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REGULAR ARTICLE

Elevated CO_2 improves root growth and cadmium accumulation in the hyperaccumulator *Sedum alfredii*

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Abstract
Aims This study examined the effect of elevated CO_2 on plant growth, root morphology and Cd accumulation in *S. alfredii*, and assessed the possibility of using elevated CO_2 as fertilizer to enhance phytoremediation efficiency of Cd-contaminated soil by *S. alfredii*.
Methods Both soil pot culture and hydroponic experiments were carried out to characterize plant biomass, root morphological parameters, and cadmium uptake in *S. alfredii* grown under ambient (350 μL L^{-1}) or elevated (800 μL L^{-1}) CO_2.
Results Elevated CO_2 prompted the growth of *S. alfredii*, shoot and root biomass were increased by 24.6–36.7% and 35.0–52.1%, respectively, as compared with plants grown in ambient CO_2. After 10 days growth in medium containing 50 μM Cd under elevated CO_2, the development of lateral roots and root hairs were stimulated, additionally, root length, surface area, root volume and tip number were increased significantly, especially for the finest diameter roots. The total Cd uptake per pot was significantly greater under elevated CO_2 than under ambient CO_2. After 60 d growth, Cd phytoextraction efficiency was increased significantly in the elevated CO_2 treatment.
Conclusions Results suggested that the use of elevated CO_2 may be a useful way to improve phytoremediation efficiency of Cd-contaminated soil by *S. alfredii*.

Keywords Cd uptake · Elevated CO_2 · Phytoextraction · Root morphology · *S. alfredii*

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Introduction

Phytoremediation of heavy metal-contaminated soil is an emerging technology (McGrath and Zhao 2003; Krämer 2005). It has attracted attention in recent years due to its low cost and environmental benefits. Phytoextraction technology makes use of pollutant-accumulating plants to extract pollutants from the soil and accumulate them in the harvestable above-ground biomass (Kumar et al. 1995; Chaney et al. 1997; McGrath and Zhao 2003; Robinson et al. 2009). Although hundreds of hyperaccumulators have been identified and many studies have been carried out to investigate the mechanisms of uptake, transport, and detoxification of heavy metal pollutants (Krämer 2005; Zhao and McGrath 2009; Barcelo and Poschenrieder 2011; Rascio and Navari-Izzo 2011), the practical application of hyperaccumulators to remediation of contaminated soils at a field scale is limited. This may be attributed to the slow-growing and limited shoot

biomass of most accumulator plants. To increase the feasibility of this technology, it is necessary to improve remediation efficiency by increasing the accumulation of heavy metals in the plants through external intervention (Zhao and McGrath 2009; Rascio and Navari-Izzo 2011). Addition of chelating reagents has been reported to enhance phytoextraction by mobilizing metals and increasing metal accumulation; however, side effects exist on soil microbial community or groundwater (Wenzel et al. 2003; Wu et al. 2004; Liu et al. 2008; Sun et al. 2009). Transferring functional genes of hyperaccumulation to crop plants with higher shoot biomass has been suggested to enhance phytoremediation (Pence et al. 2000; Banuelos et al. 2007), but progress in this area has been hindered by lack of understanding the basic molecular, biochemical, and physiological mechanisms involved in heavy metal hyperaccumulation and the probable involvement of several genes in the process.

Human activities have increased the concentration of atmospheric CO_2 from about 280 μL L^{-1} at the beginning of the nineteenth century to 367 μL L^{-1} at the end of the twentieth century, and CO_2 concentration is expected to continue to rise through the end of the current century (IPCC 2007). An increase in CO_2 concentration is expected to have a profound impact on plant growth. Previous studies have shown that elevated CO_2 concentration enhances plant growth (Deepak and Agrawal 1999; Kimball et al. 2002), water and nutrient use efficiency (Kimball et al. 1995; Hungate et al. 1997; Marinari et al. 2007; Jin et al. 2009), photosynthesis (Cousins et al. 2003; Luttge 2004), and microecological characteristics of rhizosphere (Ebersberger et al. 2003). Elevated CO_2 does enhances the growth of Indian mustard (*Brassica juncea* L. Czern.) and sunflower (*Helianthus annuus* L.) and increases copper accumulation in both plants (Tang et al. 2003). Recent studies have revealed that elevated CO_2 not only increases aboveground biomass of the *Sorghum* and *Trifolium* species, but also increases Cs hyperaccumulation in these species (Wu et al. 2009). Thus CO_2 enrichment may increase the biomass of hyperaccumulators growing on metal contaminated soils and promote accumulation of heavy metals in plants. However, minimal research has been conducted in this area, and little is known about the characteristic responses of hyperaccumulators to elevated CO_2 (Zheng et al. 2008; Li et al. 2010). Few studies have addressed heavy metal uptake by hyperaccumulators under elevated levels of CO_2 in terms of alterations in root morphological traits.

