



Article

Epichloë bromicola Enhances *Elymus dahuricus* Plant Growth and Antioxidant Capacity under Cadmium Stress

Qian Shi ¹, Wayne R. Simpson ² , Yuling Li ^{1,3}, Chengti Xu ^{1,3}, Kejia De ^{1,3} and Xiuzhang Li ^{1,3,*}¹ State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining 810016, China² AgResearch Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand³ Qinghai Academy of Animal and Veterinary Science, Qinghai University, Xining 810016, China

* Correspondence: xiuzhang@qhu.edu.cn

Abstract: *Elymus dahuricus* is an essential plant for ecological restoration in fragile ecological areas and mining area restoration. As lawn grass, it can quickly cover soil and prevent soil erosion, so it is commonly used as a pioneer grass for lawn greening and slope protection. In recent years, with the development of mineral resources, Qinghai–Tibet Plateau soil is facing the threat of heavy metal cadmium (Cd) pollution. *E. dahuricus* can host the filamentous fungus *Epichloë bromicola*. To make better use of the advantages that *Epichloë* bring to host plants to alleviate heavy metal pollution in soil, plant growth and antioxidant capacity effects on *E. bromicola* infected (E+) and uninfected (E−) *E. dahuricus* were determined under Cd stress. During Cd treatment, plant growth was decreased by Cd stress, while E+ plants exhibited equal or better growth compared to E− plants. Cd treatment induces a proline and antioxidant enzyme burst in infected plants, while malondialdehyde (MDA) increases. *E. bromicola* improved plant growth and antioxidant capacity. *E. dahuricus* breeding strategies could use the information here in efforts to improve the performance of *E. dahuricus* in both environmental protection and agronomic contexts.

Keywords: *Epichloë bromicola*; *Elymus dahuricus*; plant growth; antioxidant; interaction; cadmium stress



Citation: Shi, Q.; Simpson, W.R.; Li, Y.; Xu, C.; De, K.; Li, X. *Epichloë bromicola* Enhances *Elymus dahuricus* Plant Growth and Antioxidant Capacity under Cadmium Stress. *Agronomy* **2024**, *14*, 365. <https://doi.org/10.3390/agronomy14020365>

Academic Editor: Małgorzata Szczepanek

Received: 26 December 2023

Revised: 28 January 2024

Accepted: 2 February 2024

Published: 12 February 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The natural environment has been significantly impacted by technological advancements and societal changes in the modern era. Currently, the degradation of soil by metallic compounds is widely acknowledged as a prominent threat to agro-ecosystems worldwide, with direct implications for food safety [1,2]. Toxic metals, especially cadmium (Cd), are widely distributed in the natural environment. Cd ranks among the most significant heavy metal pollutants in soil [3,4]. Cd is harmful to various biochemical and physiological processes in plants, reducing seed germination, early seedling growth, and plant biomass. It causes changes in photosynthesis, relative water content, transpiration rate, stomatal conductance, and electrolyte leakage. Cd activates reactive oxygen species that induce chromosomal aberrations, gene mutations, and DNA damage affecting the cell cycle and cell division. The accumulation of Cd in crops and its potential entry into the food chain pose serious concerns for public health [5]. Even at very low concentrations in the body, Cd poses a significant health hazard and can lead to DNA mutation and damage, potentially resulting in carcinogenesis. Therefore, it has been classified as a human carcinogen (Group I) by the International Agency for Research on Cancer [6]. The Qinghai–Tibet Plateau is an essential area for biodiversity conservation and is rich in mineral resources, with one of the most valuable nonferrous metal deposits in the region. Presently, it is facing the threat of heavy metal Cd pollution following the development of mineral resources [7].

About one-third of C3 grass species are capable of being, or becoming, infected with *Epichloë* [8]. Anamorph-typified *Epichloë* can develop asymptomatic mutualistic interactions that feature seed transmission of the fungus [9]. The enhanced growth of *Epichloë*-

infected plants can be a feature of mutualistic interaction [10,11], along with increased plant nutrient uptake [12], the inhibition of plant pathogen growth [12], and increased fitness through improved responses to biotic [13–16] and abiotic influences [12,17–21]. However, some symbionts can produce metabolites that are harmful to grazing animals, an example being ergovaline, produced by *Epichloë coenophiala* in its host grass, which contributes to the occurrence of “fescue toxicosis” in grazing animals. theT stock ailment “ryegrass staggers” can also present due the presence of *Epichloë* within *Lolium perenne*. These *Epichloë* can produce indole diterpenes (IDTs) [22]. In China, *E. gansuensis* var. *inebrians*-infected *Achnatherum inebrians* produces ergot alkaloids and toxicity in domestic animals [23]. There are many studies examining the benefits, including stress resistance, of grasses hosting infections of *Epichloë*, and the abatement of heavy metal stress features in many of these studies [5]. The *Epichloë* hosted by tall fescue, perennial ryegrass, and drunken horse grass (*Epichloë gansuensis*) play a positive role under Cd stress [24–26]. toT make better use of the advantages that *Epichloë* bring to host plants to alleviate heavy metal pollution in soil, the performance of a large number of host plants under Cd stress needs to be studied.

The *Elymus dahuricus* growing throughout northwest China has excellent genes for disease, pest, and stress resistance and has strong drought and cold resistance, barren resistance, and high nutritional and feeding value [27–29]. It makes a key contribution to degraded grassland restoration and the sustainable development of grassland animal husbandry in northwestern China [28,30] and is also an essential plant for ecological restoration in fragile ecological areas and mining area restoration [31]. As a lawn grass, it can quickly cover soil and prevent soil erosion, so it is commonly used as a pioneer grass for lawn greening and slope protection [32]. *E. bromicola* forms host-specific symbioses with *Elymus* spp. and *E. bromicola* is commonly found in *Elymus* spp. [33–35]. Despite the presence of gene coding for ergot alkaloids, cases of livestock toxicosis are not reported [33]. In addition, there have been no studies on the effect of *E. bromicola* on *E. dahuricus* under Cd stress in the Qinghai–Tibet Plateau. To address this knowledge gap, *E. dahuricus*, both infected (E+) and uninfected (E–) with *Epichloë*, were collected from Qinghai–Tibet Plateau, forming material to study the growth indicators and antioxidant capacity of seedlings to evaluate performance under high Cd levels.

2. Material and Methods

2.1. Plant Material

In September 2020, mature individual plants of *E. dahuricus* with fully ripened seedheads were collected from 3 sites (Table 1) distributed on wild permanent grassland on the Qinghai–Tibet Plateau. Tillers of 20–40 plants were collected at each of the 3 sites. Sampling information, including spatial coordinates and elevation, were recorded, and samples were transported to the laboratory for analysis. Culms were examined using aniline blue following the staining method described by Cheplick [36].

Table 1. *E. dahuricus* collection data and infection rate.

Site	Location	Elevation (m)	Longitude	Latitude	Endophyte Infection Rate (%)
1	Qinghai Tongren	2438	102°05′07″	35°57′58″	50.0%
2	Qinghai Zeku	2876	101°56′23″	35°33′24″	33.3%
3	Qinghai Gonghe	3230	100°52′18″	36°20′17″	25.0%

Seeds sourced from the 3 sites were planted February 2021, and the emerged seedlings were examined for endophyte presence. Seeds were sown at 1 cm depth, 1600 with seed per site, and germinated in a glasshouse set to maintain 18–24 °C and 10 h light/14 h dark. *Epichloë* infection status was determined one month after sowing. Plants were separated into two populations, one confirmed *Epichloë* infected and the other uninfected [36].

2.2. Experimental Design

Infected and uninfected seedlings were potted to 35 cm × 32 cm pots using a 3:1 mix of sterilized soil and vermiculite. Pots were planted with 6 seedlings and received equal initial water treatment. CdCl₂ solutions with concentrations of 0, 40, 90, 140, and 190 µmol/L were prepared. CdCl₂ treatments were applied as 400 mL of each solution to pots at 3-day intervals, and control pots received 400 mL of distilled water. Treatments were applied over 5 replicates. Temperature was maintained at 25 ± 2 °C, and humidity was maintained at 42 ± 5%.

2.3. Measurements

About 2.0 g of leaf tissue per plant was harvested for malondialdehyde (MDA), proline content antioxidant enzyme analysis following 30 days of MDA CdCl₂ treatment. Each treatment measured 5 plants, with each plant serving as one replicate.

2.3.1. MDA Content

MDA was quantified following the protocol established by Esterbauer and Cheeseman [37] to estimate lipid peroxidation. A total of 0.5 g of sample was mixed with trichloroacetic acid solution (5 mL) to produce a 5% solution, which was subjected to centrifugation at a speed of 12,000 r/min for a duration of 25 min. A total of 2 mL of thiobarbituric acid (0.67%) was added to supernatant (2 mL) and maintained at 100 °C for 30 min. The precipitate was separated through centrifugation after the cooling process. Absorbance measurements were made at 450, 535, and 600 nm, and a blank solution was measured as a control. MDA content was determined using the following:

$$C/\mu\text{mol/L} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450} \quad (1)$$

2.3.2. Proline Content

The determination of proline content was conducted using a modified method from Li [38]. Leaves were freeze-dried, 0.5 g were finely powdered, and 5 mL of sulphosalicylic acid (3%) was added and maintained at 100 °C for 10 min. A total of 2 mL of supernatant was combined with 2 mL each of glacial acetic acid and acidic ninhydrin solution (2.5% w/v) and heated for 25 min at 100 °C. A total of 4 mL of toluene was added to the cooled mixture, and the absorbance was measured at a wavelength of 520 nm to obtain the µg/g dry matter concentration.

