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Intra- and Interspecific Competition Altered the Competitive Strategies of *Alternanthera philoxeroides* and *Trifolium regens* under Cadmium Contamination

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Abstract: Heavy metal accumulation in soils has been one of the environmental and ecological issues, as it caused life and biodiversity problems. However, many invasive plants can survive in heavy metal polluted areas, but little is known about the invasiveness while under different densities either with native species or themselves. In this study, a greenhouse experiment was performed to examine how cadmium contamination with different concentrations (0, 100, and 200 mg/kg) may influence the interspecific competition between invasive plant *Alternanthera philoxeroides* and the landscape grass *T. regens*, as well as the intraspecific competition of *A. philoxeroides* with different densities. The results showed that stronger interspecific competition would alleviate cadmium damage to both *A. philoxeroides* and *T. regens*, but the two species adopted different allocation strategies. *A. philoxeroides* allocated more biomass to belowground and less to aboveground, while *T. regens* showed exactly the opposite allocation strategy. There was a significant density effect of intraspecific competition on *A. philoxeroides*. That is to say, with the increase of *A. philoxeroides* density, the cadmium stress on the growth of *A. philoxeroides* decreased. Our findings provide a theoretical basis and technical support for the effective control of *A. philoxeroides* invasion, as well as the restoration and reconstruction of green vegetation.

Keywords: invasive plant; heavy metal pollution; biomass allocation; enzyme activity; density effect

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1. Introduction

Heavy metal accumulation in soils, whether caused by natural processes or human activity, severely impacts life and biodiversity [1]. The problem of soil heavy-metal pollution has been a focus of attention in China [2]. Heavy metal toxicity is one of the primary causes of environmental and ecological issues [3,4]. According to China's soil analysis reports, the lead, cadmium, mercury, and arsenic exceed the prescribed standard [5]. Heavy metals in soils are not only difficult to remove, but also circulate and accumulate in the food chain of the whole ecosystem, threatening the stability of the ecosystem and human health [6]. Specifically, soil heavy-metal pollution not only damages soil quality and reduces crop yields, but also exacerbates global climate change and affects the sustainable development of society [7].

However, some plants are tolerant to heavy metals and may become more competitive in contaminated sites [8]. Many invasive plants have been found to readily colonize areas with high levels of heavy metal contamination [9]. *Alternanthera philoxeroides* is a worldwide invasive plant, which is native to South America. In the 1930s, it was introduced into Shanghai as a forage plant. Due to the high invasiveness, *A. philoxeroides* has caused serious damage to many ecosystems all around China. First, *A. philoxeroides* competed with crops for fertilizer and water, which led to the loss of crop yields [10,11]. Second, it severely blocked the rivers, obstructed water traffic and transportation, and reduced species richness, causing serious damage to the local agricultural eco-economic systems [12]. *A. philoxeroides* can occupy ecological niches quickly and decrease the stability and species richness of the native community. The invasion of *A. philoxeroides* can drastically affect biodiversity and cause yield losses in agriculture. As a consequence, much attention has been paid to studying the invasion mechanism of *A. philoxeroides* and taking measures to prevent the further spread of its invasion.

Trifolium repens is famous for its ornamental value, including its long green period, easy reproduction, extensive management, drought resistance, barrenness, pruning resistance, long flowering period, and low cost [13]. Therefore, it is often planted for green lawns as well as in various sports fields and parks. *T. repens* has a good ground coverage and greening effect in soil consolidation and slope protection, which can effectively prevent soil erosion. Its leaves are rich in nutrients, which can speed up the growth rate after grazing, so it usually forms excellent pastures [14]. It also plays a good role in regulating climate and opens up avenues for improving urban soil fertility [15]. The presence of *T. repens* can repair and improve the fragile soil environment and help maintain the stability of the ecosystem. However, from field investigation, we found *A. philoxeroides* invaded most of the *T. repens* lawns. Its invasion of lawns will not only increase the consumption of human, material, and financial resources in the urban landscape, but will also have an impact on soil consolidation and slope protection.

In this study, we would like to examine the invasiveness of *A. philoxeroides* and the competitiveness of *A. philoxeroides* and *T. repens* under cadmium stress. In 2021, we conducted a pot-experiment under different concentrations of cadmium stress to predict the invasion strategy of this alien plant. We also added a factor of planting density to quantify the response of both species to cadmium stress and planting density. We hypothesized that the relative invasive dominance of *A. philoxeroides* would increase with increasing cadmium concentration, while planting density may aggravate this influence as planting density could weaken the stressful effects of cadmium on invaders thereby accelerating invader invasion. Specifically, we addressed the following questions: (1) How do the performance and dominance of *A. philoxeroides* and *T. repens* vary along with different cadmium concentrations? (2) Whether the interspecific competition change with different *T. repens* densities under cadmium stress? (3) Will the intraspecific competition of *A. philoxeroides* to cadmium stress change with its different densities?

2. Material and Methods

2.1. Overview of the Experimental Site

We conducted a competition experiment from June to August 2021 in a greenhouse at China West Normal University (106°4′1″ E, 30°48′45″ N), Nanchong, China. It has a subtropical monsoon climate with an average annual temperature of 15.6 °C and annual precipitation of 1070.5 mm. The land is dominated by brown-purple clay and reddish-brown-purple clay soils with low organic matter content. The soil used for this experiment was made by mixing local soil with nutrient soil (containing a small amount of perlite, high quality grass peat, peat, and various trace elements) in a ratio of 1:4.

2.2. Experimental Design

The soil used in this experiment was from the campus of China West Normal University, and it was artificially contaminated with Cd [100 and 200 mg·kg^{-1} supplied as $CdCl_2$]. Uncontaminated soil was used as a control. The metal concentrations were designed following the Guideline Values of Cd for agriculture used in China by ~33×, 100×, 200×, and 333× [16]. The weight of soil in each pot was constant in the experiment. Cadmium concentration= cadmium mass/ soil mass. Before the experiment, all soil was evenly sprayed with $CdCl_2$ solution containing the corresponding mass of elemental cadmium, thoroughly homogenized, and periodically mixed for 1 week.