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 of metal-contaminated soil, due to its rapid growth, asexual propagation, and high biomass yield (Yang et al. 2002; Yang et al. 2006). Research has shown that Crassulacean acid metabolism (CAM) plant species have an average increase in biomass productivity of 35% in response to a doubled atmospheric CO_2 concentration (Drennan and Nobel 2000). However, the effects of elevated CO_2 concentration on the growth and metal accumulation of *S. alfredii* have not been studied. The objective of this study was (1) to investigate the effect of elevated CO_2 on plant growth, root morphology and Cd accumulation in *S. alfredii*, and (2) to assess the possibility of using elevated CO_2 as fertilizer to enhance phytoremediation efficiency of Cd-contaminated soil by *S. alfredii*.

Materials and methods

Plant material and soil characterization

Sedum alfredii was collected from an old Pb/Zn mining area in Quzhou City(29°17′N,118°56′E), Zhejiang province, China (Yang et al. 2002). Seedlings of *S. alfredii* were cultivated according to Yang et al. (2004). Plants were chosen to grow in noncontaminated soil for several generations to minimize the internal metal contents, then uniform and healthy shoots were selected and cultivated in the basal nutrient solution containing: 2 mM Ca^{2+}, 4 mM NO_3^+, 1.6 mM K^+, 0.1 mM $H_2PO_4^-$, 0.5 mM Mg^{2+}, 1.2 mM SO_4^{2-}, 0.1 mM Cl^-, 10 μM H_3BO_3, 0.5 μM MnSO4, 1 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01 μM (NH4)6 Mo_7O_{24}, and 100 μM Fe–EDTA. Nutrient solution pH was adjusted daily to 5.8 with 0.1 M NaOH or 0.1 M HCl. Plants were grown under glasshouse conditions with natural light, day/night temperature of 26/20°C and day/night humidity of 70/85%.The nutrient solution was aerated continuously and renewed at every 3 d.

The soil used in the pot experiment was collected from a long-term experimental station in the farm of

Zhejiang University, Hangzhou, Zhejiang Province, China. Basic physical and chemical properties of the soil were analyzed according to the routine analytical methods of agricultural chemistry in soil (Lu 1999). The chemical and physical properties are shown in Table 1. The soil was slightly polluted when compared with the National Soil-Environmental Quality Standards of China (NSEQSC, GB 15618, 1995).

Soil pot culture experiment

The soil used in the pot experiment was collected from the surface layer (0–20 cm). It was air-dried, ground and sieved <4 mm. 2.5 kg of soil samples were placed in each plastic pot (15 cm in diameter and 15 cm in height) after mixing with appropriate amount of Cd ($Cd(NO_3)_2 \cdot 4H_2O$ solution). 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 represent slight, medium and severe contamination, respectively. The soil was left to equilibrate outdoors under a waterproof tarpaulin for about 2 months after being moistened to 70% field holding capacity. This period is long enough to allow natural equilibration of the various sorption mechanisms in the soil. After pre-culturing for 2 weeks in hydroponic solution, three *S. alfredii* plants were transplanted in each pot. All pots were transferred into growth chambers (Conviron® E7/2) at a humidity of 70%, with day and night temperatures of 26 and 20°C, respectively. The average light intensity was maintained at 160 μmol m^{-2} s^{-1} during a 14-h light cycle. CO_2 treatments were similarly initiated by growing the above plants in the chambers with a CO_2 concentration of either 350 (ambient) or 800 (elevated CO_2) μL L^{-1}. These pots were arranged in a randomized complete block design with 4 replicates for each treatment. Total CO_2 treatment time was 60 days. At the end of experiment, the plants were harvested and each plant was separated into root and shoot parts. These parts were washed thoroughly with tap water and rinsed with distilled water, and dried in an oven for 3 days at 65°C. The oven dried plant samples were weighed, and ground to a 60-mesh fineness prior to chemical analysis. Soil samples were collected from the pots immediately after plant harvest, air-dried and ground to pass through a 0.149 mm sieve prior to use.