2.3.3. Activity of Antioxidant Enzymes

A total of 0.5 g of leaf tissue was dissolved in a test tube, and 5 mL 50 mmol/L of sodium phosphate buffer was added into the test tube (pH 7 when catalase (CAT) was measured; superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) were determined at pH 7.8), along with 1% (w/v) of polyvinylpyrrolidone and 0.1 mmol/L Na₂EDTA. The homogenate was filtered with four layers of gauze and then centrifuged at 15,000 r/min for 20 min. The supernatant was absorbed and stored in a refrigerator at 4 °C for the determination of antioxidant enzyme activity. CAT activity was determined using Clairborne's method [39]. The enzyme solution (0.2 mL), phosphoric acid buffer solution (pH 7.8, 1.5 mL), and distilled water (1 mL) were added to the test tube. After preheating at 25 °C, hydrogen peroxide (0.3 mL of 0.1 mol/L) was added and quickly poured into a quartz colorimetric cup. Absorbance was measured at 240 nm. SOD activity was determined using a spectrophotometer, with reference to Beryer and Fridovich's method [40]. The inhibition

of SOD on the photochemical reduction of nitrogen blue tetrazole (NBT) was determined at 560 nm. One unit of SOD activity is defined as the amount of enzyme required to inhibit 50% of the photochemical reduction of NBT. POD activity was determined using guaiacol as an electron donor. According to Chance and Maehly et al. (1955) [41], 2.5 mL of 0.1 mol/L guaiacol solution and 1 mL of enzyme solution were added into the test tube. After 10 min, 0.5 mL of 1 mol/L hydrogen peroxide solution was quickly added, and the solution was put into the water bath to keep the temperature at about 30 °C for a reaction time of 15 min. The test tube was then removed, and after adding 2.5 mL of distilled water, it was shaken well before measuring the absorbance at 470 nm. APX was determined using a spectrophotometer and the method by Gupta et al. (1993) [42]. A total of 2.6 mL of reaction buffer (containing 0.1 mmol/L EDTA and 0.5 mmol/L ascorbic acid) and 0.1 mL of enzyme extract were added to a test tube, followed by the addition of 0.3 mL of 2 mmol/L H₂O₂ to initiate the enzymatic reaction. The mixture was immediately mixed, and the timing was started. The absorbance at 290 nm of the reaction system was recorded starting from 15 s after initiation.

2.3.4. Plant Growth

After 30 days of growth, plant height and tiller number were recorded. Whole plants were then carefully removed from pots, washed with distilled water, and dried on filter paper. Dry weight was obtained following oven-drying the tissue at 60 °C until a constant weight was reached. The dry aboveground and underground parts from each treatment were weighed separately to determine the total dry matter per plant.

2.4. Statistical Analyses

Data analyses were performed using SPSS (Version 24.0, Chicago, IL, USA) for Windows. One-way analysis of variance (ANOVA) was employed to analyze the effect of endophyte infection on all parameters measured. The interactions among Cd and the sampling site and endophyte infection on growth parameters, membrane lipid peroxidation, and activity of antioxidant enzymes were determined using three-way ANOVA. Statistical significance was defined at the 95% confidence level. PCA (Principal Component pcanalysis) was conducted using Canoco (ter Braak and Šmilauer 2002) to analyze the relationship among plant growth, antioxidant capacity, Cd concentration, *E. bromicola*, and sites. Means are reported with their standard errors.

3. Results

3.1. *E. dahuricus* Growth Parameters

Growth parameters are a direct reflection of plant response to stress [43]. Cd negatively affected plant growth parameters. The growth of the plants gradually weakened after the start of the Cd treatment (Figures 1–4). Therefore, Cd stress decreased plant height (Figure 1), tiller number (Figure 2), aboveground biomass (Figure 3), and underground biomass (Figure 4). In contrast, untreated control plants showed the best growth (Figures 1–4). E+ plants generally grew equal to, or better than, E− plants in the Cd treatment (Figures 1–4 and Table 2). With an increase in Cd concentration, E+ plant height, aboveground biomass, and belowground biomass were significantly higher than those of E− plants ($p < 0.05$) (Figures 1, 3 and 4 and Table 2). The site had no significant effect on the aboveground biomass but had a significant effect on the other three growth parameters ($p < 0.05$). There was also a significant interaction between the site and Cd concentration ($p < 0.05$), and the interaction between Cd concentration and endophytic fungi was weaker than that between Cd concentration and sampling site (Table 2).

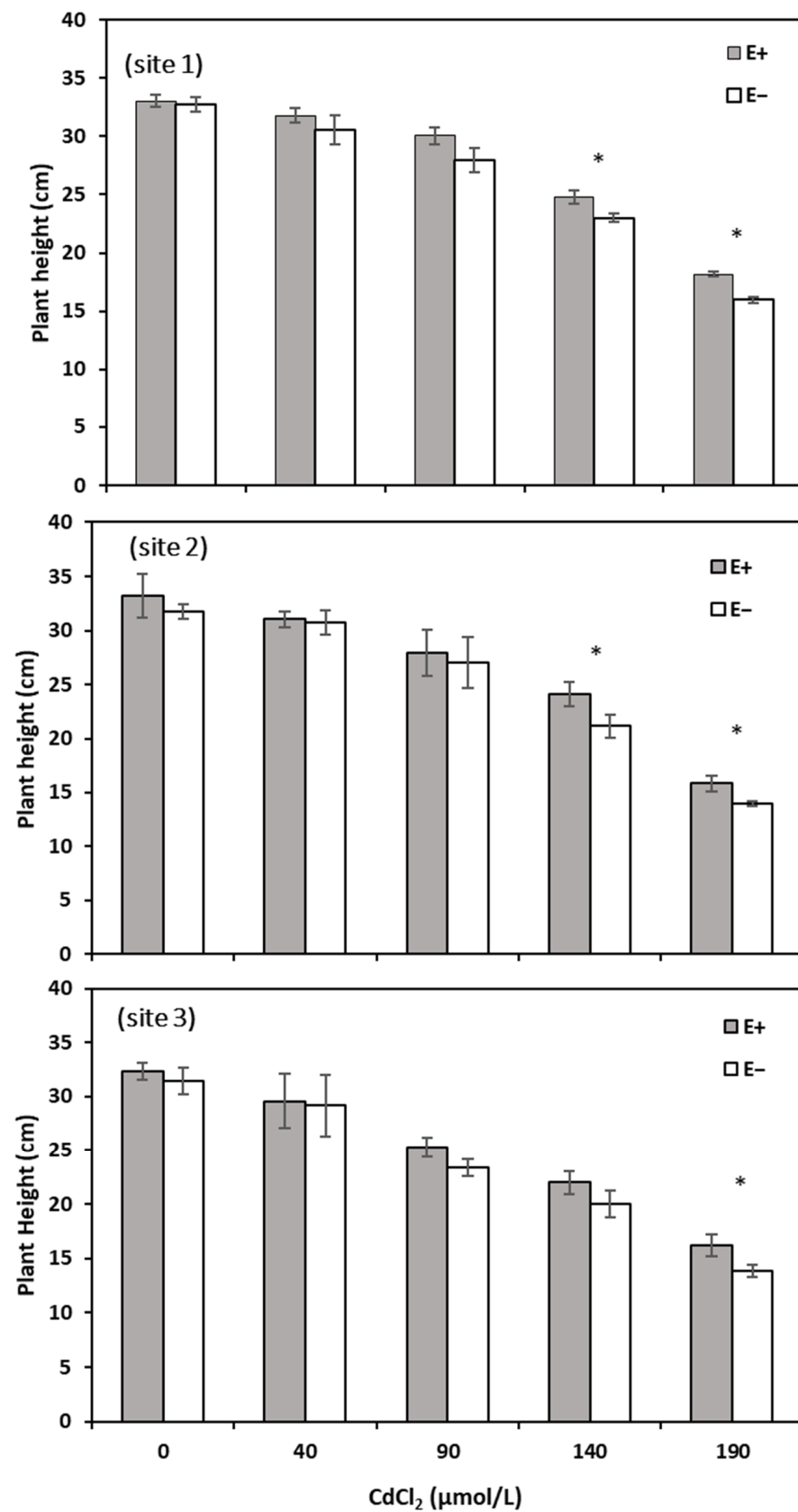


Figure 1. Plant height of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe (Site 3). * indicated significant difference between E+ and E- at 0.05 level.

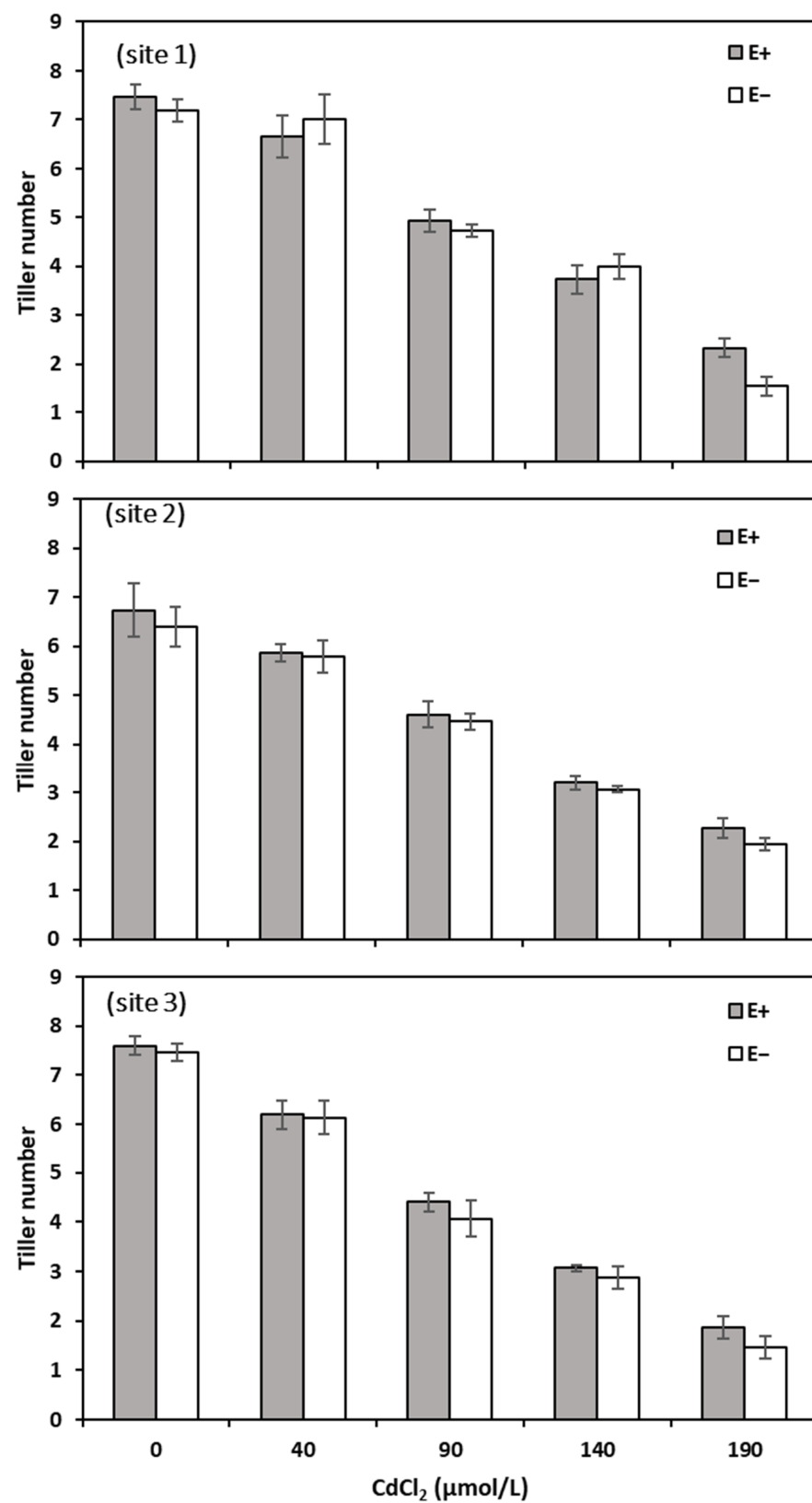


Figure 2. Tiller number of *E. dahuricus* infected with *E. bromicola* grown under different CdCl₂ treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe.

