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RESEARCH ARTICLE

Nitrate reduces copper toxicity by preventing oxidative stress and inhibiting copper translocation from roots to shoots in *Liriodendron Chinense*

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Abstract

Nitrogen forms can affect metal accumulation in plants and tolerance to metals, but a few published studies on the effects on Cu toxicity and Cu accumulation in plants are scarce. Thus, the objective of this study was to evaluate the responses of *Liriodendron chinense* to different nitrogen forms, by the oxidative stress, antioxidant enzymes system, GSH-AsA cycle, Cu uptake, translocation, and accumulation under Cu stress. We found that Cu-induced growth inhibiting was alleviated by added exclusive NO_3^--N. Adding N as NH_4^+-N with or without NO_3^--N was aggravated as evidenced by significantly elevated malonaldehyde (MDA) and hydrogen peroxide (H_2O_2) compared to N-Null. Cu exposure and adding NH_4^+-N inhibited superoxide dismutase activity, but remarkably stimulated the activities of catalase and peroxidase, the efficiency of glutathione-ascorbate (GSH-AsA) cycle, and the activity of glutathione reductase and nitrate reductase, with respect to the control. However, adding exclusive NO_3^--N progressively restored the alteration of antioxidant to prevent Cu-induced oxidative stress. Additionally, adding exclusive NO_3^--N significantly promoted the Cu uptake and accumulation in roots, but reduced Cu concentration in leaves, accompanied by the inhibited Cu translocation factor from roots to shoots by 36.7%, when compared with N-Null. Overall, adding NO_3^--N alleviated its Cu toxicity by preventing Cu-induced oxidative stress and inhibiting Cu translocation from roots to shoots, which provides an effective strategy for phytostabilization in Cu-contaminated lands.

Keywords Copper toxicity · Nitrogen form · Enzyme activity · Metal translocation

Introduction

Copper (Cu) is an essential trace element and affects plant growth and development by regulating multiple metabolic pathways (Shabbir et al.2020). However, higher level of Cu can be toxic to plants and can impair shoot growth and cause leaf chlorosis and necrosis in leaf tips and edges (Ameh and Sayes 2019; Chen et al. 2015). It also can inhibit photosynthesis and metabolic functions, interfere with nutrient absorption, trigger oxidative bursts, damage cell structure, and increase plant mortality (Bouazizi et al. 2010; Gong et al. 2019). The Cu phytotoxicity affects the efficiency of phytoremediation and phytostablization in Cu-contaminated soils, especially Cu mining and metal processing areas. Several strategies, such as breeding of resistant plant varieties, solidifying soil Cu, fertilizer management, have been developed to reduce metal toxicity in plants (Rizwan et al. 2016). Nitrogen management has been regarded as the most economical and time-saving agronomic strategy to control the accumulation of metal elements, including Cd, Mn, and Zn in plants (Gao et al. 2016; Zhao and Shen 2018; Yang et al. 2020).

Nitrogen (N) is the most abundant macro-nutrient element for plant growth and developmental processes (Makino 2011; Yang et al. 2020) and has been well-known to be related to metal accumulation in plant, as well as plant tolerance to

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metal toxicity (Mitchell et al. 2000; Wu et al. 2019). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) are the two major forms of inorganic nitrogen absorbed by the roots of higher plants, showing variant effects on uptake, translocation, and accumulation of metals in plants (Ata-UI-Karim et al. 2020; Cheng et al. 2020; Hassan et al. 2005; Jalloh et al. 2009; Yang et al. 2020). For instance, compared to NO_3^--N, NH_4^+-N was reported to accelerate Cd accumulation in tobacco (Tsadilas et al. 2005), sunflower (Zaccheo et al. 2006), *Populus* (Qasim et al. 2015), and *Carpobrotus rossii* (Cheng et al. 2016). Wang et al. (2021) found that adding NH_4^+-N induced remobilization of the immobilized Cd in soil for reduced rhizosphere pH, which resulted in Cd accumulation in *Amaranthus mangostanus.* However, the other studies showed that NO_3^--N enhanced Cd uptake and accumulation in rice (Hassan et al. 2005; Jalloh et al. 2009; Wu et al. 2019), potato (Larsson and Asp 2013), sweet sorghum (Bai et al. 2021), and *Sedum plumbizincicola* (Hu et al. 2013). Similarly, NH_4^+-N reduced the accumulation of Al and alleviated the metal's toxicity to rice (Wang et al. 2015) and *Lespedeza bicolor* (Chen et al. 2010). Therefore, the practical use of nitrogen fertilizer should take into account plant species, nitrogen forms, metal types and levels, and soil characteristics (Yang et al. 2020). However, not enough information is available about the effects of nitrogen fertilizers on Cu toxicity and stabilization capacity by pioneer plants in Cu contaminated soils.

Nitrogen plays an important role in promoting plant tolerance to toxicity of metals by regulating antioxidant system to eliminate excessive metal-induced oxidative damage (Jalloh et al. 2009; Yang et al. 2020). Giansoldati et al. (2012) found that N application ameliorated boron-induced oxidative stress in *Brassica juncea* by progressively lessening the activity of SOD, APX, PPX, and GR to the control levels. Wu et al. (2020) presented NH_4^+-N enhanced Cd tolerance in rice by increased SOD enzyme and the efficiencies of GSH-AsA cycle with increasing concentration of AsA and GSH and GR and DHAR activities. As a redox active metal, excessive Cu induced the activities of CAT, SOD, APX, and GR in *Tanzania guinea* grass, and the combination of NO_3^- with NH_4^+-N favored the antioxidant system to promote the efficiency of copper phytoextraction (de Souza Junior et al. 2019). Thus, enzymatic and non-enzymatic antioxidants were distinctly regulated by nitrogen forms in different plant species under different metals stress. However, the effects of different NH_4^+/NO_3^- proportion on plant resistance to Cu toxicity related to the antioxidant enzyme system and GSH-AsA cycle are still poorly understood, especially in trees.

Liriodendron chinense (Hemsl.) Sarg. is a widely used tree species in landscaping and afforestation programs, due to its fast growth rate, beautiful tree shape, good ornamental, and biomass accumulation (Huang and Guo 2015). *Liriodendron* species are planted as a crucial hardwood species in old field and mine wasteland (Sena et al. 2015). Previously, we performed preliminary experiments to understand how *Liriodendron chinense* responded in Cu wasteland by field planting in Dexing copper mine and found that *Liriodendron chinense* was strongly affected by Cu more than by other metals, such as Cd and Pb. Given this fact, we decided to investigate the effects of different NH_4^+/NO_3^- ratios on Cu toxicity in *Liriodendron chinense* in oxidative stress, antioxidant enzyme system, GSH-AsA cycle, Cu uptake, translocation and accumulation, and selected elements in plant tissues. To the best of our knowledge, this study is the first report to research the effects and underlying mechanisms of different nitrogen forms in protecting plants from Cu, and the potential of developing N management strategies to promote the stabilization in Cu-polluted soils.

Material and methods

Plant material and experimental design

The plant materials were derived by stem cutting *Liriodendron chinense*, which collected from an adult tree in a provenance trail plantation located in Huangma, Nanchang country, Jiangxi province of China. The tree originated from Mount Lu Nature Reserve, Jiangxi Province. Annual seedlings were transferred to pots containing soil culture medium using inert silica pellets and perlite (3:2, v/v), avoiding other factors in soils during the experiment. Each pot had one seedling. The pots were kept in a greenhouse with natural light and temperature conditions (average day, 18–30 °C; night, 16–24 °C), watered every 2 days by filtered water and added 1/4 Hoagland nutrient solution every week.

