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Effect of simulated climate warming on the morphological and physiological traits of *Elsholtzia haichowensis* in copper contaminated soil

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ABSTRACT

The aim of this study was to investigate the effects of temperature and Cu on the morphological and physiological traits of *Elsholtzia haichowensis* grown in soils amended with four Cu concentrations (0, 50, 500, and 1000 mg kg^{-1}) under ambient temperature and slight warming. At the same Cu concentration, the height, shoot dry weight, total plant dry weight, and root morphological parameters such as length, surface area and tip number of *E. haichowensis* increased due to the slight warming. The net photosynthetic rate, stomatal conductance, transpiration, light use efficiency were also higher under the slight warming than under ambient temperature. The increased Cu concentrations, total Cu uptake, bioaccumulation factors and tolerance indexes of shoots and roots were also observed at the slight warming. The shoot dry weight, root dry weight, total plant dry weight and the bioaccumulation factors of shoots and roots at 50 mg Cu kg^{-1} were significantly higher than those at 500 and 1000 mg Cu kg^{-1} under the slight warming. Therefore, the climate warming may improve the ability of *E. haichowensis* to phytoremediate Cu-contaminated soil, and the ability improvement greatly depended on the Cu concentrations in soils.

KEY WORDS
climate warming; phytoextraction; root morphology; gas exchange; *Elsholtzia haichowensis*

Introduction

Anthropogenic activities such as mining, smelting, application of sewage sludge into soil, fertilization, and reclaimed water irrigation have accelerated soil contamination by heavy metals (Khan *et al.* 2000; Terzano *et al.* 2007), which is difficult to remediate (Benavides, Gallego, and Tomaro 2005). In China, it is reported that more than 2.0 × 10^7 ha of agricultural soil is contaminated with heavy metals (Huang, Hu, and Liu 2009), that potentially affects plant growth and development, and poses possible risks to human health. A number of technologies such as excavation, soil washing, leaching with chelating agents, flocculation and reverse osmosis–ultrafiltration have been developed for the remediation of the heavy metal-contaminated soils (Khan and Scullion 2000). These methods are not suitable, however, on a large-scale or on soils with low doses of heavy metal contamination due to their high cost, low remediation efficiency, and difficulty of implementation under field conditions. Phytoremediation of heavy metal-contaminated soil is an ecological technology (McGrath and Zhao 2003; Krämer 2005), and has some advantages over the conventional approaches as it is in situ, low cost, environmentally friendly, and aesthetically pleasing. However, phytoremediation also has several disadvantages such as slow-growing, limited shoot biomass, long period of plant growth, and dependence on season.

Because of an increase in atmospheric CO_2 and other greenhouse gasses, climate warming is occurring (Oreskes 2004; Xu *et al.* 2008). According to the fourth appraisal report content of the Inter-governmental Panel on Climate Change, the global mean temperature will increase by 1.8–4.0°C by the end of this century (IPCC 2007). In the past 100 years, the mean temperature around China has increased by 0.4–0.6°C, and it is estimated to rise by 1.7°C during 2020–2030 (Qin 2003). An increase in global mean temperature is expected to have a profound impact on plant growth. Previous studies have shown that climate warming usually increases net photosynthesis rate and increases carbon assimilates in plants (Tumbull, Murthy, and Griffin 2002; Niu *et al.* 2008; Shi *et al.* 2009). Consequently, climate warming generally promotes physiological activity and growth of plants, thus increasing their biomass (Suzuki and Kudo 2000; Naoya *et al.* 2002).

Although some earlier studies have reported the effects of climate warming on plants, only a few studies have considered the effects of climate warming on the plants under heavy metals stress (Li *et al.* 2012b; Sardans and Penuelas 2007; Sardans, Peñuelas, and Estiarte 2008) and found a positive effect of increasing temperature on the heavy metal accumulation in plant tissues (Li *et al.* 2011, 2012b; Sardans *et al.* 2008). For example, Sardans *et al.* (2008) observed that the warming treatment increased the accumulation of aluminum (Al) in both *Erica multiflora* and *Globularia alypumand*, arsenic (As), chromium (Cr), and lead (Pb) in *E. multiflora* and antimony (Sb) and zinc (Zn) in *G. alypum*.

From these previous studies it is assumed that climate warming may increase the biomass of hyperaccumulators growing in heavy metal-contaminated soils and promote accumulation of heavy metals in plants. However, little is known

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about the characteristic responses of hyperaccumulators to climate warming, and few studies have addressed heavy metal uptake by hyperaccumulators under climate warming in terms of alterations in root morphological traits.