On 25 May 2021, we collected a stem cutting (of approximately the same size and length) of *A. philoxeroides* near the experimental site and grew them in an incubator for 12 d under the same external condition before transplanting them into plastic pots (top diameter: 23.5 cm; bottom diameter: 13.2 cm; height: 14 cm) which contained 3.5 kg prepared soils. On 6 June 2021, the similarly-sized ramets were transplanted to pots for one of the following two culture types: (1) *A. philoxeroides* monoculture, (2) Mixed culture with *A. philoxeroides* and *T. regens. A. philoxeroides* monoculture is available in 3 planting densities: 1, 5, and 9 plants/pot. Thus, the intraspecific competition experiment included 9 treatments (3 cadmium concentration × 3 planting density). Mixed culture is available in 2 planting densities: 5 (one *A. philoxeroides* in the center and four *T. regens* evenly surrounded) and 9 (one *A. philoxeroides* in the center and eight *T. regens* evenly surrounded) plants/pot. Thus, the interspecific competition experiment included 6 treatments (3 cadmium concentration × 2 planting density). For each treatment, we used five replicates. The pots were watered in sufficient quantity every day during the experiment to keep the soil in the pots moist, cleared of weeds in a timely manner, and moved randomly once a week to avoid position effects. All plants were harvested on 26 August 2021.

2.3. Growth and Biomass Allocation

The total plants were thoroughly washed and divided into leaves, shoots, and roots; the biomass of each part was determined using the balance after oven drying at 75 °C for 48 h. The biomass allocation traits shoot mass ratio (SMR), root mass ratio (RMR), leaf mass ratio (LMR), root to shoot ratio (RMR), and root: shoot mass (R/S) were calculated as follows:

$$\text{Total mass} = \text{leaf mass} + \text{shoot mass} + \text{root mass}$$

$$\text{SMR} = \text{shoot mass}/\text{total mass}$$

$$\text{RMR} = \text{root mass}/\text{total mass}$$

$$\text{R/S} = \text{root mass}/(\text{shoot mass} + \text{leaf mass})$$

2.4. Photosynthesis-Related Traits

Traits associated with photosynthetic activity, namely, specific leaf area (SLA), total leaf area, number of leaves, and leaf chlorophyll content. SLA and leaf area were calculated as follows:

$$\text{SLA} = \text{leaf area}/\text{dry weight of the leaves}$$

The total leaf area was measured by Image J after scanning by the scanner. The total chlorophyll content of leaves of both *A. philoxeroides* and *T. regens* in each pot was measured using the method of Gao [17]. The number of leaves was counted out by hand. We weighed 0.1 g of the fresh leaves of *A. philoxeroides* and *T. regens*, ground them in a mortar, then extracted chlorophyll with 95% ethanol, and then measured the absorbance with a spectrophotometer at 645 nm and 663 nm, respectively. The chlorophyll content was calculated as follows:

$$\text{Chl}_\text{a}\text{ content }\left(\text{mg}\cdot\text{g}^{-1}\text{ FW}\right) = \frac{(12.72 \times \text{A}_{663} - 2.59 \times \text{A}_{645}) \times \text{V}_\text{t}}{\text{FW} \times 1000} \times n$$

$$\text{Chl}_\text{b}\text{ content }\left(\text{mg}\cdot\text{g}^{-1}\text{ FW}\right) = \frac{(22.88 \times \text{A}_{645} - 4.67 \times \text{A}_{663}) \times \text{V}_\text{t}}{\text{FW} \times 1000} \times n$$

$$\text{Chl}_\text{T}\text{ content }(\text{mg}\cdot\text{g}^{-1}\text{ FW}) = \text{Chl}_\text{a} + \text{Chl}_\text{b}$$

where Chl_a, Chl_b, and Chl_T denote chlorophyll a, chlorophyll b, and the total chlorophyll content, respectively; A_{645} and A_{663} denote the absorbance at 645 nm and 663 nm, respectively; Vt denotes the total volume of chlorophyll extract solution; FW denotes the fresh weight of the leaves (g); and n denotes the dilution times. The spectrophotometric analyses were performed by UV/Vis spectrophotometry (ONDA, mod, UV-30 Scan).

2.5. H_2O_2 Content, MAD Content, and Enzyme Activity

Hydrogen peroxide (H_2O_2) content is a very important physiological indicator of plants under stress. H_2O_2 content was determined by the colorimetric method of titanium sulfate [18]. Lipid peroxidation levels in *A. philoxeroides* and *T. regens* were measured by estimating the malondialdehyde (MDA) content. The MDA content was colorimetrically determined using the thiobarbituric acid (TBA) assay. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are used to scavenge hydrogen peroxide produced in metabolism to avoid oxidative damage to cells by hydrogen peroxide accumulation, and the level of their activity is related to the resistance of plants. SOD activity was determined by photochemical reduction of nitrogen blue tetrazole (NBT), POD activity was determined by the guaiacol method, and CAT activity was determined by UV absorption. All spectrophotometric analyses were performed by UV/Vis spectrophotometry (ONDA, mod, UV-30 Scan). H_2O_2 content, MAD content, and enzyme activity were calculated as follows [17,18]:

$$H_2O_2 \text{ content } \left(\mu\text{mol}\cdot\text{g}^{-1}\text{ FW}\right) = \frac{C \times V_t}{FW \times V_s}$$

$$\text{MAD content } \left(\text{mmol}\cdot\text{g}^{-1}\text{ FW}\right) = [6.452 \times (A532 - A_{600}) - 0.559 \times A_{450}] \times \frac{V_t}{V_s \times FW}$$

$$\text{SOD activity } \left(\text{U}\cdot\text{g}^{-1}\text{ FW}\cdot\text{h}^{-1}\right) = \frac{(A_0 - A_s) \times V_t \times 60}{A_0 \times 0.5 \times FW \times V_s \times t}$$

$$\text{CAT activity } \left(\text{U}\cdot\text{g}^{-1}\text{ FW}\cdot\text{min}^{-1}\right) = \frac{\Delta A_{240} \times V_t}{0.1 \times V_s \times t \times FW}$$

$$\text{POD activity } \left(\mu\text{g}\cdot\text{g}^{-1}\text{ FW}\cdot\text{min}^{-1}\right) = \frac{(X^1 - X_0) \times V_t}{FW \times V_s \times t}$$

where C denotes the concentration of H_2O_2 in the sample found on the standard curve; V_t and V_s denote the total volume of the extract and the amount of crude enzyme solution taken for the determination, respectively; A_n denotes the absorbance at n nm; A_0 indicates the absorbance of the control tube under light; and A_s indicates the absorbance of the sample measuring tube.

