Ecological and molecular characteristics of arbuscular mycorrhizal fungi (AMF) on mercury phytoremediation

Dissertation

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There is only one heroism in the world: to see the world as it is and to love it.

Romain Rolland

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Summary

Environmental pollution caused by harmful chemicals represents a major challenge worldwide. Among these, heavy metals (HM) in soils are of particular concern due to their persistence, toxicity, and bioaccumulation which can significantly threaten human health, plant growth, and ecosystem integrity. Phytoremediation, which uses plants to extract pollutants from soils, has been recognized as a promising approach to remediate HM-contaminated soils. Arbuscular mycorrhizal fungi (AMF)-assisted phytoremediation has shown great potential to enhance plant growth and metal uptake by forming a mutual association between plant roots and AMF, which can improve nutrient uptake and tolerance to environmental stress. Despite its potential, however, the effectiveness of this approach can be limited by various factors, such as environmental and geographic factors, soil properties, and plant-microbe interactions. An advanced fundamental understanding of both ecological and molecular characteristics of this technology is thus crucial to improve its effectiveness and application potential. Therefore, the impetus of this doctoral thesis was to investigate the potential of AMF in phytoremediation of soils contaminated with HM, with a particular focus on mercury (Hg) remediation.

The first study (Chapter 2) contributes to the ecological understanding of AMF in a degraded ecosystem. In this study, two geographically distinct, abandoned gold mining locations in Ghana were selected and the genetic diversity and composition of AMF communities both in the rhizosphere and roots of the pioneer plant *Pueraria phaseoloides* (Roxb.) Benth. (tropical kudzu) were analyzed using a metagenomic sequencing approach. To determine the primary factor shaping AMF communities, both biotic (plant identity) and abiotic factors (geographic locations and soil conditions) were examined. In total, 195 amplicon sequence variants (ASV) affiliated to eight genera of the phylum *Glomeromycota* were identified. The root compartment showed a lower diversity than the rhizosphere soils and a difference of AMF compositions between the two compartments was detected irrespective of geographical location. Moreover, co-occurrence network analysis revealed two different keystone species in the two compartments, i.e., *Acaulospora* in rhizosphere soil and *Rhizophagus* in roots. The high abundance of *Rhizophagus* in the roots of *P. phaseoloides* was the result of a good match of functions between plant and AMF. Collectively, the results indicated that plant compartment (root versus rhizosphere) is the main factor shaping AMF communities associated with *P. phaseoloides*.

The second study (Chapter 3) comprises a research synthesis of the role of AMF in zinc (Zn), cadmium (Cd), and Hg

bioremediation. The study assumed that mycorrhization plays a role in modulating the uptake of Hg, facilitated by Zn and/or Cd transporters. The synthesis demonstrated that AMF have the ability to regulate the transporters responsible for Zn and Cd uptake and transport, such as ZIP (zinc-iron permease or ZRT-IRT-like protein), CDF (cation diffusion facilitator), NRAMP (natural resistance-associated macrophage proteins), and HMA (heavy metal ATPase). This regulation can either enhance or inhibit the uptake and transport of Zn or Cd. The extent of this regulation is influenced by multiple factors, such as the plant species, the species of AMF involved, and soil conditions, including pH and elements such as phosphorus (P). It was concluded that future research is needed to investigate the optimal environmental conditions under which AMF are effective in Hg remediation for appropriate application.

The third study (Chapter 4) offers essential insights into the distinct functions of AMF symbiosis in Hg partitioning in plants. This relationship was assessed in the context of Zn uptake mechanisms and the expression of two Zn transporter genes (*ZIP2* and *ZIP6*). Zn is crucial for plants and has a similar outer electronic configuration as Hg, which implies a potential competition for the same transporters. In a greenhouse experiment, plants of *Medicago truncatula* were exposed to different Hg concentrations with and without inoculation of the AMF species *Rhizophagus irregularis*. This study demonstrated that mycorrhizal symbiosis improved plant Hg tolerance under Hg exposure, but the specific roles of mycorrhizal symbiosis in Hg partitioning depended on Hg concentrations in the substrate. A negative relationship between Hg and Zn concentrations in roots was observed, although the expression of Zn transporters (*ZIP2* and *ZIP6*) by mycorrhizal inoculation was upregulated irrespective of Hg concentrations in the substrate. More importantly, mycorrhizal colonization reduced Hg concentrations in leaves compared to controls, regardless of Hg concentrations in the substrate. This study demonstrated that mycorrhizal symbiosis influences Hg uptake in *M. truncatula* and highlights the importance of AMF in phytoremediation.

Overall, this doctoral thesis extends the understanding of AMF in phytoremediation with insights from both ecological and molecular perspectives and provides a knowledge basis to realize the potential and implementation of this technology.

Zusammenfassung

Die Umweltbelastung durch schädliche Chemikalien stellt weltweit eine große Herausforderung dar. Unter diesen sind Schwermetalle in Böden aufgrund ihrer Persistenz, Toxizität und Bio-akkumulation von besonderer Bedeutung, da sie die menschliche Gesundheit, das Pflanzen-wachstum und die Funktion von Ökosystemen erheblich gefährden können. Phytoremediation, bei der Pflanzen Schadstoffe aus Böden extrahieren, wird als vielversprechender Ansatz zur Sanierung von schwermetall-kontaminierten Böden angesehen. Die durch arbuskuläre Mykorrhizapilze (AMF) unterstützte Phytosanierung hat ein großes Potenzial zur Verbesserung des Pflanzenwachstums und der Metallaufnahme, da eine wechselseitige Verbindung zwischen Pflanzenwurzeln und AMF gebildet wird, welche die Nährstoffaufnahme und die Toleranz gegenüber Umweltstress verbessern kann. Trotz seines Potenzials kann die Effektivität dieses Ansatzes jedoch durch verschiedene Faktoren begrenzt werden, wie z.B. Umwelt- und geografische Faktoren, Bodeneigenschaften und Wechselwirkungen zwischen Pflanzen und Mikroben. Ein erweitertes grundlegendes Verständnis sowohl der ökologischen als auch der molekularen Eigenschaften ist daher entscheidend für ein erhöhtes Anwendungspotenzial dieser Technologie. Der Anstoß zu dieser Doktorarbeit war daher die Untersuchung des Potenzials von AMF bei der Phytosanierung von Böden, die mit Schwermetallen kontaminiert sind, mit besonderem Fokus auf die Quecksilber (Hg)-Sanierung.

