Journal of Hazardous Materials 170 (2009) 861–870

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

# 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

Huibin Wu[a,b,c], Shirong Tang[a,b,*], Ximei Zhang[a,b], Junkang Guo[a,b], Zhengguo Song[a,b], Shuai Tian[a,b], Donald L. Smith[d]

[a] Centre for Research in Ecotoxicology and Environmental Remediation, Institute of Agro-Environmental Protection, The Ministry of Agriculture, Tianjin 300191, PR China
[b] Open Key Laboratory of Agro-environment and Agro-product Safety of the Ministry of Agriculture, Tianjin, 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 28 March 2009
Received in revised form 8 May 2009
Accepted 12 May 2009
Available online 21 May 2009

*Keywords:*
Elevated $CO_2$
Cesium
Hyperaccumulation
*Sorghum vulgare* × *Sorghum vulgare var. sudanense* hybrid
*Trifolium pratense* L.

ABSTRACT

The most important challenge to use phytoremediation is how to improve its efficiency by increasing the accumulation of metals in plants, or by improving key plant biological traits that should enhance metal uptake. In this paper, we used open-top chambers to investigate the effects of elevated $CO_2$ (860 $\mu L L^{-1}$) on biomass and Cs uptake by a *Sorghum vulgare* × *Sorghum vulgare var. sudanense* hybrid and *Trifolium pratense* L. growing on soils spiked with various levels of cesium (0, 300, 1500 and 3000 mg Cs $kg^{-1}$). The results showed that elevated $CO_2$ not only increased aboveground biomass of the *Sorghum* and *Trifolium* species by 32–111%, and by 8–11%, respectively, compared to the ambient $CO_2$ treatment, but also caused more accumulation of Cs by *Sorghum* species (up to 73%) than *Trifolium* species (up to 43%). It was speculated that the increase in biomass and the improvement in Cs accumulation ability at elevated $CO_2$ could be related to lowered soil pH values, and changes in number and kind of microorganisms in the rhizospheres of the two tested species. This is the first report of a link among elevated $CO_2$, increased biomass and hyperaccumulation of Cs by *Sorghum* and *Trifolium* species.



## 1. Introduction

With the development of the nuclear industry, soil contamination with radionuclides, especially with strontium and cesium, is receiving more attention [1–3]. A number of technologies have been developed for treating radionuclide contaminated soils, including excavation, soil washing, leaching with chelating agents, flocculation and reverse osmosis–ultrafiltration [4,5]. These methods are not suitable, however, for treating large-scale contamination of soils with low-doses of radionuclides, due to their high-cost, and difficulty of implementation at the sites. By comparison, phytoremediation technologies have several advantages over the above mentioned more conventional approaches, such as in situ, environmental friendly, cost-effective, and aesthetically pleasing. Phytoremediation also has some disadvantages like toxicity of pollutants to the plants, risks posed to consumers of plants, taking a long time, dependence on season and on root systems of remediating plants. Disposal of contaminated parts of the plants after harvest is an unsolved problem [6,7], especially for remedial plant materials containing radioactive isotopes. Despite these, more attention has been given to fundamental aspects of this technology in recent years, including screening of more hyperaccumulator plants (being capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in nonaccumulators [8,9]), plant growth enhancing mechanisms, structural and functional changes in rhizosphere microbial communities in contaminated environments, as well as plant–bacteria interactions during phytoremediation [10,11]. To increase the feasibility of this technology, we need to improve its efficiency by increasing the accumulation of radionuclides in plants, or by modifying the plant's biological traits (growth rate, the growth cycle, etc.) [12]. Our previous studies showed that elevated $CO_2$ not only increased Indian mustard (*Brassica juncea* L. Czern.) and sunflower (*Helianthus annuus* L.) biomass, but also triggered hyperaccumulation of copper by the two plants [13]. This caused us to speculate that $CO_2$ enrichment might increase the biomass of plants growing on radionuclide contaminated soils and cause increased accumulation of the radionuclide contaminants from the soils into the plants,

* Corresponding author at: Centre for Research in Ecotoxicology and Environmental Remediation, Institute of Agro-Environmental Protection, The 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 © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.05.069

in a similar way. However, to our knowledge, there has been little research conducted in this area.

Ongoing combustion of fossil fuels leads to increases in the atmospheric carbon dioxide concentration, contributing to global warming [14]. It is known that elevated $CO_2$ enhances plant growth on non-contaminated soils in terms of plant biomass [15–17], water and nutrient use efficiency [18,19], photosynthesis rate and intensity [20–22], and rhizosphere microecological environment characteristics [23,24]. A survey of the literature indicates that few studies have investigated the effect of elevated carbon dioxide on plant uptake of pollutants from contaminated environments, especially environments contaminated with radionuclides [25]. Since the behavior of radionuclides in the environment follows that of stable elements and plants do not discriminate between stable and radioactive Cs isotopes, the understanding of the fate of stable Cs in the environment could guide us to understand the behavior of radioactive isotopes [26–28], and allow us to predict the long-term transfer of radioactive isotopes in plant–soil system [29]. In this paper, we used open-top chambers (OTC) to investigate the effects of elevated $CO_2$ (860 $\mu L\,L^{-1}$) on the growth and development of a *Sorghum vulgare* × *Sorghum vulgare var. sudanense* hybrid and *Trifolium pratense*, their associated uptake of stable Cs, and important rhizosphere characteristics of the two tested species, including microorganism populations and pH values. The main objective of the current work was to assess the possibility of using elevated $CO_2$ as a gas fertilizer to increase the biomass of the *S. vulgare* × *S. vulgare var. sudanense* hybrid and *T. pratense*, and cause more accumulation of Cs by the two species, making them more effective as phytoremediation agents. To our knowledge, this is the first report on the utilization of elevated $CO_2$ as a gas fertilizer to induce phytoextraction of cesium from contaminated soils.

## 2. Materials and methods

### 2.1. Tested plant species

*S. vulgare* × *S. vulgare var. sudanense* hybrids provide high-quality forage with high biomass, are tolerant to drought and heat, and have potential for phytoremediation because they are easy to cultivate and produce large amounts of biomass. *T. pratense* is also high-quality forage grown widely in northern and central parts of China. These species were selected for this study because the *Sorghum* species is a $C_4$ plant and *Trifolium* species is a $C_3$ plant. Seeds were obtained from Beijing Feng-Nen Agricultural Technology Limited Company, PR China.

### 2.2. The tested soil and its preparation for this study

The soil used in this study was collected from a long-term experimental rice field at Shenyang Agricultural University, Liaoning Province (PR China). The Cs concentrations and physical and chemical properties of the soil are shown in Table 1. The methods for soil property determination and elemental analysis were described previously [13]. The soil is a sandy, silty loam (FAO classification) with high levels of organic matter (15.39%) and a pH of 5.53.

Fresh soil was sieved to pass a 3-mm mild sieve and kept in the dark before use. Two thousand grams of dry soil was placed in each plastic pot (15 cm diameter and 12 cm height). Cesium chloride (CsCl) was artificially added to soil to give four levels: 0, 300, 1500, and 3000 mg Cs $kg^{-1}$. The treatment with no Cs added acted as the control. Fifty mL of solution containing the required concentrations of Cs was added to, and thoroughly mixed into, the soil. The mixed soil was then transferred into each pot, under which a suitable size plastic saucer was placed. Water was added to each pot until the maximum water holding capacity (WHCmax) of the soil was reached. The soil was then kept in darkness, at room temperature, while it equilibrated; this required 2 months. Once the water in pots had naturally evaporated to soil dryness (moisture content less than 10%), the pot was again watered until it reached WHCmax. There were eight replicates of each Cs treatment. Four of the replicate pots were placed under the $CO_2$ control condition, and the other four under elevated $CO_2$.

**Table 1**
Physical and chemical characteristics of the soil used in this study.

| Analysis items | |
|---|---|
| Total N (g $kg^{-1}$) | 0.92 |
| Total P (g $kg^{-1}$) | 0.46 |
| Total K (g $kg^{-1}$) | 19.00 |
| Available K (mg $kg^{-1}$) | 98.26 |
| Cs concentration in soil ($\mu$g $kg^{-1}$) | 7.79 |
| Organic matter (g $kg^{-1}$) | 15.39 |
| CEC[a] (cmol $kg^{-1}$ soil) | 17.24 |
| pH ($H_2O$) | 5.53 |
| Particle size distribution[b] (wt%) | |
| Clay | 17.9 |
| Silt | 29.9 |
| Sand | 52.2 |
| Soil texture | Sandy silty loam |

[a] Cation exchange capacity.
[b] Sand, 0.02–2 mm; silt, 0.002–0.02 mm; clay, <0.002 mm.