Uniform seedlings (about 20–30 green leaves and 40–50 cm tall) were selected and used for Cu and N treatments after 30 days of transplantation. In a preliminary experiment, the seedlings had damage symptoms under Cu treatment (25 mg.kg^{-1}) and slight damage above 10 mg.kg^{-1} of sole supply of NH_4^+-N. In this study, we treated with 25 mg/kg Cu added as $CuCl_2$ in combination with three molar percentage ratios of NH_4^+ to NO_3^- (1:0, 1:1, 0:1). Therefore, the treatments were designed as follows: (1) CK: plants untreated with Cu and N; (2) Cu: plants treated with 25 mg kg^{-1} Cu; (3) Cu-NH_4^+: plants treated with 25 mg kg^{-1} Cu + 300 mg kg^{-1} N (NH_4Cl); (4) Cu-NH_4^+ + NO_3^-: plants treated with 25 mg kg^{-1} Cu + 150 mg kg^{-1} N (NH_4Cl) + 150 mg kg^{-1} N (KNO_3); (5) Cu-NO_3^-: plants treated with 25 mg kg^{-1} Cu + 300 mg kg^{-1} N (KNO_3). To maintain the balance of potassium (K) for the addition of NO_3^--N, KCl was added in the Cu-NH_4^+-N and Cu-NH_4^+ + NO_3^-treatments, respectively. Each treatment was replicated with six pots using a randomized complete block design. Obvious toxicity symptoms had been observed after 5th day of the treatment in Cu-treated plants,

so the experiment harvest took place at the seventh day of the treatment when we observed old leaf senescence. Leave samples were harvested with a scissor from the fifth or sixth expanded leaves (counting from the top of the plant) and stored immediately at − 80 °C for physiological and biochemical analyses. Then, different tissues (roots, stem, and leaves) were collected for measuring the concentration of N and Cu. The roots were immersed in 20 mM Na_2-EDTA for 30 min and rinsed with de-ionized water thoroughly.

Measurements of chlorophyll concentration in leaves

Chlorophyll concentration was determined following the method of Sun et al. (2015). Briefly, 0.1 g fresh leaves were added to 10 mL dimethyl sulfoxide (DMSO) and shaken in the dark for 72 h. The absorbance of the extract was measured at 663 nm and 645 nm with a spectrophotometer (SPAD-502, Minolta, Camera Co. Ltd., Osaka, Japan).

Crude enzyme extraction, MDA, and H_2O_2 contents analysis

For crude enzyme extraction, 1.0 g fresh leaves were ground in liquid nitrogen and transferred to 10-mL pre-cooling sodium phosphate buffer (100 mM, pH 7.0) and centrifuged for 20 min at 13,000 × *g* at 4 °C. Then, the supernatant was used to determine the concentrations of malondialdehyde (MDA) and reactive oxygen species (H_2O_2) and activities of antioxidant enzymes.

To measure MDA, 1 mL of the supernatant was mixed with 4 mL of reaction solution (0.5% (w/v) thiobarbituric acid and 5% (w/v) trichloroacetic acid), incubated at 95 °C for 30 min, and cooled in ice bath. The mixture was centrifuged at 10,000 × *g* for 10 min and the absorbance of the supernatant was measured at 532 nm and 600 nm (Khator and Shekhawat 2020).

H_2O_2 concentration was measured according to Shi et al. (2015). In brief, 100-µL supernatant was mixed with 1-mL solution containing 0.1% titanium sulfate and 20% (v/v) H_2SO_4 and incubated at 25 °C for 10 min. Then, the mixture was centrifuged at 12,000 × *g* for 10 min at room temperature, and the absorbance was read at 410 nm.

Measurement of antioxidant enzymes activity

The activities of four antioxidant enzymes, including superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), and ascorbate peroxidase (APX; EC1.11.1.1), were determined using a Total SOD Assay Kit (A001-3), a Plant POD Assay Kit (A084-3), a Plant CAT Assay Kit (A007-1), and a Plant APX Test Kit (A123-1–1), respectively, Xanthine oxidase method was used to measure superoxide dismutase (SOD; EC 1.15.1.1) activity by a Total SOD Assay Kit (A001-3), which was calculated by the values of inhibiting 50% initial decline of nitro blue tetrazolium at 550 nm. Peroxidase (POD; EC 1.11.1.7) activity was determined in a 3-mL reaction mixture containing 100-µL enzyme extract, 50 mM phosphate buffer (pH 7.0), 28-µL guaiacol, and 19-µL H_2O_2 by a Plant POD Assay Kit (A084-3), which was read five times at 420 nm within 2 min at 30-s intervals and measured the absorbance change of 0.01. Catalase (CAT; EC 1.11.1.6) activity was detected by a Plant CAT Assay Kit (A007-1). One unit of activity was expressed as the amount of enzymes for 1-mg tissue proteins consuming 1 µM H_2O_2 at 405 nm. Ascorbate peroxidase (APX) activity (EC1.11.1.1) was measured by a Plant APX Test Kit (A123-1–1). The reaction solution (1 mL) was composed 50 mM phosphate buffer (pH 7.8), 0.1 mM H_2O_2, 0.1 mM EDTA, 0.2 mM ascorbate (AsA), and 100µL supernatant. The reaction was started by addition of H_2O_2, and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm. APX activity was calculated by using the molar extinction coefficient for AsA (Zeng et al.2017). The four enzyme assay kits were obtained from Nanjing Jiancheng Bioengineering Institute, China.

Activity determination of nitrate reductase, glutathione reductase, dehydroascorbate reductase, AsA, and GSH

Nitrate reductase (NR) (EC.1.7.1.1) activity was determined according to the method described by Li et al. (2007). The 1-mL crude enzyme extract was mixed with a solution containing 100 mM potassium phosphate buffer (pH 7.5), 50 mM KNO_3, and 1% (v/v) iso-propanol, then incubated at 30 °C for 30 min. Subsequently, 1 mL 30% (w/v) TCA was added into the reaction solution to stop the enzyme activity. The nitrite was released to the medium by adding 1% (w/v) sulfanilamide in 2.4 M HCl, 0.02% (w/v) naphthylethylenediamine (1:1) into the reaction solution. Then, the absorbance was read at 540 nm. Controls were measured before the incubation period.

Glutathione reductase (GR) (EC1.6.4.2) activity was assayed according to Shi et al. (2015) using a Plant GR Assay Kit (A062-1–1; Nanjing Jiancheng Bioengineering Institute, China). The reaction solution was composed of 20-µL crude extract, 100-µL 2.5 mM GSSH, 10-µL 2 mM NADPH, and 70-µL GR assay solution. The oxidation rate was corrected for the non-enzymatic oxidation of NADP by GSSG. Then, GR activity was determined by a decrease in absorbance at 340 nm.

Dehydroascorbate reductase (DHAR) was assayed according to the method of Chen and Gallie (2006). Briefly, 0.1-g fresh leaves were ground in 1-mL pre-cooling extraction buffer containing 50 mM Tris–HCl, 2 mM EDTA, and 1 mM $MgCl_2$ (pH 7.5), and the homogenate

was centrifuged at 13,000 × *g* for 10 min at 4 °C. Then, 100-µL supernatant was mixed with 700 µL K_2HPO_4/KH_2PO_4 (pH 6.5, 50 mM), 100 µL DHA (0.5 mM), and 100 µL reduced glutathione (1 mM). The DHAR activity was calculated based on the absorbance at 265 nm.

AsA and glutathione (GSH) contents were determined according to the methods of Chen and Gallie (2006) and Brehe and Burch (1976), respectively. Briefly, 0.5-g leaves were ground in liquid nitrogen and transferred into 4-mL pre-chilled 5% (w/v) TCA solution, containing 2 mM Na_2-EDTA. The mixture was centrifuged at 16,500 × g for 20 min at 4 °C, and the supernatant was collected for AsA and GSH analysis. The reaction solution for measuring AsA content was prepared by mixing 1 mL of supernatant, 1 mL of ethanol, 1 mL of 0.5% (w/v) 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline), 1 mL of 5% (w/v) TCA, 0.5 mL of 0.03% (w/v) $FeCl_3$, and 0.5 mL of 0.4% (w/v) H_3PO_4. The reaction solution was incubated at 30 °C for 1 h until the color changed and used to read the absorbance at 525 nm. For GSH determination, the reaction mixture consisted of 1 mL of supernatant and 0.5 mL of 4 mM 5,5-dithiobis-(2-nitrobenzoic acid), and 1 mL of phosphate buffer (100 mM, pH 7.7) was kept at 25 °C for 10 min until the color changed. The GSH content was determined based on the absorbance at 405 nm.