Die erste Studie (Kapitel 2) trägt zum ökologischen Verständnis von AMF in einem degradierten Ökosystem bei. In dieser Studie wurden zwei geografisch unterschiedliche, aufgegebene Gold-minenstandorte in Ghana ausgewählt und die genetische Vielfalt und Zusammensetzung von AMF-Gemeinschaften sowohl in der Rhizosphäre als auch in den Wurzeln der Pionierpflanze Pueraria phaseoloides (Roxb.) Benth. (Tropischer Kudzu) unter Verwendung eines metagenomischen Sequenzierungsansatzes analysiert. Um den primären Faktor zu bestimmen, der AMF-Gemeinschaften prägt, untersuchte diese Studie sowohl biotische (Pflanzenidentität) als auch abiotische Faktoren (geografische Standorte und Bodenbedingungen). Insgesamt wurden 195 Amplikon-Sequenzvarianten (ASV) identifiziert, die zu acht Gattungen des Stammes Glomeromycota gehören. Das Wurzelkompartiment zeigte eine geringere Diversität als die Rhizosphärenböden und es wurde unabhängig von der geografischen Lage ein Unterschied in der AMF-Zusammensetzung zwischen den beiden Kompartimenten festgestellt. Darüber hinaus ergab die Co-Occurrence-Netzwerkanalyse zwei verschiedene Keystone-(Schlüssel-)Arten in den beiden Kompartimenten, d.h. Acaulospora im Boden der Rhizosphäre und Rhizophagus in Wurzeln. Das hohe Vorkommen von Rhizophagus in

den Wurzeln von P. phaseoloides war das Ergebnis einer guten Übereinstimmung der Funktionen zwischen Pflanze und AMF. Insgesamt zeigten die Ergebnisse, dass das Pflanzenkompartiment (Wurzel versus Rhizosphäre) der Hauptfaktor ist, der AMF-Gemeinschaften formt, die mit P. phaseoloides assoziiert sind.

Die zweite Studie (Kapitel 3) umfasst eine Forschungssynthese der Rolle von AMF bei der biologischen Sanierung von Zink (Zn), Cadmium (Cd) und Hg. Die Studie legte zugrunde, dass die Mykorrhizierung eine Rolle bei der Modulation der Hg-Aufnahme spielt, welche durch Zn- und/oder Cd-Transporter erleichtert wird. Die Synthese zeigte, dass AMF in der Lage sind, die Transporter zu regulieren, die für die Aufnahme und den Transport von Zn und Cd verantwortlich sind, wie ZIP (Zink-Eisen-Permease oder ZRT-IRT-ähnliches Protein), CDF (Kationendiffusionsförderer), NRAMP (natürliche resistenzassoziierte Makrophagenproteine) und HMA (Schwermetall-ATPase). Diese Regulierung kann die Aufnahme und den Transport von Zn oder Cd entweder erhöhen oder hemmen. Das Ausmaß dieser Regulierung wird von mehreren Faktoren beeinflusst, wie z.B. der Pflanzenart, der beteiligten AMF-Art und den Bodenbedingungen, einschließlich pH-Wert und Elementen wie Phosphor (P). Es wurde der Schluss gezogen, dass weitere Forschung erforderlich ist, um die optimalen Umgebungs-bedingungen zu bestimmen, unter denen AMF bei der Hg-Sanierung für eine angemessene Anwendung wirksam sind.

Die dritte Studie (Kapitel 4) gibt wesentliche Einblicke in die unterschiedlichen Funktionen der AMF-Symbiose bei der Hg-Verteilung in Pflanzen. Diese Beziehung wurde im Zusammenhang mit Zn-Aufnahmemechanismen und der Expression von zwei Zn-Transportergenen (ZIP2, ZIP6) bewertet. Zn ist ein essentielles Element für Pflanzen und hat eine ähnliche äußere elektronische Konfiguration wie Hg, was eine potenzielle Konkurrenz um dieselben Transporter impliziert. In einem Gewächshausversuch wurden Pflanzen von Medicago truncatula mit und ohne Inokulation der AMF-Spezies Rhizophagus irregularis unterschiedlichen Hg-Konzentrationen ausgesetzt. Diese Studie zeigte, dass die Mykorrhiza-Symbiose die pflanzliche Hg-Toleranz unter Hg-Exposition verbesserte. Die spezifische Rolle der Mykorrhiza-Symbiose bei der Hg-Verteilung hing jedoch von den Hg-Konzentrationen im Substrat ab. Eine negative Beziehung zwischen Hg- und Zn-Konzentrationen in den Wurzeln wurde beobachtet, obwohl die Expression von Zn-Transportern (ZIP2 und ZIP6) durch Mykorrhiza-Inokulation unabhängig von den Hg-Konzentrationen im Substrat hochreguliert wurde. Noch wichtiger ist, dass die Besiedlung mit Mykorrhiza die Hg-Konzentrationen in den Blättern im Vergleich zu den Kontrollen reduzierte, unabhängig von den Hg-Konzentrationen im Substrat. Diese Studie zeigte, dass eine Mykorrhiza-Symbiose die Hg-Aufnahme bei M. truncatula beeinflussen kann und unterstreicht die

Bedeutung von AMF bei der Phytoremediation.

Insgesamt erweitert diese Doktorarbeit das Verständnis von AMF in der Phytoremediation um Erkenntnisse sowohl aus ökologischer als auch aus molekularer Sicht und bietet eine Wissens-basis, um das Potenzial und die Umsetzung dieser Technologie voll auszuschöpfen.