Before planting, pots were fertilized with 200 mg $kg^{-1}$ of N, 100 mg $kg^{-1}$ of P, and 200 mg $kg^{-1}$ of K, watered to WHCmax, and subsequently allowed to equilibrate for two more weeks.

### 2.3. $CO_2$ purity and structure of the open-top chambers

The pot experiments were performed in six naturally lit chambers (aluminium frame and clear glass walls, 3 m diameter × 5.2 m high). Three of the chambers were ventilated with ambient air ($[CO_2] = 361\ \mu L\,L^{-1}$), the three others with ambient plus 500 $\mu L\,L^{-1}$ $CO_2$ air. $CO_2$ used in this study was supplied in a gas cylinder which was purchased from the Tianjin Saint-Nan Gases Supply Limited Company and had a purity of 99.9%. It was added to each chamber with a blower, through PVC pipes from outside that was connected to the Control Centre. The $CO_2$ flow rate was controlled automatically by a gas flow-meter controlled by the computer in the Control Centre. $CO_2$ was metered through the PVC pipes to maintain a canopy $CO_2$ level of ca. 860 $\mu L\,L^{-1}$ in each $CO_2$ treated chamber. The $CO_2$ level was monitored with an infrared gas analyzer (GMP343, VAISALA, Finland, purchased from Beijing Yi Sheng Tai He Technology Limited Company). For the non-enriched control, the canopy $CO_2$ level averaged 361 $\mu L\,L^{-1}$.

$CO_2$ concentration, temperature and humidity measurements were recorded automatically, at 1 min intervals, throughout each day from the beginning to end of the experiments (Table 2). Each chamber was fitted with a fan operated at an air speed of approximately 3500 L $min^{-1}$. The fan was fixed vertically in the center of the chamber to assure that $CO_2$ concentrations were homogeneous. Prior to the imposition of the $CO_2$ treatments, the growth chambers were tested for gas homogeneity and for $CO_2$–time relationships.

### 2.4. Conditions of plant growth

*Sorghum* and *Trifolium* seeds were surface sterilized by soaking in 1% NaClO for 10 min, rinsed in deionized water for five to seven times, and then directly sown in the soils at five per pot. Seventeen days after germination, the seedlings were thinned to 1 per pot and then allowed to grow in the open-top chambers for 47 more days. Throughout the experimental period, soils were kept at 60–70% WHC using deionized water. The pot experiments were conducted

**Table 2**
Statistical analysis of parameters measured during the pot experiments.

| Chamber no. | Temperature (°C) | Humidity (%) | $CO_2$ concentrations ($\mu L L^{-1}$) | Photosynthetic flux density ($\mu mol\ m^{-2}\ s^{-1}$) | |
|---|---|---|---|---|---|
| | | | | Cloudy | Shining |
| 1 | 28.5 ± 4.3 | 43.17 ± 8.83 | 876 ± 51 | 380 ± 168 | 713 ± 295 |
| 3 | 30.4 ± 3.9 | 42.62 ± 8.99 | 862 ± 51 | 410 ± 179 | 754 ± 306 |
| 5 | 27.7 ± 4.0 | 43.05 ± 8.66 | 853 ± 50 | 391 ± 180 | 710 ± 290 |
| 2 | 28.8 ± 4.2 | 40.29 ± 8.69 | 359 ± 17 | 394 ± 173 | 707 ± 291 |
| 4 | 30.0 ± 3.8 | 41.68 ± 8.97 | 363 ± 19 | 421 ± 179 | 726 ± 272 |
| 6 | 28.9 ± 4.0 | 41.60 ± 8.75 | 361 ± 15 | 399 ± 167 | 716 ± 277 |

Each value represents mean ± SD.

in OTCs at ambient temperature (25–30 °C) and illuminated with natural light (see Table 2). Relative humidity and temperature data during the experiment are shown in Table 2.

To minimize pot position effects inside the chambers and chamber-to-chamber effects, pots were randomly switched among chambers every 7 days and placed in a new, randomly selected position within each chamber, at each move. The pots were watered equally two or three times per week, to avoid water stress, but were never watered to the point of drainage, to avoid leaching.

$CO_2$ enrichment was applied to plants from 09:00 to 14:00 h on sunny days (no cloud cover). Total $CO_2$ treatment time was 47 days.

### 2.5. Harvest and analysis of Cs in plants and soil

After 47 days of growth, the plants in the chambers were harvested (2 cm above the soil line) and separated into shoots and roots. Individual plant samples from each pot were kept separate for further treatment. Fresh plant samples were dried in an oven at 65 °C for 72 h, then weighed and pulverized to a homogeneous powder with a stainless steel cutter blender (IKA T250D, Germany). Plant roots were removed from the pots, and soil loosely adhering to the roots was gently shaken off, back into the pot. Soils adhering to root (i.e. rhizosphere soil) were taken off with a brush. Rhizosphere soils were stored in a freezer (−4 °C) for future use in the determination of pH value and estimation of microbial biomass.

Plant samples (0.5 g) or air dried soil samples (1.0 g) were digested overnight in 2 mL of concentrated $HNO_3$ at 120 °C, on a hot plate, and then were dissolved in 5 mL of $HNO_3$–$HCl_4$ (1:1, v/v) mixed acid and digested at 220 °C overnight. The samples were filtered through Whatman No. 1 filter paper and then diluted to 25 mL with 5% $HNO_3$. The Cs 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 made up from commercial standard solutions of 1000 mg $L^{-1}$ (Sigma). The reliability of the digestion and analytical procedure was verified by including blanks and standard soil samples with every batch of sample digest, as part of the QA/QC protocol. Reagent blanks and at least two replicates of all samples were used to ensure accuracy and precision in the analysis. The AAS analysis was conducted based on US EPA Method 3050B [30]. The detection limits were 0.20 $\mu g\ L^{-1}$ for Cs. The sample extraction and analytical procedures were accompanied by running blanks and spiked standards. All samples were analyzed in three independent replicates, to ascertain the reproducibility of the analyses. Analyses of test samples replicated better than 5%. To check the analytical accuracy, Cs determination for some replicated plant and soil samples was also performed by ICP-MS (Agilent 7500a), equipped with a Babington nebulizer, a glass double path spray chamber and a standard quartz torch. The agreement between the analytical results determined by AAS and ICP-MS for Cs was satisfactory with the analytical errors being less than 10%.

### 2.6. Determination of microbial biomass

The bacterial and fungal populations of rhizosphere soil were determined by a soil dilution plate method using common laboratory nutrient agar (NA) for bacteria, potato dextrose agar medium (PDA) for fungi, and Gause's synthetic isolation agar (Difco) for actinomycetes [31]. A series of soil samples diluted in saline to different concentrations were spread on multiple plates and incubated at 30 °C. Bacterial, actinomycete, and fungal colonies with distinctive morphologies were then counted.

### 2.7. Statistical analysis

Statistical analysis was performed using the SPSS software program (SPSS Inc., Chicago, IL, Version 16.0). The data were analyzed with a two-way analysis of variance (ANOVA) approach, i.e. $CO_2$ treatment (two levels) and Cs treatment (four levels). Differences between treatments were taken to be statistically significant when they occurred at $p < 0.05$. When this occurred a Tukey's test was also performed.

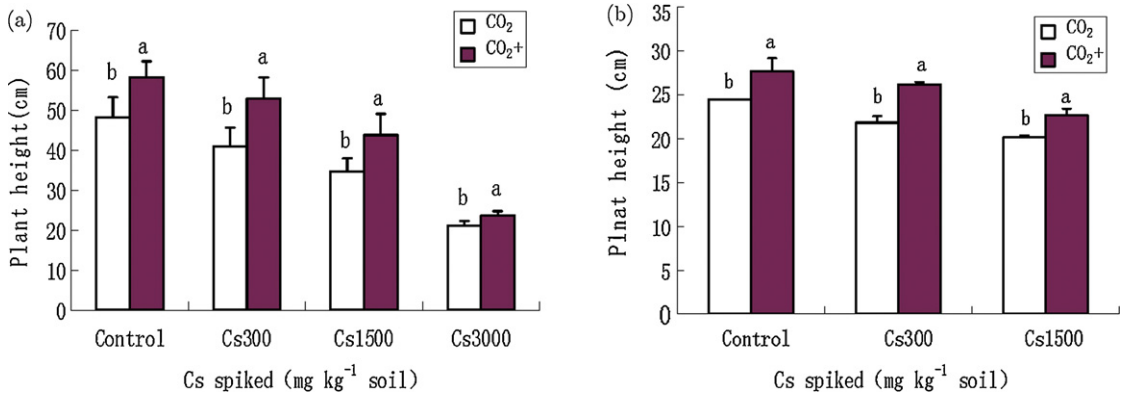


**Fig. 1.** Heights of the *S. vulgare* × *S. vulgare var. sudanense* hybrid (a) and *T. pratense* (b) growing on Cs-spiked soils at ambient and elevated $CO_2$. Different letters within the same soil treatment indicate significant differences between $CO_2$ treatments ($p < 0.05$), by analysis of variance [ANOVA]. Each value represents the mean ± SD.

