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RESEARCH ARTICLE

Elevated atmospheric CO_2 affected photosynthetic products in wheat seedlings and biological activity in rhizosphere soil under cadmium stress

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Abstract The objective of this study was to investigate the effects of elevated CO_2 (700±23 μmol mol^{-1}) on photosynthetic products in wheat seedlings and on organic compounds and biological activity in rhizosphere soil under cadmium (Cd) stress. Elevated CO_2 was associated with decreased quantities of reducing sugars, starch, and soluble amino acids, and with increased quantities of soluble sugars, total sugars, and soluble proteins in wheat seedlings under Cd stress. The contents of total soluble sugars, total free amino acids, total soluble phenolic acids, and total organic acids in the rhizosphere soil under Cd stress were improved by elevated CO_2. Compared to Cd stress alone, the activity of amylase, phenol oxidase, urease, L-asparaginase, β-glucosidase, neutral phosphatase, and fluorescein diacetate increased under elevated CO_2 in combination with Cd stress; only cellulase activity decreased. Bacterial abundance in rhizosphere soil was stimulated by elevated CO_2 at low Cd concentrations (1.31–5.31 mg Cd kg^{-1} dry soil). Actinomycetes, total microbial abundance, and fungi decreased under the combined conditions at 5.31–10.31 mg Cd kg^{-1} dry soil. In conclusion, increased production of soluble sugars, total sugars, and proteins in wheat seedlings under elevated CO_2 + Cd stress led to greater quantities of organic compounds in the rhizosphere soil relative to seedlings grown under Cd stress only. Elevated CO_2 concentrations could moderate the effects of heavy metal pollution on enzyme activity and microorganism abundance in rhizosphere soils, thus improving soil fertility and the microecological rhizosphere environment of wheat under Cd stress.

Keywords Elevated atmospheric CO_2 · Cd-contaminated soil · Wheat seedlings · Photosynthetic products · Organic compounds · Rhizosphere soil · Soil biological activity

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Introduction

Atmospheric carbon dioxide (CO_2) concentrations have increased continuously during the last two centuries, and significant effects of elevated CO_2 have been demonstrated in various plants species. These effects include increased growth rates and productivity or altered biomass allocation patterns, mainly as a result of stimulation of photosynthesis (Bunce 2004; Marchi et al. 2004; Kim and Kang 2011). Large fluxes of primary and secondary compounds into soil have been observed under elevated CO_2 (Johnson and Pregitzer 2007), resulting in increased levels of dissolved organic carbon (DOC), total soluble sugars, and soluble phenolic acids in soil (Freeman et al. 2004), which, in turn, could affect the activity and survival of soil microorganisms. In addition, elevated atmospheric CO_2 has been associated with increased microbial biomass and soil enzyme activity, such as that of urease, protease, invertase, xylanase, arylsulfatase, and alkaline phosphatase (Kandeler et al. 2006; Li et al. 2010).

Heavy metal contamination of soil is a serious global problem. Among the heavy metals, cadmium (Cd) is widespread and is one of the most toxic pollutants of the surface soil layer. Cd is principally dispersed into agricultural soil as a result of the use of phosphate fertilizers, application of sewage and industrial wastewater for irrigation, atmospheric deposition from metallurgical industries, incineration of plastics and batteries, and burning of fossil fuels (Tukaj et al. 2007; Li et al. 2013). The severity of environmental Cd pollution is increasing (Guo et al. 2011), and numerous studies have focused on the effects of Cd contamination on crop growth, development, and quality, and on soil enzyme activity and soil microorganisms (Del et al. 1999; Hinojosa et al. 2004; Zhang et al. 2012). Soil microbial biomass and carbon, and activity of enzymes including alkaline phosphomonoesterase, arylsulfatase, and protease were significantly reduced in Cd-contaminated soil (Renella et al. 2004).

As much as 40 % of the carbon fixed by plants can be lost through root exudation (Lynch and Whipps 1991), which is a major source of DOC and organic compounds in soil. These compounds, released into the rhizosphere by plant roots, create unique microenvironments for soil microorganisms and stimulate soil biological activity (Patra et al. 2006; Xu et al. 2009), thus, playing an important role in soil fertility. Cd stress has been reported to increase the concentration of root exudates in soil (Pérez-de-Mora et al. 2006), thus increasing an availability of substrates in the rhizosphere for soil microorganisms. However, elevated atmospheric CO_2 and Cd contamination of soil are concurrent environmental problems, and it is important to investigate their combined effects on plant photosynthetic products, soil biological activity, and organic compounds in rhizosphere soils. Many studies have reported the effects of either elevated CO_2 or metal contamination on these variables (Johnson and Pregitzer 2007; Kwon-Rae et al. 2007), but little is known about their combined effects, especially in crop plants. We examined these effects in wheat, a crop that forms an important part of the human diet worldwide (H gy et al. 2009).

Materials and methods

Plant species and soil preparation

Seeds of *Triticum aestivum* L. (spring wheat, No. 15 Yongliang) were obtained from the Institute of Wheat Breeding in Yongning County, Ningxia Province, China. The experimental soil was collected from the surface layer (0–20 cm) in a wheat field in central Shaanxi Province. The soil type and chemical characteristics are shown in Table 1. Fresh soil was passed through a 5-mm sieve. Four concentrations of Cd were selected according to environmental quality standards (GB 15168–1995) and current levels of Cd pollution observed in farmland in China (Song et al. 2006). The soil was artificially contaminated using $CdCl_2{\cdot}2H_2O$ solution to concentrations of 0.31 (Cd0, the control, no Cd spiked to soil); 1.31 (Cd1); 5.31 (Cd5); and 10.31 (Cd10) mg Cd kg^{-1} dry soil and incubated for 30 days.

Table 1 Type and chemical characteristics of the experimental soil

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

Experimental site and atmospheric CO_2 concentration

The study was conducted in Spring 2013 at an open-top chamber facility on the Weishui Campus of Chang'an University, Xi'an, China. The elevation of the study site is 402 m a.s.l.; mean annual temperature and precipitation (1995–2010) are 13.6 °C and 508–720 mm, respectively.

Air temperature was the same in all chambers, thus eliminating temperature as an explanatory factor for differences between treatments. Each treatment was replicated three times using a randomized complete block design. Six hexagonal open-top chambers (4.4 m dia × 1.6 m tall) were established at the experimental facility. Three were used as controls (ambient CO_2 chambers, 385 μmol mol^{-1}), and three were used for an elevated CO_2 treatment (700±23 μmol mol^{-1}). Automated measurements of CO_2 concentration, temperature, humidity, and soil water content were taken every 60 s throughout the experiment. An automatic control system was used to adjust CO_2 to the target concentration in the elevated CO_2 chambers by regulating the influx rate of CO_2 or air. Air temperature was recorded in each chamber at 10-min intervals. During the experiment, average CO_2 concentrations in the elevated and ambient CO_2 chambers were 703 and 385 μmol mol^{-1}, respectively. Average temperature in the elevated and ambient CO_2 chambers was 23.3 and 23.1 °C, respectively, and average humidity was 69.3 and 69.0 %, respectively.

