RESEARCH ARTICLE

Elevated carbon dioxide concentrations increase the risk of Cd exposure in rice

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Abstract

Since the Industrial Revolution, crops have been exposed to various changes in the environment, including elevated atmospheric carbon dioxide (CO_2) concentration and cadmium (Cd) pollution in soil. However, information about how combined changes affect crop is limited. Here, we have investigated the changes of *japonica* and *indica* rice subspecies seedlings under elevated CO_2 level (1200 ppm) and Cd exposure (5 μM Cd) conditions compared with ambient CO_2 level (400 ppm) and without Cd exposure in CO_2 growth chambers with hydroponic experiment. The results showed that elevated CO_2 levels significantly promoted seedling growth and rescued the growth inhibition under Cd stress. However, the elevated CO_2 levels led to a significant increase in the shoot Cd accumulation of the two rice subspecies. Especially, the increase of shoot Cd accumulation in *indica* rice was more than 50% compared with control. Further investigation revealed that the decreases in the photosynthetic pigments and photosynthetic rates caused by Cd were attenuated by the elevated CO_2 levels. In addition, elevated CO_2 levels increased the non-enzymatic antioxidants and significantly enhanced the ascorbate peroxidase (APX) and glutathione reductase (GR) activities, alleviating the lipid peroxidation and reactive oxygen species (ROS) accumulation induced by Cd. Overall, the research revealed how rice responded to the elevated CO_2 levels and Cd exposure, which can help modify agricultural practices to ensure food security and food safety in a future high-CO_2 world.

Keywords ASA-GSH · Cd · Elevated CO_2 concentrations · Non-enzymatic antioxidants · ROS · Rice

Introduction

The atmospheric carbon dioxide (CO_2) concentration has sharply increased from 280 ppm before industrialization to the current levels of approximately 415 ppm, primarily because of soaring human activities (e.g., excessive fossil fuel consumption and deforestation) (AbdElgawad et al. 2021; Yu and Chen 2019). Moreover, the atmospheric CO_2 concentration will rise to over 800 ppm by the end of this century (Bouain et al. 2022). Many researches have showed that elevated atmospheric CO_2 concentrations will significantly impact on plant growth, productivity, and species composition in agricultural and ecosystems (Li et al. 2023).

With the functions of photosynthetic substrates and signal transduction, CO_2 levels in plants would deeply affect the plants' photosynthesis and carbon metabolism (Loladze 2014; Sage and Khoshravesh 2016). As a matter of fact, the current CO_2 concentration does not meet the demand of the maximal photosynthetic efficiency for the C_3 species (Thompson et al. 2017). With the increase in CO_2 concentration, the CO_2 capture of the C_3 species would be significantly enhanced, leading to an improvement in biomass, which is called "CO_2 fertilization" (AbdElgawad et al. 2021; Seibert et al. 2021). Thirty years of free-air carbon dioxide enrichment (FACE) research has shown that a 35% yield increase was recorded in the rice cultivars of the highest yield potential at elevated CO_2 concentrations (Ainsworth and Long 2021).

The "CO_2 fertilization" generated by elevated CO_2 concentrations would obviously relieve the future problem of food shortages, but some negative effects have also been

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firmly recognized recently. For example, elevated CO_2 concentrations incur the reductions in most of the mineral and nutrient concentrations in plant tissues. Research has shown that the beneficial influence of elevated CO_2 concentrations on cotton, as relayed by P supplementation, might be an adverse impact in response to severe P deficiencies (Singh and Reddy 2014). With a FACE platform, a field experiment showed that elevated CO_2 reduced the prolamin, albumin, glutelin, and total protein concentrations in rice (*Oryza sativa* L.) grain (Wei et al. 2021). In wheat (*Triticum aestivum* L.) grain, the S, Zn, Fe, and protein concentrations were decreased with elevated CO_2 concentrations (Dier et al. 2020). At the Giessen FACE site, the Ca, Mg, N, P, and K concentrations of many grasses were significantly reduced (Seibert et al. 2021), and elevated CO_2 concentrations decreased the concentrations of N, P, K, S, and Mg in the edible parts of lettuce (Giri et al. 2016). Many mineral element concentrations (Mn, Fe, Zn, Mg, Mo, K, and Ca) in wheat grain were significantly decreased under increasing atmospheric CO_2 concentrations (Beleggia et al. 2018). Previously, these negative effects have been regarded as the results of the "dilution effect" because of the increase in C capture and biomass (Taub and Wang 2008). However, the "dilution effect" was not an effectively hypothesis. For instance, the changes in the different nutrient element concentrations are very different (Myers et al. 2014). The decreased efficiency in nutrient assimilation and/or acquisition appears to be the main issue of the negative effects of elevated CO_2 concentrations on plants' mineral elements (Gojon et al. 2022). Collectively, with the atmospheric CO_2 concentration increased, the yield increase but loss of mineral elements (also called "hidden hunger") would be a prominent problem in the future.

Rice, as a C_3 cereal crop species, is a staple food for over half of the world's population (Zhao and Chang 2022). Elevated CO_2 concentrations also could lead to the gain of crop yield and loss of nutrients for rice. Elevated CO_2 concentrations contributed to a significant increase in rice grain yield, and this is partly regulated by qPE9-1, a G protein γ subunit which is associated with N uptake in response to elevated CO_2 concentrations (Wang et al. 2022). Elevated CO_2 would also reduce the accumulation of P, and PHT4;3 is a P transporter that regulates the loss of P in chloroplasts in the face of elevated CO_2 to support plant growth in *Arabidopsis thaliana*, and this mechanism is conserved in rice (Bouain et al. 2022). Besides the potential loss of nutrients under elevated CO_2 concentrations, rice also faces a risk of Cd pollution. Because of the Cd pollution of paddy field caused by the industrial activities (e.g., non-ferrous metal manufacturing, energy production and mining) and application of phosphate fertilizer, livestock manure, and sewage sludge, the rice Cd exposure has become a serious public problem for plant growth and human health in many countries, and at present,

many researchers have shown that rice is the major source of human Cd intake (McGrath 2022). Rice shares over 55.8% of the Cd intake among the Chinese population (Song et al. 2017). Although many researchers have studied the influence of elevated CO_2 concentrations or Cd exposure on rice, a clear assessment is necessary when rice simultaneously faces elevated CO_2 concentrations and Cd pollution. Here, we have mainly analyzed the biomasses, Cd concentrations, gas exchange parameters, and antioxidant-related parameters of rice seedlings to illustrate the impacts of Cd exposure and elevated CO_2 concentrations on rice using hydroponics experiments in CO_2 growth chambers.

Materials and methods

Plant materials and culture conditions

Two rice cultivars, Yangdao No. 6 (YD) and Ninggeng No. 7 (NG), were used in this study. Yd belongs to the *indica* rice subspecies, and NG belongs to the *japonica* rice subspecies. Rice seeds were soaked in distilled water that was kept in a 28 °C incubator in the dark. After 2 days, the germinated seeds were transferred to home-made hydroponic grow trays containing 28 °C distilled water, where they remained for 2 days. Then, the uniform seedlings of approximately 1.5 cm in height were transferred to a hydroponic box containing 1/8 Kimura B solution for 3 days, and then 1/4 Kimura B solution was added, and they were kept for an additional 3 days. Finally, 1/2 Kimura B solution (180 μM $Ca(NO_3)_2$, 90 μM KNO_3, 90 μM KH_2PO_4, 270 μM $MgSO_4$, 180 μM $(NH_4)_2SO_4$, 3 μM H_3BO_3, 0.5 μM $MnCl_2$, 1 μM $(NH_4)_6Mo_7O_{24}$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4$, and 20 μM Fe(II)-EDTA, at a pH of 5.6–5.7) was added, and this solution was renewed in the hydroponic box every 3 days. Each hydroponic box contained 6 seedlings as one biological replicate. The rice seedlings were grown in CO_2 growth chambers (RGL-500C-CO_2, Hefei Dascate Biotechnology Co., Ltd, Hefei, China) under a daily cycle of 28 °C and 14 h day, and 26 °C and 10 h night, with 65% humidity and a light intensity of approximately 350 μmol m^{-2} s^{-1}. The CO_2 concentrations of the two CO_2 growth chambers used in this study were 400 ppm (i.e., ambient CO_2) and 1200 ppm (i.e., elevated CO_2), respectively.

Cd and CO_2 treatments

For the Cd concentration gradient experiments, 10-day-old seedlings were treated with 0, 1, 2, 5, or 10 μM of Cd. Each treatment was applied to four biological replicates.

