



# **Review Research Progress and Potential Functions of AMF and GRSP in the Ecological Remediation of Metal Tailings**

Yan-Jun Ai<sup>1</sup>, Fu-Ping Li<sup>1,2,3,\*</sup>, Jia-Qing Yang<sup>1</sup>, Sai Lu<sup>1</sup> and Hai-Hong Gu<sup>1,2,3,\*</sup>

- <sup>1</sup> College of Mining Engineering, North China University of Science and Technology, Tangshan 063210, China
- <sup>2</sup> Hebei Key Laboratory of Mining Development and Security Technology, Tangshan 063210, China
- <sup>3</sup> Hebei Industrial Technology Institute of Mine Ecological Remediation, Tangshan 063210, China
- \* Correspondence: lifuping@ncst.edu.cn (F.-P.L.); guhaihong@ncst.edu.cn (H.-H.G.)

**Abstract:** Metal mining generates a considerable amount of tailings. Arbuscular mycorrhizal fungi (AMF) have potential value for the ecological remediation of tailings from metal mining, despite problems with these tailings, such as loose structure, high heavy-metal concentration and low organic matter and microbial diversity. This review summarizes both the application and physiological functions of AMF, and plant symbiotic systems, in the ecological remediation of tailings from metal mining. The review also includes an in-depth analysis of the characteristics, structural composition, and potential functions of glomalin-related soil protein (GRSP), a release product of mycorrhizal fungi, in the ecological remediation of tailings from metal mining. This review is expected to provide a basis for the application of arbuscular mycorrhizal fungi remediation technology in the ecological remediation of tailings from metal mining.

**Keywords:** arbuscular mycorrhizal fungi; metal tailings; ecological remediation; glomalin-related soil protein



Citation: Ai, Y.-J.; Li, F.-P.; Yang, J.-Q.; Lu, S.; Gu, H.-H. Research Progress and Potential Functions of AMF and GRSP in the Ecological Remediation of Metal Tailings. *Sustainability* **2022**, *14*, 9611. https://doi.org/10.3390/ su14159611

Academic Editor: Glen Corder

Received: 1 July 2022 Accepted: 3 August 2022 Published: 4 August 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/).

# 1. Introduction

Rapid changes in modern industry have led to a sharp rise in the demand for mineral resources, which has also resulted in increased discharge of mining spoil (tailings). By the end of 2014, accumulated tailings in China reached 14.6 billion tons, 83% of which are tailings formed from iron ore, copper, and gold mining [1]. The accumulation of tailings occupies a large amount of land; tailings usually have loose structure, high heavy-metal content, and low organic matter and microbial diversity. This makes it difficult for plants to naturally colonize tailings, thereby restricting ecological remediation. The long-term accumulation of tailings can lead to a serious degradation of environment quality, and even threaten human development. Therefore, tailings from metal mining and the threat they pose to the ecological environment cannot be ignored as there is a need for the timely restoration of these mining sites.

Studies have resulted in a variety of methods for the ecological remediation of tailings, such as physical remediation, chemical remediation, bioremediation, and integrated remediation [2–4]. All these methods have certain advantages and disadvantages. For example, physical remediation has not been widely applied because of the large volumes of material and high relative cost per unit of volume. Although remediation using inorganic amendments produces rapid results, it is expensive, its action cycle is short, and its effectiveness is limited to only tailings of specific mining operations or plant species. Therefore, it is not suitable for long-term, large-scale ecological remediation of tailings. By contrast, organic matter is an important component in maintaining soil structure [5] and driving soil processes [6]. Therefore, remediation using organic amendments can establish soil structure and biological processes in tailings relatively quickly, while increasing the nutrient content in tailings. Among the available bioremediation techniques, phytoremediation is an attractive option in which vegetation can be directly applied to the surface of the tailings, reducing wind and water erosion [7,8]. In addition, phytoremediation can be used to effectively immobilize or extract heavy metals and achieve sustainable recovery of tailings [9,10]. However, phytoremediation alone still has limitations because of the characteristics of poor tailing structure and high heavy-metal content from multiple heavy-metal species. Therefore, an increasing number of studies are employing microbial-assisted phytoremediation to improve remediation efficiency [11].

Many microbial species, including bacteria and fungi, have a remarkable ability to remove heavy metals from a matrix, promote plant growth, and improve matrix structure. Arbuscular mycorrhizal fungi (AMF) are garnering increasing attention from researchers as a remediation technique. AMF are soil microorganisms that are ubiquitous in almost all habitats and climates, can form symbiotic relationships with most plants, and can be applied as a potential bioremediation technique in the ecological remediation of tailings. AMF have many functions in symbiotic systems [12–14], such as promoting plant growth, enhancing drought and disease resistance, tolerance to heavy metals, regulating changes in physiological metabolic activities of the root system, and changing rhizosphere microbial diversity and community structure. Thus, AMF have promising applications in ecological remediation of tailings.

The only protein known to be released into soil by AMF, glomalin-related soil protein (GRSP), is an important mediator of the interaction between AMF and the soil environment [15]. The role of GRSP in enhancing the stability of soil aggregates, increasing carbon and nitrogen content in soil, and sequestering heavy metals, has gradually gained recognition [16]. However, studies on the role of GRSP in ecological remediation of tailings are rarely reported. GRSP may be an important factor for a symbiotic system employing AMF in the ecological remediation of tailings.

#### 2. Overview of AMF

The concept of mycorrhiza was first proposed in the 1880s by Frank, a German plant physiologist [17]. Mycorrhiza is a Greek word combining "myco" (fungus) and "rhiza" (root). AMF is named after its physiological properties; namely, the ability of mycelium to invade the intercellular or intracellular layers of plant roots to form vesicular and arbuscular structures. It was originally named vesicle-arbuscular mycorrhizal fungi. However, it was later found that not all AMF could form vesicle structures; thus, its name was modified to "AMF" [18]. A schematic representation of the structure of AMF is shown in Figure 1. The structure of AMF may be different among different genotypes. Some AMF structures include hypha, arbuscular and vesicle, while some AMF structures only have hypha and arbuscular.



Figure 1. Schematic diagram of the structure of AMF.

AMF are ubiquitous soil organisms and their symbiosis can be found in almost all types of ecosystems. They form symbioses with more than 80% of terrestrial plants. All examined legumes have been found to be associated with mycorrhizae [19]. AMF provide a direct physical link between the soil and plant root system. Many researchers have extensively studied AMF morphological structures, the various species of fungi involved in the formation of AMF, and the physiological and ecological functions of AMF. An in-depth

3 of 15

understanding of the primary functions of AMF can help achieve improved plant growth under abiotic stress situations. Moreover, AMF can be used as a potential biotechnological tool to increase the efficiency of phytoremediation of contaminated soils [20,21].

# 3. Analysis of the Physiological Functions of AMF Symbiosis for Ecological Remediation of Tailings from Metals Mining

Under normal or stressful conditions, AMF can act as a biocatalyst, bioprotectant, and biocontrol agent to improve plant growth and metabolism and enhance plant productivity [22]. AMF has been shown to induce plant growth and increase auxin levels in symbiotic systems by increasing plant access to nutrients. They can affect plant tissue uptake and distribution [23] and enhance drought and disease resistance, as well as tolerance to heavy metals [12,24,25]. AMF can also improve the conditions of the rhizosphere soil microenvironment [26].

# 3.1. Promoting Plant Growth

The mycelial network formed by AMF in symbiosis with plants can substantially increase the area of water and nutrient uptake by host plants and promote plant growth [27]. Moreover, the active mechanism of AMF is attributed to the ability of AMF to alleviate oxidative stress in plants [28] and change the transcription levels of genes involved in signaling pathways or stress responses [29]. Different AMF genotypes can have different effects on host plants, including spreading of extraradical mycelium, nutrient uptake efficiency, and mycorrhizal-specific gene expression, resulting in various growth responses in the host plant [30,31]. The extraradical mycelium of AMF draws mineral nutrients from the soil and transports them to the host plant. Differential expression levels of phosphorus transporter and nitrogen assimilation genes have been reported to result in changes in the ability of plants to take up and transport phosphorus and nitrogen [30].