Elsholtzia haichowensis Sun, an indicator of Cu mines (Xie and Xu 1952), is widely distributed on Cu mining wastes and Cu-contaminated soils along the middle and lower reaches of Yangtze River, China (Tang, Wilke, and Huang 1999; Lou, Shen, and Li 2004) and in Zhuji of Zhejiang Province, Eastern China. It has a large biomass, and its shoot dry matter yield reaches 10 ton ha^{-1} under field conditions. Previous studies have showed that *E. haichowensis* has a high Cu accumulation and tolerance, implying that it might be a good candidate for phytoremediation of Cu-contaminated soils (Yang, Yang, and Römheld 2002; Ke *et al.* 2001; Jiang, Yang, and He 2004). However, the effects of climate warming on the growth and Cu accumulation of *E. haichowensis* have not been studied. It is hypothesized that better growth and physiological responses to climate warming will help *E. haichowensis* accumulate Cu and enhance its phytoremediation efficiency. We aimed to find out: 1) the effect of simulated climate warming on the morphological and physiological traits of *E. haichowensis* in Cu-contaminated soil; 2) whether the simulated climate warming increase the Cu accumulation in different tissues of *E. Haichowensis*; 3) whether these effects depended on the Cu concentration in soils? Our results could provide basic references to the phytoremediation of Cu-contaminated soil.

Materials and methods

Seeds collection and germination

On December 18, 2011, seeds of *E. haichowensis* were collected from a natural population near Chi Mashan Cu mine (29°59′N, 115°05′E) in Hubei Province, China, and were stored in storage chamber with a low humidity till germination. On March 21, 2012, seeds were immersed into 0.01 g ml^{-1} NaClO for 10 min for the surface sterilization, then washed three times with sterilized deionized water. Seeds were germinated in 36-well insert trays in the greenhouse with 25°C of temperature and 70% relative humidity (RH) during the day, and 20°C of temperature and 85% RH during the night. A mixture composed of peat, perlite, and vermiculite (6:3:1, v/v/v) were used as the growth substrate. The growth substrate had a pH of 5.01 ± 0.04, organic matter content of 27.16 ± 0.26 g kg^{-1}, total nitrogen content of 4.15 ± 0.23 mg kg^{-1}, alkali-hydrolyzable nitrogen content of 0.50 ± 0.03 mg kg^{-1}, available phosphorus content of 6.30 ± 0.49 mg kg^{-1} and available potassium content of 4.20 ± 0.16 mg kg^{-1}. After 40 days, the seedlings with six leaves were used for further experiment.

Experimental design

Aliquots of 2 kg growth substrate were placed in each plastic pot (15 cm in diameter and 12 cm in height), then copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) was artificially added to growth substrate to give four levels such as CK (control, no Cu), 50 mg Cu kg^{-1} (slight), 500 mg Cu kg^{-1} (medium) and 1000 mg Cu kg^{-1} (severe). 50 mL of solution containing the required concentrations of Cu was added to the growth substrate, and thoroughly mixed. The mixed growth substrate was then transferred into each pot under which a suitable size plastic saucer was placed. The treated growth substrate was left to equilibrate outdoors in a waterproof plastic tunnel for about two months after being moistened to 70% field holding capacity (Li *et al.* 2012a). Once the water in pots had been evaporated naturally, leading to dried soil with a less than 10% moisture, the pot was again watered until the field holding capacity reached 70%. This period is long enough to allow natural equilibration of the various sorption mechanisms in the growth substrate. Healthy and uniformly sized seedlings of *E. haichowensis* with six leaves were transplanted into the pots, and one seedling was transplanted in each pot. All pots were transferred into the plastic tunnel and placed randomly. Each Cu treatment was replicated with thirty pots among which fifteen pots were placed under ambient temperature, and fifteen under the warming treatment. All pots were heated continuously (24 h/day, starting on June 10, 2012) using infrared heaters (165 × 15 cm, MR-2420, Kalglo Electronics, Inc., Bethlehem, PA, USA) which were suspended 2.25 m above the ground. The radiation power was set at 1600 W. For control (ambient temperature), 'dummy' heaters of the same shape and size were suspended at the same height to simulate the shading effects of the infrared radiator. The microclimate data from June 2012 to October 2012 showed that the slight warming treatment resulted in an increase of 2.10°C in the surface soil temperature and a decrease by 3.6% in gravimetric soil water content (% volume) at 0–10 cm. The pots were watered equally with tap water two or three times per week. To avoid water stress, these pots were never watered to the point of drainage.

Parameter analysis

Photosynthetic traits

On October 12, 2012, during the fully flowering stage, three pieces of fully expanded, healthy mature leaves (leaf index number 3–6 from shoot tip) of *E. haichowensis* were chosen from each treatment for measurement of photosynthesis. Three individuals from each treatment were randomly selected and the measurements were conducted between 08:30 and 15:30 on sunny days. Gas exchange by leaves was measured with a portable gas analysis system (Licor-6400xt, Li-COR, Lincoln, Nebraska, USA). The photosynthetically active radiation (PAR) was maintained at 1,200 μmol m^{-2} s^{-1} using the LI-6400 artificial light source (6400-02B, Li-COR, Lincoln, Nebraska, USA), and temperature was maintained at 25°C with an RH of 70% inside the leaf measurement chamber. The CO_2 concentration within the chamber was maintained at 380 μmol mol^{-1}. Net photosynthetic rate (P_n), transpiration (E), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were determined under each treatment. Water use efficiency (WUE) was calculated from the ratio P_n/E (Nijs *et al.* 1997). Light use efficiency (LUE) was calculated from the ratio P_n/PAR (Long, Baker, and Rains 1993).

Biomass and root morphological traits

After growing for five months, plant samples were harvested and separated into three parts such as leaves, stems and roots.