List of Abbreviations

ABC	ATP-binding cassette transporter
AM	arbuscular mycorrhizal
AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
ASGM	small-scale gold mining
ASV	amplicon sequence variants
BLAST	basic local alignment search tool
Ca	calcium
CDF	cation diffusion facilitator
Cd	cadmium
cDNA	complementary deoxyribonucleic acid
db-RDA	distance-based redundancy analysis
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ERM	extraradical mycelium
Hg	mercury
HMA	heavy metal ATPase
HM	heavy metal
HSD	honestly significant difference
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
IRM	intraradical mycelium
IRT	iron-regulated transporter
MANOVA	multivariate analysis of variance
NCBI	National Center for Biotechnology Information
NMDS	nonmetric dimensional scaling
NRAMP	natural resistance-associated macrophage proteins

PCR	polymerase chain reaction
P	phosphorus
PT4	a mycorrhizal-induced phosphate transporter
RNA	ribonucleic acid
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
TI	tolerance index
UNEP	United Nations Environment Programme
VIF	variance inflation factor
ZIP	Zinc-Iron-Regulated Transporter
Zn	zinc

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1 General introduction

1.1 Heavy metals and their global environmental problems

Soil is a vital component for sustaining life and the production of food, timber, and fiber (Banerjee et al. 2023). Intensive agriculture, industrial activities, and waste disposal increasingly threaten the quality and multi-functionality of soil and with this, food security and human well-being (Misselhorn et al. 2012; Keesstra et al. 2016; Montanarella et al. 2016). Heavy metals (HM) pollute soil, water, and air (Rai et al. 2021; Wei et al. 2021) and are recognized as the most serious environmental problem globally due to their toxicity, persistence and bioaccumulation (Ali et al. 2019; Tan et al. 2023). HM represent a group of elements with a high atomic weight and a density of at least five times greater than that of water (Tchounwou et al. 2012). HM include both essential and non-essential elements (Alloway 2013). Essential elements, such as Zn, Fe, Mn, Cu and Ni, are required by living organisms in certain amounts for vital physiological and biochemical functions but become toxic above limits; while non-essential elements, such as Cd, Hg, As, Cr and Pb, are toxic even at very low concentrations.

Soil is considered as an ultimate sink of HM and acts as media to spread HM to water bodies, atmosphere and organisms (Ahmad et al. 2021), posing a great threat to human and environmental health. For instance, soil contamination by HM can cause vegetation loss and land degradation, affect soil organisms and consequently a reduction or loss of functions of a whole ecosystem (Wong 2003). More seriously, human exposure to HM can cause mutations of human genes and metabolic molecules, induce cancer and irreversibly damage the brain, lungs and other body organs (Godwill et al. 2019; Mitra et al. 2022).

1.1.1 Mercury pollution and artisanal gold mining: an intractable environmental problem

Mercury (Hg) is a hazardous metal with no identified biological function that is toxic to humans and environments even at very low concentrations (Zahir et al. 2005). Mercury occurs in various forms, i.e., elemental gaseous Hg (Hg^0), in ionic form (Hg^{2+}), or as a constituent in metal ores (e.g., cinnabar, HgS), or in methylated forms (CH_3Hg^{+1}, $(CH_3)_2Hg$) (Clarkson 1972). Mercury also has low melting and boiling points, making it a challenging chemical element to manage (Mukherjee et al. 2004). Due to its ability to transform from one to another, Hg can easily cycle through the atmosphere, terrestrial, and aquatic ecosystems (Obrist et al. 2018) causing global Hg pollution when in

the elemental gaseous form (Hg^0) transported over long distances (Pacyna 2020).

Mercury is the only metal in the periodic table, which has its own environmental convention, i.e., 'Minamata Convention on Mercury', highlighting the intractable problem of Hg pollution (Bank 2020). The overarching objective of this program is to control anthropogenic releases of Hg and ultimately protect human health and environments from hazards of Hg (UNEP 2013). Artisanal and small-scale gold mining (ASGM) mainly in South America and Sub-Saharan Africa is quantified as the largest global source of Hg pollution (UNEP 2019), because Hg is the necessary metal to amalgamate gold during gold mining activities (Bugmann et al. 2022). During the amalgamation process, miners extract gold from ore by the Hg amalgamation method, using an average of 2 grams of Hg per gram gold commonly (Yevugah et al. 2021). Unfortunately, there is currently no viable alternative to Hg in ASGM, and consequently, Hg usage in ASGM remains rampant (Clifford 2014).

1.2 A case study: background and problem statement

Ghana, located in West Africa on the Gulf of Guinea, is also known as Gold Coast. ASGM has been an important economic sector for centuries and both the number of activities and the intensity of operations have dramatically increased over the past decades (Yevugah et al. 2021). Although it has contributed to huge benefits of economy, this business operates at very high environmental costs. With a rate of 2600 hectares annually, natural vegetation in Ghana has been intensively converted into mining sites (Barenblitt et al. 2021). These sites are mostly free of vegetation because of inevitable loss of soil nutrients and / or Hg contamination (Mensah 2015). Consequently, these sites are subjected to soil erosion, resulting in pollution of water bodies and / or other non-contaminated areas which led to loss of biodiversity, decline of agricultural production, and severe consequences for human health (Schueler et al. 2011; Ibrahim 2018). For instance, renal disease markedly increased in past twenty years, especially in rural areas where illegal mining dominated (Adjei et al. 2018; Adjei et al. 2019). Besides, studies showed that areas of land devoid of vegetation emit more Hg than those with plants (Gworek et al. 2020). Thus, those sites represent a long-term source of Hg pollution if not managed properly.

Immediate and conscious efforts to remediate Hg contaminated mining sites, including cleaning up Hg and improving soil fertility for their respective utilization for productive cultivation of healthy food crops, are needed. Given the noted national limited capacity, significant assistance internationally is appreciated (Clifford 2014). The project

'Phytoremediation of mercury-contaminated mining sites in Ghana and Burkina Faso with arbuscular mycorrhizal fungi' (Mercury-AMF) funded by the German government was aiming to find sustainable solutions to manage Hg contaminated sites and build capacities in West Africa, especially in Ghana. Although the research focus is in West Africa, the knowledge amassed and the insights gleaned are widely applicable and transferable. Notably, there are over 3000 mining sites across the world that continually emit Hg and aggravate Hg pollution globally (Kocman et al. 2013).