Three CO_2 conditions were set as follows: (1) 4-day-old seedlings that had always been grown at an ambient CO_2 concentration (AC) as the control; (2) 4-day-old seedlings

that had been grown in a growth chamber with ambient CO_2 for 6 days and then were transferred to an elevated CO_2 concentration, named elevated CO_2 condition 1 (EC1); and (3) seedlings that had always been grown in a growth chamber with elevated CO_2, named elevated CO_2 condition 2 (EC2). To investigate the effects of Cd stress at different CO2 concentrations, 10-day-old seedlings grown in the CO_2 conditions described above were treated with 0 or 5 μM of Cd. Each treatment was applied to four biological replicates.

Measurements of the shoots, root lengths, and weights

After the rice seedlings grown at different CO_2 conditions had been treated with Cd for 9 days, the shoot and root lengths of the seedlings were measured. The rice seedling roots were then washed with 5 mM of $CaCl_2$ twice to remove the nutrient elements and Cd adhering to the root surfaces, and they were further cleaned with distilled water twice to remove any $CaCl_2$ that remained attached to the root surfaces. The seedlings were then separated into shoots and roots, and their fresh weights were weighed and totally dried at 70 °C. The dry shoot and root samples were used to measure the dry weights and Cd concentrations.

Cd concentration assay

The dry samples were sheared and put into mixed acid ($HClO_4$: HNO_3 = 15:85, v/v) overnight and then digested with a heating block (DigiBlock ED54-iTouch, LabTech, Beijing, China) that was applied using the following procedure: initial heating to 80 °C, where it was held for 1 h and then heating to 100 °C for 1 h, 120 °C for 1 h, 160 °C for 1 h, and 180 °C until the solution was clarified and further crystallized. The solution was then mixed with 10 ml of 2.5% HNO_3 and put into a water bath at 60 °C to dissolve the samples before taking the Cd concentration measurements (Fang et al. 2020).

The Cd concentrations were determined using an ICP-OES (inductively coupled plasma optical emission spectrometer, Optima 2100DV, Perkin Elmer, Gaithersburg, MD, USA). The Cd translocation factor (TF) was the ratio of the Cd concentrations in the shoots to the Cd concentrations in the roots (Wang et al. 2017). For quality control, reagent blanks and orange leaves which was a certified reference plant material (GBW10020, National Research Center for Standards, China) were applied in the assay. The Cd recovery rates of the reference materials were 95–101%.

Photosynthetic system-related assay

The contents of the photosynthetic pigments (total chlorophyll (total Chl), chlorophyll a (Chl a), chlorophyll b (Chl b), and

carotenoid (Car)) were measured as follows: approximately, 100 mg of fresh leaves was cut up and placed in 10 ml tubes containing 5 ml of 95% ethanol. The tubes containing the samples were kept in the dark until the leaves were completely decolorized. The absorbance levels of the extracted solutions at 665 nm, 649 nm, and 470 nm were measured, respectively, with a spectrophotometer (SHIMADZU UV-2450, Kyoto, Japan). The contents of the photosynthetic pigments were then calculated as follows (Huang et al. 2019):

$$\text{Chl a (mg/L)} = 13.95 \times A_{665} - 6.88 \times A_{649},$$

$$\text{Chl b (mg/L)} = 24.96 \times A_{649} - 7.32 \times A_{665},$$

$$\text{Car (mg/L)} = \left(1000 \times A_{470} - 2.05 \times \text{Chl a} - 114.7 \times \text{Chl b}\right)/245,$$

and

$$\text{total Chl (mg/L)} = \text{Chl a} + \text{Chl b}.$$

The contents of photosynthetic pigment (mg/g) $= C \times V \times N/W$. where C was the content of the photosynthetic pigment, V was the volume of extracting solution (mL), N was the dilution ratio, and W was the fresh weight (g).

The leaf gas exchange parameters (P_n, net photosynthetic rate; T_r, transpiration rate; C_i, intercellular CO_2 concentration; and G_s, stomatal conductance) were recorded with a Li-6400 photosynthesis system with an LED light source (Li-Cor Biosciences, Lincoln, NB, USA) on a flag leaf from 10:00 am to 12:00 pm (Ulfat et al. 2021). The light intensity of the leaf chamber was set to 1500 μmol m^{-2} s^{-1} with a flow rate of 500 μmol s^{-1}, the leaf temperatures were set at 28 °C, and leaf chamber conditions were set at 400 or 1200 ppm CO_2.

Analysis of the MDA contents, H_2O_2 contents, O_2^- contents, and plasma membrane integrity

To measure the MDA contents, 0.2 mg of fresh sample was triturated with liquid nitrogen and mixed with 5 ml of 5% TCA (trichloroacetic acid). After being centrifuged at 4 °C for 10 min at 8000 × g, 2 ml of the supernatant was transferred to a new tube and mixed with 5 ml of 6% TBA (thiobarbituric acid) dissolved with 10% TCA. Then, the mixture was boiled for 30 min and immediately cooled using running water (Nie et al. 2016). The absorbance levels of the mixture at 450 nm, 532 nm, and 600 nm were measured, and the MDA contents were calculated according to the following formula:

$$\text{MDA (μM)} = 6.45 \times \left(A_{532} - A_{600}\right) - 0.56 \times A_{450}.$$

The superoxide anion (O_2^-) contents were measured as follows: A quantity of 0.2 g of fresh sample was triturated with liquid nitrogen and homogenized with 1 mL of 50 mM

phosphate buffer (pH of 7.8). After being centrifuged at 4 °C for 10 min at 12000 × g, 0.5 ml of the supernatant was moved to a new tube and mixed with 0.5 ml of 50 mM phosphate buffer (pH of 7.8) and 0.1 ml of hydroxylamine hydrochloride (10 mM). The mixture was incubated at 25 °C for 1 hand further mixed with 1 ml of 17 mM P-aminobenzene sulphonic acid anhydrous and 1 ml of 7 mM a-naphthylamine. Afterward, the samples were incubated for 20 min at 25 °C, and the absorbance at 390 nm was measured. $NaNO_2$ was used to establish the standard curve (Huang et al. 2008). The histochemical stain of O_2^- of the rice roots under the Cd and CO_2 treatments was applied with NBT (nitro-blue tetrazolium) and imaged with a stereoscopic microscope (Model Stemi 2000-C; Carl Zeiss, Jena, Germany) (Huang et al. 2019).

The H_2O_2 contents were measured as follows: A quantity of 0.2 g of fresh sample was triturated with liquid nitrogen and homogenized with 5 mL of 5% TCA (trichloroacetic acid). After being centrifuged at 4 °C for 15 min at 12000 × g, 0.5 ml of the supernatant was moved to a new tube and mixed with 0.5 ml of phosphate buffer (10 mM, pH of 7.0) and 1 ml KI (1 M). The samples were then used to measure the absorbance at 390 nm to obtain the H_2O_2 content. H_2O_2 was used to establish the standard curve (Shah et al. 2020). The histochemical stain of H_2O_2 of the rice roots under the Cd and CO_2 treatments was applied with 3, 3′-diaminobenzisine (DAB) and imaged with a stereoscopic microscope (Model Stemi 2000-C; Carl Zeiss, Jena, Germany) (Huang et al. 2019).

The plasma membrane integrity was analyzed with Evans blue (Martins et al. 2013). The roots were stained with 0.25% (w/v) Evans blue solution for 10 min at room temperature, then washed with distilled water, and imaged with a stereoscopic microscope (Model Stemi 2000-C; Carl Zeiss, Jena, Germany), the equivalent root tips were sampled and soaked in N,N-dimethylformamide to release the stain, and the concentration was determined by measuring absorbance at 600 nm.

Determination of the NPT and total phenolic contents

The non-protein thiol (NPT) content was measured with Ellman's reagent (Tian et al. 2011) as follows: A quantity of 0.2 g of fresh sample was triturated with liquid nitrogen and mixed with 3 mL of sulfosalicylic acid (5%, w/v). Then, the homogenate was centrifuged at 4 °C for 30 min at 10000 × g, and 0.3 ml of the supernatant was added to a new tube and mixed with 1.2 ml of 100 mM phosphate buffer (pH of 7.6) and 25 µl of 6 mM DTNB (5,5'-dithiobis-2-nitrobenzoic acid) solution (the DNTB was dissolved with 100 mM phosphate buffer (pH of 7.6) with 5 mM EDTA). The mixed solution was incubated for 20 min at room temperature, and

then the absorbance at 412 nm was measured. Glutathione was applied to establish the standard curve.

