Journal of Hazardous Materials 180 (2010) 384–394

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Effects of elevated CO_2 on growth, photosynthesis, elemental composition, antioxidant level, and phytochelatin concentration in *Lolium mutiforum* and *Lolium perenne* under Cd stress

Yan Jia[a,b,c], Shirong Tang[a,b,*], Ruigang Wang[a,b], Xuehai Ju[a,b], Yongzhen Ding[a,b], Shuxin Tu[c], Donald L. Smith[d]

[a] Centre for Research in Ecotoxicology and Environmental Remediation, Agro-Environmental Protection Institute, Ministry of Agriculture, Tianjin 300191, PR China
[b] Key Laboratory of Production environment and Agro-product Safety of Ministry of Agriculture and Tianjin Key Laboratory of Agro-environment and Food Safety, PR China
[c] College of Resources and Environment, Huazhong Agricultural University, 430070 Wuhan, Hubei Province, PR China
[d] Plant Science Department, McGill University, Macdonald Campus, 21111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9

ARTICLE INFO

Article history:
Received 3 February 2010
Received in revised form 10 April 2010
Accepted 12 April 2010
Available online 24 April 2010

Keywords:
Cadmium
Elevated CO_2
Photosynthesis
Phytochelatin
Ryegrass

ABSTRACT

The objective of this study was to investigate combined effects of Cd and elevated CO_2 on growth, physiological and physiochemical characteristics, elemental compositions in *Lolium mutiforum* and *Lolium perenne* grown in soils amended with three Cd concentrations (0, 25, 100 mg kg^{-1}) under two CO_2 levels (375, 810 $\mu L\,L^{-1}$). Elevated CO_2 increased net assimilation rate and internal CO_2 concentration, and consequently increased total plant biomass by 51 to 31%. At same spiked Cd level, malondialdehyde content in leaves was lower under elevated than under ambient CO_2, whereas superoxide dismutase activity was higher. Elevated CO_2 decreased Cd, S, and phytochelatin concentrations in roots and shoots to a various degree, depending on plant species and element, but the PC–Cd ratio was not affected. It was concluded that elevated CO_2 ameliorated Cd toxicity in both *Lolium* species under Cd stress, and that the increase of plant biomass and the alleviation of Cd toxicity with elevated CO_2 for the *Lolium* species may be more dependent on increased photosynthesis and enhanced antioxidant capacity. Results of the study may provide insights into the interaction between soil Cd contamination and atmospheric CO_2 concentration with regard to plant ability to grow and remove the Cd from soils.

1. Introduction

The ongoing combustion of fossil fuels has increased the atmospheric carbon dioxide concentration from 280 $\mu L\,L^{-1}$ to approximately 380 $\mu L\,L^{-1}$ since the beginning of industrialization, and it is expected to continue increasing in the future, resulting in global warming and global climate change [1]. Elevated CO_2 is a new challenge for crops, and its continuing increase may cause crops to develop specific short-term acclimation and long-term adaptation responses [2]. The effects of CO_2 on crop growth and development might be more important than has been thought. Plants are sensitive and respond directly to elevated CO_2 through photosynthetic rate and stomatal conductance [3]. Elevated CO_2 increases net photosynthetic rate, increases carbon assimilation for plant growth and development [4–5], and decreases photorespiration and oxidative stress [6–7].

Mining and smelting, disposal of sewage sludge and the use of Cd rich phosphate fertilizers [8,9] have contaminated a large proportion of the agricultural land throughout the world, causing an increase in the soil concentration of many heavy metals. The situation is particularly concerning in China where about 2.0×10^7 hm^2 of agricultural land is contaminated with heavy metals [10]. Thus, elevated levels of heavy metals such as Cd in the environment are a reality today.

As one of the most toxic environmental pollutants [11] cadmium (Cd) has a strong influence on metabolic activities of crop plants by inducing a number of physiological changes, such as growth inhibition, changes in water and ion metabolism, photosynthesis inhibition, enzyme activity changes, and free radical formation [12]. Even at relatively low concentrations it can exert strong toxic effects on crops [13]. Cd induces severe oxidative stress by producing excessive ROS [14], causing severe damage to membrane systems, cell organelles and DNA [15,16]. MDA is a major product of membrane lipid peroxidation, and its level is an indicator of the extent of oxidative damage [14]. Chlorophyll a and b are important factors of photosynthesis inhibition, and their concentration in plant tissues decreases under abiotic stress environment. It is known that the decrease of chlorophyll content inhibits plant abil-

* Corresponding author at: Centre for Research in Ecotoxicology and Environmental Remediation, Agro-Environmental Protection Institute, Ministry of Agriculture, Tianjin 300191, PR China. Tel.: +86 22 23003707; fax: +86 22 23003707.
E-mail addresses: tangshir@hotmail.com, tangshirong@cae.org.cn (S. Tang).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.04.043

ity to absorb and utilize light energy, and consequently, leads to reduced photosynthesis [17]. There are many environmental factors that affect chlorophyll a and b contents. The effects of NaCl salinity, ozone and CO_2 levels on chlorophyll a and b contents have received much attention. Geissler et al. [15] found that under ambient atmospheric CO_2 concentrations, rising NaCl salinity caused a continual decrease in chlorophyll a and b. In the high salt treatments, elevated CO_2 concentration led to higher contents of all three pigments (chlorophyll a and b plus carotenoids). Similar alleviation effects of elevated CO_2 on stress were observed in plants grown under O_3 and drought stress [18,19]. It is clear that elevated CO_2 affects plant growth in either unstressed or stressed environments, but little information is available in the literature concerning the combined effect of elevated CO_2 and metal contamination on plant physiochemical processes. Previous studies showed that elevated CO_2 enhances plant growth in either uncontaminated [20,21] or contaminated environments [22,23]. Given expected global increases in CO_2 concentration and the resulting changes in plant physiological and physiochemical processes, the effects of heavy metal contamination upon plant growth, development and uptake of heavy metals may be different in the near future, a possibility already demonstrated for some plant species [22–24].

Since slightly elevated levels of heavy metals in the environment are more common than severe contamination, the effect of elevated CO_2 on growth and development of plants grown in slightly heavy metal-contaminated soils should receive attention. Currently, there are few studies reporting the effect of elevated CO_2 on plant uptake of essential micronutrients, such as Cu, Fe, Mn, Zn [22,24–30]. Little is known about possible effects of elevated CO_2 on plant uptake of non-essential elements such as Cd, especially in terms of the relationship with phytochelatin concentration in plant tissues under elevated CO_2. In spite of efforts to understand the mechanisms by which elevated CO_2 and metals such as Cu and Cs jointly affect plant growth, development, and element uptake [22,23], there is still a great deal that remains unknown. The enhanced accumulation of Cu and Cs might be related to decreased pH values [22,23] and changes in microorganisms [23] under elevated CO_2. There is no good data regarding changes in photosynthesis and enzyme activity, and element (Cd and S) and phytochelatin concentrations in plant species growing under elevated CO_2, and the effects this could have on alleviating toxicity for metal-stressed plants.

L. multiflorum and *L. perenne* are frequently studied due to their ability to survive in metal-contaminated soil and to accumulate metals [31–35]. Previous research demonstrated their potential for rehabilitation of degraded soils [34,36]. Understanding their growth, gas exchange, leaf pigment composition and metal accumulation responses to elevated CO_2 under heavy-metal stress will improve our knowledge of metal uptake by the plants and their survival in contaminated environments, and also allow comparison of the behaviors of *L. multiflorum* versus *L. perenne* with regard to the interaction between metal contamination and elevated CO_2. This can provide important information regarding food safety and the potential of plants as bioindicators and for phytoremediation. Therefore, in-depth research should be carried out to understand the tolerance of *L. multiflorum* versus *L. perenne* to Cd stress, and its interaction with elevated CO_2.

The objective of the present study is to investigate how elevated CO_2 ameliorates Cd toxicity in terms of changes in biomass, gas exchange and photosynthetic pigments, and through changes in elemental composition and phytochelatin concentration in *L. perenne* and *L. mutiforum* grown in soils artificially contaminated with three levels of Cd (0, 25, and 100 mg kg^{-1}). It is hypothesized that elevated CO_2 may increase biomass production and alleviate Cd stress by altering photosynthetic activities, antioxidant enzymes, element composition, and PC concentrations. To our knowledge, this is the first study carried out to investigate the interactive effects of Cd stress and elevated CO_2 concentration on key responses of plants, ranging from element uptake to physiological processes.

2. Materials and methods

2.1. Tested plant species

Lolium perenne L. and *Lolium multiflorum* Lam, both C_3 species, were selected for this study because of their high biomass production, extensive root system, high adaptability, low-cost management, and potential for use in the remediation of contaminated soil.