Table 1 Physical and chemical characteristics of the used soil

pH[a]	5.94
Organic matter (g kg^{-1})	12.8
Total N (g kg^{-1})	1.05
Available-P (mg kg^{-1})	13.5
Available-K (mg kg^{-1})	96.3
CEC[b] (cmol kg^{-1})	14.0
Total Cd (mg kg^{-1})	0.64
Available Cd(mg kg^{-1})	0.12
Particle size distribution(g kg^{-1})	
Sand[c]	510
Silt	330
Clay	160

[a] 1:2.5 soil/water ratio

[b] Cation exchange capacity

[c] Sand (2–0.02 mm), Silt (0.02–0.002 mm), and Clay (≤0.002 mm)

Hydroponic experiment and root morphology analysis

After pre-culturing for 1 week in hydroponic solution, plants were transferred to complete nutrient solution with 50 μM Cd. The plants were subjected to ambient and elevated CO_2 treatments. After 10 d growth under elevated CO_2, the plants were excised at their base and roots were washed with distilled water and stored in 50% isopropyl alcohol at 4°C until further processing. Root morphological parameters were determined by using a root automatism scan apparatus (MIN Mac, STD1600$^+$) equipped with WinRHIZO™2000 software offered by Regent Instruments Inc. WinRHIZO™ 2000 is a software that recognizes digital root images and analyzes root parameters (length, surface area, and volume) for defined root diameters. Root segments were then placed on STD1600$^+$ in a transparent plastic tray filled with water. A white plastic plate served as image background. Images were recorded at a resolution of 800 dpi and images were saved as TIFF (tagged image file format). For a better understanding of the root morphological characteristics of *S. alfredii*, 11 root diameter classes were defined with a width interval of 0.05 mm, for example, the finest roots had a diameter between 0–0.05 mm and were defined as diameter class 1. The thickest roots had diameters >0.5 mm and were defined as diameter class 11. Root length, surface area, volume and average root diameter

(ARD) were analyzed for each diameter class. Specific root length (SRL, root length per plant), specific root surface area (SRA, root surface area per plant) and specific root volume (SRV, root volume per plant) were calculated. For each CO_2 treatments, the roots of ten plants were analyzed.

Chemical analysis

Plants were digested with a mixture of concentrated HNO_3 (16 M) and $HClO_4$ (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. Metal concentrations in the digest were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, iCAP 6000 SERIES, Thermo Scientific). Total Cd in the soil was analyzed by digestion with concentrated HNO_3 (16 M), $HClO_4$ (12 M) and HF (40%) (5:1:1 *v*/*v*/*v*) and for determination of soil available Cd, samples were extracted with DTPA-TEA (5 mM diethylenetriaminepentaacetic acid, DTPA, 0.1 M triethanolamine, and 0.01 M $CaCl_2$ at pH7.3, respectively). The concentrations of Cd in the extracts were determined by ICP-AES (iCAP 6000 SERIES, Thermo Scientific). Two indices were calculated to evaluate plants for phytoextraction purposes. The bioconcentration factor (BCF) is calculated as the Cd concentration in shoots versus the Cd concentration in soil. Phytoextraction efficiency is calculated as total Cd uptake by *S. alfredii* to the total Cd content in soil of each pot.

Statistical analysis

All data were statistically analyzed using the SPSS package (Version 11.0). The data were analyzed with a two-way analysis of variance (ANOVA). Means of significant difference were separated at $P<0.05$ by *t* test or the least significant difference (LSD) test.

