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Arbuscular Mycorrhizal Fungi Promote the Degradation of the Fore-Rotating Crop (*Brassica napus* L.) Straw, Improve the Growth of and Reduce the Cadmium and Lead Content in the Subsequent Maize

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Abstract: Arbuscular mycorrhizal fungi (AMF) are widely present in heavy metal-polluted soils, but their effects on straw degradation and plant growth of rotated crops are poorly understood. In this study, a pot experiment was used to simulate the return of fore-rotating crop (*Brassica napus* L.) straw to farmland with a subsequent planting of maize in a lead–zinc mining area on the Yunnan Plateau, Southwest China, which included four treatments: control (CK), addition of rape straw (SR), inoculation of AMF (AMF), and both AMF inoculation and straw addition (AMF + SR). The effects of AMF on the degradation and nutrient release of the fore-rotating rape straw and the growth, mineral nutrition and the cadmium (Cd) and lead (Pb) contents of the subsequent maize were investigated. Compared with the CK treatment, AMF significantly promoted the degradation of rape straw and the release of mineral nutrients (nitrogen, phosphorus and potassium) as well as the Cd and Pb, increased the content of available nutrients in soil, and improved the mineral nutrient contents in the maize. AMF + SR significantly increased the maize height and biomass by 32–35% and decreased the available Cd and Pb contents in soil and the Cd and Pb contents in the maize by 20–30% and 18–25%, respectively. Moreover, the available Cd and Pb contents in the soil presented significant positive correlations with their contents in the maize but negative correlations with the height and biomass of the maize. Thus, AMF played an important regulatory role in the nutrient cycling and heavy metal accumulation of the crop rotation.

Keywords: arbuscular mycorrhizal fungi; rape/maize rotation; straw incorporation; mineral nutrition; heavy metal uptake

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1. Introduction

The rapid development of industry and agriculture has led to serious heavy metal pollution in soils in mining areas, among which cadmium (Cd) and lead (Pb) pollution in soil, which seriously affects crop yield and quality, are more serious [1,2]. When crops are planted in heavy metal-polluted soil, the heavy metal content of crop straw is generally high. However, crop straws are used for straw return to the field due to their rich nitrogen, phosphorus, potassium, and other mineral nutrient contents [3,4]. The decomposition of crop straw by soil microorganisms can release these nutrients for use by later crops, which is of great significance for balancing soil nutrients, effectively promoting the growth of later crops and improving their yield [5,6].

Arbuscular mycorrhizal fungi (AMF) are closely related to plants in soil and can form beneficial symbiosis with the roots of most crops, even in the presence of heavy metal pollution [7,8]. Researchers have found that AMF can form a huge extrinsic mycelium network between the same or different plant roots that can improve soil available nutrients and increase the absorption of mineral nutrients by plants [9]. It can reduce the availability of heavy metals in soil and reduce the toxic effect of heavy metals on plants [10]. Many studies have shown that AMF can accelerate the degradation of crop straw, promote the conversion of soil nutrients, improve the growth and development of crops under adverse conditions, and thus improve crop yield and quality [11,12]. Therefore, AMF can accelerate the degradation of the previous crop straw and release mineral nutrients, improve the soil nutrient content, and promote the absorption of mineral nutrients and crop growth of the later crop.

The Huize lead–zinc mine in the capital of Yunnan Province is located in Southwest China, where rapeseed maize rotation is a common cropping pattern around this lead–zinc mine [13]. Considering the sustainable development of the ecological environment, it is necessary to do a good job in the follow-up treatment of a large amount of rape straw with respect to the heavy metal pollution of the soil around the lead–zinc mines [14,15]. In terms of agricultural treatment, straw compost is mainly degraded and returned to the field as fertilizer [16–18]. However, the effects of AMF on the degradation of heavy metal-polluted straw, the release of heavy metals and nutrients in rapeseed maize rotation systems have rarely been reported and needs further exploration.

The study presented in this paper mainly considered the heavy metal pollution of soil in mining areas and the treatment of rape straw. Therefore, the Cd- and Pb-polluted soil in the farmland surrounding the Huize lead–zinc mine in Yunnan Province was selected as the test soil, and the previous crop rape straw was used as the research material to carry out the pot experiment of adding rape straw and AMF. This work studied the effects of AMF on the degradation of rape straw and the release of mineral nutrients in the previous crop and on the growth, mineral nutrition, and plant Cd and Pb contents in the later crop maize. The aim was to clarify the ecological function of AMF in regulating the mineral nutrition and plant Cd and Pb contents in the rotation system of polluted farmland and to provide a theoretical basis for improving the soil fertility of polluted farmland and reducing the absorption of Cd and Pb by crops. We hypothesized that AMF could promote the degradation and nutrient release of previous crop rape straw and improve the available soil nutrients. It can promote the growth of the later maize crop and reduce the maize contents of Cd and Pb. These are closely related to the degradation of the previous crop rape straw, its mineral nutrients, and the release of Cd and Pb.

2. Materials and Methods

2.1. Test Materials

The tested soil type was mountainous red soil, which was taken from farmland (26°34′29″ N, 103°37′17″ E) polluted by heavy metals in the lead–zinc mining area of Huize County, Yunnan Province. The soil was dried naturally, crushed, ground, and passed through a nylon screen with 2 mm apertures. The soil was put into a high-pressure steam sterilization pot and kept at 121 °C for 2 h. After natural cooling, the soil was held for 3 days prior to use. The basic physical and chemical properties of the soil are shown in Table 1.

Table 1. Soil Basic physical and chemical properties.

pH	Organic Matter Content (g/kg)	Available Nitrogen Content (mg/kg)	Available Phosphorus Content (mg/kg)	Available Potassium Content (mg/kg)	Cadmium Content (mg/kg)	Lead Content (mg/kg)
5.74	28.12	35.04	19.72	125.07	4.51	268.89

The test crop was maize (*Zea mays* L.), with the maize variety Huidan No. 4. Intact maize seeds of the same size were selected and soaked in 75% ethanol for 1 min and 10% sodium hypochlorite for 15 min for surface disinfection, washed with clean sterile water, moved into sterilized petri dishes with wet filter paper, placed in a constant temperature incubator at 28 °C for 3 days, and planted after the seeds were exposed to white light.

Funneliformis mosseae was selected as the primary fungus, and it was provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. Inoculants containing spores, mycelium, root segments, and culture substrate, with approximately 20–30 spores per gram of inoculants, were obtained by propagating the fungi on potted maize.

Preparation of test straw: rape straw from around the mining area was selected. In order to better mix with soil and obtain degradation results of rape straw in a shorter period of time, rape straw was rinsed with deionized water, dried, ground, and screened to obtain a 0.5–2 mm particle size powder. Approximately 2.0 g of rape straw powder (weighed to 0.0001 g) was sealed in a 9 cm $\times$ 5 cm nylon mesh bag with a mesh diameter of 0.2 mm. The contents of total nitrogen, total phosphorus, and total potassium in the rape straw were 2.23%, 0.15%, and 0.75%, respectively, and the total Cd and Pb contents were 1.63 mg/kg and 5.76 mg/kg, respectively.