1.3 Phytoremediation

Decontamination of HM contaminated soils is a major concern in environmental legislation. In the past decades, many technologies have been developed to decontaminate soil HM pollution. They are generally classified into two classes: *in-situ* and *ex-situ* methods (Paul et al. 2021). The *ex-situ* strategies usually excavate polluted soils to an external location, which requires intensive labor and/or sophisticated infrastructure. Although this is a rapid method for managing polluted sites, it just potentially relocates the problem and is only limited to small areas. In contrast, *in-situ* methods enable the remediation process to take place at the original location and thus overcome the weaknesses of *ex-situ* methods. Consequently, *in-situ* methods are considered to be a more suitable approach for managing soil pollution. There are three categories of *in-situ* methods, i.e., physical, chemical and biological. Physical and chemical approaches, such as ion exchange, soil washing, soil vapor extraction, chemical leaching and chemical precipitation, are expensive and impractical, thus they are not widely adopted (Paul et al. 2021). Besides, those methods cause irreversible changes on soil structure and productivity (Kuppusamy et al. 2017; Paul et al. 2021).

Phytoremediation, a plant-based method, offers an alternative sustainable solution in metal-polluted sites by using plants for removing or stabilizing HM (Yan et al. 2020; Sharma et al. 2023). Plants can act as solar-driven pumps which are capable of extracting pollutants from the environment (Ali et al. 2013). Plants can be applied *in-situ* in large-scale to cover the contaminated area which will prevent erosion and thus reducing the spread of pollutants in the environment. This can also benefit soil stabilization and soil health, thereby enhancing carbon sequestration, nutrient contents, and microbial activities (Ali et al. 2013; Yan et al. 2020; Wani et al. 2023). Nevertheless, the survival of plants is affected by the toxicity of contaminated land and general soil conditions like nutrient-poor soils (Gerhardt et al. 2017), compromising the success of phytoremediation programs in metal-contaminated sites. Plant associated root microorganisms are increasingly being used to enhance plant performance under stress conditions and consequently,

the efficiency of phytoremediation is promoted. Among those microorganisms, arbuscular mycorrhizal fungi (AMF) are of key interest, given their intimate association with most vascular plants (Moura et al. 2022).

1.4 Arbuscular mycorrhizal fungi (AMF)-assisted phytoremediation

1.4.1 A brief introduction of AMF

AMF, which are obligate biotrophs, form intimate symbiosis with over 90% of plant species which can be found across all terrestrial ecosystems (van der Heijden et al. 2015). AMF are recognized as ancestral alliances with plants, along with the migration of plants from water to land over 450 million years ago (Martin et al. 2017), highlighting their essential role in plant adaptations. Individual studies have indicated that AMF can facilitate success of plant establishment in metal-polluted sites, and even, some studies conclude that this symbiosis is partly responsible for plant survival in these harsh environmental conditions (Meier et al. 2011; Colombo et al. 2020; Moura et al. 2022). This is manifested in well-known roles of AMF for nutrients (e.g., phosphors, nitrogen) and water transfer beyond the root zone to plants which help plants to confer resistance to abiotic (e.g., drought, salt, HM) and biotic (e.g., pathogens) stresses (Bücking et al. 2015). Moreover, AMF improve the soil structure through external hyphae and glomalin production (Rillig et al. 2006), which also has the capacity to sequester metals from soils (Gil-Cardeza et al. 2014; Chen et al. 2018).

1.4.2 The importance of indigenous AMF species

It is well established that using an appropriate AMF species is the cornerstone to achieve the potential benefits of AMF in a recipient system of interest (Maltz et al. 2015). Specifically, indigenous AMF species have attracted attention because they appear to be physiologically and genetically adapted to the target environment (Miransari 2011). Indeed, AMF isolates from polluted environments are more effective to remediate pollutants than exotic commercial inocula (Redon et al. 2009; Kodre et al. 2017), which was attributed to the adaptation to local environment and correspondingly co-evolution with the surrounding environment. Another crucial point is that exotic mycorrhizal species can be invasive, whereby negatively affecting local microbial communities (Meglouli et al. 2018; Martignoni et al. 2020). Thus, the focus on indigenous AMF species to help remediating Hg contaminants is both more practical and ecologically safe. However, the precondition to isolate indigenous AMF species requires the knowledge on the composition of the local AMF communities, which provide the necessary information for selecting suitable AMF

species. Furthermore, it is well-known that AMF communities depend on both biotic factors (e.g., plant identity) and abiotic factors (e.g., soil conditions), where the relative importance of each factor is context-dependent (Vályi et al. 2015; Xu et al. 2017; Sandoz et al. 2020). More importantly, *Pueraria phaseoloides*, a perennial, N_2-fixing legume plant, was found as a pioneer plant species in post-mining sites. An earlier research demonstrated that *P. phaseoloides* failed to establish without the presence of symbiotic AMF (Waidyanatha, 1979), highlighting the importance of AMF in helping this plant to establish on post-mining sites. Understanding how factors shape the dynamics and compositions of AMF associated with *P. phaseoloides* would help to develop promising strategies to manage mining-degraded lands. Therefore, a first step of this doctoral thesis was to identify AMF communities associated with *P. phaseoloides* in selected field sites and the relative importance of biotic and abiotic factors in AMF communities (chapter 2).