## 3.2. Improving Drought Resistance in Plants

Inoculation with AMF can improve the drought resistance of plants. AMF symbiosis with plants can improve overall plant growth by increasing root length, leaf area, plant biomass, and nutrient uptake under drought conditions [32]. Improved plant growth through AMF inoculation has been attributed to the formation of an extensive mycelial network and secretion of glomalin, which in turn enhances water and nutrient uptake and improves soil structure [33]. AMF symbiosis is involved in several physiological and biochemical processes. These processes include the direct uptake and translocation of water and nutrients by AMF, increased osmoregulation, improved gas exchange and water use efficiency, and enhanced protection against oxidative damage [34,35]. As AMF-inoculated plants can absorb water from soil to meet the water requirements of their evaporating surfaces [36], these plants show an increase in transpiration rates [37]. For example, high stomatal conductance, transpiration rate, and photosynthetic rate were observed in pot experiments, whereas carbon dioxide concentrations were decreased in plants inoculated with AMF [38]. AMF can also regulate the hydraulic properties of plants by modulating plasma membrane intrinsic proteins through phytohormones [39,40]. In addition, at the molecular level, drought tolerance in plants can be improved by altering their genetic code and regulating gene expression, such as the expression of the PIP2 gene [41]. These results show that inoculation of AMF can enhance the drought tolerance of plants and thus benefit plant growth.

#### 3.3. Improving Metal Tolerance in Plants

Certain mechanisms can be used to enhance plant tolerance to heavy metals and reduce the uptake of elemental metals from plant roots. For example, AMF can enhance the metal tolerance of host plants in symbiotic systems and are widely used in the remediation of heavy-metal contaminated soils [42,43]. Mycorrhizal fungi can achieve morphological transformation of trace elements in the rhizosphere soil through various pathways, in-

cluding chemical precipitation in the soil through acidification and immobilization [44]. The AMF could improve plant tolerance to metals by different mechanisms such as (1) metal restriction by compounds (e.g., glomalin) secreted by AMF [45], Qiu et al. [46] found that the significant negative correlation between GRSP and the combined indicators of eight bioavailable metals' concentration, (2) accumulation of metals on the hyphal surface [47], metal adsorption onto substance (e.g., chitin) in the cell walls [48], to reduce the metals translocation to the host plant, (3) AMF increases the content of phosphorus in the soil, metal deposition in polyphosphate particles in the soil [49,50], (4) alteration of metals availability by changing the rhizosphere pH [51], (5) regulation of gene expression under stress conditions [52], AMF symbiosis up-regulated metallothionein *PtMT2b* in roots regardless of contamination, *PtMT2b* greatly increased Cd tolerance in transgenic yeast under Cd stress [53], AMF colonization distinctly reduced the level of *MsPCS1* and *MsMT2* genes, thereby reducing Cd translocation to the aboveground biomass [54]. One study has demonstrated a significant increase in the biomass of AMF-inoculated plants in soils contaminated with Cd. Furthermore, although Cd concentrations in the roots of the plants increased, the above ground Cd concentrations decreased compared to the control [55]. Using proton-induced X-ray emission, Wu et al. [56] proposed fungal structures in the roots sequestrated Zn (likely by binding to thiols) and the precipitation of Pb and Cu in the mycorrhizal root rhizodermis (likely by Fe compounds). In summary, AMF can adjust the growth pattern of plants in a heavy-metal contaminated environment to improve plant tolerance to these metals.

## 3.4. Altering the Microbial Composition of the Soil Environment

AMF symbionts play a key role in the modification of microorganisms in the soil environment [57]. Soil microorganisms are a critical component in maintaining soil bioactivity. Changes in microbial activity and community structure are sensitive to soil quality and health. The soil microbial population structure is one of the important markers of community structure and stability of soil ecosystems. AMF inoculation can affect the physiological metabolic activities of plant roots. This can lead to nutrient demand-mediated selection of the microbiota in the mycorrhizal root zone, altering rhizosphere microbial diversity, community structure, and microbial activity [58]. This, in turn, affects the release and transformation of soil nutrients. AMF (*Claroideoglomus etunicatum*) was inoculated to maize grown in soils spiked with Lanthanum, and it showed that AMF could significantly alter the structural diversity of soil bacterial and fungal communities [59]. Fu [60] found that soil enzyme activity was enhanced, and the abundance and diversity of microorganisms were significantly upregulated in soils inoculated with AMF.

In addition, AMF can improve soil texture and porosity [61]. AMF can contribute to soil aggregate stability directly through the physical effects of the network around soil particles [62]. Aggregated soils are resistant to wind and water erosion and have better air and water infiltration rates for plant and microbial growth [63]. In addition to affecting soil aggregation and particles in metal contaminated soils, AMF size distribution also plays an important role in the accumulation of soil organic matter and soil organic carbon [64]. All these benefits are inextricably linked to AMF-secreted GRSP.

# 4. Application of AMF Symbionts in the Ecological Remediation of Tailings from Metals Mining

In view of the various physiological functions possessed by AMF symbionts, in some heavily polluted environments, such as smelting and metal tailings, the soil–fungus–plant interactions have been extensively studied by Chinese and other scholars [65–67]. The application of AMF and phytoremediation techniques to the ecological remediation of mining areas has gradually received more attention from researchers [21]. Table 1 summarizes the research progress of AMF-phytoremediation in the ecological remediation of metal-mining wastes in recent years.

Mycorrhizal Species	Host Plants	AMF Effects	References
Funneliformis mosseae	Lolium perenne L., Festuca arundinacea, Hylotelephium spectabile (Bor.) H. Ohba, Tradescantia pallida	Increased plant biomass, decreased heavy metals uptake	[23]
Funneliformis mosseae, Diversispora spurcum	Cynodondactylon (L.) Pers.	Increased the pH, decreased Pb and Cd availability in tailings	[51]
Glomus mosses, Glomus etunicatum, Glomus versiforme	Lolium perenne L.	Increased plant growth, activities of CAT and SOD in plant	[66]
Glomus claroideum, Glomus coronatum	Kalappia celebica	Increased plant growth and N, P and K uptake	[67]
Glomus intraradices	Lolium perenne L.	Increased plant growth	[68]
Glomus mosseae, Glomus intraradices	Medicago sativa L.	Increased plant biomass and P uptake	[69]
Glomus versiforme, Glomus mosseae	Agropyron cristatum (L.) Gaertn., Elymus dahuricus Turcz.	Increased plant growth and N, P and K uptake, decreased heavy metals uptake	[70]
Glomus mosseae, Glomus intraradices	Zenia insignis Chun	Increased plant biomass and P uptake, decreased root to shoot Fe, Pb and Zn translocation	[71]
Gigaspora margarita, Rhizophagus irregularis	Allium cepa L., Lotus japonicus	Showed high signal of Cd in fungal cell	[72]
Claroideoglomus claroideum	Sorghum bicolor (L.) Moench, Trifolium repens L.	Increased plant biomass, promoted the production of photosynthetic pigments and decreased Cu availability in tailings	[73]
Glomus species	Canna indica L.	Increased plant biomass, decreased bioavailability of heavy metals	[74]

**Table 1.** An overview of AMF and phytoremediation in the ecological remediation of metal-mining wastes in recent years.

Scholars applied mycorrhizal fungi to the remediation of solid wastes from mines as early as 1977 [75]. Subsequently, researchers in various countries increasingly applied one or more AMFs or combined AMFs with plants and organic fertilizers to the ecological remediation of mining wastes and achieved promising results. By introducing AMF to promote plant growth on waste sites such as tailings [68], promote the plant uptake of nutrients such as phosphorus [76], and alleviate the concentration of metal in the rhizosphere sediments [77]. It was found that AMF is correlated with the pH, organic matter, and total phosphorus content of the matrix, AMF increased nitrogen, phosphorous and potassium uptake by the plants in the studies on AMF inoculation in gold mine tailings [67,78,79]. Studies of iron mine tailings indicated that inoculation with AMF could effectively increase the activity of acid phosphatase in the rhizosphere of plants, enhance the utilization of nitrogen, phosphorus, and potassium; and significantly reduce the heavy-metal content in plant stems and leaves in tailings by plants, and increase the biomass of plants [69,70,80]. In addition, inoculation of AMF in Pb-Zn tailings increased aboveground and root biomass by 196% and 263%, respectively [23]; effectively contributed to the utilization of phosphorus by plants [71], decreased the available metals in soils and reduced metals translocation to shoots [51]. Analysis of AMF selected from Cd contaminated soils revealed that the fungal cytoplasm had many polyphosphate particles bound to metals, such as Cd, Al, and Fe. Moreover, AMF were effective in reducing the content of heavy metals transported to plants [72]. Previous studies on AMF inoculation in Cu tailings showed that AMF inoculation promoted the production of photosynthetic pigments and plant growth, alleviated the negative effect of Cu on the reproduction output of plants, and reduced the Cu availability in mine tailings [73,81]. Phytomicrobial remediation of a mine in Morocco indicated that the presence of AMF plays an important role in protecting and maintaining vegetative cover. AMF reduced the bioavailability of heavy metals and potential phytotoxic effects while increasing the plant biomass and changing the physical, chemical, and biological properties of the soil [74].

In summary, the establishment of AMF and plant symbiosis systems help plant growth, improve the loose structure of tailings, reduce the concentrations of heavy metals in tailings, alter enzymatic activity and the microbial community in tailings, and possess potential applications for the improvement of the tailing environment.