2.2. Rape Straw Degradation Test

The device used was a four-compartment partition system, which is an improved version of the test device of Peng et al. [19]. This device was made of 3 mm thick plexiglass, and each room was 10 cm long, 5 cm wide, and 15 cm high. The mycorrhizal compartment and the root compartment were separated by a plexiglass partition 14 cm long and 10 cm wide, with the rest of the compartments separated by a nylon mesh with a mesh diameter of 37.4 μm. The pore size of the nylon mesh is required to allow mycelium and solution to penetrate, while retaining the root system.

A total of 15 container devices were used in the experiment. In each device, straw was added to the mycorrhizal chamber and AMF (AMF + SR) was inoculated, while straw (CK) was added to the root chamber. Then, 4 kg of sterilized soil was placed into each container. First, 0.3 kg sterilized soil per chamber was placed on the bottom of the fourth chamber, and then the nylon mesh bags containing rape straw were placed vertically on the closed position of the fourth chamber. Then, 30 g of AMF inoculum was added to the mycorrhizal chamber and 30 g of AMF bactericidal agent was added to the root chamber. Each maize root was divided into two parts and placed in the mycorrhizal chamber and the root chamber. Two maize seeds were planted in each container and covered with 0.2 kg sterilized soil. During the experiment, deionized water was used to irrigate the soil according to its moisture condition, and no chemical fertilizers or pesticides were applied during the experiment. At 20, 30, 40, 50, and 60 days after planting, 3 containers were randomly selected to collect plant and soil samples, and the contents of rape straw nutrients, Cd, and Pb in nylon mesh bags were determined.

2.3. Pot Experiment of Rape Straw Addition and AMF Inoculation

There were four treatments: control (CK), addition of rape straw (SR), addition of AMF (AMF), and addition of both AMF and straw (AMF + SR). Each treatment contained 4 replicates and a total of 16 pots were planted. The soil volume of each basin was 3 kg. For the AMF treatment, 2.5 kg of sterilized soil was first placed into the basin and 100 g of AMF inoculum was then added to the sterilized soil; however, the treatment without AMF inoculation was the addition of equal amounts of sterilized AMF bactericidal agent, all put into 3 seeds of germinated maize seeds, with 0.5 kg of sterilized soil used to cover the inoculum and seeds. The weight ratio of straw to soil used was 1:20 and it was evenly mixed. No pesticides or chemical fertilizers were applied during the entire test process and deionized water was applied according to the soil moisture conditions. Then, 60 days after planting, plant and soil samples were collected to determine their index values.

2.4. Determination of Maize Height Biomass, Rape Straw Degradation, and Mineral Element Release

After 60 days of planting, the heights of maize plants were measured with a ruler, and the maize plants were split into shoots and roots, washed with deionized water, and part of the fresh root samples were saved to determine the AMF colonization rate. The remaining parts were firstly oven dried at 105 °C for 30 min and then dried at 75 °C to constant weight, weighed as biomass, ground, and screened for use.

The weight and content of each nylon mesh bag were determined separately. Straw degradation amount = $B_0 - B_t$ and straw nutrients and cadmium and lead release amounts = $B_0 \times$ initial content $- B_t \times$ residual content, where B_0 is the initial weight of straw and B_t is the residual weight of straw [20].

2.5. Determination of the AMF Colonization Rate, Number of Soil Spores, and Content of Glomalin-Related Soil Protein

After 60 days of maize growth, the root colonization rate and the number of spores in the rhizosphere soil were measured. Part of the new fine roots were selected and the roots were cut into 1 cm root segments. The roots were soaked in a 10% potassium hydroxide (KOH) solution in a constant temperature water bath (90 °C) for 30 min until transparent, washed with sterile water, acidified with 2% hydrochloric acid (HCl) for 3–5 min, washed with sterile water again, and stained with an acidic fuchsin staining solution (0.01%) for 30 min. Mycelium colonization was observed under a light microscope after decolorization, and the formula to calculate the AMF colonization rate (%) is the number of colonized root segments divided by the total number of root segments multiplied by 100% [21].

The AMF spore count was determined by the wet sieve dump method: 10 g of a naturally air-dried rhizosphere soil sample was put into a beaker, then 100 mL distilled water was added, stirred clockwise for 2 min, and then left standing for 10 min. The suspension was poured into a centrifuge tube and centrifuged at 3000 r/min for 5 min. The supernatant was taken, added to a 45% sucrose solution, and then centrifuged at 3000 r/min for 5 min, with the supernatant withdrawn and filtered onto filter paper. The number of AMF spores was observed and counted under a microscope [22].

The rhizosphere soil samples were collected by the shaking root method, dried, and then passed through 1.00 and 0.15 mm sieves. Determination of glomalin-related soil protein (GRSP) content: an amount of 1 g of the naturally air-dried rhizosphere soil sample was extracted with 50 mol/L sodium citrate buffer (pH 8.0) for 60 min under extraction conditions of 0.11 MPa and 121 °C and then centrifuged at 835× *g* for 5 min to obtain the GRSP solution. The content of GRSP in the supernatant was determined by the Braford method [23].

2.6. Determination of the Nutrient, Cadmium, and Lead Contents in the Maize

Determination of the nitrogen (N), phosphorus (P), and potassium (K) contents of maize straw: dried plant samples were ground and passed through a 100 mesh sieve, 0.1 g powder was weighed in a 100 mL triangular bottle, 5 mL sulfuric acid (H_2SO_4) was added to soak overnight, and then 10 drops of hydrogen peroxide (H_2O_2) were added to digest. During the digesting process, the temperature was continuously raised (280–400 °C) until the liquid was digested transparent or colorless and then filtered to a constant volume. The contents of N, P, and K in plants were determined by Kjeldahl nitrogen determination, the vanadium–molybdenum yellow colorimetric method, and flame spectrophotometry, respectively. Determination of the Cd and Pb contents of the maize: a 0.1 g dried powder sample was weighed and digested with nitric acid (HNO_3)-H_2O_2 mixture (160 °C for 4 h), and the filtrate was diluted to 50 mL and the contents determined by flame atomic absorption spectrophotometry [24].

2.7. Determination of the Physical and Chemical Properties of the Rhizosphere Soil

The rhizosphere soil samples were collected by shaking the root method, dried, and ground by natural air and passed through 1.00 and 0.15 mm screens. The alkali-hydrolyzed nitrogen content was determined by the alkali-hydrolyzed diffusion method. The content of available phosphorus was determined by the 0.5 mol/L sodium bicarbonate–molybdenum–antimony resistance colorimetric method. The content of available potassium was determined by 1 mol/L ammonium acetate (CH_3COONH_4) extraction and flame spectrophotometry. The contents of the available Cd and Pb in the rhizosphere soil were determined by diethylenetriaminepentaacetic acid (DTPA)–triethanolamine (TEA) extraction and atomic absorption spectrophotometry [24].

2.8. Data Processing and Statistical Analysis

The test data were the mean values of 3 replicates. Microsoft Excel 2010 was used to process the data, and the mean values and standard deviations were calculated, which were expressed as the mean ± standard deviation. SPSS 23.0 and the Student—Newman—Keuls (SNK) test were used to test the significance of the data at the level of $p < 0.05$, and ANOVA two-factor analysis of variance was used to test the significance of AMF, straw addition, and their interactions. OriginPro 9.0 was used for charting.