1.4.3 The role and functions of AMF in HM phytoremediation, with a focus on Hg uptake via Zn or Cd transporters

The functions of AMF in HM phytoremediation have been extensively investigated, which can be grouped into two pathways: direct and indirect functions (Table 1.1). Direct functions usually indicate that AMF can be directly involved in the process of phytoremediation. For example, HM bind with fungal structures (spores, hyphae and glomalin), or HM sequester into fungal vacuoles and/or arbuscules. On the other hand, indirect functions mean that AMF facilitate the surrounding environment to achieve the potential functions in phytoremediation. For instance, AMF improve plant performance by increasing nutrient uptake, enhancing antioxidative stress, regulating hormonal signals, thereby enhancing the efficiency of phytoremediation. Besides, AMF facilitate the functions of other microbes, like *Rhizobia*, *Actinibacteria* and fungal endophytes which provide a pleasant environment for plants to perform better under HM toxicity. More importantly, AMF can regulate the transporter genes to facilitate or inhibit HM uptake which contribute to either phytoextraction or phytostabilization (Ferrol et al. 2016). Other functions appear in phytoextraction and phytostabilization simultaneously, however, only the regulation of transporter genes appears either in phytoextraction or phytostabilization. Because of these two contrasting performances of AMF species in HM phytoremediation, there has been limited acceptance of this biotechnology despite extensive research on the roles of AMF in phytoremediation (Table 1.1). Thus, understanding the specific role of AMF in metal transporting to plants will help to improve or refine this technology. More importantly, when it comes to Hg, the studies, however, remain scarce regarding the role of AMF in Hg phytoremediation. Thus, this requires further research to examine the role of

AMF in Hg phytoremediation with additional focus on the regulation of transporter genes.

Because Hg has no biological function in plants, this suggests that plants do not have specific transporters evolved for Hg uptake. However, it is well-known that plants uptake non-essential metals via essential nutrient transporters, such as Cs^{+} with K^{+}, and Cd^{2+} with Zn^{2+}. This is attributed to structural similarities between two metals (Shi et al. 2019; Manoj et al. 2020), namely, they are positioned in the same column in the periodic table. We thus assume that Hg may enter plants via Zn or Cd transporters because Hg is in the same column in the periodic table with Zn and Cd. Based on this assumption, knowledge of roles of AMF in regulating Zn and Cd transporter genes were compiled and knowledge gaps regarding Hg research were identified (chapter 3). Furthermore, given the fact that Zn is an essential micronutrient with vital biological functions and is always present in plant systems, plants have evolved specific membrane transporters for Zn, and among Zn transporters, Zinc-Iron-Regulated (*ZIP*) are well identified and verified for Zn transporting (Fariduddin et al. 2022). The effort of empirical evidence put first into understanding the relationship between Zn and Hg concentrations under Hg exposure, as well as the regulation of *ZIP* transporter genes (chapter 4).

Table 1.1 Summary of functions of AMF in HM phytoremediation.

Functions of AMF	**Proposed mechanisms**	**References**
Direct function		
Immobilization of HM	Binding with spores	(González-Guerrero et al. 2008; Salazar et al. 2018)
	Binding with ERM	(González-Guerrero et al. 2008; Chen et al. 2018)
	Binding with IRM	(Wu et al. 2016; Zhou et al. 2017)
	Binding with glomalin	(González-Chávez et al. 2009; Gil-Cardeza et al. 2014)
Sequestration of HM	Sequestering into vacuoles	(González-Guerrero et al. 2008)
	Sequestering into arbuscular	(Chamba et al. 2017; Kodre et al. 2017; Debeljak et al. 2018)
	Sequestering into vesicles	
Efflux HM	Efflux As	(Gonzalez-Chavez et al. 2002)

Notes: HM: heavy metals; ERM: extraradical mycelium; IRM: intraradical mycelium; As: arsenic

Table 1.1 *Continued*

Functions of AMF	**Proposed mechanism**	**References**
Indirect function		
Regulation of hormonal signals	Switch the negative feedback regulation mode of indole acetic acid upward transport and methyl jasmonate downward transmission	(Wang et al. 2022)
Regulate transporters	Downregulate: *Nramp5* and *HMA3* *GmHMA19* *MsIRT1* and *MsNramp1*	(Chen et al. 2019; Cui et al. 2019; Motaharpoor et al. 2019)
	Upregulate: *ABC* transporters *Nramp2* and *Nramp5*	(Shabani et al. 2016; You et al. 2022)
Facilitate other microbes	Increasing the abundance of Actinibacteria Enhancing rhizobia activity Improving performance of fungal endophytes	(Wężowicz et al. 2017; Chen et al. 2019; Ren et al. 2019)

Notes: *Nramp*: natural resistance-associated macrophage proteins; *HMA*: heavy metal ATPase; *IRT*: iron-regulated transporter; *ABC*: ATP-binding cassette transporter.

1.5 Research objectives and hypotheses

1.5.1 Specific objectives

1) Characterize abundance and composition of AMF communities in post-mining sites;
    a) Understand how abiotic (geographic location, soil conditions) and biotic (host plant) factors shape dynamics and function of AMF;
    b) Provide the information for selecting most powerful AMF candidates for site-adapted Hg phytoremediation.
2) Examine the specific role of AM fungal species on Hg uptake and translocation under Hg exposure;
3) Determine whether there is a *ZIP* transporter gene that is differentially-regulated upon mycorrhizal colonization and may be involved in mycorrhizal-mediated Hg uptake.

1.5.2 Guiding hypotheses

1) Host identity (biotic factor) is a major driving factor shaping AMF communities, assumably stronger than local factors (abiotic factor) related to geography and soil conditions, due to the strong adaptation potential of *Pueraria phaseoloides* in different environments.
2) The high adaptability of *Pueraria phaseoloides* may be reflected in the selection of AMF specific species from the rhizosphere soil.
3) AM fungal species may have different roles under different Hg concentrations, because plants have certain capacities to detoxify the Hg toxicity.
4) There might be a competition between Zn and Hg uptake in roots under Hg exposure, because Zn is an essential element to plants and shares the same outer electronic configuration with Hg, implying a competition for the same transporters.

1.6 Thesis outline and methods

This doctoral thesis is conceived as a cumulative thesis, where each chapter represents a journal article with the exception of the general introduction and discussion (Figure 1.1).

