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ARTICLE

The Application of Fertilizer and AMF Promotes Growth and Reduces the Cadmium and Lead Contents of Ryegrass (*Lolium multiflorum* L.) in a Copper Mining Area

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ABSTRACT

Heavy metal-polluted soil was collected from the Pulang copper mine in Shangri-La City, Yunnan Province, Southwest China. The effects of fertilizer (organic and inorganic) and arbuscular mycorrhizal fungi (AMF) on ryegrass (*Lolium multiflorum* L.) growth, root morphology, mineral nutrition and cadmium (Cd) and lead (Pb) contents were investigated by pot experiments. The results showed that both fertilizer and AMF significantly ameliorated the root morphology and mineral nutrition, reduced the Cd and Pb contents, and promoted the growth of ryegrass. Among all treatments, the combined application of organic–inorganic compound fertilizer with AMF had the highest effect, resulting in increases in root length, surface area and branch number by 2.3, 1.1, and 3.9 times, respectively; an 88% increase in plant biomass; a nitrogen content increase of 2.3 and 1.2 times, and phosphorus content increase of 62% and 68% in shoots and roots, respectively was also recorded as well as decreases in Cd content by 34% and 62% and Pb content by 47% and 34% in shoots and roots, respectively. Two-factor analysis showed that both fertilizer and AMF significantly promoted ryegrass growth (plant height, biomass, chlorophyll content, root length, nitrogen and phosphorus content) and reduced the Cd and Pb contents in roots, and there was a synergistic effect between them. Moreover, the nitrogen and phosphorus contents were very significantly positively correlated with the shoot and root biomasses but very significantly negatively correlated with the Cd and Pb contents of ryegrass. Thus, the application of fertilizer and AMF synergistically improved ryegrass growth on polluted soils in the copper mining area.

KEYWORDS

Arbuscular mycorrhizal fungi; heavy metal pollution; organic–inorganic compound fertilizer; ryegrass; synergistic effect

1 Introduction

The content of cadmium (Cd) and lead (Pb) in the soil exceeds set standards due to anthropic activities, such as mining, utilization of mineral resources, industrial and agricultural production, which are highly damaging to soil quality and plant growth [1]. The soil structure around the wasteland mining area is poor, which leads to the destruction of soil microbial activity and community structure and significantly inhibits plant growth [2,3]. Therefore, it is difficult to restore vegetation in wasteland, and it is urgent to strengthen the improvement of soil physical and chemical properties in mining areas [4]. Vegetation restoration and ecological reconstruction in wasteland have received extensive attention [5].

Fertilizer is one of the most effective and commonly used measures in soil improvement and can enhance soil fertility and nutrient contents, promote plant growth, and help restore vegetation in mining wasteland [6]. Additionally, arbuscular mycorrhizal fungi (AMF) are a group of microbes that commonly exist in plant roots and form mutualistic symbionts with host plants to help plants adapt to various adverse habitats [7]. AMF increase the absorption of mineral nutrients by plants through the extension and expansion of extraradical mycelium [8], improve soil structure and play an important role in the formation of soil aggregates [9], improve the stress resistance of host plants and promote revegetation of mining wasteland [10]. Thus, both fertilizer and AMF can promote plant growth, and there is often a synergistic effect between fertilizer and AMF.

Ryegrass (*Lolium multiflorum* L.) has characteristics of fast growth, strong regeneration ability, high yield and strong adaptability. It is often used as a pioneer plant for vegetation restoration in mining wastelands [11,12], and its growth requires a large amount of nutrients [13]. The rational application of fertilizer can improve soil fertility and the quality and yield of ryegrass [14]. Moreover, AMF can improve the resistance of plants under heavy metal stress, which is beneficial for improving the viability of ryegrass in plateau alpine mining wasteland [15]. Previous studies indicated that the single application of fertilizer or AMF was beneficial to ryegrass growth. However, the effect of the combined application of fertilizer and AMF on soil quality and vegetation restoration was relatively limited.

The Pulang copper mine is a giant copper deposit located in Shangri-La City, Yunnan Province, Southwest China. The mining area is 6.0521 km^2 at an altitude of 3600–4500 m and belongs to the cutting structure erosion landform in an alpine valley. The exploitation and utilization of Pulang copper mine resources have resulted in the destruction of the surrounding soil quality [16]. However, there are no reports on the effects of fertilizer and AMF on the growth of ryegrass in waste highland mining areas.

In the present study, polluted soil from the Shangri-La Pulang copper mine was collected, and ryegrass was used as the test plant. A pot experiment was carried out with single and combined applications of fertilizer (organic fertilizer, inorganic fertilizer, organic and inorganic compound fertilizer) and AMF. The effects of fertilizer and AMF on ryegrass growth and the contents of Cd and Pb were investigated. We hypothesized that there is a synergistic effect between fertilizer and AMF, which can collectively improve the root morphology and mineral nutrient content of ryegrass, reduce the Cd and Pb contents of plants, and then contribute to the growth of ryegrass.

2 Materials and Methods

2.1 Materials

The soil tested was the Shangri-La Plang copper mine tailings pond (28°04′31″N; 99°97′25″E; altitude 3590.1 m) soil. Its basic chemical properties are as follows: pH value is 3.6, organic matter content is 53.77 g/kg, total nitrogen, total phosphorus and total potassium contents are 1.47, 0.44, 18.89 g/kg, respectively, and the contents of Cd and Pb are 2.57 and 42.46 mg/kg, respectively.