After plant roots were cleaned with a brush, root scanning was carried out immediately, i.e., the roots were spread out on a 30 × 48 cm glass frame, and captured root images using a flatbed scanner (1680 Professional, Epson, Long Beach, CA, USA). Root length, surface area, volume and number of tips were measured and recorded using root image analysis software WinRHIZO (Pro2004b, Regent Instruments, Inc., Quebec, Canada).

Cu concentration determination

After scanning, the fresh plant samples were washed with deionized water, dried in an oven at 105°C for 30 min, and then at 75°C for 72 h. The dried samples were weighed for dry weight and ground to a homogeneous powder with a stainless steel cutter blender (XA-1, Jintan Shenglan Instrument Manufacturing Co., Ltd., Jintan, China) for analysis of Cu concentration.

Subsamples (~0.2500 g) of the oven-dried plant samples

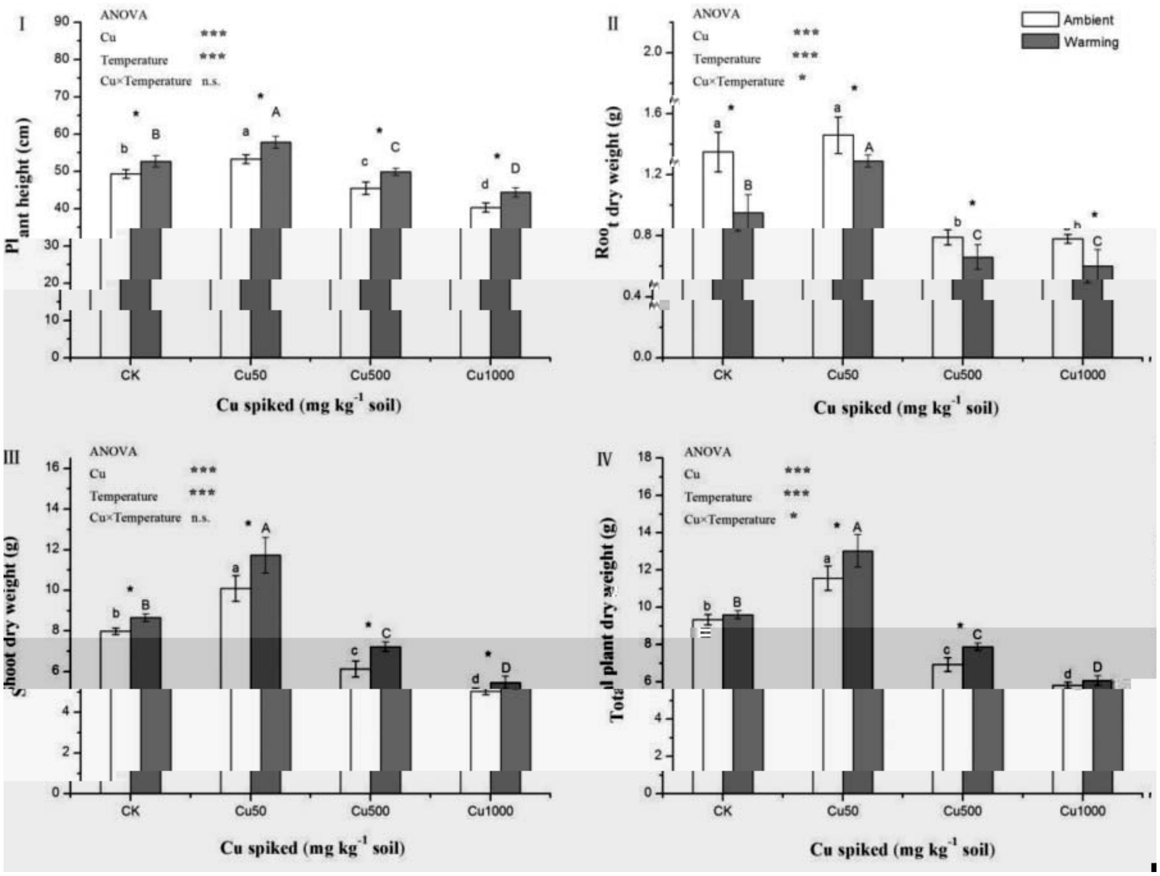

Figure 1. Effects of Cu and temperature on plant height (I), root dry weight (II), shoot dry weight (III), and total plant dry weight (IV) in *E. haichowensis*. Different capital letters indicate significant differences ($P < 0.05$) between different soil treatments in elevated temperature, different lowercase letters indicate significant differences ($P < 0.05$) between different soil treatments at ambient temperature, and * indicates significant differences ($P < 0.05$) between the two temperature treatments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