2.2. Soil preparation and plant growth

The soil used in this study was collected from a long-term experimental rice field at Guangdong province (PR China). The chemical and physical properties are shown in Table 1. Fresh soil was sieved to pass a 3-mm sieve and kept in darkness before use. Specified amounts of Cd ($CdCl_2{\cdot}2H_2O$) in the form of dissolved solution were added and thoroughly mixed into the soil to give three levels: 0, 25, and 100 mg Cd kg^{-1}. The spiked soil was then watered to field water capacity and kept in darkness for three months. Before being transferred into the pots, the artificially contaminated soil was sampled randomly and analyzed for Cd concentrations. The actual total concentration of Cd in the artificially contaminated soil was measured to be 0.03, 24.7, 99.5 mg kg^{-1}, respectively, which is very close to the three targeted levels of added Cd. The balanced soil was then fertilized with 150 mg kg^{-1} N, 100 mg kg^{-1} P and 150 mg kg^{-1} K, and transferred into plastic pots (18 cm diameter and 15 cm height), each containing 2.5 kg dry weight soil.

Seeds of ryegrass (*L. perenne* and *L. mutiforum*) were soaked in 1% NaClO for 15 min, washed several times with double-distilled water, and sown into the moist soil in the pot. Three seeds were sown in each pot. After germination of the seeds, the pots were placed into six open top chambers (OTCs), as described in details in Wu et al. [23]. Three of the chambers were maintained at ambient CO_2 (average of 375 μL L^{-1} during the daytime, the other three were ventilated with double CO_2 (average 810 μL L^{-1}) air. Six replicates were established for each treatment, with two placed in each chamber. Pots were randomly switched among the chambers with the same CO_2 concentration every seven days and placed randomly in the OTCs. CO_2 enrichment was applied to plants from 8:00 to 17:00 each day of the experiment (13th, May to 4th, July). The total time of CO_2 enrichment was 58 days.

Table 1
General physico-chemical properties of the experimental soil.

Soil properties		Values
pH (soil:water: 1:2.5)		5.24
CEC	C mol kg^{-1}	7.65
Textural class		Silty loam
Total N	g kg^{-1}	1.80
Total K	g kg^{-1}	2.75
Total P	g kg^{-1}	21.6
Total S	g kg^{-1}	0.58
Available N	mg kg^{-1}	152
Available P	mg kg^{-1}	35.5
Available K	mg kg^{-1}	52.1
Total Cd	mg kg^{-1}	0.03
Total Cu	mg kg^{-1}	41.8
Total Zn	mg kg^{-1}	136.9

2.3. Harvest and analysis of Cd in plants

After growth for 58 days, plants were harvested and separated into shoots and roots. Roots were soaked in 0.5 mmol L^{-1} $CaCl_2$ for about ten min, and then washed several times with distilled water. Parts of the plant tissues were cut into small pieces (less than 0.5 cm in length) with stainless steel scissors. For each plant, a subset of the resulting pieces was randomly selected, wrapped with tin foil, flash frozen in liquid nitrogen, and stored in darkness at −80 °C for determination of cysteine, glutathione, phytochelatins, MDA, and SOD. The residual material was dried in an oven at 75 °C for 72 h, weighed and pulverized to a homogeneous powder with a stainless steel cutter-blender (IKA T2500, Germany).

Subsamples (about 0.3000 g) of the oven-dried plant organs were digested in a mixture of HNO_3 and H_2O_2 (8:1, v/v) using a microwave digestion method (CEM, Mars: 240/50, America). The Cd content in the extractants was determined using an Atomic Absorption Spectrometer (AAS) and a graphite tube equipped with an automatic sampler (ZEEnit 700, Analytikjena, Germany). Calibration curves were prepared with commercial standard solutions of 1000 mg L^{-1} (Sigma). The reliability of the digestion and analytical procedure was assessed using blanks and standards as QA/QC samples.

2.4. Determination of chlorophyll and carotenoid content

Chlorophyll and carotenoids were extracted by homogenizing ca 0.1000 g fresh leaves in chilled ethanol solution (19:1 ethanol:water (v/v)), and with the extractant vessels filled to 25 mL. Chlorophyll and carotenoid contents in supernatants were determined spectrophotometrically (Specord S600, Analytik Jena, Germany) at 665, 649 and 470 nm; the calculation method followed Lichtenthaller and Wellburn [37].

2.5. Gas exchange

Gas exchange by leaves was measured with a Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). Measurements were carried out at ambient relative humidity (40–60%) and temperature (28–32 °C) in the open top chambers between 9:00–11:00 h. Net photosynthetic rate (P_n), transpiration (E), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were determined under ambient CO_2 (375 μL L^{-1}) and elevated CO_2 (810 μL L^{-1}) under a light intensities of 1200 μmol photon m^{-2} s^{-1}.

2.6. Determination of cysteine, glutathione and phytochelatins

Samples (approximately 0.2000 g each) of two ryegrass species, previously stored at −80 °C, were ground in liquid N_2 and then extracted in 1.8 mL of 5 mmol L^{-1} DTPA containing 0.1% TFA. The homogenate was centrifuged at 12,000 × g for 10 min at 4 °C. Twenty μL supernatant were mixed with 200 mmol L^{-1} HEPES buffer containing 5 mmol L^{-1} DTPA (pH 9.0, 650 μL) and 20 mmol L^{-1} TCEP (25 μL). This reaction mix was pre-incubated at 25 °C for 5 min. The derivatization was then carried out by incubating the samples in the dark for 30 min at 25 °C after the addition of 20 μL of 50 mmol L^{-1} mBBr, then terminated by the addition of 100 μL of 1 mol L^{-1} MSA. The derivatized samples were filtered with 0.20 μm nylon syringe filters (Millipore Corp., Bedford, MA, USA) for HPLC analyses.

The separation of thiol-bimane derivatives was performed using an Agilent Technologies 1200 series HPLC system (Agilent Technologies Inc., Germany). The column was an Agilent Zorbax Eclipse XDB-C18 (4.6 mm ID × 30 mm, 1.8 μm particle size, 80 Å; Agilent Technologies Inc., Princeton, MN, USA; part No. 924975-902). The temperature of the column oven was maintained at 25 °C. The excitation and emission wavelengths were set at 380 and 470 nm, respectively. Data were integrated using ChemStation software (Agilent Technologies Inc., Version B.03.02).

2.7. Sulfur determination

About 10.000 mg oven-dried shoot and root powder was wrapped with tinfoil, and then analyzed for sulfur by Elemental Analyzer (Vario EL II, Elementar, Germany).

2.8. Determination of MDA and SOD

Leaf material (0.2000 g) were taken from the −80 °C refrigerator, immediately ground in liquid nitrogen in a mortar and pestle, and homogenized in 3 mL 50 mM potassium phosphate buffer (pH 7.8). After centrifugation at 3500 × g for 10 min (4 °C), the supernatants were assayed for SOD and MDA. The measurement method for SOD activity follows Beyer and Fridovich [38]; a brief summary follows. Three mL 50 mM potassium phosphate buffer (pH 7.8) consisting of 13 mM L-methionine, 10 μM Na_2-EDTA, 2 μM riboflavin and 75 μM nitrotetrazolium blue chloride were mixed with 20 μL enzyme extract in a small transparent glass beaker. The mixture was illuminated under full sun for 5 min and then analyzed spectrophotometrically at 560 nm. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibited NBT reduction for every unit fresh weight (U g^{-1} FW). Lipid peroxides were measured by thiobarbituric acid test for MDA according to Heath and Packer [39]. Supernatent (1.5 mL) was added to 2.5 mL of 20% Trifuoroacetic acid containing 0.5% thiobarbituric acid. The mixture was held at 100 °C in a water bath for 20 min, cooled quickly in an ice bath, and then centrifuged at 10,000 × g for 10 min. The absorbances of the resulting solutions were determined at 450, 532 and 600 nm.

2.9. Tolerance index (T_i)

The tolerance index (T_i) was computed as the shoot or root biomass on contaminated soils divided by the shoot or root biomass on uncontaminated soils under elevated CO_2 and ambient CO_2, respectively. The tolerance index represents the ability of plants to survive on Cd contaminated soils under elevated CO_2 relative to ambient CO_2.

2.10. Statistical analysis

All experiments were conducted with six replicates, and results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out by one-way or two-way analysis using SPSS (SPSS Inc., USA, version 13.0) and OriginPro (OriginLab Corp., USA, v8.0724). Differences between treatments were resulted to be statistically significant when they occurred at $P < 0.05$.

3. Results

3.1. Growth response

Increasing the Cd concentration in soils decreased shoot and root dry weight of *L. multiflorum* and *L. perenne* regardless of CO_2 concentration (Table 2). Elevated CO_2 had positive effects on biomass production. When compared with the ambient CO_2 control, the increase in total biomass due to elevated CO_2 was about 32 and 31% for *L. multiflorum* and *L. perenne*, respectively, grown on the control soil; 37 and 45% on soil amended with 25 mg kg^{-1} Cd; 46 and 52% on soil spiked with 100 mg kg^{-1} Cd, respectively. It was clear that elevated CO_2 triggered a greater biomass increase for the two *Lolium* species grown under Cd stress than under no Cd

Table 2
Biomass of *L. mutiforum* and *L. perenne* grown on soils spiked with different Cd level under ambient and elevated CO_2. Values represent mean ± SD, with 6 replicates per treatment. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference ($P \leq 0.05$) between CO_2 treatments (within one Cd level).