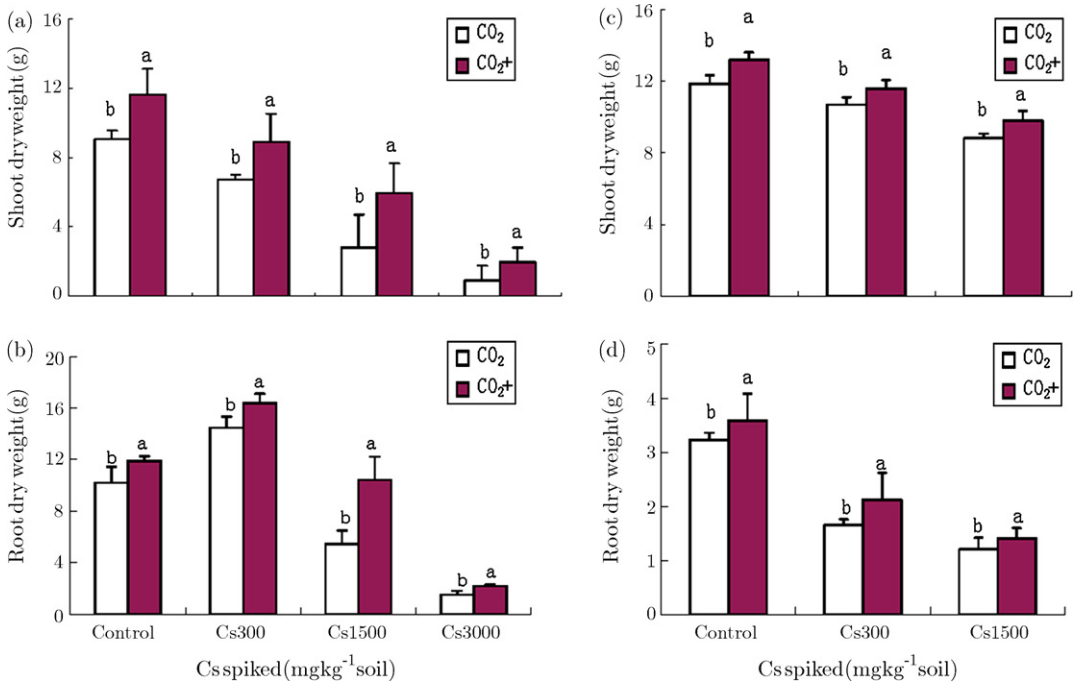


**Fig. 2.** Effects of elevated $CO_2$ on dry weight of the shoots and roots of the *S. vulgare* × *S. vulgare var. sudanense* hybrid (a and b) and *T. pratense* (c and d). Different letters within the same soil treatment indicate significant differences between $CO_2$ treatments ($p < 0.05$), by analysis of variance [ANOVA]. Each value represents the mean ± SD.

## 3. Results

### 3.1. Plant growth

Figs. 1 and 2 show the height and dry weight of the shoots and roots of the *S. vulgare* × *S. vulgare var. sudanense* hybrid and *T. pratense* growing on soils contaminated with various levels of Cs at ambient and elevated $CO_2$. The height and shoot and root dry weight of the *Sorghum* and *Trifolium* species generally decreased with increasing Cs-spiked concentrations at either ambient or elevated $CO_2$, while, after germination, *T. pratense* seedlings did not grow at all on soils spiked with 3000 mg Cs $kg^{-1}$, showing severe Cs phytotoxicity (Figs. 1 and 2). For soils contaminated with the same level of Cs, elevated $CO_2$ increased height and dry weight of the shoots and roots of the two tested species (Figs. 1a and b, 2a–d). The height, aboveground biomass, and belowground biomass of the *Sorghum* species growing on soils spiked with 0, 300, 1500, and 3000 mg Cs $kg^{-1}$ at elevated $CO_2$ increased by 21, 20, 27 and 9% (Fig. 1a); by 2, 32, 111 and 110% (Fig. 2a); by 16, 6, 26 and 6% (Fig. 2b), respectively, compared to the $CO_2$ control. For the *Trifolium* species, the corresponding increase was 13, 20 and 12% (Fig. 1b); 11, 8 and 11% (Fig. 2c); 11, 28 and 4%, respectively (Fig. 2d).

### 3.2. Soil pH variations

Table 3 shows that the pH values in the rhizosphere soils of *Sorghum* and *Trifolium* species exhibited a decreasing trend in generally with increasing Cs concentration at either ambient or elevated $CO_2$. For soil contaminated with 0, 300, 1500, and 3000 mg Cs $kg^{-1}$, elevated $CO_2$ decreased the pH values in the rhizosphere soil by 0.05, 0.16, 0.07, and 0.40 units for the *Sorghum* species, respectively, compared to the ambient $CO_2$ control. For the *Trifolium* species growing on soil contaminated with 0, 300, and 1500 mg Cs $kg^{-1}$, elevated $CO_2$ decreased pH values in the rhizosphere soil by 0.06, 0.26, and 0.08 units, respectively, compared to the ambient $CO_2$ control.

### 3.3. Cs concentrations in the shoots and roots of the *S. vulgare* × *S. vulgare var. sudanense hybrid and T. pratense*

Fig. 3 shows the Cs concentration in the shoots and roots of the *Sorghum* and *Trifolium* species at elevated $CO_2$ and ambient levels. The Cs concentrations in the shoots and roots of the *Sorghum* (Fig. 3a and b) and *Trifolium* (Fig. 3c and d) species increased with increasing Cs concentrations at both ambient and elevated $CO_2$. For

**Table 3**
Soil pH values in the Cs-spiked rhizosphere soils on which the *S. vulgare* × *S. vulgare var. sudanense* hybrid and *T. pratense* grown at ambient and elevated $CO_2$.

| Plant species | Cesium added to soil (mg $kg^{-1}$ soil) | pH | |
|---|---|---|---|
| | | Ambient $CO_2$ | Elevated $CO_2$ |
| *S. vulgare* × *S. vulgare var. sudanense* hybrid | Control | 5.57 ± 0.05 Cb | 5.52 ± 0.09 Aa |
| | Cs300 | 5.93 ± 0.02 Aa | 5.77 ± 0.06 Ab |
| | Cs1500 | 5.49 ± 0.23 Ca | 5.42 ± 0.08 Bb |
| | Cs3000 | 5.32 ± 0.04 Ba | 4.92 ± 0.03 Cb |
| *T. pratense* | Control | 5.59 ± 0.04 Aa | 5.53 ± 0.03 Ab |
| | Cs300 | 5.28 ± 0.08 Ba | 5.02 ± 0.12 Bb |
| | Cs1500 | 5.52 ± 0.06 Aa | 5.45 ± 0.10 Ab |

Different letters within the same row indicate significant differences between $CO_2$ treatments ($p < 0.05$), by analysis of variance [ANOVA]. Each value represents the mean ± SD.