between different Cu concentrations were statistically significant at either ambient temperature or slight warming ($P < 0.05$). For soils with the same level of Cu, *E. haichowensis* had higher P_n, G_s, E, and LUE at slight warming than ambient temperature, and the differences between the two temperature treatments were statistically significant ($P < 0.05$). Compared to the ambient temperature, the P_n of *E. haichowensis* grown in soils with 0, 50, 500, and 1000 mg Cu kg^{-1} under slight warming increased significantly by 7.9%, 26.9%, 13.6%, and 15.4%, respectively (Fig. 3 I), while the E increased significantly by 58.8%, 17.4%, 16.2%, and 10.5%, respectively ($P < 0.05$) (Fig. 3 III). It also increased the G_s by 23.8%, 32.5%, 27.1%, and 39.1% (Fig. 2 IV), and the LUE by 7.9%, 26.9%, 13.6%, and 15.4%, respectively (Fig. 2 VI), compared to the ambient temperature. With increasing Cu concentration in soils, the C_i of *E. haichowensis* decreased in most cases (Fig. 3 II), but the WUE of *E. haichowensis* followed the opposite trend, which increased in most cases at either ambient temperature or slight warming (Fig. 3 V). For the same level of Cu, C_i was, in general, lower under ambient temperature than under slight warming, but only the C_i at 1000 mg Cu kg^{-1} was statistically significant ($P < 0.05$). The effects of Cu concentration, temperature, and their interaction on P_n, E, and LUE were significant ($P < 0.001$). The Cu concentration or temperature individually had a significant effect for G_s and C_i ($P < 0.01$), but the interaction between them were not significant ($P > 0.05$). Besides, the effect of Cu concentration and the interaction between Cu concentration and temperature were significant ($P < 0.01$), but the effect of temperature was not significant ($P > 0.05$).

Cu concentrations in the plant parts

Regardless of temperatures, Cu concentrations in the leaves, stems and roots of *E. haichowensis* increased significantly with increasing Cu concentrations in the soils ($P < 0.05$). For the plant grown in soils spiked with Cu under either ambient temperature or slight warming, the roots had the highest Cu concentrations, followed by the stems, and the leaves had the lowest. For soils with the same level of Cu, *E. haichowensis* growing at slight warming had significantly higher Cu concentrations in leaves, stems and roots than at the ambient temperature ($P < 0.05$). The Cu concentrations in leaves, stems and roots of *E. haichowensis* growing in soils with 0, 50, 500, and 1000 mg kg^{-1} Cu at slight warming increased by 74.1, 130.0, 30.5, and 18.6% (Fig. 4 I); by 48.3, 60.7, 29.6, and 22.8% (Fig. 4 II); by 56.4, 68.6, 157.1, and 101.7% (Fig. 4 III), respectively, compared to the ambient temperature. The Cu concentration, temperature effects, and their interaction between them were significant for leaf Cu concentration and root Cu concentration ($P < 0.05$). The Cu concentration or temperature individually had a significant effect for stem Cu concentration ($P < 0.001$), but the interaction between them was not significant ($P > 0.05$).

Total Cu uptake in the shoots and roots

Fig. 5 shows the total Cu uptake in shoots and roots of *E. haichowensis* at slight warming and ambient temperature. The total Cu uptake in shoots and roots of *E. haichowensis* increased significantly with increasing Cu concentrations at both ambient temperature and slight warming ($P < 0.05$), but the increase at 50 and 500 mg Cu kg^{-1} in the slight warming treatment were not statistically significant ($P > 0.05$). For soils with the same level of Cu, *E. haichowensis* growing at slight warming had higher total Cu uptake in shoots and roots than at the ambient temperature, but the increase of total Cu uptake in roots at CK and 1000 mg Cu kg^{-1} were not statistically significant ($P > 0.05$). The total Cu uptake in shoots of *E. haichowensis* growing in soils with 0, 50, 500 and 1000 mg kg^{-1} Cu was 23.5, 94.1, 47.3, and 29.0% greater under slight warming than under ambient temperature, respectively, and those of roots increased under slight warming, with magnitude being up to 8.9, 49.8, 53.2, and 9.9%, respectively (Figs. 5 I and II). The effects of Cu concentration, temperature, and their interaction on the total Cu uptake in shoots and roots were significant according to the two-way ANOVA ($P < 0.05$).

The bioaccumulation factors and tolerance indices of shoots and roots

The bioaccumulation factors (BFs) and tolerance indices (Tis) of shoots and roots decreased with the increased Cu concentrations in the soils regardless of temperatures (Fig. 6). The BFs of shoots and roots, the Ti of roots at 50 mg Cu kg^{-1} were significantly higher than those at 500 and 1000 mg Cu kg^{-1} at either ambient temperature or slight warming, and the differences in the Tis of shoots among different Cu treatments were statistically significant ($P < 0.05$). It was clear that for the same Cu level, *E. haichowensis* had higher BFs and Tis of shoots and roots when grown under slight warming in all situations, and the differences in BFs between the two temperature treatments were statistically significant ($P < 0.05$). Compared to the ambient temperature, the BFs of shoots of *E. haichowensis* grown in soils with 50, 500, andmg Cu kg