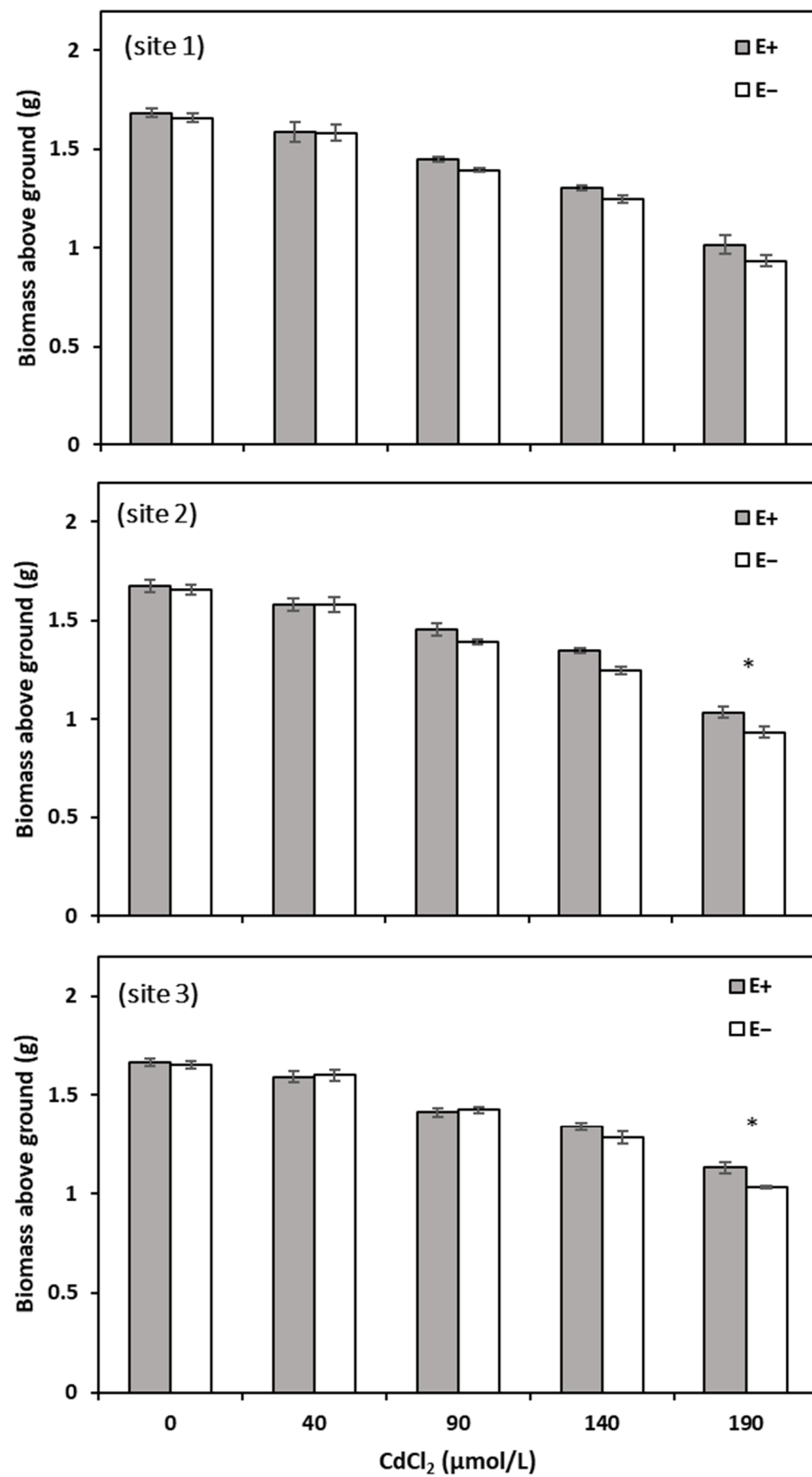


Figure 3. Biomass above ground of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at 0.05 level.

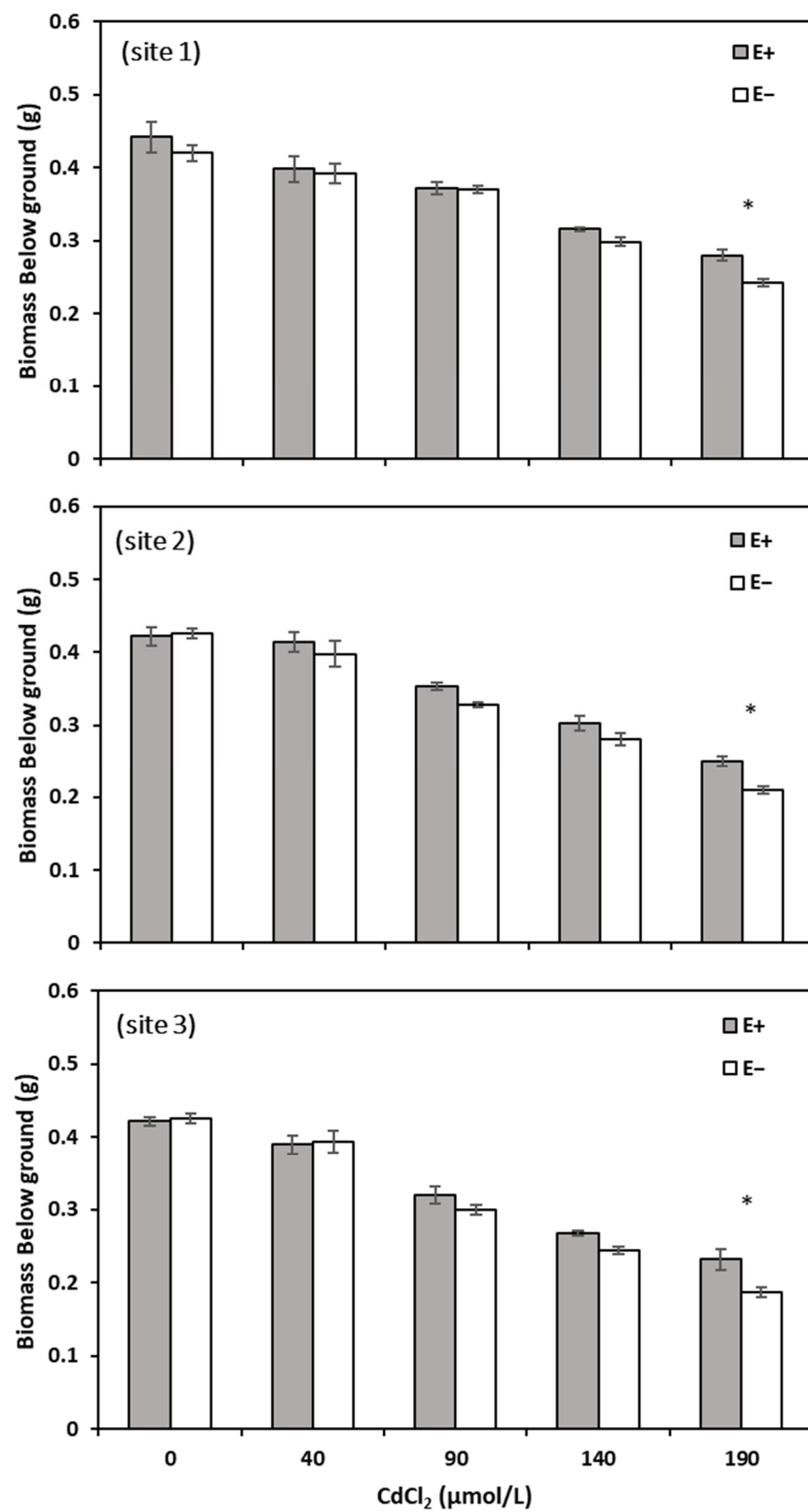


Figure 4. Biomass below ground of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at 0.05 level.

Table 2. Results of multivariate variance analysis of Cd concentration (C), endophyte (E), and site (S) on *E. dahuricus* biomass. The significant level was 0.05. Data with significant differences are marked in bold.

	df	Plant Height		Tiller Number		Biomass above Ground		Biomass below Ground	
		F-Value	P	F-Value	P	F-Value	P	F-Value	P
C	4	610.32	0.000	370.61	0.000	574.58	0.000	359.80	0.000
E	1	38.54	0.000	3.57	0.061	12.85	0.000	22.55	0.000
S	2	35.00	0.000	10.94	0.000	2.66	0.074	28.85	0.000
C × E	4	1.80	0.134	1.03	0.397	2.54	0.043	3.05	0.020
C × S	8	3.13	0.003	3.29	0.002	2.63	0.011	3.98	0.000
E × S	2	0.01	0.999	0.08	0.926	0.65	0.522	0.10	0.904
C × E × S	8	0.40	0.918	0.32	0.956	0.33	0.953	0.57	0.799

3.2. *E. dahuricus* Membrane Lipid Peroxidation

The level of membrane lipid peroxidation indicates the extent of Cd stress in plants [5]. Cd positively affected the MDA and proline contents of the plant. The contents of MDA and proline gradually increased after the start of the Cd treatment. When the concentration of Cd was 140 µmol/L and 190 µmol/L, the difference of MDA and proline between E+ and E− plants was significant (Figures 5 and 6). Cd concentration, sampling sites, endophytic fungi and their interactions had significant effects on membrane lipid peroxidation (Table 3).