Results

Plant growth

Under different Cd levels, *S. alfredii* grew normally without showing any toxic symptoms, indicating that *S. alfredii* has high tolerance to Cd toxicity. Plant growth was increased by elevated CO_2 concentration as compared to the ambient CO_2 under all three Cd treatments (CK, Cd5 and Cd50) (Fig. 1). Shoot dry weight was increased by 36.7%, 26.7% and 24.6%, respectively, and root dry weight by 52.1%, 36.7% and 35.0%, respectively, as compared with those grown in ambient CO_2, indicating that the enhancement was greater in slight contamination than those in severe contamination. The ratios of root to shoot biomass were also increased by elevated CO_2 (Fig. 1c).

Root morphological traits

After 10 days growth in medium containing 50 μM Cd, the development of lateral roots and root hairs was observed, but the growth was much greater at elevated CO_2 than at the ambient CO_2 treatment (Fig. 2). Data of specific root length (SRL), specific root surface area (SRA), specific root volume (SRV), average root diameter (ARD) and tip number are presented in Table 2. For 50 μM Cd, elevated CO_2 improved the root growth of *S. alfredii*. Additionally, SRL, SRA, SRV and tip number was increased by 31.5%, 50.5%, 35.0% and 23.8%, respectively

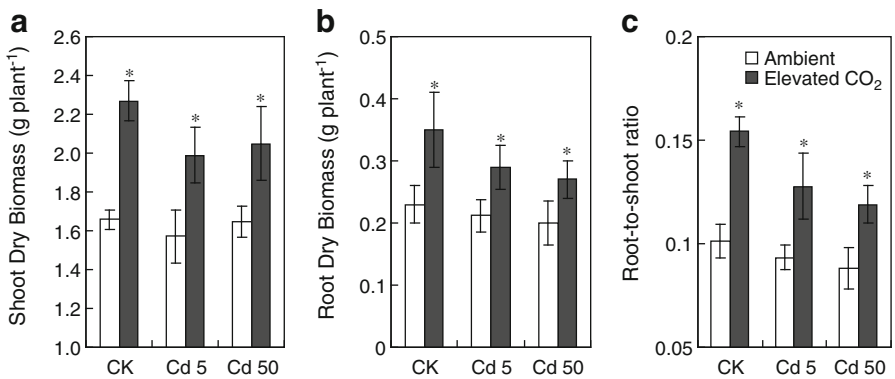

Fig. 1 Effect of elevated CO2 concentration on shoot (**a**) and root (**b**) biomass, root-to-shoot ratio (**c**) of *S. alfredii* grown in Cd contaminated soil. The plants were grown under ambient (350 μL L^{-1}) or elevated (800 μL L^{-1}) CO_2. Data are means±SD (n=4). *, Significant differences ($P<$ 0.05) between ambient and elevated CO_2 treatments

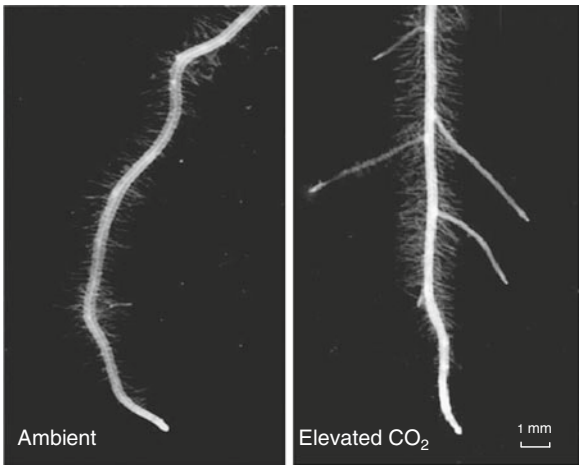

Fig. 2 Effect of elevated CO_2 concentration on development of lateral roots and root hairs of *S. alfredii* grown in 50 μM Cd medium. The plants were grown under ambient (350 μL L^{-1}) or elevated CO_2 (800 μL L^{-1})

(Table 2). No effect of elevated CO_2 on ARD of *S. alfredii* was observed under hydroponic conditions.

For a better understanding of the root morphological characteristics of *S. alfredii*, root diameter distribution of specific root length (SRL) and specific root surface area (SRA) were calculated (Fig. 3). The SRL mainly consisted of the first two diameter classes (0–0.05 mm and 0.05–0.1 mm) under both CO_2 levels; however, a significant increase in SRL of class 1 and 2 were observed due to elevated CO_2, indicating that elevated CO_2 prompted the growth of fine roots. A similar pattern was observed for root surface area.