Contents of Cu, N, P, and K in plant tissue

For Cu content analysis, 0.5-g harvested dried samples (root, stem, leave) were crushed and digested in acid mixture of HNO_3–$HClO_4$ (4:1, v/v) at 220 °C for 4 h. The concentrations of Cu in the diluted digestive solution were determined by inductive coupled plasma optical emission spectroscopy (ICP-OES) (Optima 8000; Perkin-Elmer, Waltham, Massachusetts, USA). Cu translocation from roots to shoots has been calculated by shoot-to root concentration ratio.

Total nitrogen was determined using oven dry roots, stems, and leaves according to the Kjeldahl method (Helrich 1990) after digesting with H_2SO_4 and H_2O_2. Total potassium was measured using NaOH-melting-flame photometric method (FP6431, Shanghai, China), and total phosphorus was determined using H_2SO_4–$HClO_4$ digestion-phosphomolybdate blue spectrophotometry (UV/VIS-4802, UNICO, Shanghai, China),

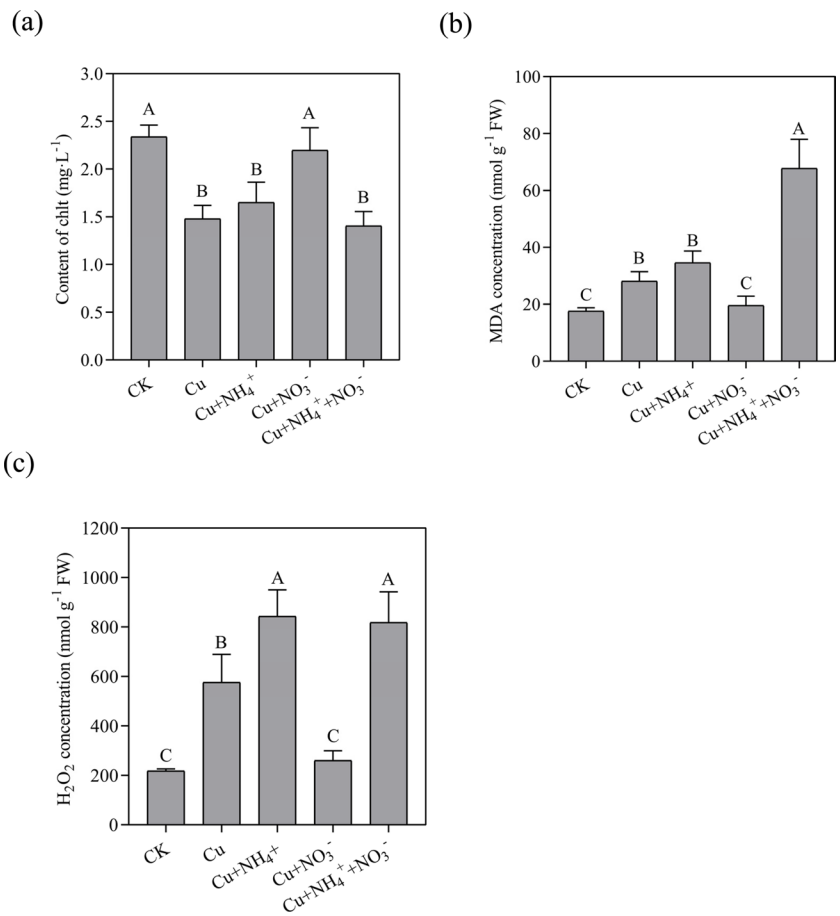

Fig. 1 Chlorophyll content, MDA level, and H_2O_2 concentration in leaves of *Liriodendron chinense*. (**a**) Total chlorophyll (Chlt) content, (**b**) malonaldehyde (MDA) concentration, (**c**) H_2O_2 concentration. Data are means ± SD ($n = 4$). Different letters indicate significant differences between different treatments at $P < 0.05$ by least significant difference test

Statistical analysis

All treatments were conducted in triplicate independent experiments, and all data were analyzed using the SPSS software (Version 18.0; IBM Corp., Armonk, NY). A one-way analysis of variance (ANOVA) was used to compare means among treatments. The significant differences among treatments were separated based on the least significant difference test (multi-range comparison) at $P < 0.05$. The data presented in tables and figures, expressed means ± SD, are based on three biological independent experiments.

Results

Supply of NO_3^--N alleviates Cu toxicity in *Liriodendron chinense*

Liriodendron chinense exposed to Cu for 7 days showed obvious toxicity symptoms, including curled, yellowed, and wilted leave. These effects were consistent with decreased chlorophyll content (Fig. 1a). Meanwhile, effects of NO_3^--N and NH_4^+-N were apparently different in Cu-treated plants. Supply of exclusive NO_3^--N showed mild toxicity symptoms, as evidenced by less leaf chlorosis and greater chlorophyll content by 1.48-fold compared to the N-Null under Cu treatment (Fig. 1a). However, regardless of the presence or absence of NO_3^--N, adding NH_4^+-N intensified the Cu-induced inhibitory effects on *L. chinense* growth in the first 3 days. Afterward, leaf necrosis and wilting occurred more quickly when ammonium was used as the nitrogen source, especially when supplied as NH_4^+-N + NO_3^--N. The chlorophyll content in the supply of NH_4^+-N and NH_4^+-N + NO_3^--N was reduced by 29.4% and 39.7%, respectively, compared to controls.

Supply of NO_3^--N reduces oxidative damage

Cu significantly increased the contents of H_2O_2 and MDA in *Liriodendron chinense* leaves, compared to controls (Fig. 1b, c). Adding NH_4 + -N, with or without NO_3^--N, significantly promoted H_2O_2 concentration by 41.8% and 46.3%, respectively, and MDA concentration was also increased compared to the N-Null. However, sole NO_3^--N significantly reduced the concentrations of H_2O_2 and MDA in leaves under treatment of Cu.