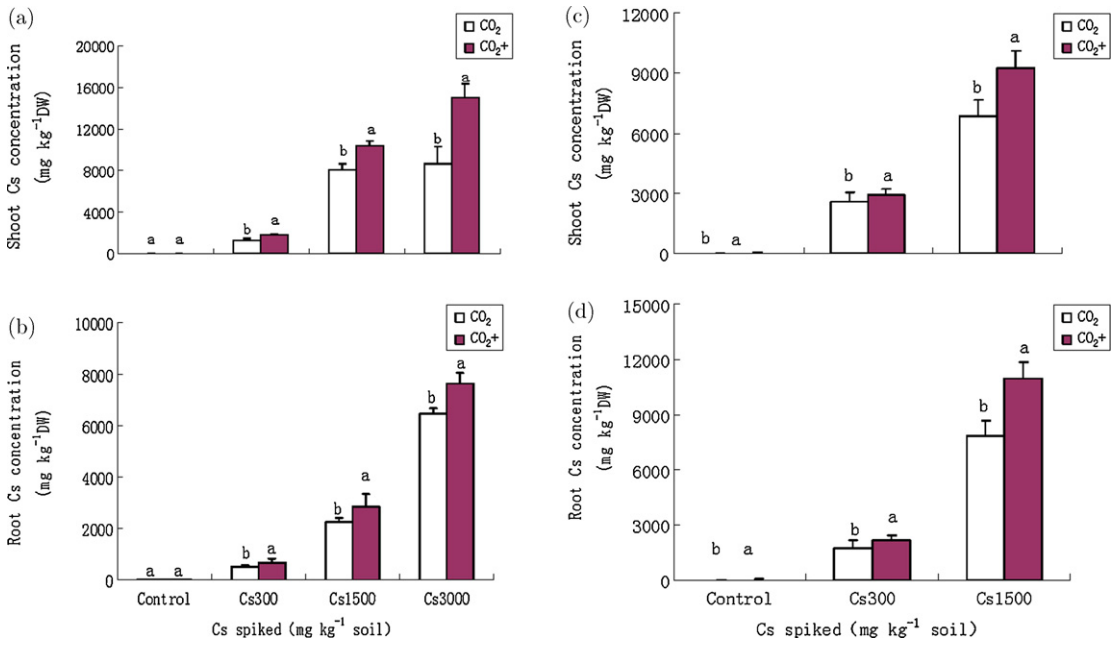


**Fig. 3.** Cs concentrations in the shoots and roots of the *S. vulgare* × *S. vulgare var. sudanense* hybrid (a and b) and *T. pratense* (c and d) growing on Cs-spiked soils at ambient and elevated $CO_2$. Different letters within the same soil treatment indicate significant differences between $CO_2$ treatments ($p < 0.05$), by analysis of variance [ANOVA]. Each value represents the mean ± SD.

the soil spiked with the same level of Cs, the *Sorghum* and *Trifolium* species growing at elevated $CO_2$ had higher Cs concentrations in the shoots and roots than at the ambient $CO_2$. The Cs concentrations in shoots of the *Sorghum* species growing on soils spiked at 0, 300, 1500 and 3000 mg Cs $kg^{-1}$ at elevated $CO_2$ increased by 31, 38, 28, and 73% (Fig. 3a) while in the roots by 33, 31, 40 and 18%, respectively, compared to the ambient $CO_2$ control (Fig. 3b). A similar trend was observed in *Trifolium* species growing on soils spiked at 0, 300, and 1500 mg Cs $kg^{-1}$ at elevated $CO_2$; the plants exhibited Cs concentration increases in the shoots of 43, 13 and 36% (Fig. 3c) and in the roots of 42, 26 and 40% (Fig. 3d), respectively, compared to the ambient $CO_2$ control.

Table 4 shows the bioaccumulation factor of the two tested plants at ambient and elevated $CO_2$. Both species had higher BF values when grown at elevated $CO_2$ than at ambient $CO_2$, in all cases, and different species had different BFs, depending on Cs concentrations and $CO_2$ treatments. The highest BF was 6.9 for the *Sorghum* species growing on soils spiked with 1500 mg Cs $kg^{-1}$ at elevated $CO_2$, and 9.8 for the *Trifolium* species growing on soils spiked with 300 mg Cs $kg^{-1}$ at elevated $CO_2$.

### 3.4. Microbial community in the rhizosphere soils of the *S. vulgare* × *S. vulgare var. sudanense* hybrid and *T. pratense*

Bacterial populations (Fig. 4a and d) and actinomycete populations (Fig. 4c and f) in the rhizosphere soil of the *Sorghum* and *Trifolium* species generally decreased with increasing Cs concentrations at either ambient or elevated $CO_2$. Fungal populations in the rhizosphere soil of the *Sorghum* species growing on soils spiked with 0 and 300 mg Cs $kg^{-1}$ (Fig. 4b) decreased, and then increased on soils amended with 1500 mg Cs $kg^{-1}$. Fungal populations growing in association with *Trifolium* species responded differently. They decreased with increasing Cs concentration under ambient $CO_2$, but increased with increasing Cs concentration in the case of elevated

**Table 4**
Cs bioaccumulation factor (BF) (calculated average, $n = 4$) of the *S. vulgare* × *S. vulgare var. sudanense* hybrid and *T. pratense* growing on Cs-spiked soils at ambient and elevated $CO_2$.

| Species | Added Cs (mg $kg^{-1}$) | [$CO_2$] (μL $L^{-1}$) | Shoot [Cs] (mg $kg^{-1}$) | | Soil [Cs] (mg $kg^{-1}$) | BF[a] |
|---|---|---|---|---|---|---|
| | | | Mean | SD | | |
| *S. vulgare* × *S. vulgare var. sudanense* hybrid | 0 | 350 | 0.016 | 0.001 | 0.008 | 2.0 |
| | | 850 | 0.021 | 0.003 | | 2.6 |
| | 300 | 350 | 1293 | 184 | 300.008 | 4.3 |
| | | 850 | 1785 | 169 | | 5.9 |
| | 1500 | 350 | 8132 | 474 | 1500.008 | 5.4 |
| | | 850 | 10,384 | 410 | | 6.9 |
| | 3000 | 350 | 8670 | 1591 | 3000.008 | 2.9 |
| | | 850 | 14,996 | 1367 | | 5.0 |
| *T. pratense* | 0 | 350 | 0.027 | 0.003 | 0.008 | 3.4 |
| | | 850 | 0.039 | 0.004 | | 4.8 |
| | 300 | 350 | 2599 | 258 | 300.008 | 8.7 |
| | | 850 | 2930 | 226 | | 9.8 |
| | 1500 | 350 | 6826 | 2080 | 1500.008 | 4.6 |
| | | 850 | 9250 | 3056 | | 6.2 |

Each value represents the mean ± SD.
[a] BF = average [Cs] in shoot/average [Cs] in soil. For the control treatments, average Cs in soil = average [Cs] determined in soil. For the added Cs treatments, average Cs in soil = [Cs] in soil + Cs added.

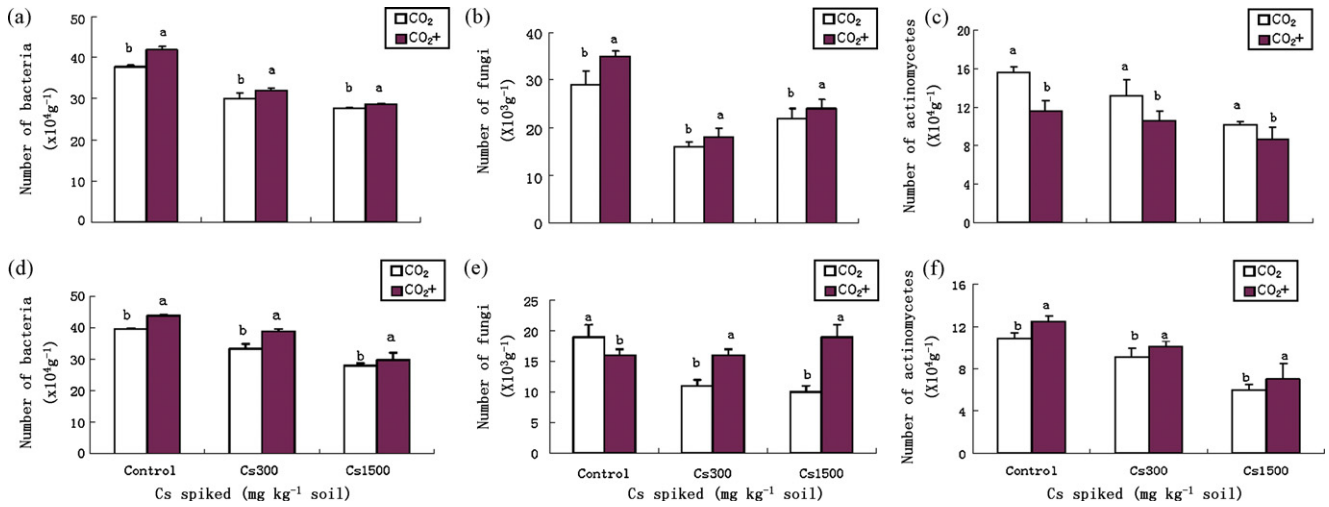


**Fig. 4.** Population of bacteria, fungi and actinomycetes in rhizosphere soils of the *S. vulgare* × *S. vulgare var. sudanense* hybrid (a–c) and *T. pratense* (d–f) growing on Cs-spiked soils at ambient and elevated $CO_2$. Different letters within the same soil treatment indicate significant differences between $CO_2$ treatments ($p < 0.05$), by analysis of variance [ANOVA]. Each value represents the mean ± SD.

