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Combined effects of elevated CO_2 and Cd-contaminated soil on the growth, gas exchange, antioxidant defense, and Cd accumulation of poplars and willows

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ABSTRACT

Few studies have explored the combined effects of elevated CO_2 (EC) and Cd treatments on poplars and willows. The objective of this study is to determine how growth and phytoremediation efficiency are enhanced by EC. For this purpose, this study investigated the combined effects of EC and Cd treatments on the growth, gas exchange, antioxidant defense, and Cd accumulation in one poplar genotype (*Populus* × *euramericana* (Dode) cv. 'Nanlin-95' (NL95)) and one willow genotype (*Salix jiangsuensis* CL. '172' (J172)), which were grown on three Cd-contaminated soil in six open-top chambers. Under Cd treatment, plant growth was decreased, Cd accumulation was increased, and the photosynthesis and malondialdehyde concentration were unchanged in leaves of two tree species. At EC levels for both species, plant growth, total Cd uptake, CO_2 assimilation rate, and intrinsic water use efficiency were increased; stomatal conductance and transpiration rate were decreased; and Cd concentrations were unchanged. EC also decreased malondialdehyde content in two species grown in Cd-contaminated soil and increased antioxidant enzymatic activities in J172 grown in high Cd-contaminated soil. At EC, plant growth and total Cd uptake exhibited greater increase in high Cd-contaminated soil than in low Cd-contaminated soil. These findings suggest that EC stimulated plant growth by increasing leaf photosynthesis and enhanced phytoremediation efficiency, particularly at high levels of Cd exposure. EC decreased oxidative damage by stimulating photosynthesis and increasing antioxidant enzyme activities. Cd treatment inhibited the growth of two tree species, and this suppression was unrelated to photosynthesis. Under Cd treatment, the well-maintained photosynthesis is assumed responsible for decreasing reactive oxygen species accumulation and avoiding membrane lipid peroxidation.

1. Introduction

The combustion of fossil fuels since the beginning of industrialization has caused the concentration of atmospheric carbon dioxide to rise from 280 $\mu l\,l^{-1}$ to approximately 380 $\mu l\,l^{-1}$, and this value is predicted to continue to increase in the future (IPCC, 2007). Numerous studies have shown that elevated CO_2 (EC) stimulates photosynthesis and plant growth in terms of plant biomass (Aranjuelo et al., 2006; Erice et al., 2006; Loladze, 2002; Long et al., 2006), increases intrinsic water use efficiency (WUE_i), and decreases stomatal conductance (g_s) in C_3 plants (Bernacchi et al., 2005; Curtis and Wang, 1998; Drake et al., 1997). Photosynthetic acclimation to long-term exposure to EC usually occurs in C_3 plants (Liberloo et al., 2006).

Wastewater discharge from mining operations and industrial emissions have resulted in more than 13,000 hm^2 of Cd-contaminated soil in China, such as the Shenyang Zhangshi irrigation area and the rice fields near the mining and smelting plants of Daye region, Hubei Province (Xiong et al., 2004; Yu and Zhou, 2009). Cd is a toxic

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and non-essential element for plants. High Cd concentrations in agricultural soils inhibit photosynthesis and growth as well as diminish water and nutrient uptake (Elobeid et al., 2012; He et al., 2011; Liu et al., 2011). Moreover, Cd induces oxidative stress and initiates membrane lipid peroxidation, thereby causing severe damage to membrane systems, cell organelles, and DNA (He et al., 2013a,b, 2015; Jin et al., 2013; Polle et al., 2013; Schützendübel and Polle, 2002; Z. Wang et al., 2008).

Many studies have investigated the effect of either EC or Cd treatment on plant growth and development; however, the combined effects of EC and heavy metals on plants remain largely unexplored (Tang et al., 2003). At present, plants are simultaneously exposed to Cd-contaminated soil and EC as a result of the continuous deterioration of the environment and the increase in atmospheric carbon dioxide concentration. As such, an increasing number of researchers are devoting their attention to the combined effects of EC and heavy metal phytotoxicity on plants (Guo et al., 2011, 2014; Jia et al., 2010; Li et al., 2010, 2012, 2014; Rajkumar et al., 2013; Song et al., 2013; Tang et al., 2003, 2011; Zheng et al., 2008). Tang et al. (2003) found that Indian mustard (*Brassica juncea* L. Czern.) and sunflower (*Helianthus annuus* L.) absorb more copper and produce more biomass when grown at EC. Similar results were tested and verified on ferns under Cu pollution (Zheng et al., 2008); *Lolium*, rice, and *Sedum alfredii* under Cd pollution (Guo et al., 2011; Jia et al., 2010; Li et al., 2010, 2012); as well as *Lolium multiflorum* and *Phytolacca americana* under combined Cd–Pb pollution (Song et al., 2013). In addition, EC improves the phytoremediation efficiency of heavy metals by enhancing root development, increasing fine root areas, and increasing heavy metal bioavailability in soil (Li et al., 2012; Song et al., 2013). Few studies have focused on the gas exchange and oxidative stress response of plants under heavy metal-contaminated soil to EC. Only Jia et al. (2010) found that the increase in biomass of two *Lolium* spp. under Cd treatment was related to the increases in photosynthetic and antioxidant defense capacity at EC. Previous studies have mainly focused on herbaceous plants; thus, little information is known about the effects of EC on the growth, gas exchange, antioxidant defense, and Cd uptake of fast-growing poplars and willows grown in contaminated soil.