The ryegrass variety used in this experiment was *Nicaragua*. Selected ryegrass seeds with full grains and the same size were soaked in H_2O_2 with a volume percentage of 10% for 10 min prior to being used to kill the bacteria on the surface of the seeds, and then rinsed with sterile water 3 times.

The tested AMF inoculant was *Funneliformis mosseae*, which was provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry (No. BGC YN05 1511C0001BGCAM0013). In the greenhouse of Yunnan Agricultural University, the propagation of potted sandy soil was adopted. Using maize as the propagation host, the rhizosphere sandy soil mixture of maize root segments, mycorrhizal fungal spores and extra root hyphae was collected after 2 months, and the spore density was 28 n/g.

The organic fertilizer was provided by Lanping Science and Technology Agricultural Development Co., Ltd., China, the organic matter content was ≥ 50%, and the total nutrients ($N + P_2O_5 + K_2O$) were ≥ 5%. The nitrogen, phosphorus and potassium fertilizers in the inorganic fertilizer were urea, sodium dihydrogen phosphate and potassium chloride, respectively, expressed as ($N + P_2O_5 + K_2O$)%, and the ratio of nitrogen, phosphorus and potassium was $N:P_2O_5:K_2O = 1:0.5:0.8$.

2.2 Experimental Treatments

The experiment used a 3-Factor 2-Level experimental design (Table 1). The 3 factors were organic fertilizer, inorganic fertilizer and AMF. Each pot soil amount was 800 g, and each treatment was set with 4 parallel pots, for a total of 32 pots. A total of 700 g of sterilized soil was placed into a pot, fertilizer and AMF were applied to the ryegrass seeds, and spread another 100 g of sterilized soil was spread to cover ryegrass seeds, fertilizer and AMF.

Table 1: Test design

Treatment	Number	Organic fertilizer	Inorganic fertilizer	AMF
Control	CK	0	0	0
Organic fertilizer	O	1	0	0
Inorganic fertilizer	I	0	1	0
AMF	F	0	0	1
Organic and inorganic compound fertilizer	OI	1	1	0
Combination of organic fertilizer with AMF	OF	1	0	1
Combination of inorganic fertilizer with AMF	IF	0	1	1
Fertilizer with AMF	OIF	1	1	1

Note: 0 means without application; 1 means application.

A single application of organic fertilizer dosage was 10 g/pot; a single application of inorganic fertilizers was as follows: N (0.24 g/kg soil), P_2O_5 (0.12 g/kg soil), K_2O (0.16 g/kg soil); in the organic–inorganic compound fertilizer, the application amount of inorganic fertilizer accounted for 9%, and the application amount of organic fertilizer was 9 g (accounting for 91%); the application rate of AMF was 40 g/pot.

2.3 Determination of the Arbuscular Mycorrhizal Fungal Colonization Rate and Spore Number

Fresh and tender roots were collected, washed with distilled water, then cut into 1 cm root segments. The root segments were decolorized in a constant-temperature (90°C) water bath of 10% KOH solution for 30 min until transparent, and washed with sterile water 3 times. Then, 2% HCl solution was added to acidify the samples for 5 min, and they were rinsed with sterile water 3 times. The roots were stained with acid fuchsin lactic acid glycerol staining solution, left overnight at room temperature, and decolorized with lactic acid. After decolorization, relatively complete root segments were selected and arranged on glass slides for microscopic examination [17].

The number of spores was detected by the wet sieve pouring sucrose centrifugation method [18]: 10 g of naturally dry rhizosphere soil was placed in a beaker, 500 mL of sterile water was added, stirred clockwise, allowed to stand for 10 s, and the upper suspension solution was passed through 80-mesh and 400-mesh sample sieves in turn. The 400-mesh residue was washed into a 50 mL centrifuge tube with sterile water and centrifuged at 3000 r/min for 5 min. The supernatant was collected, then mixed with 50% sucrose solution, and centrifuged again for 10 min. The supernatant was passed through a 400-mesh sieve and gently rinsed into a clean Petri dish with filter paper, and the number of AMF spores was counted under a microscope.

2.4 Determination of Chlorophyll Content in Plant Leaves

Chlorophyll content was determined using the 95% ethanol extraction method [19]: We weighed 0.2 g of fresh plant leaves, added a small amount of quartz sand, calcium carbonate and 3 mL of 95% ethanol, ground into a homogenate, added 10 mL of 95% ethanol, continued grinding until the tissue turned white, and transferred it all into a 25 mL brown volumetric flask, and finally filling the container to the mark with 95% ethanol. After shaking, the absorbance was measured at wavelengths of 665 and 649 nm, and the content of chlorophyll ab was calculated by the absorbance.

2.5 Determination of Plant Height, Biomass and Root Morphology

The plant height was measured with a tape measure, and the plants were divided into shoots and roots, rinsed three times with tap water and subsequent distilled water, and the complete root system was removed and evenly spread on an Epson Perfection V700 scanner (Seiko Epson, Japan) for scanning. Root length, root surface area, root mean diameter and branch number were analyzed using Win RHI-ZO PRO STD 4800 (Regent, Canada) root image processing software. The samples were fixed at 105°C for 30 min, dried at 75°C to constant weight, weighed, and their dry weight was recorded. The samples were passed through a 0.25 mm sieve after pulverization.