Cd accumulation in *S. alfredii*

Cadmium accumulation in *S. alfredii* increased significantly with increasing Cd concentrations in the soil (Fig. 4). Cadmium concentration in the shoots was much greater than in the roots, indicating that *S. alfredii* was efficient in transporting Cd from roots to the shoots. In the control treatments, Cd concentrations in both shoots and roots of *S. alfredii* were low and no difference was observed between both CO_2 levels. However, in Cd50 treatment, cadmium concentration in the plant grown in elevated CO_2 was much greater than in ambient CO_2, indicating that elevated CO_2 treatment significantly improved Cd uptake in the plants grown in Cd polluted soil.

Cadmium accumulation in the plant was significantly influenced by elevated CO_2. The total Cd uptake calculated per-pot shoot dry weight was increased by 37.5%, 48.9%, and 41.2%, respectively, as compared to the control (grown in ambient CO_2) (Table 3). The bioconcentration factor (BCF) describes the ability of plants to accumulate Cd from soil. Cadmium accumulation in plants was increased by elevated CO_2 under both Cd5 and Cd50 treatments (Table 3). After 60-d growth in soil, BCF was increased by 17% and 16%, respectively, as compared with control.

Cd removal from soil and post harvest soil available Cd

After a growth period of 60 d, Cd removal by *S. alfredii* ranged from 9.5% to 20.47%; Cd removal efficiency was increased significantly by elevated CO_2 (Table 3). Under the treatments of three Cd levels, phytoextraction efficiency of Cd was increased by 34.2%, 48.3%, and 41.3%, respectively, compared with the plants grown in ambient CO_2.

At the end of the experiment, available Cd concentration in the soil of CK treatment was very low and the difference between two CO_2 levels was not significant. However, in both Cd5 and Cd50 treatments, the concentrations of available Cd in soil were significantly reduced by elevated CO_2 which confirmed that elevated CO_2 enhanced Cd uptake by *S. alfredii*.

Table 2 Effect of elevated CO_2 treatment on specific root length (SRL), specific surface area (SRA), specific root volume (SRV), average root diameter (ARD), and tip number of the plants grown in 50 μM Cd medium. *S. alfredii* were grown under ambient (350 μL L^{-1}) or elevated (800 μL L^{-1}) CO_2. Data are means±SD (n=10)

Treatment	SRL (cm $plant^{-1}$)	SRA (cm^2 $plant^{-1}$)	SRV (cm^3 $plant^{-1}$)	ARD (mm)	Tip number
Ambient	94.72±10.18	24.04±2.38	0.23±0.03	0.085±0.015	1,850
Elevated CO_2	124.59±13.31*	36.18±8.52*	0.30±0.02*	0.096±0.023	2,291*