# 5. Environmental Functions of GRSP, a Release Product of Arbuscular Mycorrhizal Fungi

The GRSP is the only protein known to be released into soil by AMF and is an important mediator of the interaction between mycorrhizal fungi and the soil environment [15]. Glomalin was first discovered in 1996 by Wright et al. [82] as a glycoprotein containing metal ions. It has some adhesive effects produced by the roots or extraradical hyphae surfaces of the AMF host plant. It was considered as an aggregate binder, later defined by Rillig [62] as a GRSP. It was detected in large quantities in different ecosystems. Analysis of GRSP-related literature using CiteSpace [83] software revealed that it has been studied by researchers in many countries around the world, and has received particular attention from Chinese scholars. An internet search for the keyword phrase "co-occurrence network diagram" revealed that studies related to GRSP have been predominantly in the areas of agroecosystems, root morphology, carbon sequestration, plant growth, and enzymatic activity and so on (Figure 2).

# 5.1. Characterization of GRSP

Following Wright's proposed method for the extraction of glomalin from soil, glomalin has been classified into four categories: total glomalin (TG), easily extractable glomalin (EEG), immune reactive total glomalin (IRTG), and immune reactive easily extractable glomalin (IREEG) [82]. However, in-depth studies of the available literature suggest that the glomalin extracted by the above techniques is not a specific secretion of AMF and is not a glomalin. Based on these studies, Rillig et al. [62] proposed a new terminology, GRSP, to clarify that the extracted glomalin is specifically a glomalin-related soil protein.

enzyme activity community carbon growth #8 habitate arbuscular mycorrhiza diversity #3 citrus 2 root morphology hyphae #9 soil organic c plant #0 agroecosystem glomalin related soil protein #1 glomalin-related soil protein glomalin-related soil protein funa organic matter CHILE COTE NOIRE BRAZIL #6 carbon sequestration FRANCE ANADA AUSTRALIA management extraction KAZAKHSTAN ENGLAND GERMANY ..... SPAIN USA elevated co2 **PEOPLES R CHINA** INDIA ITALY CZECH REPUBLIC

**Figure 2.** Map of keyword "co-occurrence network diagram" and the distribution of countries in the studies. #7 c (carbon), #9 soil organic c (soil organic carbon).

GRSP is related to mycorrhizal colonization, spore density, mycelial length, and other soil properties [84]. It forms a symbiotic relationship with roots of most terrestrial plants [85]. Moreover, it is a major soil component in the formation and maintenance of soil structure [86]. As a glycoprotein, GRSP consists primarily of two parts: protein and carbohydrate. GRSP is insoluble in water, heat resistant and hard to degrade, not susceptible to protease hydrolysis, extremely stable in its natural state [87], and can remain in the soil for 6–42 years [88].

## 5.2. Structural Composition of GRSP

With advances in the understanding of GRSP, the structural composition of GRSP has received increasing attention from scholars. Gillespie et al. [89] used X-ray absorption nearedge structure spectroscopy and pyrolysis ionization mass spectrometry simultaneously to reveal that GRSP is a mixture of proteins, humic acids, lipids, and inorganic substances. GRSP was found to be rich in aromatic carbons as well as carboxyl carbons in NMR analysis [90]. Zhang et al. [91] analyzed the possible presence of various metals, such as Cu, Zn, Mg, Fe, Au, and Al, in GRSP using X-ray electron spectroscopy. In addition, the functional groups of GRSP can be analyzed using infrared spectroscopy. Wang et al. [92,93] found that various functional groups, such as hydroxyl and carboxyl groups, are involved in the binding of heavy metals.

# 5.3. Analysis of the Potential Functions of Glomalin-Related Soil Proteins in the Ecological Remediation of Tailings from Metal Mining

GRSP has sufficient refractoriness to remain in the soil for prolonged periods of time, plays a key role in long-term carbon and nitrogen storage, metal sequestration, and enhances the stability of soil aggregates [16]. High AMF spore density and glomalin content are associated with improved soil fertility and can be used as important indicators of the environmental condition of ecosystems spoiled by mining [94]. These characteristics of AMF provide potential value in its application for the ecological remediation of tailings from metal mines.

### 5.3.1. Carbon Fixation Function

GRSP plays a particularly important role in the ecosystem carbon cycle, contributing up to 4–5% to the soil carbon pool [95]. It also has a strong carbon fixation potential [96,97], which benefits soil carbon content [98]. The study by Guo [99] showed that GRSP played an important function in soil carbon pooling. In addition, many experimental studies

suggest that GRSP content is positively correlated with soil organic carbon content and is a key component of soil organic carbon [100–102]. Kumar et al. [103] found a correlation between GRSP and soil organic carbon accumulation in a study on soils in coal mining areas. Because GRSP is a product secreted by AMF, GRSP content and AMF abundance are intimately associated. One study found a significant positive correlation between soil organic carbon, AMF biomass, and surface GRSP [104]. Inhibition of AMF activity in soil can reduce carbon and nitrogen content in soil, mainly because of the reduced mycelial and GRSP contents [105]. These studies suggest that increasing the GRSP content by inoculating AMF is an effective method to increase the carbon content in tailings where carbon sources are relatively scarce.

# 5.3.2. Immobilizing Heavy Metals

GRSP can adsorb or chelate metal cations, such as Fe, Mn, Cu, and Zn, from the soil [106]. This action occurs at the matrix–mycelium junction (i.e., before the metals enter the fungal-plant symbiosis) [107] and plays an equally vital role in sorbent compartmentalization of toxic metals and the reduction of their bioavailability [108]. GRSP can effectively sequester or immobilize different metals from the cell walls of fungal hyphae in soil [46,109]. Even in heavy-metal contaminated soils, fungi are able to associate with the roots of plants [110]. The high content of heavy metals in GRSP extracted from contaminated soils indicates that GRSP is able to adsorb or chelate heavy-metal ions [111,112] and reduce the bioavailability of metals [113,114]. This serves to alleviate the contamination of the surrounding environmental fungi and harm to plants by heavy metals in the soil, helps buffer against metal elements, and protects fungi [115–117]. It was reported that inoculation of Glomus mosseae and Glomus intraradices during ecological remediation in an iron mine in Harbin, China, effectively reduced the total content of heavy metals-Pb, Zn, Cd, Cu, and Fe-in the soil. For example, in the control group, the total Cd concentration was 3.45 mg  $kg^{-1}$ . Following inoculation with *Glomus mosseae* and *Glomus intraradices*, the concentration of Cd was reduced to 2.10 mg·kg<sup>-1</sup> and 2.90 mg·kg<sup>-1</sup>, respectively. However, their diethylenetriaminepentaacetic acid (DTPA) extracted content was higher than that in the control [118]. Siani et al. [119] studied the effects of inoculation of Glomus intraradices on the growth of fenugreek. They found that inoculation with AMF promoted the secretion of rhizosphere GRSP and metal chelation, thereby significantly reducing plant Zn uptake. Because GRSP is able to adsorb and chelate heavy metals, it has the potential to reduce metal toxicity in mine tailings. Furthermore, it shows potential as an indicator of ecosystem remediation of tailings.

# 5.3.3. Increasing the Stability of Soil Aggregates

GRSP can increase the stability of soil aggregates and improve soil quality [120,121]. Glycoproteins cover soil aggregates and form a characteristic coating on their surface, binding them together [122]. The viscous GRSP acts as a bio-gel and helps to bind the tiny soil particles into small aggregates of varying size [123,124]. Inoculation with AMF increases the content of GRSP in the soil and has a positive effect on water-stable aggregates that are <1 mm in size [125]. GRSP polymeric aggregates can greatly improve infiltration of soil water, soil stability, and the ability to prevent natural erosion. Its relatively porous structure provides the necessary space, and better gas exchange channels, for plant root growth [126,127]. In turn, this is favorable for plant and microbial growth [128]. Miller et al. showed that GRSP can adhere to the ectomycorrhizal mycelium of AMF and form aggregates with them to change the permeability of the soil, and enhance the stability of the soil structure, while providing erosion resistance [129]. They demonstrated that GRSP is important for altering the stability of soil aggregates. This function is beneficial in the remediation of tailings with loose structure.