Table 3. Results of multivariate variance analysis of Cd concentration (C), endophyte (E), and site (S) on MDA and proline content. The significant level was 0.05. Data with significant differences are marked in bold.

	df	MDA		Proline	
		F-Value	P	F-Value	P
C	4	1235.72	0.000	30159.36	0.000
E	1	110.53	0.000	3072.03	0.000
S	2	170.57	0.000	24.43	0.000
C × E	4	6.46	0.000	1100.54	0.000
C × S	8	24.58	0.000	9.63	0.000
E × S	2	4.16	0.018	29.47	0.000
C × E × S	8	4.09	0.000	9.22	0.000

3.3. *E. dahuricus* Activity of Antioxidant Enzymes

protectiveP enzymes can protect plants against Cd stress damage [5]. The effect of Cd on antioxidant activity was consistent with the effect of Cd on proline and MDA contents. With an increase in Cd concentration, the activity of antioxidant enzymes generally showed a tendency that E+ was higher than E−, and the difference was significant under the Cd concentration of 140 µmol/L and 190 µmol/L (Figures 7–10). Cd concentration, sampling points, endophytic fungi, and their interactions had significant effects on SOD, POD, and CAT activity. The interaction between endophytic fungi and sampling site had no significant effect on APX activity, but other factors and interactions had significant effect on APX activity (Table 4).

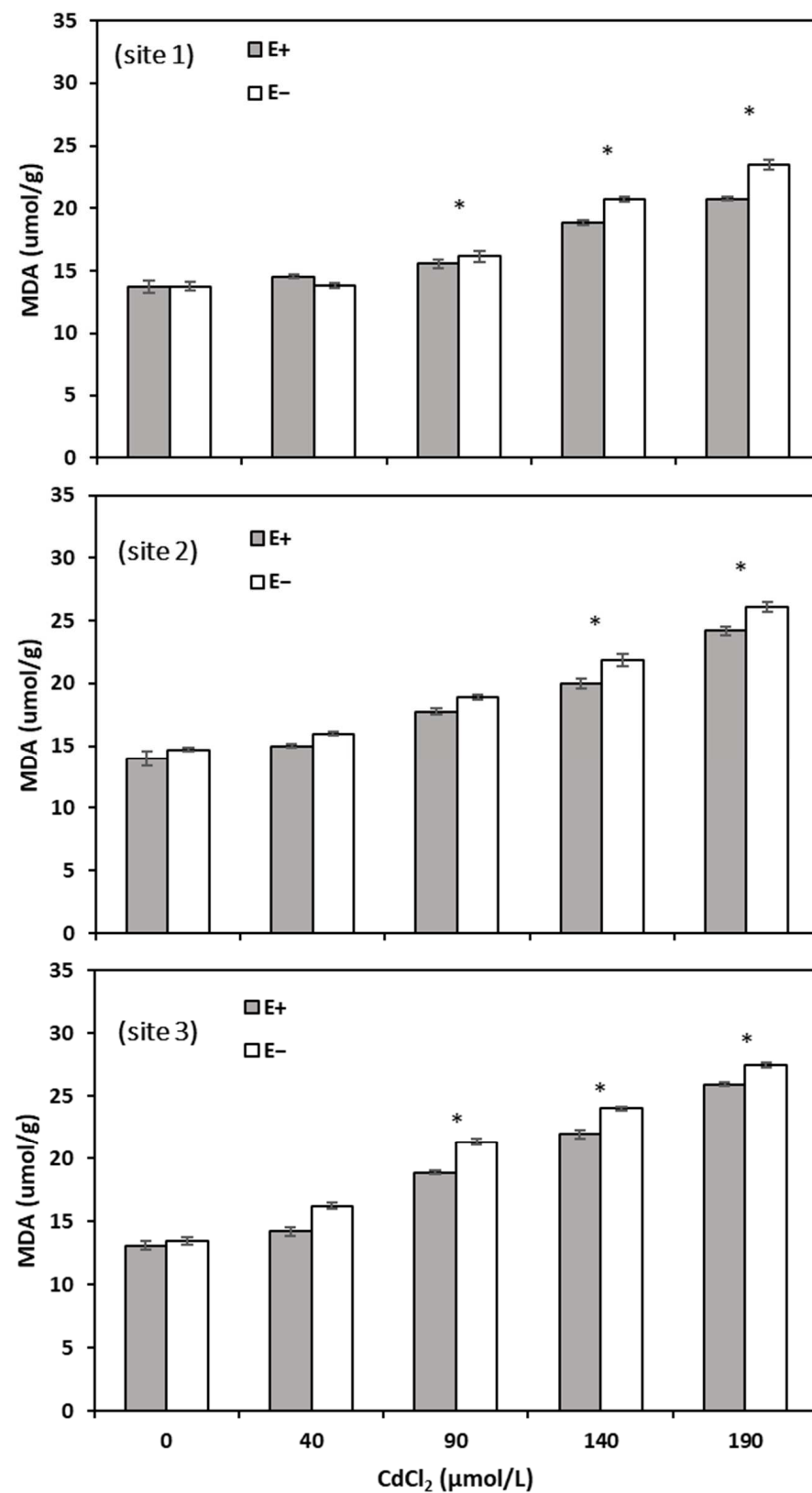


Figure 5. MDA content of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at 0.05 level.

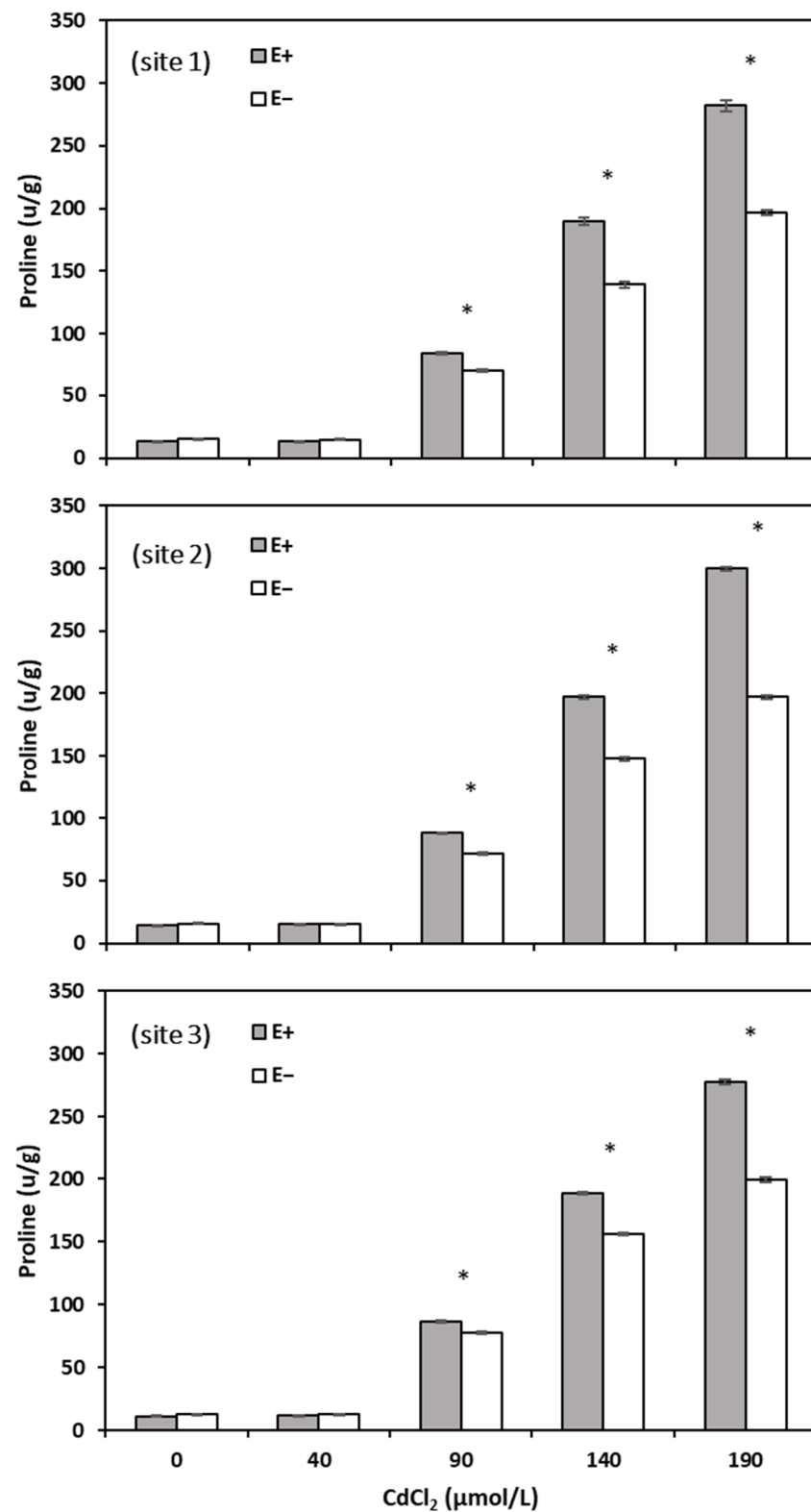


Figure 6. Proline content of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at 0.05 level.

