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Original Research

Effect of Arbuscular Mycorrhizal Fungi on Switchgrass Growth and Mineral Nutrition in Cadmium-Contaminated Soil

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Abstract

The effect of arbuscular mycorrhizal fungi (AMF) and cadmium (Cd) treatment on switchgrass *(Panicum virgatum*) growth, Cd accumulation, mineral element concentrations and soil Cd bioavailability were investigated in a greenhouse experiment. The results showed that switchgrass biomass increased with AMF inoculation at all Cd levels except the 40 mg/kg Cd treatment. AMF decreased the shoots and roots Cd accumulation for the 10 and 40 mg/kg Cd treatments and limited Cd translocation from root to shoot, but enhanced the phosphorus concentrations for all Cd treatments. Furthermore, the presence of AMF reduced the reducible-extractable and acid-extractable Cd concentrations in the 10 and 40 mg/kg Cd soils, respectively. AMF inoculation significantly increased the shoot Ca, Mg, K, and Na concentrations without Cd addition; it decreased Ca, Mg, and Zn concentrations in the roots and Fe in the shoots with the low Cd addition; it increased K, Mg, and Na in the shoots with the 10 mg/kg Cd addition; and it increased Ca and Mg in the shoots and Na in the roots with the 40 mg/kg Cd addition. In summary, AMF inoculation decreased Cd absorption and translocation, improved P absorption, and took a different strategy to elements absorption under different Cd levels. The improved biomass and decreased Cd concentrations with AMF assistance increased the potential for switchgrass use as a phytoremediation and bioenergy crop in a contamination site.

Keywords: energy grass, AMF, heavy metal, nutrition element, bioavailability

Introduction

With increasing levels of economic development, pollution containing heavy metals originating from industry, mining and agriculture is becoming a serious threat to the environment and humans – with Cd being the most toxic contaminant. Cd has the potential to inhibit plant growth by destroying the photosynthetic structure, disturbing nutrition absorptihe, decreasing root and leaf extension and even leading to death [1-2]. Cd can enter animals and humans through the food chain, causing kidney and reproductive function damage, osteoporosis and hypertension [3]. Thus, the remediation of Cd-polluted soil to avoid Cd damage

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to plant growth and human health was an urgent undertaking.

Phytoremediation, a promising technology that uses plants to extract/stabilize contaminants from/in the soil is a low-cost, environmentally friendly technique compared to traditional remediation methods [4]. Most selected hyperaccumulators have low biomass and slow growth rates, restricting their long-term utilization and resulting in low economic benefit. The discovery of a plant species that could balance these features, decreasing time cost and promoting economic development – all while providing phytoremediation – would be a favorable solution.

Energy crops have been introduced to produce biofuels to substitute for traditional fossil fuels in recent decades [5-6]. With the decreasing area of cultivated land available in the world, it is an excellent choice to cultivate energy crops on abandoned or contaminated land to extract/stabilize Cd, improve the local ecological environment, produce energy biomass and prevent Cd from entering the food chain [7]. Consequently, many studies have been conducted on how to phytoremediate contaminated soil using energy grass, and a consensus has been reached that the utilization of high-biomass energy grass as phytoremediation material could be a profit-making operation [2, 8]. Switchgrass, a perennial rhizomatous grass, has a broad climate tolerance, rapid growth rate, high biomass yield, strong tolerance to low fertilizer level and varied abiotic and biotic stress; as proposed by Koçar and Civaş, these characteristics make up perfect phyroremediation materials [6]. Several studies have demonstrated that switchgrass has a strong tolerance to water and salinity stress [9-10]. Some studies have reported that switchgrass absorbed excessive Cs, Rb, Cd, Cu, Zn, and Pb in the portion belowground, where the accumulation capacity greatly relied on switchgrass cultivar, soil pH and duration [1, 8, 11-13]. Although relevant studies found that switchgrass has a certain tolerance to Cd, the sharp growth inhibition by high Cd toxicity cannot be ignored [1, 14]. In the search for a rapid solution to increase energy crop (switchgrass) biomass to compensate for low Cd accumulation in the portion above might be a new direction for sustainable bioenergy feedstock in contaminated soils.