*Significant differences (P<0.05) between ambient and elevated CO_2 treatments

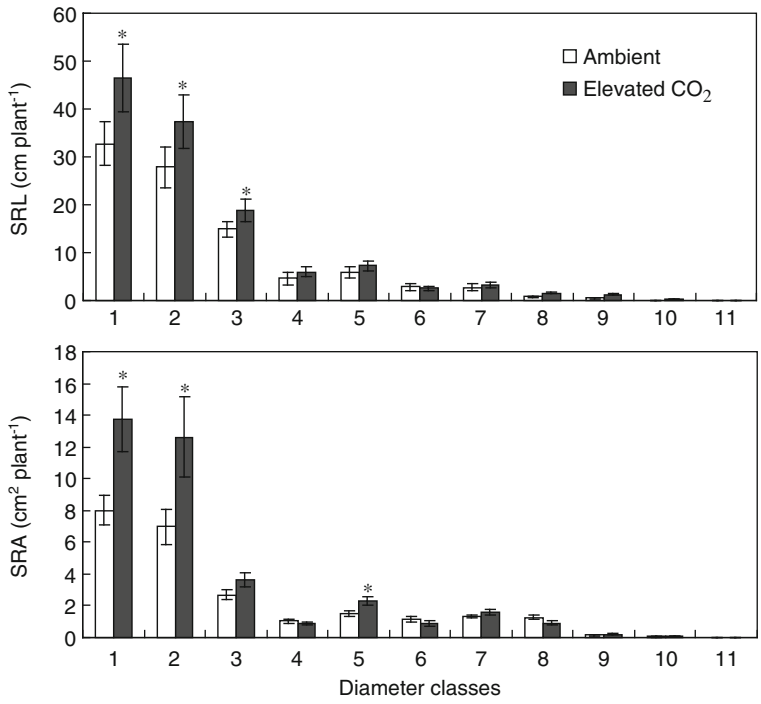

Fig. 3 Root diameter distribution of specific root length (SRL) and specific root surface area (SRA) of *S. alfredii* grown in 50 μM Cd medium. The plants were grown under ambient (350 μL L^{-1}) or elevated (800 μL L^{-1}) CO_2. Bars represent standard deviation (SD) of ten replicates. *, Significant differences ($P<0.05$) between ambient and elevated CO_2 treatments

Mineral nutrient accumulation in *S. alfredii*

Elevated CO_2 had a significantly positive effect on the accumulation of four mineral nutrients in the plant (Table 4). In Cd5 treatment, Zn, Fe, Mg and Mn accumulation in the shoot of *S. alfredii* increased significantly, by 33%, 79%, 18% and 17%, respectively, as compared to the control. A similar trend was observed for Cd50 treatment.

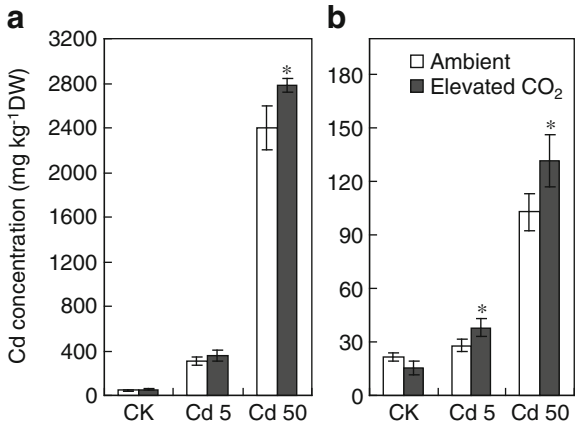

Fig. 4 Effect of elevated CO_2 treatment on Cd concentration in shoot (**a**) and root (**b**) of *S. alfredii* plants grown in Cd contaminated soil. Data are means±SD ($n=4$). *, Significant differences ($P<0.05$) between ambient and elevated CO_2 treatments

Discussion

Elevated CO_2 favors plant growth under cadmium pollution condition

Enhanced plant growth under elevated levels of CO_2 and uncontaminated soil conditions has been widely documented, and the magnitude of increase is dependent on plant species and genotype (Drennan and Nobel 2000; Kimball et al. 2002; Cousins et al. 2003; Hogy et al. 2009; Jia et al. 2010; Li et al. 2010). Crassulacean acid metabolism (CAM) plant species show an average increase in biomass productivity of 35% in response to a doubled atmospheric CO_2 concentration (Drennan and Nobel 2000). *S. alfredii* is a natural Zn/Cd hyperaccumulator belonging to the crassulaceae family (Yang et al. 2002). In this study, a significant effect of elevated levels of CO_2 on the dry weights *S. alfredii* in pot experiment was observed (Fig. 1), and this result is in accordance with previous studies on hybrid orchid 'Mokara Yellow' (Gouk et

Table 3 Effect of elevated CO_2 treatment on total Cd uptake, bioconcentration factor (BCF), phytoextraction efficiency, and post harvest available Cd in the soil. *S. alfredii* were grown under ambient (350 μL L^{-1}) or elevated (800 μL L^{-1}) CO_2. Data are means±SD (n=4)

Soil	CO_2	Total Cd uptake (mg pot^{-1})	BCF	Phytoextraction efficiency (%)	Post harvest available Cd (mg kg^{-1})
CK	Ambient	0.24±0.01	76.6±2.9	15.25±1.07	0.08±0.01
	Elevated CO_2	0.33±0.02*	78.6±1.8	20.47±1.80*	0.06±0.02
Cd5	Ambient	1.92±0.09	61.4±2.1	11.58±1.29	1.43±0.04
	Elevated CO_2	2.86±0.19*	71.9±6.6*	17.18±1.88*	0.99±0.15*
Cd50	Ambient	11.88±1.05	48.0±3.1	9.50±0.89	17.61±1.43
	Elevated CO_2	16.78±1.39*	55.7±3.9*	13.43±1.06*	13.83±2.04*