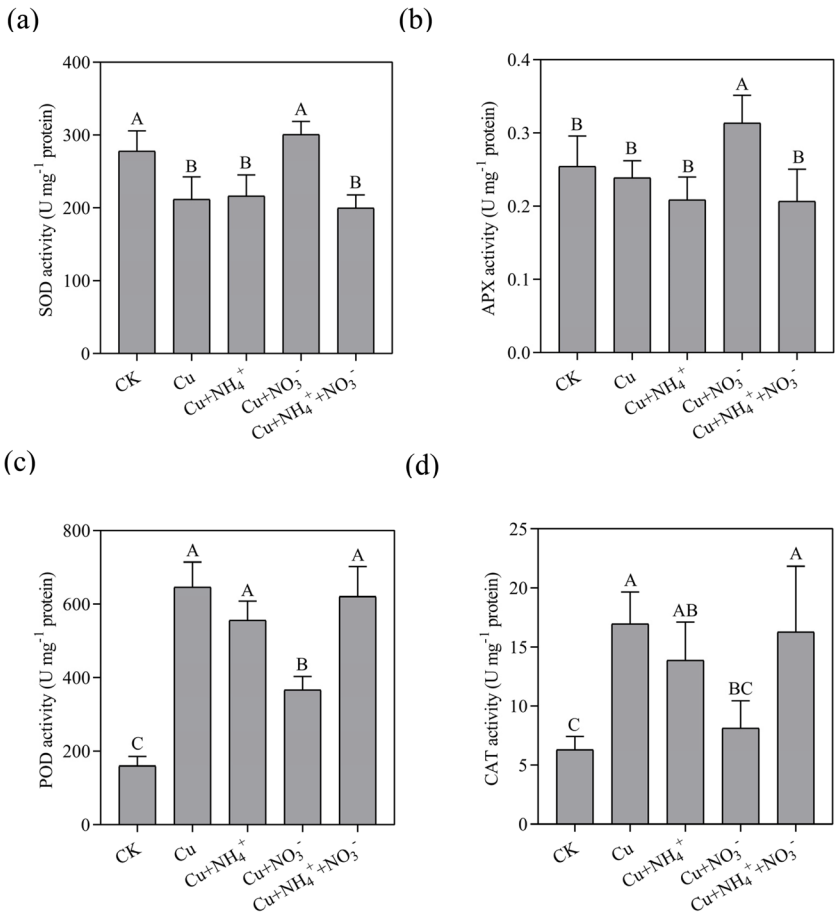

Fig. 2 Activity of leaf (**a**) superoxide dismutase (SOD), (**b**) ascorbate peroxidase (APX), (**c**) peroxidase (POD) and (**d**) catalase (CAT) in *L. chinense*. Data are means ± SD ($n = 4$). Different letters indicate significant differences between different treatments at $P < 0.05$ by least significant difference test

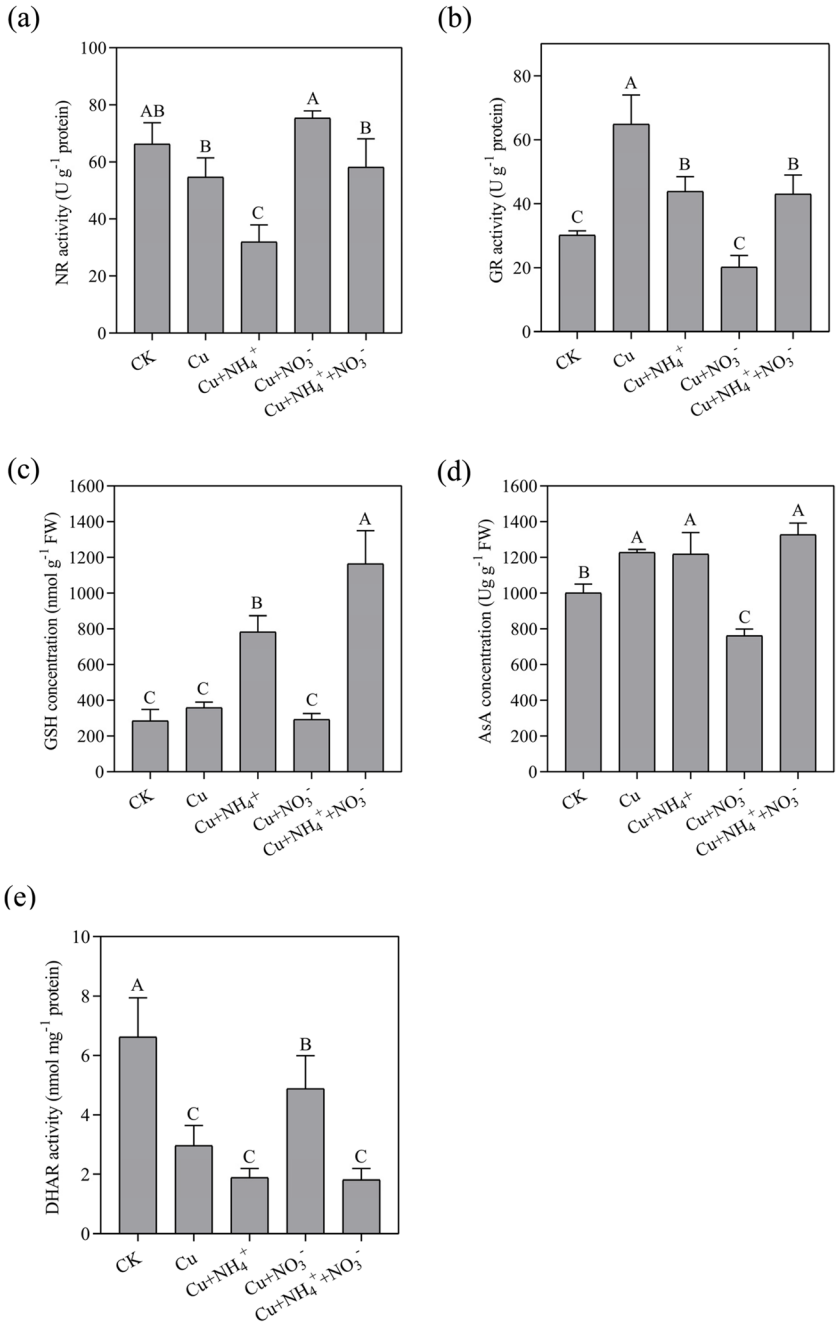

Fig. 3 Activity of leaf (**a**) nitrate reductase (NR), (**b**) glutathione reductase (GR), (**c**) glutathione (GSH), (**d**) ascorbic acid (AsA) concentration, and (**e**) dehydroascorbate reductase (DHAR) in *L. chinense*. Data are means ± SD ($n=4$). Different letters indicate significant differences between different treatment at $P<0.05$ by least significant difference test

Effects of nitrogen forms on antioxidant enzymes in response to Cu

Cu significantly inhibited SOD activity, compared to controls (Fig. 3). Sole NO_3^--N significantly increased the SOD activity by 25.4%, compared to the N-Null (Fig. 3a). Supply of $NH4^+$-N, with or without NO_3^--N, had no apparent effects. Cu significantly inhibited APX activity. Surprisingly, NO_3^--N treatments significantly stimulated APX activity in *L. chinense* leaves by 23.2% and 31.0%, respectively, compared to control and N-Null treatments (Fig. 3b). Cu treatments and adding NH_4^+-N, with or without NO_3^--N, markedly increased POD and CAT activities, but NO_3^--N restored the Cu-induced activities of POD and CAT (Fig. 2c, d).

Effects of nitrogen forms on NR activity and GSH-AsA cycle under Cu stress

Sole NO_3^--N significantly stimulated NR activity in Cu-treated plants; however, supply NH_4^+-N alone significantly