AMF could form symbiotic relationships with 80% vascular plants on the earth, and have been widely recommended in recent years to promote the host plant phytoremediation process in contaminated polluted soil. AMF could connect the root with rhizosphere by fungal mycelium enhancing plant nutrient exploitation, especially P [15-16]. Nutrition improvement greatly increased plant biomass, which had the benefit of diluting the contaminants [15, 17]. The mode of heavy metal absorption and translocation in roots and shoots was regulated by AMF based on the concentration in the soil and plant species [14, 18-20]. Simultaneously, heavy metals could be immobilized in the fungal structure or on the fungal surface through the formation of polyphosphates [16, 21-23]. In addition, AMF produced glomaline to cheale contaminate, and improved organic matter content and soil pH to decreasing the heavy metal bioavailability [24]. Therefore, AMF has been used as a biological fertilizer to enhance plant Cd tolerance by promoting nutrition absorption, affecting heavy metal absorption/translocation and improving soil characteristics [25].

However, there has been limited information available on AMF improving switchgrass tolerance to Cd. Researchers have found that AMF could improve switchgrass growth in acidic soil and help use more nitrogen fertilizer with increasing temperatures [26-27]. Arora et al. reported that inoculated AMF could promote switchgrass germination, survival and biomass under various added Cd concentrations [14]; however, further mechanisms are needed to explain the function of AMF in the interaction between switchgrass and Cd stress. Therefore, in the present study we grew switchgrass cv. Summer under three Cd levels and inoculated with *Rhizophagus irregularis* (RI). We detected plant growth, Cd, mineral element, and three soil Cd species concentration, and analyzed the mycorrhizal response of these factors to explore if AMF could increase the growth and tolerance of switchgrass seedlings to Cd, change Cd translocation, affect the relationship between Cd and elements nutrition concentrations, and provide useful information for the sustainable use of this bioenergy feedstock on contaminated land.

Materials and Methods

Growth Media Preparation

Soil was collected from the Shangzhuang Experimental Station of China Agriculture University. The soil chemical properties were as follow: pH 6.8 (1:2.5 in water), available P 1.8%, and cadmium 0.01 mg/kg. The soil was air-dried and passed through a 2 mm mesh sieve. The sand (diameter<0.5 mm) was bought from Dasenlin Gardon material station, washed several times, and rinsed three times with deionized water. The soil and sand were mixed uniformly (w/w = 2:1), and sterilized by gamma rays (25 kGy, 10MeVelecton beam). Then the mixture was divided into three parts, to which was added one of the three concentrations of Cd (in the form of $CdCl_2.5H_2O$): 0 mg/kg, 10 mg/kg, and 40 mg/kg. Before planting, the medium was combined with basal nutrition consisting of 30 mg/kg P, 120 mg/kg N, and 120 mg/kg K.

Host Plant

Seeds of switchgrass were provided by the Breeding Laboratory of Animal Science and Technology College of China Agriculture University. The seeds were sterilized with 10% H_2O_2 for 20 minutes, washed clean using tap water, and rinsed three times with deionized water. The seeds were germinated until the radicles reached 3cm, and each pot had sown 10 seeds. The pots were thinned to 5 seeds in each pot two weeks later.

AMF Preparation

The *Rhizophagus irregularis* (*R. irregularis*) (BGC BJ09) was provided by Jingping Gai from the Resource and Environment College of China Agriculture College. The harvested inoculum consisted of a mixture of spores, hyphae and plant root fragments. 1 g inoculum included 150 spores.

Pot Experiment

The experiment was designed with three Cd levels (0, 10, 40 Cdmg/kg) and two AMF treatments (inoculation or non-inoculation with *R. irregularis*). Each treatment was replicated 6 times.

The 1.2 kg soil filled the plastic pots (φ19 cm×height 17 cm). When the soil reached 2/3 of the pot, we added 25 g inoculum uniformly, then covered the rest of the culture on the top. The inoculum was sterilized at 121ºC for 4 hours as uninoculated treatment. To keep the same soil bacteria series except AMF, the uninoculated treatment was supplied by 5 ml filtrate that was passed through a 40 mesh sieve. The pots were watered by deionized water to maintain moisture content of 15% on a dry soil basis (55% water-holding capacity). The experiment was conducted in a greenhouse with 16 h/27ºC day time and 8 h/22ºC night under natural light conditions.