$CO_2$ (Fig. 4e). For the soil spiked with the same level of Cs, the number of bacteria and fungi in the rhizosphere soil of the *Sorghum* species growing at elevated $CO_2$ was higher than that under ambient $CO_2$, but the number of actinomycetes was lower under elevated $CO_2$ than ambient $CO_2$. For the *Trifolium* species, the number of all three types of microorganisms was higher at elevated $CO_2$ than that at ambient $CO_2$, regardless of the Cs concentrations in the soil, with the exception of the Cs control (no Cs added) soil for fungal populations.

## 4. Discussion

### 4.1. Effects of elevated $CO_2$ on plant height and biomass

Previous studies revealed the effects of elevated $CO_2$ on the growth and development of plants [18,19,21,32]. The most noticeable effect is that $CO_2$ enrichment increases plant biomass and yields. Poorter [33] reported an average biomass increase of 41% for $C_3$ plants, 22% for $C_4$ plants and 15% for CAM species, so that the growth stimulation for $C_3$ species was substantially larger than for $C_4$ plants. After investigating the effect of elevated $CO_2$ on the short grass prairie, Morgan et al. [32] showed that doubling the $CO_2$ concentrations increased the $C_3$ and $C_4$ plants aboveground biomass by 54 and 44%, respectively. Tang et al. [13] showed that elevated $CO_2$ concentrations not only improved plant growth and biomass production for Indian mustard (*B. juncea* L. Czern.) and sunflower (*H. annuus* L.) in non-contaminated soil environments, but also increased aboveground biomass of the tested species on copper contaminated soils. Our present study also showed that elevated $CO_2$ increased aboveground biomass of the *Sorghum* and *Trifolium* species growing on Cs-spiked soils by 32–111% (Fig. 2a), and by 8–11% (Fig. 2c), respectively, compared to the ambient $CO_2$, suggesting that elevated $CO_2$ is beneficial to growth and development of the tested species. It was noted that the growth stimulation was larger for the *Sorghum* than for *Trifolium* species. Since the efficiency of the phytoremediation of contaminated soil is often connected with high biomass production by plant species and their accumulation of the contaminants, the increase of the biomass of the *Sorghum* and *Trifolium* species growing at elevated $CO_2$ implies improvement of phytoremediation efficiency if they are applied to field practice. We also observed that elevated $CO_2$ had more effect on shoot than root development. It was speculated that the *S. vulgare* × *S. vulgare var. sudanense* hybrid is more tolerant to Cs stress than *T. pratense* since the former grew well on soils spiked with 3000 mg Cs $kg^{-1}$ while the latter did not grow at all at this level.

Although non-FACE experiments are generally considered to overestimate the effects of $CO_2$ concentration on plant growth [34–36], there are still some people who argue against this view [37]. Despite this, the validity of our findings still needs to be tested in FACE or for simplified greenhouses before it is applied to the field.

### 4.2. Impact of elevated $CO_2$ and on uptake of Cs by plants growing on soils contaminated with various levels of Cs

No plant species has been reported to hyperaccumulate stable Cs in its biomass under natural environmental conditions. All stable Cs concentrations in plants collected in various natural habitats were reported to be low, being almost within the normal range. Wallace [38] reported Cs concentrations for the leaves of nine species of desert plants collected in Nevada as ranging from 0.029 to 0.373 mg $kg^{-1}$. Varskog et al. [39] found Cs concentrations varying between 0.03 and 0.08 mg $kg^{-1}$ in the leaves of *Betula nana* and shoots of *Juncus trifidus* collected at 11 Norwegian forest sites. Willey and Martin [30] reported Cs concentrations for eight upland plant species in England as ranging from 0.01 to 0.29 mg $kg^{-1}$. Cs concentrations in 29 mushrooms (fungi) and 8 plants collected at a pine forest site in Tokai-mura (Japan) averaged 0.043 mg $kg^{-1}$ (dry weight) [40]. Polished rice (*Oryza sativa*) collected from two sites in Japan contained Cs ranging from 0.0005 to 0.0065 mg $kg^{-1}$ [29]. Low Cs concentrations varying from 0 and 0.365 mg $kg^{-1}$ were reported in 330 plant species collected from 27 sites on the eastern Snake River plain in Idaho by Cook et al. [1] who showed that the mean values for the 18 most frequent species ranged from 0 to 0.179 mg $kg^{-1}$. Mushrooms contained higher stable Cs concentrations, being two orders of magnitude greater than plants such as rice, potato, cabbage, and leafy vegetables produced in Japan [41]. Several other researches showed that plants growing on Cs-spiked soil might have higher Cs concentrations and bioaccumulation ratio than naturally occurring vegetation. For example, Chiang et al. [42] reported that rape growing on Cs-spiked soils had Cs concentrations in the shoots and roots reaching up to 70 mg Cs $kg^{-1}$. Their reported BF values for Cs in shoots of pot grown plants are also very high, ranging between 9 and 31 [42], whereas ours varied from 2.9 to 9.8 (Table 4), exhibiting a trend of $CO_2$-induced increase compared to the ambient $CO_2$ control. However, both sets of the BF values for

plants generated in potted greenhouse experiments were higher than those for plants sampled in the field [1,29,43].

At the same time, the effects of elevated $CO_2$ on plants growing in uncontaminated environments have been thoroughly studied [44], and it is known that elevated atmospheric $CO_2$ concentrations enhance plant water and/or nutrient use efficiency, and nutrient demand [45–47]. However, the effects of elevated $CO_2$ on plant uptake of environmental contaminants, in terms of food safety and phytoremediation efficiency, have received very little attention. The potential for interaction effects between elevated $CO_2$ and soil contaminants on plant growth and development is evident in comparisons of plants growing in uncontaminated soil in the presence and absence of elevated $CO_2$. Our previous study showed that Indian mustard (*B. juncea* L. Czern.) and sunflower (*H. annuus* L.) growing on copper contaminated soils at elevated $CO_2$ took up more copper and produced more biomass than those at ambient $CO_2$ [13]. Another study of ours showed that the ferns *Pteridium revolutum* and *Pteridium aquilinum* growing at elevated $CO_2$ removed more copper from contaminated soils than those at ambient $CO_2$ [48]. There may be similar effects of elevated $CO_2$ and radionuclides on plant growth and development, but little research has been conducted, especially on the specific effects of elevated $CO_2$ on plant uptake of radionuclides. We speculated that the physiological changes which plants undergo due to an elevated atmospheric $CO_2$ levels, are likely to affect Cs uptake by plant. Our present study showed that elevated $CO_2$ increased the Cs concentrations in the shoots and roots of the *S. vulgare* × *S. vulgare var. sudanense* hybrid and *T. pratense* compared to ambient $CO_2$ for the soil spiked with the same level of Cs. The increase for the roots and shoots of the *S. vulgare* × *S. vulgare var. sudanense* hybrid ranged from 18 to 40% and 28 to 73%, respectively, while for the roots and shoots of *T. pratense* it ranged from 26 to 42% and 13 to 43%, respectively. It was noted that the stimulation of Cs accumulation was much larger for the *Sorghum* than *Trifolium* species, implying that the former species might have greater potential to remediate Cs-contaminated soils if assisted with $CO_2$ fertilization, and could also aid in the studies of Cs uptake, translocation, speciation, distribution and detoxification in plants. This finding is of great importance since the increase in both biomass and Cs uptake at elevated $CO_2$ implies that the use of elevated $CO_2$ might be a way to improve phytoremediation efficiency. However, the use of the *S. vulgare* × *S. vulgare var. sudanense* hybrid for phytoremediation of radiocesium contaminated soils might pose hazards to animals through food chain bio-magnification.

### 4.3. Elevated $CO_2$ effects on soil pH

Tang et al. [13] reported that elevated $CO_2$ decreased soil pH values by 0.2 units in sunflower and Indian mustard rhizosphere soils contaminated with copper. The decrease of the pH values in the rhizosphere soil probably increased plant uptake of heavy metals. Giannakopoulou et al. [49] reported that soil adsorption of Cs decreased with decreasing rhizosphere pH, with the adsorption being maximum at pH 8. Our present results showed that elevated $CO_2$ decreased the pH values by 0.2–0.4 units compared to the ambient $CO_2$ control, implying that the lowering pH in the rhizosphere zone due to elevated $CO_2$ could help the plants take up more Cs. A similar mechanism was reported in the case of heavy metals where the decrease in pH reduced the negative charges on the surface of soil organic matter and clay mineral particles, resulting in more release of heavy metals into the soil solution [50]. Chiang et al. [42] showed that the low-molecular-weight organic acids (LMWOAs) in rhizosphere soils increased with increasing Cs addition to the soil, resulting in more release of Cs into soil solutions, and was also favorable to Cs uptake by rape. Decreasing soil pH values promotes the production of organic acids in rhizosphere soil and, as a result, Cs bioavailability was increased, which favors uptake of Cs by plants [49]. In our case, whether there were more organic acids produced in the rhizosphere soil of the *Sorghum* and *Trifolium* species growing at elevated $CO_2$ than at ambient $CO_2$ needs to be further investigated since the pH values in rhizosphere soils of both tested species decreased with increasing Cs concentrations at either ambient or elevated $CO_2$.