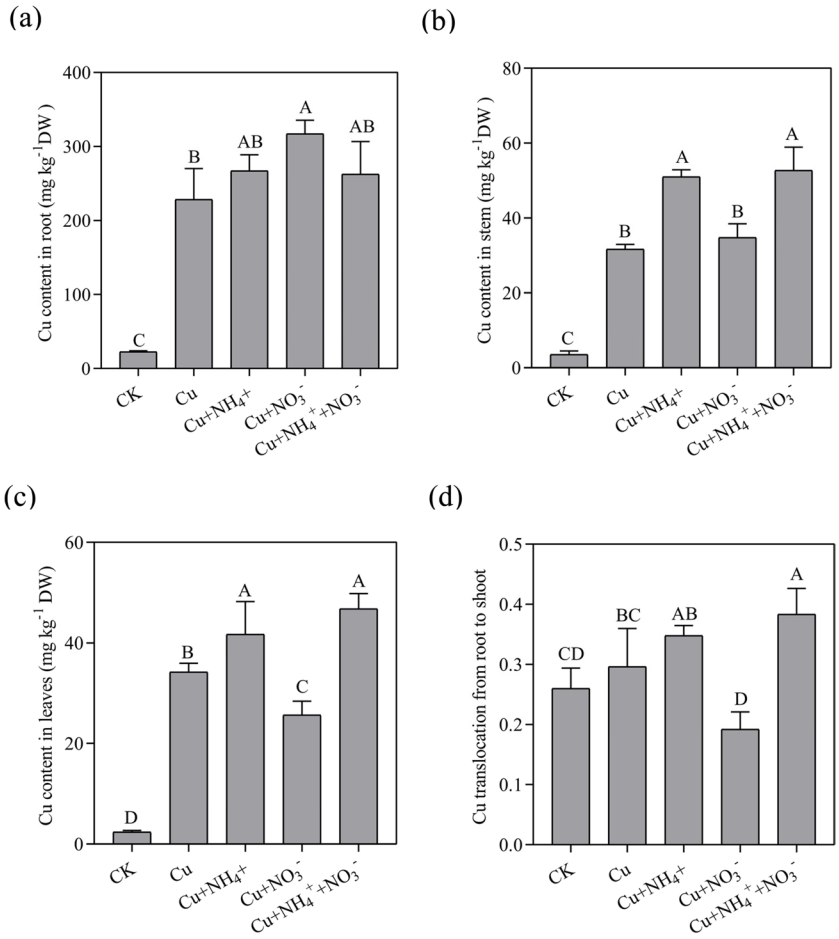

Fig. 4 Copper (Cu) accumulation and translocation in *L. chinense*. Cu concentration in root (**a**), stem (**b**), leaves (**c**), and Cu translocation from root to shoot (**d**). Data are means ± SD ($n = 4$). Different letters indicate significant differences between different treatments at $P < 0.05$ by least significant difference test

inhibited NR activity, when compared to N-Null (Fig. 3a). Cu treatment significantly activated GR activity and increased concentration of GHS and AsA in leaves (Fig. 3b, c, d), but significantly inhibited the DHAR activity ($p < 0.05$, Fig. 3e), which was 2.2-fold compared to controls. NO_3^--N alone dramatically increased the DHAR activity by 64.4%, whereas NH_4^+-N, with or without NO_3^--N, did not affect DHAR activities, compared with N-Null. Interestingly, adding N in two forms reduced the GR activity in *Liriodendron chinense leaves* compared to N-Null (Fig. 3b). Moreover, NH_4^+-N, with or without NO_3^--N, greatly increased the GSH concentration in Cu-treated plant leaves, by 3.2- and 2.2-fold, respectively, compared to the N-Null (Fig. 3c). In contrast, NO_3^--N alone restored Cu-induced GSH content compared to the N-Null. Similarly, NH_4^+-N, with or without NO_3^--N, showed no remarkable effect in AsA concentrations compared to the N-Null (Fig. 3d), although NO_3^--N alone significantly reduced AsA content to levels lower than those of controls.

Table 1 Nitrogen (N), potassium (K), and phosphorus (P) accumulations in *L. chinense*

	N (g/kg)			K (g/kg)			P (g/kg)		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
CK	6.24a	3.80b	12.61ab	17.55ab	13.04b	17.97ab	11.93a	13.10a	8.75a
Cu	5.11b	3.56b	11.40b	15.06b	16.93a	18.29a	13.81a	18.30a	10.18a
Cu-NH_4^+	5.21b	4.60a	11.56b	20.58a	11.59ab	9.81c	10.50a	14.53a	7.29a
Cu-NO_3^-	5.20b	3.67b	13.85a	21.71a	15.42a	15.32ab	13.29 a	14.94 a	10.24a
Cu-NH_4^++$_{NO3}^-$	5.08b	4.40a	12.44ab	15.03b	8.91c	13.71bc	14.56a	15.50a	8.52a

Data are means ± SD ($n = 4$). Different letters indicate significant differences between different treatments at $P < 0.05$ by least significant difference test

Nitrogen forms regulate Cu uptake, accumulation, and translocation from roots to shoots

Different forms of N differently influence Cu concentrations in roots, stems, and leaves and changed Cu translocation from roots to shoots, compared to N-Null treatment (Fig. 4). The greatest Cu concentration in roots was occurred when N was added as NO_3^--N, which was 1.39-fold higher than that of N-Null. However, Cu concentrations in leaves were 24.8% lower compared to N-Null, in agreement with a significant lower translocation factor from roots to shoots by 35.2 (Fig. 4d). NH_4^+-N, with or without NO_3^--N, increased Cu accumulation in roots, stem, and leaves, with increasing by 17.0%, 61.1%, and 21.9% in NH_4^+-N alone and 15.1%, 66.6%, and 36.9% in NH_4^+-N + NO_3^--N; respectively, when compared with N-Null. Thus, compared to the N-Null treatment, adding NH_4^+-N, with or without NO_3^--N, promoted Cu translocation from roots to shoots by 17.4% and 29.4%, respectively.

NO_3^--N regulates nutrient uptake in *Liriodendron Chinense*

Cu treatment inhibited nitrogen (N) uptake, increased slightly phosphorus (P) uptake, and reduced potassium (K) concentration of root in *Liriodendron chinense* (Table 1). Compared to the Cu only treatment, NO_3^--N obviously increased N content in leaves and increased K content in roots. However, NO_3^--N had little effect on N content in roots and stems, or on the content of K in stems and leaves, or on P content in roots and leaves. On the other hand, NH4$^+$-N, with or without NO_3^--N, markedly decreased K and P concentrations in *L. chinense* leaves. Overall, the concentrations of N in the leaves of Cu-treated plants were consistently higher in NO_3^--N treatment than those in NH4$^+$-N treatments.

Discussion

Copper is essential for plant growth, but only in small quantity (5–20 mg kg^{-1} DW in leaves). Cu levels above 20–30 mg.kg^{-1} DW in plant leaves can damage many plant species (Marschner 2011). In our study, the Cu concentration in *L. Chinense* leaves in the sole Cu treatment was 34.2 mg kg^{-1}, and plants in this treatment showed toxicity symptoms and indeed reduced chlorophyll contents. Excessive Cu in leaves causes reactive oxygen species bursts, which can trigger lipid peroxidation, disturb integrity of thylakoid membranes, and destroy chlorophyll structure (Khator and Shekhawat 2020). Interestingly, NO_3^--N exclusively alleviated the Cu-induced toxicity in *L. chinense*, in which the Cu concentration of leaves was 22.4 mg kg^{-1}. Conversely, NH_4^+N, with or without NO_3^--N, stimulated Cu accumulations in plant tissues, leading to greater toxic effects in leaves. Such different effects in NO_3^--N and NH_4^+-N may provide a new vision to phytostabilize or phytoextract on Cu-contaminated soils.

Effects of NO_3^--N/NH_4^+-N on oxidative stress indicators

Cu catalyzes the generation of reactive oxygen species (Li et al. 2007), and Cu treatment increased H_2O_2 and MDA production in the *L. chinense* seedlings (Fig. 2). These findings suggest that Cu-induced ROS accumulation could cause oxidative damage and disturb cellular homeostasis (Cui et al. 2010; Khator and Shekhawat 2020; Shabbir et al. 2020). Evidence suggests that nitrogen plays important roles in defensing oxidative stress, and additional nitrogen prevents Cd-induced oxidative stress in rice (Wu et al. 2020), boron-induced toxicity in *Brassica juncea* (Giansoldati et al. 2012), and reduced Al-toxicity in *Lespedeza bicolor* (Chen et al. 2010). Furthermore, the form of N could influence cellular homeostasis and plant tolerance to Cd, Mn, Cu, and Al (Cheng et al. 2020; de Souza Junior et al. 2018, 2019; Jalloh et al. 2009). In our study, we observed that adding NO_3^--N ameliorated Cu-induced oxidative damage in *L. chinense* leaves by reducing the levels of H_2O_2 and MDA to those of the non-Cu treatment. These observations suggest that ROS bursts were not induced by adding NO_3^--N under Cu treatments and indicated that nitrate had a positive role in mitigating Cu toxicity to *L. chinense*. Conversely, NH_4^+-N, alone or combined with NO_3^--N (1:1), further aggravated Cu toxicity in *L. chinense* seedlings, evidenced by significantly higher MDA and H_2O_2 contents compared to those in the alone Cu treatment. Similar results were reported by Zhu et al. (2016) in submerged plants *Vallisneria natans*. Also, it is well known that if the molar ratio of NH_4^+ to NO_3^- in a solution exceeds 1:1, NH_4^+-N can cause toxicity by increasing oxidative stress and lipid peroxidation in *Panicum maximum* (Santos et al. 2013). de Souza Junior et al. (2019) also reported that greater oxidative stress and Cu toxicity was induced by NH_4^+ above 50% of applied N rate in Tanzania guinea grass. However, NH_4^+-N-preferring plant species such as rice and *Lespedeza bicolor* are native to acidic soils and have evolved mechanisms to use NH_4^+-N efficiently, so N added as NH_4^+-N might alleviate Cd and Al toxicity, respectively (Wu et al. 2020; Jalloh et al. 2009; Zhao et al. 2009). For these reasons, we speculate that the effects of different N forms on metal toxicity should take into consideration the specificity of plant species.