2.6. Quality Control (QC)

Whole analytical procedures were monitored using strict quality control measures. Repeated tests were used for each set of experiments for monitoring interference. The standard errors (SE) were all below 25%.

2.7. Statistical Analysis

Before analysis, data of each variable were divided by the number of plants initially grown in each pot so that the data were scaled to per unit plant level. The experimental data were statistically analyzed by SPSS statistics (version 20.0; IBM Corp, Armonk, NY, USA) for each measurement and the mean and standard error were calculated. The variability of the effects of different planting densities and cadmium concentrations on the physiological and ecological characteristics of *A. philoxeroides* and *T. repens* was analyzed by two-way ANOVA. Multiple comparisons were performed by the Duncan test to examine the level of significant differences in the corresponding data ($p < 0.05$). Use origin to complete the drawing.

3. Results

3.1. Effects of Cadmium Concentration and Planting Density on Plant Growth

The cadmium concentration was a significant factor affecting the total biomass, biomass allocation (including SMR, RMR, LMR, and R/S), and leaf traits of plants (including chlorophyll content, SLA, and the number of leaves, excepting the total leaf area) in the process of *A. philoxeroides*'s invasion. Planting density only had a significant effect on chlorophyll content, the total leaf area, and the number of leaves of *A. philoxeroides* ($p < 0.001$), and the interaction between cadmium concentration and planting density on total biomass, chlorophyll content, total leaf area, and the number of leaves was significant ($p < 0.005$) (Table 1). The same trend was found in total biomass of *A. philoxeroides* and *T. regens* when *A. philoxeroides* invaded *T. regens* population. When the planting density of *T. regens* was low, the total biomass of both species showed a trend of "low promotion and high suppression" with the increase of cadmium concentration, but when the planting density of *T. regens* was high, both total biomass showed a trend of "first decrease then increase" with the increase of cadmium concentration (Figure 1A,B). Cadmium concentration and planting density significantly reduced the total biomass of *A. philoxeroides*. The total biomass of *A. philoxeroides* decreased progressively with increasing planting density at each cadmium concentration, and the total biomass decreased progressively with increasing cadmium concentration at each planting density (Figure 1C). The intraspecific competition of *A. philoxeroides* increased with increasing density, thus affecting the material accumulation.

Table 1. F-values and significance levels for factorial ANOVA of the effects of density and cadmium on morphological traits and biomass of *A. philoxeroides* and *T. regens*.

Variables	Cadmium Concentration		Planting Density		Interaction	
	F	P	F	P	F	P
A. philoxeroides in mixed culture						
Total biomass	30.123	<0.001	0.042	0.840	14.702	<0.001
SMR	10.734	<0.001	0.482	0.494	0.301	0.743
RMR	8.564	0.002	0.517	0.479	0.173	0.842
LMR	27.967	<0.001	0.080	0.780	2.414	0.111
R/S	7.501	0.003	1.255	0.274	0.388	0.683
Chlorophyll content	32.391	<0.001	192.178	<0.001	338.890	<0.001
SLA	7.501	0.003	1.255	0.274	0.388	0.683
Total leaf area	1.849	0.179	14.334	0.001	6.782	0.005
Number of leaves	204.118	<0.001	989.807	<0.001	253.300	<0.001
T. regens in mixed culture						
Total biomass	1.506	0.242	0.055	0.817	4.508	0.022
SMR	0.769	0.475	1.133	0.298	1.009	0.379
RMR	1.803	0.186	1.326	0.261	4.303	0.025
LMR	1.023	0.375	0.661	0.808	1.699	0.204
R/S	2.224	0.130	0.155	0.697	4.525	0.022
Chlorophyll content	7.219	0.004	0.008	0.929	20.774	<0.001
SLA	2.224	0.130	0.155	0.697	4.525	0.022
Total leaf area	453.896	<0.001	6.791	0.015	1148.826	<0.001
Number of leaves	86.771	<0.001	43.102	<0.001	7.980	0.002
A. philoxeroides in monoculture						
Total biomass	51.019	<0.001	39.612	<0.001	15.517	<0.001
SMR	27.296	<0.001	4.166	0.024	1.928	0.127
RMR	26.027	<0.001	6.583	0.004	1.748	0.161
LMR	18.900	<0.001	16.409	<0.001	3.908	0.01
R/S	17.800	<0.001	4.478	0.018	2.108	0.100
Chlorophyll content	11.951	<0.001	15.468	<0.001	34.875	<0.001
SLA	21.138	<0.001	10.731	<0.001	13.753	<0.001
Total leaf area	7.295	0.002	4476.921	<0.001	37.028	<0.001
Number of leaves	13.587	<0.001	5.304	0.030	55.681	<0.001

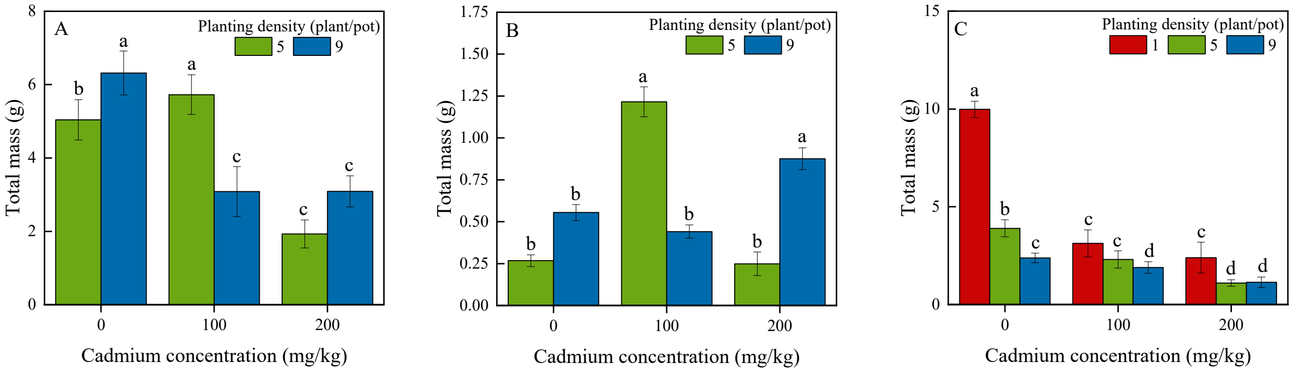

Figure 1. Total biomass of *A. philoxeroides* and *T. regens* subjected to cadmium concentration and planting density. (**A**–**C**) were the total biomass of *A. philoxeroides* in mixed culture, the total biomass of *T. regens* in mixed culture, and the total biomass of *A. philoxeroides* in monoculture, respectively. The data represents the means ± SE (n = 5). Lowercase letters indicate a significant difference ($p < 0.05$) in *A. philoxeroides* or *T. regens* present on each treatment based on post hoc multiple comparisons in Duncan with cadmium concentration and planting density as fixed factors.

