

Effect of elevated CO₂ concentration on photosynthetic characteristics of
hyperaccumulator *Sedum alfredii* under cadmium stress

Running Title: Photosynthetic characteristics of *Sedum alfredii*

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Abstract The combined effects of elevated CO₂ and cadmium (Cd) on photosynthetic rate, chlorophyll fluorescence and Cd accumulation in hyperaccumulator *Sedum alfredii* were investigated to predict plant growth under Cd stress with rising atmospheric CO₂ concentration. Both pot and hydroponic experiments were conducted and the plants were grown under ambient (350 μL L⁻¹) or elevated (800 μL L⁻¹) CO₂. Elevated CO₂ significantly ($P<0.05$) increased Pn (105%-149%), Pn_{max} (38.8%-63.0%) and AQY (20.0-34.8%) of *S. alfredii* in all the Cd treatments, but reduced chlorophyll concentration, dark respiration and photorespiration. After 10 days growth in medium with 50 μM Cd under elevated CO₂, PSII activities were significantly enhanced ($P<0.05$) with Pm, Fv/Fm, Φ(II) and qP increased by 66.1%, 7.5%, 19.5% and 16.4%, respectively, as compared with ambient-grown plants. Total Cd uptake in shoot of *S. alfredii* grown under elevated CO₂ was increased by 44.1-48.5%, which was positively correlated with the increase in Pn. These results indicate that elevated CO₂ promoted the growth of *S. alfredii* due to increased photosynthetic carbon uptake rate and photosynthetic light-use efficiency, and showed great potential to improve the phytoextraction of Cd by *S. alfredii*.

Abbreviations

AQY, apparent quantum yield of CO₂ fixation; Fv/Fm, maximum quantum yield of PSII; Pn, net photosynthetic rate; Pn_{max}, light-saturated maximum of net photosynthetic rate; Pm, photosynthetic capacity at saturation; Φ(II), effective quantum yield of PSII.

INTRODUCTION

Heavy metal pollution of soil is one of the most important environmental problems in the world. Anthropogenic activities in modern society including mining, smelting, land application of sewage sludge, fertilization, and reclaimed water irrigation, have accelerated soil contamination by heavy metals (Terzano et al. 2007). Heavy metals at excessive levels have a strong influence on metabolic activities of plants. For instance, heavy metals can damage nucleoli, inhibit ribonuclease activity, induce oxidative stress, and affect photosynthesis (Di Toppi and Gabbriellini 1999; DalCorso et al. 2008; Nagajyoti et al. 2010).

Industrial activities have increased the atmospheric carbon dioxide concentration from about $280 \mu\text{L L}^{-1}$ at the beginning of the nineteenth century to $380 \mu\text{L L}^{-1}$ at the end of the twentieth century, and this concentration is expected to continue increasing in the future (IPCC 2007). An increase in CO_2 concentration has profound influences on plant growth. Previous studies have shown elevated CO_2 concentration enhances plant growth in terms of plant biomass (Drennan and Nobel 2000; Cousins et al. 2003), water and nutrient use efficiency (Wu et al. 2004; Jin et al. 2009), photosynthesis rate and intensity (Cousins et al. 2003; Lüttge 2004) and rhizospheric microecological environment characteristics (Ebersberger et al. 2003; Kandeler et al. 2006).

In natural ecosystem, both elevated carbon dioxide concentration in the atmosphere and heavy metal pollution in soil often co-exist. Thus the interaction of elevated CO_2 and heavy metal pollution may have an effect on plant growth, plant development and even heavy metal uptake of plant, which may influence the hazardous role of heavy metals in human health through food chain. Consequently, the impacts of elevated CO_2 and heavy metal contamination on plants are receiving more and more attentions (Yu et al. 2006; Li et al. 2013). However, minimal information is available in literature concerning the combined effects of elevated CO_2 and heavy metal contamination on hyperaccumulator physiochemical processes (Tuba and Lichtenthaler 2007; Jia et al. 2011). To most plants, increased photosynthesis is the fundamental response to elevated CO_2 , which affects all other aspects, such as growth stimulation (Long et al. 2004; Ainsworth and Rogers 2007). Analyses on parameters of gas exchange and chlorophyll fluorescence provide insights into photosynthesis under elevated CO_2 and representatives of plant states under heavy metal stresses, since photosynthetic reactions belong to the

most important sites of inhibition by heavy metals and photosystem II (PSII) in the thylakoids has frequently been identified as the main target of heavy metal stresses (Küpper et al. 2007). Currently, a few studies have reported the effect of elevated CO₂ on plant uptake of essential micronutrients, such as Cu, Fe, Mn, and Zn (Jia et al. 2007; Jia et al. 2010). However, little is known about possible effects of elevated CO₂ on plant uptake of non-essential elements such as Cd, especially in terms of the relationship with chlorophyll fluorescence parameters and photosynthetic rate of plant under elevated CO₂.