3. Results

3.1. The Colonization Characteristics of AMF in Maize Roots

As shown in Table 2, no mycorrhizal colonization or number of spores were detected in the CK and SR (without AMF) treatments. Under the AMF and AMF + SR treatments, the root colonization rates were 48.53% and 50.30%, and the numbers of spores per gram of soil were 44.50 and 50.71, respectively, with the number of spores under AMF + SR treatment significantly higher than that under AMF inoculation alone. In conclusion, AMF can form an effective symbiotic relationship with maize roots and AMF + SR treatment can significantly promote spore formation.

Table 2. Effects of straw addition and AMF on colonization rate in maize root and its spore number. AMF—treatment of AMF inoculation; AMF + SR—treatment of addition straw and AMF inoculation; different lowercase letters indicate significant differences among treatments ($p < 0.05$).

Treatment	Colonization Rate (%)	Spore Number (n/g)
AMF	48.53 ± 5.93^{a}	44.50 ± 3.58^{b}
AMF + SR	50.30 ± 1.67^{a}	50.71 ± 3.58^{a}

Compared with CK, both AMF and AMF + SR treatments significantly increased the content of soil glomalin-related protein (GRSP) by 95% and 104%, respectively, but there was no significant difference between the two treatments. The SR treatment increased the GRSP content in soil, but the effect was not significant. Two-factor analysis of variance showed that AMF had a very significant effect on the soil GRSP content and straw addition had a significant effect on the soil GRSP content, but the interaction between the two factors was not significant (Figure 1). Therefore, both AMF and straw addition can increase the GRSP content in soil.

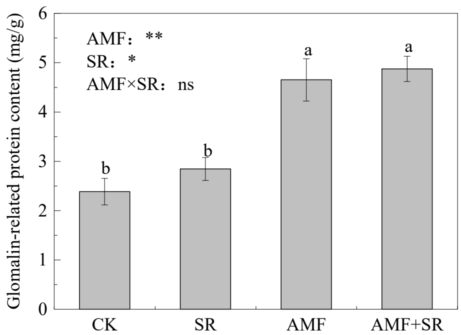

Figure 1. Effects of straw addition and AMF on soil glomalin-related protein. CK—control; SR—treatment of rape straw addition; AMF—treatment of AMF inoculation; AMF + SR—treatment of addition straw and AMF inoculation; different lowercase letters indicate significant differences among treatments ($p < 0.05$). "ns", "*", and "**" indicate not significant, $p < 0.05$, and $p < 0.01$ according to two-way ANOVA, respectively.

3.2. Effects of AMF on the Degradation of Rape Straw and Release of Mineral Elements

As shown in Figure 2, the amounts of rape straw degradation and the amounts of mineral elements released into the soil increased with the extension of cultivation days. At the 20th, 30th, 40th, and 60th days, compared with the CK control, the rape straw degradation amounts increased by 13–14% with AMF (Figure 2A). The contents of N (Figure 2B), P (Figure 2C), and K (Figure 2D) in rape straw increased by 13–14%. The Cd (Figure 2E) and Pb (Figure 2F) releases of rape straw increased by 11–13% and 13–14%, respectively. In conclusion, AMF can promote the degradation of rape straw and the release of mineral elements to different degrees.

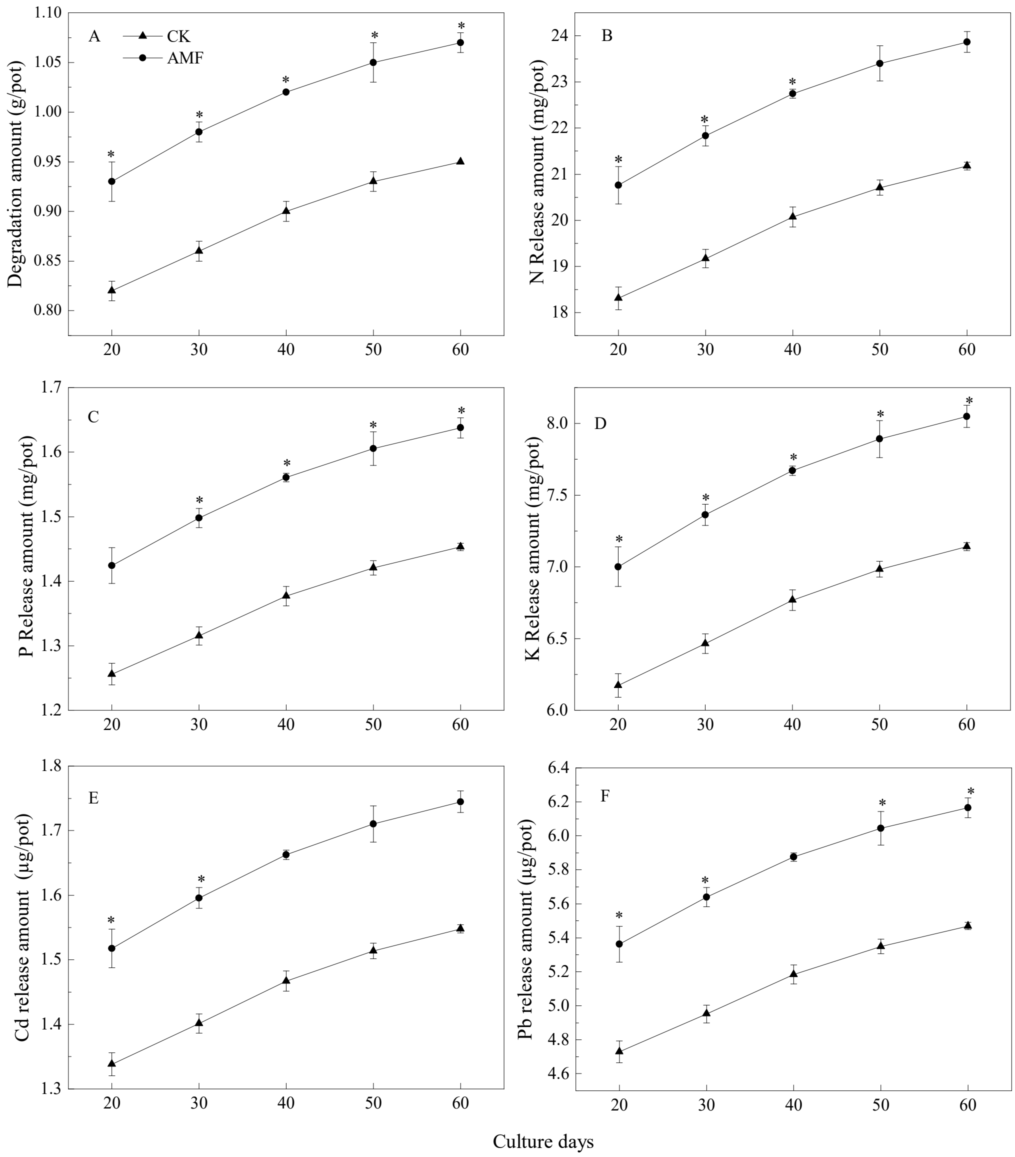

Figure 2. Effects of AMF on rape straw degradation and mineral element release. (**A**): rape straw degradation amount with and without AMF inoculation; (**B**): N release amount with and without AMF inoculation; (**C**): P release amount with and without AMF inoculation; (**D**): K release amount with and without AMF inoculation; (**E**): Cd release amount with and without AMF inoculation; (**F**): Pb release amount with and without AMF inoculation. CK—control; AMF—treatment of AMF inoculation. "*" indicate significant difference among treatments for the same culture days ($p < 0.05$).