The total phenolic content was measured using Folin–Ciocalteu reagent (Li et al. 2012) as follows: A quantity of 0.2 g of fresh sample was triturated with liquid nitrogen and mixed with 10 ml of 50% ethanol, and then the mixture was ultrasound treated for 1.5 h. After being centrifuged for 15 min at 4000 × g, 1 ml of the supernatant was homogenized with 1 ml of Folin–Ciocalteu reagent and 2 ml of 15% Na_2CO_3 (w/v). After incubation at room temperature in the dark for 50 min, the absorbance at 765 nm was detected. Pyrogallol was used to obtain the standard curve.

Determination of the ascorbate and glutathione contents

The ascorbate (ASA) and glutathione (GSH) contents were measured as follows: A quantity of 0.2 g of fresh sample was triturated with liquid nitrogen and mixed with 3 ml of chilled extraction buffer (5% meta-phosphoric acid dissolved with 1 mM EDTA solution). After centrifugation at 4 °C for 20 min at 12000 g, the supernatants were moved to new tubes and used for detecting the ascorbate and glutathione contents (Hasanuzzaman et al. 2011).

For ASA the content measurements, 0.3 ml of the supernatant was mixed with 750 µl of 0.15 M phosphate buffer (pH of 7.4, with 5 mM EDTA), 0.6 ml of TCA (10%), 0.6 ml of H_3PO_4 (44%), 0.6 ml of 2,2′-dipyridyl (4%, dissolved in 70% ethanol), and 0.15 ml of $FeCl_3$ (0.3%) before being incubated for 30 min at 40 °C. The absorbance at 525 nm was recorded. For the GSH content measurements, 2.6 ml of 150 mM phosphate buffer (pH of 7.7) and 0.15 ml of DTNB were mixed with 0.25 ml of supernatant. After incubation for 5 min at 30 °C, the absorbance at 412 nm was recorded. ASA and GSH were used to establish the respective standard curves.

Determination of APX and GR activity

0.2 g of fresh sample was triturated with liquid nitrogen and mixed it with 2 ml of chilled phosphate buffer (100 mM, pH of 7.0, containing 20 mM EDTA-Na_2 and 10% polyvinyl pyrrolidone) before centrifuging it at 4 °C for 15 min at 12000 × g. The supernatant (i.e., the enzyme extract) was then used to analyze the activities of ascorbate peroxidase (APX) and glutathione reductase (GR) (Hasanuzzaman et al. 2017).

To measure the APX activity, the supernatant was reacted with 100 mM phosphate buffer (pH of 7.0), 0.1 mM EDTA, 0.1 mM H_2O_2, and 0.5 mM AsA, and the changes in the absorbance at 412 nm were recorded.

To measure the GR activity, enzyme extract was reacted with 100 mM phosphate buffer (pH of 7.0), 20 mM

EDTA-Na$_2$, 5 mM GSSG, and 1.5 mM NADPH, and then it was used to measure the changes in the absorbance at 340 nm.

Statistical analysis

The data were analyzed by two-way analysis of variance by Duncan's multiple comparison test ($P < 0.05$) using with SPSS 20.0 (SPSS, Inc., Chicago, IL, USA).

Results

Elevated CO$_2$ attenuated the growth inhibition of the rice caused by Cd

Here, the effects on the rice of different Cd concentrations were analyzed, and their growth was inhibited when treated with 1 μM Cd, and the relative shoot elongation and relative root elongation were inhibited approximately 50% in two cultivars (Fig. S1). The Cd concentrations in the roots and shoots of both cultivars showed that YD had the stronger ability to transport Cd from the roots to the shoots, although the Cd concentrations in the roots of the YD rice were

significantly higher than those of the NG rice (Fig. S2). With the changes of relative root elongation under different Cd concentrations together, we selected 5 μM of Cd to proceed follow-up experiments.

To identify the combined effects of the elevated CO$_2$ and Cd exposure on the rice, we used Yangdao No. 6 (YD) and Ninggeng No. 7 (NG), which belong to the *indica* and *japonica* rice subspecies, respectively. The seedlings were grown at three different CO$_2$ conditions as follows: (1) grown at ambient CO$_2$ levels (AC); (2) grown at ambient CO$_2$ levels for 6 days and then at elevated CO$_2$ levels, which we named elevate CO$_2$ condition 1 (EC1); and (3) grown at elevated CO$_2$ levels, which we named elevate CO$_2$ condition 2 (EC2).

The fresh weights and dry weights of the shoots of two cultivars were significantly increased by approximately 20% for both EC1 and EC2 compared to the AC rice ($P < 0.05$) (Fig. 1 a–c). The different elevated CO$_2$ conditions (EC1 and EC2) did not result in significant differences in the fresh weights and dry weights of the shoots of the two rice cultivars. A similar trend was observed for the root weights, but the increases in root weights enhanced for the EC1 and EC2 samples were not as big as the increases in the shoot weights (Fig. 1d–e). Elevated CO$_2$ conditions (EC1 and EC2) attenuated the inhibition caused by the Cd treatment (Fig. 1a, b,
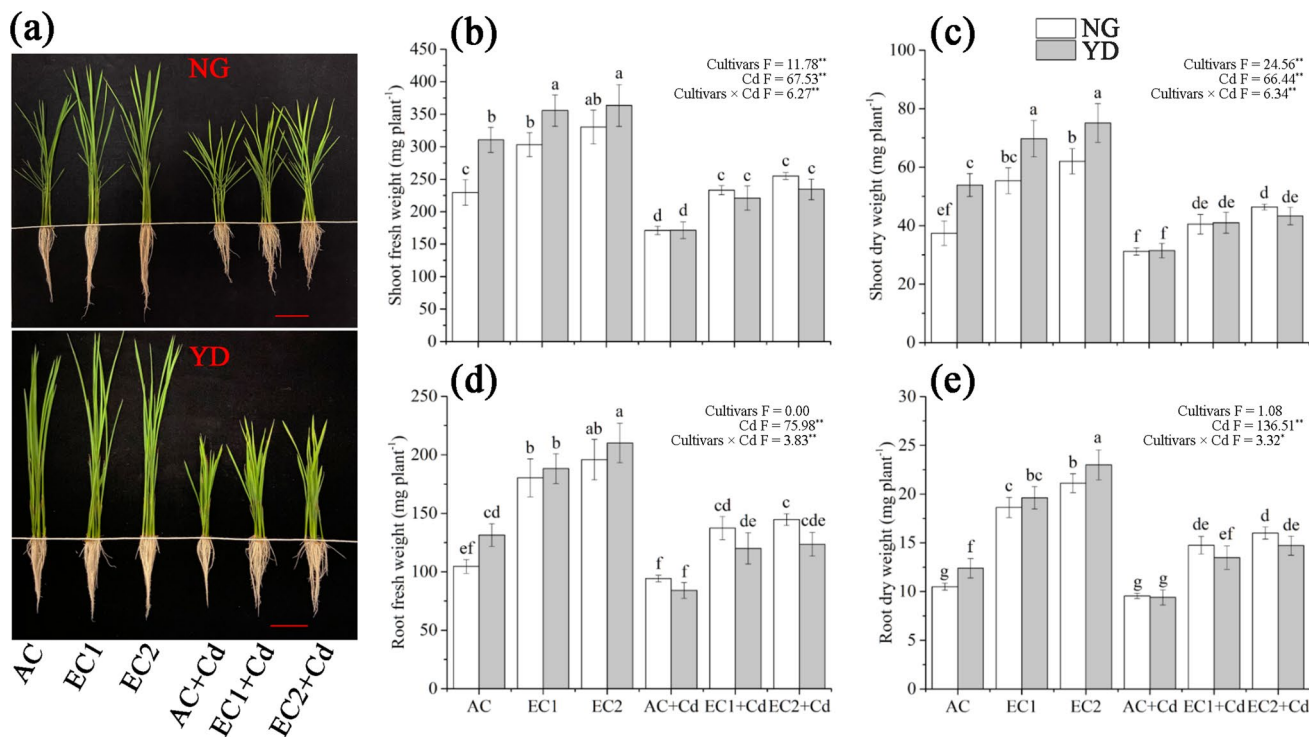

Fig. 1 Phenotypes and biomasses of the rice under the Cd and CO$_2$ treatments: **a** phenotypes, **b** fresh weights of the shoots, **c** dry weights of the shoots, **d** fresh weights of the roots, and **e** dry weights of the roots. NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice; AC, ambient concentration; EC1, growth in the chamber of

ambient CO$_2$ concentration and then transferred to elevated CO$_2$ concentration chamber treated with Cd; EC2, growth in the chamber of elevated CO$_2$ concentration from beginning to end. The different letters above the bars indicate significant differences with Duncan's test ($P < 0.05$). The values shown are the means ± SDs ($n = 4$)

and d). In the indica rice, NG, under Cd stress, the elevated CO_2 treatment restored the fresh weights of both the shoots and roots to the levels of the rice grown at ambient CO_2 without Cd treatment (Fig. 1b and d). These results suggested that elevated CO_2 attenuated the growth inhibition of the rice caused by Cd treatment.

