably higher than photochemical utilization, which causes inhibition of photosynthetic capacity (Huang et al. 2014). In contrast to its higher $A_{\max}$ and F_v/F_m , the invasive species exhibited lower NPQ compared with its native congeners at three irradiances, indicating that high levels of light energy that exceed photosynthetic capability are not transformed into thermal dissipation (Huang et al. 2011). Thus, *A. formosana* may protect the photosynthetic apparatus via increased $A_{\max}$. However, the opposite trends occurred in the native species; thus, part of the EEE in the PSII antennae was potentially dissipated as heat via the activation of non-radiative dissipation (Demmig-Adams, 2003).

Guard cell protoplasts contain both PSI and PSII, and stomata are formed by two guard cells. The stomata minimize water loss and optimize the CO₂ absorption to acclimate to light-induced stress (Araújo et al. 2011). Studies have demonstrated that sunlight plays a vital role in the control of stomatal behavior. In this study, A_n was highly and positively correlated with G_s ($r = 0.78^{**}$) and also highly and negatively correlated with C_i ($r = -0.76^{**}$) and L_s ($r = -0.75^{**}$). Thus, we speculate that the decrease in A_n that occurs with decreasing irradiance might be due to stomatal factors. As reported by Farquhar and Sharkey (1982), C_i and L_s can assess whether G_s is the main factor

inhibiting A_n . They hypothesized that C_i and A_n decreased (in the same direction) and that L_s increased because the decrease in A_n is the main result of the decrease in G_s ; however, this is referred to the rate of CO_2 assimilation versus the intercellular partial pressure of CO_2 response curves. By contrast, fluctuations in P_n but not in G_s are determined by the photosynthetic activity of the mesophyll cells. With decreasing irradiance, G_s , A_n , and T_r in both studied species gradually decreased, possibly resulting from an increase in $[\text{Ca}^{2+}]_{\text{cyt}}$. Changes in $[\text{Ca}^{2+}]_{\text{cyt}}$ are important for light-induced stomatal opening. Light sensing is transduced by phototropins, which are plasma membrane-associated serine/threonine photoreceptor kinases undergoing autophosphorylation and regulating $[\text{Ca}^{2+}]_{\text{cyt}}$ (O'Carrigana et al. 2014). Generally, the stomata of well-adapted plants play a relatively small role in determining the photosynthetic rate, composing less than ca. 20% of the total photosynthetic limitation (Jones, 1985). Thus, G_s was not important in explaining the interspecific difference in A_{max} , although it is significantly correlated with A_{max} ($r=0.89^{**}$). In this study, *A. formosana* had a higher G_s than its native congener, whereas its C_i was not higher. A_{max} decreased significantly with increasing C_i . The results further indicated that the ability to use intercellular CO_2 was more important than the ability to supply CO_2 (associated with G_s) for explaining the difference in A_{max} between invasive and native species, thus again confirming the importance of N allocation (Zheng et al. 2012; Huang et al. 2014).

Leaf Chl content is well established as a common reference system when physiological reactions are quantified. Light energy absorbed by Chl molecules can undergo three fates: (1) the molecules can be used to drive photosynthesis; (2) excess energy can be dissipated as heat; or (3) excess energy can be re-emitted as light-Chl fluorescence. Thus, photoprotection mechanisms could differ between leaves in the full sun and those in shade. Many studies have demonstrated that plants grown under shaded conditions optimize their effectiveness of light absorption by increasing the pigment density per unit leaf area (e.g., Dai et al. 2009; Favaretto et al. 2011; Huang et al. 2011). In this study, the shaded plants at 56.2% and 12.5% irradiances in both studied species had higher Chl levels (Chla, Chlb, and Chl) with high levels of LHC to absorb as much light as possible. In general, increased irradiance resulted in a reduction in the Chl content, especially the Chlb content (Fig. 5). Lower Chlb levels in high-light leaves facilitate the photoprotection of the reaction center of PSII by reducing the light absorption (Walters, 2005). Further, Chlb is most abundant in the antennae of the LHCII, whereas Chla is involved in determining the photosynthetic activity. Compared with its

native congener, *A. formosana* exhibited significantly higher Chl_a only at 12.5% irradiance, at least partially explaining the higher $A_{\max}$, LSP, and LCP (Fig. 2). The reductions we observed in the Chl_a/*b* ratio in *A. formosana* plants grown at 12.5% irradiance were due primarily to increases in Chl_b content (Fig. 5C) and are most likely due to changes in the organization of both light harvesting and electron transport components (Dai et al. 2009). The marked increase in leaf Chl content only at 12.5% irradiance demonstrated the invasive species' ability to maximize the light harvesting capacity in low-light growth conditions (Fig. 5E).