Species	Tissue	Cd	Biomass (g)		T_i (%)		Biomass Increase rate (%)
			Ambient	Elevated	Ambient	Elevated	
Mutiforum	Shoot	0	7.89 ± 0.63 a	10.20 ± 1.02 a*	1.00	1.00	29.3
		25	6.58 ± 0.35 b	9.10 ± 0.77 b*	0.83	0.89	38.3
		100	2.65 ± 0.67 c	3.80 ± 0.55 c*	0.34	0.37	43.4
	Root	0	4.23 ± 0.65 a	5.67 ± 0.81 a*	1.00	1.00	34.0
		25	3.32 ± 0.47 a	4.48 ± 0.44 b*	0.78	0.79	34.9
		100	1.01 ± 0.07 b	1.56 ± 0.25 c*	0.24	0.28	54.5
Perenne	Shoot	0	9.08 ± 0.98 a	11.71 ± 1.58 a*	1.00	1.00	29.0
		25	5.64 ± 1.07 b	8.19 ± 1.21 b*	0.62	0.70	45.2
		100	1.90 ± 0.52 c	2.93 ± 0.67 c*	0.21	0.25	54.2
	Root	0	3.69 ± 0.35 a	4.98 ± 0.39 a*	1.00	1.00	34.4
		25	2.08 ± 0.48 b	2.83 ± 0.50 b*	0.56	0.57	36.0
		100	0.65 ± 0.04 c	0.94 ± 0.18 c*	0.18	0.19	44.6

stress. It should be noted that elevated CO_2 generally had a stronger positive influence on biomass for *L. perenne* than for *L. multiflorum*. The tolerance index (T_i) showed a greater ability to grow in the presence of Cd under elevated CO_2 than ambient CO_2 (Table 2).

3.2. Photosynthetic characteristics

3.2.1. Gas exchange

Cd affects gas exchange under both ambient and elevated CO_2. We observed a decrease in net assimilation rate of CO_2 (P_n) and stomatal conductance (G_s) in both species with increasing Cd concentration in soils (Table 3) ($R^2 > 0.48$ for P_n and $R^2 > 0.52$ for G_s for regression equations of P_n-soil Cd concentration and G_s-soil Cd concentration, respectively, $P < 0.05$). There was also a numerical, but not statistical ($P < 0.05$) decrease in the transpiration (E) and WUE (Table 3). The P_n and C_i increased under elevated CO_2. Under elevated CO_2 G_s and E were lower than under the ambient CO_2 (control) level for the same Cd exposure.

3.2.2. Chlorophyll and carotenoid content

Increasing Cd concentrations in soil caused a continual decrease in the contents of chlorophyll a, chlorophyll b, and carotenoids, irrespective of CO_2 treatment (Fig. 1). With increasing Cd concentration in soils, the chlorophyll a/b ratio decreased ($R^2 > 0.58$ for regression equations between the ratio and soil Cd concentration, $P < 0.05$). There were no differences in all three pigments for plants grown on soils amended with 0 and 25 mg Cd kg^{-1} under either elevated or ambient CO_2, but plants grown on soils amended with of 100 mg Cd kg^{-1} had higher contents of chlorophyll a and b under elevated CO_2 than ambient CO_2 (Fig. 1A, B). The chlorophyll a/b ratio was not influenced by elevated CO_2 (Fig. 1C, D).

3.3. Cadmium and sulfur concentrations

Regardless of plant species and CO_2 concentration, Cd concentration in shoots and roots of the two *Lolium* species increased with increasing soil Cd concentration. Accumulation of Cd by plants was found to be Cd concentration and CO_2 concentration dependent (Fig. 2). For both plants grown on soils spiked with Cd under either ambient or elevated CO_2, the roots had much higher concentrations of Cd than the shoots (Fig. 2). In roots of *L. perenne*, the maximum amount of accumulated metal was 763 mg kg^{-1} dw at 100 mg kg^{-1} Cd treatment. For *L. multiflorum* and *L. perenne* grown under ambient CO_2 on soil amended with 100 mg kg^{-1} Cd, the Cd concentration was 150 and 179 mg kg^{-1} in the shoots; and 672 and 763 mg kg^{-1} in the roots, respectively. Compared to the ambient CO_2 control, under elevated CO_2 both *Lolium* species had decreased Cd concentrations in shoots and roots (Fig. 2). The decreased magnitude of Cd concentration in *L. multiflorum* and *L. perenne* grown on soil spiked with 25 mg kg^{-1} Cd was 10.3 and 3.8% for the shoots, and 18.6 and 14.7% for the roots, respectively; for those grown on soil spiked with 100 mg kg^{-1} Cd, it was 8.4 and 8.9% for the shoots, and 12.5 and 13.9% for the roots, respectively. It is obvious that the magnitude of Cd reduction due to elevated CO_2 was greater in the roots than in the shoots.

Sulfur concentration in the roots and shoots increased with increasing Cd concentration in soils irrespective of plant species and CO_2 concentration ($R^2 > 0.86$ for regression equations between S and soil Cd concentration, $P < 0.05$). For the shoots of *L. mutiforum* and *L. perenne* growing on soils contaminated with 100 mg Cd kg^{-1}, the sulfur concentration increased by 11.6 and 11.9%, and for the roots by 14.7 and 15.1%, respectively. For the same Cd treatments, sulfur concentration in the plant organs was lower under elevated CO_2 than under ambient CO_2 (Fig. 3).

3.4. Cys, GSH and phytochelatins

As soil Cd concentration increased Cys, GSH, and PC concentrations in plant tissues increased ($R^2 > 0.79$, 0.52, and 0.89 for regression equations of Cys-soil Cd concentration, GSH- soil Cd concentration, and PCs-soil Cd concentration, respectively, $P < 0.05$) regardless of plant species and CO_2 treatment (Table 4, Fig. 4). Cys concentration in the roots and shoots of *L. perenne* was higher than that of *L. mutiforum* in most cases. For the same level of Cd treatment, the concentration of Cys in either roots or shoots was, in general, lower under elevated CO_2 than under ambient CO_2. Elevated CO_2 had no influence on GSH content in the shoots of *L. mutiforum*, but it did affect GSH levels in the shoots of *L. perenne*, with GSH being higher under elevated CO_2 for each Cd treatment level than under ambient CO_2.

PCs were barely detected in the shoots of the control plants, and minor amounts of PCs were found in the roots of these plants. With increasing soil Cd levels, PC concentration in the plant tissues of both *Lolium* species increased. For the same level of Cd treatment, the PC concentration was lower under elevated CO_2 than under ambient CO_2 (Fig. 4), but the PC–Cd ratios under Cd stress was not affected (Table 5). It is interesting to note that PC concentration in the shoot of *L. mutiforum* is much higher than that in *L. perenne*, but the reverse occurred in the roots (Fig. 4).

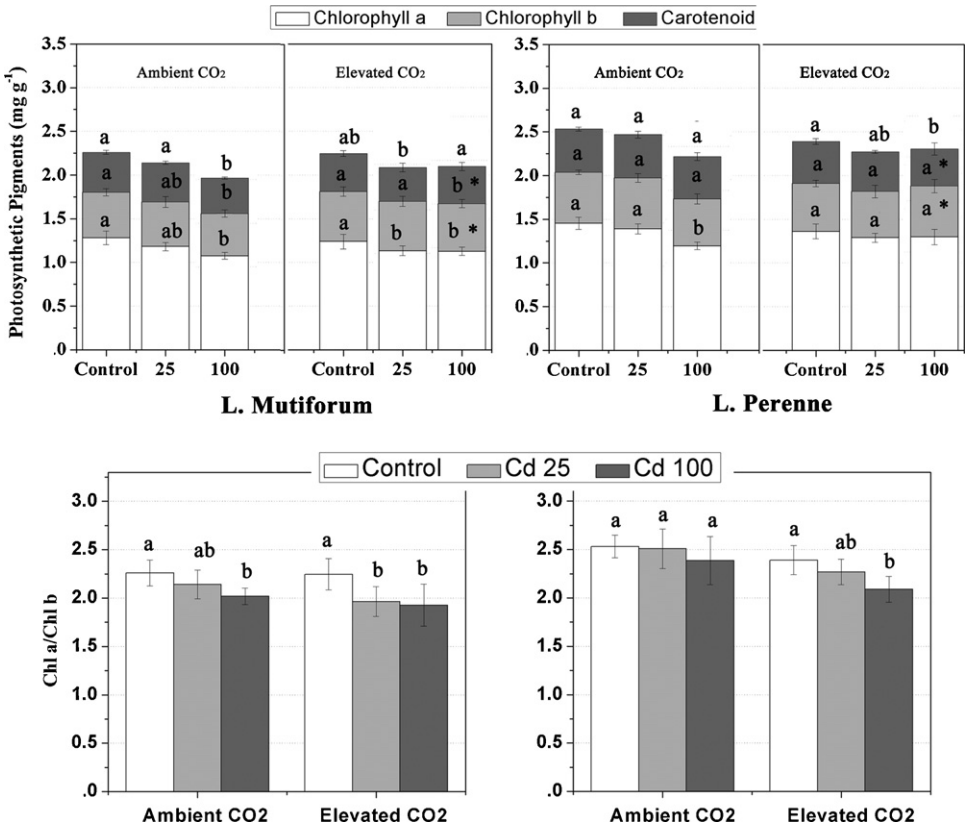

Fig. 1. Photosynthetic pigments in leaves of plants grown on soils spiked with different Cd levels under ambient and elevated CO_2. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference between CO_2 treatments (within one Cd level).