2.6 Determination of Nitrogen and Phosphorus Contents in Plants

We weighed 0.1 g of the ground and dried plant sample, placed it in a 100 mL Kelvin bottle, wetted the sample with distilled water, added 5 mL of concentrated sulfuric acid, shook gently overnight, and placed a curved neck funnel on a bottle. The samples were slowly heated in a digestion furnace and gradually heated when the concentrated sulfuric acid decomposed and emitted white smoke. When the solution was brown–black, the Kettle bottle was removed, 10 drops of 30% hydrogen peroxide were added after cooling, and the mixture was shaken. The mixture was heated to a slight boiling for 20 min, which was repeated 2~3 times until the solution was colorless or clear, filtered into a 50 mL volume, again filled to the mark, then the filtrate was used to measure the contents of nitrogen and phosphorus. The plant nitrogen content was determined using the distillation method, and the plant phosphorus content was determined by the molybdenum antimony resistance colorimetric method [20].

2.7 Determination of Cadmium and Lead in Plants

The Cd and Pb contents in plants were determined by the HNO_3-H_2O_2 digestion method [20]: 0.5 g of sample powder was weighed into a digestion tank, 5 mL of nitric acid was added and soaked overnight, 3 mL of 30% hydrogen peroxide solution was added, the inner cover was covered, the stainless steel jacket was tightened, and the sample was placed into a constant temperature drying box and kept at 160°C for 4 h, resulting in a transparent digestive juice after digestion. After opening, the digestion solution was washed into a 50 mL volumetric flask with distilled water, and the inner tank and the inner lid were washed 3 times and brought up to the mark with distilled water. After mixing, the contents of Cd and Pb in plants were determined by flame atomic absorption spectrophotometry.

2.8 Statistical Analysis

The test data were the mean value of 4 replicates, and Microsoft Excel 2013 was used to calculate the mean value and standard error, which were expressed as the mean ± standard deviation. IBM SPSS 21.0 software was used to conduct univariate analysis of the data. Duncan's method was used to test the differences at the 0.05 level, and Pearson's correlation analysis was conducted between plant nitrogen and phosphorus contents and Cd and Pb contents. Two-way ANOVA was performed on the data with fertilizer and AMF as factors. Graphs were drawn using Origin Pro 9.0.

3 Results

3.1 Colonization of Arbuscular Mycorrhizal Fungi

Table 2 shows that fertilizer significantly reduced the infection rate of AMF on the roots of ryegrass and the spore content in the soil. Compared with the inoculation of AMF alone, the infection rates of the combination of organic fertilizer with AMF, combination of inorganic fertilizer with AMF and combination of organic–inorganic compound fertilizer with AMF were reduced by 17%, 39%, and 19%, respectively, and the number of spores was reduced by 27%, 50%, and 28%, respectively. Among the treatments inoculated with AMF, the combination of inorganic fertilizer with AMF treatment was the most unfavorable for the colonization of AMF.

Table 2: Colonization rate of AMF in ryegrass roots and spore number

Treatment	Colonization rate (%)	Spore number (n/g)
F	19.5 ± 1.3 a	12.3 ± 1.1 a
OF	16.2 ± 0.9 b	9.0 ± 0.8 b
IF	11.8 ± 1.1 c	6.2 ± 1.1 c
OIF	15.7 ± 0.7 b	8.8 ± 1.1 b

Note: The data in the table are the average of 4 replicates ± standard deviation. Different lowercase letters indicate significant differences between treatments (P < 0.05). F: AMF; OF: combination of organic fertilizer with AMF; IF: combination of inorganic fertilizer with AMF; OIF: Fertilizer with AMF.

3.2 Effects of Fertilizer and Arbuscular Mycorrhizal Fungi on Ryegrass Growth

As shown in Fig. 1, compared with CK, both applications of fertilizer and AMF significantly increased the plant height and shoot and root biomass of ryegrass. AMF, organic fertilizer, inorganic fertilizer and organic–inorganic compound fertilizer increased plant height by 34%, 33%, 51%, and 36%, respectively, and increased shoot biomass by 23%, 21%, 32%, and 31%, respectively, and root biomass by 320%, 112%, 144%, and 176%, respectively.

Compared with no AMF inoculation, the combination of organic fertilizer with AMF treatment significantly increased plant height and root biomass, the increase was 9% and 105%, respectively; the combination of inorganic fertilizer with AMF treatment increased by 4% and 83%, respectively; the increase of combination of organic–inorganic compound fertilizer with AMF treatment was 18% and 70%, respectively. It can be seen that applications of fertilizer and AMF synergistically promote the growth of ryegrass. Two-way ANOVA showed that fertilizer and AMF had highly significant effects on ryegrass plant height and biomass, and there was an interaction between the two on ryegrass height and biomass.

3.3 Effects of Fertilizer and Arbuscular Mycorrhizal Fungi on the Chlorophyll Content of Ryegrass

As shown in Fig. 2, both fertilizer and AMF significantly enhanced the chlorophyll content of ryegrass leaves compared with CK. AMF, organic fertilizer, inorganic fertilizer and organic–inorganic compound fertilizer increased the content of chlorophyll a by 129%, 143%, 549%, and 382%, respectively, and increased the content of chlorophyll b by 135%, 156%, 613%, and 422%, respectively.