Hyperaccumulators are used for phytoremediation of heavy metal-contaminated soil and have attracted attention in recent years for low cost application of implementation and environmental benefits (McGrath and Zhao 2003; Yang et al. 2005). However, their practical application is limited, because many of hyperaccumulator species are slow-growing and produce limited shoot biomass. *Sedum alfredii* Hance, a Crassulaceae species, originally grown in a Pb/Zn mined area of south China, is the first non-Brassicaceae Zn/Cd hyperaccumulator identified so far (Yang et al. 2004). It is a good candidate for phytoremediation practices of metal-contaminated soil, with the characteristics of rapid growth, asexual propagation, and perennial growth (Yang et al. 2004; Liu et al. 2008; Li et al. 2013). Previous research has indicated that the development of lateral roots and subapical root hairs of *S. alfredii* are stimulated by elevated CO₂ (800 µL L⁻¹); and both plant growth and Cd accumulation are improved even under Cd-stressed conditions (Li et al. 2012). This result does suggest that elevated CO₂ may effectively improve plant growth and its phytoextraction efficiency. However, the effect of elevated CO₂ on plant physiological and physiochemical processes has rarely been examined in hyperaccumulators under heavy-metal stress (Pongrac et al. 2009; Jia et al. 2010; Jia et al. 2011). Therefore, the aim of this study was to assess the combined effects of elevated CO₂ and Cd on photosynthetic rate, chlorophyll concentration and chlorophyll fluorescence parameters in *S. alfredii* and their relationships with Cd accumulation. Results from this study should help to predict plant growth under Cd stress in the future with increase of atmospheric CO₂ concentration, and provide scientific evidences for application of CO₂-triggered phytoextraction in environmental remediation.

RESULTS

Biomass and Cd accumulation in shoot

Plant growth was significantly promoted by elevated CO₂ as compared to ambient CO₂ (Table 1). Shoot fresh weights for all Cd treatments (CK, Cd5 and Cd50) at elevated CO₂ increased by 55.0%, 49.9% and 54.9%, respectively, as compared to those grown in ambient CO₂, and the corresponding values for dry weight were 36.7%, 26.8% and 24.2%, respectively. In the control treatment, no differences of Cd concentrations in the shoots of *S. alfredii* were observed between both CO₂ levels. In Cd5 and Cd50 treatment, total Cd uptake in shoots at elevated CO₂ was significantly larger ($P<0.05$) than that at ambient CO₂, indicating that elevated CO₂ treatment significantly improved Cd accumulation in the plants grown in Cd polluted soil. In addition, total Cd accumulation in shoots showed higher enhancement ratios (48.5% for Cd5 and 44.1% for Cd50) than Cd concentration (17.3% for Cd5 and 16.0% for Cd50).

Gas exchange

Net photosynthetic rates (Pn) of *S. alfredii* for all Cd treatments were increased by elevated CO₂ concentration as compared to the ambient CO₂ (Figure 1A). At the growth irradiance of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Pn was increased by 149%, 137% and 105%, respectively, indicating that the enhancement was greater in slight contamination than those in middle and severe contamination and Cd-stressed plants had significant reductions in Pn. In contrast, transpiration rate was significantly reduced by elevated CO₂ ($P<0.05$) (Figure 1B). Plants grown at elevated CO₂ had a significant increases in the maximum net photosynthetic rate (Pn_{max}) (38.8%–63.0%), the apparent quantum yield of CO₂ fixation (AQY) (20.0%–34.8%) and the light saturation point (Lsp) (10.1%–17.8%) for each Cd treatment, while there were reductions in dark respiration rate (Rd) (17.4%–67.3%) and light compensation point (Lcp) (38.0%–75.8%) at elevated CO₂ (Table 2).

Photosynthetic pigments in leaves

S. alfredii grown at elevated CO₂ had significant reductions ($P<0.05$) in chlorophyll a and chlorophyll b compared with plant grown at ambient CO₂ (Table 2). However, the ratio of chlorophyll a to chlorophyll b was

not affected by elevated CO₂. On the other hand, cadmium treatment resulted in an enhancement of chlorophyll concentrations in *S. alfredii* under both CO₂ levels. Cd had no significant effects on chlorophyll a/b ratio regardless of CO₂ level.

Chlorophyll fluorescence

After 10 days of growth in a medium containing 50 µM Cd, the chlorophyll fluorescence parameters of *S. alfredii* were measured (Figure 3). The maximum quantum yield of PSII (Fv/Fm), the effective quantum yield of PSII (Φ(II)) and the photochemical quenching (qP) were increased by 7.5%, 19.5% and 16.4%, respectively, as compared to ambient-grown plants. However, the minimum fluorescence yield (Fo) and the non-photochemical quenching (NPQ) were significantly ($P < 0.05$) decreased. Fm was not different between the two CO₂ levels (data not shown), so a marked increase in Fv/Fm, calculated from (Fm-Fo)/Fm, at elevated CO₂ was probably due to a significant increase in Fo ($P < 0.05$). From the RLCs, Pm at saturation radiation and ETR at higher radiation (above 80 photon µmol m⁻² s⁻¹) were significantly increased ($P < 0.05$) by elevated CO₂ in *S. alfredii* under 50 µM Cd stress (Figure 4)

DISCUSSION

Elevated CO₂ enhances photosynthetic carbon uptake in *S. alfredii* under Cd stress

In this study, the effects of elevated CO₂ on photosynthesis of *S. alfredii* were similar at all Cd levels (Figure 1 and Table 2), and Cd stress did not inhibit the growth of *S. alfredii* or significantly changed photosynthetic responses of *S. alfredii* to elevated CO₂. Similar to most CAM plants and C3 plants, net photosynthetic rates (Pn) of *S. alfredii*, i.e. net CO₂ uptake rates, significantly increased ($P < 0.05$) under elevated CO₂ levels (Figure 1), with much greater increment than other plants (Drennan and Nobel 2000; Springer and Thomas 2007). This result was associated with more CO₂ for carboxylation reaction and in accordance with higher apparent quantum yield of CO₂ fixation (AQY), i.e. maximum photosynthetic light-use efficiency, calculated from the Pn/PAR curve in *S. alfredii* (Figure 2), which is also mentioned by Drake et al. (1997). Moreover, enhanced Pn_{max} under light-saturation conditions was also found at elevated CO₂ levels, which is a common phenomenon in other plants (Long et al. 2004), this result might also be associated with

raised light saturation point (Lsp) (Table 2), including more CO₂ substrate and larger AQY. Increased net CO₂ uptake rates under both light-limiting and light-saturation conditions contributed to more carbon fixation of *S. alfredii* at elevated CO₂ and led to the plant growth enhancement. Reductions in energy costs of both dark respiration (Rd) and photorespiration (Lcp) (Table 2) result in less carbon loss and also contributed to the growth enhancement of *S. alfredii* at elevated CO₂.