Harvest and Samples Analysis

Nine weeks later, all the plants were harvested and separated by shoots and roots. The roots were carefully washed with tap water and deionized water. The roots were divided into two parts: the fresh part was used to determine colonization and the remainder was used to determine the dry weight, heated at 65ºC for 48 h. Forty-five 1-cm fine root fragments were collected from switchgrass roots for each replicate to determine the rate of colonization stained with aniline blue [28-29]. The dried materials were milled and digested by HNO_3 and H_2O_2 (3:1 v/v) in a microwave-accelerated reaction system (MarsX; CEM) with a three-step digestion process. The concentrations of Cd, P, Ca, K, Na, Mg, and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS; model 7500a; Agilent Technologies). The samples without plant material addition were used for quality control to determine Cd and nutrition concentration. The soil Cd speciation was determined by the European Community Bureau of Reference (BCR) 3-step sequential extraction procedure.

Statistical Analysis

The translocation factor (TF) (Equation 1), the mycorrhizal response of Cd, P, Ca, Cu, Fe, K, Mg, Na, Zn and the mycorrhizal biomass response (Equation 2) were calculated to evaluate plant Cd tolerance and accumulation ability by below equations.

$$\text{Translocation factor} = \frac{\text{shoot metal concentration of dry plant}}{\text{root metal concentration of dry plant}} \quad (1)$$

$$\text{Mycorrhizal response(\%)} = \frac{\text{elements content (M plant)} - \text{mean element content (NM plant)}}{\text{mean element content (NM plant)}} \times 100 \quad (2)$$

The data mean values for all treatments were compared using Duncan's multiple range test with SPSS17.0 software. The Cd concentration, soil Cd species, and nutrition concentration (P, Ca, Fe, K, Mg, Na, and Zn) were analyzed with a two-way ANOVA to evaluate the influences of mycorrhizal inoculation treatment and Cd addition.

Results and Discussion

Root Colonization and Biomass

The root colonization under three Cd addition levels is shown in Fig. 1a). With 0 and 10 mg/kg Cd addition, the switchgrass roots were greatly colonized by AMF, which reached 80%; however, colonization decreased to 41% with the 40 mg/kg Cd addition ($P<0.01$).

The shoot and root biomass were significantly affected by the AMF inoculation under different Cd treatments ($P<0.001$, Fig. 1b). The biomass of inoculated switchgrass was significantly higher than in the corresponding noninoculated treatment with the 0 and 10 mg/kg Cd addition, and was increased by 320% and 477% in the shoots, and 270% and 858% in the roots, respectively. There were no significant differences with AMF absence and presence under the 40 mg/kg Cd addition level ($P>0.05$).

Biomass is the most important factor for evaluating whether a plant could be an energy crop or phytoremediation species. Compared with noninoculated treatment, the shoot and root biomass in the presence of *R. irregularis* increased by 320% and 270%, respectively, for the 0 mg/kg Cd treatment, and increased by 477% and 858% for the 10 mg/kg Cd treatment. RI-symbiosis growth acceleration was observed in *Helianthus annuus*, *Lotus japonicas*, and *Oryza sativa* under different Cd addition levels [17, 30-31]. AMF inoculation could improve plant photosynthesis and, through external hyphae, expand root surface to improve plant nutrition status. However, there were no significant differences between inoculated and non-inoculated samples with the 40 mg/kg Cd treatment; even the biomass mycorrhizal response was negative when soil Cd reached 40 mg/kg. Zhang et al. found the host plants growth promotion blocked when the soil Cd reached