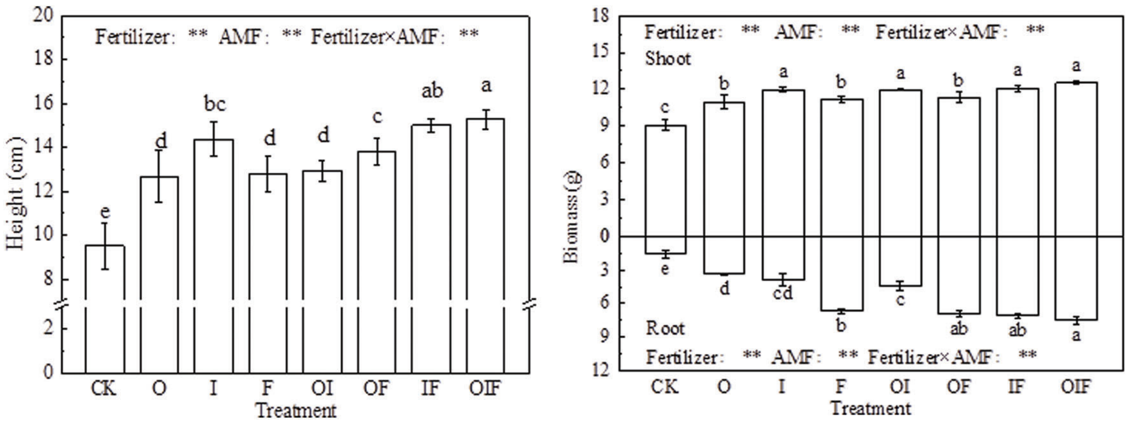

Figure 1: Effects of fertilizer and AMF on the growth of ryegrass; according to the results of AMF and fertilizer two-factor analysis of variance, ns: not significant; *: $P < 0.05$; **: $P < 0.01$. CK: control; O: organic fertilizer; I: inorganic fertilizer; F: AMF; OI: organic and inorganic compound fertilizer; OF: combination of organic fertilizer with AMF; IF: combination of inorganic fertilizer with AMF; OIF: fertilizer with AMF

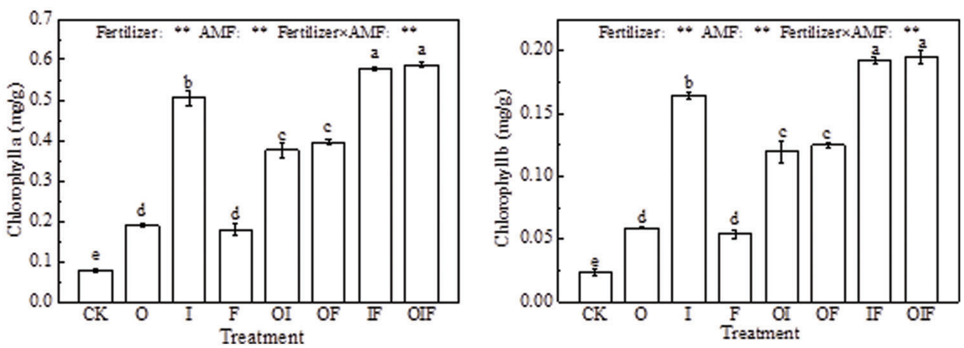

Figure 2: Effects of fertilizer and AMF on the chlorophyll content of ryegrass; according to the results of AMF and fertilizer two-factor analysis of variance, ns: not significant; *: $P < 0.05$; **: $P < 0.01$. CK: control; O: organic fertilizer; I: inorganic fertilizer; F: AMF; OI: organic and inorganic compound fertilizer; OF: combination of organic fertilizer with AMF; IF: combination of inorganic fertilizer with AMF; OIF: fertilizer with AMF

Compared with no AMF inoculation, the combination of organic fertilizer with AMF significantly increased chlorophyll a and chlorophyll b content by 108% and 112% compared with the single application of organic fertilizer; the increase of the combination of inorganic fertilizer with AMF was 14% and 17%, respectively; the combination of organic–inorganic compound fertilizer with AMF treatment increased by 56% and 63%, respectively. It can be seen that applications of fertilizer and AMF synergistically increased the chlorophyll content of ryegrass leaves. Two-way ANOVA showed that both fertilizer and AMF had highly significant effects on the chlorophyll content of ryegrass leaves, and there was an interaction between the two on the chlorophyll content of ryegrass leaves.

3.4 Effects of Fertilizer and Arbuscular Mycorrhizal Fungi on the Root Morphology of Ryegrass

Fig. 3 shows that compared with CK, AMF, organic fertilizer, inorganic fertilizer and organic–inorganic compound fertilizer treatments all increased root length, root surface area and branch number but decreased the average root diameter. Among them, root length increased by 186%, 39%, 93%, and 41%, respectively; the root surface area increased by 93%, 29%, 53%, and 12%, respectively; the number of branches increased by 261%, 109%, 131%, and 87%, respectively; and the average diameter of roots decreased by 33%, 7%, 21%, and 21%, respectively.

