



Elevated ambient carbon dioxide and *Trichoderma* inoculum could enhance cadmium uptake of *Lolium perenne* explained by changes of soil pH, cadmium availability and microbial biomass



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ABSTRACT

Trichoderma inoculum may mediate plant growth and uptake of heavy metals, but the combined effects and the mechanisms of *Trichoderma* sp. and elevated ambient carbon dioxide (CO₂) on the cadmium (Cd) availability and plant uptake are far less investigated. A pot experiment was conducted to investigate plant growth, Cd uptake, and chemical and microbial biomass characteristics of the rhizosphere of *Lolium perenne* grown on three Cd-contaminated soils under combined effects of elevated ambient CO₂ and *Trichoderma virens* strain F7 inoculum. Rhizosphere soil samples were analyzed for pH, concentrations of available Cd measured by diffusive gradients in thin-films (DGT-Cd) and microbial biomass carbon and nitrogen. It was found that the elevated ambient CO₂ and *Trichoderma* inoculation, in combination or alone, significantly promoted plant biomass and total Cd uptake by plants with decreased soil pH (by 0.08–0.32 units) and increased microbial biomass carbon (by 21.54–121.21%) and nitrogen (by 21.88–112.95%) in the rhizosphere. Also, the concentration of DGT-Cd in the rhizosphere under elevated ambient CO₂ and *Trichoderma* inoculum treatments was increased by 15.45–152.48%, indicating that there was more available Cd for plant uptake under effects of elevated ambient CO₂ and *Trichoderma* inoculum. Results suggested that the acidulated rhizosphere, enhanced microbial activities and increased Cd availability may have contributed to the efficiency of plants in Cd accumulation under effects of elevated ambient CO₂ and *Trichoderma* inoculum.

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1. Introduction

As an eco-friendly and cost-effective technology, phytoextraction is being developed as one of the most promising methods of removing heavy metals from contaminated soils (Ma et al., 2011). However, most of the metal accumulating plants are characterized by slow growth, small biomass yields and often incapable of accumulating high concentrations of potentially phytotoxic metals (Ruiz et al., 2009; Li and Wong, 2012). Two ways have been suggested to break through the application limitation of phytoextraction technology: enriching atmospheric carbon dioxide (CO₂) in a simplified greenhouse to enhance the biomass of

hyperaccumulator plants or inoculation of plants with microorganisms such as fungi to increase bioavailability of low-solubility metals in soils (Wang et al., 2006; Jia et al., 2010; Kavamura and Esposito, 2010). Interestingly, elevated CO₂ not only increased aboveground biomass of metal-accumulator plants such as *Phytolacca americana* Linn and *Amaranthus cruentus* L., but also increased the diversity of rhizosphere soil microbial communities, and this process may contribute to enhanced metal accumulation by plants (Song et al., 2012). Some studies have been carried out to examine the combination effects of elevated CO₂ and microbial inoculation on the phytoextraction, and found that their combination was more effective on the metal uptake by plants (Tang et al., 2012; Song et al., 2013). *Trichoderma* species are opportunistic, avirulent plant symbionts and common in soil and root ecosystems (Harman et al., 2006). Its high colonization ability together with its action in promoting plant growth allowed a significant increase in heavy metal uptake in a wide range of plant species (Fiorentino

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et al., 2013). However, to date, there is little information on the potential use of *Trichoderma* inoculum combined with elevated ambient CO₂ for phytoextraction of heavy metals. In addition, relative to the well demonstrated role of endophytic fungi to increase heavy metal uptake (Ren et al., 2006, 2011; Wan et al., 2012), the mechanisms that may regulate heavy metal accumulation phenomenon under combined effects of elevated ambient CO₂ and *Trichoderma* inoculum are far less investigated.

Rhizosphere, as a dynamic micro-environment where the root, the soil and the microorganisms are interacting (Lynch and Whipps, 1990), plays a significant role in controlling the uptake and accumulation of heavy metals by plants (Wang et al., 2009). Root-associated microorganisms including *Trichoderma* in the rhizosphere soil provide a link between roots and the soil. Any variation in abundance or diversity of microorganisms may affect plant growth, the cycling of plant nutrients and the fate of heavy metals in soil by altering the soil physicochemical conditions (Giller et al., 1998; Song et al., 2012). Elevated ambient CO₂ and inoculation of microorganisms may affect the soil microbial community and activity (Nguyen et al., 2011; Cordier and Alabouvette, 2009; Zhang et al., 2010), and therefore the uptake of heavy metals by plant roots. Unfortunately, most previous studies about the effect of elevated ambient CO₂ and microbial inoculation on heavy metal uptake by plants did not include the measurement of soil biological parameters such as microbial biomass (Jia et al., 2010; Li and Wong, 2012; Tang et al., 2012). Thus, the information about the change of microbial biomass and microbial activity in rhizosphere may improve our understanding of the mechanisms by which elevated ambient CO₂ and *Trichoderma* inoculum might influence the uptake processes of heavy metals by plant roots.