The allocation strategy of *A. philoxeroides* was decreasing SMR and increasing RMR, LMR, and R/S with the addition of cadmium. RMR and R/S of *A. philoxeroides* significantly increased with increasing density of *T. regens*, while the effect on SMR was not significant under each cadmium concentration stress; RMR and R/S of *A. philoxeroides* showed a trend of "low promotion and high suppression" with the increase of cadmium concentration, while the leaf mass ratio showed a continuously increasing trend under each planting density (Figure 2A–C,J). SMR of *T. regens* showed a trend of increasing and then decreasing with the increase of cadmium concentration. The trends of changes in RMR and R/S were the same for different planting densities under low cadmium concentration (100 mg/kg), while the trends of changes in their effects were opposite for different planting densities under high cadmium concentration (200 mg/kg). It is noteworthy that the trends of RMR, SMR, LMR, and R/S with increasing planting density under each cadmium stress were exactly opposite to those presented without cadmium stress (Figure 2D–F,K). SMR of *A. philoxeroides* decreased and RMR and R/S increased with increasing cadmium concentration. In the absence of cadmium stress, the greater the intraspecific competition of *A. philoxeroides*, the greater RMR and R/S, and the smaller SMR and LMR. Overall, RMR, LMR, and R/S of *A. philoxeroides* increased with increasing cadmium concentration at each planting density, while SMR decreased with increasing cadmium concentration (Figure 2J–I,L).

A. philoxeroides tended to reduce leaf numbers and increase total chlorophyll content, while total leaf area and SLA showed a trend of "low promotion and high suppression" with increasing cadmium concentration when *A. philoxeroides* invaded the low-density *T. regens* population. The interspecific competition between *A. philoxeroides* and *T. regens* increased when the planting density was high, causing the number of leaves of *A. philoxeroides* to decrease continuously with increasing cadmium concentration. The trend of total leaf area and SLA was opposite to that of low planting density, i.e., the total leaf area and SLA showed a trend of decrease and then increase with increasing cadmium concentration. The chlorophyll content of *A. philoxeroides* in general continued to decline with increasing cadmium concentration. Under the same cadmium stress, the stronger the interspecific competition between *A. philoxeroides* and *T. regens*, the more *A. philoxeroides* tended to increase the number of leaves and decrease the total leaf area (Figure 3A–C,J–L). At low planting density, *T. regens* tended to reduce SLA and chlorophyll content, while leaf number and total leaf area showed a trend of "low promotion and high inhibition" with increasing cadmium concentration. The number of leaves, SLA, and chlorophyll content of *T. regens* tended to "first decrease then increase" with the increase of cadmium concentration when planting density was high (Figure 3D–F,M–O). When *A. philoxeroides* successfully invaded

T. regens habitat, both cadmium concentration and density decreased leaf number and total leaf area of *A. philoxeroides*, and cadmium addition increased the SLA of *A. philoxeroides*. In the absence of cadmium stress, chlorophyll content of *A. philoxeroides* decreased with increasing intraspecific competition; the higher the population density, the more chlorophyll content under low cadmium concentration stress (100 mg/kg); while chlorophyll content showed a trend of first decreasing and then increasing with increasing intraspecific competition under high cadmium concentration stress (200 mg/kg) (Figure 3G–I,P–R).