*Significant differences (P<0.05) between ambient and elevated CO_2 treatments

al. 1997). Elevated CO_2 concentrations had a positive effect on the biomass of *S. alfredii* even under Cd-stressed conditions (Fig. 1), showing the possibility of using elevated CO_2 as fertilizer to promote growth of *S. alfredii* in phytoremediation practices. Similar results have been reported in *L. multiflorum* and *L. perenne* with Cd stress (Tang et al. 2010), *Pteridium revolutum*, sunflower and Indian mustard with Cu stress (Tang et al. 2003; Zheng et al. 2008) as well as for a *Sorghum* species and a *Trifolium* species with Cs stress (Wu et al. 2009), but all of them are C_3 plants other than CAM plants. The stimulating effects of elevated CO_2, were usually higher under non-Cd stressed than Cd stressed conditions for *S. alfredii*, indicating that excess cadmium in soil might limit the positive effect of elevated CO_2 on plant biomass of *S. alfredii* in some way, which needs to be further investigated. The present research demonstrated that elevated CO_2 concentration promoted growth of *S. alfredii* within the range of Cd treatments, which may be explained by some factors such as mineral nutrition and root morphology.

Elevated CO_2 enhances root elongation and branching

Root length, surface area, volume, root diameter, and tip number are important parameters for understanding how root systems respond to environmental changes, such as increases in CO_2 levels and heavy metal stress. *S. alfredii* had higher values of these root morphological parameters (specific root length, specific surface area, specific root volume, and tip number) at elevated CO_2 levels (Table 2), showing that elevated levels of CO_2 increased root elongation and root branching. It is worth to point out that SRL, SSA, and SRV are more sensitive to elevated CO_2 than root tip number. It is widely documented that elevated levels of CO_2 increase fine root numbers (Pritchard and Rogers 2000). In this study, significant increases (P<0.05) in both SRL and SRA for the first two diameter classes (0–0.05 mm and 0.05–0.1 mm) were observed under elevated CO_2, indicating that fine roots were more stimulated by elevated CO_2 even under Cd-stressed conditions (Fig. 3). Moreover, lateral roots and subapical root hair development

Table 4 Effect of elevated CO_2 treatment on Zn, Fe, Mg and Mn accumulation in shoots of *S. alfredii* which were grown under ambient (350 μL L^{-1}) or elevated (800 μL L^{-1}) CO_2. Data are means±SD (n=4)

*Significant differences (P<0.05) between ambient and elevated CO_2 treatments

Soil	CO_2	Zn (μg $plant^{-1}$)	Fe	Mg	Mn
CK	Ambient	4130±187	235.6±13.6	5780±175 d	41.72±11.47
	Elevated CO_2	5246±282*	309.1±13.1*	9353±828*	62.26±4.70*
Cd5	Ambient	5068±691	258.6±33.6	7910±1530	50.43±8.14
	Elevated CO_2	6726±135*	461.6±78.4*	9327±76*	58.87±5.88*
Cd50	Ambient	4258±332	467.3±25.1	8118±746	79.74±10.54
	Elevated CO_2	6864±539*	501.5±45.9	12826±794*	80.83±5.64

were also enhanced by elevated CO_2 (Fig. 2). The changes in root morphology greatly enlarge the root surface area, and thus aid the nutrient and water uptake. This can explain why elevated CO_2 triggered a significant increase in plant biomass under Cd stress.

Root/shoot ratio is commonly used to assess compensatory changes in root growth characteristics in response to CO_2 enrichment (Bassirirad et al. 1996). CAM plants typically have extremely low root/shoot ratios, about 0.08–0.12 (Drennan and Nobel 2000). However, elevated CO_2 stimulated root growth and resulted in a greater root-to-shoot ratio even in soil contaminated with 5 or 50 mg kg^{-1}Cd (Fig. 1c). This result is consistent with previous studies on other CAM plants (Cui et al. 1993; Gouk et al. 1997), indicating that roots of *S. alfredii* are generally stimulated by elevated CO_2 more than shoots (Kimball et al. 2002).