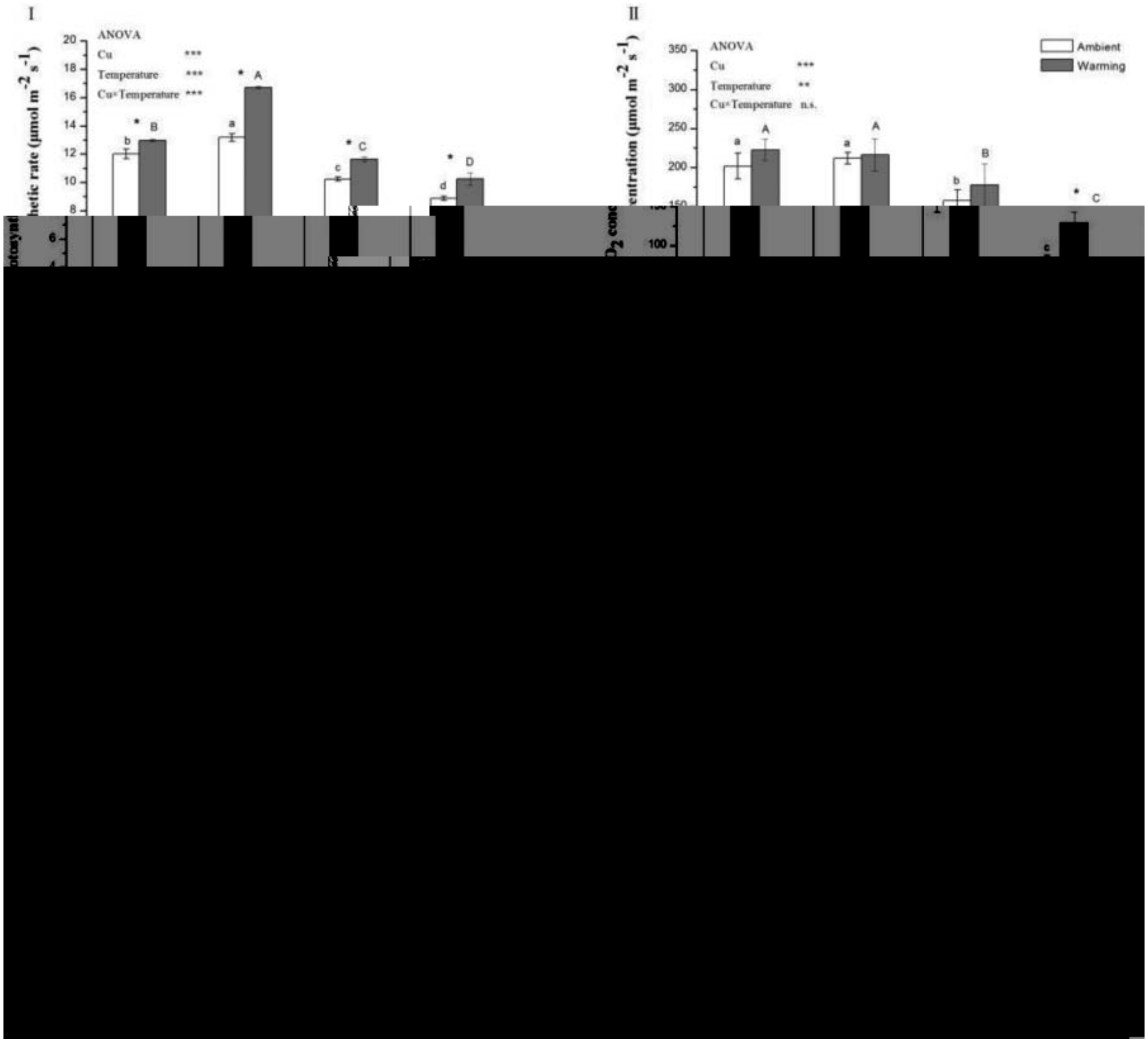

Figure 3. Effects of Cu and temperature on gas exchange parameters in *E. haichowensis*. Different capital letters indicate significant differences ($P < 0.05$) between different soil treatments in elevated temperature, different lowercase letters indicate significant differences ($P < 0.05$) between different soil treatments at ambient temperature, and * indicates significant differences ($P < 0.05$) between the two temperature treatments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

the Tis of shoots and roots were 1.36 and 1.35, respectively. Based on the two-way ANOVA, there were significant effects of Cu concentration ($P < 0.001$), temperature ($P < 0.001$), and their interaction on the BFs of shoots, BFs of roots, and Tis of shoots ($P < 0.05$). The Cu concentration or temperature individually had a significant effect for Tis of roots ($P < 0.05$), but the interaction between them was not significant ($P > 0.05$).

Discussion

Previous studies revealed that *E. Haichowensis* is a Cu-tolerant plant (Tang *et al.* 1999; Xiao *et al.* 2008), and its tolerance mechanisms to Cu possibly include: (1) Cu was accumulated in the apoplast space of *E. Haichowensis* due to the cation exchange process; (2) Cu could be bound to thiols, hydroxyl or other molecules of membrane surface; (3) the metal-chelating molecules by plants decreased the ability of Cu to penetrate the plasma membrane; (4) Cu induced the synthesis of intracellular metal-binding compounds such as Cu-binding protein (Lou *et al.* 2004). Based on these mechanisms, the roots of *E. Haichowensis* contain much higher Cu concentrations than the shoots (Song *et al.* 2004; Lou *et al.* 2004). In the present study, the roots of *E. Haichowensis* had much higher Cu concentrations than the stems and the leaves under either ambient temperature or slight warming. Most of the Cu was accumulated in the root, thus prevented Cu transport to the shoot. This may provide at least a partial explanation for *E. haichowensis* survival on severely Cu-contaminated soils.