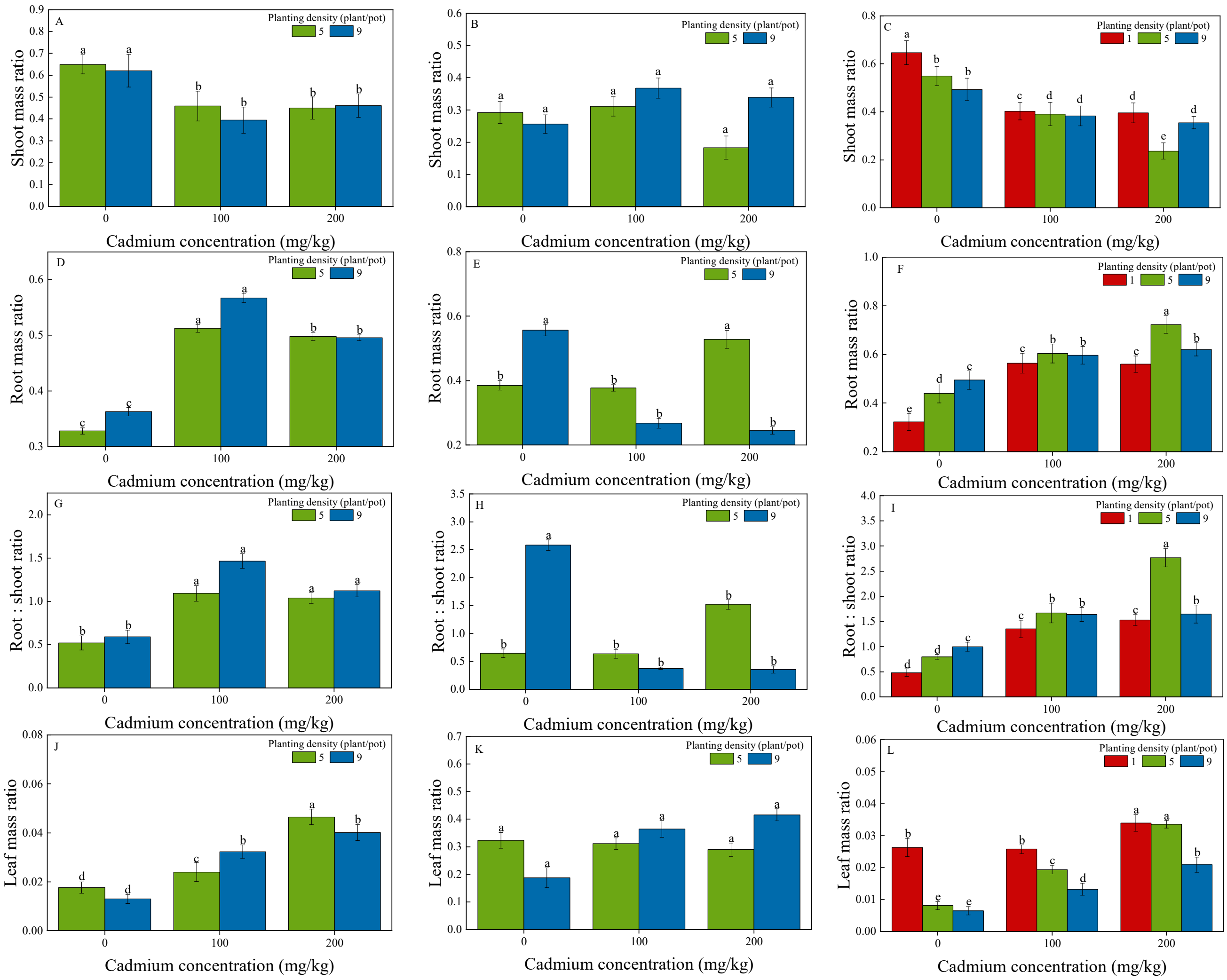

Figure 2. Biomass allocation of *A. philoxeroides* and *T. regens* in monoculture and mixed culture. (**A**,**D**,**G**,**J**), (**B**,**E**,**H**,**K**), and (**C**,**F**,**I**,**L**) were the biomass allocation of *A. philoxeroides* in mixed culture, the biomass allocation of *T. regens* in mixed culture, and the biomass allocation of *A. philoxeroides* in monoculture, respectively. Lowercase letters indicate a significant difference ($p < 0.05$) in *A. philoxeroides* or *T. regens* present on each treatment based on post hoc multiple comparisons in Duncan with cadmium concentration and planting density as fixed factors.

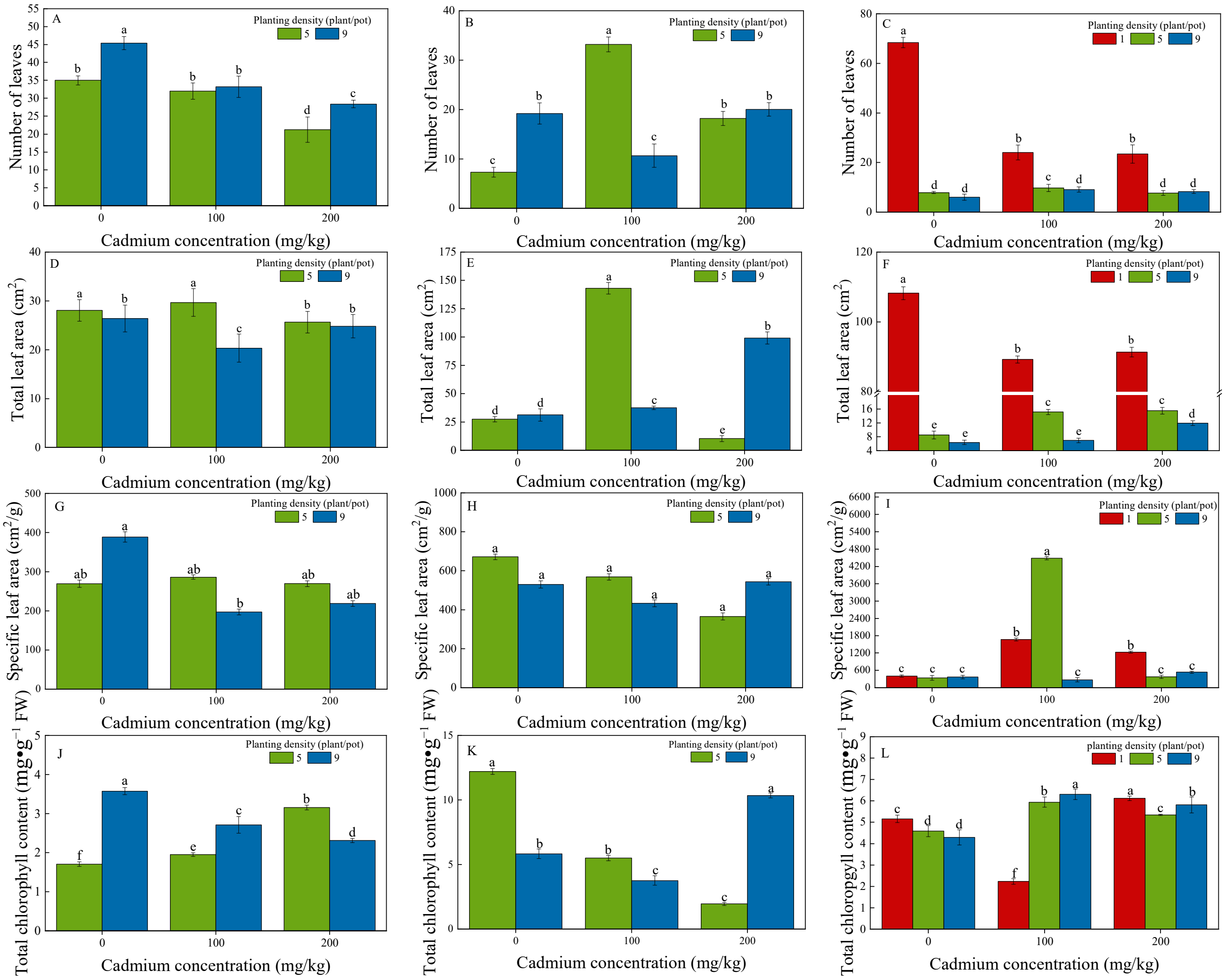

Figure 3. Traits related to photosynthesis of *A. philoxeroides* and *T. regens* subjected to cadmium concentration and planting density. (**A**,**D**,**G**,**J**), (**B**,**E**,**H**,**K**), and (**C**,**F**,**I**,**L**) were the traits related to photosynthesis of *A. philoxeroides* in mixed culture, the traits related to photosynthesis of *T. regens* in mixed culture, and the traits related to photosynthesis of *A. philoxeroides* in monoculture, respectively. Lowercase letters indicate a significant difference ($p < 0.05$) in *A. philoxeroides* or *T. regens* present on each treatment based on post hoc multiple comparisons in Duncan with cadmium concentration and planting density as fixed factors.