Pot experiment

The experiment was performed using plastic pots (45 cm dia × 50 cm tall) and root bags to maximize the reliability of the

results for rhizosphere soil samples. Each nylon root bag was filled with 800 g Cd-contaminated soil, and seven root bags were placed in each pot and covered with 19.4 kg soil. Three replicate pots were prepared for each Cd concentration. The pots were placed in the open-top chambers, and *T. aestivum* seeds were planted in 2013 April to obtain 91 seedlings per pot after emergence. Litter and weeds were monitored and removed from pots by hand to reduce effects of them on variables measured in this experiment. The treatments were consisted of ambient CO_2 chambers + Cd-contaminated soils and elevated CO_2 chambers + Cd-contaminated soils. Soil water content was measured at 5-cm depth by randomly selecting three pots; water content was 15.4 % higher in elevated CO_2 chambers than in ambient CO_2 chambers. Based on these measurements, the pots were maintained at 60 % field capacity by watering during the seedling growth.

The influence of elevated CO_2 alone was examined by comparing samples under elevated and ambient CO_2. The influence of Cd stress alone was examined by comparing samples under Cd stress (Cd1, Cd5, and Cd10) with samples not exposed to Cd (Cd0) in the ambient CO_2 chambers. The combined effects of elevated CO_2 and Cd-contaminated soil were assessed by comparing samples under elevated CO_2 + Cd with samples under ambient CO_2 + Cd.

Rhizosphere soil sampling

Soil collected from the rhizosphere of 3-week-old wheat seedlings was used for analysis of organic compounds, enzyme activity, and microbial abundance and activity. Soil that was strongly adhered to roots or that was within the space penetrated by roots in the root bags was considered rhizosphere soil (Sinha et al. 2009) and was carefully collected, mixed, homogenized, and divided into two subsamples. One subsample was stored at 4 °C for analysis of organic compounds, enzyme activity, cultivable microorganism populations, and fluorescein diacetate (FDA) hydrolysis activity. The other subsample was air dried for analysis of soil pH, DOC, and available nitrogen (AN).

Analysis of photosynthetic products

Wheat seedlings (3 weeks old) were harvested, separated from roots, and immediately immersed in liquid nitrogen, for analysis of photosynthetic products. Soluble carbohydrates were extracted according to the method of Rogers et al. (2004). Reducing and total sugar contents were measured with phenol–sulfuric acid (Dubois et al. 1956). Starch was extracted as described by Vu et al. (2001), and starch content was determined by glucose oxidase–peroxidase (GOD-POD)/2-2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay (Bergmeyer 1974) using glucose as a standard. Soluble sugar content was determined according to Dubois et al. (1956). Soluble proteins were extracted as described by Katny et al. (2005), and soluble protein content was determined according to Bradford (1976). Soluble amino acids were analyzed using the ninhydrin colorimetric method (Moore and Stein 1954).

Enzyme assays

The activity of eight enzymes was measured and reported on a gram per dry soil equivalent basis. Amylase activity was measured by starch hydrolysis (Cole 1977) and expressed as micrograms maltose per day. Cellulase activity was determined by hydrolysis of carboxymethyl cellulose (CMC) following the method of Pancholy and Rice (Pancholy and Rice 1973) and was expressed as micrograms glucose per day. Phenol oxidase activity was determined using L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate (Sinsabaugh et al. 1993) and was expressed as micrograms DOPA converted per hour. Urease activity was assessed by measuring the release of NH_4^+ from urea hydrolysis (Tabatabai and Bremmer 1972) and was expressed as milligrams NH_4^+–N per hour. The activity of L-asparaginase was measured according to Frankenberger and Tabatabai (Frankenberger and Tabatabai 1991) and expressed as micrograms ammonia per hour. Invertase activity was measured according to Xu and Zheng (Xu and Zheng 1986) and was expressed as micrograms glucose per hour. Activity of β-glucosidase was determined by spectrophotometric assay by incubating 1 g soil (air-dry basis) for 1 h with p-nitrophenyl-β-D-glucoside at pH 6.0 (Eivazi and Tabatabai 1988) and was expressed as micrograms p-nitrophenol per day. Neutral phosphatase activity was determined spectrophotometrically following Wu's disodium phenyl phosphate method (Wu et al. 2004) and expressed as micrograms phenol per day.

Culturable microorganisms and FDA hydrolysis activity

Colony-forming units of bacteria, actinomycetes, and fungi were determined using a modified plate-dilution technique on meat–peptone agar, Gause's starch agar, and Thayer–Martin agar, respectively (Yang et al. 2009). The FDA hydrolysis activity (U) was determined according to Mora et al. (2005) and expressed as micrograms fluorescein released per minute per grams dry soil equivalent.

Soil properties and organic compounds in rhizosphere soil

Soil DOC content was measured in a soil and distilled water slurry (1:10 *w*/*v*) and analyzed using an automated TOC

Analyzer (Shimadzu TOC-500). Rhizosphere soil pH was measured using a pH meter in a soil and distilled water slurry (1:2.5 *w/v*, without CO_2) (Tukaj et al. 2007). Soil AN concentration was determined by persulfate digestion (Sollins et al. 1999). Total soluble sugars were extracted according to Johnson and Pregitzer (2007) and measured using phenol–sulfuric acid colorimetry (Dubois et al. 1956). Total free amino acid concentrations were measured using the ninhydrin colorimetric method (Moore and Stein 1954), total soluble phenolic acid content was analyzed using the Folin–Ciocalteu method (DeForest et al. 2005), and total organic acid content was measured by spectrophotometry with $FeCl_3$ (Liu and Mo 1985).

Statistical analyses

Effects of elevated CO_2 or Cd-contaminated soil on soil properties, photosynthetic products, organic compounds in rhizosphere soil, enzyme activity, abundance of cultivable microorganisms, and FDA hydrolysis activity were analyzed using one-way analysis of variance (ANOVA). Two-way ANOVA was used to assess the effects of elevated CO_2 in combination with Cd-contaminated soil on these parameters. The distribution of the data was assessed by Kolmogorov–Smirnov (KS) tests. All statistical tests were performed using SPSS (SPSS Inc., version 16.0).

Results

DOC, pH, and soil AN in rhizosphere soil

The DOC content increased by 1.6 % relative to the control (Cd0 in ambient chambers) under elevated CO_2 alone (Fig. 1a). When compared to the control, DOC content under Cd1, Cd5, and Cd10 decreased significantly by 8.3, 16.2, and 24.1 %, respectively, and showed a decreasing trend with increasing Cd concentration. In contrast, DOC increased under elevated CO_2 in combination with Cd stress but showed an obvious decreasing trend with increasing Cd concentration. The effect of elevated CO_2 combined with Cd stress on DOC content was significant ($p<0.01$, Table 2).