### 4.4. Impact of elevated $CO_2$ on the microbial activity in soils contaminated with various levels of Cs

Microorganisms play an important role in organic matter decomposition and nutrient cycling in soils [51,52]. Their activity and functional diversity is also important for phytoremediation. Several studies showed that high concentrations of heavy metals decreased the microbial biomass and functional diversity of soil microorganisms, as well as the biological activity of soils [5,53–59]. Numerous factors, such as plant and root exchanges of chemical compounds, have been shown to affect microbial activity in contaminated and uncontaminated environments. Plants tolerant of contamination could provide favorable conditions for microbial activity [58,59]. For example, Chen et al. [59] reported higher soil microbial biomass and phosphatase activity in the rhizosphere of a copper (Cu)-accumulator, *Elsholtzia splendens* than those of a non-Cu-accumulating plant, *Trifolium repens*, and also showed that the addition of Cu decreased the number of bands in PCR-denaturing gradient gel electrophoresis (PCR-DGGE) fingerprint analysis of bare soil and soil with *T. repens*, and significantly increased the number of bands in soil with *E. splendens* with either 200 or 500 mg $kg^{-1}$ Cu incorporated. Nichols et al. [60] reported increasing number of hydrocarbon-degrading microorganisms under the influence of the "rhizosphere effect" in the root zone of the plants investigated for the bioremediation of hydrocarbon-contaminated soil. There is, however, little information concerning the effect of plants adapted to Cs contamination on the microbial activity of soils, especially in the presence of elevated $CO_2$.

Plant root systems can be affected by elevated $CO_2$ concentrations, and as a result, plant growth and the living conditions for associated soil biota are altered, leading to shifts in the size and composition of the soil microbial communities [61]. Increased atmospheric $CO_2$ concentrations alter the weight, length and architecture of plant roots [62], and affect the biotic and abiotic environment of the root system, which results in increased total rhizo-deposition [63], and variations in the microbial community structure and the activities of rhizosphere microorganisms. O'Neill et al. [64] reported that elevated atmospheric $CO_2$ enhanced mycorrhizal colonisation of pine and oak seedlings. Rogers et al. [63] reported an increase in *Rhizoctonia* infestation and larger numbers of saprophagous nematodes in the rhizosphere of cotton growing under elevated $CO_2$ and dry soil conditions, but they did not find obvious changes in bacterial numbers in the cotton rhizosphere. Yeates et al. [65] found that elevated $CO_2$ could affect the total number of microbes, with some groups of microbes being closely related to plant growth, such as phosphate-solubilizing bacteria and nitrifying bacteria. Jia et al. [66] showed that elevated $CO_2$ promoted the growth of bacteria in white pine rhizosphere soils, with positive effects on the fungal population in Korean pine rhizosphere soils and little effect of actinomycetes.

Despite effects of elevated $CO_2$ on plants growing in uncontaminated environments, there are limited data on the effects of $CO_2$ on rhizosphere microorganisms associated with plants growing in contaminated environments. We showed that the bacterial and fungal populations associated with the *Sorghum* and *Trifolium* species under elevated $CO_2$ were higher when the soils were spiked with Cs. We also found that the *S. vulgare* × *S. vulgare var. sudanense* hybrid had remarkably reduced actinomycete populations under elevated

$CO_2$ conditions when the soil was spiked with Cs, with respect to $CO_2$ control Cs-spiked soil samples (Fig. 4), while *T. pratense* had larger actinomycete populations at elevated $CO_2$ than that at ambient $CO_2$ under the Cs-spiked soil condition. It was more likely that the elevated $CO_2$ stimulated the development of bacteria and fungi and increased the activity of the microorganisms due to altered rhizosphere conditions resulting from elevated atmospheric $CO_2$. It was also more likely that the rhizosphere zone was more suitable for microorganisms under conditions of Cs contamination associated with elevated $CO_2$. The present experiments indirectly confirmed the enhanced development of microorganism populations (probably bacteria and fungi) in the rhizosphere under the influence of elevated $CO_2$. One possible explanation for this effect of elevated $CO_2$ on rhizosphere microorganisms in Cs-contaminated soil is that, elevated $CO_2$ might allow the plant to support greater microbial and fungal populations or/and protect the microorganisms against the impact of Cs pollution. The activation of microbial populations in the rhizosphere may be a mechanism of plant defense against cesium contaminants, as described by Walton et al. [67]. Detailed investigation, however, needs to be carried out, specifically, into the extent to which rhizosphere conditions are altered in the presence of elevated $CO_2$ and high Cs concentrations.

Our estimates of the fungal populations in the rhizosphere soil exposed at either ambient or elevated $CO_2$ are numerically comparable to those of Edvantoro et al. and Chopra et al. [68,69]. The fungal counts reported by Edvantoro et al. [68] ranged from 20 to $80 \times 10^3$ $g^{-1}$ for contaminated soil, and 7 and $13 \times 10^3$ $g^{-1}$ for Chopra et al. [69], compared with 10 to $35 \times 10^3$ $g^{-1}$ in the Cs-spiked soil associated with the *Sorghum* and *Trifolium* species exposed at either ambient or elevated $CO_2$. De Souza et al. [70] reported that rhizosphere bacteria enhanced selenium and mercury uptake by wetland plants. In our case, the increase in bacterial and fungal populations in the elevated $CO_2$ treated plants and their associated soils, compared to the $CO_2$ controls, may suggest alleviation of Cs phytotoxicity by the microbes as described by Denton [71], preserving them for possible roles in excreting acids, proteins, plant antibiotics and other chemicals, which can help to alleviate plant heavy metal phytotoxicity. Soil-bound cesium may be mobilized, and thus made available to plants, by changes in soil pH and by microbial action, possibly increased by elevated $CO_2$. However, the mechanism mediated by rhizosphere microorganisms, in terms of Cs accumulation by plants growing at elevated $CO_2$, is poorly understood, and needs to be further investigated. Since a single-step increase in $CO_2$ and a gradual increase over several decades may result in different structural and functional community responses to increased $CO_2$ [72], the soil microbial strain(s) in the rhizosphere soils of the *Sorghum* and *Trifolium* species growing on Cs-spiked soils at either ambient and elevated $CO_2$ in this study remains to be identified, and compared.

## 5. Conclusions

The results obtained in our study suggest that elevated $CO_2$ not only resulted in an increase of aboveground biomass of the *S. vulgare* × *S. vulgare var. sudanense* hybrid and *T. pratense* by 32–111%, and by 8–11%, respectively, compared to the ambient $CO_2$, but also enhanced its Cs resistance when the growth was conducted in Cs artificially contaminated soil and under elevated $CO_2$. The effect of elevated $CO_2$ on Cs uptake by the *Sorghum* and *Trifolium* species was evident with the maximum increase reaching 73% for the *S. vulgare* × *S. vulgare var. sudanense* hybrid and 43% for *T. pratense*, compared to the ambient $CO_2$ control. It was concluded that the *S. vulgare* × *S. vulgare var. sudanense* hybrid has greater potential to remediate Cs-contaminated soils than *T. pratense*, if assisted with elevated $CO_2$ fertilization, and could also aid in the studies of Cs uptake, translocation, speciation, distribution and detoxification in plants. It was also observed that the bacterial and fungal populations in the elevated $CO_2$ exposed Cs-spiked soil samples for both *Sorghum* and *Trifolium* species were higher than those in the $CO_2$ control Cs-spiked soil samples. It was also shown that the *S. vulgare* × *S. vulgare var. sudanense* hybrid had remarkably smaller associated actinomycete populations in the elevated $CO_2$ exposed Cs-spiked rhizosphere soil samples than in the $CO_2$ control Cs-spiked soil samples while *T. pratense* had higher associated actinomycete populations at elevated $CO_2$ than that at ambient $CO_2$ in all Cs-spiked soil samples. Our findings suggested that microbial associations with the *Sorghum* and *Trifolium* species growing on Cs-spiked soils at elevated $CO_2$ might result in enhanced Cs accumulation by the plant without inhibition of plant growth. Further studies are needed to identify the specific microorganisms associated with elevated $CO_2$, and to determine their effect on plant uptake of Cs and soil health. It was speculated that the improvement in biomass and Cs accumulation ability at elevated $CO_2$ could be related to lowered soil pH as well as changes in the number of microorganisms in the rhizosphere soils of the tested plant species, due to elevated $CO_2$. The results described above led us to conclude that the use of elevated $CO_2$ to increase biomass of the *Sorghum* and *Trifolium* species and to enhance accumulation of Cs by the tested species may provide a new method for phytoremediation of radionuclide contaminated soils due to the improved efficiencies presented by these associations.