3.2. Effects of Cadmium Concentration and Planting Density on H_2O_2 Content, MAD Content, and Antioxidant Enzyme Activity

Cadmium concentration had significant effects on the H_2O_2 content, MAD content, and antioxidant enzyme activities (CAT and POD) of *A. philoxeroides* and *T. regens* in mixed culture. The planting density had a significant effect on enzyme activities of *A. philoxeroides* in mixed culture, while it had little effect on the enzyme activities of *T. regens*. The interaction of cadmium concentration and planting density only had significant effects on MAD and POD of *A. philoxeroides* and *T. regens* (Table 2). Both cadmium concentration and planting density significantly increased the content of H_2O_2 and MAD, and CAT activity of *A. philoxeroides* and *T. regens*. Specifically, the content of H_2O_2 and MAD, and CAT activity gradually increased with increasing cadmium concentration for both *A. philoxeroides* and *T. regens* at each planting density. They all gradually decreased with increasing planting density at two cadmium concentrations (Figure 4A,B,D,E,J,K). The SOD activity of *A. philoxeroides* showed a trend of increasing and then decreasing with the increase of cadmium concentration at low planting density, while it showed a completely opposite trend of decreasing and then increasing at high planting density (Figure 4G). Although planting density had little effect on SOD activity of *T. regens* under each cadmium stress, SOD activity showed a trend of decreasing and then increasing with cadmium concentration increase at two planting densities, while it increased with increasing planting density at two cadmium concentration (Figure 4H). The POD activity of *A. philoxeroides* decreased with increasing planting density at each cadmium concentration, while the same trend was observed for *T. regens* only under cadmium contamination (Figure 4M,N).

Cadmium concentration, planting density, and their interaction had significant effects on enzyme activities (except for MAD content) of *A. philoxeroides* in monoculture (Table 2). Similarly, both cadmium concentration and planting density significantly increased the content of H_2O_2 and MAD, and CAT activity of *A. philoxeroides.* Both H_2O_2 and MAD contents of *A. philoxeroides* decreased with increasing planting density under two cadmium contamination concentrations, while they both increased with increasing cadmium addition concentrations at different planting densities (Figure 4C,F,L). When *A. philoxeroides* successfully invaded uncontaminated cadmium habitats of *T. regens*, it gradually reduced CAT activity as its intraspecific competition increased. Under each cadmium stress, CAT activity of *A. philoxeroides* showed a trend of increasing and then decreasing with the increase of intraspecific competition; under each planting density, CAT activity of *A. philoxeroides* increased with the increase of cadmium concentration. The SOD activity gradually increased with the increase of planting density (Figure 4I). The POD activity of *A. philoxeroides* showed a continuously increasing trend with increasing cadmium concentration at low planting density (5 plants/pot), while its activity showed a trend of increasing and then decreasing at high planting density (9 plants/pot). And the trend of POD activity at low cadmium addition concentration was exactly opposite to that at high cadmium addition concentration. Under low cadmium stress, POD activity of *A. philoxeroides* gradually increased with increasing planting density, while under high cadmium stress, POD activity gradually decreased with increasing planting density.

Table 2. F-values and significance levels for factorial ANOVA of the effects of density and cadmium on enzyme activity of *A. philoxeroides* and *T. regens*.

Variables	Cadmium Concentration		Planting Density		Interaction	
	F	P	F	P	F	P
A. philoxeroides in mixed culture						
H_2O_2 content	2293.342	<0.001	60.202	<0.001	35.337	<0.001
MAD content	147.702	<0.001	27.136	<0.001	28.955	<0.001
SOD activity	35.528	<0.001	18.818	<0.001	106.077	<0.001
CAT activity	255.588	<0.001	18.811	<0.001	0.520	0.601
POD activity	121.129	<0.001	106.096	<0.001	24.361	<0.001
T. regens in mixed culture						
H_2O_2 content	41.094	<0.001	0.241	0.628	1.524	0.238
MAD content	50.144	<0.001	0.013	0.909	15.849	<0.001
SOD activity	3.276	0.055	0.117	0.736	0.327	0.724
CAT activity	370.694	<0.001	4.100	0.054	0.405	0.672
POD activity	34.128	<0.001	5.879	0.023	19.753	<0.001
A. philoxeroides in monoculture						
H_2O_2 content	220.223	<0.001	4.551	0.017	3.220	0.023
MAD content	1257.838	<0.001	2.494	0.097	32.358	<0.001
SOD activity	37.376	<0.001	21.781	<0.001	15.891	<0.001
CAT activity	737.560	<0.001	16.871	<0.001	21.122	<0.001
POD activity	445.598	<0.001	14.189	<0.001	90.811	<0.001