The pH of rhizosphere soil decreased significantly under either elevated CO_2 or Cd stress. However, the combination of elevated CO_2 and Cd stress did not affect pH significantly (Table 2). Compared to Cd stress alone, rhizosphere soil pH decreased under elevated CO_2 + Cd stress (Fig. 1b). Moreover, pH showed a decreasing trend with increasing Cd concentration under the combined conditions.

Rhizosphere soil AN decreased by 26.5 % under elevated CO_2 alone and decreased significantly ($p<0.05$) at Cd1 and Cd5 levels and increased significantly ($p<0.05$) at Cd10 (Fig. 1c). Compared to Cd stress alone, the soil AN content was reduced by the combined conditions and decreased with increasing Cd concentration (Fig. 1c).

Photosynthetic products in wheat seedlings

Treatment with elevated CO_2 alone resulted in significantly increased quantities of reducing sugars (9.2 %), soluble sugars (5.8 %), and total sugars (3.2 %), and to a significant decrease in soluble amino acids (−6.9 %) compared to the control (Cd0 in ambient chambers) (Fig. 2). Reducing sugars under Cd1, Cd5, and Cd10 increased significantly by 8.7 % ($p<0.05$), 30.0 % ($p<0.01$), and 31.8 % ($p<0.01$), respectively, while starch, total sugars, soluble sugars, soluble amino acids, and soluble protein contents in seedlings decreased relative to their contents in plants grown in uncontaminated soil (Fig. 2). Starch, total and soluble sugars, and soluble amino acids also showed a decreasing trend with increasing Cd under Cd stress alone (Fig. 2).

The combination of elevated CO_2 and Cd stress had a significant effect on sugars, starch, and soluble amino acids and proteins (Table 2). The combined treatment resulted in decreased contents of reducing sugars, starch, and soluble amino acids (Fig. 2a, b, f); however, soluble sugars, total sugars, and soluble proteins increased (Fig. 2c–e). Although Cd stress lead to decreased sugar and protein contents, elevated CO_2 counteracted these effects. However, quantities of reducing sugars, starch, and soluble amino acids were lower under elevated CO_2 + Cd stress than those under either condition alone. In addition, the contents of photosynthetic products in wheat seedlings decreased with increasing soil Cd concentration under the combined conditions (Fig. 2).

Organic compounds in rhizosphere soil

Elevated atmospheric CO_2 significantly increased the contents of total soluble sugars, total free amino acids, total soluble phenolic acids, and total organic acids in rhizosphere soil, by 35.9, 2.6, 3.3, and 5.5 %, respectively, compared to the control (Cd0 in ambient chambers) (Fig. 3a–d). Under Cd stress, soluble sugar and free amino acid contents increased while total soluble phenolic and organic acids decreased compared to the control (Fig. 3c, d). In addition, total soluble sugars and free amino acids increased as Cd concentration increased (Fig. 3a, c).

The content of soluble sugars, free amino acids, soluble phenolic acids, and organic acids in rhizosphere soil increased under elevated CO_2 combined with Cd stress relative to Cd stress alone (Fig. 3a–d). Under the combined conditions, total soluble sugars and free amino acids increased gradually, and soluble phenolic acids decreased gradually with increasing Cd concentration (Fig. 3a–c). In addition, total soluble sugars and free amino acids in rhizosphere soil were greater under elevated CO_2 + Cd stress than under either condition alone; soluble