On the other hand, as lipid-soluble antioxidants, Car performs three major functions in plants: (1) absorbs light at a wavelength and transfer it to the Chl (an accessory light harvesting role); (2) protects the photosynthetic apparatus by quenching harmful free radicals (an antioxidant function); and (3) assembles PSI and stabilizes light harvesting complex proteins (a structural role) (Gill and Tuteja, 2010; Zheng et al. 2012; Rodríguez-López et al. 2014). Cars within the photosynthetic apparatus are known to quench ¹O₂, but the photosynthetic growth of cells lacking Car suggests the presence of other mechanisms to protect cells from ¹O₂ damage. In this study, the relative increment in Car with increasing irradiance was higher for the invasive species than the native species (at 100% irradiance) (Fig. 5D), contributing to photoprotection. Numerous studies reported that Cars have a role in thermal dissipation of EEE (Huang et al. 2011; Giorio et al. 2012; Zheng et al. 2012). The higher Car/Chl ratios found in invasive species developed under high-sunlight conditions (at 100% and 56.2% irradiance) compared with plants in the shade resulted from both a reduction in the total chlorophyll content and an increase in Car content (Demmig-Adams, 2003). Thus, the high plasticity of Cars may contribute to the invasion success of the invasive at high irradiance.

Photosynthesis is a well-established source of ROS in plants. The photosynthetic electron transport (PET) chain operates in an aerobic environment; thus, regulatory systems are required to minimize ROS production (Foyer and Shigeoka, 2011). Efficient regulation of PET serves to minimize the production of singlet oxygen at PSII as well as the generation of superoxide and H₂O₂, which occurs predominantly on the reducing side of PSI. Similar results were also documented in our work (Fig. 6); EEE promotes the TBARS accumulation (except MDA for invasive species) due to the increased LPO. H₂O₂ is a potent inhibitor of photosynthesis (Shi et al. 2006) because even at low concentrations it can inhibit CO₂ fixation by 50% due to the oxidation of the thiol-modulated enzymes of the Calvin cycle. Therefore, the balance between ROS production and ROS scavenging

is delicate and must be strictly controlled. In this study, irradiances increased SOD, CAT, and APX activities, consistent with many previous reports (Jaleel et al. 2009; Gill and Tuteja, 2010). SOD forms the first line of defense to remove $O_2^{\bullet-}$ via the metal-catalyzed Haber-Weiss-type reaction. The higher SOD level at high irradiance (100% irradiance) but lower SOD level at low irradiance (Fig. 6D) is likely due to temperature. Peltzer and Polle (2001) revealed that short-term changes in foliar antioxidant systems correlate more with temperature than light, indicating that temperature is an important factor modulating antioxidants. Meanwhile, the increase in CAT activity with increasing irradiance may be related to the photorespiratory detoxification of H_2O_2 through the mitochondrial electron transport systems (Huylensbroeck et al. 2000). By contrast, in some pioneer species, CAT activity decreases at high irradiance (Favaretto et al. 2011) because CAT is light-sensitive and suffers from photoinactivation with subsequent degradation in intact leaves. Thus, we postulated that CAT inactivation could also be mediated by photooxidative events initiated through light absorption by chlorophyll. In the ascorbate-glutathione cycle, the first step is the APX-catalyzed reduction of H_2O_2 to water, where APX serves as an electron donor (Zheng et al. 2012). An increase in APX activity was observed at high irradiances (Fig. 6E), indicating the important role of APX in removing H_2O_2 from the cell. Conversely, plants at low irradiances were less exposed to an overenergization (lowest NPQ) and in turn do not show an enhanced oxidative burst. Indeed, harvested excitation energy is mostly used in photosynthesis. However, Peltzer and Polle (2001) observed that APX activity does not fluctuate according to light exposure in field-grown beech trees.

The above results confirm the importance of antioxidative enzymes and the value of genetically enhanced antioxidants as a mechanism for stabilizing photosynthesis in stress situations. Enhanced antioxidant capacity provides added protection to photosynthesis and illustrates the point that an excess capacity does not exist for ROS destruction. Rather, such findings suggest that the capacity for ROS detoxification is balanced with regard to ROS production. This idea is perhaps surprising given the susceptibility of the photosynthetic processes to oxidative inactivation. However, if one considers that ROS generated in photosynthesis participate in a redox signaling cascade, then the importance of the regulation of both ROS abundance and the lifetime of the ROS signal becomes apparent (Foyer and Shigeoka, 2011). Acclimation to high light is known to involve increases in antioxidants and decreases in light-harvesting antenna size that are likely due to changes in gene

expression (Rodríguez-López et al. 2014). Moreover, ROS are considered to act as second messengers in the pathways responsible for high-light stress perception and long-distance signal transduction (Szechyńska-Hebda et al. 2010). Consequently, antioxidant systems have evolved in a manner that does not completely remove ROS but allows these signals to persist within the cellular environment.