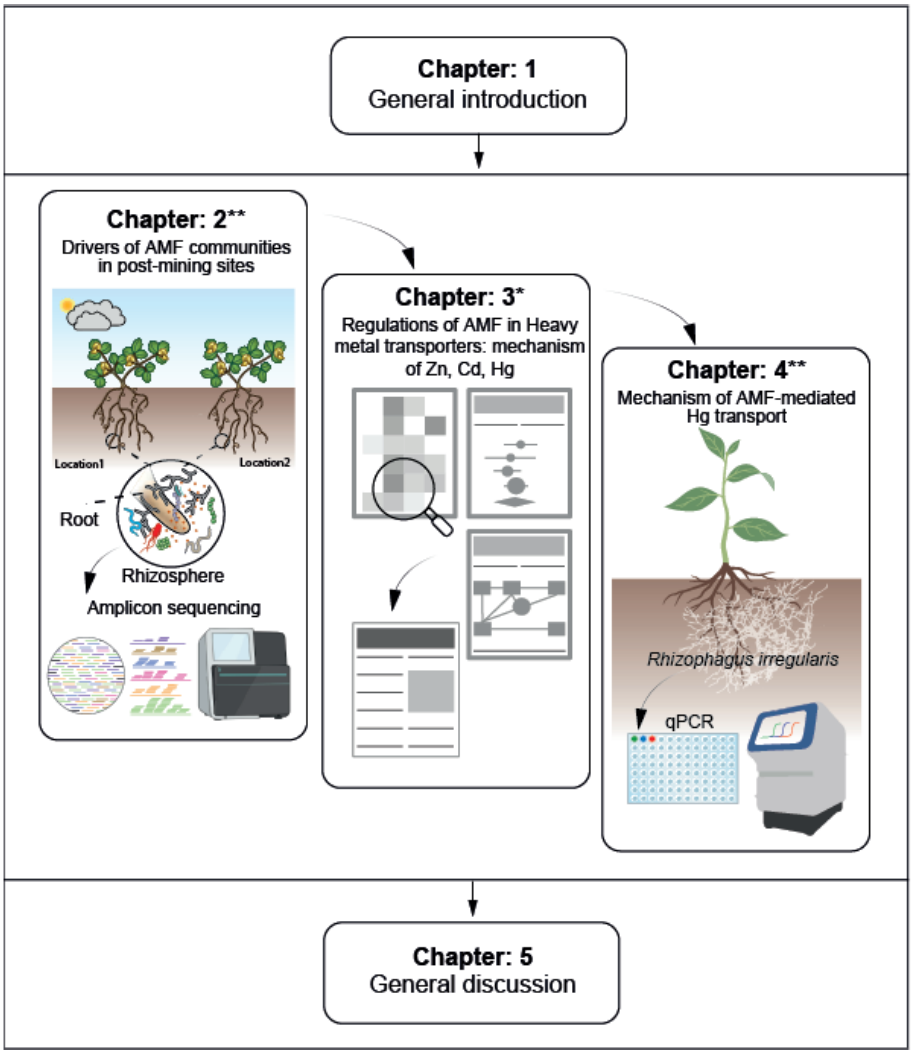

Figure 1.1 Schematic outline of this thesis elaborating the methods used in each chapter. Double asterisk (**) indicates a published chapter, while a (*) denotes a submitted manuscript.

Chapter 2 aims to characterize the abundance and composition of AMF communities and the driving factor on these communities under post-mining sites which will provide the necessary information for selecting the most powerful AMF candidate for site adapted Hg phytoremediation. Chapter 3 provides a comprehensive overview of the current understanding of three elements (Zn, Cd, Hg), specifically focusing on metal transporters. Since the research on Hg is still limited, there is a need to leverage the knowledge from the other elements with similar electronic configurations to advance the study of Hg and facilitate the remediation of Hg-contaminated lands. Thus, this chapter specifically focuses on these three elements to provide insights into metal transporter mechanisms that could aid in Hg research. Chapter 4 bridges the knowledge gaps identified with previous chapter. Specifically, this study aims to investigate *Rhizophagus irregularis* an AMF species, in facilitating Hg translocation. Moreover, this study explores the relationship between Hg and Zn elements by using the model legume plant species *Medicago truncatula.* Finally, chapter 5 provides a general discussion and synthesis of the findings obtained from this doctoral thesis into a broader context and provides directions for progressive research.

1.7 References

Adjei D N, Adu D, Quayson S E, Kardaun J W P F, Erskine I J, Lartey I S, Agyemang C (2019) 20 year trends in renal disease mortality in Ghana: A review of autopsies. Nephrology 24:387-394. https://doi.org/10.1111/nep.13255

Adjei D N, Stronks K, Adu D, Beune E, Meeks K, Smeeth L, Addo J, et al. (2018) Chronic kidney disease burden among African migrants in three European countries and in urban and rural Ghana: the RODAM cross-sectional study. Nephrology Dialysis Transplantation 33:1812-1822. https://doi.org/10.1093/ndt/gfx347

Ahmad W, Alharthy R D, Zubair M, Ahmed M, Hameed A, Rafique S (2021) Toxic and heavy metals contamination assessment in soil and water to evaluate human health risk. Scientific reports 11:17006. https://doi.org/10.1038/s41598-021-94616-4

Ali H, Khan E, Ilahi I (2019) Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. Journal of Chemistry 2019:6730305. https://doi.org/10.1155/2019/6730305

Ali H, Khan E, Sajad M A (2013) Phytoremediation of heavy metals—Concepts and applications. Chemosphere 91:869-881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Alloway B J (2013) Introduction. Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability. Springer Netherlands, Dordrecht, 3-9. https://doi.org/10.1007/978-94-007-4470-7_1

Banerjee S, van der Heijden M G A (2023) Soil microbiomes and one health. Nature Reviews Microbiology 21:6-20. https://doi.org/10.1038/s41579-022-00779-w

Bank M S (2020) The mercury science-policy interface: History, evolution and progress of the Minamata Convention. Science of the Total Environment 722:137832. https://doi.org/10.1016/j.scitotenv.2020.137832