In this study, we found that slight warming increased plant height, shoot dry weight, and total plant dry weight of *E. haichowensis* growing in soils with different Cu levels (Fig. 1 I, III, and IV), suggesting that slight warming is beneficial to the growth and development of *E. haichowensis*. Previous studies have shown that climate warming affects plant growth (Luo 2007; Shah and Paulsen 2003), plant photosynthetic physiology (Niu *et al.* 2008; Zhou *et al.* 2007), plant phenology (Dunne, Harte, and Taylor 2003), species composition (Wang *et al.* 2012b), and soil characteristics (Zhang *et al.* 2005), and the effects depend on specific plant species and ecosystem types. However, most researchers have suggested that moderate warming can enhance plant growth. Slight warming has been shown to increase plant photosynthesis (Tumbull *et al.* 2002;

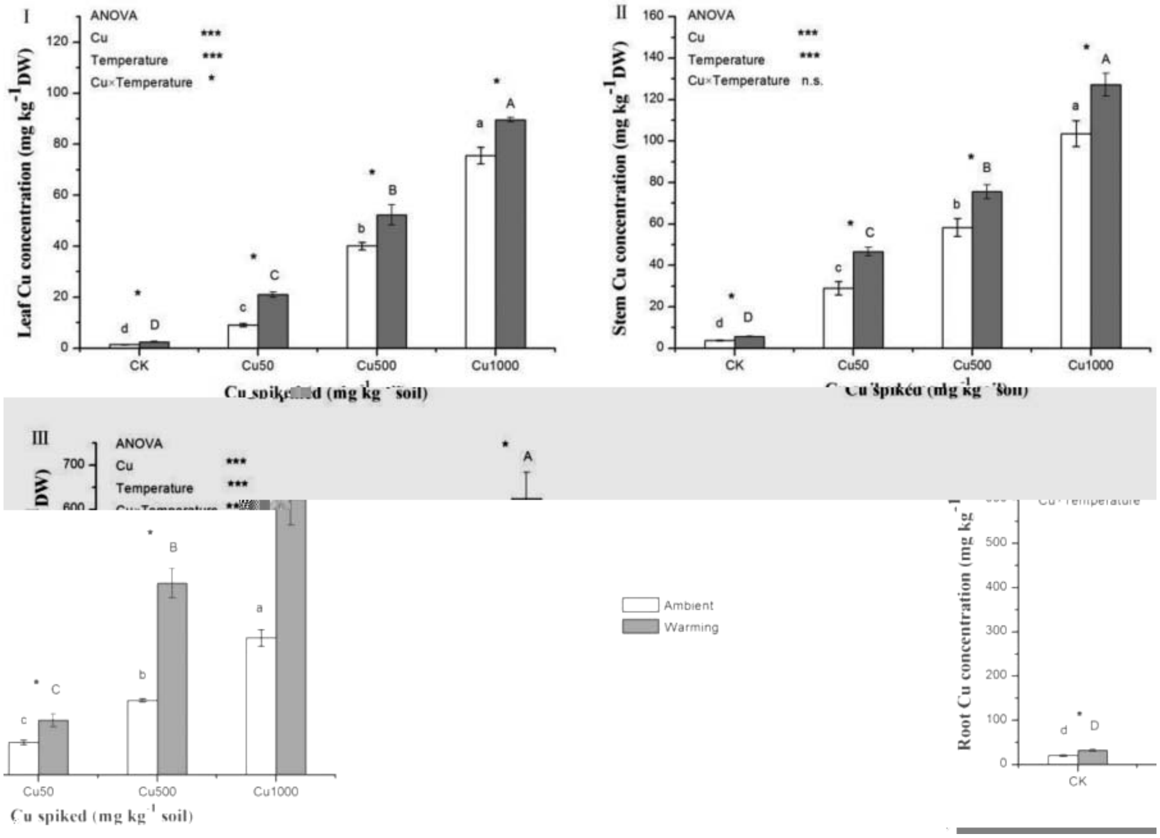

Figure 4. Effects of Cu and temperature on Cu concentration in leaf (I), stem (II) and root (III) of *E. haichowensis*. Different capital letters indicate significant differences ($P < 0.05$) between different soil treatments in elevated temperature, different lowercase letters indicate significant differences ($P < 0.05$) between different soil treatments in ambient temperature, and * indicates significant differences ($P < 0.05$) between the two temperature treatments. $^* P < 0.05$, $^{**} P < 0.01$, $^{***} P < 0.001$.

Niu *et al.* 2008; Shi *et al.* 2009), enhance soil enzyme activity (Xu *et al.* 2010), and thus result in greater biomass production (Suzuki and Kudo 2000; Naoya *et al.* 2002). Even in the slight warming could increase the growth of plants on heavy metal contaminated soil (Li *et al.* 2012b; Sardans *et al.* 2008). Because the efficiency of the phytoremediation of contaminated soil is often connected with a high biomass production of plant species and their accumulation of the contaminants, the increase in biomass of *E. haichowensis* at slight warming implies improvement of its phytoremediation efficiency if it is applied to field practice.

