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Concentrations of secondary metabolites in tissues and root exudates of wheat seedlings changed under elevated atmospheric CO_2 and cadmium-contaminated soils

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ABSTRACT

We investigated the short-term effects of elevated atmospheric CO_2 and cadmium (Cd)-contaminated soils on wheat seedlings root exudates and secondary metabolites in tissues. On average, total soluble sugar, total free amino acid, total phenolic acid, and total organic acid in root exudates were 45.7%, 63.2%, 40.0%, and 89.5% higher ($p < 0.01$), respectively, under elevated CO_2 + Cd when compared to ambient CO_2 chambers + Cd, which were greater than those under either elevated CO_2 or Cd stress. Total phenolic acid, condensed tannin, and total flavonoid in tissues of wheat seedlings were 18.1%, 15.7%, and 21.9% ($p < 0.01$) lower, respectively, on average under elevated CO_2 combined with Cd stress, which were lower than those under either elevated CO_2 or Cd stress. Furthermore, the content of indole alkaloids in tissues at 1.0, 5.0, and 10.0 Cd mg Cd kg soil^{-1} levels differed by −11.8%, −4.2%, and 37.3% ($p < 0.01$), respectively, under elevated CO_2. The combination of elevated CO_2 and Cd stress significantly influenced free amino acid, total phenolic acid, and organic acid in root exudates and condensed tannin, total flavonoid, and indole alkloids in tissues; however, the effect on total soluble sugar in root exudates and total phenolic acid in tissues was insignificant. Overall, elevated CO_2 improved the concentration of root exudates of wheat seedlings under Cd-stressed conditions. However, elevated CO_2 inhibited the accumulation of total phenolic acid, total flavonoid, and condensed tannin in tissues under Cd-stressed conditions. In addition, there might be a threshold in the response of indole alkaloids in tissues to elevated CO_2 with increasing Cd levels.

Introduction

Ongoing combustion of fossil fuels has led to an increase in atmospheric CO_2 levels since the advent of the industrial revolution (Johnson and Pregitzer, 2007). It is well known that elevated CO_2 affects global ecosystems. Numerous studies have revealed significant effects of elevated CO_2 on a wide variety of plants (Medlyn et al., 2001; Gunderson et al., 2002; Marchi et al., 2004), and some have shown increased rates of plant growth (Kim and Kang, 2011). Increases in plant growth under elevated CO_2 can enhance carbon deposition in soil and change the rhizosphere conditions by increasing root exudates (Freeman et al., 2004; Hill et al., 2007). Heavy metal pollution in soils is another serious environmental problem. In China, more than 2.0×10^9 ha of agricultural land is reportedly contaminated with heavy metals (Guo et al., 2011). Among the numerous heavy metals contaminants, cadmium (Cd) is one of the most toxic and prevalent pollutants of surface soils. Cd is principally dispersed into agricultural soils via the use of phosphate fertilizers, application of sewage and industrial wastewater for irrigation, atmospheric deposition from metallurgical industries, incineration of plastics and batteries, and the burning of fossil fuels (Tukaj et al., 2007; Li et al., 2011). Because of its prevalence, substantial attention has been paid to the effects of Cd contamination on crop growth, development, and quality (Maksymiec, 2007; Huang et al., 2009). Further increases in atmospheric CO_2 and Cd contamination of soils are expected to occur in the future; therefore, an understanding of the combined effects of these factors on plants, especially crops, is crucial.

It has been shown that under elevated CO_2 a significant proportion of the net primary production is allocated to root systems,

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resulting in large fluxes of organic compounds into the soil (King et al., 2004; Johnson and Pregitzer, 2007). Estimates indicate that up to 40% of the carbon fixed by plants can be released through root exudation (Lynch and Whipps, 1990). Some studies have shown that the concentrations of root exudates increase significantly in Cd-contaminated soils (Xu et al., 2006; Pérez-de-Mora et al., 2006). Organic compounds derived from root exudates play important roles in soil processes (Walker et al., 2003). It is well understood that microbial biomass and activity are strongly influenced by the physicochemical and biological characteristics of the rhizosphere and impact soil processes and soil fertility (Pearce et al., 1995; Sorensen, 1997). Root exudates are one of the most important factors affecting these microbiological parameters (Yao and Allen, 2006; Qu and Wang, 2008). Because root exudates consist of a mixture of organic acids, phenolic compounds, sugars, vitamins, amino acids, inorganic molecules, enzymes, and root border cells (Dakora and Phillips, 2002), their availability and utilization are one of the most crucial factors for successful bacterial establishment in the rhizosphere (Oksinska et al., 2013). Therefore, changes in root exudate concentration, which are expected under elevated CO_2 and Cd-contaminated soils, would influence soil biological characteristics and rhizosphere soil fertility. Li et al. (2013) found that elevated CO_2 combined with Cd and Zn contamination changes soil pH, the dissolved organic matter, and microbial biomass carbon in the rhizosphere of *Sedum alfredii*. However, to our knowledge, no studies have examined the effects of the combination of elevated CO_2 and heavy metal contaminates on root exudates in particular.