## Acknowledgements

The work was financially supported from the National Science Foundation of China (Grant No. 40773078). The authors acknowledge the partial funding from Central Public Research Institutes Basic Funds for Research and Development. We also want to thank Dr. Paul N. Williams from University of Aberdeen, U.K. for critically reviewing the initial manuscript, and the anonymous reviewers for their constructive suggestions.

## References

[1] L.L. Cook, R.S. Inouye, T.P. McGonigle, G.J. White, The distribution of stable cesium in soils and plants of the eastern Snake River Plain in southern Idaho, J. Arid Environ. 69 (2007) 40–64.
[2] A. Takeda, H. Tsukada, Y. Takaku, N. Akata, S. Hisamatsu, Plant induced changes in concentrations of caesium, strontium and uranium in soil solution with reference to major ions and dissolved organic matter, J. Environ. Radioact. 99 (2008) 900–911.
[3] C. Tamponnet, A. Martin-Garin, M.A. Gonze, N. Parekh, R. Vallejo, T. Sauras-Yera, J. Casadesus, C. Plassard, S. Staunton, M. Norden, R. Avila, G. Shaw, An overview of BORIS: bioavailability of radionuclides in soils, J. Environ. Radioact. 99 (2008) 820–830.
[4] S.D. Cunningham, W.R. Berti, J.W. Huang, Phytoremediation of contaminated soils, Trends Biotechnol. 13 (1995) 393–397.
[5] M. Khan, J. Scullion, Effect of soil microbial responses to metal contamination, Environ. Pollut. 110 (2000) 115–125.
[6] S.D. Cunningham, W.R. Berti, Remediation of contaminated soils with green plants: an overview, In Vitro Cell. Dev. Biol. Plant 29 (1993) 207–212.
[7] A. Sas-Nowosielska, R. Kucharski, E. Malkowski, M. Pogrzeba, J.M. Kuperberg, K. Krynski, Phytoextraction crop disposal—an unsolved problem, Environ. Pollut. 128 (2004) 373–379.
[8] I.D. Pulford, C. Watson, Phytoremediation of heavy metal-contaminated land by trees—a review, Environ. Int. 29 (2003) 529–540.
[9] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediation, Annu. Rev. Plant Physiol. 49 (1998) 643–668.
[10] M.R. Banerjee, B.K. Dey, Effects of different pesticides on microbial populations, nitrogen mineralisation, and thiosulphate oxidation in the rhizosphere of jute (*Corchotus capsularis* L. cv.), Biol. Fertil. Soils 14 (1992) 213–218.
[11] S.D. Siciliano, J.J. Germida, Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria, Environ. Rev. 6 (1998) 65–79.
[12] S.R. Tang, The Principle and Methods of Phytoremediation of Contaminated Environment, Scientific Press, Beijing, 2006.
[13] 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.