On the contrary, chlorophyll (a and b) concentrations were found lower at elevated CO₂ under all Cd stress conditions (Table 2), consistent with other CAM plants under normal conditions (Croonenborghs et al. 2009). Lower chlorophyll concentrations may not inhibit the photosynthesis promotion at elevated CO₂ attributed to a dilution effect from increased fresh weight. Furthermore, it means a decrease in chlorophyll density and indicates a likely reduced need for chlorophyll molecules and an increased photosynthetic efficiency of chloroplast at elevated CO₂, which may lead to higher rate of photosynthetic carbon uptake. It seems that photosynthetic and growth promotion of *S. alfredii* by elevated CO₂ under Cd stress conditions has little relationship to chlorophyll concentrations.

In non-accumulator species, photosynthesis is the most sensitive process negatively affected by heavy metals, and Pn is significantly inhibited by Cd stress in other studies (Pietrini et al. 2003; Rosso et al. 2005; Solti et al. 2008). In this study, however, photosynthesis and growth of *S. alfredii* were not significantly influenced by Cd stress (Figure 1 and Table 2), indicating that Cd/Zn hyperaccumulator *S. alfredii* has a strong tolerance to Cd stress. Moreover, the positive effect of elevated CO₂ on the photosynthetic carbon uptake of *S. alfredii* was comparatively more crucial than the effect of Cd stress and the former should help the plant to resist Cd stress. In addition, 5mg kg⁻¹ Cd treatments increased chlorophyll concentrations at both CO₂ levels (Table 1), this result is consistent with Zhou and Qiu (2005), who also found an increase of chlorophyll content in leaves of *S. alfredii* under Cd treatment, suggesting that Cd could act as a promoter to produce more chlorophyll in leaves of *S. alfredii*. However the related molecular mechanisms need to be further studied.

Elevated CO₂ influences electron transport and PSII activity in *S. alfredii* under Cd stress

PSII has frequently been identified as the main target of heavy metal stresses (Küpper et al. 2007).

Chlorophyll fluorescence analysis has become one of the most powerful technique used to estimate the photochemical activities of PSII in leaves and the operating quantum efficiency of electron transport through PSII (Baker and Rosenqvist 2004), which can provide insight of intra-cellular photosynthetic responses to abiotic stress (Seaton and Walker 1990; Redondo-Gómez et al. 2010). Elevated CO₂ increased the quantum yield of PSII electron transport ($\Phi(II)$) of *S. alfredii* under Cd stress and thus stimulated the actual photosynthetic process, which was accompanied by an increase in the consumption of NADPH and ATP (larger qP) and by a reduction in non-radiative energy loss (smaller NPQ) (Figure 3). These results were similar to an epiphytic CAM orchid exposed to super-elevated CO₂ (Gouk et al. 1999). Moreover, elevated CO₂ enhanced relative electron transport rate (rETR) of *S. alfredii* under Cd stress at a wide irradiancy range (100 ~ 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Figure 4), suggesting that elevated CO₂ not only improved actual photosynthetic activities under high-irradiancy condition, taking the photosynthetic capacity at saturation (Pm) (Figure 4) as an example, but also improved actual photosynthetic activities under low-irradiancy condition. In view of larger $\Phi(II)$ and larger rETR, it can be concluded that elevated CO₂ can drive resource reallocation from carboxylation to electron transport, similar to other studies (Bernacchi et al. 2005; Ainsworth and Rogers 2007) and improved electron transport activities helped to stimulate photosynthetic CO₂ uptake by *S. alfredii* and benefit plant growth, especially under Cd stress.

The enhancement of maximum quantum yield of PSII (Fv/Fm) (Figure 3) has a positive correlation with the enhancement of quantum yield of net photosynthesis (AQY) under Cd stress (Table 2), which has also been reported by Bolhar-Nordenkamp et al. (1989) and indicates a relationship between chlorophyll fluorescence and gas exchanges as two ways to measure photosynthesis. Differing from other plants whose Fv/Fm decreased by Cd stress (Rosso et al. 2005; Redondo-Gómez et al. 2010), *S. alfredii* had an increase in Fv/Fm after CO₂ concentration rose, suggesting that there was an increase in light-harvesting complexes relative to PSII reaction centers (Gutiérrez et al. 2009) and intra-cellular photosynthetic activities were improved again by elevated CO₂, facilitating *S. alfredii* to keep normal physiological functions under Cd stress at elevated CO₂. Therefore, elevated CO₂ might alleviate Cd toxicity to *S. alfredii* and assist the plant to thrive in Cd-polluted soil.