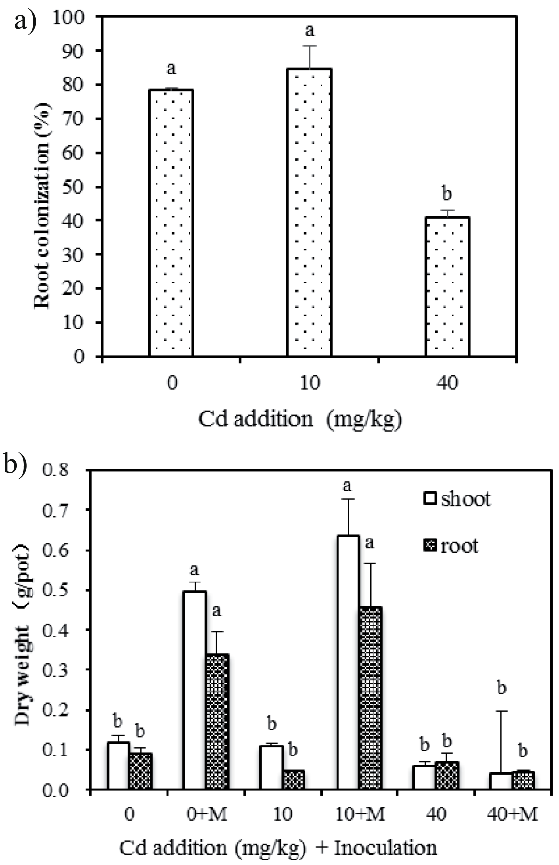

Fig. 1. The root colonization and dry weights of switchgrass with AMF inoculation under three Cd levels. M represent inoculation with *Rhizophagus irregularis*. Bars above the columns represent the standard errors (n = 3). Different letters above the column represent the significant differences under three Cd level, regardless of inoculations. These descriptions are same to the blow figures.

50 mg/kg, which indicated that AMF acted as an obstruction in the plant under high Cd stress [17].

Cd and P Accumulation in Shoots and Roots

The Cd and P concentrations of switchgrass in shoots and roots under three Cd addition levels with inoculation and noninoculation are shown in Table 1. The results show that these concentrations were significantly influenced by Cd addition and AMF inoculation. P concentrations were affected by their interaction ($P<0.01$, $P<0.001$, Table 2), whereas nonsignificant differences were observed in root Cd concentration by inoculation and their interaction, and were the same as the effect of Cd addition on the shoot P concentrations ($P>0.05$, Table 2).

As the soil Cd levels increased, the shoot and root Cd concentrations decreased with AMF symbiosis, with the 10 and 40 mg/kg Cd additions, the Cd levels decreased by 68%, 36% in the shoots, and -8% and 11% in the roots, respectively. The Cd translocation factors (TFs) were higher in the 0 and 10 mg/kg Cd treatment without AMF symbiosis, and the Cd TFs for the AMF-inoculated samples were lower than those of noninoculated samples irrespective of Cd addition. Substantial increases were observed in shoot and root P concentrations with AMF presence under all Cd treatments, with increases of 702%, 271%, and 332% in the shoots, and 285%, 99%, 143% in the roots for the 0,10 and 40 mg/kg Cd treatments, respectively. The TFs of P remained stable under three Cd concentrations, irrespective of inoculation; however, the inoculated TFs of P were higher than those of the corresponding noninoculated sample under the three Cd concentrations.

Table 1. The concentration and translocation factor of Cd (mg/kg) and P (g/kg) in switchgrass with AMF inoculation under three Cd levels.

Cd level(mg/kg)	AMF	Cd Concentration			P concentration		
		Shoot	Root	TF-Cd	Shoot	Root	TF-P
0	NM	0.20±0.0.17b	0.63±0.09d	0.68±0.63	0.47±0.14c	1.02±0.02d	0.47±0.14b
	M	0.07±0.03b	0.73±0.13d	0.11±0.03	3.77±0.39a	3.93±0.07a	1.11±0.09a
10	NM	21.73±4.17a	68.43±12.05c	0.38±0.03	0.59±0.13c	0.81±0.06d	0.72±0.14b
	M	7.03±1.60b	73.77±3.62c	0.09±0.02	2.19±0.25b	1.61±0.08c	1.36±0.11a
40	NM	36.74±3.8a	281.97±14.48a	0.13±0.02	0.59±0.11c	0.79±0.08e	0.78±0.19ab
	M	23.43±7.25a	250.47±20.44b	0.09±0.02	2.55±0.55b	1.92±0.17b	1.15±0.35a
Significance of							
Cadmium (Cd)		***	***	ns	ns	***	**
Inoculation (I)		**	ns	ns	***	***	ns
Cd×I		ns	ns	ns	*	***	ns

NM and M represent noninoculated and inoculated with *Rhizophagus irregularis*, respectively. Different letters in the same column represent the significant differences among three Cd levels, regardless of inoculation. ns represent $P>0.05$, * represent $P<0.05$, ** represent $P<0.01$,*** represent $P<0.001$. It is same in the below tables.