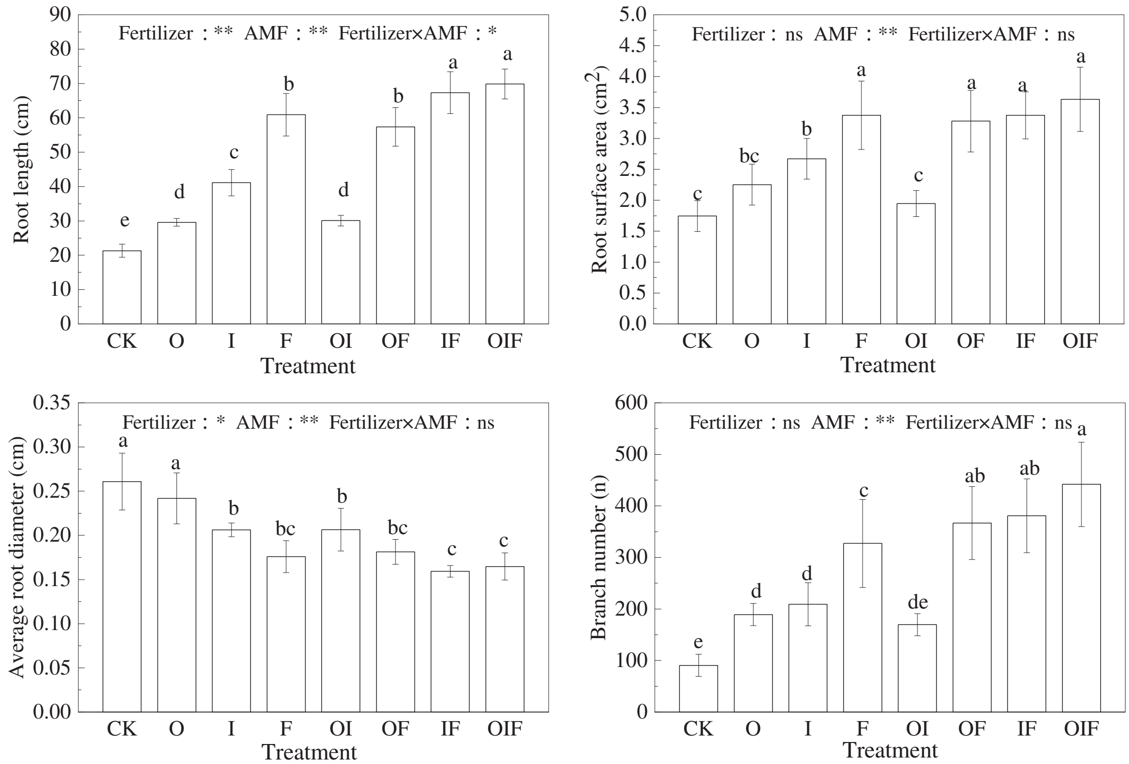

Figure 3: Effects of fertilizer and AMF on the root morphology of ryegrass; according to the results of AMF and fertilizer two-factor analysis of variance, ns: not significant; *: $P < 0.05$; **: $P < 0.01$. CK: control; O: organic fertilizer; I: inorganic fertilizer; F: AMF; OI: organic and inorganic compound fertilizer; OF: combination of organic fertilizer with AMF; IF: combination of inorganic fertilizer with AMF; OIF: fertilizer with AMF

Compared with no AMF inoculation, the combination of organic fertilizer with AMF significantly increased the root length, root surface area and branch number of ryegrass by 94%, 46%, and 94%, respectively; the combination of inorganic fertilizer with AMF treatment increased by 64%, 26%, and 82%, respectively; the combination of organic–inorganic compound fertilizer with AMF increased by 132%, 86%, and 160%, respectively. Compared with the single fertilizer treatment, the combination of organic fertilizer with AMF, the combination of inorganic fertilizer with AMF and organic–inorganic compound fertilizer with AMF reduced the average root diameter by 25%, 23%, and 20%, respectively. Two-factor ANOVA showed that AMF had significant effects on root length, root surface area, root average diameter and branch number of ryegrass; fertilizer had significant or highly significant effects on root length and average root diameter of ryegrass; and there was an interaction between the two on ryegrass root growth.

3.5 Effects of Fertilizer and Arbuscular Mycorrhizal Fungi on Nitrogen and Phosphorus in Ryegrass

As shown in Fig. 4, the fertilizer and AMF had significant effects on the nitrogen and phosphorus contents in ryegrass shoots and roots. Compared with CK, AMF, organic fertilizer, inorganic fertilizer and organic–inorganic compound fertilizer enhanced the nitrogen content of shoots by 20%, 23%, 92%, and 52%, respectively; the increase in root nitrogen content was 88%, 94%, 169%, and 119%, respectively; the increase in phosphorus content in shoots was 34%, 34%, 62%, and 38%, respectively; and the increase in phosphorus content in roots was 32%, 26%, 50%, and 39%, respectively.