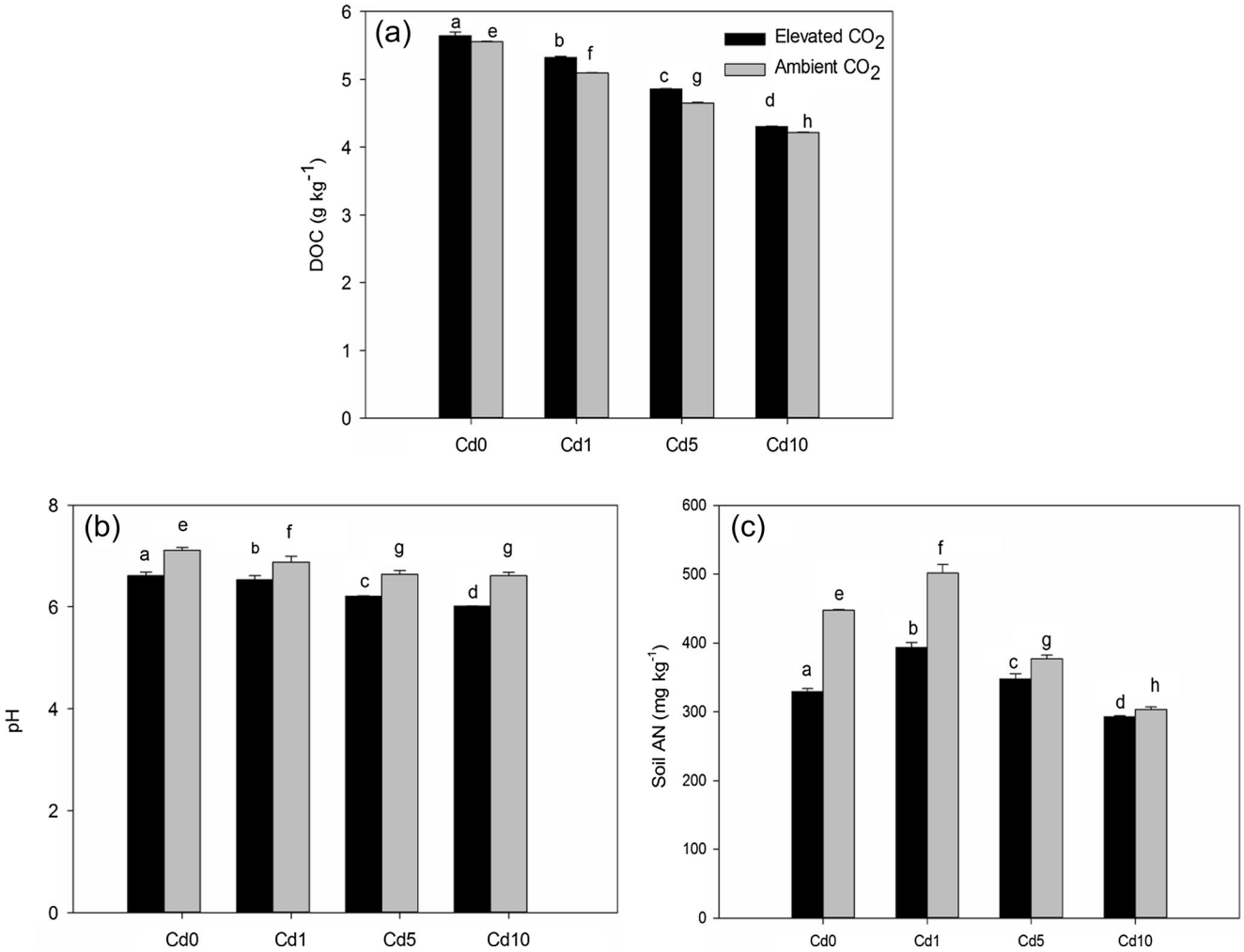


Fig. 1 DOC (**a**), pH (**b**), and soil AN (**c**) in rhizosphere soil of wheat seedlings. Data are means± SE (n=9). Cd0, Cd1, Cd5, and Cd10 in the figure were 0.31, 1.31, 5.31, and 10.31 mg Cd kg^{-1} dry soil, respectively; Different *small letters* in the same group indicates significant difference between elevated CO_2 and ambient CO_2 for the same Cd level at $p<0.05$; *a–d* Significant differences between different Cd levels under elevated CO_2 at $p<0.05$; *e–h* Difference between Cd levels under ambient CO_2 at $p<0.01$

phenolic acid and total organic acid contents were lower under the combined conditions than under elevated CO_2 and were higher than the contents under Cd stress (Fig. 3a–d). The combination of elevated CO_2 + Cd stress significantly affected the contents of soluble sugars, free amino acids, soluble phenolic acids, and organic acids in rhizosphere soil (Table 2).

Enzyme activity

Under elevated CO_2 alone, the activity of amylase, cellulase, phenol oxidase, urease, and L-asparaginase increased significantly (Fig. 4a–e). In contrast, the activity of invertase, β-glucosidase, and neutral phosphatase decreased significantly (Fig. 4f–h). With Cd stress only, amylase, cellulase, urease, L-asparaginase, β-glucosidase, and neutral phosphatase activity decreased (Fig. 4e). Phenol oxidase activity increased at lower concentrations of Cd (Cd1 and Cd5) and decreased at Cd10 level (Fig. 4c). Invertase activity decreased significantly at Cd1 level but increased significantly at the higher Cd concentrations (Fig. 4f).

Under elevated CO_2 + Cd stress, the activity of amylase, phenol oxidase, urease, L-asparaginase, β-glucosidase, and neutral phosphatase increased, and the activity of cellulase and invertase decreased relative to Cd stress alone (Fig. 4). The amylase, urease, L-asparaginase, and β-glucosidase activity was greater under elevated CO_2 + Cd stress than under Cd stress only but lower than under elevated CO_2 only (Fig. 4a, d, e, and g). Phenol oxidase and neutral phosphatase activity were greater, and cellulase and invertase activity were lower, under the combined conditions than under either condition alone (Fig. 4). The combination of elevated CO_2 + Cd stress had a significant effect on amylase, phenol oxidase, L-asparaginase, β-glucosidase, cellulase, and neutral phosphatase activities ($p<0.01$, Table 2).

Table 2 Results of two-way ANOVA (*F* value) examining effects of elevated CO_2 and Cd pollution on photosynthesis products levels in wheat seedlings, organic compounds in rhizosphere soil, and rhizosphere soil biological activities of wheat seedlings

		Elevated CO_2	Cd	Elevated CO_2 × Cd
Photosynthesis products	Reducing sugar	(31.50)**	(258.59)**	(143.72)**
	Starch	(36.11)**	(28.50)**	(10.47)**
	Soluble sugar	(127.38)**	(93.66)**	(22.33)**
	Total sugar	(227.56)**	(19.40)*	(11.48)*
	Soluble protein	(106.40)**	(103.85)**	(16.16)**
	Soluble amino acid	(231.50)**	(33.79)*	(22.37)**
Soil properties and organic compounds in the rhizosphere soil	DOC	(147.54)**	(101.55)**	(6.16)**
	pH	(24.26)**	(85.85)**	(0.97) p=0.43, NS
	Soil AN	(68.64)**	(64.40)**	(14.51)**
	Soluble sugars	(219.30)**	(275.82)**	(19.34)**
	Free amino acids	(156.87)**	(550.83)**	(51.03)**
	Soluble phenolic acids	(212.41)**	(372.59)**	(3.50)*
	Organic acids	(155.04)**	(470.84)**	(89.66)**
Enzyme activities	Amylase	(25.29)**	(227.84)**	(31.29)**
	Cellulase	(43.57)**	(21.09)**	(46.58)**
	Phenol oxidase	(28.94)**	(10.99)**	(11.17)**
	Urease	(52.17)**	(156.69)**	(3.22) p=0.05, NS
	L-Asparaginase	(6.27)**	(8.31)*	(11.86)**
	Invertase	(13.26)**	(8.73)**	(1.34) p=0.29, NS
	β-Glucosidase	(247.19)**	(15.82)*	(42.16)**
	Neutral phosphatase	(58.91)**	(90.32)**	(35.82)**
Microbial abundance and FDA activity	Bacteria	(6.75)**	(29.62)**	(14.71)**
	Actinomycetes	(57.44)**	(44.36)**	(223.57)**
	Total microorganism	(6.61)**	(29.28)**	(14.43)**
	Fungi	(29.62)**	(0.68) p=0.42, Ns	(93.69)**
	FDA activity	(20.37)**	(16.44)*	(22.69)**

NS not significant

*p<0.05; **p<0.01

Abundance of culturable microorganisms and FDA hydrolysis activity

Elevated CO_2 was associated with significantly greater abundance of bacteria, actinomycetes, and total microorganisms and with decreased fungi abundance (Fig. 5). Under Cd stress alone, bacteria, fungi, and total microbial abundance decreased, and the abundance of actinomycetes increased (Fig. 5).

Elevated CO_2 significantly stimulated bacteria abundance at low Cd concentrations (Cd1 and Cd5) and significantly decreased bacteria abundance at Cd10 level (Fig. 5). The abundance of actinomycetes, total microorganism, and fungi was significantly higher under elevated CO_2 at Cd1 level and was significantly lower at Cd5 and Cd10 levels. Interactive effects of elevated CO_2 and Cd on the abundance of bacteria, actinomycetes, total microorganisms, and fungi were significant (Table 2, p<0.01).

FDA hydrolysis activity increased under elevated CO_2 alone but decreased under Cd stress alone. Under Cd stress, FDA hydrolysis activity was higher under elevated than under ambient CO_2 (Fig. 5e); the combination of elevated CO_2 + Cd had a significant effect on FDA hydrolysis activity (Table 2, p<0.01).