Effect of NO_3^--N/NH_4^+-N on antioxidant systems

Many studies have reported the crucial roles of different antioxidant enzymes including SOD, POD, and CAT in scavenging Cu-induced ROS accumulation (Buapet et al. 2019). SOD is considered as the first line of defense against ROS bursts (Chen et al.2015), and in our study, SOD enzyme was greatly reduced in the sole-Cu treatment and in the NH_4^+-N treatment. However, the inhibitory effects of Cu were alleviated by NO_3^--N by increasing the enzyme activity to the level of the control. Indeed, the positive effect of NO_3^--N on SOD activity was also found in APX activity and suggested a higher capacity to eliminate ROS as well as a protective role of NO_3^--N to Cu stress.

In general, after ROS catalyzes into H_2O_2 by SOD enzyme, CAT and POD have been converted H_2O_2 to molecular oxygen and water (Fidalgo et al. 2013; Bai et al. 2021). Here, Cu stress and adding NH_4^+-N caused increased CAT and POD activities in leaves, which was concomitant with higher H_2O_2 accumulation. Cu-induced activation of POD and CAT enzymes also occurred in *Solanum lycopersicum* (Nazir et al. 2019) and *Lemna minor* (Hu et al. 2018), suggesting oxidative damage in plant leaves. However, N added as NO_3^--N restored the Cu-induced increases in POD and CAT activities, accompanying with the lower H_2O_2 concentration in leaves. The CAT and POD inhibition by NO_3^--N may be contributed to the low oxidative stress.

GSH-AsA cycle is also important to ROS detoxification in plants, which included the main enzyme (GR and DHAR) sustaining high efficiency of generation of GSH and AsA to catalyze excess H_2O_2 (Wu et al. 2020). In this cycle, GR plays important role in maintaining a high GSSG/GSH ratio under metal-induced oxidative stress by catalyzing to synthesize GSH (Giansoldati et al. 2012). Also, DHAR regulates the cellular AsA redox state by regenerating AsA from an oxidized state, thus enhancing tolerance to ROS (Chen and Gallie 2006). We found that GSH-AsA was activated by Cu, and adding NH_4^+-N increased GR activity and AsA and GSH concentrations. But NO_3^--N alone recovered Cu-induced GR activity with lower levels of AsA and GSH concentration and alleviated the inhibition of Cu-induced DHAR activity. These findings suggest that NO_3^--N modulated the levels of antioxidants and activities of antioxidase to provide greater ROS-scavenging capacity (Song et al. 2019). Another possible mechanism of NH_4^+-N in promoting efficiency of GSH-AsA cycle may contribute to an urgent demand of phytochelatin synthesis induced by Cu stress (Finkemeier et al. 2003). GSH not only has crucial role in scavenging metal-induced ROS, but also chelates toxic metals in cytoplasm of plant cells by compartmentalizing metals in vacuoles (Xu et al. 2010). Cd^{2+} is transported from roots to aerial organs via xylem and phloem in the form of GSH-Cd and PC-Cd complexes (Mendoza-Cózatl et al. 2008). Thus, the concomitant increased GR activity in *L. chinense* leaves could be due to the increased demand for reduced GSH to binding excessive Cu (Giansoldati et al. 2012).

Nitrate reductase is sensitive to oxidative stress (Campbell 1999), which could be as a result of the Cu-SH formation or oxidative damage to NR enzymes (Burzyński 2001; Li et al. 2007). In the present study, adding NH_4^+-N aggravated the Cu-induced inhibition of NR activity; however, adding sole NO_3^--N significantly promoted the activity of NR, suggesting adding NO_3^--N alleviated the oxidative stress by exposure to Cu. However, few studies explain the effects of different nitrogen forms on enzymatic and non-enzymatic antioxidant responses to Cu stress in plants. Thus, further study about mechanism of metal detoxification of nitrogen forms in enzyme system is needed to secure more detail.

Different effects of NO_3^--N/NH_4^+-N on Cu uptake, translocation and accumulation of *L. chinense* seedlings

In general, restrained Cu translocation from roots to shoots by sequestrating excessive Cu in the roots is a vital mechanism of plant defense against Cu stress (Brunner et al. 2008; Konno et al. 2010; Sahi et al. 2007). In this study, adding exclusive NO_3^--N obviously inhibited Cu translocation from roots to shoots, which resulted in a remarkably reduced Cu concentration in leaves. Conversely, adding NH_4^+-N, with or without NO_3^--N, enhanced Cu translocation and increased Cu concentration in leaves. These changes were concomitant with the greater Cu toxicity in *L. chinense*, compared with N-Null. Therefore, the stronger affinities for Cu^{2+} of roots and lower Cu translocation from roots to shoots in NO_3^--N treatments may have helped protect *L. chinense* from Cu.

NO_3^- and NH_4^+ could change the rhizosphere pH in different ways by regulating release of OH^- and H^+ ions, which affects the availability of metals in soil and solution (Hinsinger et al. 2003; Jalloh et al. 2009). Many pot experiments found that NH_4^+-N can enhance Cd accumulation in plants by regulating ion exchange reactions, availability of metal elements, and rhizosphere pH (Qasim et al. 2015; Cheng et al. 2020). However, other studies showed that supply of NO_3^--N encourages accumulation of metals such as Cd, Cu, Mn, Al, and Mg in roots (Chen et al. 2010; Hu et al. 2019; de Souza Junior et al. 2018, 2019; Yang et al. 2020). NO_3^--N hyperpolarizes the plasma membrane potential that facilitates the membrane transport of Cd, thus promoting Cd uptake by roots of *Sedum plumbizincicola* (Hu et al. 2013). Similar result was noted in *Triticum polonicum* L. (Cheng et al. 2020). Furthermore, Zhao and Shen (2018) also found that NH_4^+-N promoted the solubilization of Al compared to NO_3^--N, but inhibited Al accumulation in rice

roots. This result was explained by that the NH_4^+-N-induced protons compete with Al^{3+} for adsorption sites on root surfaces (Cumming 1990). In our study, both nitrogen forms promoted Cu uptake and accumulation in roots and stem, compared with plants in the N-Null treatment, but had different effects on Cu translocation from roots to shoots. So, the discrepant effects of different N forms on Cu uptake of plant roots take into account plant species, metal types and levels, soil properties, and so on.