#### 5.3.4. Improving Drought Tolerance in Plants

GRSP increases soil water retention and improves plant drought tolerance. GRSP covers the surface of mycelium and acts as a mycelial coating to regulate the entry and exit of water and nutrients in plants [130]. It prevents water loss and by forming a hydrophobic layer on the surface of soil aggregates and it reduces water loss under drought conditions [131,132]. GRSP has extremely similar amino acid sequences to heat shock protein 60 (HSP60), produced by eukaryotic and prokaryotic cells under environmental stress conditions. It can act as a stress-inducible protein that is strongly expressed in response to various adverse environmental stresses [133]. HSP21 is a molecular chaperone that can maintain the PSII complex and vesicle-like membrane stability under high temperature stress [134]. Therefore, GRSP can play a key role as a molecular chaperone to improve photosynthetic efficiency under drought conditions, thereby improving plant drought tolerance. In addition, GRSP can strongly promote the activity of root superoxide dismutase under drought stress [135], which contributes to plant antioxidant activity and thus improves plant drought tolerance. Therefore, GRSP may improve the water retention properties of tailings and contribute to the growth of plants in tailings.

Moreover, a study has shown that the stability of soil aggregates has an important influence on soil carbon storage, and that reduced aggregate stability leads to reduced protection of soil carbon and nitrogen by soil macroparticles [136]. The distribution of aggregates affects the carbon and nitrogen cycle within them. Aggregates of different particle sizes have different carbon contents [137]. In addition, aggregates greatly affect the transport as well as the bioavailability of heavy metals in soils, and have important effects on heavy-metal enrichment characteristics and morphological distribution [138]. The enrichment of soil heavy metals is related to the particle size of soil aggregates [139]. There are significant differences in the distribution of heavy metals in soils with different particle size aggregates because of the considerable differences in the nature and composition of the aggregate particles [140]. Thus, GRSP, heavy-metal concentrations, carbon content, and aggregate stability are closely related to each other. However, few studies have combined these four factors for elucidating the effector mechanism of GRSP on tailings in rhizosphere aggregates.

In summary, the potential functions of AMF and GRSP on the ecological remediation of metal tailings mainly affect the following aspects: organic carbon accumulation, aggregate aggregation, nutrient absorption, heavy metal migration, microbial regulation, and plant growth. The schematic diagram of the functions of AMF and GRSP is shown in Figure 3.



**Figure 3.** Schematic diagram of the functions of AMF and GRSP. AMF (arbuscular mycorrhizal fungi), GRSP (glomalin-related soil protein), N (nitrogen), P (phosphorus), A (aggregate), HM (heavy metal), SM (soil microflora).

# 6. Conclusions and Outlook

The establishment of a symbiotic system between AMF and plants has potential applications for the improvement of tailings from metal mining. There is an inextricable relationship between GRSP, heavy metals, carbon content, and aggregate stability. Improving environmental quality by increasing the GRSP content in the soil through inoculation with AMF is important for ecological remediation of the fragile tailings from metal mining.

The release and accumulation of GRSP may be an important mechanism for the ecological remediation of tailings from metal mining. Examining the active mechanisms between various factors such as GRSP, heavy metals, carbon content, and aggregate stability may provide a basis for future application of AMF in the remediation of tailings.

Although the structural characteristics of GRSP are understood, the active mechanism of GRSP in chelating heavy metals still requires further study.

**Author Contributions:** Y.-J.A. was associated with validation, the original draft and literature collection. F.-P.L. was associated with validation, framework, and supervision. J.-Q.Y. was associated with software. S.L. was associated with literature collection. H.-H.G. was associated with validation, framework, and correction process. All authors have read and agreed to the published version of the manuscript.

**Funding:** This project was supported by the Key Project of Science and Technology Commission Foundation of Hebei Province, China (No. 19224204D); the Program Project of Innovation Capability Enhancement of Hebei Province, China (No. 20534201D); the Natural Science Foundation of Hebei Province, China (No. E2021209152); the Science and Technology Planning Key Project of Tangshan, China (No. 19150247E); and Science and Technology Innovation Team Project of Tangshan, China (No. 19130206C).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

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

# References

- 1. Sun, Z.Y. Study on Process and Properties of Porous Ceramics Prepared by Fine Grained Iron Tailing from Beijing Area. Master's Thesis, Beijing Jiaotong University, Beijing, China, 2017. (In Chinese)
- Sun, W.; Ji, B.; Khoso, S.A.; Tang, H.H.; Liu, R.Q.; Wang, L.; Hu, Y.H. An extensive review on restoration technologies for mining tailings. *Environ. Sci. Pollut. R* 2018, 25, 33911–33925. [CrossRef]
- Gao, Y.; Wu, P.; Jeyakumar, P.; Bolan, N.; Wang, H.; Gao, B.; Wang, S.; Wang, B. Biochar as a potential strategy for remediation of contaminated mining soils: Mechanisms, applications, and future perspectives. *J. Environ. Manag.* 2022, 313, 114973. [CrossRef] [PubMed]
- 4. Nguyen, T.H.; Won, S.; Ha, M.G.; Nguyen, D.D.; Kang, H.Y. Bioleaching for environmental remediation of toxic metals and metalloids: A review on soils, sediments, and mine tailings. *Chemosphere* **2021**, *282*, 131108. [CrossRef] [PubMed]
- 5. Oades, J.M. Soil organic matter and structural stability: Mechanisms and implications for management. *Plant Soil* **1984**, *76*, 319–337. [CrossRef]
- 6. Schnitzer, M. Soil organic matter: The next 75 years. Soil Sci. 1991, 151, 41–58. [CrossRef]
- Gil-Loaiza, J.; Field, J.P.; White, S.A.; Csavina, J.; Felix, O.; Betterton, E.A.; Saez, A.E.; Maier, R.M. Phytoremediation reduces dust emissions from metal(ioid)-contaminated mine tailings. *Environ. Sci. Technol.* 2018, 52, 5851–5858. [CrossRef] [PubMed]
- Madejón, P.; Domínguez, M.T.; Girón, I.; Burgos, P.; López-Fernández, M.T.; Porras, Ó.G.; Madejón, E. Assessment of the phytoremediation effectiveness in the restoration of uranium mine tailings. *Ecol. Eng.* 2022, 180, 106669. [CrossRef]
- Babu, A.G.; Shim, J.; Shea, P.J.; Oh, B.T. Penicillium aculeatum pdr-4 and *trichoderma* sp. Pdr-16 promote phytoremediation of mine tailing soil and bioenergy production with sorghum-sudangrass. *Ecol. Eng.* 2014, 69, 186–191. [CrossRef]
- Afonso, T.F.; Demarco, C.F.; Pieniz, S.; Camargo, F.A.O.; Quadro, M.S.; Andreazza, R. Potential of solanum viarum dunal in use for phytoremediation of heavy metals to mining areas, southern brazil. *Environ. Sci. Pollut. R* 2019, 26, 24132–24142. [CrossRef]
- Ali, A.; Guo, D.; Mahar, A.; Wang, Z.; Muhammad, D.; Li, R.; Wang, P.; Shen, F.; Xue, Q.; Zhang, Z. Role of streptomyces pactum in phytoremediation of trace elements by brassica juncea in mine polluted soils. *Ecotox. Environ. Safe* 2017, 144, 387–395. [CrossRef]
- 12. Ziedan, E.S.; Elewa, I.; Mostafa, M.; Sahab, A. Application of mycorrhizae for controlling root diseases of sesame. *J. Plant Prot. Res.* 2011, *51*, 355–361. [CrossRef]
- 13. Begum, N.; Qin, C.; Ahanger, M.A.; Raza, S.; Khan, M.I.; Ashraf, M.; Ahmed, N.; Zhang, L.X. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Front. Plant Sci.* **2019**, *10*, 1068. [CrossRef]