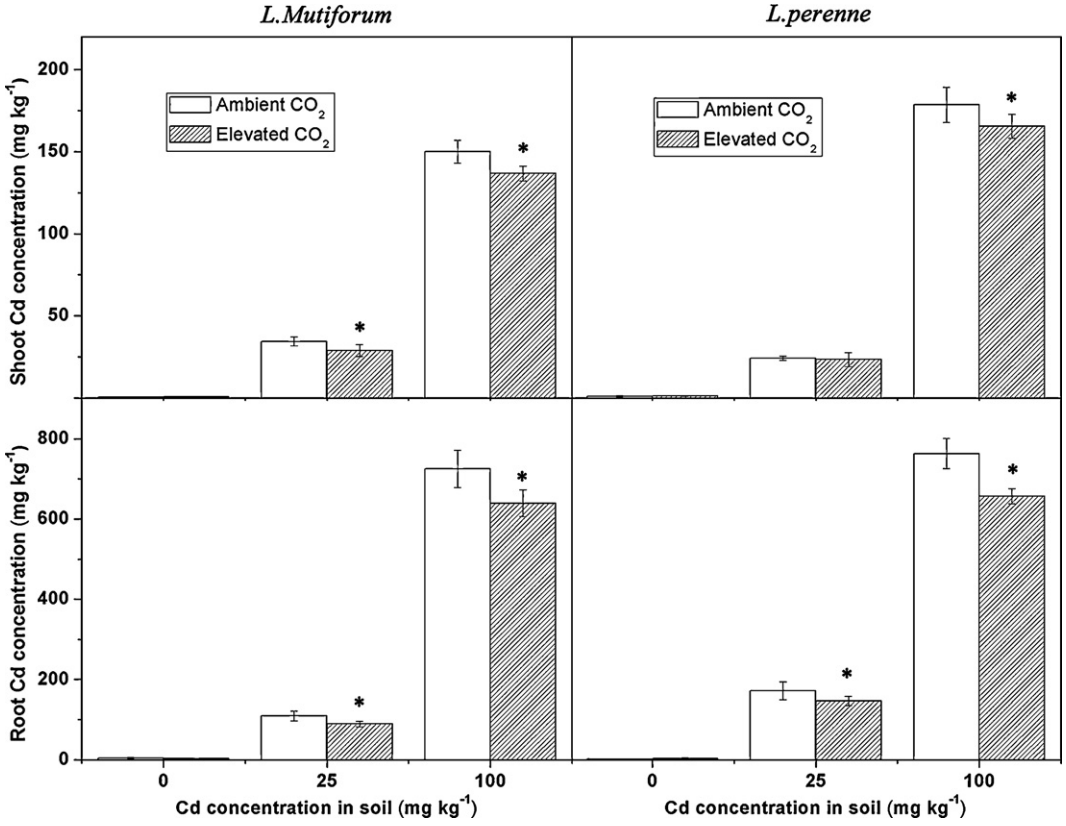

Fig. 2. Cd concentrations in the shoots and roots of *L. mutiforum* and *L. perenne* grown on soils spiked with different Cd levels under ambient and elevated CO_2. An asterisk indicates significant difference between CO_2 treatments (within one Cd level).

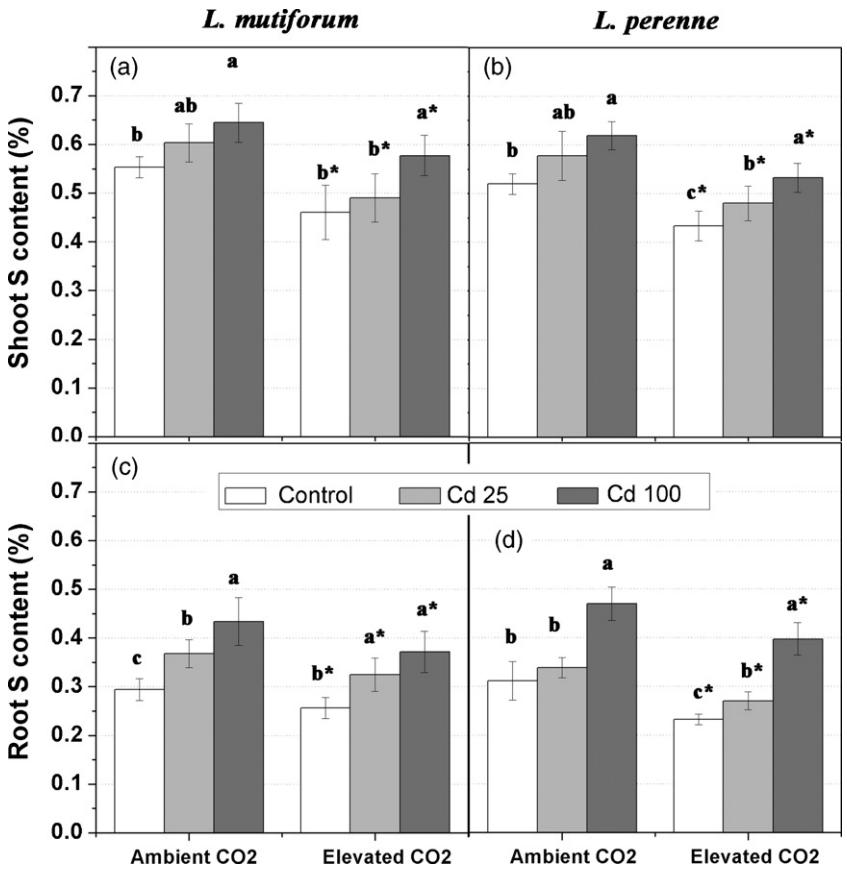

Fig. 3. Sulfur concentrations in the shoots and roots of *L. mutiforum* and *L. perenne*. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference between CO_2 treatments (within one Cd level).

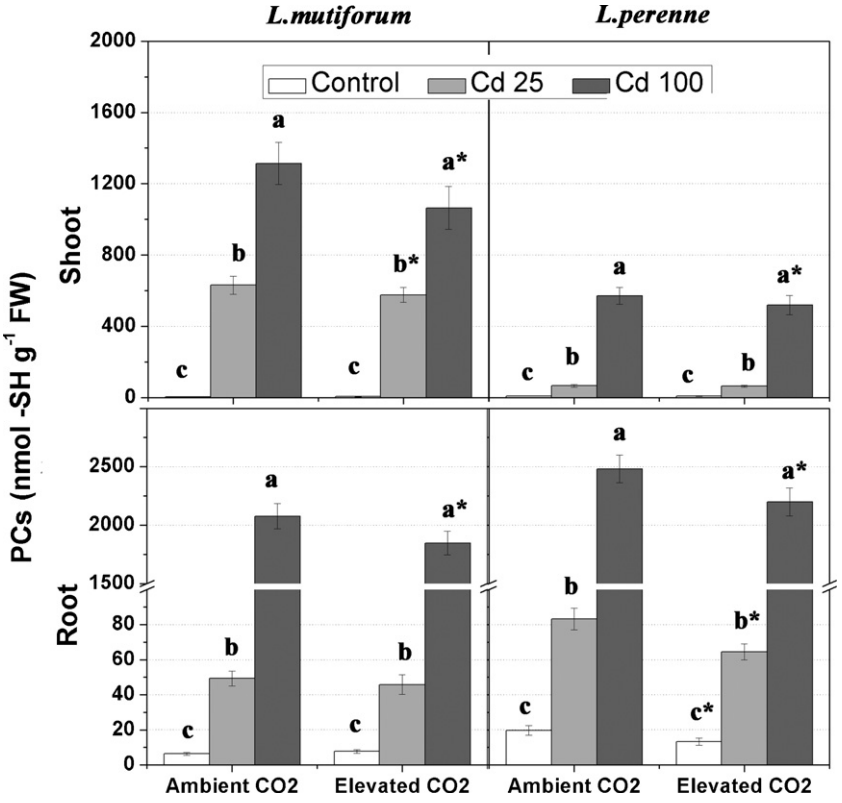

Fig. 4. PCs concentration in *L. mutiforum* and *L. perenne* grown under ambient and elevated CO_2. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference between CO_2 treatments (within one Cd level).