Elevated CO_2 levels promoted Cd transportation from the roots to the shoots

The Cd concentrations in the shoots and roots of the rice exposed to Cd were analyzed (Fig. 2). The Cd concentrations in the NG shoots were significantly lower than those of the YD shoots, regardless of the CO_2 condition used (Fig. 2a). The Cd concentrations in the shoots of the NG rice grown at elevated CO_2 conditions (EC1 and EC2) were significantly increased compared with the NG rice

grown at an ambient CO_2 condition (AC). Interestingly, the Cd concentrations in the NG roots grown at an ambient CO_2 condition (AC) were higher than those of the YD rice grown at elevated CO_2 conditions (EC1 or EC2) (Fig. 2b), resulting in the lowest Cd translocation factor (the Cd concentration ratio between the shoots and roots) at AC (Fig. 2c). Although a similar trend was observed for the Cd concentrations in the YD rice roots, the Cd concentrations in the YD shoots did not show significant differences at either CO_2 condition, leading to similar Cd translocation factors for the YD rice for all the three CO_2 conditions. In addition, the Cd translocation factors for the YD rice at all three CO_2 conditions were significantly higher than those of the NG rice (Fig. 2c). The Cd accumulations in the shoots (Fig. 2d), roots (Fig. 2e), and whole seedling (Fig. 2f) significantly increased at the elevated CO_2 concentrations (EC1 and EC2). This revealed that elevated
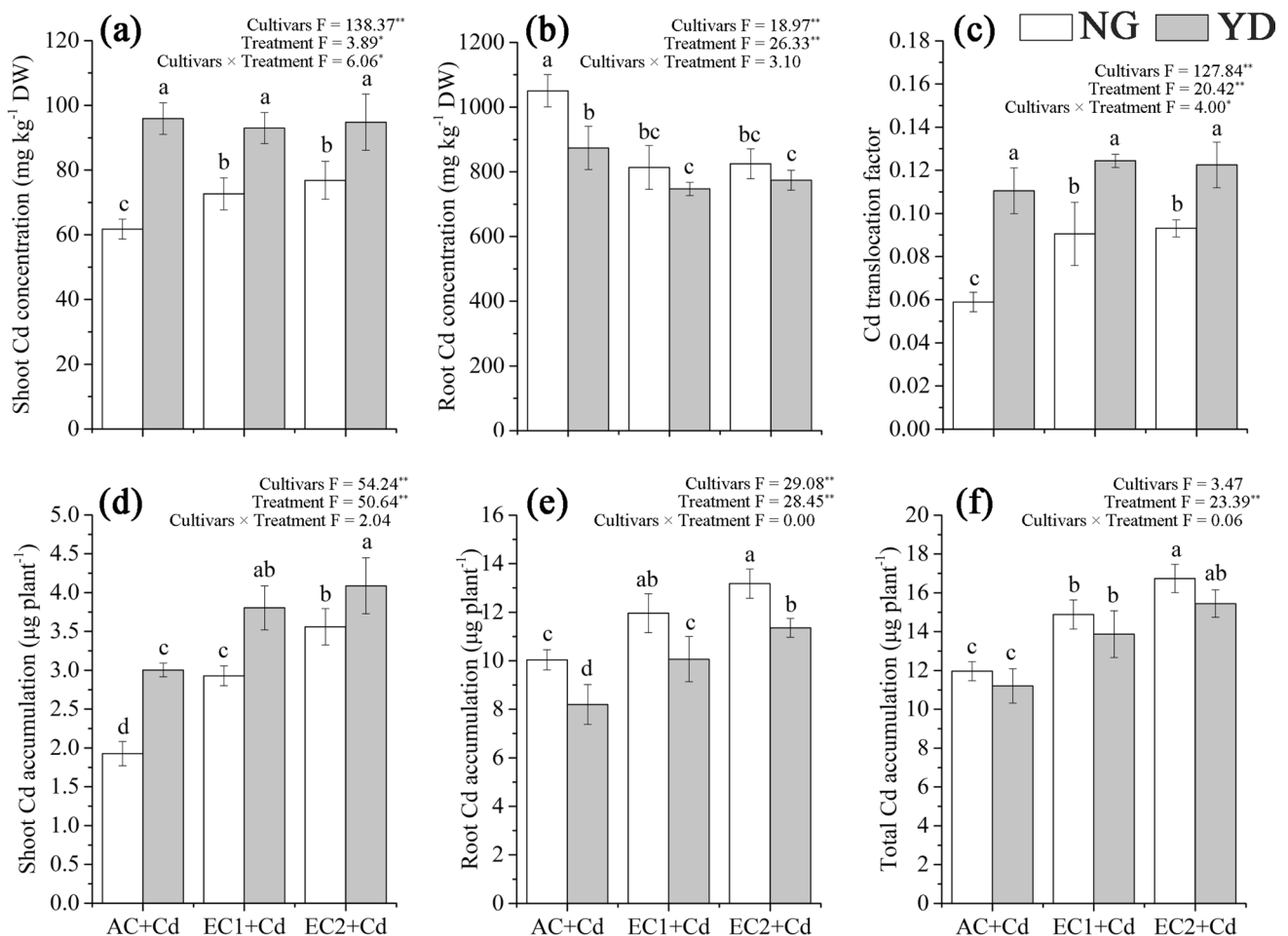

Fig. 2 Cd concentrations, translocation factors, and accumulations of rice under the Cd and CO_2 treatments. **a** Cd concentrations in the shoots, **b** Cd concentrations in the roots, **c** Cd translocation factors, **d** Cd accumulations in the shoots, **e** Cd accumulations in the roots, and **f** total Cd accumulations. NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice; AC, ambient concentration; EC1,

growth in the chamber of ambient CO_2 concentration and then transferred to elevated CO_2 concentration chamber treated with Cd; EC2, growth in the chamber of elevated CO_2 concentration from beginning to end. The different letters above the bars indicate the significant differences with Duncan's test ($P < 0.05$). The values shown are the means ± SDs ($n = 4$)

CO_2 conditions enhanced Cd accumulation and promoted Cd translocation from the roots to the shoots of the rice.

Elevated CO_2 concentrations increased the contents of the photosynthetic pigments

Compared with the AC treatment, the elevated CO_2 concentrations (EC1 and EC2) significantly increased the contents of the photosynthetic pigments in the two cultivars, and the contents of the photosynthetic pigments in the two cultivars were significantly reduced in response to Cd stress (AC + Cd), while the elevated CO_2 concentration significantly boosted the contents of the photosynthetic pigments of the two cultivars under Cd stress (Fig. 3). In particular, the carotenoid contents of the two cultivars did not show any significant differences for the AC, EC1, or EC2 treatments, but when the seedlings were stressed by the Cd treatment, the carotenoid contents of the NG rice were significantly higher than those of the YD rice for each of the AC + Cd, EC1 + Cd, and EC2 + Cd treatments (Fig. 3c). The two cultivars showed similar patterns.

Elevated CO_2 concentrations changed the gas exchange parameters of the rice leaves

The net photosynthetic rates (P_n) (Fig. 4a) and intercellular CO_2 concentrations (C_i) (Fig. 4b) of the rice leaves were significantly increased under the elevated CO_2 concentrations (EC1 and EC2) compared with the AC treatment (Fig. 4a).

While the P_n of the AC treatment was significantly greater than the P_n of the AC + Cd treatment and the elevated CO_2 concentrations (EC1 + Cd and EC2 + Cd) significantly contributed to the P_n but could not rescue the concentrations of only the elevated CO_2 treatments (EC1 and EC2), the P_n values of the elevated CO_2 concentrations (EC1 + Cd and EC2 + Cd) remained significantly lower compared to those without elevated CO_2 concentrations and without Cd exposure (EC1 + Cd and EC2 + Cd) (Fig. 4a). The intercellular CO_2 concentration (C_i) met the concentration of the CO_2 treatment (Fig. 4b). Only the elevated CO_2 concentration, the stomatal conductance (g_s) (Fig. 4c), and transpiration rate (T_r) (Fig. 4d) were significantly reduced, and we found the same results for the Cd exposure cases (AC + Cd compared with the EC1 + Cd or EC2 + Cd). The g_s of the AC + Cd treatment was significantly lower compared with that of the control (AC), but the elevated CO_2 concentrations did not significantly affect the g_s under Cd stress (EC1 compared with EC1 + Cd and EC2 compared with EC2 + Cd) (Fig. 4c). The T_r showed a similarly tendency, but Cd stress did not significantly inhibit the T_r (AC compared with AC + Cd) (Fig. 4d). The two cultivars showed similar patterns.