Root morphology parameters more accurately reflect the physiological and ecological function of root systems than root biomass (Robinson, Hodge, and Fitter 2003). Previous studies have showed that slight warming increases total root length, total root surface area, total number of root tips, and volume (Zhang 2010), whereas Cu stress has been shown to decrease root morphology variables (Kulikova, Kuznetsova, and

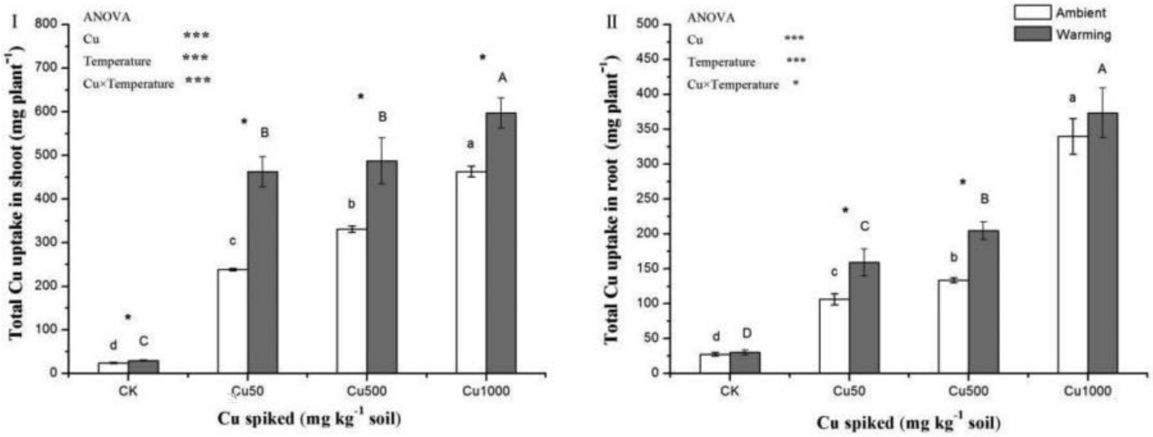

Figure 5. Effects of Cu and temperature on total Cu uptake in shoot (I) and root (II) of *E. haichowensis*. Different capital letters indicate significant differences ($P < 0.05$) between different soil treatments in elevated temperature, different lowercase letters indicate significant differences ($P < 0.05$) between different soil treatments in ambient temperature, and * indicates significant differences ($P < 0.05$) between the two temperature treatments. $^* P < 0.05$, $^{**} P < 0.01$, $^{***} P < 0.001$.