Table 3
Gas exchange parameters of *L. mutiforum* and *L. perenne* grown different Cd level under ambient and elevated CO_2. P_n, net assimilation rate; G_s, stomatal conduction; E, transpiration; WUE, water use efficiency of photosynthesis; C_i, internal CO_2 concentration. Values represent mean ± SD, values of 6 measurements per treatment. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference between CO_2 treatments (within one Cd level).

Species	Cd	CO_2	P_n (μmol m^{-2} s^{-1})	G_s (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	*WUE* (μmol mmol)	C_i (ppm)
Mutiforum	0	Ambient	13.7 ± 1.2 a	0.160 ± 0.005 a	4.36 ± 0.51 a	2.55 ± 0.20 a	227.7 ± 15.4 a
	25	Ambient	11.8 ± 1.1 b	0.143 ± 0.012 b	4.30 ± 0.92 a	2.68 ± 0.38 a	225.7 ± 21.8 a
	100	Ambient	10.8 ± 2.1 b	0.142 ± 0.016 b	4.48 ± 0.50 a	2.41 ± 0.26 a	218.0 ± 24.3 a
	0	Elevated	16.5 ± 1.2 a*	0.100 ± 0.021 a*	3.36 ± 0.32 a*	4. 58 ± 0.39 a*	481.6 ± 30.7 a*
	25	Elevated	14.5 ± 1.4 b*	0.088 ± 0.017 a*	3.30 ± 0.25 a*	4.44 ± 0.38 a*	454.1 ± 51.5 a*
	100	Elevated	14.7 ± 0.9 b*	0.071 ± 0.009 b*	3.31 ± 0.39 a*	4. 43 ± 0.43 a*	466.3 ± 65.2 a*
Perenne	0	Ambient	10.0 ± 1.1 a	0.103 ± 0.017 a	2.89 ± 0.24 a	3.32 ± 0.41 a	194.8 ± 39.6 a
	25	Ambient	8.5 ± 0.7 ab	0.086 ± 0.022 ab	2.84 ± 0.22 a	3.25 ± 0.37 a	186.2 ± 30.7 a
	100	Ambient	8.0 ± 0.6 b	0.067 ± 0.025 b	2.62 ± 0.42 a	3.32 ± 0.53 a	180.1 ± 33.2 a
	0	Elevated	14.8 ± 1.0 a*	0.077 ± 0.018 a*	2.25 ± 0.34 a*	5.24 ± 0.54 a*	410.3 ± 42.0 a*
	25	Elevated	12.5 ± 1.8 ab*	0.064 ± 0.013 ab*	2.24 ± 0.28 a*	5.17 ± 0.56 a*	389.6 ± 34.3 ab*
	100	Elevated	11.1 ± 0.8 b*	0.057 ± 0.007 b	2.10 ± 0.32 a*	5.07 ± 0.86 a*	357.0 ± 32.1 b*

Table 4
Cys and GSH content in *L. mutiforum* and *L. perenne* grown under ambient and elevated CO_2. Values represent mean ± SD, values of 6 measurements per treatment. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference between CO_2 treatments (within one Cd level).

	Species	Cd	Cys (nmol g^{-1} FW)		GSH (nmol g^{-1} FW)	
			Ambient	Elevated	Ambient	Elevated
Shoot	*Mutiforum*	0	19.7 ± 2.1 c	18.0 ± 1.0 c	124.8 ± 13.8 b	118.2 ± 8.4 b
		25	37.0 ± 4.0 b	31.9 ± 2.8 b*	160.1 ± 8.7 a	164.7 ± 7.2 a
		100	53.0 ± 5.2 a	51.4 ± 2.1 a	162.6 ± 13.6 a	163.7 ± 12.2 a
	Perenne	0	42.6 ± 3.2 c	33.0 ± 4.3 c*	52.7 ± 3.8 b	62.4 ± 5.3 c*
		25	69.0 ± 2.7 b	45.6 ± 4.1 b*	166.2 ± 10.2 a	206.8 ± 12.6 b*
		100	105.8 ± 8.9 a	100.0 ± 8.5 a	171.0 ± 8.1 a	228.8 ± 10.5 a*
Root	*Mutiforum*	0	6.1 ± 0.8 c	5.4 ± 0.4 c	76.7 ± 8.2 c	79.6 ± 5.4 c
		25	15.3 ± 0.9 b	12.8 ± 1.0 b*	102.1 ± 8.7 b	90.6 ± 7.2 b
		100	31.6 ± 2.0 a	21.0 ± 3.4 a*	150.4 ± 13.6 a	132.1 ± 12.2 a
	Perenne	0	18.6 ± 2.0 c	15.7 ± 1.2 c*	46.3 ± 3.8 c	48.5 ± 3.1 c
		25	26.2 ± 2.4 b	18.9 ± 1.3 b*	69.9 ± 6.2 b	69.5 ± 4.2 b
		100	30.2 ± 3.8 a	30.7 ± 2.6 a	124.2 ± 8.1 a	120.2 ± 10.5 a

3.5. MDA and SOD

MDA levels in the shoots and roots of *L. mutiforum* and *L. perenne* increased ($P < 0.01$) as Cd concentration increased ($R^2 > 0.80$ for regression equation of MDA-soil Cd concentration, $P < 0.05$) in soils (Fig. 5). For *L. mutiforum* and *L. perenne* grown on soil amended with 100 mg kg^{-1} Cd, MDA level in the shoots increased by 29.1 and 37.0% under ambient CO_2; and 25.2 and 26.9% under elevated CO_2, respectively, compared to the control Cd treatment. There was a decrease in Cd induced MDA under elevated CO_2 in comparison with the ambient CO_2 control.

Increasing Cd concentration in soil increased SOD activity in plant tissues ($R^2 > 0.87$ for regression equation of SOD-soil Cd concentration, $P < 0.05$) (Fig. 6) regardless of plant variety and CO_2 level. The SOD activity for the same level of Cd was higher under elevated CO_2 than ambient CO_2. For *L. mutiforum* and *L. perenne*, the increasing magnitude of SOD activity in the shoots was 59.1 and 61.5% under ambient CO_2; 89 and 104% under elevated CO_2, respectively.

4. Discussion

4.1. Plant growth, tolerance index and Cd accumulation

In this study, increasing Cd concentration in soil decreased the biomass of the two species irrespective of CO_2 concentration, especially for those grown on soil spiked with 100 mg Cd kg^{-1}. We observed an increase in plant biomass under elevated CO_2, and the relative increase in biomass was greater under elevated CO_2 when soils were contaminated with Cd. The tolerance index for both *Lolium* species was greater at elevated than ambient CO_2 (Table 2). These lines of evidence suggested an improvement in Cd tolerance

Table 5
PCs–Cd ratio of *L. mutiforum* and *L. perenne* grown on soils spiked with different Cd level under ambient and elevated CO_2. Values represent mean ± SD, with 6 replicates per treatment. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference ($P \leq 0.05$) between CO_2 treatments (within one Cd level).

	Cd	Shoot		Root	
		Ambient	Elevated	Ambient	Elevated
Mutiforum	25	16.50 ± 1.20 a	16.81 ± 0.81 a	0.47 ± 0.02 b	0.50 ± 0.05 b
	100	8.01 ± 0.51 b	7.77 ± 0.63 b	2.58 ± 0.32 a	2.43 ± 0.25 a
Perenne	25	1.77 ± 0.34 a	1.76 ± 0.22 a	0.40 ± 0.04 b	0.39 ± 0.04 b
	100	1.85 ± 0.14 a	1.80 ± 0.15 a	2.06 ± 0.15 a	2.24 ± 0.20 a

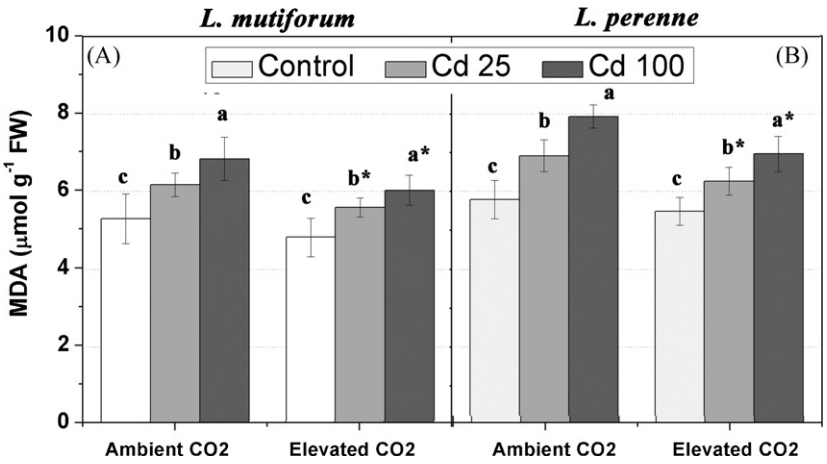

Fig. 5. MDA contents in *L. mutiforum* and *L. perenne* grown under ambient and elevated CO_2. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference between CO_2 treatments (within one Cd level).

when plants were grown on Cd contaminated soil under elevated CO_2.