- 14. Kaur, S.; Suseela, V. Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. *Metabolites* **2020**, *10*, 335. [CrossRef]
- 15. Singh, P.K.; Singh, M.; Tripathi, B.N. Glomalin: An arbuscular mycorrhizal fungal soil protein. *Protoplasma* **2013**, 250, 663–669. [CrossRef]
- 16. Malekzadeh, E.; Aliasgharzad, N.; Majidi, J.; Abdolalizadeh, J.; Aghebati-Maleki, L. Contribution of glomalin to pb sequestration by arbuscular mycorrhizal fungus in a sand culture system with clover plant. *Eur. J. Soil Biol.* **2016**, *74*, 45–51. [CrossRef]
- 17. Yang, H.J. The Study on the Role and Effects of AMF in Iron Tailings Reclamation of Inner Mongolia Grasslands. Master's Thesis, Inner Mongolia University, Huhehaote, China, 2012. (In Chinese)
- 18. Koide, R.T.; Mosse, B. A history of research on arbuscular mycorrhiza. Mycorrhiza 2004, 14, 145–163. [CrossRef]
- Estaún, V.; Calvet, C.; Camprubí, A. Effect of differences among crop species and cultivars on the arbuscular mycorrhizal symbiosis. In *Arbuscular Mycorrhizas: Physiology and Function*; Springer: Dordrecht, The Netherlands, 2010; pp. 279–295.
- Mathur, N.; Bohra, J.S.S.; Quaizi, A.; Vyas, A. Arbuscular mycorrhizal fungi: A potential tool for phytoremediation. *J. Plant Sci.* 2007, 2, 127–140. [CrossRef]
- 21. Wężowicz, K.; Rozpądek, P.; Turnau, K. Interactions of arbuscular mycorrhizal and endophytic fungi improve seedling survival and growth in post-mining waste. *Mycorrhiza* **2017**, 27, 499–511. [CrossRef]
- 22. Diagne, N.; Ngom, M.; Djighaly, P.I.; Fall, D.; Hocher, V.; Svistoonoff, S. Roles of arbuscular mycorrhizal fungi on plant growth and performance: Importance in biotic and abiotic stressed regulation. *Diversity* **2020**, *12*, 370. [CrossRef]
- Gu, H.H.; Zhou, Z.; Gao, Y.Q.; Yuan, X.T.; Ai, Y.J.; Zhang, J.Y.; Zuo, W.Z.; Taylor, A.A.; Nan, S.Q.; Li, F.P. The influences of arbuscular mycorrhizal fungus on phytostabilization of lead/zinc tailings using four plant species. *Int. J. Phytoremediat.* 2017, 19, 739–745. [CrossRef]
- 24. Bahadur, A.; Batool, A.; Nasir, F.; Jiang, S.J.; Qin, M.S.; Zhang, Q.; Pan, J.B.; Liu, Y.J.; Feng, H.Y. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. *Int. J. Mol. Sci.* **2019**, *20*, 4199. [CrossRef] [PubMed]
- Khalid, M.; Ur-Rahman, S.; Hassani, D.; Hayat, K.; Zhou, P.; Hui, N. Advances in fungal-assisted phytoremediation of heavy metals: A review. *Pedosphere* 2021, 31, 475–495. [CrossRef]
- Miransari, M. Interactions between arbuscular mycorrhizal fungi and soil bacteria. *Appl. Microbiol. Biot.* 2011, *89*, 917–930. [CrossRef] [PubMed]
- 27. Pepe, A.; Giovannetti, M.; Sbrana, C. Appressoria and phosphorus fluxes in mycorrhizal plants: Connections between soil- and plant-based hyphae. *Mycorrhiza* **2020**, *30*, 589–600. [CrossRef] [PubMed]
- 28. Abdel Latef, A.A.H.; He, C. Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. *Acta Physiol. Plant.* **2011**, *33*, 1217–1225. [CrossRef]
- 29. Sun, M.F.; Yuan, D.; Hu, X.C.; Zhang, D.J.; Li, Y.Y. Effects of mycorrhizal fungi on plant growth, nutrient absorption and phytohormones levels in tea under shading condition. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2020**, *48*, 2006–2020. [CrossRef]
- Lee, B.R.; Muneer, S.; Avice, J.C.; Jung, W.J.; Kim, T.H. Mycorrhizal colonisation and p-supplement effects on n uptake and n assimilation in perennial ryegrass under well-watered and drought-stressed conditions. *Mycorrhiza* 2012, 22, 525–534. [CrossRef]
- Mitra, D.; Saritha, B.; Janeeshma, E.; Gusain, P.; Khoshru, B.; Abo Nouh, F.A.; Rani, A.; Olatunbosun, A.N.; Ruparelia, J.; Rabari, A.; et al. Arbuscular mycorrhizal fungal association boosted the arsenic resistance in crops with special responsiveness to rice plant. *Environ. Exp. Bot.* 2022, 193, 104681. [CrossRef]
- 32. Kumar, B.; Rathore, M.; Ranganatha, A.R.G. Weeds as a source of genetic material for crop improvement under adverse conditions. In *Plant Acclimation to Environmental Stress*; Springer: New York, NY, USA, 2013; pp. 323–342.
- Pagano, M.C. Drought stress and mycorrhizal plant. In Use of Microbes for the Alleviation of Soil Stresses, Volume 1; Springer: New York, NY, USA, 2014; pp. 97–110.
- Rapparini, F.; Peñuelas, J. Mycorrhizal fungi to alleviate drought stress on plant growth. In Use of Microbes for the Alleviation of Soil Stresses, Volume 1; Springer: New York, NY, USA, 2014; pp. 21–42.
- 35. Begum, N.; Ahanger, M.A.; Su, Y.Y.; Lei, Y.F.; Mustafa, N.S.A.; Ahmad, P.; Zhang, L.X. Improved drought tolerance by amf inoculation in maize (zea mays) involves physiological and biochemical implications. *Plants* **2019**, *8*, 579. [CrossRef]
- Abdalla, M.; Ahmed, M.A. Arbuscular mycorrhiza symbiosis enhances water status and soil-plant hydraulic conductance under drought. *Front. Plant Sci.* 2021, 12, 722954. [CrossRef]
- Shi, S.M.; Chen, K.; Gao, Y.; Liu, B.; Yang, X.H.; Huang, X.Z.; Liu, G.X.; Zhu, L.Q.; He, X.H. Arbuscular mycorrhizal fungus species dependency governs better plant physiological characteristics and leaf quality of mulberry (*Morus alba* L.) seedlings. *Front. Microbiol.* 2016, 7, 1030. [CrossRef]
- Yang, Y.R.; Tang, M.; Sulpice, R.; Chen, H.; Tian, S.; Ban, Y.H. Arbuscular mycorrhizal fungi alter fractal dimension characteristics of robinia pseudoacacia l. Seedlings through regulating plant growth, leaf water status, photosynthesis, and nutrient concentration under drought stress. J. Plant Growth Regul. 2014, 33, 612–625.
- Fan, Q.J.; Liu, J.H. Colonization with arbuscular mycorrhizal fungus affects growth, drought tolerance and expression of stress-responsive genes in poncirus trifoliata. *Acta Physiol. Plant.* 2011, 33, 1533–1542. [CrossRef]
- Aganchich, B.; Wahbi, S.; Yaakoubi, A.; El-Aououad, H.; Bota, J. Effect of arbuscular mycorrhizal fungi inoculation on growth and physiology performance of olive tree under regulated deficit irrigation and partial rootzone drying. *S. Afr. J. Bot.* 2022, 148, 1–10. [CrossRef]