Elevated CO₂ promote plant growth and Cd accumulation of *S. alfredii* through photosynthesis improvement

Elevated atmospheric CO₂ concentrations showed a positive effect on the shoot biomass of *S. alfredii* even under Cd-stressed conditions (Table 3), in agreement with the results reported by Drennan and Nobel (2000) and other hyperaccumulators grown in heavy metal-contaminated soils (Tang et al. 2003; Wu et al. 2009), showing the possibility of using CO₂ as fertilizer to promote growth of *S. alfredii* in phytoremediation practice. On the other hand, elevated CO₂ increased Cd concentration in the shoot of *S. alfredii*, especially under the condition with severe Cd contamination (Table 3) and this result was consistent with the positive effect of elevated CO₂ on Cd concentration in sunflower and Indian mustard (Tang et al. 2003) and Cs concentration in *Sorghum* species and *Trifolium* species (Wu et al. 2009). Elevated CO₂ also significantly increased ($P<0.05$) total Cd uptake in shoot of *S. alfredii* (Table 3), in agreement with other previous studies (Tang et al. 2003; Wu et al. 2009; Jia et al. 2010).

Previous studies have found that elevated CO₂ under Cd-stress conditions enhances fine root growth, root hair development, and mineral nutrient uptake, and thus enables *S. alfredii* to access and accumulate more Cd (Li et al. 2012). In this study, we found that the magnitude of increment in total Cd accumulation in shoot was much greater than the Cd concentration (Table 3), indicating that greater Cd accumulation in *S. alfredii* at elevated CO₂ was mainly due to the growth promotion by elevated CO₂ rather than enhanced Cd concentration. Plant growth promotion directly results from increased photosynthetic rate at elevated CO₂ (Drennan and Nobel 2000). In this study, a positive correlation between photosynthetic rate and Cd accumulation in shoot of *S. alfredii* was observed (Table 3 and Figure 1), thus the increased Cd accumulation in *S. alfredii* at elevated CO₂ could be mainly attributed to photosynthetic improvement. On the contrary, the reduced transpiration rate at elevated CO₂ (Figure 1) indicated that the positive effect of elevated CO₂ on Cd accumulation in *S. alfredii* was likely unrelated to the transpiration-driving mechanism (Marchiol et al. 2004). Study on *A. halleri* has shown that it can reinforce its photosynthetic mechanism by over expressing proteins involved in photosynthesis in order to afford the high energetic cost of hyperaccumulating Cd and Zn (Farinati et al. 2009). So there may be

some molecular relationship between intra-cellular photosynthesis and Cd accumulation in *S. alfredii*, which needs to be further studied.

MATERIALS AND METHODS

Plant material and soil characterization

The hyperaccumulating ecotype of *S. alfredii* was collected from an old Pb/Zn mining area in Quzhou City (29°17'N, 118°56'E), Zhejiang province, China. Seedlings of *S. alfredii* were cultivated according to Yang et al. (2004).

The soil used in the pot experiment was collected from the surface layer (0–20 cm) of a long-term experimental station in the farm of Zhejiang University, Hangzhou, Zhejiang Province, PR China. Soil samples were air-dried, ground and sieve to <4-mm, and stored in plastic bags until use. The chemical and physical properties are: pH (1:2.5 soil/water ratio), 5.96; organic matter, 12.8 g kg⁻¹; particle size distribution – clay (≤0.002 mm), 16%, silt (0.002–0.02 mm), 33% and sand (0.02–2 mm), 51%; total N, 1.05 g kg⁻¹; available P, 13.5 mg kg⁻¹; available K, 96.3 mg kg⁻¹; available Cd, 0.12 mg kg⁻¹.

Pot experiment

Soil samples of 2.5 kg each mixed with a known amount of Cd [Cd (NO₃)₂·4H₂O solution] were placed in plastic pots (15 cm in diameter and 15 cm in height). Three treatments were applied: CK (the control, no Cd spiked to soil), Cd5 and Cd50 (5 mg kg⁻¹ and 50 mg kg⁻¹, respectively). The three levels represented slight, medium and severe contamination, respectively. Each treatment had 4 replicates. The soil was left to equilibrate outdoors under a waterproof tarpaulin for about two months after being moistened to 70% field water holding capacity. This period is long enough to allow natural equilibration of the various sorption mechanisms in the soil. After pre-cultured for 2 weeks in hydroponic solution, three *S. alfredii* plants were transplanted into each pot. All pots were transferred to growth chambers (Conviron[®] E7/2, Canada) with average day/night temperature of 26 /20 °C, and day/night humidity of 70/85%. The average light intensity was maintained at 100 μmol m⁻² s⁻¹ during a 14-h light cycle. CO₂ treatments were similarly initiated by growing the above plants in the chambers with the CO₂ concentration of either 350 (ambient CO₂) or 800 (elevated CO₂) μL L⁻¹. To

minimize the effect of non-uniform illumination on plant growth, the position of these pots were adjusted periodically. Total CO₂ treatment time was 60 days.

Gas exchange and photosynthetic pigments measurement

The net photosynthetic rate (P_n), the intercellular CO₂ concentration (C_i) and the transpiration rate were measured with a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE) at the end of the pot experiment in the daytime. External air was scrubbed of CO₂ and mixed with a supply of pure CO₂ to result in concentrations of 350 μL L⁻¹ (for the plants with the ambient treatment) and 800 μL L⁻¹ (for the plants with the elevated CO₂ treatment). Light was photosynthetically active radiation (PAR) of 100 μmol m⁻² s⁻¹ provided by the light source, temperature was 20 °C and relative humidity was 75% (the same conditions as one of growth chambers).