Elevated CO_2 concentrations reduced the MDA, O_2^-, and H_2O_2 accumulations in the rice under Cd stress

Cd stress caused MDA, O_2^-, and H_2O_2 to accumulate in the rice (Fig. 5). In detail, compared with the AC treatment, only the EC2 elevated CO_2 concentration did not significantly

Fig. 3 Contents of the photosynthetic pigments of the rice under the Cd and CO_2 treatments: **a** chlorophyll a (Chl a), **b** chlorophyll b (Chl b), **c** carotenoid (Car), and **d** total chlorophyll (total Chl). NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice; AC, ambient concentration; EC1, growth in the chamber of ambient CO_2 concentration and then transferred to elevated CO_2 concentration chamber treated with Cd; EC2, growth in the chamber of elevated CO_2 concentration from beginning to end. The different letters above each bar indicate the significant differences with Duncan's test ($P < 0.05$). The values shown are the means ± SDs ($n = 4$)
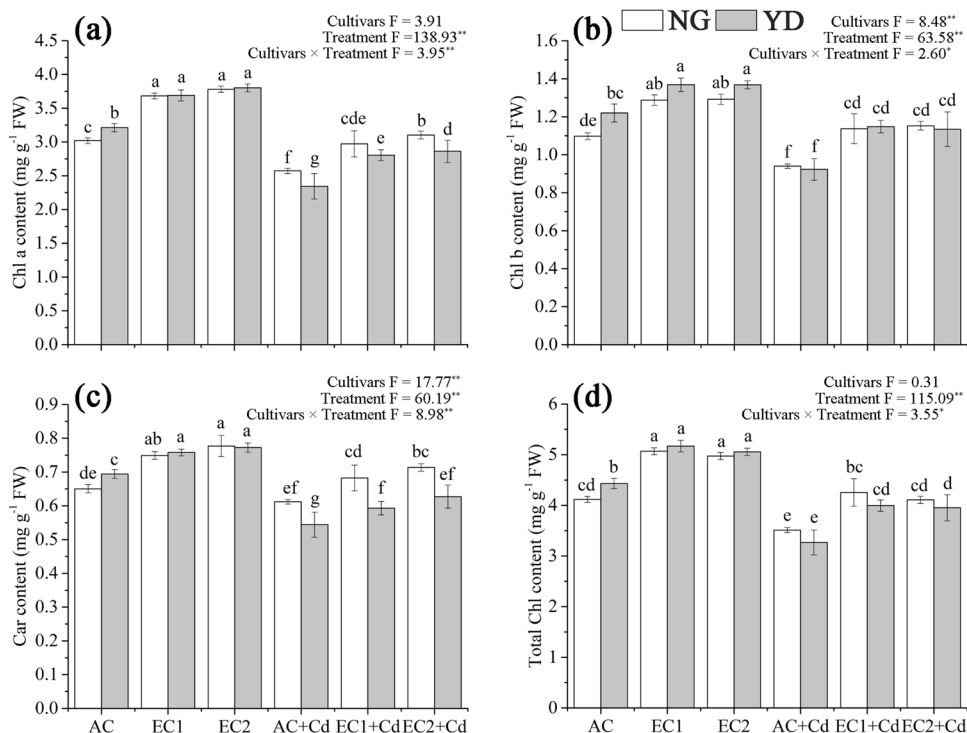
Fig. 4 The gas exchange parameters of rice leaf under Cd and CO_2 treatment. **a** Pn (net photosynthetic rate), **b** C_i (intercellular CO_2 concentration), **c** g_s (stomatal conductance), **d** T_r (transpiration rate). NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice; AC, ambient concentration; EC1, growth in the chamber of ambient CO_2 concentration and then transferred to elevated CO_2 concentration chamber treated with Cd; EC2, growth in the chamber of elevated CO_2 concentration from beginning to end. The different letters above each bar indicated significant differences at Duncan's test ($P < 0.05$). Values are means $\pm$ SD ($n = 4$)
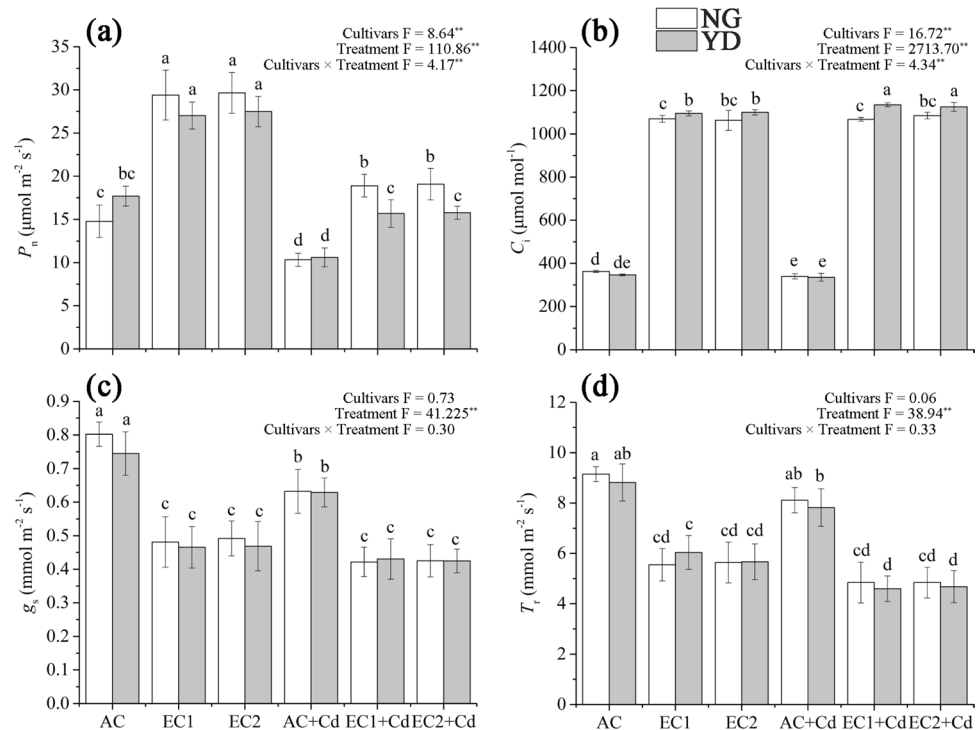
influence the MDA, O_2^-, and H_2O_2 accumulations in the rice shoots and roots. However, when the seedlings were exposed to Cd, the elevated CO_2 concentrations significantly reduced the MDA, O_2^-, and H_2O_2 accumulations in the rice shoots and roots, except the root O_2^- contents of the NG rice, which were lower than those of the AC + Cd treatment. The two cultivars showed similar patterns, whereas when the seedlings were treated with Cd, the MDA, O_2^-, and H_2O_2 accumulations in the NG rice were significantly lower than those of the YD rice, except for the shoots' MDA and H_2O_2 contents under the EC2 + Cd treatment. Also, the roots of rice seedlings were stained by DAB, NBT, and Evans blue to analyze the O_2^-, and H_2O_2 accumulations and plasma membrane integrity. The images of histochemical staining roots were shallower treated with EC2 compared with control (AC), but darker treated with Cd (AC+Cd) compared with control (AC), and further treated with elevated CO_2 (EC2+Cd) rescued the darker tendence, and the two cultivars showed similar patterns (Fig. S3).

Elevated CO_2 concentrations increased the NPT and total phenolic contents

The NPT contents of the shoots were significantly increased in the rice treated with elevated CO_2 concentrations compared with the AC treatment. Moreover, under Cd stress, the NPT contents of the shoots (AC + Cd) were further significant increased compared with the AC and EC2 treatments, and the NPT contents of the EC2 + Cd treatment

were significantly higher than those of the AC + Cd treatment (Fig. 6a). For the rice treated with elevated CO_2 concentrations, the NPT contents of the roots showed consistent tendencies compared to those of the shoots, but under Cd stress, at either of the elevated CO_2 concentrations, the NPT contents of the roots did not show any significant differences between the AC + Cd and EC2 + Cd treatments, and the NPT contents of the roots of the YD rice were significantly lower than those of the NG rice (Fig. 6c).