Discussion

Elevated CO_2 changed concentrations of photosynthetic products under Cd stress

Elevated CO_2 alone significantly increased the levels of reducing sugars, soluble sugars, total sugars, and soluble proteins in wheat seedlings (Fig. 2), consistent with previous studies (Li et al. 2007; Kim and Kang 2011). Accumulation of Cd in plants inhibits photosynthesis by

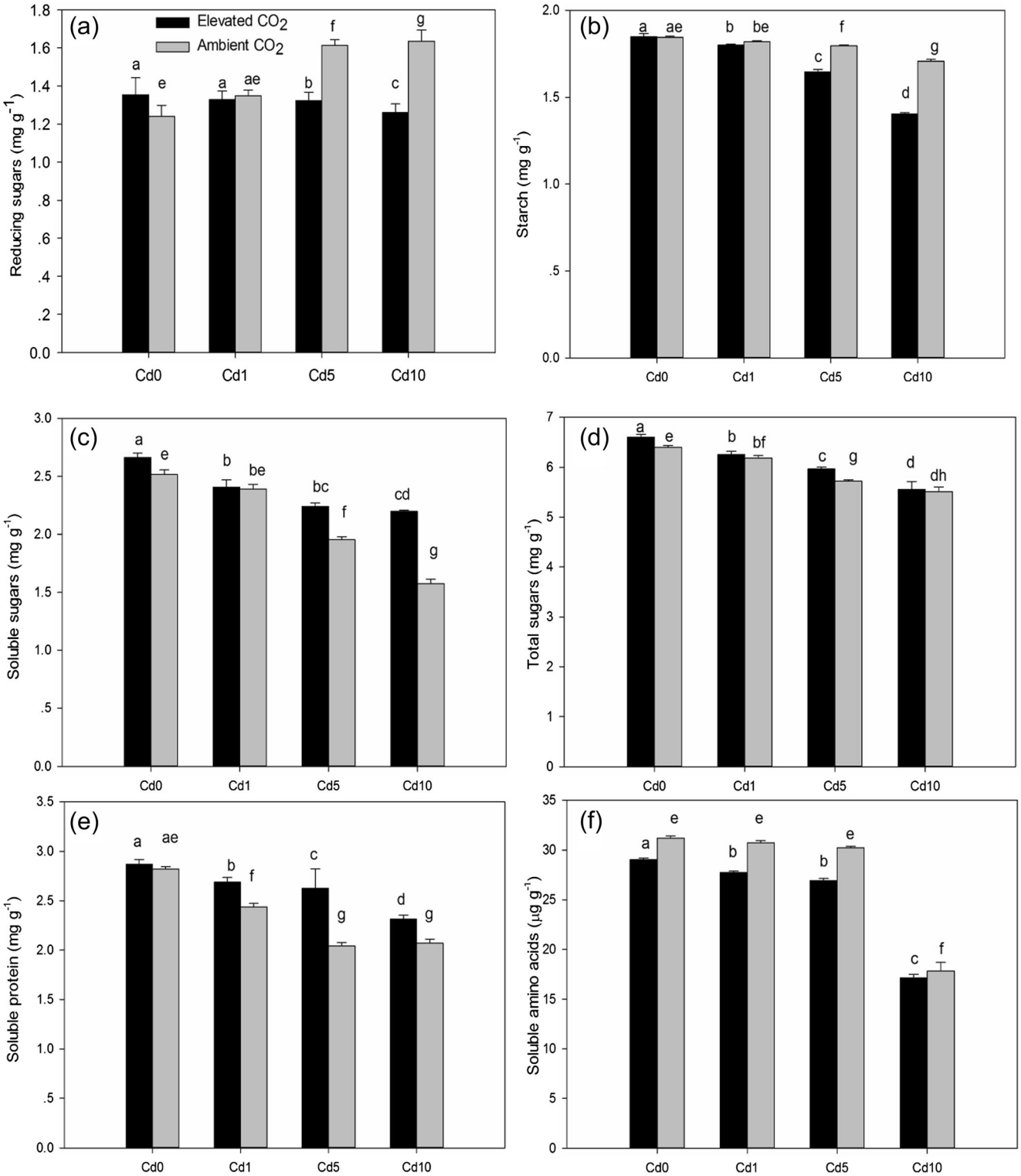


Fig. 2 Photosynthesis products in wheat seedlings Data are means±SE (n=9). **a**–**f** Reducing sugars, starch, soluble sugars, total sugars, soluble protein, and soluble amino acids, respectively; Cd0, Cd1, Cd5, and Cd10 in the figure were 0.31 1.31, 5.31, and 10.31 mg Cd kg^{-1} dry soil, respectively; Different *small letters* in the same group indicates significant difference between elevated CO_2 and ambient CO_2 for the same Cd level at $p<0.05$; *a–d* Significant differences between different Cd levels under elevated CO_2 at $p<0.05$; *e–h* Difference between Cd levels under ambient CO_2 at $p<0.05$

affecting the contents of chlorophyll *a* and *b* (Jia et al. 2010). Thus, with the exception of reducing sugars, the quantities of photosynthetic products decreased under Cd stress in this study.