- 41. Azcón, R.; Medina, A.; Aroca, R.; Ruiz-Lozano, J.M. Abiotic Stress Remediation by the Arbuscular Mycorrhizal Symbiosis and Rhizosphere Bacteria/Yeast Interactions, Molecular Microbial Ecology of the Rhizosphere, 1 & 2; Wiley: New York, NY, USA, 2013.
- 42. Hashem, A.; Abd Allah, E.F.; Alqarawi, A.A.; Egamberdieva, D. Bioremediation of adverse impact of cadmium toxicity on cassia italica mill by arbuscular mycorrhizal fungi. *Saudi J. Biol. Sci.* **2016**, *23*, 39–47. [CrossRef]
- 43. Wang, H.R.; Zhao, X.Y.; Zhang, J.M.; Lu, C.; Feng, F.J. Arbuscular mycorrhizal fungus regulates cadmium accumulation, migration, transport, and tolerance in *Medicago sativa*. J. Hazard. Mater. **2022**, 435, 129077. [CrossRef]
- 44. Upadhyaya, H.; Panda, S.K.; Bhattacharjee, M.K.; Dutta, S. Role of arbuscular mycorrhiza in heavy metal tolerance in plants: Prospects for phytoremidiation. *J. Phytol.* **2010**, *2*, 16–27.
- Riaz, M.; Kamran, M.; Fang, Y.Z.; Wang, Q.Q.; Cao, H.Y.; Yang, G.L.; Deng, L.L.; Wang, Y.J.; Zhou, Y.Y.; Anastopoulos, I.; et al. Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. *J. Hazard. Mater.* 2021, 402, 123919. [CrossRef]
- Qiu, L.; Lin, H.Z.; Song, B.R.; Kong, T.L.; Sun, W.M.; Sun, X.X.; Zhang, Y.X.; Li, B.Q. Glomalin-related soil protein (grsp) in metal sequestration at pb/zn-contaminated sites. J. Soil Sediment 2022, 22, 577–593. [CrossRef]
- 47. Toler, H.D.; Morton, J.B.; Cumming, J.R. Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. *Water Air Soil Pollut.* 2005, 164, 155–172. [CrossRef]
- 48. Galli, U.; Schuepp, H.; Brunold, C. Heavy-metal binding by mycorrhizal fungi. Physiol. Plant. 1994, 92, 364–368. [CrossRef]
- 49. Nafady, N.A.; Elgharably, A. Mycorrhizal symbiosis and phosphorus fertilization effects on zea mays growth and heavy metals uptake. *Int. J. Phytoremediat.* **2018**, *20*, 869–875. [CrossRef]
- Lance, A.C.; Burke, D.J.; Hausman, C.E.; Burns, J.H. Microbial inoculation influences arbuscular mycorrhizal fungi community structure and nutrient dynamics in temperate tree restoration. *Restor. Ecol.* 2019, 27, 1084–1093. [CrossRef]
- Zhan, F.D.; Li, B.; Jiang, M.; Li, T.G.; He, Y.M.; Li, Y.; Wang, Y.S. Effects of arbuscular mycorrhizal fungi on the growth and heavy metal accumulation of bermudagrass *cynodon dactylon* (L.) pers. Grown in a lead-zinc mine wasteland. *Int. J. Phytoremediat.* 2019, 21, 849–856. [CrossRef]
- 52. Malekzadeh, E.; Alikhani, H.A.; Savaghebifiroozabadi, G.R.; Zarei, M. Influence of arbuscular mycorrhizal fungi and an improving growth bacterium on cd uptake and maize growth in cd-polluted soils. *Span. J. Agric. Res.* **2011**, *9*, 1213–1223. [CrossRef]
- 53. De Oliveira, V.H.; Ullah, I.; Dunwell, J.M.; Tibbett, M. Mycorrhizal symbiosis induces divergent patterns of transport and partitioning of cd and zn in populus trichocarpa. *Environ. Exp. Bot.* **2020**, *171*, 103925. [CrossRef]
- Motaharpoor, Z.; Taheri, H.; Nadian, H. Rhizophagus irregularis modulates cadmium uptake, metal transporter, and chelator gene expression in *Medicago sativa*. *Mycorrhiza* 2019, 29, 389–395. [CrossRef] [PubMed]
- 55. Liu, L.Z.; Zhang, Y.L.; Li, P.J.; Gong, Z.Q. Effects of arbuscular mycorrhizal fungi (*Glomus mosseae*) on Cd Accumulation in Maize Plants. *Chin. J. Soil Sci.* 2011, 42, 568–572. (In Chinese)
- Wu, S.L.; Vosatka, M.; Vogel-Mikus, K.; Kavcic, A.; Kelemen, M.; Sepec, L.; Pelicon, P.; Skala, R.; Powter, A.R.V.; Teodoro, M.; et al. Nano zero-valent iron mediated metal(loid) uptake and translocation by arbuscular mycorrhizal symbioses. *Environ. Sci. Technol.* 2018, 52, 7640–7651. [CrossRef]
- 57. Luo, L.; Guo, M.; Wang, E.; Yin, C.; Wang, Y.; He, H.; Zhao, C. Effects of mycorrhiza and hyphae on the response of soil microbial community to warming in eastern Tibetan Plateau. *Sci. Total Environ.* **2022**, *837*, 155498. [CrossRef]
- 58. Fabianska, I.; Sosa-Lopez, E.; Bucher, M. The role of nutrient balance in shaping plant root-fungal interactions: Facts and speculation. *Curr. Opin. Microbiol.* **2019**, *49*, 90–96. [CrossRef]
- 59. Hao, L.J.; Zhang, Z.C.; Hao, B.H.; Diao, F.W.; Zhang, J.X.; Bao, Z.H.; Guo, W. Arbuscular mycorrhizal fungi alter microbiome structure of rhizosphere soil to enhance maize tolerance to La. *Ecotox. Environ. Safe* **2021**, 212, 111996. [CrossRef]
- 60. Fu, L. The Remediation of Copper Contaminated Soils Combined with *Tagetes patula*, L., Earthworm and Arbuscular Mycorrhizal Fungus. Ph.D. Thesis, Nanjing Agricultural University, Nanjing, China, 2016. (In Chinese).
- 61. Gao, Y.Z.; Cheng, Z.X.; Ling, W.T.; Huang, J. Arbuscular mycorrhizal fungal hyphae contribute to the uptake of polycyclic aromatic hydrocarbons by plant roots. *Bioresour. Technol.* **2010**, *101*, 6895–6901. [CrossRef]
- 62. Rillig, M.C. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can. J. Soil Sci. 2004, 84, 355–363. [CrossRef]
- 63. Xia, J.B.; Zhao, Z.G.; Fang, Y. Soil hydro-physical characteristics and water retention function of typical shrubbery stands in the yellow river delta of China. *Catena* **2017**, *156*, 315–324. [CrossRef]
- 64. Yang, Y.R.; He, C.J.; Huang, L.; Ban, Y.H.; Tang, M. The effects of arbuscular mycorrhizal fungi on glomalin-related soil protein distribution, aggregate stability and their relationships with soil properties at different soil depths in lead-zinc contaminated area. *PLoS ONE* **2017**, *12*, 19. [CrossRef]
- 65. Meier, S.; Borie, F.; Bolan, N.; Cornejo, P. Phytoremediation of metal-polluted soils by arbuscular mycorrhizal fungi. *Crit. Rev. Environ. Sci. Technol.* **2012**, *42*, 741–775. [CrossRef]
- 66. Yang, Q.; Zhao, Z.; Bai, Z.; Hou, H.; Yuan, Y.; Guo, A.; Li, Y. Effects of mycorrhizae and water conditions on perennial ryegrass growth in rare earth tailings. *RSC Adv.* **2019**, *9*, 10881–10888. [CrossRef]
- 67. Husna Tuheteru, F.D.; Arif, A. The potential of arbuscular mycorrhizal fungi to conserve kalappia celebica, an endangered endemic legume on gold mine tailings in sulawesi, indonesia. *J. For. Res.* **2021**, *32*, 675–682. [CrossRef]
- Verdugo, C.; Sánchez, P.; Santibáñez, C.; Urrestarazu, P.; Bustamante, E.; Silva, Y.; Gourdon, D.; Ginocchio, R. Efficacy of lime, biosolids, and mycorrhiza for the phytostabilization of sulfidic copper tailings in chile: A greenhouse experiment. *Int. J. Phytoremediat.* 2011, 13, 107–125. [CrossRef]