Barenblitt A, Payton A, Lagomasino D, Fatoyinbo L, Asare K, Aidoo K, Pigott H, et al. (2021) The large footprint of small-scale artisanal gold mining in Ghana. Science of the Total Environment 781:146644. https://doi.org/10.1016/j.scitotenv.2021.146644

Bugmann A, Brugger F, Zongo T, van der Merwe A (2022) “Doing ASGM without mercury is like trying to make omelets without eggs”. Understanding the persistence of mercury use among artisanal gold miners in Burkina Faso. Environmental Science & Policy 133:87-97. https://doi.org/10.1016/j.envsci.2022.03.009

Chamba I, Rosado D, Kalinhoff C, Thangaswamy S, Sánchez-Rodríguez A, Gazquez M J (2017) Erato polymnioides – A novel Hg hyperaccumulator plant in ecuadorian rainforest acid soils with potential of microbe-associated phytoremediation. Chemosphere 188:633-641. https://doi.org/10.1016/j.chemosphere.2017.08.160

Chen B, Nayuki K, Kuga Y, Zhang X, Wu S, Ohtomo R (2018) Uptake and Intraradical Immobilization of Cadmium by Arbuscular Mycorrhizal Fungi as Revealed by a Stable Isotope Tracer and Synchrotron Radiation μX-Ray Fluorescence Analysis. Microbes Environ 33:257-263. https://doi.org/10.1264/jsme2.ME18010

Chen X W, Wu L, Luo N, Mo C H, Wong M H, Li H (2019) Arbuscular mycorrhizal fungi and the associated bacterial community influence the uptake of cadmium in rice. Geoderma 337:749-757. https://doi.org/10.1016/j.geoderma.2018.10.029

Clarkson T W (1972) The biological properties and distribution of mercury. Biochem J 130:61p-63p. https://doi.org/10.1042/bj1300061pb

Clifford M J (2014) Future strategies for tackling mercury pollution in the artisanal gold mining sector: Making the Minamata Convention work. Futures 62:106-112. https://doi.org/10.1016/j.futures.2014.05.001

Colombo R P, Benavidez M E, Fernandez Bidondo L, Silvani V A, Bompadre M J, Statello M, Scorza M V, Scotti A, Godeas A M (2020) Arbuscular mycorrhizal fungi in heavy metal highly polluted soil in the Riachuelo river basin. Revista Argentina de Microbiologia 52:145-149. https://doi.org/10.1016/j.ram.2019.05.001

Cui G, Ai S, Chen K, Wang X (2019) Arbuscular mycorrhiza augments cadmium tolerance in soybean by altering accumulation and partitioning of nutrient elements, and related gene expression. Ecotoxicology and Environmental Safety 171:231-239. https://doi.org/10.1016/j.ecoenv.2018.12.093

Debeljak M, van Elteren J T, Špruk A, Izmer A, Vanhaecke F, Vogel-Mikuš K (2018) The role of arbuscular mycorrhiza in mercury and mineral nutrient uptake in maize. Chemosphere 212:1076-1084. https://doi.org/10.1016/j.chemosphere.2018.08.147

Fariduddin Q, Saleem M, Khan T A, Hayat S (2022) Zinc as a Versatile Element in Plants: An Overview on Its Uptake, Translocation, Assimilatory Roles, Deficiency and Toxicity Symptoms. Microbial Biofertilizers and Micronutrient Availability: The Role of Zinc in Agriculture and Human Health. Springer International Publishing, Cham, 137-158. https://doi.org/10.1007/978-3-030-76609-2_7

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. Journal of Experimental Botany 67:6253-6265. https://doi.org/10.1093/jxb/erw403

Gerhardt K E, Gerwing P D, Greenberg B M (2017) Opinion: Taking phytoremediation from proven technology to accepted practice. Plant Science 256:170-185. https://doi.org/10.1016/j.plantsci.2016.11.016

Gil-Cardeza M L, Ferri A, Cornejo P, Gomez E (2014) Distribution of chromium species in a Cr-polluted soil: presence of Cr(III) in glomalin related protein fraction. Science of the Total Environment 493:828-833. https://doi.org/10.1016/j.scitotenv.2014.06.080

Godwill A E, Paschaline U F, Friday N N, Marian N U (2019) Mechanism and Health Effects of Heavy Metal Toxicity in Humans. Poisoning in the Modern World. IntechOpen, Rijeka, Ch. 5. https://doi.org/10.5772/intechopen.82511

Gonzalez-Chavez C, D'Haen J, Vangronsveld J, Dodd J C (2002) Copper sorption and accumulation by the extraradical mycelium of different Glomus spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant and Soil 240:287-297. https://doi.org/10.1023/A:1015794622592

González-Chávez M C, Carrillo-González R, Gutiérrez-Castorena M C (2009) Natural attenuation in a slag heap contaminated with cadmium: the role of plants and arbuscular mycorrhizal fungi. J Hazard Mater 161:1288-1298. https://doi.org/10.1016/j.jhazmat.2008.04.110

González-Guerrero M, Melville L H, Ferrol N, Lott J N, Azcón-Aguilar C, Peterson R L (2008) Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus Glomus intraradices. Can J Microbiol 54:103-110. https://doi.org/10.1139/w07-119

Gworek B, Dmuchowski W, Baczewska-Dąbrowska A H (2020) Mercury in the terrestrial environment: a review. Environmental Sciences Europe 32:128. https://doi.org/10.1186/s12302-020-00401-x

Ibrahim I (2018) Gold Exports and Cost Implication of Illegal Gold Mining in Ghana. British Journal of Economics, Finance and Management Sciences 15:1-18. https://www.researchgate.net/publication/329714559

Keesstra S D, Bouma J, Wallinga J, Tittonell P, Smith P, Cerdà A, Montanarella L, et al. (2016) The significance of soils and soil science towards realization of the United Nations Sustainable Development Goals. SOIL 2:111-128. https://doi.org/10.5194/soil-2-111-2016

Kocman D, Horvat M, Pirrone N, Cinnirella S (2013) Contribution of contaminated sites to the global mercury budget. Environmental Research 125:160-170. https://doi.org/10.1016/j.envres.2012.12.011