These results showed that AMF symbiosis took a contrasting strategy to P and Cd translocation.

In our study, AMF inoculation reduced switchgrass shoot Cd concentrations with the 10 and 40 mg/kg Cd additions (Table 1). These results were consistent with those of Aghababaei et al. and Jiang et al., who reported that inoculated R. irregularis obviously decreased Cd concentration in the shoots of *Zea mays* and *Lonicera japonica* under the 10 and 20 mg/kg Cd conditions [32-33]. The decreased Cd concentrations might be a result of the dilution effects of biomass promotion by AMF inoculation. However, the findings disagreed with the above results reported by Zhang et al. and Liu et al. who found AMF could improve Cd concentrations in both shoots and roots in *Lotus japonica*s and *Solanum nigrum* with different Cd addition levels [17, 19]. It is possible that the contradictory Cd concentration in plants may be explained by the varied plant and AMF species used or due to the different soil characteristics (pH, Cd concentration) [34].

AMF symbiosis not only affected the absolute Cd concentration in the tissue, but also alerted their translocation. In the present study, the TF of Cd with AMF symbiosis was lower than that of the corresponding non-inoculated treatment at every Cd level, and the TF values were much lower than 1. It was the consensus that AMF could alleviate Cd direct damage to photosynthetic organs by inhibiting Cd translocation from the root to shoot compared with AMF absence. AMF helped the plant to retain most of the Cd in mycorrhizal roots by compartmenting Cd in root cell walls and vacuoles or directly immobilizing the contaminants in the structure of AMF, such as the extra- or intra-radical mycelia, arbuscular or vesicles [22-23, 35]

AMF are known to increase plant nutrition status, especially for P levels. In the present study, the P concentrations with AMF inoculation were higher than with noninoculated treatment for all Cd levels regardless of plant tissue, and a positive mycorrhizal of P response was observed in each level (Table 1, Table 3). Previous results suggested that the increased plant tissue P concentrations could be another strategy to counteract Cd toxicity, regardless of inoculation [17, 36]. In addition to accelerating plant growth, P could alleviate Cd toxicity by decreasing tissue Cd uptake or forming phosphate complexes to chelate Cd on the root surface [16]. P could also enhance the sequestration of Cd in AMF by the accumulation of polyphosphate in the AMF extraradical hyphae [22, 23]. Therefore, the regulation of P nutrition could be a mutually beneficial action for plant growth and Cd detoxification in plant tissue.

Soil Cd Sspecies

The amounts of acid-extractable, reducible Cd in the plants were significantly affected by Cd addition and inoculation treatment ($P<0.001$, $P<0.05$), and the concentrations of acid-extractable, reducible and oxidizable Cd were increased with the soil Cd increasing (Table 2). The reducible-Cd content with 10 mg/kg Cd addition and acid-extractable content with 40 mg/kg Cd addition with *R. irregularis* symbiosis were both lower than that of the corresponding noninoculated treatment. However, no significant difference was observed for other soil Cd species between the noninoculated and inoculated samples under different Cd addition levels ($P>0.05$).

The bio-toxicity of heavy metals was regulated by the total metal amount and metal species, with the latter determining metal bioavailability. Our results showed that the main Cd species was acid-extractable, as shown by Chen et al. [1]. Although the inoculated treatment did not affect the total Cd concentration in soil, the present study found that *R. irregularis* could change soil Cd species, decreasing acid-extractable Cd at low Cd levels and reducible-Cd at high Cd stress with RI symbiosis. Previous studies indicated that soil Cd species might change into more stable forms, but owing to the limitations of our experimental apparatus, we could not confirm whether the Cd transformed into an exchangeable or residual form [37-38]. AMF could

Table 2. The concentration of three Cd species (acid-extractable, reducible and oxidizable Cd) in the soil with AMF inoculation under three Cd levels.

Cd level	Acid-extractable Cd		Reducible-Cd		Oxidizable-Cd	
	NM	M	NM	M	NM	M
0	0	0	0	0	0	0
10	10.41b	10.61b	11.67a	10.86 b	0.00 c	1.05c
40	44.93a	41.8 8b	8.97c	9.68c	3.59d	4.97d
Significance of						
Cd	***		***		***	
I	**		*		ns	
Cd×I	**		ns		ns	

Different letters in the same Cd level represent the significant differences among three Cd levels, regardless of inoculation.