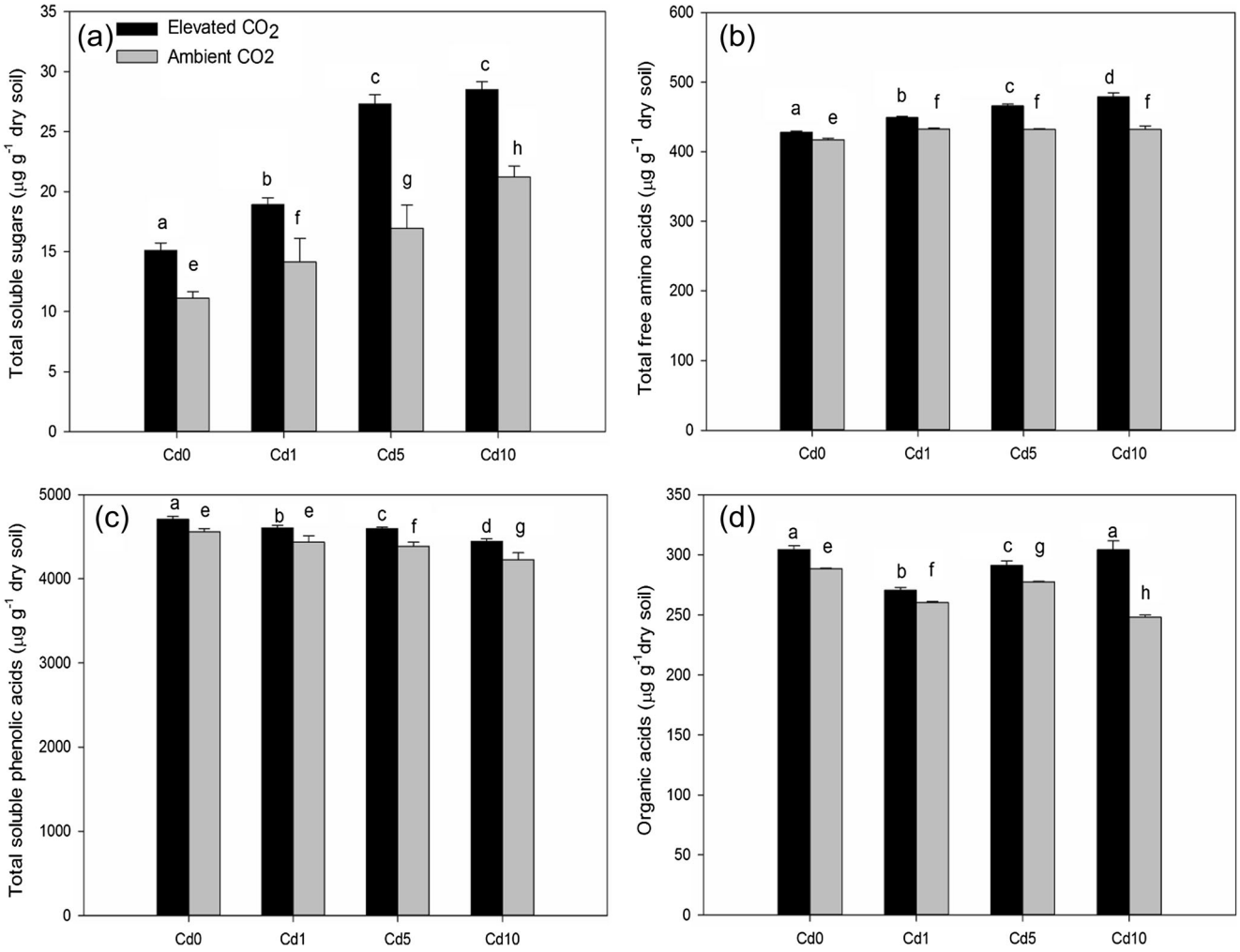
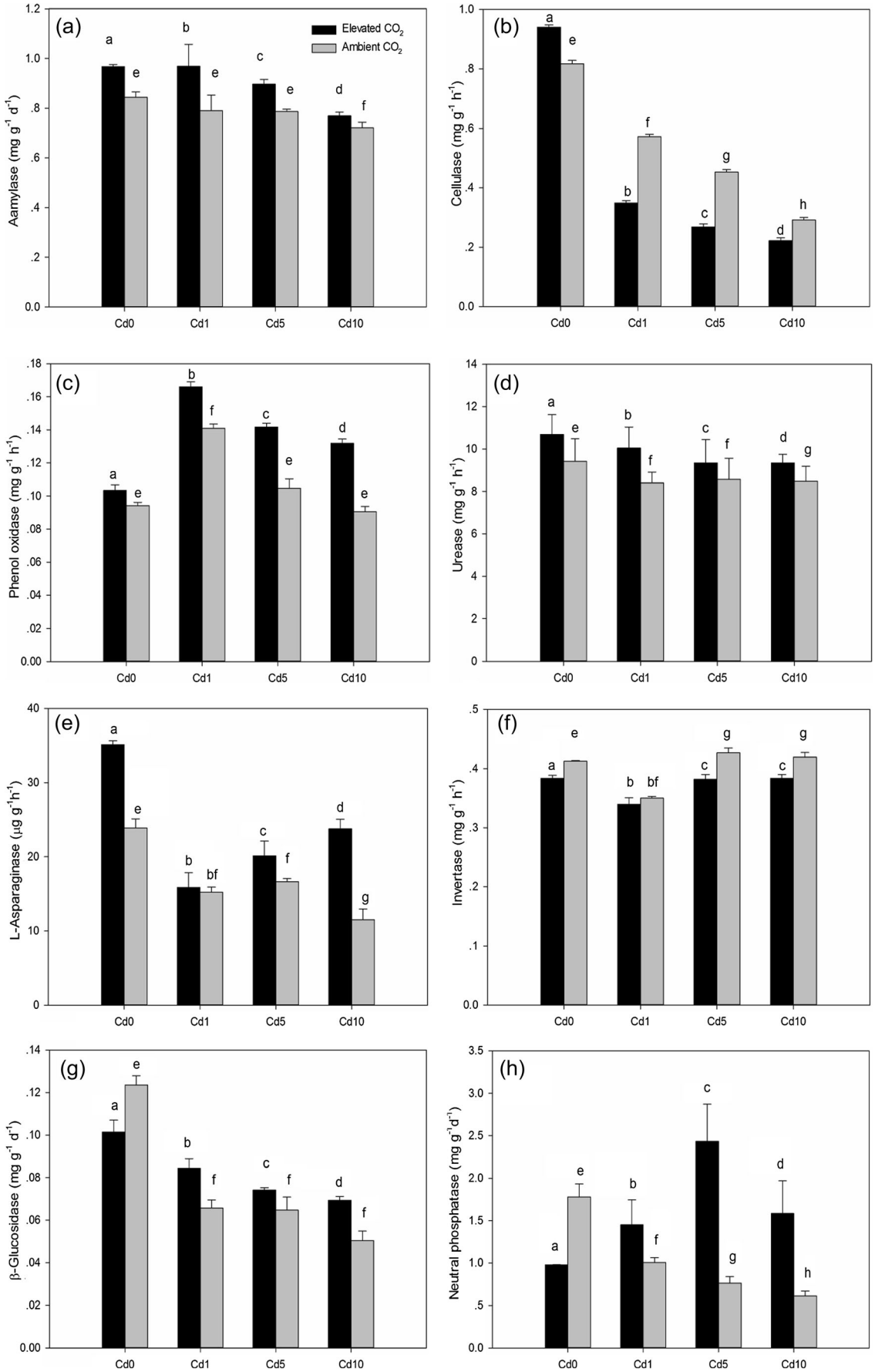


Fig. 3 Organic compounds (μg g^{-1} dry soil) in rhizosphere soil of wheat seedlings. Data are means±SE (n=9). **a**–**d** Total soluble sugars, total free amino acids, total soluble phenolic acids, and organic acids in the rhizosphere soil, respectively; Cd0, Cd1, Cd5, and Cd10 in the figure were 0.31, 1.31, 5.31, and 10.31 mg Cd kg^{-1} dry soil, respectively; Different *small letters* in the same group indicates significant difference between elevated CO_2 and ambient CO_2 for the same Cd level at $p<0.05$; *a–d* Significant differences between different Cd levels under elevated CO_2 at $p<0.01$; *e–h* represent the difference between Cd levels under ambient CO_2 at $p<0.01$

Higher concentrations of soluble sugars, total sugars, and soluble proteins in wheat seedlings under the combined conditions relative to Cd stress alone (Fig. 2) suggested that elevated CO_2 stimulated the biosynthesis of these compounds under Cd stress. However, inhibitory effects of Cd on carbohydrate and protein biosynthesis were observed, where the accumulation of these compounds in wheat seedlings was lower under elevated CO_2 + Cd stress than under elevated CO_2 alone. Lower concentrations of organic compounds under the combined conditions compared to either condition alone indicated that inhibition of biosynthesis by Cd would be greater than the stimulation by elevated CO_2. Gradual decreases in organic compounds with increasing Cd concentration indicated that photosynthesis was increasingly inhibited and that the effect of the combined treatments on photosynthesis was stronger at higher concentrations of Cd.