3.3. Effects of Rape Straw Addition and AMF on Available Nutrient Content in Soil

As shown in Figure 3, compared with CK, the SR, AMF, and AMF + SR treatments increased the content of nitrogen, phosphorus, and potassium of available nutrients in the soil rhizosphere to varying degrees, and the contents of alkali-hydrolyzed nitrogen significantly increased by 3%, 8%, and 15%, respectively (Figure 3A). The contents of available phosphorus were significantly increased by 3%, 12%, and 14%, respectively (Figure 3B). The available potassium contents were significantly increased by 8%, 23%, and 24%, respectively (Figure 3C). In conclusion, the AMF + SR treatment significantly increased the contents of soil alkali-hydrolyzed nitrogen, available phosphorus, and available potassium compared with the other treatments. Two-factor analysis of variance showed that the addition of AMF and rape straw had significant effects on the soil available nitrogen, phosphorus, and potassium contents, but the interaction between the factors did not reach a significant level.

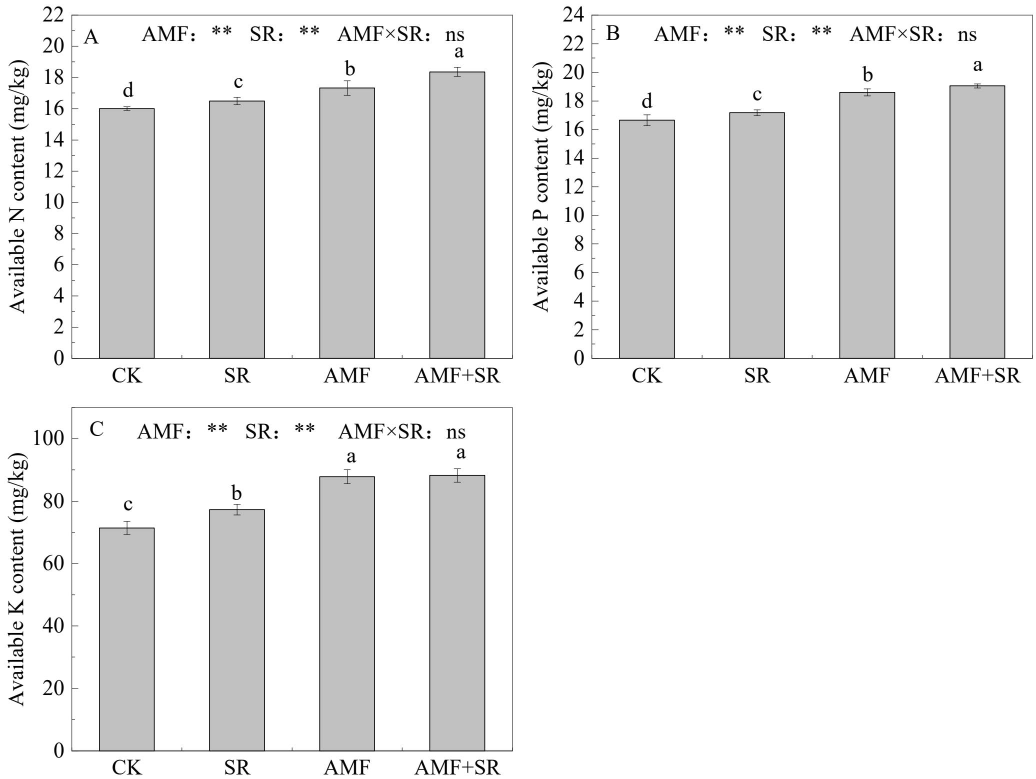

Figure 3. Effects of adding rape straw and AMF on soil available nutrient content. (**A**): effects of adding rape straw and AMF on soil available N content; (**B**): effects of adding rape straw and AMF on soil available P content; (**C**): effects of adding rape straw and AMF on soil available K content. CK—control; SR—treatment of rape straw addition; AMF—treatment of AMF inoculation; AMF + SR—treatment of addition straw and AMF inoculation. Different lowercase letters indicate significant differences among treatments ($p < 0.05$). "ns" and "**" indicate not significant and $p < 0.01$ according to two-way ANOVA, respectively.

3.4. Effects of Rape Straw Addition and AMF on Available Cadmium and Lead Contents in the Soil

As shown in Figure 4, compared with CK, the AMF and AMF + SR treatments significantly reduced the contents of available Cd and Pb in the soil, with the contents of effective Cd reduced by 19% and 20%, respectively (Figure 4A), and the contents of effective Pb reduced by 27% and 30%, respectively (Figure 4B), but there was no significant difference between the two treatments. Two-factor analysis of variance showed that AMF had a very significant effect on available Cd and Pb in the soil, but the addition of rape straw had no significant effect, and the interaction between the two factors was not significant. Therefore, the decrease in available Cd and Pb content in soil was caused by AMF.

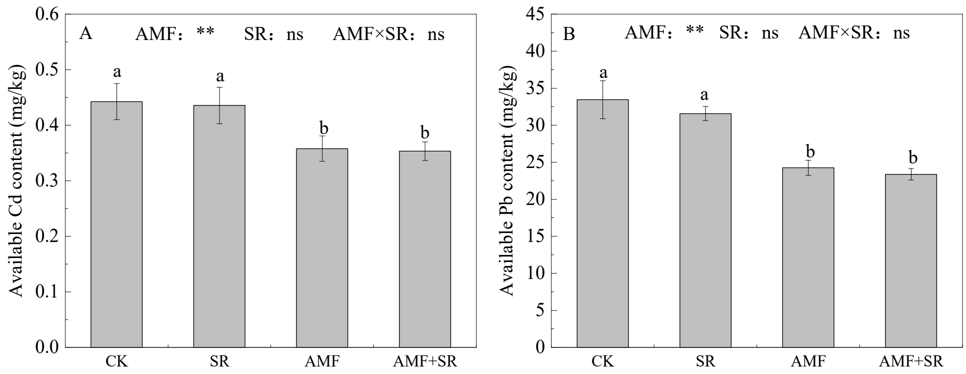

Figure 4. Effects of adding rape straw and AMF on soil available cadmium and lead content. (**A**): effects of adding rape straw and AMF on soil available Cd content; (**B**): effects of adding rape straw and AMF on soil available Pb content. CK—control; SR—treatment of rape straw addition; AMF—treatment of AMF inoculation; AMF + SR—treatment of addition straw and AMF inoculation. Different lowercase letters indicate significant differences among treatments ($p < 0.05$). "ns" and "**" indicate not significant and $p < 0.01$ according to two-way ANOVA, respectively.