The total phenolic contents in the shoots of the rice under the EC2 treatment were significantly higher than those of the AC treatment for both two cultivars, and under Cd stress, the elevated CO_2 concentrations still significantly increased the total phenolic contents (Fig. 6b). The total phenolic contents in the roots of the NG rice showed similar patterns to those of the shoots, but regardless of the treatment, the total phenolic contents in the roots of the NG rice were significantly higher than those of the YD rice (Fig. 6d).

Elevated CO_2 concentrations increased the ASA and GSH contents of the rice

The ASA contents in the roots and shoots of both cultivars treated with elevated CO_2 concentrations were significantly increased compared with the control (AC), and under Cd stress, the elevated CO_2 concentration (EC2 + Cd) significantly increased the ASA contents in the roots and shoots compared with the Cd treatment (AC + Cd) (Fig. 7a and c). In addition, Cd exposure affected the ASA contents, and
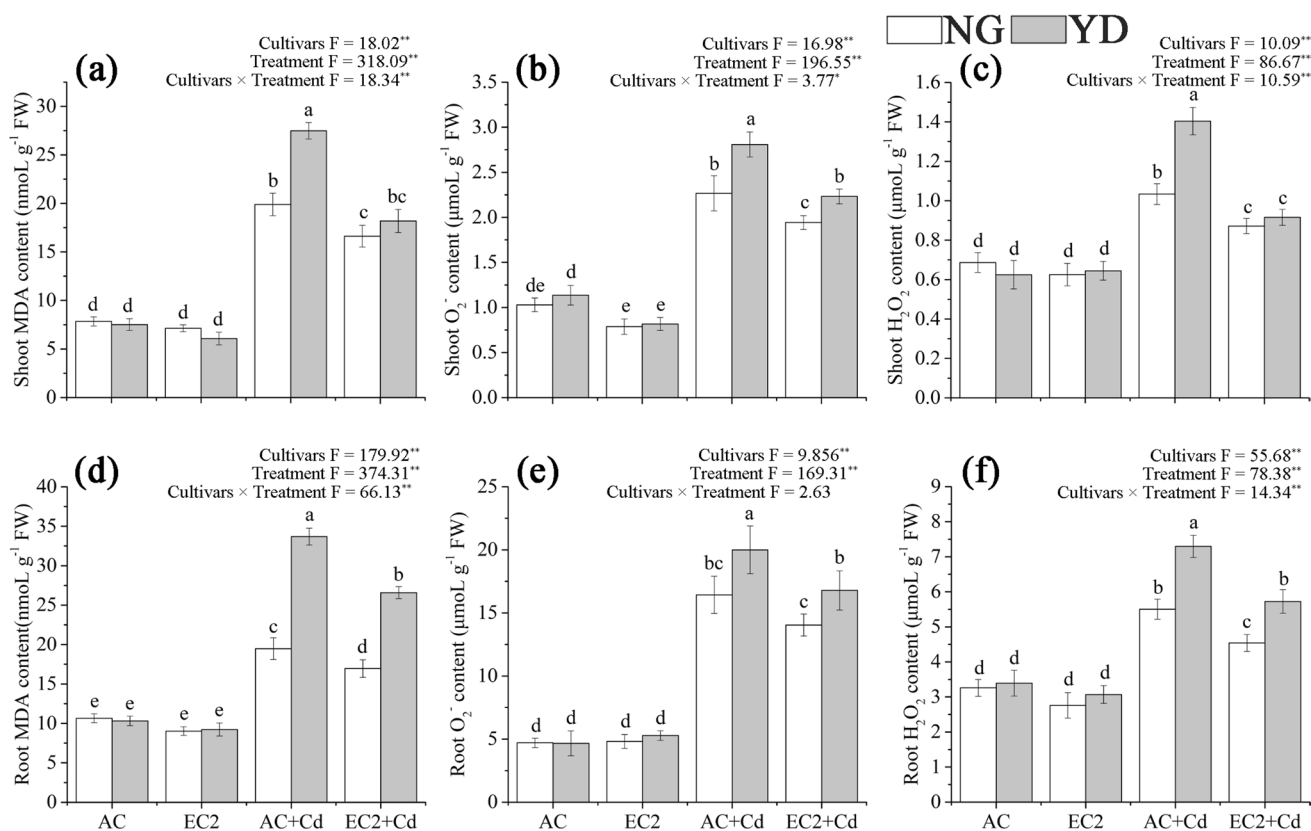
Fig. 5 The MDA, H$_2$O$_2$, and O$_2^-$ contents of the rice under the Cd and CO$_2$ treatments: **a** MDA contents of the shoots, **b** H$_2$O$_2$ contents of the shoots, **c** O$_2^-$ contents of the shoots; **d** MDA contents of the roots, **e** H$_2$O$_2$ contents of the roots, and **f** O$_2^-$ contents of the roots. NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice;

AC, ambient concentration; EC2, growth in the chamber of elevated CO$_2$ concentration from beginning to end. The different letters above each bar indicate significant differences with Duncan's test ($P < 0.05$). The values shown are the means ± SDs ($n = 4$)

regardless of whether the AC or elevated CO$_2$ concentration treatments were applied, the ASA contents in the roots and shoots of the YD rice were significantly reduced (Fig. 7a and c).

The GSH contents in the shoots and roots of the two rice cultivars were significantly increased when treated with Cd, and the elevated CO$_2$ concentrations increased the GSH contents in the two cultivars (Fig. 7b and d). The GSH contents in the roots of the two cultivars treated with EC2 were significantly increased compared to those of the AC treatment (Fig. 7d), but they did not show significant increases in the shoots (Fig. 7b).
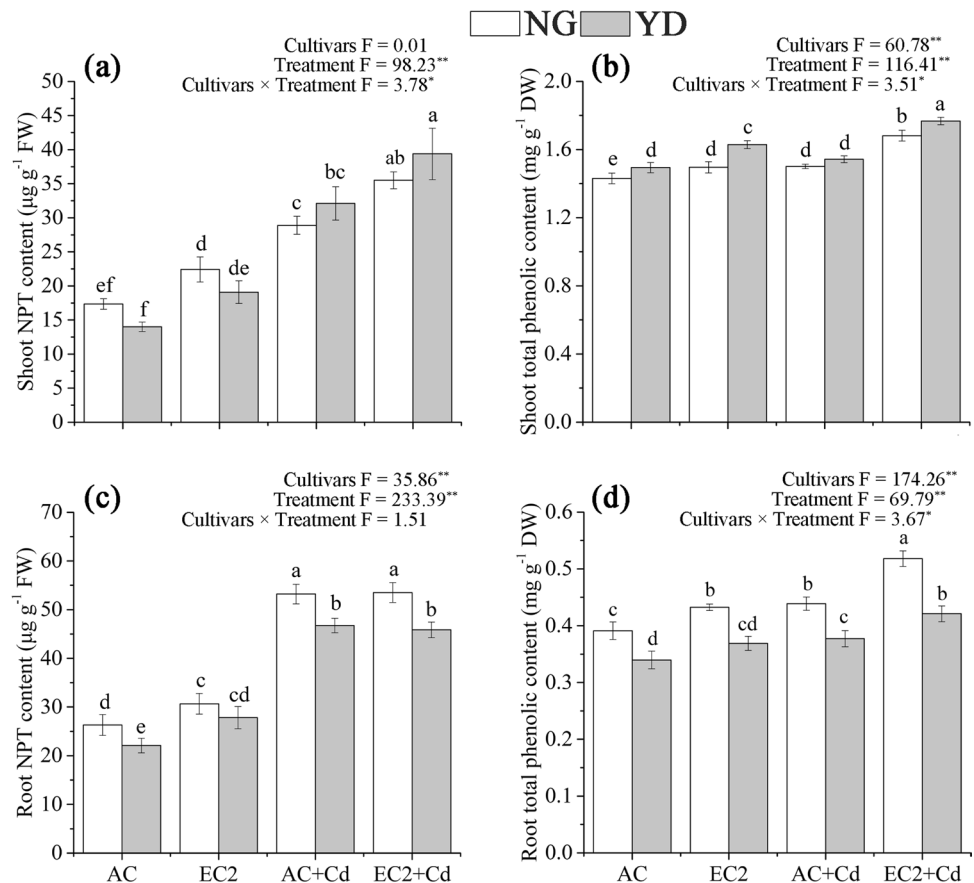
Elevated CO$_2$ concentrations enhanced the APX and GR activities

The APX activity in the shoots of the NG rice did not show significantly differences among the AC, EC2, and AC + Cd treatments, but the EC2 + Cd treatment significantly promoted APX activities in the shoots, and only the elevated CO$_2$ concentrations significantly enhanced the APX

activities in the shoots of the YD rice. Cd exposure affected APX activity, but the APX activity in the shoots of the YD rice treated with EC2 + Cd was significantly higher than the other treatments of the YD rice (Fig. 8a). There were no significant differences between the APX activities of the AC and AC + Cd treatments of the NG rice, and the EC2 and EC2+Cd treatments of the NG rice showed the same patterns. Elevated CO$_2$ concentrations (EC2) significantly increased the APX activity in the roots of the two rice cultivars, but the APX activity in the roots of the YD rice was significantly inhibited under Cd stress (AC + Cd), and when further treated with elevated CO$_2$ concentrations (EC2 + Cd), the APX activity significantly increased compared with the AC + Cd treatment, though this increase was not significantly higher than that of the control (AC) (Fig. 8c).