Contrary to the increase in biomass, Cd concentrations in roots and shoots of both *Lolium* species were smaller under elevated CO_2 than ambient CO_2 control (Fig. 2). The decrease in Cd concentration for both *Lolium* species might result from the dilution effect, resulting from rapid plant growth, and this might be an important explanation of the increased tolerance index. Loladze [40] showed that dilution is a commonly seen phenomenon, well documented in the literature for the pot studies on the effects of elevated CO_2 on plants where nutrient supplies are limited. Previous research has shown that elevated CO_2 resulted in decreased concentrations of all essential micro-elements in plants [21,24–26,28]. Our present results showed that for heavy metals, elevated CO_2 caused a reduction in Cd concentration in the two *Lolium* species, which is in agreement with the report of Guo et al. [27]. Given expected global increases in CO_2 concentration, elevated CO_2 may help plants better survive in contaminated soil and reduce the food safety risk due to CO_2-induced reduction and dilution in heavy metal concentration.

4.2. Gas exchange and chlorophyll content

The stimulation of C_3 plant growth by elevated CO_2 was closely associated with the increase in photosynthesis [3,41–42]. For plants grown under unstressed environments, exposure to elevated CO_2 increased P_n, WUE, and C_i, and decreased G_s [43–45]. The increase in leaf photosynthesis due to the higher C_i under elevated CO_2 was sufficient to offset the decrease in leaf photosynthesis resulting from the lower G_s [45]. The stimulation of P_n by elevated CO_2, however, was different under stress conditions. For example, ozone and drought tended to enhance the response whereas low N reduced the responses [44,46]. In general, decreases in G_s under elevated CO_2 were exacerbated by low N and drought stress [44,46,47]. In our present experiment, the P_n, WUE and C_i values in leaves of *L. perenne* and *L. multiflorum* under elevated CO_2 were higher than under ambient CO_2, while the G_s and E were lower under elevated CO_2 than ambient CO_2 for the same level of Cd treatment (Table 3). This indicated that elevated CO_2 increased P_n of the two *Lolium* species irrespective of Cd treatment, although G_s and E decreased to some degree. We showed that the stimulation of P_n in the two *Lolium* species under elevated CO_2 caused increased growth (Table 2). For plants grown at the same level of CO_2, Cd stress reduced the stimulation of photosynthesis by elevated CO_2 and exacerbated the decrease in G_s (Table 3). Enhanced photosynthesis under elevated CO_2 could be favorable for ryegrass growth and development, and beneficial to alleviate the negative effects of Cd stresses to a certain extent. Similar results were reported in Li et al. [48] who found that enhanced photosynthesis under elevated CO_2 alleviate the negative effects of drought stresses in cucumber.

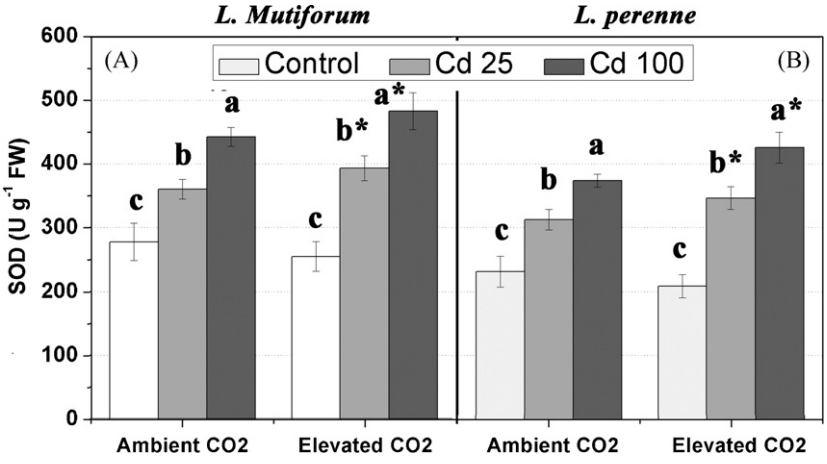

Fig. 6. SOD activities in *L. mutiforum* and *L. perenne* grown under ambient and elevated CO_2. Different letters indicate significant differences ($P \leq 0.05$) between Cd levels (within one CO_2 treatment level), an asterisk indicates significant difference between CO_2 treatments (within one Cd level).

A survey of published literature revealed that little information is available concerning the effect of metal and elevated CO_2 on chlorophyll a and b contents. Our present study showed that with increasing Cd concentration in soils, chlorophyll a and b contents in both of the tested *Lolium* species decreased regardless of CO_2 concentration (Fig. 1). When compared with the ambient CO_2 level, plants grown under elevated CO_2 on soils not amended with Cd or amended at the lowest level of Cd (i.e. 25 mg kg^{-1} Cd) showed no statistically detectable variation in chlorophyll a and b contents, but for those grown on soils amended with a high level of Cd (i.e. 100 mg kg^{-1} Cd), chlorophyll a and b contents were higher under elevated than under ambient CO_2. This suggested that elevated CO_2 improved plant ability to absorb and utilize more light energy at high levels of Cd, and as result, enhances photosynthesis, which provides a partial explanation for the biomass stimulation under elevated CO_2 at higher Cd levels.

4.3. Oxidative stress

In the present study, we showed that MDA concentration increased under Cd stress regardless of CO_2 concentration (Fig. 5), showing that increasing Cd enhanced membrane lipid peroxidation in the two evaluated *Lolium* species. For the same level of Cd treatment, a clear decrease in MDA content under elevated CO_2 was noted, suggesting that elevated CO_2 could help alleviate Cd induced oxidative damage.

ROS can be efficiently eliminated by non-enzymatic (glutathione, ascorbate, α-tocopherol and carotenoids) and enzymatic defense systems, such as SOD, and peroxidase (POD) [12,49–51]. These ROS-eliminating materials protect plants against oxidative damage [51]. Superoxide dismutase, the first major antioxidant enzyme in the detoxifying process [52], converts superoxide radicals to H_2O_2 at a very fast rate [53]. Carotenoids are considered to be antioxidants against free radicals since they can remove oxygen from the excited chlorophyll–oxygen complex [54]. GSH is a key factor in defense against hydrogen peroxide. It is an important intermediate material during the cycle of GSH-GSSH and it can effectively remove H_2O_2 [55,56]. The increase in antioxidant compounds and related enzyme activities under heavy-metal stress can help plants to better survive stressed environments. We showed that elevated CO_2 increased SOD activities in leaves of two *Lolium* species and GSH concentration in leaves of *L. perenne* (Fig. 6 and Table 4). This evidence suggested that elevated CO_2 could alleviate Cd induced oxidative damage by increasing the expression of genes related to production of antioxidant molecules and antioxidant enzymes activities. Similar studies can be found in the literature, showing the effect of elevated CO_2 on oxidative damage caused by other environmental stressed factors such as salt, drought and high temperature [57,58].

4.4. Cd and PCs

Plants have evolved numerous defense systems that can be activated to deal with against Cd stress [59]. Of the detoxification pathways activated in plants under Cd stress, phytochelatin synthesis is of prime importance for allowing plants to tolerate and survive in Cd-stressed environments, and cysteine and glutathione act as the most important precursors for PC synthesis [60,61]. It is now known that PC synthesis is related to Cd content in plant tissues [62,63], and includes chelation of Cd by PCs and compartmentalization of the PCs–Cd compounds into the vacuoles [64,65]. In our study, the decrease of PC under elevated CO_2 was probably due to the lower Cd concentration, compared with the ambient CO_2 control. Plants grown on Cd spiked soil under elevated CO_2 had lower Cd concentrations than the ambient CO_2 control, and consequently, less PC was needed to complex Cd in plant tissues for Cd detoxification in the case of elevated CO_2. This may explain why plants exposed to the same Cd level and grown under elevated CO_2 had greater biomass increases than those under ambient CO_2 (see Table 2). Since the synthesis of PCs is energy expensive and requires consumption of significant growth limiting elements such as sulfur and nitrogen [64], less synthesis of PCs under elevated CO_2 might save more energy to the benefit of the plant growth and, consequently, the plants might grow much better under conditions of elevated CO_2 and Cd stress. This may provide a partial explanation for the alleviation of Cd toxicity for Cd-stressed plants growing under elevated CO_2, and the plant biomass increase as well. More work is needed to investigate this phenomenon. It should be noted that phytochelatin concentration in the shoots of *L. mutiforum* is much higher than that of *L. perenne* for the same Cd level, but the Cd concentration in the shoots of both plant species is generally within the same range. It was speculated that PC synthesis is related not only to Cd concentration in plant tissues, but also plant genotype (species).