Decreases in rhizosphere soil pH (Fig. 1b) under elevated CO_2 + Cd stress could be induced by increased exudation of organic acids and other compounds by roots into rhizosphere soil as a result of greater soluble sugar and protein contents in wheat seedlings (Fig. 3). The accumulation of Cd in wheat seedlings would be predicted to increase under elevated CO_2 as a result of lower soil pH and increased DOC in the rhizosphere, and the stimulatory effect of elevated CO_2 on

Fig. 4 Enzymes activities in rhizosphere soil of wheat seedlings Data are means±SE (n=9) **a**–**h** Amylase, cellulase, phenol oxidase, urease, L-asparaginase, invertase, β-glucosidase, and neutral phosphatase, respectively; Cd0, Cd1, Cd5, and Cd10 in the figure were 0.31, 1.31, 5.31, and 10.31 mg Cd kg^{-1} dry soil, respectively; Different *small letters* in the same group indicates significant difference between elevated CO_2 and ambient CO_2 for the same Cd level at $p<0.05$; *a–d* Significant differences between different Cd levels under elevated CO_2 at $p<0.05$; *e–h* Difference between Cd levels under ambient CO_2 at $p<0.05$ ▶


(a)
Elevated CO_2
Ambient CO_2
Aamylase (mg g^{-1} d^{-1})
(b)
Cellulase (mg g^{-1} h^{-1})
(c)
Phenol oxidase (mg g^{-1} h^{-1})
(d)
Urease (mg g^{-1} h^{-1})
(e)
L-Asparaginase (μg g^{-1} h^{-1})
(f)
Invertase (mg g^{-1} h^{-1})
(g)
β-Glucosidase (mg g^{-1} d^{-1})
(h)
Neutral phosphatase (mg g^{-1} d^{-1})
Cd0
Cd1
Cd5
Cd10

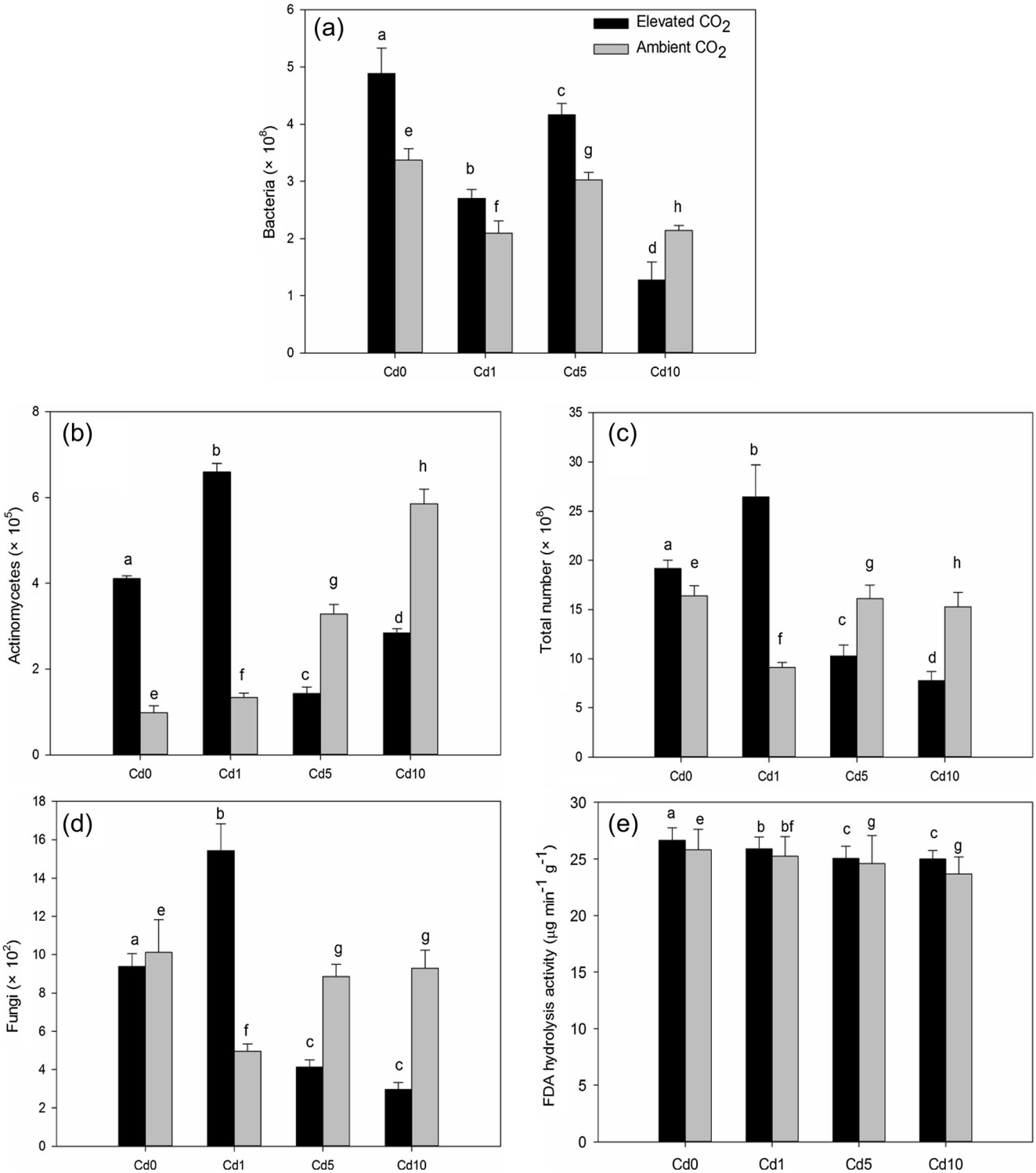


Fig. 5 Microbial number (colony-forming units per gram dry soil) and FDA hydrolysis activity (μg min^{-1} g^{-1}) in rhizosphere soil of wheat seedlings. Data are means±SE (n=9). **a**–**e** Bacteria, actinomycetes, total microorganisms, fungi, and FDA hydrolysis activity, respectively; Cd0, Cd1, Cd5, and Cd10 in the figure were 0.31, 1.31, 5.31, and 10.31 mg Cd kg^{-1} dry soil, respectively; Different *small letters* in the same group indicates significant difference between elevated CO_2 and ambient CO_2 for the same Cd level at $p<0.05$; *a*–*d* indicate significant differences between different Cd levels under elevated CO_2 at $p<0.05$; *e*–*h* represent the difference between Cd levels under ambient CO_2 at $p<0.05$

photosynthesis would be lessened by Cd accumulation, especially at higher soil Cd concentrations.