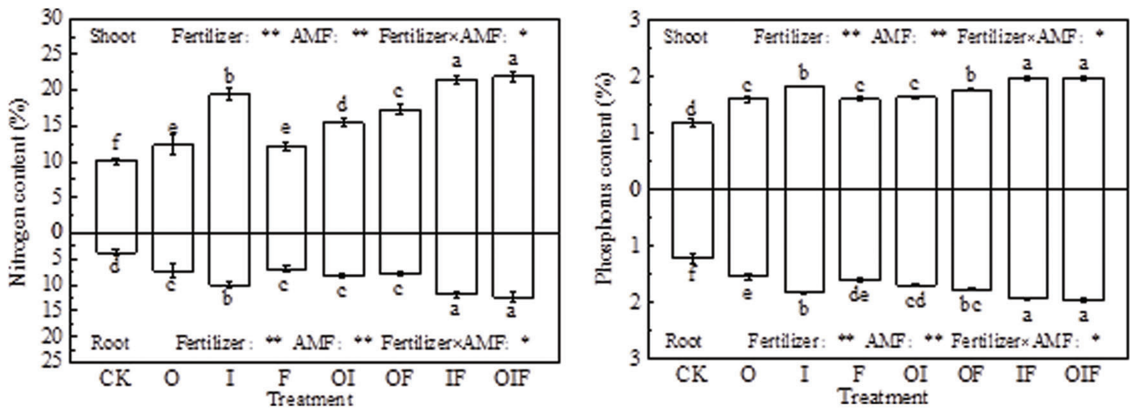

Figure 4: Effects of fertilizer and AMF on the nitrogen and phosphorus contents of ryegrass; according to the results of AMF and fertilizer two-factor analysis of variance, ns: not significant; *: $P < 0.05$; **: $P < 0.01$. CK: control; O: organic fertilizer; I: inorganic fertilizer; F: AMF; OI: organic and inorganic compound fertilizer; OF: combination of organic fertilizer with AMF; IF: combination of inorganic fertilizer with AMF; OIF: fertilizer with AMF

Compared with the single fertilizer treatment, the combination of organic fertilizer with AMF treatment significantly increased the nitrogen content in the shoots of the ryegrass and the phosphorus content in the shoots and roots by 40%, 11%, and 15%, respectively; the combination of inorganic fertilizer with AMF significantly increased the nitrogen content in the shoots and roots, the phosphorus content in the shoots and roots of ryegrass by 11%, 19%, 19%, and 6%, respectively; the combination of organic–inorganic compound fertilizer with AMF treatment significantly enhanced the nitrogen content in the shoots and roots, and the phosphorus content in the shoots and roots of ryegrass by 42%, 51%, 21%, and 17%, respectively. Two-way ANOVA showed that both AMF and fertilizer had highly significant effects on ryegrass nitrogen and phosphorus contents, and there was an interaction between the two on ryegrass nitrogen and phosphorus contents.

3.6 Effects of Fertilizer and Arbuscular Mycorrhizal Fungi on the Cadmium and Lead Contents of Ryegrass

As shown in Fig. 5, both fertilizer and AMF significantly reduced the Cd and Pb contents in the shoots and roots of ryegrass. Compared with CK, organic fertilizer, inorganic fertilizer, AMF and organic–inorganic compound fertilizer treatments decreased the Cd content in the shoots of ryegrass by 13%, 11%, 15%, and 18%, respectively; the decrease rates of Cd content in ryegrass roots were 19%, 21%, 22%, and 36%, respectively; the decrease rates of Pb content in ryegrass shoots were 18%, 10%, 17%, and 32%, respectively; and the decrease in Pb content in ryegrass roots was 15%, 21%, 16%, and 18%, respectively.

Compared with the fertilizer treatment, the combination of organic fertilizer with AMF significantly reduced the Cd and Pb contents in the shoots and roots of the ryegrass compared with the single application of the organic fertilizer treatment. The Cd content in the shoots and roots decreased by 22% and 21%, respectively, and the Pb content in the shoots and roots decreased by 21% and 20%, respectively. The combination of inorganic fertilizer with AMF treatment decreased the Cd content in the shoots and roots of ryegrass by 24% and 49%, and the Pb content decreased by 45% and 31%, respectively. The combination of organic–inorganic compound fertilizer with AMF treatment reduced the Cd content in the shoots and roots by 34% and 62%, and the Pb content by 47% and 34%, respectively. Two-way ANOVA showed that fertilizer and AMF had extremely significant effects on Cd and Pb contents in the shoots and roots of ryegrass; there was an interaction between the two on Cd contents in roots and Pb contents in the shoots and roots of ryegrass.