- 69. Zhang, C.Q.; Bi, Y.L.; Yu, M.; Chen, B.D. The modified effect of arbuscular mycorrhizal fungi on the Fe tailings substrate. *Met. Mine* **2010**, *39*, 171–174. (In Chinese)
- 70. Guo, W.; Zhao, R.X.; Yang, H.J.; Zhao, J.; Zhang, J. Using native plants to evaluate the effect of arbuscular mycorrhizal fungi on revegetation of iron tailings in grasslands. *Biol. Fert. Soils* **2013**, *49*, 617–626. [CrossRef]
- 71. Li, X.; Peng, X.W.; Wu, S.L.; Li, Z.R.; Feng, H.M.; Jiang, Z.P. Effect of arbuscular mycorrhizae on growth, heavy metal uptake and accumulation of Zenia insignis chun seedlings. *Environ. Sci.* **2014**, *1*, 3142–3148. (In Chinese)
- 72. Nayuki, K.; Chen, B.; Ohtomo, R.; Kuga, Y. Cellular imaging of cadmium in resin sections of arbuscular mycorrhizas using synchrotron micro x-ray fluorescence. *Microbes Environ.* **2014**, *29*, 60–66. [CrossRef]
- Perez, R.; Tapia, Y.; Antilen, M.; Casanova, M.; Vidal, C.; Santander, C.; Aponte, H.; Cornejo, P. Interactive effect of compost application and inoculation with the fungus claroideoglomus claroideum in oenothera picensis plants growing in mine tailings. *Ecotox Environ. Safe* 2021, 208, 111495. [CrossRef]
- 74. El, F.A.; Duponnois, R.; Winterton, P.; Ouhammou, A.; Meddich, A.; Boularbah, A.; Hafidi, M. Effect of different amendments on growing of canna indica l. Inoculated with amf on mining substrate. *Int. J. Phytoremediat.* **2015**, *17*, 503–513.
- Daft, M.J.; Hacskaylo, E. Growth of endomycorrhizal and nonmycorrhizal red maple seedlings in sand and anthracite spoil. *For. Sci.* 1977, 23, 207–216.
- 76. Victoria, K.S.; Aggangan, N.S. Effect of mycorrhizal inoculation on growth, nutrient status, and rhizosphere microbes of acacia mangium and eucalyptus urophylla. *Philipp. J. Crop Sci.* **2019**, *44*, 9–17.
- 77. Di, L.; Zheng, K.Y.; Wang, Y.; Zhang, Y.; Lao, R.M.; Qin, Z.Y.; Li, T.; Zhao, Z.W. Harnessing an arbuscular mycorrhizal fungus to improve the adaptability of a facultative metallophytic poplar (*Populus yunnanensis*) to cadmium stress: Physiological and molecular responses. *J. Hazard. Mater.* 2022, 424, 127430.
- Straker, C.J.; Weiersbye, I.M.; Witkowski, E.T.F. Arbuscular mycorrhiza status of gold and uranium tailings and surrounding soils of South Africa's deep level gold mines: I. Root colonization and spore levels. S. Afr. J. Bot. 2007, 73, 218–225. [CrossRef]
- 79. Straker, C.J.; Freeman, A.J.; Witkowski, E.T.F.; Weiersbye, I.M. Arbuscular mycorrhiza status of gold and uranium tailings and surrounding soils of South Africa's deep level gold mines. II. Infectivity. *S. Afr. J. Bot.* **2008**, *74*, 197–207. [CrossRef]
- 80. Zanchi, C.S.; Batista, E.R.; Silva, A.O.; Barbosa, M.V.; Pinto, F.A.; dos Santos, J.V.; Carneiro, M.A.C. Recovering soils affected by iron mining tailing using herbaceous species with mycorrhizal inoculation. *Water Air Soil Poll.* **2021**, 232, 110. [CrossRef]
- 81. Jin, Z.X.; Li, J.M.; Li, Y.L. Interactive effects of arbuscular mycorrhizal fungi and copper stress on flowering phenology and reproduction of elsholtzia splendens. *PLoS ONE* **2015**, *10*, e0145793. [CrossRef] [PubMed]
- 82. Wright, S.F.; Upadhyaya, A. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci.* **1996**, *161*, 575–586. [CrossRef]
- 83. Chen, C.M.; Song, M. Visualizing a field of research: A methodology of systematic scientometric reviews. *PLoS ONE* **2019**, *14*, e0223994. [CrossRef]
- 84. Wu, Q.S.; Cao, M.Q.; Zou, Y.N.; He, X.H. Direct and indirect effects of glomalin, mycorrhizal hyphae, and roots on aggregate stabililety in rhizosphere of trifoliate orange. *Sci. Rep. UK* **2014**, *4*, 5823. [CrossRef]
- 85. Wright, S.F.; Nichols, K.A.; Schmidt, W.F. Comparison of efficacy of three extractants to solubilize glomalin on hyphae and in soil. *Chemosphere* **2006**, *64*, 1219–1224. [CrossRef]
- 86. Emran, M.; Gispert, M.; Pardini, G. Patterns of soil organic carbon, glomalin and structural stability in abandoned mediterranean terraced lands. *Eur. J. Soil Sci.* 2012, *63*, 637–649. [CrossRef]
- 87. Wright, S.F.; Upadhyaya, A.; Buyer, J.S. Comparison of n-linked oligosaccharides of glomalin from arbuscular mycorrhizal fungi and soils by capillary electrophoresis. *Soil Biol. Biochem.* **1998**, *30*, 1853–1857. [CrossRef]
- 88. Tian, H.; Liu, X.L.; Gai, J.P.; Zhang, J.L.; Li, X.L. Review of glomalin-related soil protein and its function. *Chin. J. Soil Sci.* 2009, 40, 1215–1220. (In Chinese)
- Gillespie, A.W.; Farrell, R.E.; Walley, F.L.; Ross, A.R.S.; Leinweber, P.; Eckhardt, K.U.; Regier, T.Z.; Blyth, R.I.R. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. *Soil Biol. Biochem.* 2011, 43, 766–777. [CrossRef]
- 90. Zhang, J.; Tang, X.; Zhong, S.; Yin, G.; Gao, Y.; He, X. Recalcitrant carbon components in glomalin-related soil protein facilitate soil organic carbon preservation in tropical forests. *Sci. Rep. UK* 2017, *7*, 2391. [CrossRef]
- 91. Zhang, Z.; Wang, Q.; Wang, H.; Nie, S.; Liang, Z. Effects of soil salinity on the content, composition, and ion binding capacity of glomalin-related soil protein (grsp). *Sci. Total Environ.* 2017, *581*, 657–665. [CrossRef]
- 92. Wang, Q.; Chen, J.Y.; Chen, S.; Qian, L.; Yuan, B.; Tian, Y.; Wang, Y.Z.; Liu, J.C.; Yan, C.L.; Lu, H.L. Terrestrial-derived soil protein in coastal water: Metal sequestration mechanism and ecological function. *J. Hazard. Mater.* **2020**, *386*, 121655. [CrossRef]
- Wang, Q.; Lu, H.L.; Chen, J.Y.; Jiang, Y.C.; Williams, M.A.; Wu, S.J.; Li, J.W.; Liu, J.C.; Yang, G.S.; Yan, C.L. Interactions of soil metals with glomalin-related soil protein as soil pollution bioindicators in mangrove wetland ecosystems. *Sci. Total Environ.* 2020, 709, 136051–136059. [CrossRef]
- 94. Xu, J.; Tang, M. Relationship between arbuscular mycorrhizal fungi and soil factors in the rhizosphere of different tree species in Pb-Zn polluted mine. *J. Northwest A F Univ.* **2013**, *5*, 75–80. (In Chinese)
- 95. Rillig, M.C.; Wright, S.F.; Nichols, K.A.; Schmidt, W.F.; Torn, M.S. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* **2001**, 233, 167–177. [CrossRef]