Soil available nitrogen includes soluble amino acids, amines, and amide compounds. Although soluble amino acids

increased in rhizosphere soil (Fig. 3b), Soil AN, which was mainly related to amine and amide compounds, decreased under elevated CO_2 + Cd stress (Fig. 1c). The lower soil AN under the combined conditions relative to Cd stress alone suggested that elevated CO_2 could not counteract the inhibition by Cd stress (Fig. 1c). In addition, the inhibitory effects of Cd stress on soil AN were strengthened by higher Cd concentrations in the combined treatments. Nitrogen is an essential nutrient and a limiting factor for plant growth in natural and agricultural ecosystems (Ma et al. 2010). Therefore, gradual decreases in soil AN with increasing Cd concentrations in the rhizosphere could lead to decreased photosynthetic production in plants, even under elevated CO_2.

Changes in organic compounds in rhizosphere soil

Organic compounds in soil are derived from root exudates, root residues, microbial metabolism, and aboveground litter (Walker et al. 2003). Aboveground litter could not have contributed organic soil compounds under the short-term growth conditions in this experiment, so soluble sugars, organic acids, and amino acids in rhizosphere soil were mainly derived from root exudates of wheat seedlings. Because a significant proportion of net primary production is allocated to root systems, resulting in large fluxes of organic compounds into the soil (King et al. 2004), the increased quantities of carbohydrates and proteins in wheat seedlings indirectly indicated that organic compounds in rhizosphere soil could be increased by elevated CO_2.

Although soluble sugar and amino acid contents in wheat seedlings decreased under Cd stress (Fig. 2c, f), the concentrations of these compounds in rhizosphere soil increased (Fig. 3a, b), which suggested that organic compounds in root exudates had little relationship to concentrations of photosynthetic products in plants under these conditions. In addition, root exudates can bind metal ions such as Cd^{2+} (Kuzyakov and Raskatov 2008), which probably resulted in decreased soluble phenolic and organic acids in rhizosphere soil under Cd stress (Fig. 3c, d).

The increase in soluble sugar content in rhizosphere soil by elevated CO_2 under Cd stress was associated with increased soluble sugars in wheat seedlings (Fig. 2c), which could result in large fluxes of root exudates into rhizosphere soil (King et al. 2004). In addition, it is likely that increased soluble and total sugars in wheat seedlings under elevated CO_2 + Cd stress (Fig. 2c, d) would provide carbon needed for synthesis of secondary metabolites (Gleadow et al. 1998), thus increasing root exudates such as amino acids, phenolic acids, and organic acids (Fig. 3b–d). The ability of phenolic and organic acids to bind metal ions such as Cd^{2+} might have explained their lower concentrations under elevated CO_2 + Cd than under elevated CO_2 alone (Kuzyakov and Raskatov 2008); increased root exudation under elevated CO_2 could help to counteract Cd stress.

Elevated CO_2 affected biological activity of rhizosphere soil under Cd stress

Dissolved organic carbon includes macromolecules containing acidic groups such as carboxyl and phenolic OH functional groups (Hofrichter and Fakoussa 2001), and these molecules play important roles in the transport, bioavailability, and solubility of heavy metals (Kim and Kang 2011). Phenolic compounds can increase the mobility of metals in soil (Kim and Kang 2011). When metals form complexes with DOC, their mobility and availability for uptake by plant roots increases (Chen and Zhu 2006). Thus, increased DOC under elevated CO_2 + Cd stress might stimulate Cd bioavailability in rhizosphere soil. Soil pH is also an important factor controlling the availability of heavy metals (Tukaj et al. 2007; Li et al. 2013). Thus, increases in DOC content and decreases in soil pH under elevated CO_2 + Cd stress (compared to Cd stress alone) affected soil biological activity (e.g., enzyme activity and microbial abundance) by changing the bioavailability of Cd.

Increased DOC and lower pH in rhizosphere soil would lead to increased Cd bioavailability, which could reduce Cd levels in the rhizosphere as a result of plant uptake (Tukaj et al. 2007; Li et al. 2013). Decreased soil Cd was associated with increased enzyme activity under elevated CO_2 + Cd stress, compared to Cd stress alone. However, the effects of heavy metal pollution on soil enzyme activity are complex. The response of different enzymes to a given pollutant can vary greatly (Fig. 4), and the same enzyme may respond differently to different pollutants (Kim and Kang 2011). The substrate availability affects the synthesis of extracellular enzymes (Larson et al. 2002), so enzyme activity in the wheat rhizosphere should be affected by changes in DOC and soil AN under elevated CO_2 combined with heavy metal pollution.

Chen et al. (2014) found that Cd stress increased fungi abundance in unplanted soils under elevated CO_2, which was in contrast to our results and was probably due to the effects of plants on soil microorganisms in our study. Because elevated CO_2 can affect concentrations of starch, sugar, and nonstructural carbohydrates in various plant species (Katny et al. 2005), the degree to which elevated CO_2 modifies primary production and the chemistry of root exudates should have direct effects on soil microorganisms. The stimulatory effect of elevated CO_2 on bacteria at low soil Cd concentrations was caused by increased availability of substrates in the rhizosphere (Fig. 3). However, direct effects (e.g., stimulation or inhibition) of Cd on microorganisms may also occur under elevated CO_2. Thus, inhibition of rhizosphere bacteria by Cd was greater than their stimulation by organic compounds at higher Cd concentrations (10.31 mg

Cd kg^{-1} dry soil weight) under elevated CO_2, which resulted in decreased bacteria abundance. Several rhizosphere bacterial species produce antifungal molecules (Matthias et al. 1997), which may explain the decreased fungal abundance under elevated CO_2 + 5.31 and 10.31 mg Cd kg^{-1} dry soil weight relative to either elevated CO_2 or Cd stress. The response of culturable microorganisms to elevated CO_2 + Cd stress varied with increasing Cd levels in the rhizosphere. FDA hydrolysis activity is generally used as an indicator of total microbial activity (Yang et al. 2009). In this study, the increase in FDA hydrolysis activity in the combined treatments suggested that stimulation of overall microbial activity by elevated CO_2 could counteract the inhibitory effects of Cd.

Conclusions

In conclusion, the stimulatory effect of elevated CO_2 on photosynthesis would be lessened by Cd accumulation, especially at higher soil Cd concentrations. In addition, the changes in biological activity and organic compounds in rhizosphere soil suggest that elevated CO_2 improved soil fertility and the microecological environment under Cd stress.

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