Table 3. Mycorrhizal response of biomass (%MGR), Cd (%MCdR), P(%MPR), Ca(%MCaR), Cu(%MCuR), Fe(%MFeR), K(%MKR), Mg(%MMgR), Na(%MNaR), Zn(%MZnR) in the soil under three Cd additions.

	0	10	40
%MGR	298.5±59b	589.8±155c	-35.4±5a
%MCdR	-28±13	8±6	-14±6
%MPR	381±31a	170±23b	196±38b
%MCaR	2±5a	-40±10b	23±4a
%MFeR	-15±9	-23±13	6±20
%MKR	112±7a	57±18b	9±8c
%MMgR	1±5	-8±9	10±5
%MNaR	104±11	74±4	68±15
%MZnR	3±7a	-44±10b	-63±2b

Different letters in the line represent the significant differences among three Cd levels.

secrete glomalin to chelate toxic elements and alter the soil pH in order to influence metal availability [24]. Some researchers also found that AMF symbiosis raised metal bioavailability in plants. Subramanian et al. reported that under different Zn addition levels, inoculated AMF increased organically bound Zn with decreasing residual and crystalline oxides of Zn [39]. Wu et al. reported that with AMF inoculation treatment, the main Cr species in soil was oxidizable-Cr, and acid-extractable Cr concentrations decreased in dandelion (*Taraxacum mongolicum*) in the presence of *R. irregularis* presence [40]. In addition, Kumar et al. reported that AMF could regulate plants to secrete various compounds to alter the soil metal species, encourage translocation of essential metals (e.g., Zn and Cu) to shoots, and fix toxic metals (e.g., Cd and Pb) in roots or the rhizosphere to avoid destroying photosynthetic organs [41].

Mineral Elements Concentration

The concentrations of Ca, Fe, K, Mg, Na, and Zn in the shoots and roots of switchgrass are shown in Fig. 2. The concentrations of Ca, Fe, K, Mg, and Na in the shoots and K and Mg in the roots were significantly affected by Cd addition. AMF inoculation significantly affected concentrations of Ca, K, Mg, and Na in the shoots and Ca, K, Mg, Na, and Zn in the roots; Cd addition and AMF inoculation showed significant observable influences on Ca and K contents the in shoots and roots of K and Mg content in the roots ($P<0.05$; $P<0.01$; $P<0.001$, Table 4).

The concentrations of Ca, K, Mg, and Na in the shoots, and Na and K in the roots with AMF symbiosis were higher than those in the corresponding noninoculated samples under the three Cd addition levels, the exception was for Ca and K contents in the shoots with the 10 mg/kg Cd treatment and K content in the roots with the 40 mg/kg Cd treatment – none of which had any significant differences either with or without AMF inoculation. However, diminishing trends were observed in roots for the Mg content at all Cd levels and for the Zn content with the 10 and 40 mg/kg Cd addition: in the roots, the Mg levels decreased by 28%, 35% and 5%, for 0,10 and 40 mg/kg Cd additions, respectively; and the Zn levels decreased by 18%, 24% in the shoots, 28% and 24% in the roots with the 10 and 40 mg/kg Cd additions, respectively. For the inoculated treatment with 10mg/kg Cd, the roots Ca content and shoots Fe content were significantly lower than non-inoculated treatment, which decreased by 57% and 29%, respectively.