Effects of NO_3^--N on Cu uptake and Cu translocation from roots to shoots were different, depending upon the presence or absence of NH_4^+-N, because adding NO_3^--N inhibited Cu translocation from roots to shoots to reduce Cu accumulation in leaves in the absence of NH_4^+-N, but promoted those in the presence of NH_4^+-N. These results indicate that adding NO_3^--N alone may favor Cu accumulation in roots, whereas NH_4^+-N, with or without NO_3^--N, increased Cu translocation from to shoots considering aggravating symptoms of Cu toxicity. The similar observations were confirmed by de Sousa Leite and Monteiro (2019) in *Tanzania guinea* exposed to Cd; in the experiment, NO_3^--N mitigated Cd phytotoxicity by favoring Cd accumulation in roots and inhibiting Cd translocation from roots to shoots. Meanwhile, the uptake, translocation, and accumulation of Cd in *Carpobrotus rossii* and *S. nigrum* were promoted by NH_4^+-N (Cheng et al. 2016). There are many possible reasons for the different effects. Cheng et al. (2020) suggested that NO_3^--N regulated the accumulation of lactose in roots and expression of metal transport genes to promote more Cd binding in cell walls and higher Cd sequestration in vacuoles, which result in increasing Cd uptake and accumulation in root and limiting Cd translocation from roots to shoots. These results also suggest addition of NO_3^--rich fertilizers might enhance the Cu phytoextraction efficiency by *L. chinense* in areas that are moderate copper contaminated.

Conclusion

We demonstrate the alleviating effects of NO_3^--N on Cu toxicity to *L. chinens*e, likely due to inhibiting Cu transportation from roots to shoots and reducing oxidative stress. In contrast, NH_4^+-N alone, or combined with NO_3^--N, intensified oxidative stress caused by Cu, despite the increased activities of CAT and POD and greater efficiency of GSH-AsA cycle. These results suggest that NO_3^--N could ensure *L. chinense* grown in Cu-contaminated soil, which can be used for efficient metal phytostabilization. Also, considering the increase of Cu accumulation in roots, stems, and leaves caused by NH_4^+-N alone or combined with NO_3^--N, NH_4^+-N can contribute to metal phytoextraction by promoting Cu accumulation in *L. chinense*.

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Data availability Due to the nature of this research, participants of this study did not agree for their data to be shared publicly.

Declarations

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References

Ameh T, Sayes CM (2019) The potential exposure and hazards of copper nanoparticles: a review. Environ Toxicol Phar 71:103220

Ata-UI-Karim ST, Cang L, Wang Y, Zhou D (2020) Interactions between nitrogen application and soil properties and their impacts on the transfer of cadmium from soil to wheat (*Triticum aestivum* L.) grain. Geoderma 357:113923

Bai ZQ, Li D, Zhu L, Tang XY, Wang YF, Mao RJ, Wu JW (2021) Nitrate increases cadmium accumulation in sweet sorghum for improving phytoextraction efficiency rather than ammonium. Front Plant Sci 12:643116

Bouazizi H, Jouili H, Geitmann A, El Ferjani E (2010) Copper toxicity in expanding leaves of *Phaseolus vulgaris* L.: antioxidant enzyme response and nutrient element uptake. Ecotox Environ Safe 73(6):1304–1308

Brehe JE, Burch HB (1976) Enzymatic assay for glutathione. Anal Biochem 74(1):189–197

Brunner I, Luster J, Günthardt-Goerg MS, Frey B (2008) Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. Environ Pollut 152(3):559–568

Buapet P, Mohammadi NS, Pernice M, Kumar M, Kuzhiumparambil U, Ralph PJ (2019) Excess copper promotes photoinhibition and modulates the expression of antioxidant-related genes in *Zostera muelleri*. Aquat Toxicol 207:91–100

Burzyński M (2001) Influence of pH on Cd and Cu uptake, distribution and their effect on nitrate reductase activity in cucumber (*Cucumis sativus* L.) seedling roots. Acta Physiol Plant 23(2):201–206

Campbell WH (1999) Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. Annu Rev Plant Phys 50(1):277–277

Chen Z, Gallie DR (2006) Dehydroascorbate reductase affects leaf growth, development, and function. Plant Physiol 142(2):775–787

Chen ZC, Zhao XQ, Shen RF (2010) The alleviating effect of ammonium on aluminum toxicity in *Lespedeza bicolor* results in decreased aluminum-induced malate secretion from roots compared with nitrate. Plant Soil 337:389–398

Chen J, Shafifi M, Li S, Wang Y, Wu J, Ye ZQ, Peng D, Yan WB, Liu D (2015) Copper induced oxidative stresses, antioxidant responses and phytoremediation potential of Moso bamboo (*Phyllostachys pubescens*). Sci Rep -UK 5(1):1–9

Cheng M, Wang P, Kopittke PM, Wang A, Sale PWG, Tang C (2016) Cadmium accumulation is enhanced by ammonium compared to nitrate in two hyperaccumulators, without affecting speciation. J Exp Bot 67(17):5041–5050

Cheng Y, Bao Y, Chen X, Yao Q, Wang C, Chai S, Zeng J, Fan X, Kang H, Sha L, Zhang H, Zhou Y, Wang Y (2020) Different nitrogen forms differentially affect Cd uptake and accumulation in dwarf Polish wheat (*Triticum polonicum* L.) seedlings. J Hazard Mater 400:123209

Cui XM, Zhang YK, Wu XB, Liu CS (2010) The investigation of the alleviated effect of copper toxicity by exogenous nitric oxide in tomato plants. Plant Soil Environ 56(6):274–281

Cumming JR (1990) Nitrogen source effects on Al toxicity in nonmycorrhizal and mycorrhizal pitch pine (*Pinus rigida*) seedlings. II. Nitrate reduction and NO_3^- uptake. Can J Bot 68:2653–2659

de Sousa Leite T, Monteiro FA (2019) Nitrogen form regulates cadmium uptake and accumulation in *Tanzania guine*a grass used for phytoextraction. Chemosphere 236:124324

de Souza Junior JC, Nogueirol RC, Monteiro FA (2018) NO_3^-/NH_4^+ ratios affect nutritional homeostasis and production of *Tanzania guinea* grass under Cu toxicity. Environ Sci Pollu Res 25(14):14083–14096

de Souza Junior JC, Nogueirol RC, Monteiro FA (2019) Nitrate and ammonium proportion plays a key role in copper phytoextraction, improving the antioxidant defense in *Tanzania guinea* grass. Ecotox Environ Safe 171:823–832

Fidalgo F, Azenha M, Silva AF, Sousa A, Santiago A, Ferraz P, Teixeira J (2013) Copper-induced stress in *Solanum nigrum* L. and antioxidant defense system responses. Food Energy Secur 2(1):70–80

Finkemeier I, Kluge C, Metwally A, Georgi M, Grotjohann N, Dietz KJ (2003) Alterations in Cd-induced gene expression under nitrogen deficiency in *Hordeum vulgare*. Plant Cell Environ 26(6):821–833

Gao L, Chang J, Chen R, Li H, Lu H, Tao LX, Xiong J (2016) Comparison on cellular mechanisms of iron and cadmium accumulation in rice: prospects for cultivating Fe-rich but Cd-free rice. Rice 9(1):1–2

Giansoldati V, Tassi E, Morelli E, Gabellieri E, Pedron F, Barbafieri M (2012) Nitrogen fertilizer improves boron phytoextraction by *Brassica juncea* grown in contaminated sediments and alleviates plant stress. Chemosphere 87:1119–1125

Gong Q, Wang L, Dai T, Zhou JY, Kang Q, Chen HB, Li K, Li ZH (2019) Effects of copper on the growth, antioxidant enzymes and photosynthesis of spinach seedlings. Ecotox Environ Safe 171:771–780

Hassan MJ, Wang F, Ali S, Zhang G (2005) Toxic effect of cadmium on rice as affected by nitrogen fertilizer form. Plant Soil 277(1):259–365

Helrich K (1990) Official methods of analysis of the association of official analytical chemists. Association of official analytical chemists

Hinsinger P, Plassard C, Tang C, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248(1–2):43–59

Hu P, Yin Y, Ishikawa S, Suzui N, Kawachi N, Fujimaki S, Igura M, Yuan C, Huang J, Li Z, Makino T, Luo Y, Christie P, Wu L (2013) Nitrate facilitates cadmium uptake, transport and accumulation in the hyperaccumulator *Sedum plumbizincicola*. Environ Sci Pollu Res 20(9):6306–6316