Overall, elevated CO$_2$ concentrations increased GR activity in the two cultivars in both the shoots and roots, and Cd exposure also increased GR activity. The two factors worked together and further enhanced GR activity (Fig. 8b and d). In detail, the GR activity in the shoots of the NG rice between the AC and EC2 treatments did not show significant

Fig. 6 NPT and total phenolic contents of the rice under the Cd and CO_2 treatments: **a** NPT contents of the shoots, **b** total phenolic contents of the shoots, **c** NPT contents of the roots, and **d** total phenolic contents of the roots. NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice; AC, ambient concentration; EC2, growth in the chamber of elevated CO_2 concentration from beginning to end. The different letters above each bar indicate significant differences with Duncan's test ($P < 0.05$). The values shown are the means $\pm$ SDs ($n = 4$)

differences, but the GR activity was significantly increased under Cd stress (AC + Cd). In addition, when treated with elevated CO_2 concentrations and Cd, the GR activity was significantly enhanced (Fig. 8b). For the YD cultivar, the GR activity was significantly increased step by step when consecutively treated with EC2, Cd, and EC2 + Cd (Fig. 8b). The GR activity in the roots of the two cultivars showed a similar pattern to the GR activity in the shoots, whereas the GR activity in the roots of the YD rice under the AC + Cd treatment was not significantly higher than that of the EC treatment (Fig. 8d).

Discussion

In this study, we selected an *indica* cultivar (YD) and a *japonica* cultivar (NG). Both two cultivars are widely planted in the Middle and Lower Yangtze River areas of China. We analyzed the effects on the two rice cultivars under different Cd concentrations. The two cultivars showed significant differences in shoot elongation, root elongation, shoot biomass, root biomass, shoot Cd concentration, root Cd concentration, and Cd translocation factor. The Cd concentrations in the roots and shoots of the two cultivars also indicated that the *indica* rice could accumulate more Cd

in its grain than that of the *japonica* rice (Yan et al. 2019). The roots were firstly faced with Cd exposure (Song et al. 2018). We based on treatment volumes on root lengths and chose 5 μM Cd as the Cd stress treatment for the follow-up experiments.

To analyze the rice growth differences between the elevated CO_2 concentrations, combined with the elevated CO_2 concentration and the Cd stress treatments, we applied two elevated CO_2 concentration approaches (EC1 and EC2). According to the biomass analysis, the elevated CO_2 concentrations both promoted plant growth, and the "CO_2 fertilization" has been widely proven, with previous studies including *Eucalyptus* trees (Wujeska-Klause et al. 2019), grass (Andresen et al. 2018), and so on. We then detected the Cd concentrations, which showed that elevated CO_2 concentrations reduced Cd concentrations in the roots but promoted Cd translocation from the roots to the shoots. Although plants could block largely Cd in root tissues by the mechanisms of cell-wall binding, complexation, and vacuolar sequestration (Tang et al. 2023), the Cd TFs in this study were quite low, but still some Cd could be transfer to shoot and reached to the toxic level to rice. In addition, the elevated CO_2 concentrations increased the biomasses of shoot and root. As a result, elevated CO_2 concentrations led to the significantly increases of the Cd accumulations

Fig. 7 ASA and GSH contents of the rice under the Cd and CO_2 treatments: **a** ASA contents in the shoots, **b** GSH contents in the shoots, **c** ASA contents in the roots, and **d** GSH contents in the roots. NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice; AC, ambient concentration; EC2, growth in the chamber of elevated CO_2 concentration from beginning to end. The different letters above each bar indicate significant differences with Duncan's test ($P < 0.05$). The values shown are the means $\pm$ SDs ($n = 4$)
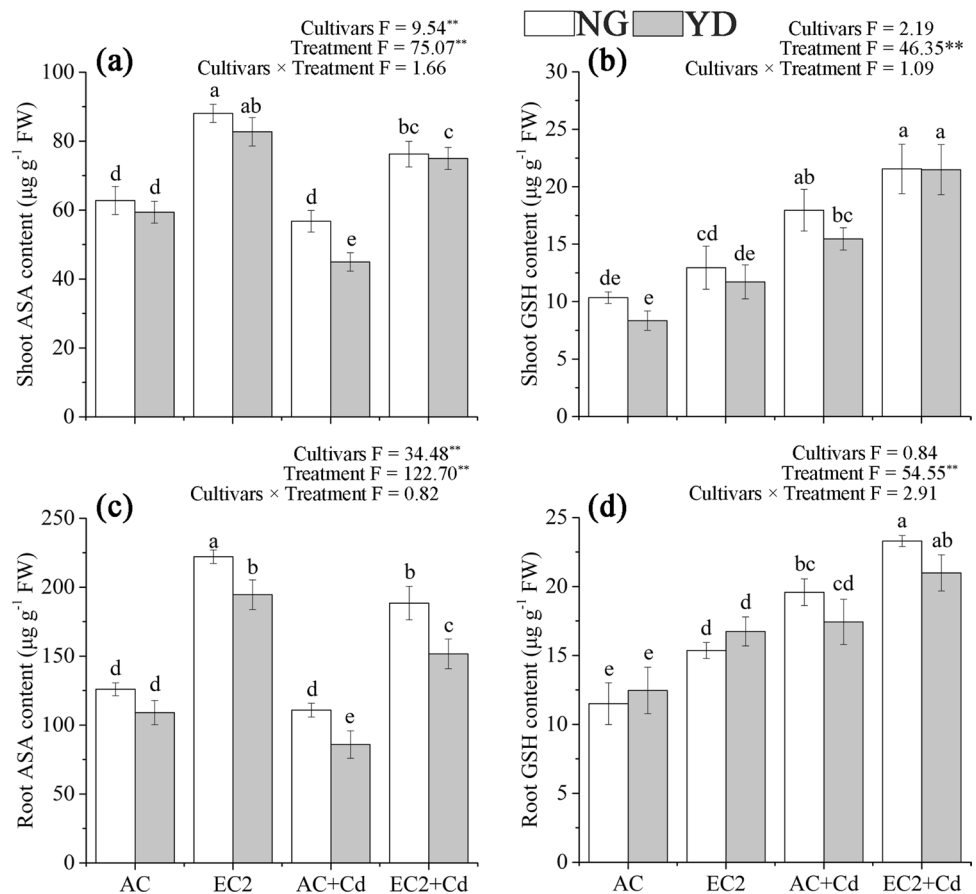
both in the shoots and roots of the rice, and total Cd accumulations of whole plant were also significantly increased compared with control. Up to here, we had a simple but serious problem that elevated CO_2 concentrations would enhance the ability of rice to absorb Cd and transport Cd to shoot. Also, previous studies found the similar results. For *Lolium perenne*, researchers found that applying CO_2 enhanced the Cd uptake (Song et al. 2015) and enhanced the phytoremediation efficiency of ryegrass in Cd-polluted soils (Guo et al. 2014). But here, we used hydroponics experiments; the results cannot be clearly explained by the changes of the bioavailability of Cd, soil pH, and microbial biomasses as previous studies. And another toxic metal, arsenic (As), its change of bioavailability was opposite with Cd in response to the change of soil pH; the As concentration of rice grain would be also increased by elevated CO_2 (Muehe et al. 2019). In addition, the "dilution effect" could not answered the results yet, because the correlation of different element concentrations depended ever more on plant species and cultivars (Yi et al. 2023). So, it was thought that CO_2 maybe provides a signal which motivated the Cd uptake by root and Cd transport within the plants, or CO_2 induced signal molecule (e.g. NO) accumulation to answer this effect (Wang et al. 2013). Because of the serious toxicity of Cd,

many researches have tried to reduce the Cd accumulation in edible parts of crops to protect people from Cd, especially for rice (McGrath 2022). But Cd pollution of paddy field was not easily scavenged; the Cd accumulated in edible parts of crops can only try to reduce. With the atmosphere CO_2 concentrations increased in the future, the Cd pollution of crops should threat human health. Thus, Cd pollution should receive attention in future studies on elevated CO_2 concentrations.