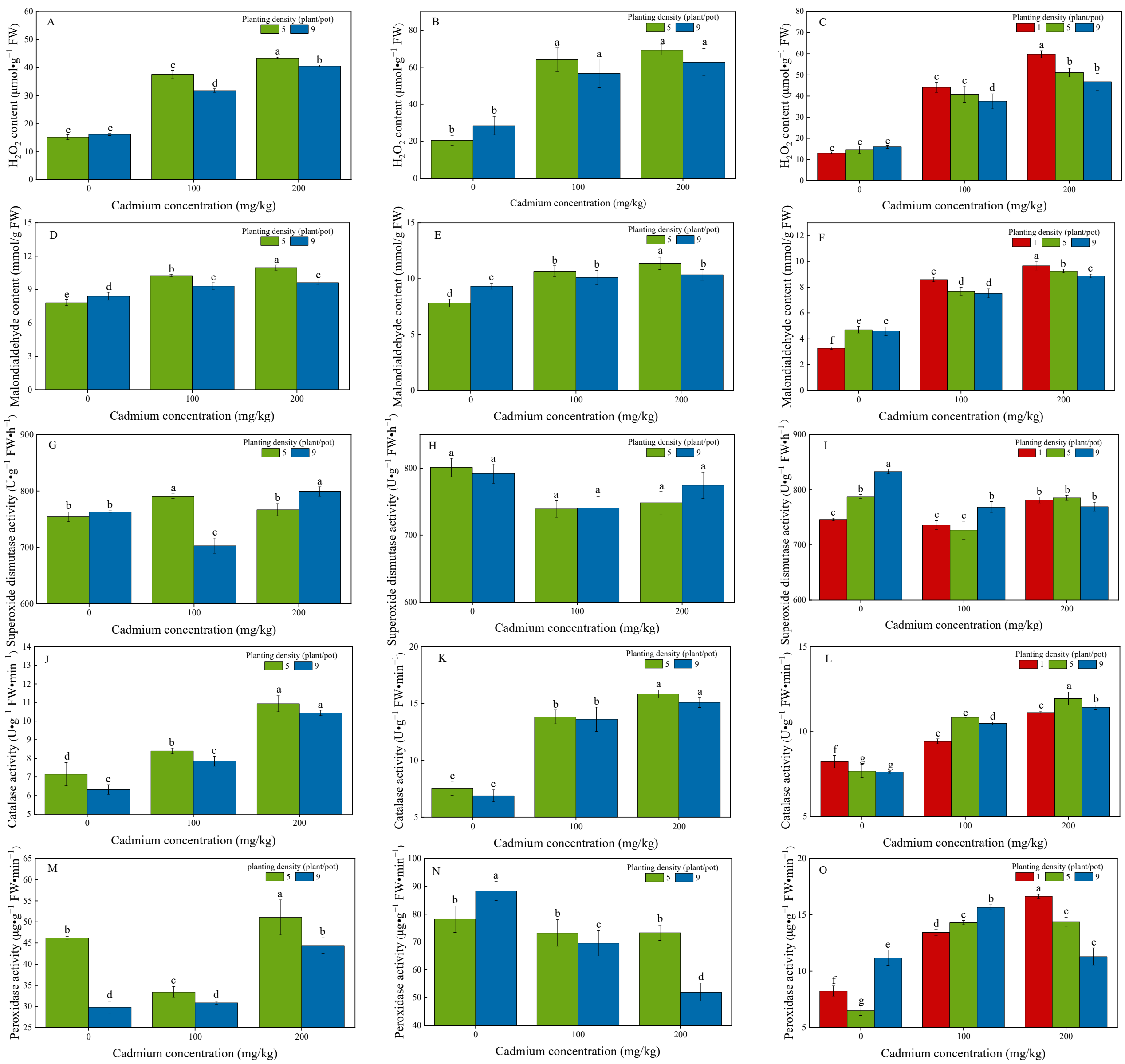

Figure 4. Enzyme activity, including H_2O_2 content, MAD content, SOD activity, CAT activity, and POD activity, of *A. philoxeroides* and *T. regens* subjected to cadmium concentration and planting density. (**A**,**D**,**G**,**J**,**M**), (**B**,**E**,**H**,**K**,**N**), and (**C**,**F**,**I**,**L**,**O**) were the enzyme activity of *A. philoxeroides* in mixed culture, the enzyme activity of *T. regens* in mixed culture, and the enzyme activity of *A. philoxeroides* in monoculture, respectively. Lowercase letters indicate a significant difference ($p < 0.05$) in *A. philoxeroides* or *T. regens* present on each treatment based on post hoc multiple comparisons in Duncan with cadmium concentration and planting density as fixed factors.

4. Discussion

Competitive ability and heavy metal tolerance are two of the most important factors that contribute to the capacity of invasive plants to colonize polluted habitats [9,19,20]. The two species *A. philoxeroides* and *T. regens* examined in this study are both characterized by a high tolerance to heavy metals [21,22], and the competition between them represents a typical interaction between invasive and native plants in polluted sites [19]. Biomass is an important basis for the study of plant ecology and functional traits, a fundamental expression of energy accumulation [23,24], and an important parameter for measuring interspecific plant competition [25]. The total biomass of both *A. philoxeroides* and *T. regens* was promoted by low cadmium concentration (100 mg/kg) and low planting density

(5 plants/pot), indicating that energy accumulation was promoted under low interspecific competition intensity and low cadmium stress. This may be since both *A. philoxeroides* and *T. regens* are tolerant of cadmium and that low interspecific competition intensity stimulated their nutrient uptake. However, their total biomass was suppressed when interspecific competition increased, probably because the nutrients they could uptake decreased with increased interspecific competition in a limited resource environment. Plant biomass decreases with increasing density, exhibiting a clear density-constrained effect to adapt to different competitive pressures and environmental conditions [26]. The total biomass of both *A. philoxeroides* and *T. regens* increased with planting density under high concentrations of cadmium stress (200 mg/kg), which may be due to the uptake of cadmium by multiple plants, thus alleviating the stressful effect of cadmium on their growth.

Allocation plasticity is a major adaptive strategy deployed by plants to counter the adverse effects of environmental stress. As for biomass allocation, *A. philoxeroides* tended to show a higher degree of allocation plasticity, and RMR and LMR rapidly increased with the cadmium addition, and the R/S ratio also increased. Plants develop high levels of root biomass in metalliferous soils, resulting in a higher R/S ratio, which not only enhances the capacity to store toxic metal ions but also facilitates the biosynthesis of diverse defense-related cellular biomolecules in roots [27]. Root mass allocation under mixed culture condition is indicative of an increased requirement of *A. philoxeroides* for nutrients or other limited resources under these circumstances [28,29]. *A. philoxeroides* adjusted root length, root mass distribution, and leaf mass distribution, thus increased competition for soil resources and light. Since *T. regens* were affected not only by cadmium concentration and interspecific competition but also by intraspecific competition, biomass allocation varied with different cadmium concentrations at different planting densities. Interspecific competition stimulated shoot biomass allocation and leaf biomass allocation, while suppressed root mass allocation of *T. regens* under cadmium stress. Such adaptive mechanism facilitated *T. regens* to occupy more space above ground and compete for more light resources. In the absence of cadmium stress, *T. regens* increased the biomass allocation of the belowground part and decreased the aboveground part as planting density increased, indicating that plant competition for belowground resources such as water, nutrients, and physical space increased with density, while competition for aboveground light resources decreased with density. This is consistent with the findings of Fan [30]. The high planting density of *T. regens* inhibited biomass allocation to roots and promoted biomass allocation to stems and leaves under cadmium stress, indicating that interspecific competition gradually dominated under the drive of cadmium stress, causing the density effect on biomass allocation to change. Overall, the R/S of *A. philoxeroides* was significantly greater than that of *T. regens*, suggesting that *A. philoxeroides* has more phenotypic competitive advantages.