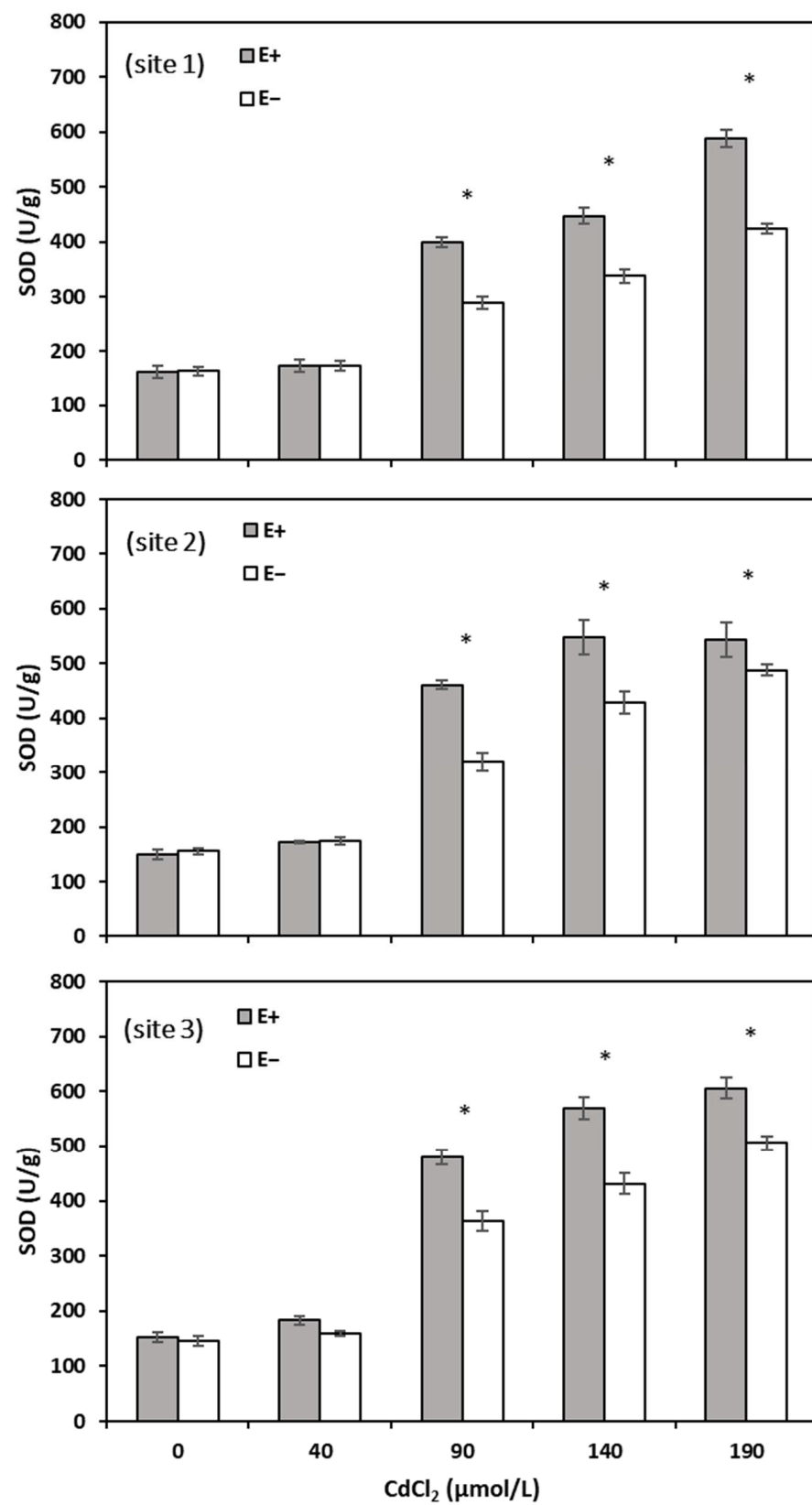


Figure 7. SOD activity of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at the 0.05 level.

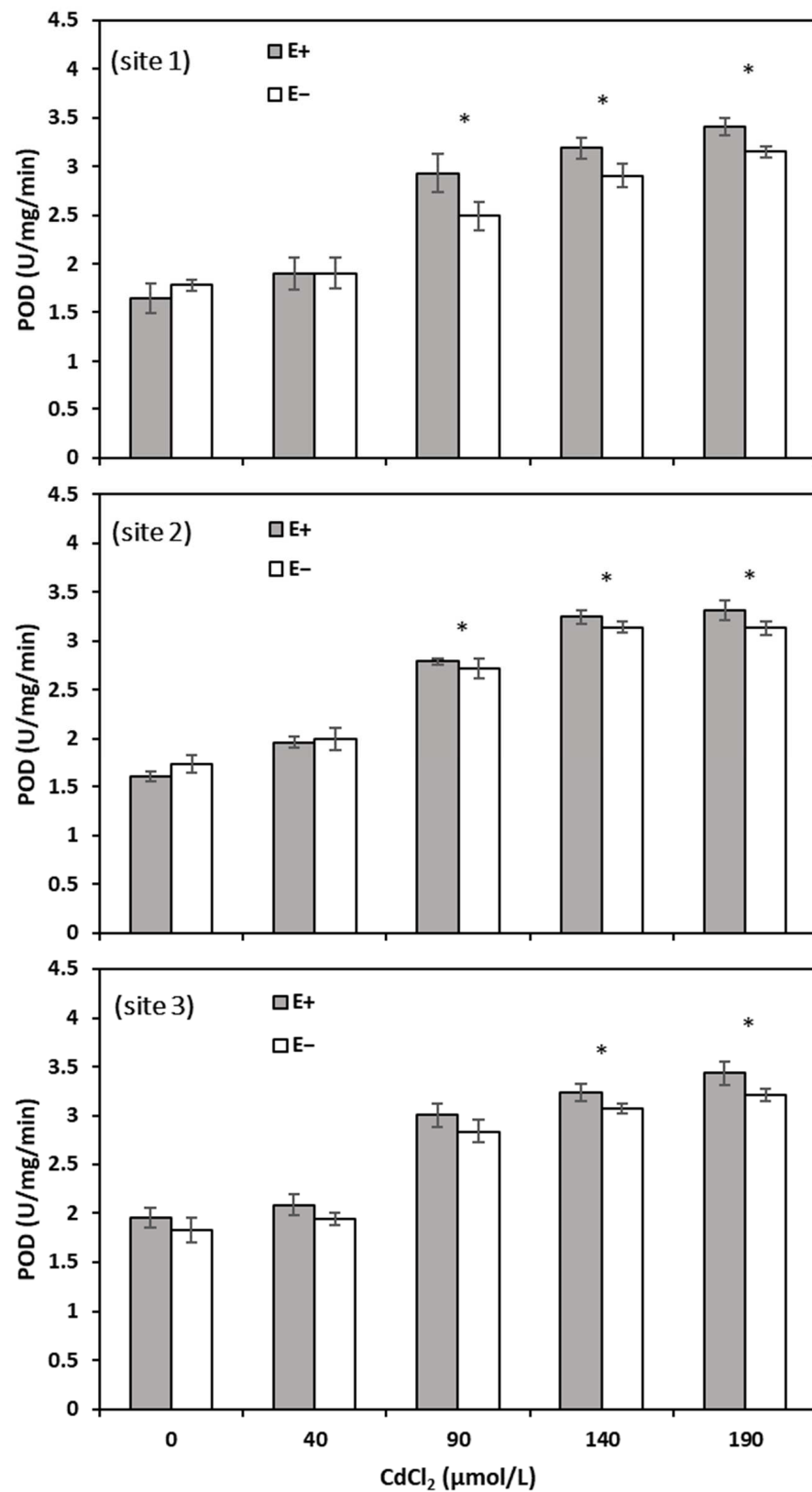


Figure 8. POD activity of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at 0.05 level.

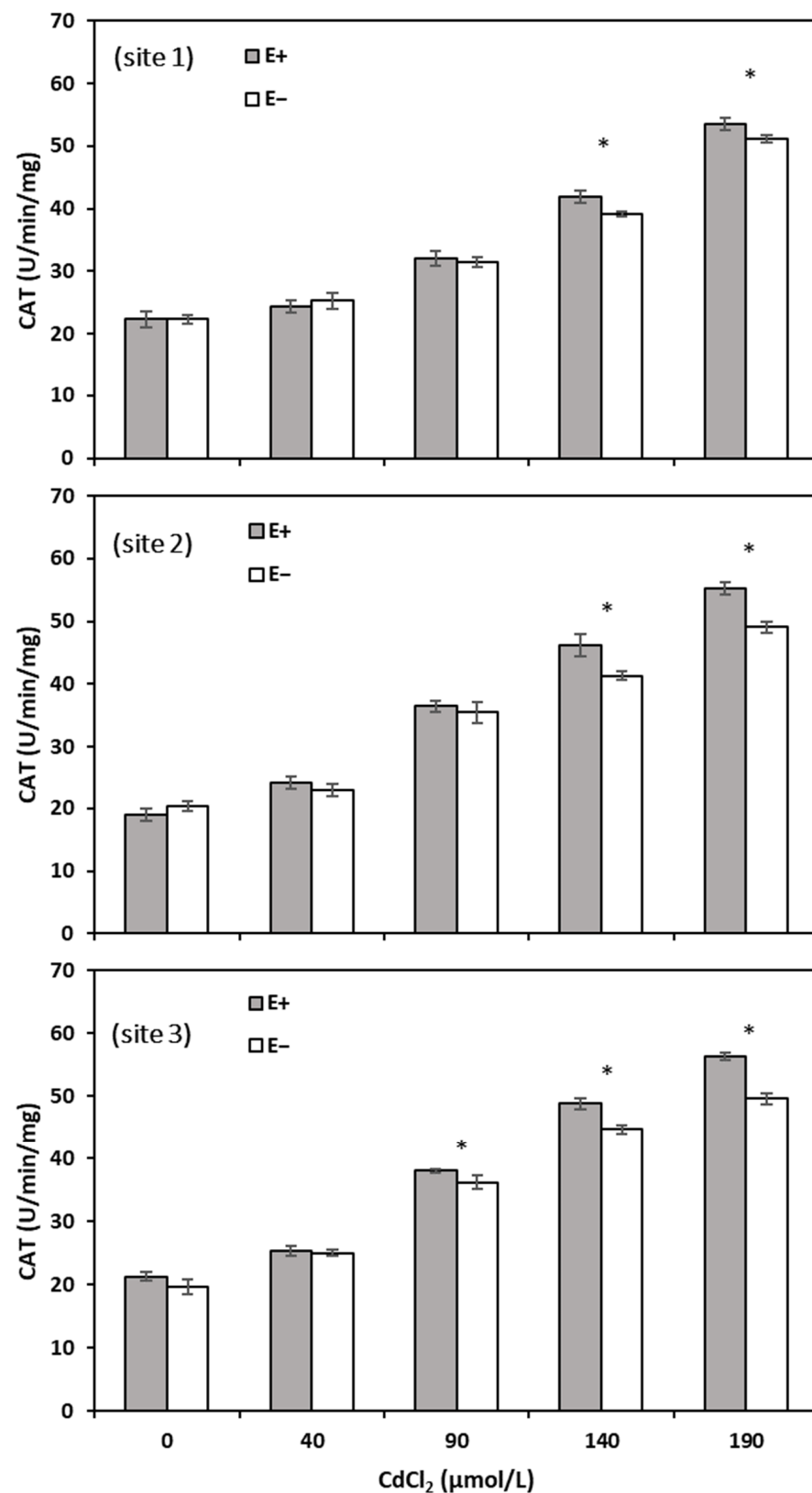


Figure 9. CAT activity of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at 0.05 level.