Determination of photosynthetic light response curves (P_n/PAR curves): Response of P_n to photosynthetically active radiation (PAR) was measured with the LI-6400 by illuminating the leaf at decreasing PAR (from 2000 μmol m⁻² s⁻¹ to 0 μmol m⁻² s⁻¹) within 30 min. CO₂ was maintained at two constant levels of 350 μL L⁻¹ (for the plants with the ambient treatment) and 800 μL L⁻¹ (for the plants with the elevated CO₂ treatment) with a high pressure liquefied CO₂ cartridge source. Data were mathematically fitted to a nonrectangular hyperbola equation (Prioul and Chartier 1977) :

$$P_n = \frac{AQY \times PAR + P_{n_{\max}} - \sqrt{(AQY \times PAR + P_{n_{\max}})^2 - 4 \times AQY \times PAR \times K \times P_{n_{\max}}}}{2 \times K} - R_d \quad (1)$$

where AQY is the apparent quantum yield of CO₂ fixation (mol mol⁻¹), P_{n_{max}} is the maximal net photosynthetic rate at light saturation (μmol CO₂ m⁻² s⁻¹), R_d is the dark respiration rate (μmol m⁻² s⁻¹) and K is the convexity of the fitting curve. On the P_n/PAR fitting curve, the light compensation point (Lcp) were estimated from axis intercepts and the light saturation point (Lsp) was calculated by extrapolating the linear function described by AQY and R_d, to its intersection with P_{n_{max}} (Walker, 1989).

Leaves of four to six storeys from the top were sampled for measure of chlorophyll pigments at the end of the experiment. The calculation method of chlorophyll concentration followed Jia et al. (2010).

Chlorophyll fluorescence measurement

After being precultured for 1 week in hydroponic solution, plants were transferred to complete nutrient solution with 50 μM Cd ($\text{Cd}(\text{NO}_3)_2$). The plants were subjected to ambient and elevated CO_2 treatments for 10 days.

To examine effects of elevated CO_2 on PSII electron transport, dark-adapted minimum fluorescence (F_o), dark-adapted maximum fluorescence (F_m) and maximum quantum yield of PSII (F_v/F_m) after 0.5 h-dark adaptation, and effective quantum yield of PSII ($\Phi(\text{II})$), photochemical quenching (qP) and non-photochemical quenching (NPQ) after 10 min- $186 \mu\text{mol m}^{-2} \text{s}^{-1}$ actinic radiation (state was steady) were monitored in leaves *in vivo* by analysis of the chlorophyll a fluorescence with an IMAGING-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany). All the leaves were measured with the same ambient CO_2 concentration. Colour-coded images of fluorescence parameters can be scaled on demand using a rainbow lookup table (Fig. 3), which allows a more detailed visual depiction of differences, gradients and heterogeneities in $\Phi(\text{II})$, qP and NPQ compared with the colour-code depiction options of the manufacturer. Some of the fluorescence parameters were displayed clearer in the images with a false color code ranging from 0 (black) to 1 (purple).

Rapid light curves (RLCs): Response of relative electron transport rate ($r\text{ETR}$) to PAR ($r\text{ETR}/\text{PAR}$ curve) was measured after 10 min- $186 \mu\text{mol m}^{-2} \text{s}^{-1}$ actinic radiation (state was steady) with the IMAGING-PAM by increasing PAR (from $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $1075 \mu\text{mol m}^{-2} \text{s}^{-1}$) within 150s in the identical leaves measured by parameters above. Data were mathematically fitted to a double exponential decay function (Platt et al., 1980), using a Marquardt–Levenberg regression algorithm:

$$r\text{ETR} = P_m (1 - e^{(-a\text{PAR}/P_m)})e^{(-b\text{PAR}/P_m)} \quad (2)$$

where P_m is the photosynthetic capacity at saturating light, a is the initial slope of the RLC before the onset of saturation and b is the slope of the RLC where PSII declines (Henley, 1993).

Plant harvest and Cd analysis

At the end of experiment, the shoots were harvested, washed thoroughly with tap water, rinsed with distilled water, and dried in an oven for 3 days at 65°C . The oven dried shoot samples were weighed, and ground to a

60-mesh fineness and digested with a mixture of concentrated HNO₃ (16 M) and HClO₄ (12 M) (5:1 v/v) in PTFE vessels. The digest was transferred to a 50 ml volumetric flask, filled to 50 mL and filtered. Cd concentration in the digest was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, iCAP 6000, Thermo Scientific).

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Table 1. Effect of elevated CO₂ treatment on biomass, Cd concentration and total Cd accumulation in shoots of *S. alfredii* grown in Cd contaminated soil

Soil	CO ₂	Fresh weight	Dry weight	Cd concentration (mg kg ⁻¹ DW)	Total Cd accumulation (μg shoot ⁻¹)
		(g shoot ⁻¹)			
CK	Ambient	19.35 ± 1.61	1.66 ± 0.05	49 ± 6	81 ± 12
	Elevated CO ₂	29.99 ± 2.60*	2.27 ± 0.10*	50 ± 7	111 ± 20
Cd5	Ambient	21.61 ± 1.25	1.57 ± 0.14	307 ± 40	482 ± 39
	Elevated CO ₂	28.29 ± 0.60*	1.99 ± 0.15*	360 ± 47*	716 ± 78*
Cd50	Ambient	18.72 ± 0.94	1.65 ± 0.08	2399 ± 201	3958 ± 424
	Elevated CO ₂	28.98 ± 1.41*	2.05 ± 0.19*	2783 ± 61*	5705 ± 935*

The plants were grown under ambient (350 μL L⁻¹) or elevated (800 μL L⁻¹) CO₂. Data are means ± SE (n=8).