As part of the redox signaling system, the concentrations of antioxidants and ROS are therefore key elements in understanding the adaptations of invasive plants to new environments and predicting the responses of native species. Recently, a few studies compared native to invasive species in relation to antioxidants and therefore sought to determine a mechanistic link between antioxidant protection and invasion success (Pintó-Marijuan and Munné-Bosch, 2013). A native (*Ulex europaeus*) and an invasive (*Acacia longifolia*) species were subjected to salt stress, and antioxidant enzymes were analyzed (Morais, et al. 2012). The results suggest that the invasive coped better with salinity in part due to higher CAT and glutathione reductase that allowed better scavenging of the ROS generated by salt stress. In this study, compared with its native congeners, *A. formosana* exhibited significantly higher SOD and APX at three irradiances and CAT only at 12.5% irradiance (Fig. 6). With better antioxidant protection, the invasive exhibit an advantage when competing with the natives. On the other hand, high-light acclimation of plants enhances photosynthetic capacity while avoiding the deleterious effects of ROS accumulation under excess light energy (Walters, 2005; Nishiyama et al. 2011). Because the photosynthetic energy utilization in invasive and native species exhibited some differences, it was assumed that higher antioxidant protection would be necessary to compensate for higher light-mediated oxidative stress (Huylensbroeck et al. 2000; Favaretto et al. 2011). However, more studies are needed to better characterize cellular redox homeostasis in other invasive species and compare these processes with that of native species to provide better insights into the mechanistic link between antioxidant protection and invasion success in particular ecosystems.

Conclusion

In conclusion, invasive *A. formosana* may occupy new habitat successfully through tolerating shading at low irradiance and out-compete native species by improving the light energy utilization via allocating a higher $A_{\max}$, LSP, and Cars to photosynthesis and detected antioxidants when

irradiance is increased; however, native *A. cremastogyne* may avoid photoinhibition mainly by dissipating EEE as heat in the form of non-radiative dissipation. Our study is the first to compare the ecophysiological traits of invasive *Alnus* species and its native congeners, and few studies have compared the plasticity in fitness-related plant traits. Our results indicated that the relationship between the invasiveness and plasticity of specific traits was complex. Plastic responses of plants depend on their intrinsic characteristics as well as the category and range of target environments. A species may exhibit higher plasticity in some traits than another species in a certain set of environments, but this plasticity may not be evident in other traits or for the same traits in a different environment (Palacio-López and Gianoli, 2011). Thus, further comparative studies on more invasive and native congeners at a wide range of irradiances are necessary to understand the generality of the patterns and fully determine the competitive advantages provided by these ecophysiological features.

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Figure captions

Fig. 1. Response of net photosynthetic rate (A_n) to incident photosynthetic photon flux density (PPFD) for leaves of invasive *A. formosana* (A) and native *A. cremastogyne* (B) exposed to 12.5% (Triangles), 56.2% (Circles) and 100% (Squares) irradiances. Data are the means of five independent experiments with SE shown by vertical bars.

Fig. 2. Apparent quantum yield (AQY, A), light saturation point (LSP, B), light-saturated photosynthetic rate (A_{max} , C) and light compensation point (LCP, D) in the seedlings of invasive *A. formosana* (black bars) and native *A. cremastogyne* (red bars) grown at 12.5%, 56.2% and 100% irradiances. Different capital and small letters indicate significant differences ($P < 0.05$) among species at the same irradiance and among irradiances for the same species, respectively. *, $P < 0.05$; **, $P < 0.01$; ns, $P > 0.05$. Data are the means of five independent experiments with SE shown by vertical bars.

Fig. 3. Maximum photochemical efficiency of PSII (F_v/F_m) and non-photochemical quenching (NPQ) in the seedlings of invasive *A. formosana* (A and C) and native *A. cremastogyne* (B and D) grown at 12.5%, 56.2% and 100% irradiances. Data are the means of six independent experiments with SE shown by vertical bars.

Fig. 4. Net photosynthetic rate (A_n , A), transpiration rate (T_r , B), stomatal conductance (G_s , C), intercellular CO₂ concentration (C_i , D), light use efficiency (LUE, E) and stomatal limitation (L_s , F) in the seedlings of invasive *A. formosana* (black bars) and native *A. cremastogyne* (red bars) grown

at 12.5%, 56.2% and 100% irradiances. Data are the means of five independent experiments with SE shown by vertical bars. Other details are the same as in Fig. 2.

Fig. 5. Chlorophyll *a* (Chl*a*, A), chlorophyll *b* (Chl*b*, B), total chlorophyll (Chl, C), chlorophyll *a*/chlorophyll *b* (Chl*a*/*b*, D), carotenoid (Car, E), and carotenoid/total chlorophyll (Car/Chl, F) in the seedlings of invasive *A. formosana* (black bars) and native *A. cremastogyne* (red bars) grown at 12.5%, 56.2% and 100% irradiances. Data are the means of five independent experiments with SE shown by vertical bars. Other details are the same as in Fig. 2.

Fig. 6. Hydrogen peroxide (H₂O₂, A), malondialdehyde (MDA, B), catalase (CAT, C), catalase (SOD, D) and ascorbate peroxidase (APX, E) in the seedlings of invasive *A. formosana* (black bars) and native *A. cremastogyne* (red bars) grown at 12.5%, 56.2% and 100% irradiances. Data are the means of 4–6 independent experiments with SE shown by vertical bars (n = 4 for H₂O₂ and MDA; n = 5 for others). Other details are the same as in Fig. 2.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6