Plant secondary metabolites, including phenolic compounds, flavonoids, tannins, and alkaloids, play a pivotal role in growth regulation, antioxidant activity, enzyme inhibition, pigment development, and in blocking UV light. Some secondary metabolites interact with herbivores, microbes, fungi, and nematodes as chemical signals and toxins (Koes et al., 1994; Seigler, 2001). Rapid changes in environmental conditions are likely to affect the formation of secondary metabolites (Morison and Lawlor, 1999; Räisänen et al., 2008). Several studies have reported that elevated CO_2 may alter the levels of chemical defense substances in leaves, including flavonoids, total phenolics, and condensed tannins (Levine et al., 2008; Koike et al., 2006; Novriyanti et al., 2012). In addition, the effects of heavy metals on plant secondary metabolites including flavonoids and phenolic acids in *Lupinus albus L.*, phenolic compounds in *Phyllanthus tenellus* Roxb. leaves and *Azolla imbricate*, and anthocyanins have been confirmed (Chalker-Scott, 1999; Santiago et al., 2000; Jung et al., 2003; Dai et al., 2006). While many studies have reported separately on effects of elevated CO_2 or metal contamination on plant secondary metabolites, the response of secondary metabolites to these compounded factors has not been carried out.

As a major crop of global importance, wheat provides nutrition to a large portion of the world's population and is among the most widely studied crop (Högy et al., 2009). Thus, it is important to understand the influence of various environmental stressors on this crop. This study uses the open-top chamber (OTC) method to focus on the responses of concentrations of main secondary metabolites in tissues and root exudates of wheat seedlings to the combination of elevated CO_2 and Cd-contaminated soils. Due to the improvements of plant growth and photosynthesis products under elevated CO_2 and the stress of Cd on plant physiology (Maksymiec, 2007; Wang et al., 2013), we hypothesized that: (1) the concentration of root exudates of wheat seedlings will be higher under elevated CO_2 combined with Cd-contaminated soils than that under either elevated CO_2 or Cd stress; (2) elevated CO_2 enhances the accumulation of main secondary metabolites in tissues under Cd-contaminated soils when compared to either elevated CO_2 or Cd stress.

Table 1
Type and chemical characteristics of the used soil.

Soil type	Leached brown soil (according to Chinese soil classification)
pH	8.41
Organic matter content ($g\,kg^{-1}$)	11.43 ± 0.97
Organic carbon ($g\,kg^{-1}$)	6.73 ± 0.06
Total nitrogen ($mg\,kg^{-1}$)	1.13 ± 0.12
Soluble salts ($mg\,kg^{-1}$)	383.52 ± 1.03
Available N ($mg\,kg^{-1}$)	0.06 ± 0.01
Available P ($mg\,kg^{-1}$)	4.42 ± 0.35
Available K ($mg\,kg^{-1}$)	133.38 ± 0.89
Cation exchange capacity (meq $100\,g^{-1}$)	26.40 ± 1.11
Total Cd ($mg\,kg^{-1}$)	0.31 ± 0.02

Materials and methods

Plant species and experimental soils

Triticum aestivum L. seeds (spring wheat, No.15 Yongliang) were obtained from the institute of Wheat Breeding in Yongning Country, Ningxia Province, China.

The experimental soils were collected from the surface layer (0–20 cm) within a wheat field in Central Shaanxi, China (34°16′ N, 108°54′ E). The soil type and chemical characteristics are shown in Table 1. Fresh soil was passed through a 5-mm sieve. Four soil concentrations of Cd (0.0, 1.0, 5.0, and 10.0 $mg\,kg^{-1}$ dry weight soil) were selected according to the environmental quality standard (GB 15168-1995) and the relevant Cd pollution levels of farmland at present in China (Song et al., 2006). The soils were artificially contaminated using $CdCl_2{\cdot}2H_2O$ to obtain different soil Cd levels (0.0 (Cd0), 1.0 (Cd1), 5.0 (Cd5), and 10.0 (Cd10) mg Cd kg^{-1} dry soil) and incubated for 30 days.

Experimental site and design of CO_2 concentration

In the spring of 2013, the study was conducted at an open-top chamber (OTC) facility located in the Weishui Campus of Chang'an University, Xi'an, China (34°15′ N, 108°55′ E). The elevation of the study site is 402 m a.s.l. and the mean annual temperature (1995–2010) is 13.6 °C. CO_2 treatments were elevated CO_2, ambient CO_2 chambers, and open plots. Each treatment was replicated three times using a randomized complete block design. Six hexagonal open-top chambers (4.4 m dia × 1.6 m tall) were established in the experimental garden. Three of them were used as controls (ambient CO_2 chambers, 350 $\mu mol\,mol^{-1}$) while the three others were exposed to elevated CO_2 (700 ± 23 $\mu mol\,mol^{-1}$). Open plots (385 $\mu mol\,mol^{-1}$, area of each open plot is 15 m^2) were also established and monitored nearby. Automated measurements of CO_2 concentration, temperature, humidity, and soil water content were taken every minute each day for the duration of the experiments. An automatic control system adjusted the actual CO_2 concentrations to the desired CO_2 concentrations in each elevated CO_2 chamber by regulating the influx rate of CO_2 or air. Air temperature and soil water content (at a depth of 5 cm) were recorded at 10 min intervals for each chamber or open plot. Soil water content was measured by randomly selecting three pots of seedlings. The pots were maintained at 60% field capacity (using constant weight) by watering during the seedling growth stage.

Design of the pot experiment

The experiments were conducted in the experimental pots (45 cm dia × 50 cm tall; n = 108). Seven root bags, which were made out of nylon, were placed in each plastic pot. Each root-bag was then filled with 800 g of Cd-contaminated soil and the pots were filled with 25 kg of Cd-contaminated soil. Three replicates of each Cd level