Except for P, inoculation with AMF also influenced the plant strategy for element nutrient uptake. In the absence of Cd, the concentrations of Ca and Mg in the shoots, and K and Na in the shoots and roots with AMF symbiosis were higher than non-inoculated treatment. A previous study showed that under acid stress AMF could increase switchgrass absorption of P, Ca, Mg and Zn to reduce Fe absorption [26]. Sarkar et al. reported that K, Mg, Fe, Cu, and Zn concentrations increased in *Miscanthus sacchariflorus* with AMF symbiosis in river sand [42]. For now, a consensus has not been reached on the relationship between Cd and the mineral elements due to synergy or competition, not to mention the interaction from Cd and AMF. Wang et al. found that for *Phragmites Australis*, inoculation with *R. irregularis* increased the total concentrations of Cu, Fe, and Ca when the soil Cd is in the range of 0.01-20 mg/L [43]. In our study, the root Ca, Mg, and Zn levels decreased with AMF symbiosis under the 10mg/kg Cd addition, and the same trends were detected for the shoots Fe and Mg concentrations. The reason for this observation may be because of the “dilution effect” of plant biomass promotion with AMF. In addition, the shoot Ca, Mg, and Na with 40 mg/kg Cd in the soil were higher than with 0 mg/kg Cd. These results suggested that AMF could enhance Ca, Mg, and Na translocations in the shoots to limit Cd translation from roots to shoots under high Cd levels, which is similar to the results reported by de Andrade et al. in sunflowers with AMF symbiosis [30]. The K concentrations with 10 and 40 mg/kg Cd addition were lower than with the 0 mg/kg Cd additions, similar to the results reported by Wang et al. [43]. This finding might be related to the relationship between Cd and the channel that regulates K^+ transmembrane transport from solutions, which would open when Cd^{2+} were the binding metal ion instead of K^+ [44-45]. Thus, excessive Cd obstructed K absorption by binding competition within the channel, resulting in decreased K absorption, regardless of inoculation.

Mycorrhizal Response

The Mycorrhizal response of total biomass and the Cd, P, Ca, Cu, Fe, K, Mg, Na, and Zn concentrations under the three Cd addition levels are shown in Table

3. The mycorrhizal response of the biomass initially increased and then decreased when the soil Cd reached 40mg/kg. The mycorrhizal responses of P, Ca, K and Zn significantly decreased with soil Cd increases ($P<0.05$), but the mycorrhizal response of Cd, Fe, Mg and Na had no significant differences among three Cd levels ($P>0.05$). The total %MCdR were positive under the 10 mg/kg Cd and negative under the 40 mg/kg Cd addition levels.

Wu et al. reported the negative mycorrhizal response of Cr in Bermudagrass with *R irregularis* presence [40]. This outcome was in contrast to that of Zhang et al., who found the positive response to Cd in *Lotus japonicas* with *R irregularis* symbiosis. This suggested that mycorrhizal response to heavy metal depends on the contaminant and plant species [17]. In addition, the %MCdR was negative in shoots for 10 mg/kg and positive in roots for 10 mg/kg Cd levels, and the results further verified that AMF accelerated the Cd accumulation in the roots under low Cd addition. Negative responses to Ca (10 mg/kg), Mg (10 mg/kg) and Zn (10 and 40 mg/kg) were detected, possibly because AMF inhibited their absorption to inhibit Cd intake. Considering the negative response observed for Fe with AMF symbiosis, the correlation analysis showed that Fe was significantly negative