As an essential macronutrient, sulfur plays an important role in regulation of plant growth and development [65]. It is now known that S metabolism tightly regulates the biosynthesis of PCs in plants. Phytochelatin synthesis involves regulation of sulfate assimilation-reduction and GSH metabolism when plants are stressed by Cd [66]. The addition of S may enhance the stability of the PC–Cd^{2+} complex and the tolerance of plants to Cd [67]. Our results indicated that sulfur concentration in the roots and shoots of both *Lolium* species increased with increasing Cd concentration in soil regardless of CO_2 concentration, suggesting that with increasing Cd concentration in soil both S assimilation and PC synthesis were enhanced, as shown by the increased PC concentration in the plant tissues. In the case of elevated CO_2, sulfur concentration in the roots and shoots of both species was lower under elevated than under ambient CO_2. This reduction relationship correlated with PC concentration, suggesting that the decrease in sulfur in the tissues of Cd-stressed plants under elevated CO_2 was closely related to reduction in PC concentration.

Unfortunately, very limited data are available in the literature regarding the dilution phenomenon, especially regarding possible relationships between elemental composition and PC concentration. Our present experiment showed that elevated CO_2 decreased the concentration of two-elements (Cd and S) and phytochelatins in plant tissues, in comparison with the CO_2 control. Although the magnitude varied substantially, the synchronous changes in elemental concentration and phytochelatin levels in plant tissues might partially explain the mechanism by which elevated CO_2 reduced Cd toxicity in both *Lolium* species when grown under Cd stress.

5. Conclusions

The results of this study showed that elevated CO_2 increased biomass production and tolerance index values of *L. multiflorum* and *L. perenne* compared to the ambient CO_2 control conditions, suggesting reduced Cd toxicity for plants grown on Cd contaminated soils under elevated CO_2. The stimulation of growth under elevated CO_2 was closely associated with the increase in photosynthetic rate, perhaps related to the increase in chlorophyll a and b contents. For both *Lolium* species grown on soils contaminated with various levels of Cd, MDA content and element (Cd and S) concentrations were lower but the photosynthetic pigment levels and SOD activity in the leaves were higher under elevated CO_2 than under ambient CO_2. These findings suggested that elevated CO_2 increased antioxidant gene expression and enzyme activities and therefore, alleviated Cd induced oxidative damage. PC synthesis was only related to Cd content in plant tissues, but elevated CO_2 did not change the PC:Cd

ratio. This leads us to speculate that plant biomass increase and the alleviation of Cd toxicity for Cd-stressed plants under elevated CO_2 was more dependent on increased photosynthesis and enhanced antioxidant capacity than PC synthesis.

The knowledge gained in this investigation constitutes an important advancement in our understanding of the interaction between soil metal contamination and atmospheric CO_2 concentration with regard to plant ability to grow and remove the Cd from soils. However, similar research with a wider range of plant species is required to provide an in-depth understanding of the mechanisms involved, and more work needs to be done to investigate the potential applications of these findings to food safety or for phytoremediation practices.

Acknowledgements

This work was supported by Central Public Research Institute Basic Funds for Research and Development (Agro-Environmental Protection Institute, Ministry of Agriculture).