Generally, the mobility and availability of heavy metals in soils, particular at the rhizosphere where root uptake or exclusion takes place, are critical factors that affect phytoextraction (Zhang and Shan, 2000). For this purpose, the technique of diffusive gradients in thin-films (DGT) which could account for both the soil solution concentration and the dynamic supply of heavy metals from the solid phase, has been widely used to evaluate the mobility and availability of heavy metals in soil science (Zhang et al., 1998). Elevated ambient CO₂ and *Trichoderma* inoculum might modify rhizosphere directly or indirectly to enhance the uptake of metals by different mechanisms such as acidification, redox changes and enhancing the release of root for ensuring the iron availability, or mobilizing the metal phosphates (Li et al., 2010; Kacprzak et al., 2014). The DGT device is a promising tool for the assessment of bioavailable fractions of metals in soil because the concentrations of the metal measured by DGT were highly correlated with the concentrations of the metal taken up by plants (Tian et al., 2008). This could help us to precisely characterize the variations among bioavailable metal pools in rhizospheric soil under conditions of elevated ambient CO₂ and *Trichoderma* inoculum.

In this study, we used open-top chambers (OTCs) to investigate the effects of elevated ambient CO₂ and inoculation with a cadmium (Cd) resistant fungi *Trichoderma virens* strain F7 on Cd uptake and rhizosphere system of *Lolium perenne* grown on soils with three levels of Cd. The main objectives of this study were (1): to assess the possibility of using *Trichoderma* inoculum and elevated ambient CO₂, either alone or in combination, to increase Cd uptake by *L. perenne*; (2) to determine the changes in pH and microbial biomass carbon (C) and nitrogen (N) in the rhizosphere of *L. perenne* with different treatments; (3) to examine how those parameters influence Cd availability in the rhizosphere and Cd accumulation in the plant. The results of this study may help us to understand the key aspects of Cd uptake by *L. perenne* under effects of elevated ambient CO₂ and *Trichoderma* inoculum.

2. Materials and methods

2.1. Plant material and soil characterization

The seeds of *L. perenne* were obtained from Beijing Feng-Nen Agriculture Technology Limited Company, PR China. As a model plant, *L. perenne* was selected for this study because of its low management requirement, extensive root system, wide adaptability, and ability to survive in metal contaminated soil and to accumulate metals (Jia et al., 2010).

The soils used in this study were collected from three Cd-contaminated sites in Tianjin, PR China, which is one of the preventative sites of the north China. Determination of the physical and chemical properties of the soils followed the methods of Tang et al. (2003). The Cd concentrations, physical and chemical properties of the soils are shown in Table 1. The soil texture of Soil 1 was classified as clay loam, and the soil textures of Soils 2 and 3 were clay according to the USDA soil classification system. The concentrations of Cd in soils are 0.27, 2.65 and 10.84 mg/kg, respectively. Soils were sieved to pass a 3-mm mild sieve and kept in the dark before use.

2.2. Isolation and identification of fungi

T. virens strain F7 was isolated from a Cd-contaminated site of Daxing Prefecture, Guangxi Zhuang Autonomous Region, PR China. The genes of the strain F7 were amplified, sequenced and the accession number in GenBank is JX993849. Strain F7 was tested and shown to be tolerant to 1 g Cd²⁺/kg. The F7 cultures were maintained on slants of potato dextrose agar (PDA) at 4 °C.

2.3. Pot experiment

The experiment had a completely randomized block design with four replications that had the following treatments: control (CK), elevated ambient CO₂ (E), *Trichoderma* inoculum (F), and elevated ambient CO₂ + *Trichoderma* inoculum (E + F).

The dry soil sample of 1 kg was placed in each plastic pot (1 L). Soil samples were then applied with N, phosphate (P) and potassium (K) fertilizers with 200 mg N/kg, 100 mg P/kg and 200 mg K/kg, respectively, watered to maximum water holding capacity (MWHC), and subsequently allowed to equilibrate for 2 weeks. The seeds of *L. perenne* were surface-sterilized with 0.5% sodium hypochlorite (NaClO) and washed several times with distilled water subsequently, and germinated at 25 °C for 48 h before sowing. Five seeds were sown in each pot, and thinned to three seedlings per pot after germination.

For inoculum preparation, strain F7 was grown on PDA slants at 30 °C for 5 days, and the conidia were harvested from PDA surface

Table 1
Physio-chemical properties of soils used in this study.

Soil properties	Values		
	Soil1	Soil2	Soil3
Total Cd (mg/kg)	0.27	2.65	10.84
pH (H ₂ O)	8.36	7.51	7.36
CEC (cmol/kg)	15.98	16.59	16.92
OM (g/kg)	23.24	27.45	31.14
Total N (g/kg)	0.75	1.12	1.34
Total P (g/kg)	0.34	0.36	0.37
Total K (g/kg)	22.14	21.36	21.78
CaCO ₃ (%)	2.12	0.25	0.18
Sand (%)	29.91	27.61	26.95
Silt (%)	33.62	32.34	32.34
Clay (%)	36.47	40.05	40.71
Texture (USDA classification)	clay loam	clay	clay

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