3.5. Effects of Rape Straw Addition and AMF on Maize Height and Biomass

Compared with the CK treatment, the AMF and AMF + SR treatments significantly increased maize plant heights and biomass amounts, including 32% and 35% in the plant height, 31% and 32% in the shoot biomass, and 34% and 33% in the root biomass, respectively. There was no significant difference in the effects of the two treatments on plant height and biomass values. Two-factor analysis of variance showed that AMF had extremely significant effects on the plant height and shoot and root biomass values of the maize, but the addition of rape straw had no significant effects on the plant height and shoot and root biomass values of the maize, and the interaction between the addition of AMF and straw was not significant (Figure 5). Thus, the good growth of maize plants was mainly caused by AMF.

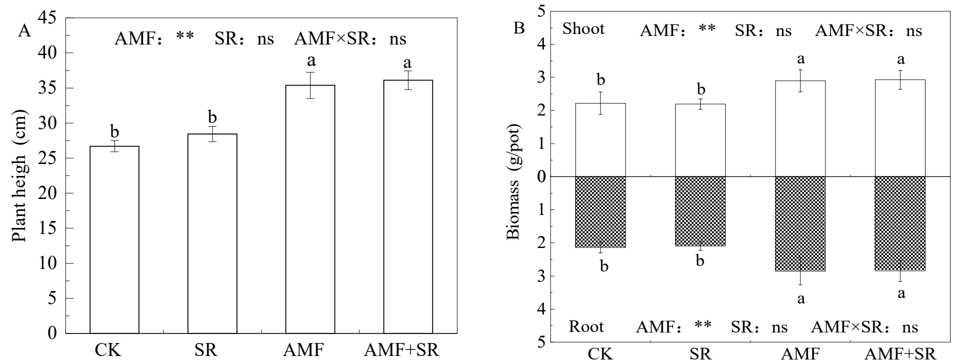

Figure 5. Effects of adding rape straw and AMF on maize height and biomass. (**A**): effects of adding rape straw and AMF on maize height; (**B**): effects of adding rape straw and AMF on maize biomass in the shoot and root. CK—control; SR—treatment of rape straw addition; AMF—treatment of AMF inoculation; AMF + SR—treatment of addition straw and AMF inoculation. Different lowercase letters indicate significant differences among treatments ($p < 0.05$). "ns" and "**" indicate not significant and $p < 0.01$ according to two-way ANOVA, respectively.

3.6. Effects of Rape Straw Addition and AMF on Nutrient Content in Maize

Compared with CK, the SR treatment significantly increased the potassium content in the maize shoots and roots by 4% and 8%, respectively. AMF and AMF + SR significantly increased the contents of nitrogen, phosphorus, and potassium in the shoots and roots of maize by 9% to 27%, and the effect of the AMF + SR treatment was better than that of the AMF treatment (Table 3). Two-factor analysis of variance showed that the contents of nitrogen, phosphorus, and potassium in the maize were significantly affected by AMF, while the contents of nitrogen in the shoots of maize and potassium in the shoots and roots of maize were significantly or extremely significantly affected by the addition of rape straw, but the interaction between the two factors was not significant.

Table 3. Effects of straw addition and AMF on mineral nutrient content of maize (%). CK—control; SR—treatment of rape straw addition; AMF—treatment of AMF inoculation; AMF + SR—treatment of addition straw and AMF inoculation. Different lowercase letters indicate significant differences among treatments ($p < 0.05$). "ns", "*", and "**" indicate not significant, $p < 0.05$, and $p < 0.01$ according to two-way ANOVA, respectively.

Treatment	N Content		P Content		K Content	
	Shoot	Root	Shoot	Root	Shoot	Root
CK	2.06 ± 0.02 ^{c}	2.18 ± 0.05 ^{b}	0.38 ± 0.02 ^{b}	0.45 ± 0.01 ^{c}	0.66 ± 0.02 ^{d}	0.67 ± 0.04 ^{c}
SR	2.11 ± 0.04 ^{c}	2.22 ± 0.02 ^{b}	0.39 ± 0.02 ^{b}	0.46 ± 0.01 ^{c}	0.69 ± 0.01 ^{c}	0.73 ± 0.02 ^{b}
AMF	2.24 ± 0.05 ^{b}	2.39 ± 0.03 ^{a}	0.47 ± 0.04 ^{a}	0.50 ± 0.02 ^{b}	0.76 ± 0.02 ^{b}	0.82 ± 0.03 ^{a}
AMF + SR	2.31 ± 0.03 ^{a}	2.40 ± 0.03 ^{a}	0.48 ± 0.03 ^{a}	0.51 ± 0.01 ^{a}	0.79 ± 0.0.2 ^{a}	0.85 ± 0.03 ^{a}
Two-way analysis of variance						
AMF	**	**	**	**	**	**
SR	**	ns	ns	ns	**	*
AMF × SR	ns	ns	ns	ns	ns	ns

3.7. Effects of Rape Straw Addition and AMF on Content of Cadmium and Lead in Maize

Compared with CK, both AMF and AMF + SR treatments significantly reduced the shoot and root Cd and Pb contents of maize; the Cd contents in the shoots were reduced by 19% and 18%, and the Cd contents in the roots were reduced by 20% and 22%, respectively (Figure 6A). The shoot Pb contents decreased by 24% and 25%, and the root Cd contents decreased by 18% and 19%, respectively (Figure 6B). However, there was no significant difference between the two treatments. Two-factor analysis of variance showed that AMF had a very significant effect on the contents of Cd and Pb in shoot and root maize, while the addition of rape straw had no significant effect on the contents of Cd and Pb in maize plants, and the interaction between the two factors was not significant. Therefore, the contents of Cd and Pb in maize plants were decreased by AMF.

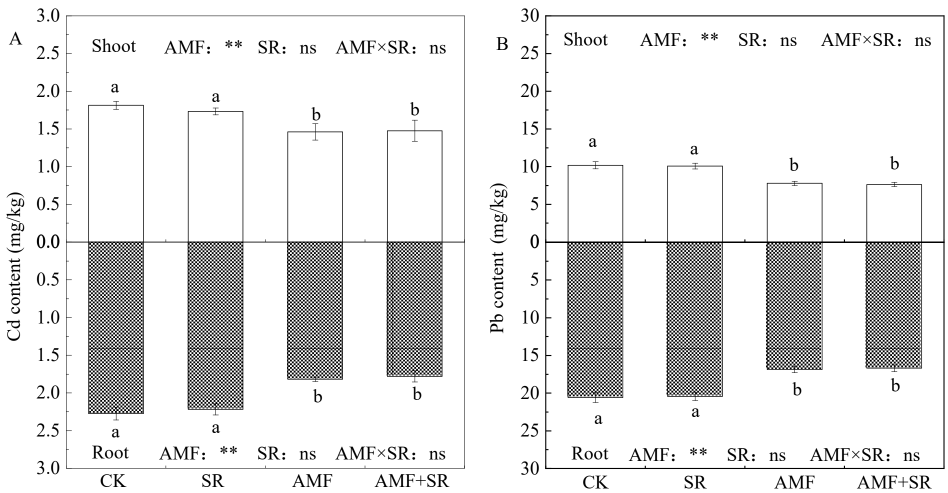

Figure 6. Effects of adding rape straw and AMF on the content of cadmium and lead in maize. (**A**): effects of adding rape straw and AMF on Cd content in maize shoots and roots; (**B**): effects of adding rape straw and AMF on Pb content in maize shoots and roots. CK—control; SR—treatment of rape straw addition; AMF—treatment of AMF inoculation; AMF + SR—treatment of addition straw and AMF inoculation. Different lowercase letters indicate significant differences among treatments ($p < 0.05$). "ns" and "**" indicate not significant and $p < 0.01$ according to two-way ANOVA, respectively.