*, Significant differences ($P < 0.05$) between ambient and elevated CO₂ treatment.

Table 2. Effect of elevated CO₂ treatment on the concentrations of chlorophyll a and b and a/b in fresh leaves of *S. alfredii* grown in Cd contaminated soil, and on apparent quantum yield of CO₂ fixation (AQY), the light-saturated maximum (Pn_{max}), dark respiration rate (Rd), light saturation point (Lsp) and light compensation point (Lcp) estimated from the Pn-PAR fitting curves of intact leaves of *S. alfredii*.

Soil	CO ₂	Chlorophyll a	Chlorophyll b	a/b ratio	AQY	Pn _{max}	Rd	Lsp	Lcp	R ²
		(mg g ⁻¹ FW)			(mol mol ⁻¹)	(μmol m ⁻² s ⁻¹)				
CK	Ambient	0.441 ± 0.008b	0.177 ± 0.003bc	2.49 ± 0.09ab	0.040 ± 0.010	6.49 ± 0.39	0.66 ± 0.26	179 ± 11	16.50 ± 2.27	0.983
	Elevated CO ₂	0.369 ± 0.015c	0.142 ± 0.006d	2.59 ± 0.13a	0.048 ± 0.008	9.01 ± 0.40	0.45 ± 0.28	197 ± 17	9.38 ± 2.24*	0.989
Cd5	Ambient	0.527 ± 0.012a	0.214 ± 0.005a	2.47 ± 0.07ab	0.036 ± 0.013	8.04 ± 0.64	0.46 ± 0.29	236 ± 29	12.78 ± 1.26	0.99
	Elevated CO ₂	0.420 ± 0.024b	0.177 ± 0.011bc	2.37 ± 0.14b	0.048 ± 0.009	12.96 ± 0.73	0.38 ± 0.43	278 ± 16*	7.92 ± 1.33*	0.981
Cd50	Ambient	0.496 ± 0.062a	0.195 ± 0.028ab	2.54 ± 0.08a	0.046 ± 0.022	7.10 ± 0.61	0.49 ± 0.35	165 ± 5	10.65 ± 1.08	0.986
	Elevated CO ₂	0.420 ± 0.014b	0.164 ± 0.008cd	2.56 ± 0.03a	0.062 ± 0.014	11.57 ± 0.72	0.16 ± 0.47	189 ± 9	2.58 ± 0.97*	0.982

The plants were grown under ambient (350 μL L⁻¹) or elevated (800 μL L⁻¹) CO₂. Values followed by the same letter within the same column are not significantly different at $P < 0.05$ as determined by LSD test. Values of AQY, Pn_{max} and Rd are values ± S.E. from the regression analysis, and Values of Lsp and Lcp are means ± SE ($n = 8$). *, Significant differences ($P < 0.05$) between ambient and elevated CO₂ treatments.

Figure legends

Figure 1. Effect of elevated CO₂ treatment on (A) net photosynthetic rate (Pn) and (B) transpiration rate of intact leaves of *S. alfredii* grown in Cd contaminated soil

(PAR: 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The plants were grown under ambient (350 $\mu\text{L L}^{-1}$) or elevated (800 $\mu\text{L L}^{-1}$) CO₂. Data are means \pm SE (n=8). *, Significant differences ($P<0.05$) between ambient and elevated CO₂ treatment.

Figure 2. Effect of elevated CO₂ treatment on Pn/PAR curves of *S. alfredii* grown in Cd contaminated soil

The plants were grown under ambient (350 $\mu\text{L L}^{-1}$) or elevated (800 $\mu\text{L L}^{-1}$) CO₂. Data are means from 8 individual replicates of intact leaves of *S. alfredii* and are fitted to Function (1).

Figure 3. Effect of elevated CO₂ treatment on the maximum quantum yield of PSII (Fv/Fm), dark-adapted minimum fluorescence (Fo), effective quantum yield of PSII ($\Phi(\text{II})$), photochemical quenching (qP) and non-photochemical quenching (NPQ) of intact leaves of *S. alfredii* in 50 μM Cd medium

The plants were grown under ambient (350 $\mu\text{L L}^{-1}$) or elevated (800 $\mu\text{L L}^{-1}$) CO₂. Data are means \pm SE (n=8). *, Significant differences ($P<0.05$) between ambient and elevated CO₂ treatment.

Figure 4. Effect of elevated CO₂ treatment on rapid light curves (rETR/PAR curves) of *S. alfredii* in 50 μM Cd medium

The plants were grown under ambient ($350 \mu\text{L L}^{-1}$) or elevated ($800 \mu\text{L L}^{-1}$) CO_2 .

Data are fitted to Function (2). rETR, relative electron transport rate; P_m , photosynthetic capacity at saturating light; a , the initial slope of the RLC before the onset of saturation; b , the slope of the RLC where PSII declines. Bars represent standard errors (SE) of 8 individual replicates of intact leaves.