Willows and poplars have attracted attention because of their capability to accumulate metals from contaminated soils. These species can also adapt to ecological niches, such as nutrient-poor, dry, wet, or metal-contaminated environments (Luo et al., 2014; Zacchini et al., 2009), and to biological features, such as deep root system and high biomass production (Licht and Isebrands, 2005). These characteristics suggest that these plants have potential economic and ecological benefits, and that they can be used for phytoremediation.

Compared with herbaceous species, willows and poplars are more responsive to EC (Ainsworth and Long, 2005), as exhibited by their plant height, biomass, and light-saturated carbon assimilation rate (A_{sat}) (Curtis and Wang, 1998; Nowak et al., 2004). Photosynthetic acclimation to long-term exposure to EC seldom occurs in fast-growing willows and poplars, but is common in other C_3 plants (Liberloo et al., 2006). Few studies have investigated the effects of EC on the growth and development of willows and poplars grown in Cd-contaminated soil. Only our previous study has shown that EC enhanced photosynthesis and root development, increased biomass and total Cd uptake, but did not change the Cd concentration of poplar and willow species grown in Cd-contaminated soil (Wang et al., 2012). However, our previous study was unable to demonstrate the effects of the interaction between EC and Cd treatments on the plant growth, gas exchange, and Cd uptake of willow and poplar species, because only a single Cd treatment was conducted in the previous study (Wang et al., 2012). Therefore, the current study focuses mainly on how EC and Cd treatments jointly affect the plant growth, gas exchange, antioxidant defense abilities, and Cd uptake of willow and poplar species. Open-top chambers were used in this study to investigate the effects of EC on the poplar genotype *Populus* × *euramericana* (Dode) cv. 'Nanlin-95' (NL95) and the willow genotype *Salix jiangsuensis* CL. '172' (J172) that were grown in three Cd-contaminated soils. The results of this study are expected to improve our understanding of the mechanisms by which EC enhances the plant growth and phytoremediation efficiency of poplars and willows grown in Cd-contaminated soil.

2. Materials and methods

2.1. Soil preparation and plant growth

Soil samples were collected from a long-term experimental rice field at Shenyang Agricultural University, Liaoning Province. The physical and chemical properties of the soil samples are shown in Table 1. Soil property determination and elemental analysis were performed as described previously (Tang et al., 2003; Wei et al., 2009). Fresh soil was sieved to pass a 3-mm sieve and stored in the dark 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 produce three concentration levels: 0, 5, and 25 mg Cd kg^{-1} soil. The spiked soils were then watered to field water capacity and stored in the dark for three months. The artificially contaminated soils were sampled randomly and analyzed for Cd concentration before transferring them into the pots. The actual total concentrations of Cd in the artificially contaminated soils were 0.06, 5.2, and 25.3 mg kg^{-1}, which are close to the three targeted levels of added Cd. The balanced soils were fertilized with 56, 32, and 6.22 mg kg^{-1} of N, P_2O_5, and K_2O, respectively.

Hardwood cuttings of *Populus* × *euramericana* (Dode) cv. 'Nanlin-95' (NL95) and *Salix jiang*suensis CL. '172' (J172) were obtained from the Chinese Academy of Forestry and Jiangsu Academy of Forestry, respectively. Each cutting was planted in a pot (16 cm diameter; 18 cm height) containing a moist mixture of perlite and vermiculite (1:1). The potted plants were precultured in a greenhouse and maintained well-watered. After 40 days, 72 rooted cuttings with similar sizes were selected and transferred to larger pots (25 cm diameter; 30 cm high) containing 9 kg dry weight soil. After one week of adaptation, potted plants were

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

Soil property	
Total Cd (mg kg^{-1})	0.06
Total Cr (mg kg^{-1})	6.71
Total Ni (mg kg^{-1})	6.25
Total Cu (mg kg^{-1})	36.83
Total Zn (mg kg^{-1})	56.47
Total As (mg kg^{-1})	3.25
Total Hg (mg kg^{-1})	0.15
Total Pb (mg kg^{-1})	23.75
Total N (g kg^{-1})	0.96
Total P (g kg^{-1})	0.58
Total K (g kg^{-1})	19.60
Organic matter (g kg^{-1})	16.32
Cation exchange capacity (CEC) (cmol kg^{-1})	17.85
pH (H_2O)	5.63
Particle size distribution[a] (wt %)	
Clay (%)	17.6
Silt (%)	29.7
Sand (%)	52.7
Soil texture	Sandy silty loam

[a] Sand, 0.02–2 mm; silt, 0.002–0.02 mm; clay, <0.002 mm.