- Wu, Q.S.; Li, Y.; Zou, Y.N.; He, X.H. Effects of arbuscular mycorrhizal fungi on aggregate stability, GRSP, and carbohydrates of white clover. *Mycorrhiza* 2015, 25, 121–130. [CrossRef]
- 97. Staunton, S.; Saby, N.P.A.; Arrouays, D.; Quiquampoix, H. Can soil properties and land use explain glomalin-related soil protein (grsp) accumulation? A nationwide survey in france. *Catena* **2020**, *193*, 104620. [CrossRef]
- Matos, P.S.; Figueira Da Silva, C.; Pereira, M.G.; da Silva, E.M.R.; Tarré, R.M.; Custódio Franco, A.L.; Zonta, E. Short-term modifications of mycorrhizal fungi, glomalin and soil attributes in a tropical agroforestry. *Acta Oecol.* 2022, 114, 103815. [CrossRef]
- 99. Guo, L.D.; Tian, C.J. Progress of the function of mycorrhizal fungi in the cycle of carbon and nitrogen. *Microbiol. China* **2013**, *1*, 158–171. (In Chinese)
- Bai, C.M.; He, X.L.; Tang, H.T.; Shan, B.Q.; Zhao, L.L. Spatial distribution of arbuscular mycorrhizal fungi, glomalin and soil enzymes under the canopy of astragalus adsurgens pall. In the mu us sandland, China. *Soil Biol. Biochem.* 2009, 41, 941–947. [CrossRef]
- 101. Buyer, J.S.; Zuberer, D.A.; Nichols, K.A.; Franzluebbers, A.J. Soil microbial community function, structure, and glomalin in response to tall fescue endophyte infection. *Plant Soil* 2011, 339, 401–412. [CrossRef]
- 102. Singh, A.K.; Jiang, X.J.; Yang, B.; Li, H.M.; Liu, W.J.; Singh, N. Effect of root-glomalin on soil carbon storage in trees' rhizosphere and interspace of a tropical dry forest. *Land Degrad. Devt.* **2021**, *32*, 5281–5291. [CrossRef]
- 103. Kumar, S.; Singh, A.K.; Ghosh, P. Distribution of soil organic carbon and glomalin related soil protein in reclaimed coal mine-land chronosequence under tropical condition. *Sci. Total Environ.* **2018**, *625C*, 1341–1350. [CrossRef]
- Li, B.W. Effect of Fertilization on GRSP and Environmental Factors in Alpine Meadows on the Qinghai-Tibetan Plateau. Master's Thesis, Lanzhou University, Lanzhou, China, 2016. (In Chinese)
- 105. Wang, J.; Zhou, Z.Y.; Ling, W.T. Disrtibution and environmental function of glomalin-related soil protein: A review. *Chin. J. Appl. Ecol.* **2016**, *27*, 634–642. (In Chinese)
- 106. Wang, M.Y.; Xia, R.X.; Wang, P. Effects of arbuscular mycorrhizal fungi on available iron and metals sequestered by glomalin in different rhizospheric soil of Poncirus trifoliata. J. Fujian Agr. For. Univ. (Nat. Sci. Edit.) 2010, 39, 42–46. (In Chinese)
- 107. Khan, A.G. Mycorrhizoremediation-an enhanced form of phytoremediation. J. Zhejiang Univ. Sci. B 2006, 7, 503–514. [CrossRef]
- 108. Jia, X.; Zhao, Y.H.; Liu, T.; Huang, S.P.; Chang, Y.F. Elevated CO<sub>2</sub> increases glomalin-related soil protein (grsp) in the rhizosphere of robinia pseudoacacia l. Seedlings in Pb- and Cd-contaminated soils. *Environ. Pollut.* **2016**, *218*, 349–357. [CrossRef]
- 109. Bano, S.A.; Ashfaq, D. Role of mycorrhiza to reduce heavy metal stress. Nat. Sci. 2013, 5, 16–20. [CrossRef]
- 110. Yuan, W.; Su, Q.; Sun, Z.J.; Shen, Y.Q.; Li, J.N.; Zhu, X.L.; Hong, H.; Chen, Z.P.; Feng, C.W. The role of arbuscular mycorrhizal fungi in plant uptake, fractions, and speciation of antimony. *Appl. Soil. Ecol.* **2016**, *107*, 244–250.
- 111. Tang, H.L.; Liu, L.; Wang, L.; Ba, C.J. Effect of land use type on profile distribution of glomalin. *Chin. J. Eco-Agric.* 2009, 17, 1137–1142. (In Chinese) [CrossRef]
- 112. Gujre, N.; Agnihotri, R.; Rangan, L.; Sharma, M.P.; Mitra, S. Deciphering the dynamics of glomalin and heavy metals in soils contaminated with hazardous municipal solid wastes. *J. Hazard. Mater.* **2021**, *416*, 125869. [CrossRef] [PubMed]
- 113. Zhao, X.G.; Wang, H.; Gao, B.; Yuan, Z.L. Effects of inoculation with *Glomus mosseae* on toxicity in tobacco under Pb stress. *J. Henan Agric. Univ.* **2015**, *2*, 153–157. (In Chinese)
- 114. Chen, H.; Xiong, J.; Fang, L.; Han, F.; Zhao, X.; Fan, Q.; Tan, W. Sequestration of heavy metals in soil aggregates induced by glomalin-related soil protein: A five-year phytoremediation field study. *J. Hazard. Mater.* **2022**, 437, 129445. [CrossRef]
- 115. Wright, S.F.; Upadhyaya, A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* **1998**, *198*, *97*–107. [CrossRef]
- 116. Gohre, V.; Paszkowski, U. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* **2006**, 223, 1115–1122. [CrossRef]
- 117. Wang, Q.; Hong, H.L.; Yang, D.; Li, J.W.; Chen, S.; Pan, C.L.; Lu, H.L.; Liu, J.C.; Yan, C.L. Health risk assessment of heavy metal and its mitigation by glomalin-related soil protein in sediments along the south china coast. *Environ. Pollut.* **2020**, *263*, 114565. [CrossRef]
- 118. Abbaslou, H.; Bakhtiari, S.; Hashemi, S.S. Rehabilitation of iron ore mine soil contaminated with heavy metals using rosemary phytoremediation-assisted mycorrhizal arbuscular fungi bioaugmentation and fibrous clay mineral immobilization. *Iran J. Sci. Technol.* **2018**, *A42*, 431–441. [CrossRef]
- Siani, N.G.; Fallah, S.; Pokhrel, L.R.; Rostamnejadi, A. Natural amelioration of zinc oxide nanoparticle toxicity in fenugreek (trigonella foenum-gracum) by arbuscular mycorrhizal (*Glomus intraradices*) secretion of glomalin. *Plant Physiol. Biochem.* 2017, 112, 227–238. [CrossRef]
- Singh, G.; Bhattacharyya, R.; Das, T.K.; Sharma, A.R.; Ghosh, A.; Das, S.; Jha, P. Crop rotation and residue management effects on soil enzyme activities, glomalin and aggregate stability under zero tillage in the indo-gangetic plains. *Soil Till. Res.* 2018, 184, 291–300. [CrossRef]
- 121. Huang, B.; Yan, G.; Liu, G.; Sun, X.; Wang, X.; Xing, Y.; Wang, Q. Effects of long-term nitrogen addition and precipitation reduction on glomalin-related soil protein and soil aggregate stability in a temperate forest. *Catena* **2022**, 214, 106284. [CrossRef]
- 122. Wright, S.F.; Anderson, R.L. Aggregate stability and glomalin in alternative crop rotations for the central great plains. *Biol. Fert. Soils* **2000**, *31*, 249–253. [CrossRef]
- 123. Rillig, M.C.; Mummey, D.L. Mycorrhizas and soil structure. New Phytol. 2006, 171, 41–53. [CrossRef]

- 124. Fokom, R.; Adamou, S.; Teugwa, M.C.; Boyogueno, A.D.B.; Nana, W.L.; Ngonkeu, M.E.L.; Tchameni, N.S.; Nwaga, D.; Ndzomo, G.T.; Zollo, P.H.A. Glomalin related soil protein, carbon, nitrogen and soil aggregate stability as affected by land use variation in the humid forest zone of south cameroon. *Soil Till. Res.* 2012, *120*, 69–75. [CrossRef]
- 125. Wu, Q.S.; Yuan, F.Y.; Fei, Y.J.; Li, L.; Huang, Y.M. Effects of mycorrhizal fungi on the stability of rhizosphere aggregates, soil proteins and saccharides related to the white trifoliate. *Acta Prataculturae Sin.* **2014**, *4*, 269–275. (In Chinese)
- 126. Li, T.; Zhao, Z.W. Advances in researches on glomalin produced by arbuscular mycorrhizal fungi. *Chin. J. Ecol.* **2005**, *24*, 1080–1084. (In Chinese)
- 127. Sekaran, U.; Sagar, K.L.; Kumar, S. Soil aggregates, aggregate-associated carbon and nitrogen, and water retention as influenced by short and long-term no-till systems. *Soil Till. Res.* **2021**, *208*, 104885. [CrossRef]
- 128. Bronick, C.J.; Lal, R. Soil structure and management: A review. Geoderma 2005, 124, 3–22. [CrossRef]
- 129. Wright, S.F.; Starr, J.L.; Paltineanu, I.C. Changes in aggregate stability and concentration of glomalin during tillage management transition. *Soil Sci. Soci. Am. J.* **1999**, *63*, 1825–1829. [CrossRef]
- 130. Galazka, A.; Gawryjolek, K. Glomalin-soil glicoprotein produced by arbuscular mycorhizal fungus. *Postepy Mikrobiol.* **2015**, *54*, 331–343.
- 131. Nichols, K.A. Indirect contributions of am fungi and soil aggregation to plant growth and protection. In *Mycorrhizae: Sustainable Agriculture and Forestry*; Springer: Dordrecht, The Netherlands, 2008; pp. 177–194.
- 132. Ji, L.L.; Tan, W.F.; Chen, X.H. Arbuscular mycorrhizal mycelial networks and glomalin-related soil protein increase soil aggregation in calcaric regosol under well-watered and drought stress conditions. *Soil Till. Res.* **2019**, *185*, 1–8. [CrossRef]
- 133. Purin, S.; Rillig, M.C. The arbuscular mycorrhizal fungal protein glomalin: Limitations, progress, and a new hypothesis for its function. *Pedobiologia* **2008**, *51*, 123–130. [CrossRef]
- 134. Chen, S.T.; He, N.Y.; Chen, J.H.; Guo, F.Q. Identification of core subunits of photosystem II as action sites of hsp21 that is activated by the gun5-mediated retrograde pathway in arabidopsis. *Plant J.* **2017**, *89*, 1106–1118. [CrossRef]
- Chi, G.G.; Srivastava, A.K.; Wu, Q.S. Exogenous easily extractable glomalin-related soil protein improves drought tolerance of trifoliate orange. Arch. Agron Soil Sci. 2018, 64, 1341–1350. [CrossRef]
- Lobe, I.; Sandhage-Hofmann, A.; Brodowski, S.; Preez, C.C.D.; Amelung, W. Aggregate dynamics and associated soil organic matter contents as influenced by prolonged arable cropping in the south african highveld. *Geoderma* 2011, 162, 251–259. [CrossRef]
- 137. Yang, H.; Wang, J.; Zhang, F. Soil aggregation and aggregate-associated carbon under four typical halophyte communities in an arid area. *Environ. Sci. Pollut. Res. Int.* 2016, 23, 23920–23929. [CrossRef]
- 138. Lu, Y.N.; Xu, D.D.; Cheng, H.X.; Zhou, G.H.; Ma, L.L. Recent advances in studying characteristics of heavy metals enriched in soil aggregates. *Chin. J. Soil Sci.* 2014, 45, 1008–1013. (In Chinese)
- Deng, A.M.; Wang, L.; Chen, F.; Li, Z.G.; Liu, W.Z.; Liu, Y. Soil aggregate-associated heavy metals subjected to different types of land use in subtropical china. *Glob. Ecol. Conserv.* 2018, 16, e00465. [CrossRef]
- 140. Sun, W.J.; Huang, Y.; Zhang, W.; Yu, Y.Q. Key issues on soil carbon sequestration potential in agricultural soils. *Adv. Earth Sci.* **2008**, *23*, 996–1004. (In Chinese)