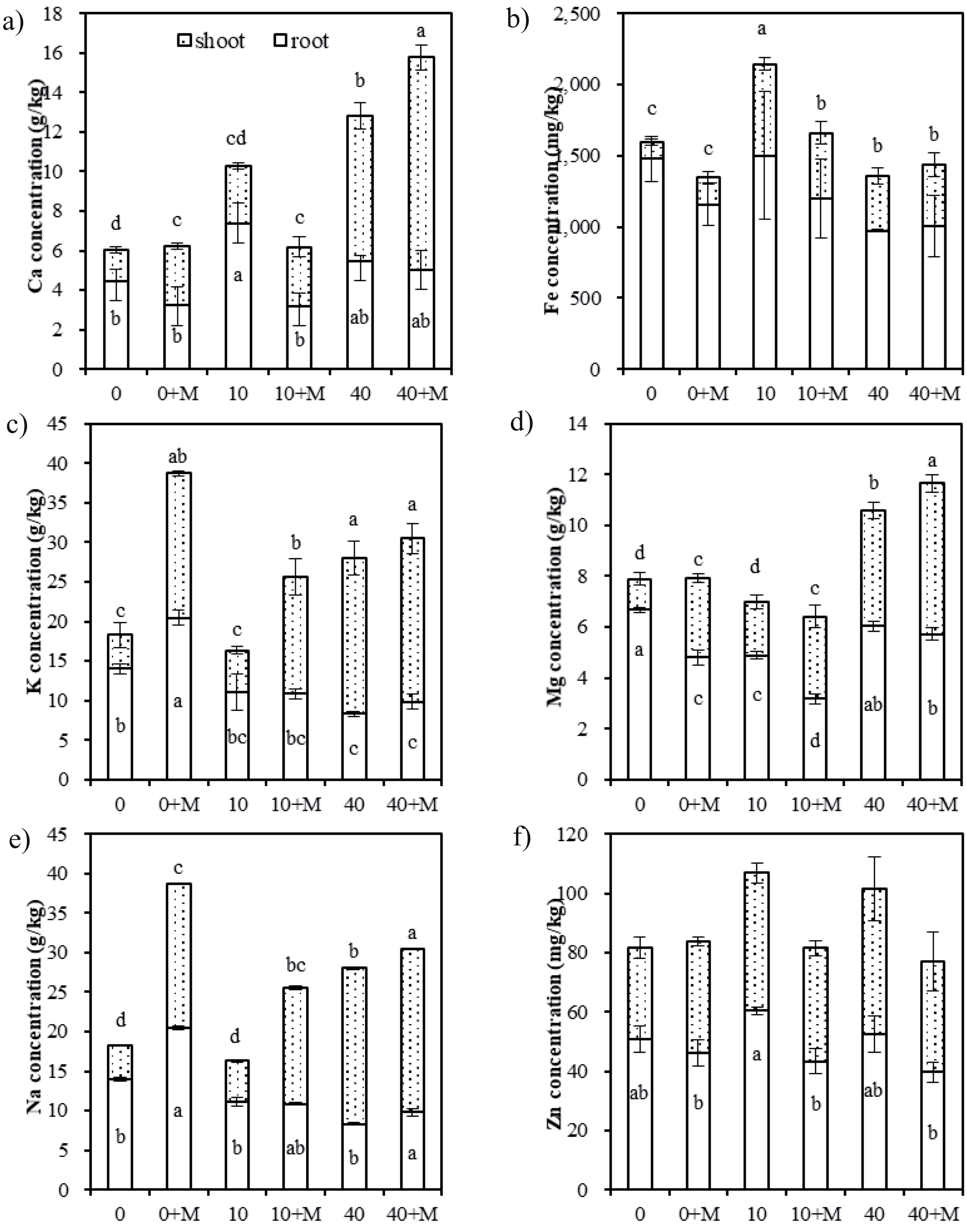

Fig. 2. The concentrations of Ca, Cu, Fe, K, Mg, Na, and Zn in switchgrass with AMF inoculation under three Cd levels.

Table 4. ANOVA analysis the effect of Cd addition and inoculation treatment on Ca, Fe, K, Mg, Na and Zn concentrations.

	Cd		Inoculation		Cd×I	
	Shoot	Root	Shoot	Root	Shoot	Root
Ca	0.000***	0.222	0.001**	0.020*	0.009**	0.127ns
Fe	0.000***	0.311ns	0.697ns	0.354ns	0.085ns	0.735ns
K	0.000***	0.000***	0.000***	0.017*	0.006**	0.036*
Mg	0.000***	0.000***	0.000***	0.000***	0.455ns	0.006**
Na	0.000***	0.495ns	0.000***	0.000***	0.167ns	0.380ns
Zn	0.341	0.413ns	0.409	0.006**	0.338ns	0.356ns

ns represent P>0.05, * represent P<0.05, ** represent P<0.01,*** represent P<0.001.

associated with P concentration. A previous study found that P deficiency induced the formation of brown iron plaques in the *Oryza sativa* root surface and accelerated plant P absorption [46], therefore, it was assumed that adequate P with AMF presence might have antagonistic effects on Fe absorption in return.

Conclusions

The main contributions of RI inoculation for switchgrass, in our study, were enhanced biomass and P nutrition, decreased Cd absorption and translocation from root to shoot, passivated soil Cd bioavailablility, decreased Cd and nutrient elements levels under a low Cd addition, and enhanced Cd antagonism effects with mineral elements under high Cd addition. Therefore, inoculation with AMF could relieve Cd toxicity in the switchgrass seedling stage. Since our study was conducted in the short-term in greenhouse conditions, more studies are needed to compare the different growth periods of switchgrass inoculated with AMF under artificial or field conditions and Cd stress. More physiological and molecular mechanisms are needed to illuminate AMF function under Cd stress.

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Conflict of Interest

The authors declare no conflict of interest.

Reference

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