Hu C, Liu L, Li X, Xu Y, Ge Z, Zhao Y (2018) Effect of graphene oxide on copper stress in *Lemna minor* L.: evaluating growth, biochemical responses, and nutrient uptake. J Hazard Mater 341:168–176

Hu AY, Zheng MM, Sun LM, Zhao XQ, Shen RF (2019) Ammonium alleviates manganese toxicity and accumulation in rice by down-regulating the transporter gene OsNramp5 through rhizosphere acidification. Front Plant Sci 10:1194

Huang SQ, Guo YH (2015) Variation of pollination and resource limitation in a low seed-set tree, *Liriodendron chinense* (Magnoliaceae). Bot J Linn Soc 140(1):31–38

Jalloh MA, Chen J, Zhen F, Zhang G (2009) Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress. J Hazard Mater 162(2–3):1081–1085

Khator K, Shekhawat GS (2020) Cd- and Cu-induced phytotoxicity on 2–3 leaf stage of *Cyamopsis tetragonoloba* and its regulation by nitrate reductase and ROS quenching enzyme. Acta Physiol Plant 42(7):1–14

Konno H, Nakashima S, Katoh K (2010) Metal-tolerant moss *Scopelophila cataractae* accumulates copper in the cell wall pectin of the *protonema*. J Plant Physiol 167(5):358–364

Larsson JEH, Asp H (2013) Effects of pH and nitrogen on cadmium uptake in potato. Biol Plant 57:788–792

Li MJ, Xiong ZT, Dai LP, Huang Y (2007) Effects of copper on nitrogen assimilation in copper-tolerant and non-tolerant populations of *Elsholtzia haichowensis* S. Water Air Soil Pollu 184(1–4):323–333

Makino A (2011) Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. Plant Physiol 155(1):125–129

Marschner H (ed.) (2011) Marschner's mineral nutrition of higher plants. Academic press

Mendoza-Cózatl DG, Butko E, Springer F, Torpey JW, Komives EA, Kehr J, Schroeder JI (2008) Identification of high levels of phytochelatins, glutathione and cadmium in the phloem sap of *Brassica napus*. A role for thiol-peptides in the long-distance transport of cadmium and the effect of cadmium on iron translocation. Plant J 54(2):249–259

Mitchell LG, Grant CA, Racz GJ (2000) Effect of nitrogen application on concentration of cadmium and nutrient ions in soil solution and in durum wheat. Can J Soil Sci 80(1):107–115

Nazir F, Hussain A, Fariduddin Q (2019) Hydrogen peroxide modulate photosynthesis and antioxidant systems in tomato (*Solanum lycopersicum* L.) plants under copper stress. Chemosphere 230:544–558

Qasim B, Motelica-Heino M, Bourgerie S, Gauthier A, Morabito D (2015) Effect of nitrate and ammonium fertilization on Zn, Pb, and Cd phytostabilization by *Populus euramericana* Dorskamp in contaminated technosol. Environ Sci Pollu Res 22(23):18759–18771

Rizwan M, Ali S, Adrees M, Rizvi H, Zia RM, Hannan F, Qayyum MF, Hafeez F, Ok YS (2016) Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. Environ Sci Pollu Res 23(18):17859–17879

Sahi SV, Israr M, Srivastava AK, Gardea-Torresdey JL, Parsons JG (2007) Accumulation, speciation and cellular localization of copper in *Sesbania drummondii*. Chemosphere 67(11):2257–2266

Santos JHS, De Bona FD, Monteiro FA (2013) Growth and productive responses of tropical grass *Panicum maximum* to nitrate and ammonium supply. Rev Bras Zootecn 42(9):622–628

Sena K, Barton C, Hall S, Angel P, Agouridis C, Warner R (2015) Influence of spoil type on afforestation success and natural vegetative recolonization on a surface coal mine in Appalachia, United States. Restor Ecol 23(2):131–138

Shabbir Z, Sardar A, Shabbir A, Abbas G, Shamshad S, Khalid S, Natasha N, Murtaza G, Dumat C, Shahid M (2020) Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. Chemosphere 259:127436

Shi H, Jiang C, Ye TT, Tan DX, Reiter RJ, Zhang H, Liu RY, Chan ZL (2015) Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L). Pers.] by exogenous melatonin. J Exp Bot 66(3):681–694

Song J, Finnegan PM, Liu WH, Li X, Yong JWH, Xu JT, Zhang Q, Wen YX, Qin KX, Guo JZ, Li T, Zhao C, Zhang Y (2019) Mechanisms underlying enhanced Cd translocation and tolerance in roots of *Populus euramericana* in response to nitrogen fertilization. Plant Sci 287:110206

Sun X, Du Z, Ren J, Amombo E, Hu T, Fu J (2015) Association of SSR markers with functional traits from heat stress in diverse tall fescue accessions. BMC Plant Biol 15:116

Tsadilas CD, Karaivazoglou NA, Tsotsolis NC, Stamatiadis S, Samaras V (2005) Cadmium uptake by tobacco as affected by liming, N form, and year of cultivation. Environ Pollut 134:239–246

Wang W, Zhao XQ, Shen RF, Dong XY, Lan P, Ma JF, Shen RF (2015) Altered cell wall properties are responsible for ammonium-reduced aluminium accumulation in rice roots. Plant Cell Environ 38(7):1382–1390

Wang JF, Li WL, Li QS, Wang LL, He T, Wang FP, Xu ZM (2021) Nitrogen fertilizer management affects remobilization of the immobilized cadmium in soil and its accumulation in crop tissues. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-021-12868-z

Wu Z, Zhang W, Xu S, Shi H, Wen D, Huang Y, Peng L, Deng T, Du R, Li F, Wang X, Wang F (2019) Increasing ammonium nutrition as a strategy for inhibition of cadmium uptake and xylem transport in rice (*Oryza sativa* L.) exposed to cadmium stress. Environ Exp Bot 155:734–741

Wu ZC, Jiang Q, Yan T, Zhang X, Xu SJ, Shi HZ, Deng T, Li FR, Du YQ, Du RY, Hu CX, Wang X, Wang FH (2020) Ammonium nutrition mitigates cadmium toxicity in rice (*Oryza sativa* L.) through improving antioxidase system and the glutathione-ascorbate cycle efficiency. Ecotox Environ Safe 189:110010

Xu J, Yin HX, Liu XJ, Li X (2010) Salt affects plant Cd-stress responses by modulating growth and Cd accumulation. Planta 231(2):449–459

Yang YJ, Xiong J, Tao LX, Cao ZZ, Tang W, Zhang JP, Yu XY, Fu GF, Zhang XF, Liu YL (2020) Regulatory mechanisms of nitrogen (N) on cadmium (Cd) uptake and accumulation in plants: a review. Sci Total Environ 708:135186

Zaccheo P, Crippa L, Pasta VD (2006) Ammonium nutrition as a strategy for cadmium mobilisation in the rhizosphere of sunflower. Plant Soil 283:43–56

Zeng Q, Ling Q, Hu F, Wu J, Yang Z, Qi Y, Li Q (2017) Genotypic differences in growth and antioxidant enzyme activities under cadmium stress in sugarcane. Bull Environ Contam Toxicol 99:607–613

Zhao XQ, Shen RF (2018) Aluminum-nitrogen interactions in the soil-plant system. Front Plant Sci 9:807

Zhao XQ, Shen RF, Sun QB (2009) Ammonium under solution culture alleviates aluminum toxicity in rice and reduces aluminum accumulation in roots compared with nitrate. Plant Soil 315(1–2):107–121

Zhu ZJ, Song SY, Li PS, Jeelani N, Wang PH, Yuan HZ, Zhang JH, An SQ, Leng X (2016) Growth of the submerged aquatic plant Vallisneria and its physiological response to water *ammonia* nitrogen and sediment copper. Peer J 4:e1953