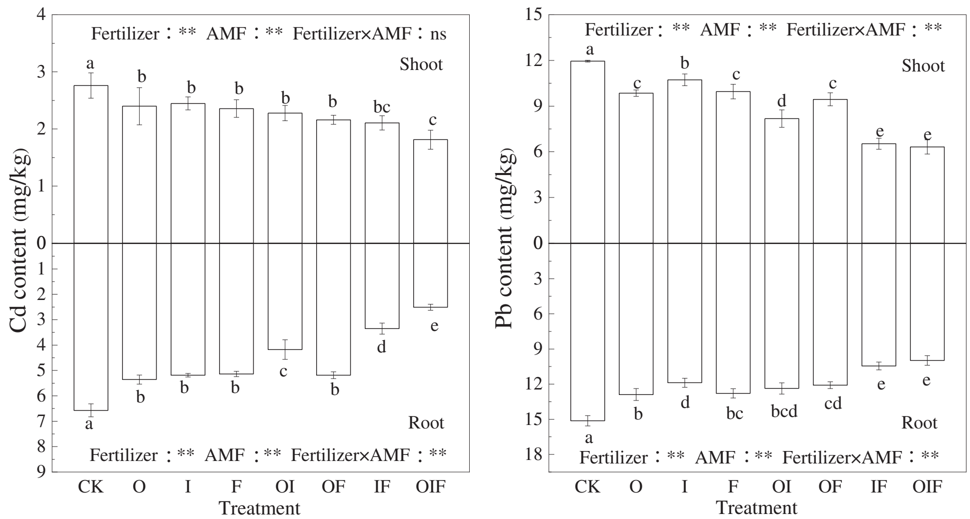

Figure 5: Effects of fertilizer and AMF on the content of cadmium and lead in ryegrass; according to the results of AMF and fertilizer two-factor analysis of variance, ns: not significant; *: $P < 0.05$; **: $P < 0.01$. CK: control; O: organic fertilizer; I: inorganic fertilizer; F: AMF; OI: organic and inorganic compound fertilizer; OF: combination of organic fertilizer with AMF; IF: combination of inorganic fertilizer with AMF; OIF: fertilizer with AMF

3.7 Correlation Analysis

Correlation analysis showed that the phosphorus content in the shoots of the ryegrass and the nitrogen and phosphorus contents in the roots were significantly or extremely significantly positively correlated with the root length and branch number, and the phosphorus content in the shoots and roots had a significant or extremely significant positive correlation with root surface area, but the nitrogen and phosphorus contents were significantly or extremely significantly negatively correlated with the average root diameter. The plant nitrogen and phosphorus contents were significantly positively correlated with biomass, but extremely significantly negatively correlated with the Cd and Pb contents (Table 3). Therefore, both the application of fertilizer and AMF synergistically improved the root morphology of ryegrass, thereby increasing the mineral nutrition, reducing the content of Cd and Pb in plants, and promoting the growth of ryegrass.

4 Discussion

4.1 Effects of Fertilizer and Arbuscular Mycorrhizal Fungi on the Growth of Ryegrass on Heavy Metal-Polluted Soil in a Copper Mining Area

AMF infection of host plants was affected by soil physical and chemical properties and nutrient content, and AMF infection was more significant under certain soil nutrient contents. In this study, fertilizer significantly reduced the infection rate of AMF on the roots of ryegrass and the content of spores in the soil, indicating that under a certain amount of heavy metal stress, fertilizer would affect the physical and chemical properties of the soil, change the infection of AMF on plants, and reduce the growth of extraradical mycelium, thereby affecting the development, sporulation and infection of AMF. Studies have shown that both applications of fertilizer and AMF can improve soil nutrient content in mining areas and increase plant tolerance to heavy metals, thereby promoting plant growth and playing an important role in vegetation restoration in mining wasteland [21]. Researchers found that fertilizer and AMF could increase growth indicators such as the biomass and mineral element content of chicory and reduce the content of heavy metals in plants, thereby promoting plant growth [22], which is consistent with the results of this study on ryegrass growth. In addition to fertilizer, AMF are natural biological soil nutrients that provide hosts with water, nutrients, and pathogen protection, and when they are absent or infertile, they lead to less efficient ecosystem functions [23]. AMF itself can supply a limited amount of nutrients required for plant growth and development, but AMF can be mixed with organic fertilizer and inorganic fertilizer to develop new bio-organic–inorganic compound fertilizers. Zhang et al. reduced nitrogen runoff from paddy fields through fertilizer management and AMF inoculation, reducing fertilizer application while protecting the environment [24]. AMF do not have strict specificity to plants. It can connect plant roots of the same or different species through mycelium to form an underground mycorrhizal network system for resource redistribution [25]. This creates the possibility for the large-scale application of bio-organic–inorganic compound fertilizers.

Table 3: Correlation of nitrogen and phosphorus contents in ryegrass with root morphology, biomass, cadmium and lead contents

Plant part	Nutrient content	Root length	Root surface area	Average root diameter	Branch number	Biomass	Cd content	Pb content
Shoot	Nitrogen content	0.690	0.644	−0.746*	0.704	0.799**	−0.719**	−0.743**
	Phosphorus content	0.779*	0.760*	−0.838**	0.803*	0.897**	−0.805**	−0.779**
Root	Nitrogen content	0.724*	0.681	−0.780*	0.736**	0.680**	−0.877**	−0.942**
	Phosphorus content	0.769*	0.741*	−0.857**	0.787*	0.774**	−0.831**	−0.956**

Note: "*" and "**" means $P < 0.05$ and $P < 0.01$ according to correlation analysis, respectively. n = 40. Plant nitrogen and phosphorus content, biomass, cadmium and lead content were the same parts for correlation analysis.

Although AMF was inoculated under the treatments of inorganic fertilizer and organic–inorganic compound fertilizer in this study, there was no significant difference in the effects of the two on plant height, biomass and chlorophyll of ryegrass, but the cost of inorganic fertilizer was higher than that of organic–inorganic compound fertilizer. Long-term excessive application of inorganic fertilizer can also lead to a series of problems, such as soil compaction and even desertification, increased activity of heavy metals in soil, and reduced soil microbial content [26]. Additionally, long-term application of inorganic fertilizer will make nutrients such as nitrogen, phosphorus and potassium easily fixed by the soil, thereby reducing the utilization rate of fertilizer. This will also lead to an imbalance in the inherent nutrient structure in the soil and changes in the physical and chemical properties of the soil [27]. Therefore, in terms of both economic economy and environmental impacts, the combination of organic–inorganic compound fertilizer with AMF treatment is a better choice. The selection of organic–inorganic compound fertilizer and AMF should be based on the different physical and chemical properties of plants and soils, and appropriate diversified combinations of microorganisms and fertilizer should be selected to maximize the effect of fertilizer and AMF [28]. Some researchers found that the combined applications of AMF and selenium fertilizer promoted the accumulation of organic selenium in rice grains [29]. The use of improved genotypic varieties and mineral fertilizer greatly increased cassava yields without compromising AMF richness or diversity [30]. The effect of the coapplication of amendments and AMF was also significant, and the addition of AMF, biochar and phosphorus fertilizer significantly increased the stem and leaf biomass accumulation of white clover on low Cd-polluted soils [31]. Thus, there is still much room for research on the effects of different fertilizers and AMF on plant growth.