The photosynthetic pigments and leaf gas exchange parameters showed that the elevated CO_2 concentrations caused increased photosynthesis in the plants (Thompson et al. 2017). The patterns for the changes of photosynthetic pigment contents were consistent with the changes of P_n values. Moreover, because of the treatments of elevated CO_2 concentrations, C_i and its related attributes, g_s and T_r, showed main variances between control and Cd stress; furthermore, the tendency of P_n, g_s, and T_r indicated the decrease of water loss and enhancement of the water use efficiency. Elevated CO_2 concentrations and Cd altered the carbon gain and water loss abilities of plants. Overall, elevated CO_2 concentrations would improve the photosynthesis efficiency, but Cd exposure weakened the benefits of elevated CO_2.

Fig. 8 The APX and GR activities of the rice under the Cd and CO$_2$ treatments: **a** APX activity in the shoots, **b** GR activity in the shoots, **c** APX activity in the roots, and **d** GR activity in the roots. NG, Ninggeng No. 7, *indica* rice; YD, Yangdao No. 6, *japonica* rice; AC, ambient concentration; EC2, growth in the chamber of elevated CO$_2$ concentration from beginning to end. The different letters above each bar indicate significant differences with Duncan's test ($P < 0.05$). The values shown are the means ± SDs ($n = 4$)
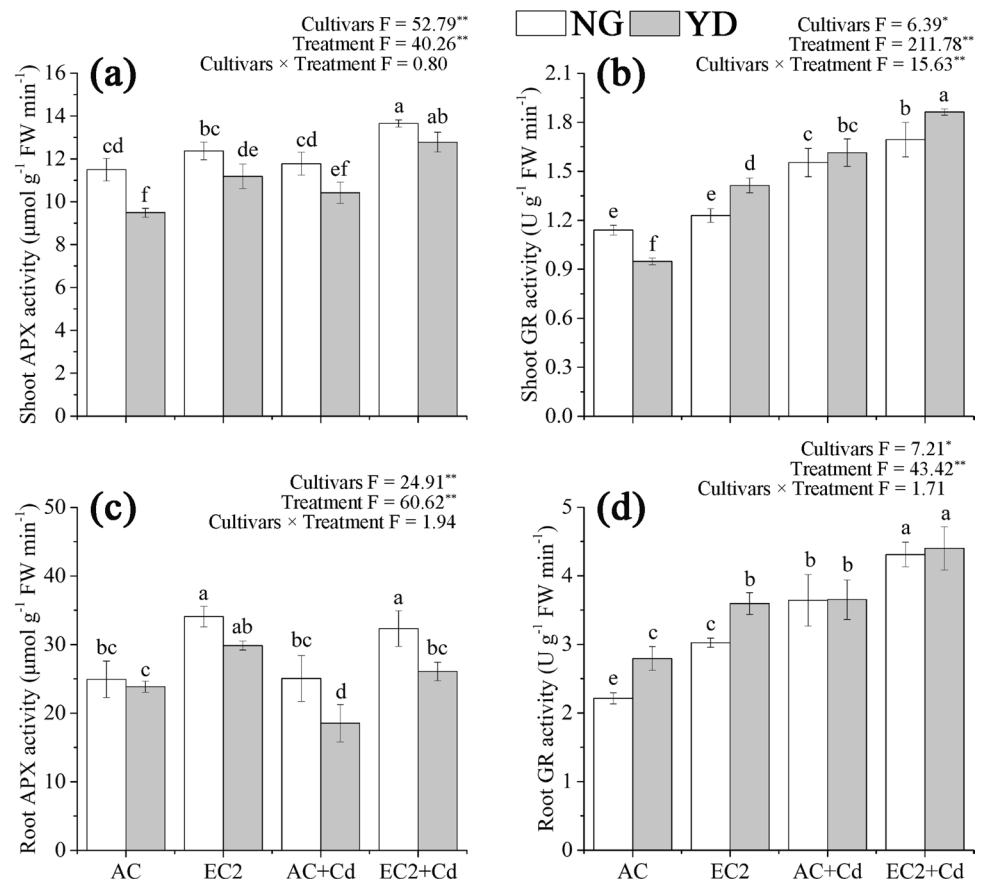
The approaches of two elevated CO$_2$ concentrations showed similar results, and elevated CO$_2$ concentration in advance showed greater risks than the treatments with simultaneous Cd exposure; in addition, the culture condition of elevated CO$_2$ concentration in advance was more aligned with the future environment. Therefore, we treated the seedlings with the elevated CO$_2$ concentrations when the seedlings were transferred to hydroponic boxes for the experiments. Cd toxicity causes the overproduction of ROS which would cause plants suffer from ROS stress (Huang et al. 2019). We measured the MDA contents and ROS accumulations. Also, we analyzed plasma membrane integrity of roots by Evans blue. The MDA measurements were an index of lipid peroxidation (Ali et al. 2014). Cd stress significantly induced an over-accumulation of O$_2^-$ and H$_2$O$_2$ and an increase in the MDA contents in the roots and shoots of the plants and caused the cell death of plasma membrane. To deal with the ROS stress, plants evolved the antioxidant systems to scavenging ROS. The plant's antioxidant systems include non-enzymatic antioxidants and antioxidant enzymes (Nahar et al. 2016). Many studies have reported that antioxidant enzymes play important roles in Cd detoxification (Naliwajski and Sklodowska 2021). The presence of antioxidant enzymes suggested that the plants' growth was affected when they were treated by elevated CO$_2$

concentrations (Gillespie et al. 2011). Cd-induced oxidative stress has been shown to be modulated by the AsA-GSH pathway (Hasanuzzaman et al. 2017). NPTs including several acid-soluble sulfhydryl components such as GSH, PC (phytochelatins), and cysteine, have been shown to play an important role in plant detoxification (in response to toxic metal exposure) (Shi et al. 2022). Here, we found that the NTPs mainly functioned in rice seedling Cd detoxification, and elevated CO$_2$ concentrations had litter effect on NPT content. Both the Cd stress and the elevated CO2 concentrations increased phenolic accumulation in this study. Yet, there were evidences suggested that the phenolic accumulation was likely to happen during the rice maturity stage (Goufo et al. 2014). As for the litter attention on non-enzymatic antioxidants, we analyzed the non-enzymatic antioxidants and the APX and GR activities in the AsA-GSH cycle. The ASA and GSH contents showed that the elevated CO$_2$ concentrations mainly affected the ASA contents and the GSH contents were mainly affected by the Cd exposure. The APX and the GR activities also indicated that the elevated CO$_2$ concentrations mainly enhanced the plants' non-enzymatic antioxidant systems. Past research has also indicated that plants have reduced the negative effects of drought mainly by maintaining their total antioxidants (Ulfat et al. 2021). Taken together, elevated CO$_2$ concentrations could

enhance the antioxidant capacity ability of rice to promote plant growth, Cd would cause rice suffer ROS stress, and elevated CO_2 concentrations alleviated the stress by scavenging ROS.

Conclusions

Although many previous studies have indicated that elevated CO_2 concentrations have a strong positive impact on crop yields due to the "CO_2 fertilization" effect, a growing number of studies have shown the challenge of the negative impacts on crop quality where the crop could not cope with the demand for nutrients under elevated CO_2 concentrations. In addition, through large-scale and long-term experiments, the seasonal precipitation (Hovenden et al. 2019) and intensifying drought cycles (Gray et al. 2016) may eliminate growth in crop yields, and predicting the carbon feedback to plants, even with the best-supported short-term models, might not effectively predict long-term results (Reich et al. 2018).

In our study, elevated CO_2 concentrations enhanced the photosynthetic capacity to promote plant growth, and the ASA–GSH cycle enhanced the activity of antioxidants induced by elevated CO_2 concentrations which contributed to Cd detoxification. In addition, elevated CO_2 concentrations affected Cd translocation, especially in the *indica* rice. We suggested that CO_2 played a role of a signal molecule which regulated the ion transportation and distribution mechanisms in rice. In conclusion, a promising strategy for identifying the mechanisms of Cd uptake and transfer is still needed for a future high-CO_2 world to maintain food security and food safety.

Declarations

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