[14] IPCC, Climate Change 2007—The Physical Science Basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Intergovernmental Panel on Climate Change, Cambridge University Press, 2007.
[15] W.A.J.M. De Costa, W.M.W. Weerakoon, H.M.L.K. Herath, K.S.P. Amaratunga, R.M.I. Abeywardena, Physiology of yield determination of rice under elevated carbon dioxide at high temperatures in a subhumid tropical climate, Field Crops Res. 96 (2006) 336–347.
[16] 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.
[17] P. Högy, A. Fangmeier, Atmospheric $CO_2$ enrichment affects potatoes. 1. Aboveground biomass production and tuber yield, Eur. J. Agron. 30 (2009) 78–84.
[18] B.A. Kimball, K. Kobayashi, M. Bindi, Response of agricultural crops to free-air $CO_2$ enrichment, Adv. Agron. 77 (2002) 293–368.
[19] J. Franzaring, I. Holz, A. Fangmeier, Different responses of *Molinia caerulea* plants from three origins to $CO_2$ enrichment and nutrient supply, Acta Oecol. 33 (2008) 176–187.
[20] G. Bowes, Facing the inevitable: plants and increasing atmospheric $CO_2$, Annu. Rev. Plant Physiol. Plant Mol. Biol. 44 (1993) 309–332.
[21] A.B. Cousins, N.R. Adam, G.W. Wall, B.A. Kimball, P.J. Pinter Jr., M.J. Ottman, S.W. Leavitt, A.N. Webber, Development of $C_4$ photosynthesis in sorghum leaves grown under free-air $CO_2$ enrichment (FACE), J. Exp. Bot. 54 (2003) 1969–1975.
[22] S.H. Kim, D.C. Gitz, R.C. Sicher, J.T. Baker, D.J. Timlin, V.R. Reddy, Temperature dependence of growth, development, and photosynthesis in maize under elevated $CO_2$, Environ. Exp. Bot. 61 (2007) 224–236.
[23] R. Ronn, M. Gavito, J. Larsen, I. Jakobsen, H. Frederiksen, S. Christensen, Response of free-living soil protozoa and microorganisms to elevated atmospheric $CO_2$ and presence of mycorrhiza, Soil Biol. Biochem. 34 (2002) 923–932.
[24] D. Ebersberger, P.A. Niklaus, E. Kandeler, Long-term $CO_2$ enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland, Soil Biol. Biochem. 35 (2003) 965–972.
[25] M. Dowdall, W. Standring, G. Shaw, P. Strand, Will global warming affect soil-to-plant transfer of radionuclides? J. Environ. Radioact. 99 (2008) 1736–1745.
[26] P.J. White, M.R. Broadley, Mechanisms of caesium uptake by plants, New Phytol. 147 (2000) 241–256.
[27] H. Tsukada, H. Hasegawa, S. Hisamatsu, S. Yamasaki, Rice uptake and distributions of radioactive $^{137}$Cs, stable $^{133}$Cs and K from soil, Environ. Pollut. 117 (2002) 403–409.
[28] K.A. Payne, H.C. Bowen, J.P. Hammond, C.R. Hampton, J.R. Lynn, A. Mead, K. Swarup, M.J. Bennett, P.J. White, M.R. Broadley, Natural genetic variation in caesium (Cs) accumulation by *Arabidopsis thaliana*, New Phytol. 162 (2004) 535–548.
[29] H. Tsukada, H. Hasegawa, S. Hisamatsu, S. Yamasaki, Transfer of $^{137}$Cs and stable Cs from paddy soil to polished rice in Aomori, Japan, J. Environ. Radioact. 59 (2002) 351–363.
[30] N.J. Willey, M.H. Martin, Annual patterns of Cs-133 concentration in British upland vegetation, Chemosphere 30 (1995) 717–724.
[31] B.F. Woolfrey, R.T. Lally, M.N. Ederer, M. Gresser-Burns, Oxacillin killing curve patterns of *Staphylococcus aureus* isolates by agar dilution plate count method, Am. Soc. Microbiol. 31 (1987) 16–20.
[32] J.A. Morgan, D.R. Lecain, A.R. Mosier, D.G. Milchunas, Elevated $CO_2$ enhances water relations and productivity and affects gas exchange in $C_3$ and $C_4$ grasses of the Colorado shortgrass steppe, Global Change Biol. 7 (2001) 451–466.
[33] H. Poorter, Interspecific variation in the growth response of plants to an elevated ambient $CO_2$ concentration, Plant Ecol. 104/105 (1993) 77–97.
[34] A.D.B. Leakey, M. Uribelarrea, E.A. Ainsworth, S.L. Naidu, A. Rogers, D.R. Ort, S.P. Long, Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of $CO_2$ concentration in the absence of drought, Plant Physiol. 140 (2006) 779–790.
[35] S.P. Long, E.A. Ainsworth, A.D.B. Leakey, J. Nösberger, D.R. Ort, Food for thought: lower-than-expected crop yield stimulation with rising $CO_2$ concentrations, Science 312 (2006) 1918–1921.
[36] E.A. Ainsworth, A.D.B. Leakey, D.R. Ort, S.P. Long, FACE-ing the facts: inconsistencies and interdependence among field, chamber and modeling studies of elevated [$CO_2$] impacts on crop yield and food supply, New Phytol. 179 (2008) 5–9.
[37] F.N. Tubiello, J.S. Amthor, K.J. Boote, M. Donatelli, W. Easterling, G. Fischer, R.M. Gifford, M. Howden, J. Reilly, C. Rosenzweig, Crop response to elevated $CO_2$ and world food supply. A comment on 'Food for Thought . . .' by Long et al., Science 312 (2006) 1918–1921 (Eur. J. Agron. 26 (2007) 215–233).
[38] A. Wallace, Regulation of the Micronutrient Status of Plants by Chelating Agents and Other Factors, Edwards Bros Inc., Los Angeles, 1971.
[39] P. Varskog, R. Naeumann, E. Steinnes, Mobility and plant availability of radioactive Cs in natural soil in relation to stable Cs, other alkali elements and soil fertility, J. Environ. Radioact. 22 (1994) 43–53.
[40] S. Yoshida, Y. Muramatsu, M. Steiner, M. Belli, A. Pasquale, B. Rafferty, W. Ruhm, A. Rantavaara, I. Linkov, A. Dvornik, T. Zhuchenko, Relationship between radiocesium and stable cesium in plants and mushrooms collected from forest ecosystems with different contamination levels, in: Proceedings of the 10th Congress of the International Radiation Protection Association, Hiroshima, Japan, May 14–19, 2000, pp. 11–244.
[41] C. Kuwahara, A. Fukumoto, A. Ohsone, N. Furuya, H. Shibata, H. Sugiyama, F. Kato, Accumulation of radiocesium in wild mushrooms collected from a Japanese forest and cesium uptake by microorganisms isolated from the mushroom-growing soils, Sci. Total Environ. 345 (2005) 165–173.
[42] P.N. Chiang, M.K. Wang, J.J. Wang, C.Y. Chiu, Low-molecular-weight organic acid exudation of rape (*Brassica campestris*) roots in cesium-contaminated soils, Soil Sci. 170 (2005) 726–733.
[43] J.R. Twining, T.E. Payne, T. Itakura, Soil–water distribution coefficients and plant transfer factors for $^{134}$Cs, $^{85}$Sr and $^{65}$Zn under field conditions in tropical Australia, J. Environ. Radioact. 71 (2004) 71–87.
[44] I. Loladze, Rising atmospheric $CO_2$ and human nutrition: toward globally imbalanced plant stoichiometry? Trends Ecol. Evol. 17 (2002) 457–461.
[45] C.E. Owensby, P.I. Coyne, L.M. Auen, Nitrogen and phosphorus dynamics of a tallgrass prairie ecosystem exposed to elevated carbon dioxide, Plant Cell Environ. 16 (1993) 843–850.
[46] R.B. Jackson, O.E. Sala, C.B. Field, H.A. Mooney, $CO_2$ alters water use, carbon gain, and yield for the dominant species in a natural grassland, Oecologia 98 (1994) 257–262.
[47] G.M. Berntson, F.A. Bazzaz, Belowground positive and negative feedbacks on $CO_2$ growth enhancement, Plant Soil 187 (1996) 119–131.
[48] 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. Phytoremediation 10 (2008) 161–172.
[49] F. Giannakopoulou, C. Haidouti, A. Chronopoulou, D. Gasparatos, Sorption behavior of cesium on various soils under different pH levels, J. Hazard. Mater. 149 (2007) 553–556.
[50] J. Yanai, F.J. Zhao, S.P. McGrath, T. Kosaki, Effect of soil characteristics on Cd uptake by the hyperaccumulator *Thlaspi caerulescens*, Environ. Pollut. 139 (2006) 167–175.
[51] H. Insam, C.C. Mitchell, J.F. Dormaar, Relationship of soil microbial biomass and activity with fertilization practice and crop yield of three ultisols, Soil Biol. Biochem. 23 (1991) 459–464.
[52] S.C. Srivastava, J.S. Singh, C. Microbial, N and P in dry tropical forest soils: effects of alternate land-uses and nutrient flux, Soil Biol. Biochem. 23 (1991) 117–124.
[53] E. Kandeler, C. Kampichler, O. Horak, Influence of heavy metals on the functional diversity of soil microbial communities, Biol. Fertil. Soils 23 (1996) 299–306.
[54] M.A.R. Khan, N.S. Bolan, A.D. Mackay, Soil test to predict the copper availability in pasture soils, Commun. Soil Sci. Plant Anal. 36 (2005) 2601–2624.
[55] J. Mertens, D. Springael, I. De Troyer, K. Cheyns, P. Wattiau, E. Smolders, Long-term exposure to elevated zinc concentrations induced structural changes and zinc tolerance of the nitrifying community in soil, Environ. Microbiol. 8 (2006) 2170–2178.
[56] M.S. Vásquez-Murrieta, I. Migueles-Garduño, O. Franco-Hernández, B. Govaerts, L. Dendooven, C and N mineralization and microbial biomass in heavy-metal contaminated soil, Eur. J. Soil Biol. 42 (2006) 89–98.
[57] T.A. Anderson, E.A. Guthrie, B.T. Walton, Bioremediation in the rhizosphere: plant roots and associated microbes clean contaminated soil, Environ. Sci. Technol. 27 (1993) 2630–2636.
[58] A. Muratova, T. Hübner, N. Narula, H. Wand, O. Turkovskaya, P. Kuschk, R. Jahn, W. Merbach, Rhizosphere microflora of plants used for the phytoremediation of bitumen-contaminated soil, Microbiol. Res. 158 (2003) 151–161.
[59] Y.X. Chen, Y.P. Wang, W.X. Wu, Q. Lin, S.G. Xue, Impacts of chelate-assisted phytoremediation on microbial community composition in the rhizosphere of a copper accumulator and non-accumulator, Sci. Total Environ. 356 (2006) 247–255.
[60] T.D. Nichols, D.C. Wolf, H.B. Rogers, C.A. Beyrouty, C.M. Reynolds, Rhizosphere microbial populations in contaminated soils, Water Air Soil Pollut. 95 (1997) 165–178.
[61] M. Schortemeyer, U.A. Hartwig, G.R. Hendrey, M.J. Sadowsky, Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to Free-Air Carbon Dioxide Enrichment (FACE), Soil Biol. Biochem. 28 (1996) 1717–1724.
[62] M. Jongen, M.B. Jones, Effects of elevated carbon dioxide on plant biomass production and competition in a simulated neutral grassland community, Ann. Bot. 82 (1998) 111–123.
[63] H.H. Rogers, G.B. Runion, S.V. Krupa, Plant responses to atmospheric $CO_2$ enrichment with emphasis on roots and the rhizosphere, Environ. Pollut. 83 (1994) 155–189.
[64] E.G. O'Neill, R.J. Luxmoore, R.J. Norby, Increases in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched $CO_2$ atmosphere, Can. J. Forest Res. 17 (1987) 878–883.
[65] G.W. Yeates, P.C.D. Newton, D.J. Ross, Response of soil nematode fauna to naturally elevated $CO_2$ levels influenced by soil pattern, Nematology 1 (1999) 285–293.
[66] X. Jia, S.J. Han, Y.M. Zhou, J.H. Zhang, C.J. Zou, Effects of elevated $CO_2$ concentration on rhizosphere soil microbes under *Pinus koraiensis* and *Pinus sylvestriformis* seedlings, Chin. J. Appl. Ecol. 16 (2005) 1295–1298.
[67] B.T. Walton, A.M. Hoylman, M.M. Perez, T.A. Anderson, T.R. Johnson, E.A. Guthrie, R.F. Christman, Rhizosphere microbial communities as a plant defense against toxic substances in soils, in: T.A. Anderson, J.R. Coats (Eds.), Bioremediation Through Rhizosphere Technology, American Chemical Society, Washington, DC, 1994, pp. 82–92.
[68] B.B. Edvantoro, R. Naidu, M. Megharaj, I. Singleton, Changes in microbial properties associated with long-term arsenic and DDT contaminated soils at disused cattle dip sites, Ecotoxicol. Environ. Saf. 55 (2003) 344–351.
[69] B.K. Chopra, S. Bhat, I.P. Mikheenko, Z. Xu, Y. Yang, X. Luo, H. Chen, L. van Zwieten, R.McC. Lilley, R. Zhang, The characteristics of rhizosphere microbes

associated with plants in arsenic-contaminated soils from cattle dip sites, Sci. Total Environ. 378 (2007) 331–342.
[70] M.P. De Souza, C.P.A. Huang, N. Chee, N. Terry, Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants, Planta 209 (1999) 259–263.
[71] B. Denton, Advances in phytoremediation of heavy metals using plant growth promoting bacteria and fungi, MMG445 Basic Biotechnol. eJ. 3 (2007) 1–5.
[72] J.N. Klironomos, M.F. Allen, M.C. Rillig, J. Piotrowski, S. Makvandi-Nejad, B.E. Wolfe, J.R. Powell, Abrupt rise in atmospheric $CO_2$ overestimates community response in a model plant–soil system, Nature 433 (2005) 621–624.