3.8. Correlation Analysis

The correlation analyses showed that the available Cd and Pb contents in soil were positively correlated with the Cd and Pb contents in plants (r = 0.997, $p < 0.01$; r = 0.992, $p < 0.01$) and negatively correlated with plant height (r = −0.996, $p < 0.01$; r = −1.000, $p < 0.01$), shoot biomass (r = −0.996, $p < 0.01$; r = −0.984, $p < 0.05$), and root biomass (r = −0.994, $p < 0.01$; r = −0.978, $p < 0.05$), showing a significant or extremely significant negative correlation. In conclusion, the decrease in Cd and Pb contents in maize plants and the increase in maize plant height biomass by AMF were closely related to the decrease in soil available Cd and Pb contents.

4. Discussion

4.1. Effects of AMF on the Degradation and Mineral Nutrition, as Well as the Cadmium and Lead Releases from Rape Straw

The effect of AMF on the degradation and release of mineral nutrients in rape straw was studied by a pot experiment under the condition of compartment screen culture. The results showed that AMF could better promote the degradation of rape straw and the release of mineral nutrients. This may be because AMF changes the mycelial mesh density, enhances the soil enzyme activity, increases the soil microbial quantity, participates in the straw mineralization process, and promotes straw degradation and nutrient release [25,26]. Hydrolases released by mycelium outside the root of AMF can directly degrade plant residues [27,28]. In this study, it was found that even under the same culture days, the degradation of straw and the release of mineral nutrients in the mycorrhizal chamber were higher than those in the root chamber, indicating that AMF played a key role in the degradation of rape straw and the release of mineral nutrients.

Studies have found that rape straw can be used as a green fertilizer because it is rich in nitrogen, phosphorus, and potassium, and therefore it is an important nutrient resource to promote crop growth [29], and when applied in soil it is conducive to the survival of soil organic matter and microbial populations [30]. However, the degradation of straw is accompanied by the return of heavy metals to the soil, increasing the contents of heavy metals in the soil. In this study, the releases of Cd and Pb from rape straw after inoculation with AMF were higher than that of the control treatment, but inoculation with AMF and AMF + SR could reduce the contents of Cd and Pb in the soil, while straw addition alone had no significant effect on the Cd and Pb contents. The increases in Cd and Pb released from rape straw may be because during the degradation of rape straw, the low molecular weight organic acids secreted by the mycelia outside the plant roots and AMF roots, such as malic acid, oxalic acid, and acetic acid, chelate with cadmium and lead in the straw to form "cadmium–lead low molecular organic acid" complex, thus promoting the release and migration of cadmium and lead [31,32]. Therefore, it is necessary to further consider the environmental risk of heavy metal spillover caused by AMF promoting crop straw decomposition.

Studies have shown that heavy metal pollution of farmland soil is common in mining areas, leading to a decrease in farmland soil fertility, by affecting the absorption and utilization of nutrients by crops, and thus inhibiting the growth and development of crops [1]. Therefore, adding crop straw to improve soil nutrient status under heavy metal pollution is of great significance for the growth and development of later crops [33,34]. Studies have shown that the colonization of AMF in crop roots has a large mycelium network, which can improve soil enzyme activity and increase microbial biomass and richness, thus promoting straw degradation; the mineral nutrients in the straw will be released or transferred to the soil by the AMF root mycelia to increase the content of available nutrients in the soil, and then the mycelia will be used to promote the absorption and utilization of nutrients by the roots of later crops [12,35]. In this study, it was found that compared with CK, both AMF and AMF + SR treatments could significantly increase the available nutrient soil contents, and the AMF + SR treatment had a better effect on the improvement of soil available nitrogen, phosphorus, and potassium, indicating that

AMF could exert its ecological function to a greater extent after adding straw. Therefore, the combined treatment of heavy metal-contaminated rape straw and AMF inoculation is beneficial to the fertilizing of heavy metal-contaminated soil and the absorption of crop nutrients, which is of great significance to the growth and development of later crops.

4.2. Effects of AMF on the Growth and Heavy Metal Content of Crops

Studies have shown that AMF can promote material circulation and maintain ecosystem balance. AMF can significantly increase the degradation rate of plant residues, promote the decomposition of plant residues, and help host plants absorb nitrogen, phosphorus, potassium, and other mineral nutrients that are in the soil [36,37]. In this study, the nitrogen, phosphorus, and potassium contents, as well as the plant height and biomass values of the maize plants, increased significantly under the AMF and AMF + SR treatments, and the increase effect of the AMF + SR treatment was better. The possible reason is that AMF can colonize the maize roots, and the extrinsic mycelium increases the contact area between the roots and the soil, reduces nutrient losses, improves its utilization efficiency, and directly promotes the growth of maize [38]. AMF can also indirectly promote plant growth by promoting the degradation of crop residues and improving the soil nutrient status [25]. Some studies have found that organic matter generated by straw decomposition has a significant effect on AMF mycelial extension and an increase in spore number [39], and mycelial extension can expand the contact area of mineral nutrients in soil absorbed by crop roots, thus improving crop development ability [11,40,41].

In this study, it was found that the addition of AMF and rape straw could significantly reduce the contents of available Cd and Pb in maize rhizosphere soil and plant Cd and Pb. This may be because compounds such as AMF mycelium and its secreted glomalin-associated protein can effectively bind heavy metal ions in the soil, thus reducing the availability of heavy metals [42]. In particular, the glomalin-related proteins secreted by AMF mycelium have special biological, physical, and chemical properties complexing large quantities of heavy metal ions in soil and significantly improving the adsorption and binding of heavy metal ions to soil particles [43,44], reduce the available state contents and bioavailability of heavy metals in soil, reduce the transfer of heavy metals from soil to plants, and reduce the contents of heavy metals in the shoots of plants [45,46]. In conclusion, the combined treatment of rape straw polluted with heavy metals and AMF can reduce the contents of heavy metals in crops, which has important implications for the safe production of crops.

This experiment was conducted under controlled experimental equipment and indoor pot studies. Although there was no statistically significant interaction between straw addition and AMF, the combined treatment was the best for enhancing soil fertility and maize mineral nutrition, promoting maize growth, and enhancing maize resistance. The influencing factors of AMF on rape straw degradation and maize growth are relatively complex. The number of research days of this experiment on the growth of maize were limited, and the results obtained are only useful as the basic data for later applications combined with field experiments. Therefore, it is necessary to carry out positioning research on long-term combined treatment combined with field trials in the future. At the same time, research on straw–soil nutrient cycling and the mechanism of reducing heavy metal absorption by crops should be further strengthened.

5. Conclusions

Under the stress of Cd and Pb pollution, AMF can promote the degradation of rape straw and the release of mineral elements, thus increasing the content of the available nitrogen, phosphorus, and potassium in the soil, while reducing the contents of the available Cd and Pb in the soil, alleviating the toxic effect of Cd and Pb on maize and promoting the absorption of mineral nutrients by maize, thus improving the growth of maize. Therefore, the combined addition of crop straw and AMF has a synergistic effect on improving the mineral nutrition and growth of later crops and reducing the contents of Cd and Pb, which is conducive to soil fertilizer cultivation and safe crop production of AMF and heavy metal-polluted straw returning to the field, revealing the key role of AMF in crop rotation growth and heavy metal absorption and accumulation in heavy metal-polluted soil.