Fertilizer and AMF had an interactive effect on plant growth, and applications of AMF and fertilizer mainly promoted plant growth by enhancing their nutritional status. The study showed that AMF and fertilizer promoted the increase in nitrogen and phosphorus content in plants, thereby promoting the growth and nutrition of seedlings [32], which was consistent with the results of this study. AMF can also increase the chlorophyll content of ryegrass to a certain extent and promote photosynthesis, phosphorus absorption and ryegrass growth [33]. The study found that there was a significant interaction effect between AMF and phosphorus application level, which could significantly increase onion biomass, chlorophyll content and mineral nutrition, thereby increasing onion yield [34]. The soil has a strong adsorption and fixation effect on phosphorus, and the applied phosphorus fertilizer will be quickly fixed by the soil and exist in the form of insoluble minerals or organic states [35]. Nevertheless, AMF can decompose insoluble inorganic phosphorus and mineralized organic phosphorus to improve the availability of soil phosphorus, thereby promoting the absorption of soil phosphorus by host plants [36].

As a nutrient source, organic fertilizer may have a synergistic effect on the development of AMF, but the effect of organic fertilizer is slow, and the application of AMF promotes the utilization of organic fertilizer by host plants. AMF and organic fertilizer treatments improved the growth parameters and biomass of *Centella asiatica*, resulting in increased N, P and K uptake per hectare in this species [37]. Studies have shown that the application of inorganic fertilizer significantly reduced AMF α-diversity, which showed similar levels to control samples after organic fertilizer [38]. In long-term field experiments, organic and inorganic fertilizers increased and decreased the development of AMF, respectively [39].

4.2 Effects of Fertilizer and Arbuscular Mycorrhizal Fungi on Cadmium and Lead Contents of Ryegrass on Heavy Metal-Polluted Soil in a Copper Mining Area

Fertilizer measures are one of the most important ways to manage crops. The application of fertilizer can not only improve the performance structure of the soil, but also improve the physical and chemical properties of the soil, affecting the absorption and accumulation of heavy metals in the plant roots and thereby increasing the biomass and yield of crops [40]. A study found that while fertilizing crops, fertilizer is not only a fertility factor for crop growth, but also plays a role in the chemical remediation of heavy metal-polluted soil, effectively reducing the effectiveness of heavy metals and reducing the toxicity of heavy metals to plants [41]. The fertilizer and AMF in heavy metal-polluted soil can effectively promote plant growth and improve plant stress resistance. Applying a certain amount of phosphorus fertilizer after inoculation with AMF can significantly improve the absorption of phosphorus by plants, minimize the absorption of heavy metals by plants and promote plant growth. This is because the chemical reaction between phosphorus and heavy metals reduces the effectiveness of heavy metals [42]. In this study, it was found that compared with CK, other treatments could significantly reduce the Cd and Pb contents of plants, among which AMF inoculated with organic–inorganic compound fertilizer had the largest decrease, and the synergistic effect was better than other treatments. This result indicated that under certain heavy metal stress, applying organic–inorganic compound fertilizer and then inoculating AMF could minimize the toxicity of heavy metals to ryegrass and promote its growth. Hence, fertilizer and AMF have a certain application value in the vegetation reconstruction of ryegrass in wasteland.

In this study, although AMF reduced the infection and spore count of ryegrass after applying organic, inorganic and organic–inorganic compound fertilizers to inoculate AMF, it improved the nutrient status of heavy metal-polluted soil in the Pulang copper mine to a certain extent. Different fertilizers and AMF could improve the root morphology of ryegrass to different degrees, promote the absorption of mineral nutrients by ryegrass, reduce the Cd and Pb contents of plants, and reduce the toxicity of heavy metals. The interaction between mineral elements such as nitrogen, phosphorus and potassium in fertilizer and AMF is relatively complex. Overall, the mechanism of synergy between fertilizer and AMF on heavy metal-polluted soil in copper mining areas needs to be further studied.

5 Conclusion

The application of fertilizer and AMF synergistically improved ryegrass growth to a certain extent, and reduced the Cd and Pb contents of the ryegrass. Among them, the effects of the combined application of organic–inorganic compound fertilizer with AMF and inorganic fertilizer with AMF were better than those of the remaining treatments. AMF had minimal short-term effects on ryegrass growth. Moreover, the nitrogen and phosphorus contents were very significantly positively correlated with the shoot and root biomasses but very significantly negatively correlated with the Cd and Pb contents. The results indicated that ryegrass mineral nutrition and Cd and Pb contents were important factors affecting ryegrass growth by the application of fertilizer and AMF, and there was a synergistic effect between fertilizer and AMF.

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