References

[1] IPCC (Intergovernmental Panel on Climate Change), Summary for Policymakers, Climate Change 2007: The Physical Science Basis, Available on: http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-spm.pdf, 2007.
[2] Z. Tuba, H.K. Lichtenthaler, Long-term acclimation of plants to elevated CO_2 and its interaction with stresses, Ann. N. Y. Acad. Sci. 1113 (2007) 135–146.
[3] S.P. Long, E.A. Ainsworth, A.D.B. Leakey, J. Nosberger, D.R. Ort, Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentration, Science 312 (2006) 1918–1921.
[4] B.A. Kimball, K. Kobayashi, M. Bindi, Responses of agricultural crops to free-air CO_2 enrichment, Adv. Agron. 77 (2002) 293–368.
[5] J. Li, J.M. Zhou, Z.Q. Duan, Effects of elevated CO_2 concentration on growth and water usage of tomato seedlings under different ammonium/nitrate ratios, J. Environ. Sci. 19 (2007) 1100–1107.
[6] O. Urban, Physiological impacts of elevated CO_2 concentration ranging from molecular to whole plant responses, Photosynthetica 4 (2003) 9–20.
[7] A. Rogers, D.J. Allen, P.A. Davey, P.B. Morgan, E.A. Ainsworth, C.J. Bernacchi, Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their lifecycle under Free-Air Carbon Dioxide Enrichment, Plant Cell Environ. 27 (2004) 449–458.
[8] G.J. Wagner, Accumulation of cadmium in crop plants and its consequences to human health, Adv. Agron. 51 (1993) 173–212.
[9] Y. Liu, X. Wang, G. Zeng, D. Qu, J. Gu, M. Zhou, L. Chai, Cadmium-induced oxidative stress and response of the ascorbate-glutathione cycle in *Bechmeria nivea* (L.), Gaud, Chemosphere 69 (2007) 99–107.
[10] Y.Z. Huang, Y. Hu, Y.X. Liu, Combined toxicity of copper and cadmium to six rice genotypes (*Oryza sativa* L.), J. Environ. Sci. 21 (2009) 647–653.
[11] F.Q. Zhang, H.X. Zhang, G.P. Wang, L.L. Xu, Z.G. Shen, Cadmium-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes, J. Hazard. Mater. 168 (2009) 76–84.
[12] Y. Ekmekçi, D. Tanyolaç, B. Ayhan, Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars, J. Plant Physiol. 165 (2008) 600–611.
[13] I.V. Seregin, V.B. Ivanov, Physiological aspects of cadmium and lead toxic effects on higher plants, Russ. J. Plant Physiol. 48 (2001) 523–544.
[14] X.X. Zhang, C.J. Li, Z.B. Nan, Effects of cadmium stress on growth and antioxidative systems in *Achnatherum inebrians* symbiotic with *Neotyphodium gansuense*, J. Hazard. Mater. 175 (2010) 703–709.
[15] N. Geissler, S. Hussin, H.W. Koyro, Elevated atmospheric CO_2 concentration ameliorates effects of NaCl salinity on phytosynthesis and leaf structure of *Aster tripolium* L, J. Exp. Bot. 60 (2009) 137–151.
[16] N.V. Laspina, M.D. Groppa, M.L. Tomaro, M.P. Benavides, Nitric oxide protects sun flower leaves against Cd-induced oxidative stress, Plant Sci. 169 (2005) 323–330.
[17] J. Flexas, H. Medrano, Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stamotal limitations revisited, Ann. Bot. 89 (2002) 183–189.
[18] C.L.M. Sgherri, M.F. Quartacci, M. Menconi, A. Raschi, F. Navari-Izzo, Interactions between drought and elevated CO_2 on alfalfa plants, J. Plant Physiol. 152 (1998) 118–124.
[19] A. Donnelly, J. Craigon, C.R. Black, J.J. Colls, G. Landon, Does elevated CO_2 ameliorate the impact of O_3 on chlorophyll content and photosynthesis in potato (*Solanum tuberosum*)? Physiol. Plant. 111 (2001) 501–511.
[20] D.B. Lobell, C.B. Field, Estimation of the carbon dioxide (CO_2) fertilization effect using growth rate anomalies of CO_2 and crop yields since 1961, Global Change Biol. 14 (2008) 39–45.
[21] P. Högy, A. Fangmeier, Atmospheric CO_2 enrichment affects potatoes: 1. Aboveground biomass production and tuber yield, Eur. J. Agron. 30 (2009) 78–84.
[22] S.R. Tang, L. Xi, J.M. Zheng, H.Y. Li, Response to elevated CO_2 of Indian mustard and sunflower growing on copper contaminated soil, Bull. Environ. Contam. Toxicol. 71 (2003) 988–997.
[23] H.B. Wu, S.R. Tang, X.M. Zhang, J.K. Guo, Z.G. Song, S. Tian, D.L. Smith, Using elevated CO_2 to increase the biomass of a *Sorghum vulgare* × *Sorghum vulgare* var. sudanense hybrid and *Trifolium pratense* L. and to trigger hyperaccumulation of cesium, J. Hazard. Mater. 170 (2009) 861–870.
[24] J.M. Zheng, H.Y. Wang, Z.Q. Li, S.R. Tang, Z.Y. Chen, Using elevated carbon dioxide to enhance copper accumulation in *Pteridium revolutum*, a copper-tolerant plant, under experimental conditions, Int. J. Phytoremediat. 10 (2008) 161–172.
[25] H.X. Jia, H.Y. Guo, Y. Yin, Q. Wang, Q. Sun, X.R. Wang, J.G. Zhu, Responses of rice growth to copper stress under free-air CO_2 enrichment (FACE), Chinese Sci. Bull. 52 (2007) 2636–2641.
[26] L.X. Yang, Y.L. Wang, G.C. Dong, H. Gu, J.Y. Huang, J.G. Zhu, H.J. Yang, G. Liu, Y. Han, The impact of free-air CO_2 enrichment (FACE) and nitrogen supply on grain quality of rice, Field Crops Res. 102 (2007) 128–140.
[27] H.Y. Guo, H.X. Jia, J.G. Zhu, X.R. Wang, Influence of the environmental behavior and ecological effect of cropland heavy metal contaminants by CO_2 enrichment in atmosphere, Chinese J. Geochem. 25 (2006) 212.
[28] S.P. Seneweera, J.P. Conroy, Growth, grain yield and quality of rice (*Oryza sativa* L.) in response to elevated CO_2 and phosphorus nutrition, Soil Sci. Plant Nutr. 43 (1997) 1131–1136.
[29] M. Lieffering, H.Y. Kim, K. Kobayashi, M. Okada, The impact of elevated CO_2 on the elemental concentrations of field-grown rice grains, Field Crops Res. 88 (2004) 279–286.
[30] C.W. Jin, S.T. Du, W.W. Chen, G.X. Li, Y.S. Zhang, S.J. Zheng, Elevated carbon dioxide improves plant Fe nutrition through enhancing the Fe-deficiency-induced responses under Fe-limited conditions in tomato (*Lycopersicon esculentum* Mill), Plant Physiol. 150 (2009) 272–280.
[31] F. Marseille, C. Tiffreau, A. Laboudigue, P. Lecomte, Impact of vegetation on the mobility and bioavailability of trace elements in a dredged sediment deposit: a greenhouse study, Agronomie 20 (2000) 547–556.
[32] Z. Kiss, É. Lehoczky, T. Németh, Testing of available heavy metal content of soils in long-term fertilization trials with ryegrass (*Lolium perenne* L.), Acta Biol. Szeged. 46 (2002) 107–108.
[33] A.J. Palazzo, T.J. Cary, S.E. Hardy, C.R. Lee, Root growth and metal uptake in four grasses grown on zinc-contaminated soils, J. Environ. Qual. 32 (2003) 834–840.
[34] M. Arienzo, P. Adamo, V. Cozzolino, The potential of *Lolium perenne* for revegetation of contaminated soil from a metallurgical site, Sci. Total Environ. 319 (2004) 13–25.
[35] R. Caggiano, M. D'Emilio, M. Macchiato, M. Ragosta, Heavy metals in ryegrass species versus metal concentrations in atmospheric particulate measured in an industrial area southern Italy, Environ. Monit. Assess. 102 (2005) 67–84.
[36] J. Pichtel, C.A. Salt, Vegetative growth and trace metal accumulation on metal-liferous waste, J. Environ. Qual. 27 (1998) 618–624.
[37] H.K. Lichtenthaler, A.R. Wellburn, Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, Biochem. Soc. Trans. 603 (1983) 591–592.
[38] W.F. Beyer, I. Fridovich, Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions, Anal. Biochem. 161 (1987) 559–566.
[39] R.L. Heath, L. Packer, Photoperoxidation in isolated chloroplasts 1. Kinetics and stoichiometry of fatty acid peroxidatoin, Arch. Biochem. Biophys. 125 (1968) 189–198.
[40] I. Loladze, Rising atmospheric CO_2 and human nutrition: toward globally imbalanced plant stoichiometry? Trends Ecol. Evol. 17 (2002) 457–461.
[41] I. Aranjuelo, J.J. Irigoyen, P.P. érez, R. Martínez-Carrasco, M. Sánchez-Díaz, Response of nodulated alfalfa to water supply, temperature and elevated CO_2: productivity and water relations, Environ. Exp. Bot. 55 (2006) 130–141.
[42] G. Erice, J.J. Irigoyen, P. Pérez, R. Martínez-Carrasco, M. Sánchez-Díaz, Effect of elevated CO_2, temperature and drought on dry matter partitioning and photosynthesis before and after cutting of nodulated alfalfa, Plant Sci. 170 (2006) 1059–1067.
[43] P.S. Curtis, X. Wang, A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology, Oecologia 113 (1998) 299–313.
[44] E.A. Ainsworth, S.P. Long, What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2, New Phytol. 165 (2005) 351–372.
[45] C.J. Bernacchi, P.B. Morgan, D.R. Ort, S.P. Long, The growth of soybean under free air [CO_2] enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity, Planta 220 (2005) 434–446.
[46] A. Robredo, U. Pérez-López, H.S. de la Maza, B. González-Moro, M. Lacuesta, A. Mena-Petite, A. Muñoz-Rueda, Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis, Environ. Exp. Bot. 59 (2007) 252–263.
[47] R.F. Sage, Acclimation of photosynthesis to increasing atmospheric CO_2: the gas-exchange perspective, Photosynth. Res. 39 (1994) 351–368.
[48] Q.M. Li, B.B. Liu, Y. Wu, Z.Y. Zou, Interactive effects of drought stresses and elevated CO_2 concentration on photochemistry efficiency of cucumber seedlings, J. Integr. Plant Biol. 50 (2008) 1307–1317.
[49] S. Baccouch, A. Chaoui, E. El Ferjani, Nickel-induced oxidative damage and antioxidant responses in *Zea mays* shoots, Plant Physiol. Biochem. 36 (1998) 689–694.
[50] S. Baccouch, A. Chaoui, E. El Ferjani, Nickel toxicity induced oxidative damage in *Zea mays* roots, J. Plant Nutr. 24 (2001) 1085–1097.

[51] O. Blokhina, E. Virolainen, K.V. Fagerstedt, Antioxidants, oxidative damage and oxygen deprivation stress: a review, Ann. Bot. 91 (2003) 179–194.
[52] D. Tanyolaç, Y. Ekmekçi, Ş. Ünalan, Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper, Chemosphere 67 (2007) 89–98.
[53] P.L. Gratão, A. Polle, P.J. Lea, R.A. Azevedo, Making the life of heavy-metal stressed plants a little easier, Funct. Plant Biol. 32 (2005) 481–494.
[54] A.J. Young, The photoprotective role of carotenoids in higher plants, Physiol. Plant 183 (1991) 702–703.
[55] G. Kocsy, Role of glutathione in plants under abiotic stress conditions, in: S.S. Purohit, S. Agarwal, S.P. Vyas, H.S. Gehlot (Eds.), Annual Review of Plant Physiology, India and Printed at Anees Offset Press, New Delhi, India, 1999, pp. 215–225.
[56] G. Kocsy, G. Galiba, C. Brunold, Role of glutathione in adaptation and signalling during chilling and cold acclimation in plants, Physiol. Plant 113 (2001) 158–164.
[57] J.S. Lin, G.X. Wang, Doubled CO_2 could improve the drought tolerance better in sensitive cultivars than in tolerant cultivars in spring wheat, Plant Sci. 163 (2002) 627–637.
[58] U. Pérez-López, A. Robredo, M. Lacuesta, C. Sgherri, A. Muñoz-Rueda, F. Navari-Izzo, A. Mena-Petite, The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO_2, Physiol. Plant. 135 (2009) 29–42.
[59] M. Pál, E. Horvath, T. Janda, E. Paldi, G. Szalai, Physiological changes and defense mechanisms induced by cadmium stress in maize, Int. J. Plant Sci. 169 (2006) 239–246.
[60] T. Rausch, A. Wachter, Sulfur metabolism: a versatile platform for launching defense operations, Trends Plant Sci. 10 (2005) 503–509.
[61] S. Clemens, Evolution and function of phytochelatin synthases, J. Plant Physiol. 163 (2006) 319–332.
[62] W.G. Keltjens, M.L. van Beusichem, Phytochelatins as biomarkers for heavy metal stress in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.): combined effects of copper and cadmium, Plant Soil 203 (1998) 119–126.
[63] J.P. Stolt, F.E.C. Snellerc, T. Bryngelsson, T. Lundborg, H. Schat, Phytochelatin and cadmium accumulation in wheat, Environ. Exp. Bot. 49 (2003) 21–28.
[64] Y.P. Tong, R. Kneer, Y.G. Zhu, Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation, Trends Plant Sci. 9 (2004) 7–9.
[65] S. Clemens, D. Peršoh, Multi-tasking phytochelatin synthases, Plant Sci. 177 (2009) 266–271.
[66] W.H.O. Ernst, Sulfur metabolism in higher plants: potential for phytoremediation, Biodegradation 9 (1998) 311–318.
[67] M.C. David, L.T. Herminia, H.N. Andrea, M.S. Rafael, Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants, FEMS Microbiol. Rev. 29 (2005) 653–671.