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References

1. Wu, J.N.; Long, J.; Liu, L.F.; Li, J.; Liao, H.K.; Zhang, M.J.; Zhao, C.; Wu, Q.S. Risk Assessment and Source Identification of Toxic Metals in the Agricultural Soil around a Pb/Zn Mining and Smelting Area in Southwest China. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1838. [CrossRef] [PubMed]
2. Gurajala, H.K.; Cao, X.R.; Tang, L.; Ramesh, T.M.; Lu, M.; Yang, X. Comparative assessment of Indian mustard (*Brassica juncea* L.) genotypes for phytoremediation of Cd and Pb contaminated soils. *Environ. Pollut.* **2019**, *254*, 113085. [CrossRef]
3. Shan, A.Q.; Pan, J.Q.; Kang, K.J.; Pan, M.H.; Wang, G.; Wang, M.; He, Z.L.; Yang, X.E. Effects of straw return with N fertilizer reduction on crop yield, plant diseases and pests and potential heavy metal risk in a Chinese rice paddy: A field study of 2 consecutive wheat-rice cycles. *Environ. Pollut.* **2021**, *288*, 117741. [CrossRef] [PubMed]
4. Zhou, J.; Chen, L.H.; Peng, L.; Luo, S.; Zeng, Q.R. Phytoremediation of heavy metals under an oil crop rotation and treatment of biochar from contaminated biomass for safe use. *Chemosphere* **2020**, *247*, 125856. [CrossRef] [PubMed]
5. Zhang, X.Y.; Gu, P.X.; Liu, X.Y.; Huang, X.; Wang, J.Y.; Zhang, S.Y.; Ji, J.H. Effect of crop straw biochars on the remediation of Cd-contaminated farmland soil by hyperaccumulator *Bidens pilosa* L. *Ecotoxicol. Environ. Saf.* **2021**, *219*, 112332. [CrossRef]
6. Dong, Y.; Wang, H.; Chang, E.; Zhao, Z.J.; Wang, R.H.; Xu, R.K.; Jiang, J. Alleviation of aluminum phytotoxicity by canola straw biochars varied with their cultivating soils through an investigation of wheat seedling root elongation. *Chemosphere* **2019**, *218*, 907–914. [CrossRef]
7. Ma, Y.; Zhang, H.; Wang, D.; Guo, X.; Yang, T.; Xiang, X.; Walder, F.; Chu, H. Differential responses of arbuscular mycorrhizal fungal communities to long-term fertilization in the wheat rhizosphere and root endosphere. *Appl. Environ. Microbiol.* **2021**, *87*, e0034921. [CrossRef]
8. Zhan, F.D.; Chen, J.X.; Zeng, W.Z.; Liang, X.R.; He, Y.M.; Zu, Y.Q. Suppression of arbuscular mycorrhizal fungi increased lead uptake in maize leaves and loss via surface runoff and interflow from polluted farmland. *Environ. Res.* **2022**, *212*, 113594. [CrossRef]
9. Montesinos-Navarro, A.; Segarra-Moragues, J.G.; Valiente-Banuet, A.; Verdú, M. The network structure of plant-arbuscular mycorrhizal fungi. *New Phytol.* **2012**, *194*, 536–547. [CrossRef]
10. Chen, J.X.; Guo, J.F.; Li, Z.R.; Liang, X.R.; You, Y.H.; Li, M.R.; He, Y.M.; Zhan, F.D. Effects of an arbuscular mycorrhizal fungus on the growth of and cadmium uptake in maize grown on polluted wasteland, farmland and slopeland soils in a lead-zinc mining area. *Toxics* **2022**, *10*, 359–372. [CrossRef]
11. Xiao, Y.; Zhao, Z.J.; Chen, L.; Li, Y. Arbuscular mycorrhizal fungi mitigate the negative effects of straw incorporation on *Trifolium repens* in highly Cd-polluted soils. *Appl. Soil Ecol.* **2021**, *157*, 103736. [CrossRef]
12. Ma, K.; Song, L.L.; Wang, M.G.; Ma, Z.Q.; An, Y.Y. Effects of returning corn stalks to the field on soil arbuscular mycorrhizal fungal communities. *Chin. J. Appl. Ecol.* **2019**, *30*, 2746–2756.
13. Li, Z.R.; Colinet, G.; Zu, Y.Q.; Wang, J.X.; An, L.Z.; Li, Q.; Niu, X.Y. Species diversity of *Arabis alpina* L. communities in two Pb/Zn mining areas with different smelting history in Yunnan Province, China. *Chemosphere* **2019**, *233*, 603–614. [CrossRef]
14. Liu, M.Y.; Zhu, J.; Yang, X.; Fu, Q.L.; Hu, H.Q.; Huang, Q.Y. Biochar produced from the straw of common crops simultaneously stabilizes soil organic matter and heavy metals. *Sci. Total Environ.* **2022**, *828*, 154494. [CrossRef]
15. Li, B.; Yang, J.X.; Wei, D.P.; Chen, S.B.; Li, J.M.; Ma, Y.B. Field evidence of cadmium phytoavailability decreased effectively by rape straw and/or red mud with zinc sulphate in a Cd-contaminated calcareous soil. *PLoS ONE* **2014**, *10*, e109967. [CrossRef]
16. Igiehon, N.O.; Babalola, O.O. Biofertilizers and sustainable agriculture: Exploring arbuscular mycorrhizal fungi. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 4871–4881. [CrossRef]
17. Zhao, X.L.; Li, B.Q.; Ni, J.P.; Xie, D.T. Effect of four crop straws on transformation of organic matter during sewage sludge composting. *J. Integr. Agric.* **2016**, *15*, 232–240. [CrossRef]