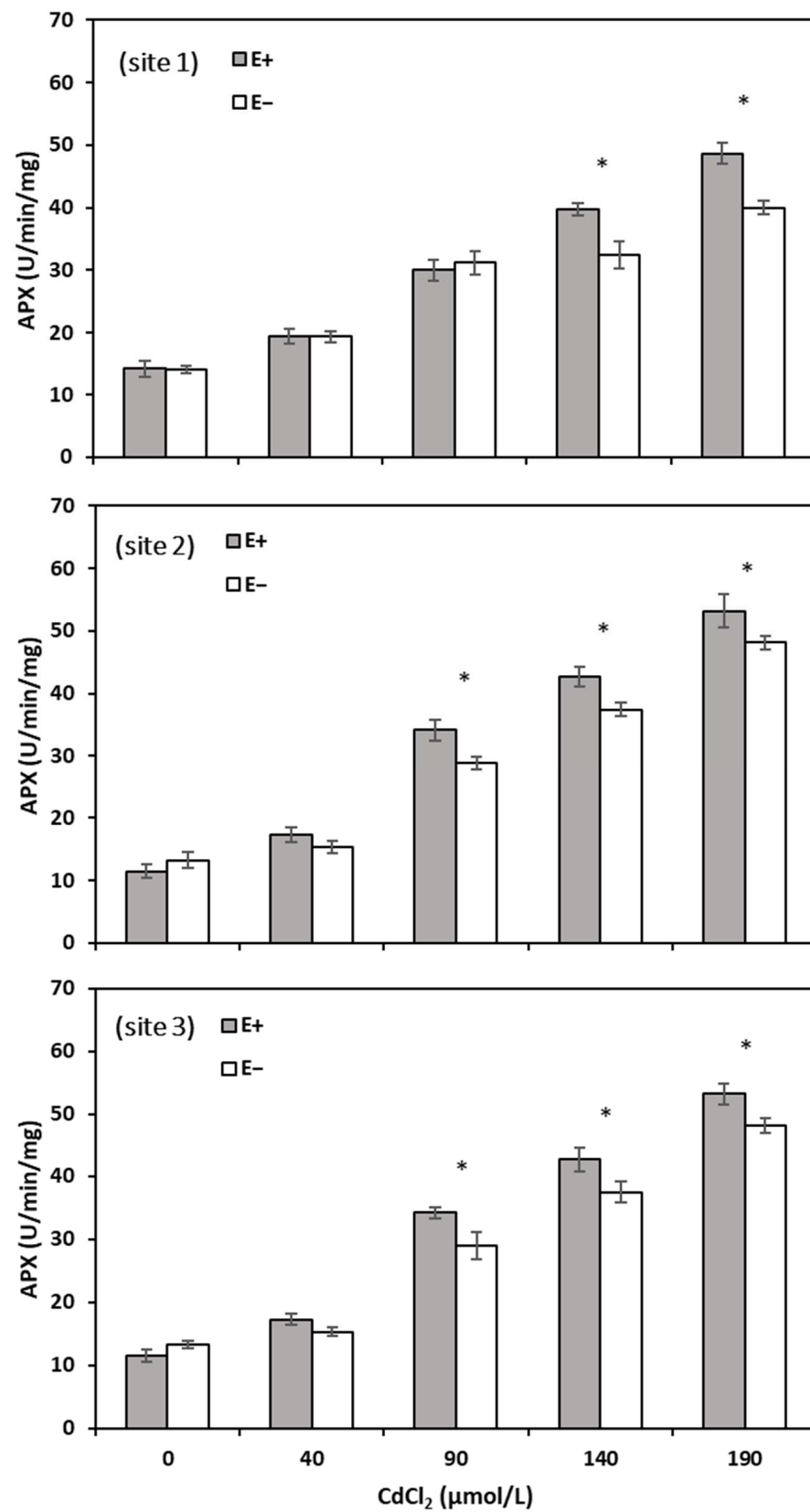


Figure 10. APX activity of *E. dahuricus* infected with *E. bromicola* grown under different CdCl_2 treatments. Seeds were collected from Qinghai Tongren (Site 1), Qinghai Zeku (Site 2), and Qinghai Gonghe. * indicated significant difference between E+ and E- at 0.05 level.

Table 4. Results of multivariate variance analysis of Cd concentration (C), endophyte (E), and site (S) on antioxidant enzyme activity. The significant level was 0.05. Data with significant differences are marked in bold.

	df	SOD		POD		CAT		APX	
		F-Value	P	F-Value	P	F-Value	P	F-Value	P
C	4	4058.84	0.000	1256.79	0.000	5341.59	0.000	2404.36	0.000
E	1	924.71	0.000	51.19	0.000	167.26	0.000	121.47	0.000
S	2	117.61	0.000	21.12	0.000	64.68	0.000	6.82	0.002
C × E	4	141.48	0.000	9.89	0.000	39.80	0.000	22.83	0.000
C × S	8	39.56	0.000	3.26	0.002	37.58	0.000	21.21	0.000
E × S	2	5.16	0.007	5.98	0.003	13.47	0.000	0.21	0.811
C × E × S	8	9.85	0.000	2.73	0.009	3.56	0.001	7.20	0.000

3.4. Relationship among Cd Concentration, *E. bromicola*, Site, Plant Growth, and Antioxidant Capacity

Generally, attention should be paid to the effects of Cd, *E. bromicola*, and sites on the growth and antioxidant capacity of *E. dahuricus*. The first and second axes are 97.4% and 2.1%, respectively (Figure 11). Cd stress had the strongest effect on *E. dahuricus* growth and antioxidant capacity. It was negatively associated with plant growth and positively associated with antioxidant capacity. *E. bromicola* mainly promoted plant growth and antioxidant capacity by enhancing plant aboveground biomass, plant height, and the activity of SOD and POD. Generally, the influence of sample sites on antioxidant enzyme activity was stronger (negative effect) than plant growth.

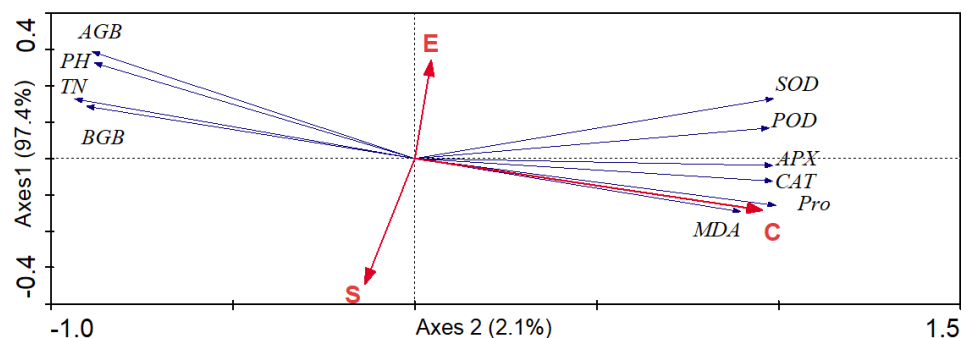


Figure 11. The result of PCA analysis among Cd concentration, *Epichloë*, site, plant growth, and antioxidant capacity. Note: AGB indicated aboveground biomass; PH indicated plant height; TN indicated tiller number; BGB indicated belowground biomass; S indicated site; E indicated *Epichloë bromicola*; C indicated Cd concentration; Pro indicated proline.

4. Discussion

Under Cd stress, plant growth and development are affected by the adaptability and response to this stress [5]. Our experimental results show that *E. bromicola* can improve the resistance of host plants to Cd stress, E+ plants have higher plant height and biomass, especially under high Cd concentration. Similarly, Ren et al. (2006) found that endophyte infection enhanced *L. perenne* tillering ability under conditions of Cd stress [25].

When Bacon et al. (1977) found that alkaloids produced by fungal endophytes in tall fescue can lead to livestock toxin [44], defensive mutualism had been the predominant framework for studies on endophyte–grass symbiosis [45–50]. Defensive mutualism involves intricate signaling and chemical communication between endophyte and host cells, such as reactive oxygen species and antioxidants, as well as between plants, herbivores, and their natural enemies through volatile organic compounds, salicylic acid, and jasmonic acid pathways. This reveals a significantly more complex network. The significance of reactive oxygen species (ROS) antioxidants in endophyte–grass symbiosis was emphasized

by Hamilton et al. (2012) [51]. Lipid peroxidation is a marker of oxidative stress [52], and the MDA content in plants increases in the presence of Cd stress, indicating oxidative stress [53,54]. Cd treatment leads to an increase in MDA content in various plant species. Cd-induced lipid peroxidation impairs membrane structures, which play a crucial role in maintaining plant metabolism. [5]. Proline is a nonessential amino acid [55] that is biosynthesized in chloroplasts and plant cell cytoplasm [5,56]. Plants may synthesize proline in either the absence or presence of abiotic stress (e.g., metal exposure) [57]. It has been reported that in Cd exposure, proline content increases in different plant species. Proline is an important metabolite for plant adaptation, protection, and tolerance to Cd stress. The accumulation of proline in plants is recognized as a strategy counteracting Cd stress by adjusting osmotic potential, the stabilization of membrane structures [5,58,59], and the reduction of oxidative stress [60]. Cd stress in plants also results in drastic changes in enzyme activity [61] due to oxidative stress in the plant cells [62]. Antioxidant enzymes can remove intracellular reactive oxygen species to reduce the damage of Cd to plants [63]. Our results support these conclusions and suggest that pronounced Cd treatment induces a burst of proline and antioxidant enzyme activity in E+ plants, as well as significant increases in MDA levels in E− plants. These findings also indicate that endophyte infection affects the lipid peroxidation and oxidative balance of the host plant. Consistent with our results, Song et al. (2015) found that waterlogging induced an increase in proline production and antioxidant enzyme activity, particularly in E+ plants, and had lower MDA content [18]. In Zhang et al. (2010) study, the proline content of *Achnatherum inebrians* seedlings increased, while the MDA content decreased with increasing Cd concentration [14]. The oxidative balance is likely to play a pivotal role in conferring the resistance of the endophyte–plant symbiont against a wide array of environmental stresses [51,63]. Additionally, there is a negative correlation between biomass and MDA and proline, as well as antioxidant enzyme activity. These results indicated that an increase in MDA and proline content and the enhancement of antioxidant enzyme activity were not conducive to the growth of the plant. However, an increase in MDA and proline content occurred with an increase in the Cd concentration. This result also supported the conclusion that an increase in Cd concentration is not conducive to the growth of calcareous grass. The results in Figure 11 further showed that *E. bromicola* can promote the growth of and increase the antioxidant capacity of the *E. dahuricus*.