Plant leaves can respond sensitively to environmental changes. Both the number of leaves and total leaf area can characterize the plant's ability to capture light and the area of photosynthesis. The total leaf area of *A. philoxeroides* and *T. regens* showed consistent trends with increasing cadmium stress when the intensity of interspecific competition is certain, indicating that heavy metal pollution caused plants to make a consistent stress response, i.e., to affect the capture of light resources and the accumulation of organic matter. Interspecific competition reduced the area of photosynthesis of *A. philoxeroides* in each cadmium stress. For *T. regens*, interspecific competition and cadmium stress generally increased its leaf number and total leaf area, while decreased SLA (although there was no significant difference between treatments). This is well in-line with the effects of density constraints and cadmium stress on biomass allocation between *A. philoxeroides* and *T. regens.* SLA is correlated with leaf thickness [31], the thicker the leaf the more it helps to preserve water in the plant in case of water shortage, ensuring that the plant body can survive harsh and extreme environments [32]. SLA also means that the plant has more resources for photosynthesis [33]. The SLA of *T. regens* was generally larger than that of *A. philoxeroides*, but it decreased at high concentrations of individual stresses, indicating that the invasiveness of *T. regens* community increased. Moreover, cadmium stress and interspecific competition increased total chlorophyll content of *A. philoxeroides*, while inhibited total chlorophyll content of *T. regens,* which indicates that interspecific competition and cadmium stress stimulated the photosynthetic capacity of *A. philoxeroides* and inhibited the photosynthetic capacity of *T. regens.*

Interspecific competition increased the H_2O_2 content of *A. philoxeroides* and *T. regens* when they were not subjected to cadmium stress; their H_2O_2 content increased significantly under heavy metal stress, but interspecific competition decreased the hydrogen peroxide content. This may be because the experiment was conducted in pots with a certain amount of cadmium, and the effect of cadmium stress on each plant was alleviated when planting density was higher. Under the same cadmium stress, the H_2O_2 content of *A. philoxeroides* was lower than that of *T. regens*, and therefore *A. philoxeroides* had better tolerance to the heavy metal cadmium. Due to the production of reactive oxygen species–the H_2O_2, the plants underwent membrane lipid peroxidation [34], but the difference in malondialdehyde content between *A. philoxeroides* and *T. regens* was not significant, indicating that the antioxidant enzymes of *T. regens* responded better to interspecific competition and cadmium stress. SOD, CAT, and POD are the main enzymes that scavenge H_2O_2 [35–37], and their previous synergistic effects allow for the maintenance of normal growth and development of *A. philoxeroides* and *T. regens.*

Overall, *A. philoxeroides* increased the number of leaves, RMR, LMR, R/S, and total chlorophyll content with increasing planting density, while *T. regens* increased SMR and LMR, to increase competitive advantage under low concentration of cadmium stress. *T. regens* increased total biomass, SMR, LMR, number of leaves, total leaf area, SLA, and total chlorophyll content with increasing planting density, while *A. philoxeroides* increased total biomass, number of leaves, and R/S, to increase competitive advantage under high cadmium stress. That is, *T. regens* stimulates leaf development to capture more light resources at high cadmium contamination and planting density, resulting in a superior competitive advantage.

Both intraspecific competition and cadmium stress in *A. philoxeroides* significantly reduced its biomass accumulation, and its total biomass decreased with increasing density or cadmium stress. The essence of density effect is the competition for space and resources among individuals within a plant population due to increased density and the mutual interference among individuals, which leads to a decrease in plant biomass accumulation [38,39]. *A. philoxeroides* adapts to intraspecific competition and cadmium-stressed environments by regulating the biomass allocation of above- and below-ground parts. Density showed a significant density effect on above- and below-ground biomass of *A. philoxeroides*, i.e., increased root biomass allocation and decreased stem and leaf biomass allocation under low concentration and no cadmium stress. The above- and below-ground biomass allocation of *A. philoxeroides* showed a compensating effect under high cadmium stress, allowing it to better absorb and capture resources for its normal growth and development. Similarly, both density effect and cadmium stress reduced the photosynthetic area and leaf number of *A. philoxeroides*. The chlorophyll content of *A. philoxeroides* decreased with the increase of intraspecific competition under low concentration of cadmium stress, indicating that cadmium in the soil was absorbed by the plant under high density and alleviated the stress effect. Cadmium stress significantly increased H_2O_2 content of *A. philoxeroides*, and it decreased with increased intraspecific competition under cadmium contamination, which was consistent with the trend of malondialdehyde. This suggests that there is also a density effect of planting density on H_2O_2 content and malondialdehyde content, and that the stressing effect of cadmium may be alleviated by the uptake of a certain amount of cadmium by more plants in a pot, which reduced the production of reactive oxygen species and inhibited the membrane lipid peroxidation reaction. H_2O_2 content increased slightly with intraspecific competition without cadmium stress, indicating that intraspecific competition is a stress at this time. The production of hydrogen peroxide caused the production of antioxidant enzymes, but the trends of SOD, CAT, and POD were different, implying the existence of synergistic effects of each antioxidant enzyme to reduce the damage of reactive oxygen species to plants.

5. Conclusions

The results from this study showed that both *A. philoxeroides* and *T. regens* increased their competitive advantage by changing the biomass accumulation as well as the trade-offs of each functional trait when *A. philoxeroides* invaded *T. regens.* In the presence of heavy metal cadmium stress, stronger interspecific competition would alleviate cadmium damage to both *A. philoxeroides* and *T. regens*, but their allocation strategies were different. *A. philoxeroides* increased its competitive ability by increasing belowground biomass allocation. *T. regens* increased competitive ability by increasing aboveground biomass allocation. In terms of enzyme activity, *T. regens* was more sensitive to cadmium stress and had stronger activity of individual antioxidant enzymes, making its membrane lipidation similar to that of *A. philoxeroides*. Overall, *T. regens* is more competitive at high cadmium stress and planting densities. Intraspecific competition and cadmium stress affected the material accumulation and nutrient uptake patterns of *A. philoxeroides.* Such findings provide insights into the invasion mechanism of *A. philoxeroides* during phytoremediation with *T. regens* at heavy metal cadmium-contaminated sites and some theoretical basis and technical support for the restoration and reconstruction of green vegetation.

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