18. Siedt, M.; Schäffer, A.; Smith KE, C.; Nabel, M.; Roß-Nickoll, M.; van Dongen, J.T. Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides. *Sci. Total Environ.* **2021**, *751*, 141607. [CrossRef]
19. Peng, S.L.; Shen, H.; Yuan, J.J.; Wei, C.F.; Guo, T. Impacts of arbuscular mycorrhizal fungi on soil aggregation dynamics of neutral purple soil. *Acta Ecol. Sin.* **2011**, *31*, 498–505.
20. Shen, Y.; Yang, H.M.; He, W.M. Nutrient availability in habitats affects carbon and nitrogen releases of litter in winter wheat. *Chin. J. Plant Ecol.* **2010**, *34*, 498–504.
21. Li, J.M.; Liang, H.J.; Yan, M.; Chen, L.X.; Zhang, H.T.; Liu, J.; Wang, S.Z.; Jin, Z.X. Arbuscular mycorrhiza fungi facilitate rapid adaptation of Elsholtzia splendens to copper. *Sci. Total Environ.* **2017**, *599*, 1462–1468. [CrossRef] [PubMed]
22. Gerdemann, J.W.; Nicolson, T.H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **1963**, *46*, 235–244. [CrossRef]
23. Wright, S.F.; Franke-Snyder, M.; Morton, J.B.; Upadhyaya, A. Time course study and partial characterization of a protein on hyphae of arbuscular mcyrrohzal fungi during active colonization of roots. *Plant Soil* **1996**, *181*, 193–203. [CrossRef]
24. Bao, S.D. *Soil Agrochemical Analysis*, 3rd ed.; China Agricultural Press: Beijing, China, 2000.
25. Kong, X.S.; Jia, Y.Y.; Song, F.Q.; Tian, K.; Lin, H.; Bei, Z.L.; Jia, X.Q.; Yao, B.; Guo, P.; Tian, X.J. Insight into litter decomposition driven by nutrient demands of symbiosis system through the hypha bridge of arbuscular mycorrhizal fungi. *Environ. Sci. Pollut. Res.* **2018**, *25*, 5369–5378. [CrossRef]
26. Hodge, A.; Campbell, C.D.; Fitter, A.H. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **2001**, *413*, 297–299. [CrossRef]
27. Atul-Nayyar, A.; Hamel, C.; Hanson, K.; Germida, J. The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. *Mycorrhiza* **2009**, *19*, 239–246. [CrossRef]
28. Luo, Z.; Zhu, M.; Wang, X.F.; Liu, X.L.; Guo, T. Influence of arbuscular mycorrhizal inoculation on maize straw degradation and soil microbial biomass carbon and nitrogen, and enzyme activity in di-chamber split-root device. *Chin. J. Eco-Agric.* **2013**, *21*, 149–156. [CrossRef]
29. Lyu, C.H.; Li, L.; Liu, X.W.; Zhao, Z.Q. Rape straw application facilitates Se and Cd mobilization in Cd-contaminated seleniferous soils by enhancing microbial iron reduction. *Environ. Pollut.* **2022**, *310*, 119818. [CrossRef]
30. Ok, Y.S.; Usman AR, A.; Lee, S.S.; Abd El-Azeem SA, M.; Choi, B.; Hashimoto, Y.; Yang, J.E. Effects of rapeseed residue on lead and cadmium availability and uptake by rice plants in heavy metal contaminated paddy soil. *Chemosphere* **2011**, *85*, 677–682. [CrossRef]
31. Li, S.B.; Shi, J.J.; He, Y.M.; Li, B.; Yan, K.; Zhan, F.D.; Duan, H.P. Effects of arbuscular mycorrhizal fungi on the degradation of broad bean straw and the absorption of nutrients, cadmium and lead in corn. *J. Plant Nutr. Fertil.* **2021**, *27*, 684–694.
32. Bai, Y.C.; Gu, C.H.; Tao, T.Y.; Chen, G.H.; Shan, Y. Straw incorporation increases solubility and uptake of cadmium by rice plants. *Acta Agric. Scand.* **2013**, *63*, 193–199. [CrossRef]
33. Li, K.Y.; Zhao, T.T.; Chen, J.; Zhao, X.L. Effects of different organic materials on absorption and translocation of arsenic and cadmium in rice. *Environ. Sci.* **2021**, *42*, 2047–2055.
34. Li, M.; Tian, X.; Liu, R.Z.; Chen, W.L.; Huang, Q.Y. Combined application of rice straw and fungus Penicillium chrysogenum to remediate heavy-metal-contaminated soil. *J. Soil Contam.* **2014**, *23*, 328–338. [CrossRef]
35. Liu, L.; Li, J.W.; Yue, F.X.; Yan, X.W.; Wang, F.Y.; Bloszies, S.; Wang, Y.F. Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil. *Chemosphere* **2018**, *194*, 495–503. [CrossRef]
36. Veresoglou, S.D.; Chen, B.; Rillig, M.C. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biol. Biochem.* **2012**, *46*, 53–62. [CrossRef]
37. Gao, X.; Akhter, F.; Tenuta, M.; Flaten, D.N.; Gawalko, E.J.; Grant, C.A. Mycorrhizal colonization and grain Cd concentration of field -grown durum wheat in response to tillage, preceding crop and phosphorus fertilization. *J. Sci. Food Agric.* **2010**, *90*, 750–758. [CrossRef]
38. He, Y.M.; Yang, R.; Lei, G.; Li, M.R.; Li, T.G.; Zhan, F.D.; Li, Y. Arbuscular mycorrhizal fungus–induced decrease in phosphorus loss due to leaching in red soils under simulated heavy rainfall. *J. Soils Sediments* **2020**, *21*, 881–889. [CrossRef]
39. Aleklett, K.; Wallander, H. Effects of organic amendments with various nitrogen levels on arbuscular mycorrhizal fungal growth. *Appl. Soil Ecol.* **2012**, *60*, 71–76. [CrossRef]
40. Li, H.; Wang, C.; Li, X.L.; Christie, P.; Dou, Z.X.; Zhang, J.L.; Xiang, D. Impact of the earthworm Aporrectodea trapezoids and the arbuscular mycorrhizal fungus Glomus intraradices on 15N uptake by maize from wheat straw. *Biol. Fertil. Soils* **2013**, *49*, 263–271. [CrossRef]
41. Kohler, J.; Caravaca, F.; Azcon, R.; Diaz, G.; Roldan, A. The combination of compost addition and arbuscular mycorrhizal inoculation produced positive and synergistic effects on the phytomanagement of a semiarid mine tailing. *Sci. Total Environ.* **2015**, *514*, 42–48. [CrossRef]
42. Yu, Z.H.; Zhao, X.L.; Su, L.; Yan, K.; Li, B.; He, Y.M.; Zhan, F.D. Effect of an arbuscular mycorrhizal fungus on maize growth and cadmium migration in a sand column. *Ecotoxicol. Environ.Saf.* **2021**, *225*, 112782. [CrossRef] [PubMed]
43. Li, Y.N.; Xu, J.Z.; Hu, J.; Zhang, T.Y.; Wu, X.F.; Yang, Y.R. Arbuscular mycorrhizal fungi and glomalin play a crucial role in soil aggregate stability in Pb-Contaminated soil. *Int. J. Environ. Res. Public Health.* **2022**, *19*, 5029. [CrossRef]

44. Salazar, M.J.; Menoyo, E.; Faggioli, V.; Geml, J.; Cabello, M.; Rodriguez, J.H.; Marro, N.; Pardo, A.; Pignata, M.L.; Becarra, A.G. Pb accumulation in spores of arbuscular mycorrhizal fungi. *Sci. Total Environ.* **2018**, *643*, 238–246. [CrossRef]
45. He, Y.M.; Yang, R.; Lei, G.; Li, B.; Jiang, M.; Yan, K.; Zu, Y.Q.; Zhan, F.D.; Li, Y. Arbuscular mycorrhizal fungi reduce cadmium leaching from polluted soils under simulated heavy rainfall. *Environ. Pollut.* **2020**, *263*, 114406. [CrossRef]
46. Shahabivand, S.; Maivan, H.Z.; Goltapeh, E.M.; Sharifi, M.; Aliloo, A.A. The effects of root endophyte and arbuscular mycorrhizal fungi on growth and cadmium accumulation in wheat under cadmium toxicity. *Plant Physiol. Biochem.* **2012**, *60*, 53–58. [CrossRef]