Previous research has shown that the direction and strength of endophyte–host plant interactions depend on plant and endophyte genotype, as well as environmental conditions [64]. In this study, the results showed that, in addition to *E. bromicola* and Cd concentration, the sites were also an important factor in *E. dahuricus* growth and antioxidant enzyme activity under Cd stress. The effect of the sites on antioxidant capacity aspects was stronger. This may be due to host genotype variation because the wild *E. dahuricus* used in this study were collected from wild grasslands on the Qinghai–Tibet Plateau. Tian et al. (2018) [65] reported that natural and wild forage species have great genetic diversity and have not undergone domestication, and this diversity of host plants may have induced different physiological and biochemical response. Our results support this view. The Qinghai–Tibet Plateau climate is harsh, extreme, and changeable [66]. Different geographical coordinates determine the climate of plant growth areas. The adaptation mechanism and survival strategy of plants in this area are the comprehensive effects of reactive oxygen species scavenging, osmosis regulation, and photosynthetic capacity [67]. Therefore, in our study, prevailing climatic factors at different sampling sites may explain the effects of the site on plant antioxidant capacity and growth.

A great deal of research has been conducted examining the effects of *Epichloë* endophyte infection on host abiotic stress responses. Over 40 years of study on the benefits of symbiotic *Epichloë* fungal endophytes for host grasses, investigations have focused on the major agricultural species, tall fescue and perennial ryegrass [68]. However, many other grass species remain to be evaluated for the effects of *Epichloë* endophytes on abiotic stress. In our study, it was confirmed that *E. bromicola* has an important ecological

function for improving *E. dahuricus* seedling resistance to Cd stress. Before this, Zhang et al. (2012) [30], confirmed that the *Epichloë* endophyte can improve the *E. dahuricus* seed germination rate under Cd stress. Therefore, we propose that the higher tolerance of E+ host plants to Cd stresses due to the presence of *Epichloë* endophytes should be acknowledged in *E. dahuricus* breeding strategies on the Qinghai–Tibet Plateau.

5. Conclusions

This is the first study on how *E. bromicola* enhances *E. dahuricus* resistance to Cd stress on the Qinghai–Tibet Plateau. This provides basic data for reducing the threat of Cd on the Qinghai–Tibet Plateau through grass endophytic fungi restoration. In the future, we believe that a combination of different techniques will help elucidate the phenomena and mechanisms of *Epichloë*-induced resistance to Cd stress in host grass. Microbiome, metabolomics, soil science methods, and especially, molecular biology will be used to clarify how *Epichloë* can improve the biochemistry mechanisms of the host for Cd resistance, which will provide the basis for improving land use efficiency and possibly working toward ensuring food safety.

Author Contributions: Conceptualization, Q.S. and X.L.; methodology, Y.L. and C.X.; software, Q.S. and X.L.; validation, Y.L. and K.D.; formal analysis, X.L.; investigation, Q.S. and Y.L.; data curation, Q.S. and Y.L.; writing—original draft preparation, Q.S.; writing—review and editing, W.R.S. and Q.S.; visualization, W.R.S. and Q.S.; supervision, X.L. and Y.L.; project administration, X.L., Y.L. and Q.S.; funding acquisition, Q.S. and X.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by The Open Project of the State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University (2022-ZZ-12), and the National Natural Science Foundation of China (32360349).

Data Availability Statement: The datasets presented in this article are not readily available because the data are part of an ongoing study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kopittke, P.M.; Menzies, N.W.; Wang, P.; McKenna, B.A.; Lombi, E. Soil and the intensification of agriculture for global food security. *Environ. Int.* **2019**, *132*, 105078. [CrossRef]
2. Wiewióra, B.; Żurek, G. The response of the associations of grass and *Epichloë* endophytes to the increased content of heavy metals in the soil. *Plants* **2021**, *10*, 429. [CrossRef]
3. Zhao, H.; Wu, Y.; Lan, X.; Yang, Y.; Wu, X.; Du, L. Comprehensive assessment of harmful heavy metals in contaminated soil in order to score pollution level. *Sci. Rep.* **2022**, *12*, 3552. [CrossRef]
4. Carvalho, M.E.A.; Gaziola, S.A.; Carvalho, L.A.; Azevedo, R.A. Cadmium effects on plant reproductive organs: Physiological, productive, evolutionary and ecological aspects. *Ann. Appl. Biol.* **2021**, *178*, 2. [CrossRef]
5. El Rasafi, T.; Oukarroum, A.; Haddioui, A.; Song, H.; Kwon, E.E.; Bolan, N.; Rinklebe, J. Cadmium stress in plants: A critical review of the effects, mechanisms, and tolerance strategies. *Crit. Rev. Environ. Sci. Technol.* **2022**, *52*, 675–726. [CrossRef]
6. Suhani, I.; Sahab, S.; Srivastava, V.; Singh, R.P. Impact of cadmium pollution on food safety and human health. *Curr. Opin. Toxicol.* **2021**, *27*, 1–7. [CrossRef]
7. Schardl, C.L. *Epichloë festucae* and related mutualistic symbionts of grasses. *Fungal Genet. Biol.* **2001**, *33*, 69–82. [CrossRef]
8. Luo, Q.Z. Selection of Cadmium-Tolerant Forage Varieties Suitable for Planting in Qinghai–Tibet Plateau. Master's Thesis, Gansu Agricultural University, Lanzhou, China, 2018.
9. Leuchtmann, A.; Bacon, C.W.; Schardl, C.L.; White, J.F.; Tadych, M. Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*. *Mycologia* **2014**, *106*, 202–215. [CrossRef]
10. Gibert, A.; Volaire, F.; Barre, P.; Hazard, L. A fungal endophyte reinforces population adaptive differentiation in its host grass species. *New Phytol.* **2012**, *194*, 561–571. [CrossRef]
11. Schardl, C.L.; Leuchtmann, A.; Spiering, M.J. Symbioses of grasses with seed-borne fungal endophytes. *Annu. Rev. Plant Biol.* **2004**, *55*, 315–340. [CrossRef]
12. Song, M.L.; Chai, Q.; Li, X.Z.; Yao, X.; Li, C.L.; Christensen, M.J.; Nan, Z.B. An asexual *Epichloë* endophyte modifies the nutrient stoichiometry of wild barley (*Hordeum brevisubulatum*) under salt stress. *Plant Soil* **2015**, *387*, 153–165. [CrossRef]
13. Xia, C.; Li, N.N.; Zhang, Y.W.; Li, C.J.; Zhang, X.X.; Nan, Z.B. Role of *Epichloë* endophytes in defense responses of cool-season grasses to pathogens: A review. *Plant Dis.* **2018**, *102*, 2061–2073. [CrossRef]

14. Zhang, X.X.; Xia, C.; Li, C.J.; Nan, Z.B. Chemical composition and antifungal activity of the volatile oil from *Epichloë gansuensis*, endophyte-infected and non-infected *Achnatherum inebrians*. *Sci. China Life Sci.* **2015**, *58*, 512–514. [[CrossRef](#)] [[PubMed](#)]
15. Ruppert, K.G.; Matthew, C.; McKenzie, C.M.; Popay, A.J. Impact of *Epichloë* endophytes on adult Argentine stem weevil damage to perennial ryegrass seedlings. *Entomol. Exp. Appl.* **2017**, *163*, 328–337. [[CrossRef](#)]
16. Faeth, S.H.; Oberhofer, M.; Saari, S.; Haskins, K.E.; Shymanovich, T. Does hybridization of endophytic symbionts in a native grass increase fitness in resource-limited environment? *Ecology* **2017**, *98*, 138–149. [[CrossRef](#)]
17. Oberhofer, M.; Güsewell, S.; Leuchtmann, A. Effects of natural hybrid and non-hybrid *Epichloë* endophytes on the response of *Hordelymus europaeus* to drought stress. *New Phytol.* **2014**, *201*, 242–253. [[CrossRef](#)] [[PubMed](#)]
18. Song, M.L.; Li, X.Z.; Saikkonen, K.; Li, C.J.; Nan, Z.B. An asexual *Epichloë* endophyte enhances waterlogging tolerance of *Hordeum brevisubulatum*. *Fungal Ecol.* **2015**, *13*, 44–52. [[CrossRef](#)]
19. Zhang, X.X.; Fan, X.M.; Li, C.J.; Nan, Z.B. Effects of cadmium stress on seed germination, seedling growth and antioxidative enzymes in *Achnatherum inebrians* plants infected with a *Neotyphodium* endophyte. *Plant Growth Regul.* **2010**, *60*, 91–97. [[CrossRef](#)]
20. Gibert, A.; Hazard, L. Endophyte infection of *Festuca eskia* enhances seedling survival to drought and cutting at the expense of clonal expansion. *J. Plant Ecol.* **2011**, *4*, 201–208. [[CrossRef](#)]
21. Peng, Q.Q.; Li, C.J.; Song, M.L.; Nan, Z.B. Effects of seed hydropriming on growth of *Festuca sinensis*, infected with *Neotyphodium*, endophyte. *Fungal Ecol.* **2013**, *6*, 83–91. [[CrossRef](#)]
22. Croy, R.G.; Sutherland, B.L.; Hume, D.E.; Mace, W.J.; van Koten, C.; Finch, S.C. Animal safety of a tall fescue endophyte (*Epichloë* sp.) in a perennial ryegrass (*Lolium perenne*) host. *N. Z. Vet. J.* **2022**, *70*, 165–176. [[CrossRef](#)]
23. Li, C.J.; Nan, Z.B.; Zhang, C.J.; Zhang, C.Y.; Zhang, Y.H. Effects of drunken horse grass infected with endophyte on Chinese rabbit. *J. Agric. Sci. Technol.* **2009**, *11*, 84–90.
24. Soleimani, M.; Hajabbasi, M.A.; Afyuni, M.; Mirlohi, A.; Borggaard, O.K.; Holm, P. Effect of endophytic fungi on cadmium tolerance and bioaccumulation by *Festuca arundinacea* and *Festuca pratensis*. *Int. J. Phytoremediat.* **2010**, *12*, 535–549. [[CrossRef](#)] [[PubMed](#)]
25. Ren, A.; Gao, Y.; Zhang, L.; Xie, F.X. Effects of cadmium on growth parameters of endophyte-infected endophyte-free ryegrass. *J. Plant Nutr. Soil Sci.* **2006**, *169*, 857–860. [[CrossRef](#)]
26. Zhang, X.X.; Li, C.J.; Nan, Z.B. Effects of cadmium stress on growth and anti-oxidative systems in *Achnatherum inebrians* symbiotic with *Neotyphodium gansuense*. *J. Hazard. Mater.* **2010**, *175*, 703–709. [[CrossRef](#)] [[PubMed](#)]
27. Liu, Y.P.; La, B.; Su, X. Current taxonomic status and major existing problems in *Elymus* (Poaceae). *J. Qinghai Norm. Univ.* **2014**, *31*–37.
28. Shao, X.O.; Wang, K.; Dong, S.K.; Huang, X.X.; Kang, M.Y. Regionalisation of suitable herbage for grassland reconstruction in agro-pastoral transition zone of northern China. *N. Z. J. Agric. Res.* **2006**, *49*, 73–84.
29. Wang, P.; Chen, J.H.; Wang, P.; Ma, Q.; Tian, L.H.; Chen, Y.J.; Zhou, Q.P. Status of research into the abiotic stress tolerance of *Elymus* species. *Acta Prataculturae Sin.* **2019**, *28*, 151–162.
30. Zhang, X.X.; Li, C.J.; Nan, Z.B. Effects of cadmium stress on seed germination and seedling growth of *Elymus dahuricus* infected with the *Neotyphodium* endophyte. *Sci. China Life Sci.* **2012**, *55*, 793–799. [[CrossRef](#)]
31. Liu, Z.I. Research on Limiting Factors of Plant Growth in Lawu Mine. Master's Thesis, Xizang University, Linzhi, China, 2011.
32. Zhu, S.Y. Comparative Study on the Response of Three Kinds of Lawn Grasses to Soil Layer Thickness and Planting Density on Slope. Master's Thesis, Sichuan Agricultural University, Wenjiang, China, 2023.
33. Shi, C.; An, S.Z.; Yao, Z.P.; Young, C.A.; Panaccione, D.G.; Lee, S.T.; Schardl, L. Toxin-producing *Epichloë bromicola* strains symbiotic with the forage grass *Elymus dahuricus* in China. *Mycologia* **2017**, *109*, 847–859. [[CrossRef](#)]
34. Song, H. Phylogeny of Nine *Elymus* Species and Related Asexual *Epichloë* Endophyte. Ph.D. Thesis, Lanzhou University, Lanzhou, China, 2015.
35. Song, H.; Nan, Z.B. Origin, divergence, and phylogeny of asexual *Epichloë*-endophyte in *Elymus* species from Western China. *PLoS ONE* **2015**, *10*, e0127096.
36. Cheplick, G.P. Persistence of endophytic fungi in cultivars of *Lolium perenne* grown from seeds stored for 22 years. *Am. J. Bot.* **2017**, *104*, 627–631. [[CrossRef](#)] [[PubMed](#)]
37. Esterbauer, H.; Cheeseman, K.H. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1990; Volume 186, pp. 407–421.
38. Li, H.S. *Principle and Techniques of Botanic, Chemical and Physiological Experiments*; Senior Education Press: Beijing, China, 2000; pp. 164–169+194–197.
39. Clairborne, A. *Catalase Activity, Handbook of Methods for Oxygen Radical Research*; Greenwald, R.A., Ed.; CRC Press: Boca Raton, FL, USA, 1985; pp. 283–284.
40. Beyer, W.F.; Fridovich, I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Anal. Biochem.* **1987**, *161*, 559–566. [[CrossRef](#)] [[PubMed](#)]
41. Chance, B.; Maehly, A.C. Assay of catalase and peroxidases. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1955; Volume 1, pp. 764–775.
42. Gupta, A.S.; Robert, P.; Webb, A.; Scott, H.; Allen, R.D. Overexpression of superoxide dismutase protects plants from oxidative stress. *Plant Physiol.* **1993**, *103*, 1067–1073. [[CrossRef](#)]