Effects of elevated CO_2 on Cd uptake and implication for phytoremediation

As is expected, elevated CO_2 increased both shoot and root Cd concentration of *S. alfredii* under Cd5 and Cd50 treatments in the pot experiment (Fig. 4), and this result is similar to the positive effect of elevated CO_2 on Cd concentration in sunflower and Indian mustard (Tang et al. 2003) and Cs concentration in *Sorghum* and *Trifolium* (Wu et al. 2009). Other studies have shown that elevated CO_2 decreases Cu concentration in three fern species (*Pteridium revolutum, P. aquilinum*, and *Pteris vittata*) (Zheng et al. 2008) and Cd concentration in two ryegrass species (*L. perenne* and *L. mutiforum*) (Tang et al. 2010). The similar or conflicting observations are mainly associated with plant species that have different metal acquisition strategies (Rascio and Navari-Izzo 2011). Some species like *S. alfredii* have the hyperaccumulation trait of capturing Cd or other heavy metals from soil, while other plant species have the excluder trait of avoiding uptake heavy metals as a protection strategy. Elevated CO_2 can presumably strengthen these two different traits by improving plant morphology and physiology.

Development of dense subapical root hairs is a typical morphological response of plants to elevated CO_2. More fine roots or a greater root hair density greatly enlarges the root surface area and allows for a larger soil volume to be explored. Therefore, fine root and subapical root hair development enhanced by elevated CO_2 (Figs. 2 and 3) may contribute partly to the increased Cd accumulation in plants grown in the Cd-polluted soil. Furthermore, elevated CO_2 stimulated root growth and resulted in a significantly greater root-to-shoot ratio (Fig. 1). Similar changes in root-to-shoot ratio have been interpreted as a mechanism by which plants can take up more nutrients when growth is stimulated by the elevated CO_2 concentration (Rogers et al. 1996). In this study, elevated CO_2 had a significant positive effect on the accumulation of four mineral nutrient elements in plant (Table 4), especially for Zn. Previous studies have shown that Cd always shares the identical transporters with Zn in plant cells (Murphy et al. 2011) and most of the Cd hyperaccumulators also hyperaccumulate Zn (Rascio and Navari-Izzo 2011). As elevated CO_2 increases Zn accumulation in plant tissues, it may increase the generation of more Zn transporters, thus transportation and accumulation of Cd in plant tissues is increased at the same time. In conclusion, the elevated CO_2 under Cd-stress conditions enhances fine root growth, root hair development, and mineral nutrient uptake, all of which enable plants to access and accumulate more Cd.

Calculation of total Cd uptake in the plant from each pot showed that there was higher total Cd uptake at elevated levels of CO_2 than at ambient CO_2 (Table 3). This result is in accordance with previous reports (Zheng et al. 2008; Wu et al. 2009; Tang et al. 2010). Furthermore, phytoextraction efficiency of Cd was increased significantly by elevated CO_2. Tang et al. (2003) have suggested that the CO_2-triggered phytoextraction is a way to facilitate phytoremediation. The present study on *S. alfredii* further confirmed that the use of elevated CO_2 may be an effective way to enhance phytoextraction efficiency.

Conclusions

This study demonstrated that *S. alfredii* growth was promoted by elevated CO_2 concentration (800 μL L^{-1}), both shoot and root biomass being increased significantly. The root-to-shoot ratio of *S. alfredii* was greater at elevated CO_2, indicating that roots of *S. alfredii* were more stimulated than the shoots. Elevated CO_2 concentration increased the values of

the root morphological parameters (SRL, SSA, SRV, and tip number), especially for fine roots. Elevated CO_2 enhanced the development of lateral roots and root hairs. The Cd accumulation in both shoot and root of *S. alfredii* was increased by elevated CO_2, which can be attributed to the enhancement of fine root growth, root hair development, and mineral nutrient uptake under elevated CO_2. These results imply that use of elevated CO_2 may be a useful way to improve phytoremediation efficiency of Cd-contaminated soil by *S. alfredii*.

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