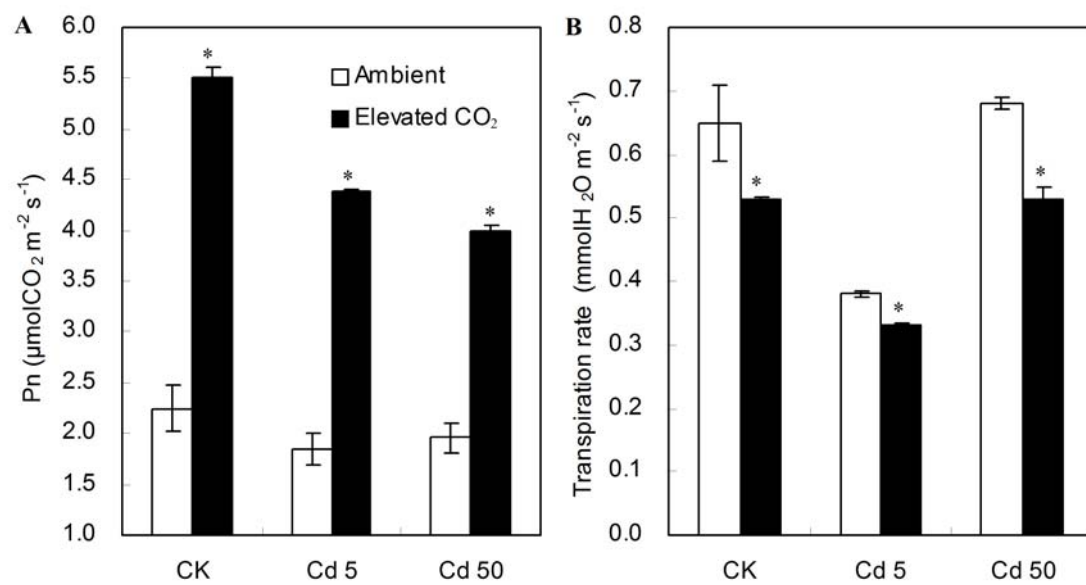


Figure 1

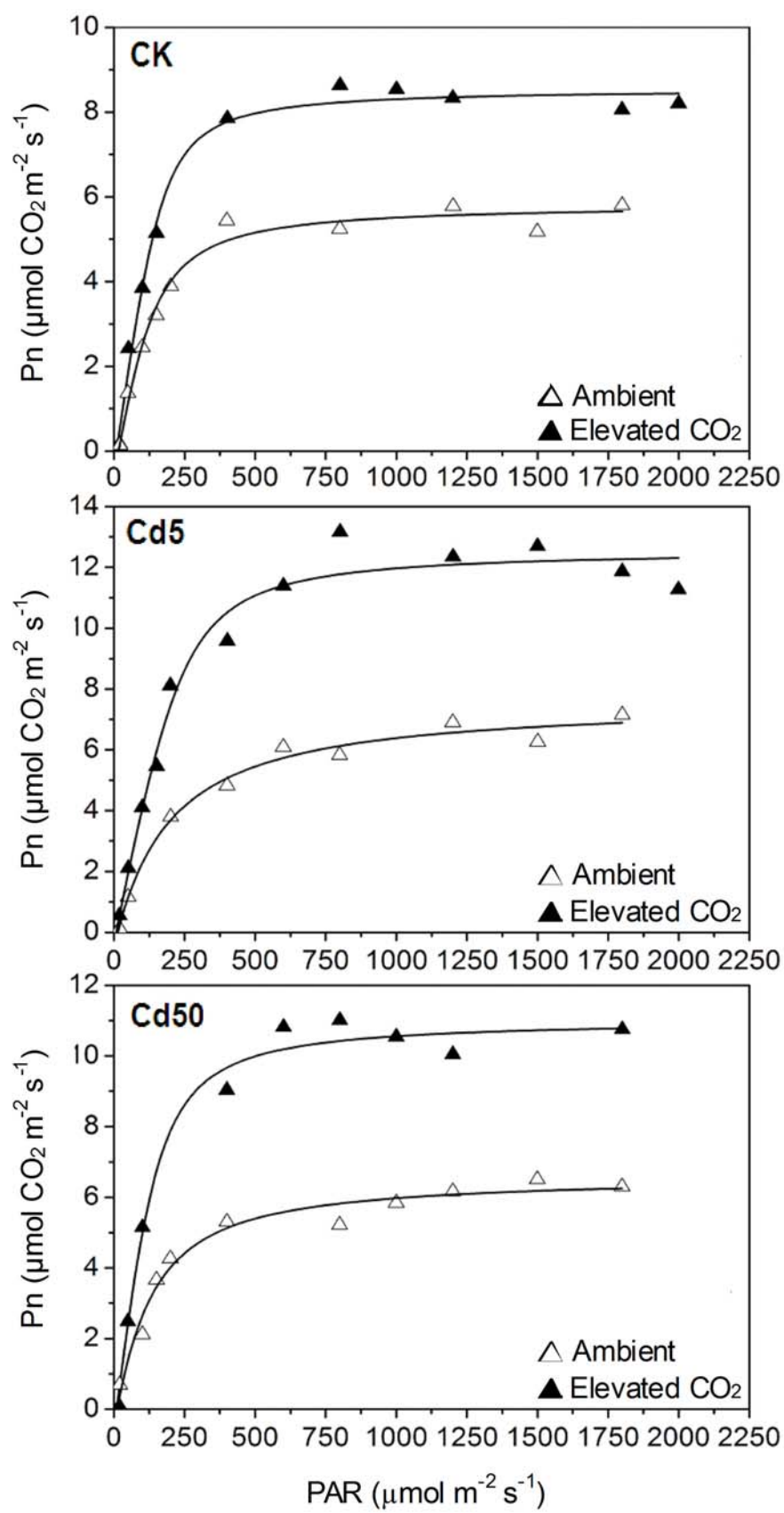


Figure 2

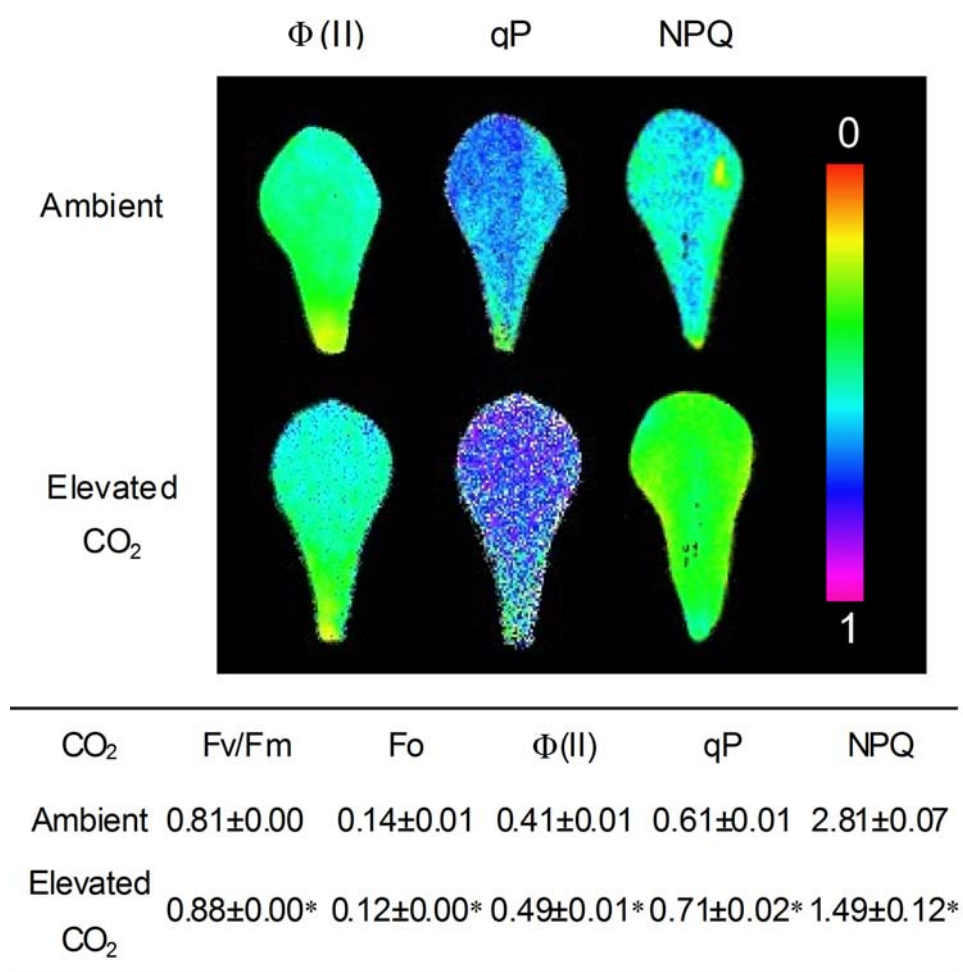
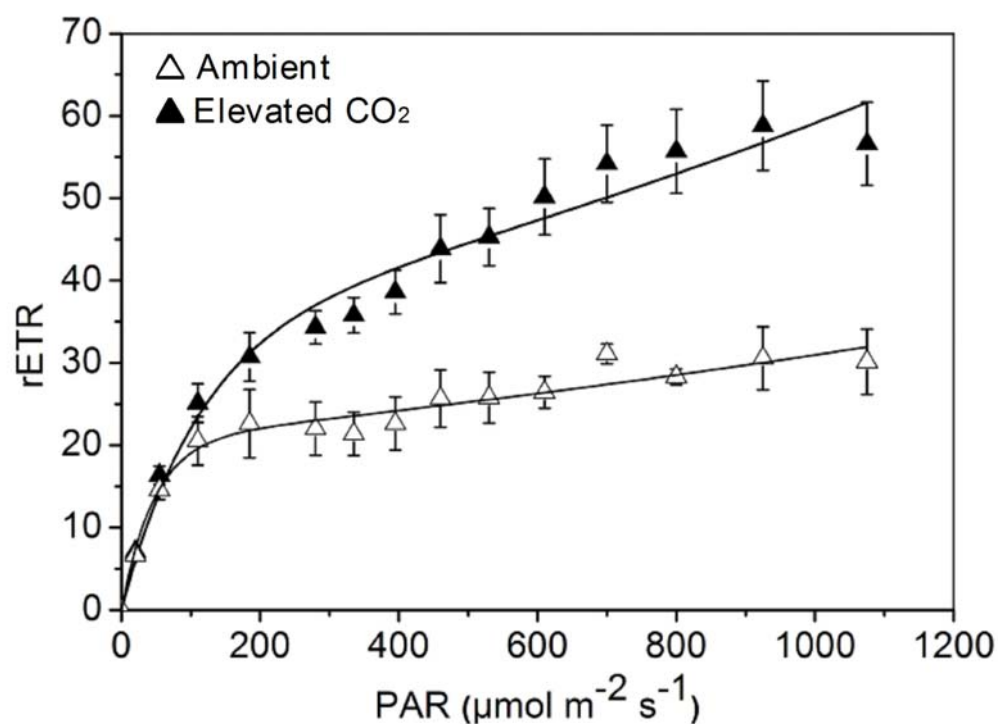


Figure 3



CO ₂	Pm	a	b	R ²
Ambient	20.56 ± 0.93	0.45 ± 0.07	-0.01 ± 0.00	0.97
Elevated CO ₂	34.16 ± 2.50*	0.32 ± 0.05*	-0.02 ± 0.00*	0.98

Figure 4