43. Potters, G.; Pasternak, T.P.; Guisez, Y.; Palme, K.J.; Jansen, M.A. Stress-induced morphogenic responses: Growing out of trouble? *Trends Plant Sci.* **2007**, *12*, 98–105. [\[CrossRef\]](#)
44. Bacon, C.W.; Porter, J.K.; Robbins, J.D.; Luttrell, E.S. *Epichloë typhina* from toxic tall fescue grasses. *Appl. Environ. Microb.* **1977**, *34*, 576–581. [\[CrossRef\]](#)
45. Cheplick, G.P.; Faeth, S. *Ecology and Evolution of the Grass Endophyte Symbiosis*; Oxford University Press: New York, NY, USA, 2009.
46. Clay, K. Fungal endophytes of grasses: A defensive mutualism between plants and fungi. *Ecology* **1988**, *69*, 10–16. [\[CrossRef\]](#)
47. Clay, K. Defensive mutualism and grass endophytes: Still valid after all these years? In *Defensive Mutualism in Symbiotic Association*; Torres, M., White, J.F., Eds.; Taylor and Francis Publications: New York, NY, USA, 2009; pp. 27–38.
48. Saikkonen, K.; Lehtonen, P.; Helander, M.; Koricheva, J.; Faeth, S.H. Model systems in ecology: Dissecting the endophyte-grass literature. *Trends Plant Sci.* **2006**, *11*, 428–433. [\[CrossRef\]](#)
49. Saikkonen, K.; Saari, S.; Helander, M. Defensive mutualism between plants and endophytic fungi? *Fungal Divers.* **2010**, *41*, 101–113. [\[CrossRef\]](#)
50. Saikkonen, K.; Gundel, P.E.; Helander, M. Chemical ecology mediated by fungal endophytes in grasses. *J. Chem. Ecol.* **2013**, *39*, 962–968. [\[CrossRef\]](#)
51. Hamilton, C.; Gundel, P.E.; Helander, M.; Saikkonen, K. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: A review. *Fungal Divers.* **2012**, *54*, 1–10. [\[CrossRef\]](#)
52. Rizwan, M.; Ali, S.; Rehman, M.Z.; Rinklebe, J.; Tsang, D.C.W.; Bashir, A.; Maqbool, A.; Tack, F.M.G.; Ok, Y.S. Cadmium phytoremediation potential of Brassica crop species: A review. *Sci. Total Environ.* **2018**, *631*, 1175–1191. [\[CrossRef\]](#)
53. Guo, T.R.; Zhang, G.P.; Zhou, M.X.; Wu, F.B.; Chen, J.X. Influence of aluminum and cadmium stresses on mineral nutrition and root exudates in two barley cultivars. *Pedosphere* **2007**, *17*, 505–512. [\[CrossRef\]](#)
54. Tamas, L.; Dudikova, J.; Durcekova, K.; Haluskova, L.; Huttova, J.; Mistrik, I.; Olle, M. Alterations of the gene expression, lipid peroxidation, proline and thiol content along the barley root exposed to cadmium. *J. Plant Physiol.* **2008**, *165*, 1193–1203. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Kumar, V.; Sharma, A.; Kaur, R.; Thukral, A.K.; Bhardwaj, R.; Ahmad, P. Differential distribution of amino acids in plants. *Amino Acids* **2017**, *49*, 821–869. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Szepesi, A.; Szollosi, R. *Plant Metabolites and Regulation under Environmental Stress*; Academic Press: Pittsburgh, PA, USA, 2018; pp. 337–353.
57. Kishor, P.B.K.; Hima, K.P.; Sunita, M.S.L.; Sreenivasulu, N. Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. *Front. Plant Sci.* **2015**, *6*, 544. [\[CrossRef\]](#)
58. Semida, W.M.; Hemida, K.A.; Rady, M.M. Sequenced ascorbate-proline-glutathione seed treatment elevates cadmium tolerance in cucumber transplants. *Ecotox. Environ. Saf.* **2018**, *154*, 171–179. [\[CrossRef\]](#)
59. Zouari, M.; Ben, A.C.; Elloumi, N.; Bellassoued, K.; Delmail, D.; Labrousse, P.; Ben, A.F.; Ben, R.B. Impact of proline application on cadmium accumulation, mineral nutrition and enzymatic antioxidant defense system of *Olea europaea* L. cv Chemlali exposed to cadmium stress. *Ecotoxicol. Environ. Saf.* **2016**, *128*, 195–205. [\[CrossRef\]](#)
60. Rady, M.M.; Elrys, A.S.; El-Maati, M.F.A.; Desoky, E.S.M. Interplaying roles of silicon and proline effectively improve salt and cadmium stress tolerance in *Phaseolus vulgaris* plant. *Plant Physiol. Biochem.* **2019**, *139*, 558–568. [\[CrossRef\]](#)
61. Rellán-Álvarez, R.; Ortega-Villasante, C.; Álvarez-Fernández, A.; Campo, F.F.D.; Hernández, L.E. Stress responses of *Zea mays* to cadmium and mercury. *Plant Soil* **2006**, *279*, 41–50. [\[CrossRef\]](#)
62. Hasanuzzaman, M.; Nahar, K.; Fujita, M. *Responses, Tolerance and Remediation*; Springer Nature: Singapore, 2018; pp. 77–106.
63. Sahu, P.K.; Jayalakshmi, K.; Tilgam, J.; Gupta, A.; Nagaraju, Y.; Kumar, A.; Hamid, S.; Singh, H.V.; Minkina, T.; Rajput, V.D.; et al. ROS generated from biotic stress: Effects on plants and alleviation by endophytic microbes. *Front. Plant Sci.* **2022**, *13*, 1042936. [\[CrossRef\]](#)
64. Faeth, S.H.; Fagan, W.F. Fungal endophytes: Common host plant symbionts but uncommon mutualists. *Integr. Comp. Biol.* **2002**, *42*, 360–368. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Tian, P.; Kuang, Y.; Lin, W.H.; Wang, J.J.; Nan, Z.B. Shoot morphology and alkaloid content of epichlo endophyte—*Festuca sinensis* associations. *Crop Pasture Sci.* **2018**, *69*, 430–438.
66. Ge, Q.; Zheng, J.; Hao, Z.; Liu, Y.; Li, M. Recent advances on reconstruction of climate and extreme events in China for the past 2000 years. *J. Geogr. Sci.* **2016**, *26*, 827–854. [\[CrossRef\]](#)
67. Cui, G.W.; Ji, G.X.; Liu, S.Y.; Li, B.; Lian, L.; He, W.H.; Zhang, P. Physiological adaptations of *Elymus dahuricus* to high altitude on the Qinghai–Tibetan Plateau. *Acta Physiol. Plant.* **2019**, *41*, 115. [\[CrossRef\]](#)
68. Hume, D.E.; Ryan, G.D.; Gibert, A.; Helander, M.; Mirlohi, A.; Sabzalian, M.R. *Epichloë* fungal endophytes for grassland ecosystems. In *Sustainable Agriculture Reviews*; Springer International Publishing: Cham, Switzerland, 2016; pp. 233–305.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.