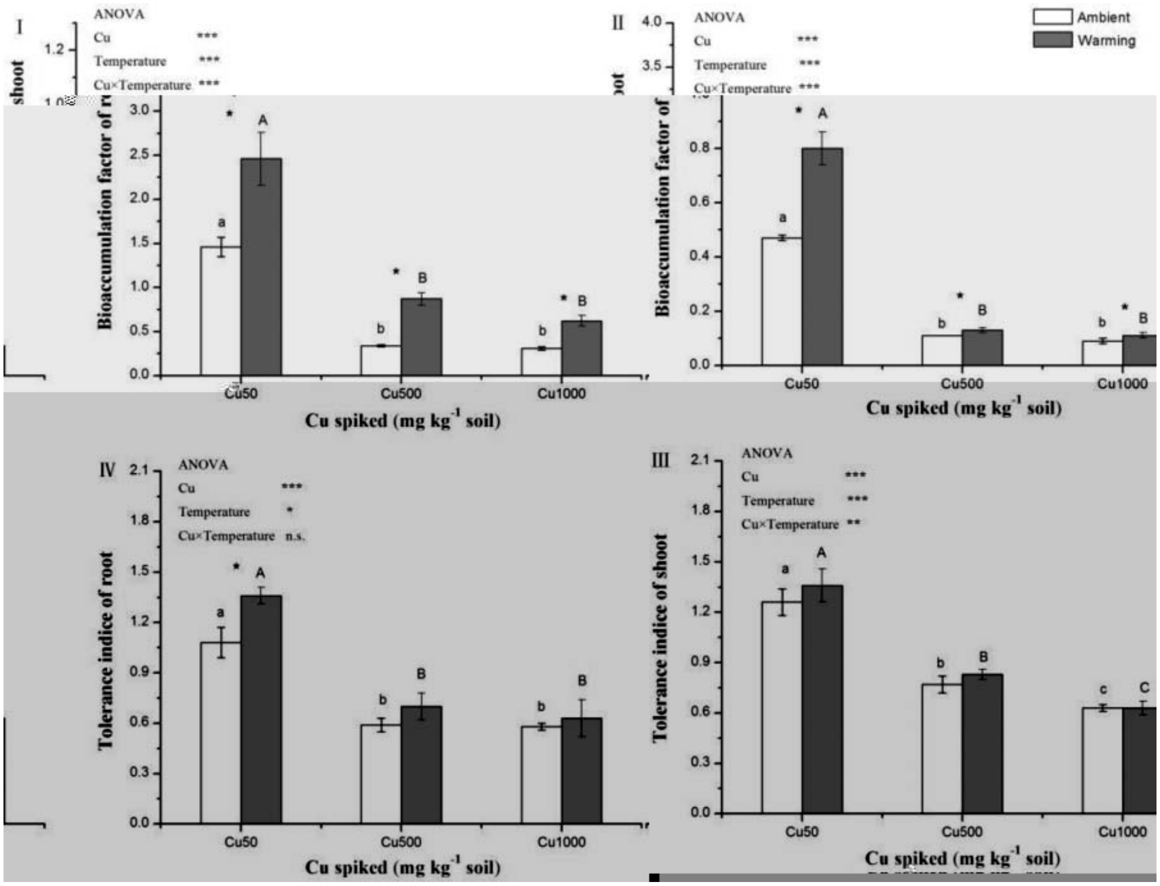

Figure 6. Effects of Cu and temperature on the Cu bioaccumulation factor (BF) of shoot (I), root (II) and tolerance index (Ti) of shoot (III), root (IV) in *E. haichowensis*. Different capital letters indicate significant differences (

Kholodova 2011). In this study, we found that *E. haichowensis* had higher values of these root morphological parameters at slight warming in all treatments, showing that slight warming increased root elongation and root branching. The changes in root morphology greatly enlarge the root surface area, and thus aid nutrient and water uptake. This can explain why slight warming triggered a significant increase in plant biomass under Cu stress. Correspondingly, the increase in root surface area enhanced the ability to capture Cu in root systems, which probably leads to increased total Cu uptake in all plant parts (Fig. 5 I and II).

Field experiments have suggested that moderate warming can enhance the photosynthesis and growth of plants (Read *et al.* 1997; Sage and Kubien 2003). Exposure to climate warming increased P_n, E, and G_s for plants under unstressed conditions (Xu *et al.* 2012). However, a survey of published literature revealed that little information is available concerning the effect of heavy metal and climate warming on plant photosynthesis. In the current study, the P_n, E, G_s, and LUE of *E. haichowensis* were higher under slight warming than under ambient temperature at the same level of Cu treatment (Figs. 3 I, III, IV, and VI). This indicated that slight warming increased photosynthetic capability of *E. haichowensis* irrespective of the Cu treatment. We also showed that the stimulation of photosynthetic capability in *E. haichowensis* under slight warming caused an increase in plant growth (Fig. 1). Enhanced photosynthesis under slight warming could be favorable for the growth and development of *E. haichowensis*, and beneficial to alleviate the negative effect of Cu stress to a certain extent.

The results of this study showed that slight warming increased the Cu concentrations in leaves, shoots and roots of *E. Haichowensis* compared to ambient temperature in the soil with the same level of Cu. The increases in Cu concentrations of leaves, shoots and roots ranged from 18.6 to 130.0%, 22.8 to 60.7%, and 56.4 to 157.1%, respectively. The total uptake of Cu in shoots and roots of *E. haichowensis* under slight warming exhibited a similar trend, ranging from 23.5 to 94.1% in the shoots and 8.9 to 53.2% in the roots, respectively. These results indicated that slight warming could increase the Cu uptake ability of *E. haichowensis* and benefit *E. haichowensis* in the phytoremediation of Cu. Similar results have been reported: Sardans *et al.* (2008) found that warming increased the accumulation of Al in both *E. multiflora* and *G. alypumand*, As, Cr and Pb in *E. multiflora* and Sb and Zn in *G. alypum* in a Mediterranean shrubland. Li *et al.* (2012b) also observed that slight warming increased Cu, Zn, and Fe concentrations by 25, 27, and 24% in *Solanum tuberosum* leaf, respectively. A recent study showed that wheat growing in Cd-contaminated soils at higher temperature took up more Cd in roots (Li *et al.* 2011).

It was interesting to note that the growth stimulation caused by slight warming was greater at slight Cu contamination stress (50 mg Cu kg^{-1}) than those at medium and severe Cu contamination stresses (500 and 1000 mg Cu kg^{-1}) (Fig. 1). It seemed that *E. haichowensis* depicted low-Cu-concentration stimulatory and high-Cu-concentration inhibitory responses. Moreover, under slight warming, BFs in shoot and root tissues at 50 mg Cu kg^{-1} were significantly higher than those at 500 and 1000 mg Cu kg^{-1}(Figs. 6 I, II). These results indicated that the toxicity of high dosage of Cu could affect the accumulation of Cu in *E. haichowensis*. Therefore, *E. haichowensis* will be a very effective candidate to treat large-scale soils with low doses of Cu contamination under the predicted climate warming in the future.

Conclusions

This study demonstrated that slight warming increased plant height, shoot dry weight, and total plant dry weight of *E. haichowensis*, and the biomass increase was closely associated with the stimulation of leaf photosynthesis induced by slight warming. Slight warming also increased the values of the root morphological parameters (length, surface area, volume, and tip numbers) of *E. haichowensis* grown in Cu-contaminated soil, and consequently, enhanced its ability to capture Cu in root systems and led to increased total Cu uptake in all plant parts. Total Cu uptake in shoots and roots of *E. haichowensis* was increased by slight warming, which can be attributed to the increased biomass under slight warming. All these results imply that climate warming may have positive implications for improving the phytoremediation efficiency of hyperaccumulators used to treat heavy metal contaminated soils under the predicted climate warming in the future.

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