Kodre A, Arčon I, Debeljak M, Potisek M, Likar M, Vogel-Mikuš K (2017) Arbuscular mycorrhizal fungi alter Hg root uptake and ligand environment as studied by X-ray absorption fine structure. Environmental and Experimental Botany 133:12-23. https://doi.org/10.1016/j.envexpbot.2016.09.006

Kuppusamy S, Thavamani P, Venkateswarlu K, Lee Y B, Naidu R, Megharaj M (2017) Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. Chemosphere 168:944-968. https://doi.org/10.1016/j.chemosphere.2016.10.115

Maltz M R, Treseder K K (2015) Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis. Restoration Ecology 23:625-634. https://doi.org/10.1111/rec.12231

Manoj S R, Karthik C, Kadirvelu K, Arulselvi P I, Shanmugasundaram T, Bruno B, Rajkumar M (2020) Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. Journal of Environmental Management 254:109779. https://doi.org/10.1016/j.jenvman.2019.109779

Martignoni M M, Garnier J, Hart M M, Tyson R C (2020) Investigating the impact of the mycorrhizal inoculum on the resident fungal community and on plant growth. Ecological Modelling 438:109321. https://doi.org/10.1016/j.ecolmodel.2020.109321

Martin F M, Uroz S, Barker D G (2017) Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. Science 356:eaad4501. https://doi.org/10.1126/science.aad4501

Meglouli H, Lounès-Hadj Sahraoui A, Magnin-Robert M, Tisserant B, Hijri M, Fontaine J (2018) Arbuscular mycorrhizal inoculum sources influence bacterial, archaeal, and fungal communities' structures of historically dioxin/furan-contaminated soil but not the pollutant dissipation rate. Mycorrhiza 28:635-650. https://doi.org/10.1007/s00572-018-0852-x

Meier S, Azcón R, Cartes P, Borie F, Cornejo P (2011) Alleviation of Cu toxicity in Oenothera picensis by copper-adapted arbuscular mycorrhizal fungi and treated agrowaste residue. Applied Soil Ecology 48:117-124. https://doi.org/10.1016/j.apsoil.2011.04.005

Mensah K A (2015) Role of revegatation in restoring fertility of degraded mined soils in Ghana: A review. International Journal of Biodiversity and Conservation 7:57-80. https://doi.org/10.5897/IJBC2014.0775

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnology Advances 29:645-653. https://doi.org/10.1016/j.biotechadv.2011.04.006

Misselhorn A, Aggarwal P, Ericksen P, Gregory P, Horn-Phathanothai L, Ingram J, Wiebe K (2012) A vision for attaining food security. Current Opinion in Environmental Sustainability 4:7-17. https://doi.org/10.1016/j.cosust.2012.01.008

Mitra S, Chakraborty A J, Tareq A M, Emran T B, Nainu F, Khusro A, Idris A M, et al. (2022) Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science 34:101865. https://doi.org/10.1016/j.jksus.2022.101865

Montanarella L, Pennock D J, McKenzie N, Badraoui M, Chude V, Baptista I, Mamo T, et al. (2016) World's soils are under threat. SOIL 2:79-82. https://doi.org/10.5194/soil-2-79-2016

Motaharpoor Z, Taheri H, Nadian H (2019) Rhizophagus irregularis modulates cadmium uptake, metal transporter, and chelator gene expression in Medicago sativa. Mycorrhiza 29:389-395. https://doi.org/10.1007/s00572-019-00900-7

Moura M A d, Oki Y, Arantes-Garcia L, Cornelissen T, Nunes Y R F, Fernandes G W (2022) Mycorrhiza fungi application as a successful tool for worldwide mine land restoration: Current state of knowledge and the way forward. Ecological Engineering 178:106580. https://doi.org/10.1016/j.ecoleng.2022.106580

Mukherjee A B, Zevenhoven R, Brodersen J, Hylander L D, Bhattacharya P (2004) Mercury in waste in the European Union: sources, disposal methods and risks. Resources, Conservation and Recycling 42:155-182. https://doi.org/10.1016/j.resconrec.2004.02.009

Obrist D, Kirk J L, Zhang L, Sunderland E M, Jiskra M, Selin N E (2018) A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. Ambio 47:116-140. https://doi.org/10.1007/s13280-017-1004-9

Pacyna J M (2020) Recent advances in mercury research. Science of the Total Environment 738:139955. https://doi.org/10.1016/j.scitotenv.2020.139955

Paul O, Amrita Jasu, Dibyajit Lahiri, Moupriya Nag, Ray. R R (2021) In Situ and Ex Situ Bioremediation of Heavy Metals: The Present Scenario. Journal of Environmental Engineering and Landscape Management 29:454-469. https://doi.org/10.3846/jeelm.2021.15447

Rai G K, Bhat B A, Mushtaq M, Tariq L, Rai P K, Basu U, Dar A A, Islam S T, Dar T U H, Bhat J A (2021) Insights into decontamination of soils by phytoremediation: A detailed account on heavy metal toxicity and mitigation strategies. Physiologia Plantarum 173:287-304. https://doi.org/10.1111/ppl.13433

Redon P-O, Béguiristain T, Leyval C (2009) Differential effects of AM fungal isolates on Medicago truncatula growth and metal uptake in a multimetallic (Cd, Zn, Pb) contaminated agricultural soil. Mycorrhiza 19:187-195. https://doi.org/10.1007/s00572-009-0230-9

Ren C G, Kong C C, Wang S X, Xie Z H (2019) Enhanced phytoremediation of uranium-contaminated soils by arbuscular mycorrhiza and rhizobium. Chemosphere 217:773-779. https://doi.org/10.1016/j.chemosphere.2018.11.085

Rillig M C, Mummey D L (2006) Mycorrhizas and soil structure. New Phytologist 171:41-53. https://doi.org/10.1111/j.1469-8137.2006.01750.x

Salazar M J, Menoyo E, Faggioli V, Geml J, Cabello M, Rodriguez J H, Marro N, Pardo A, Pignata M L, Becerra A G (2018) Pb accumulation in spores of arbuscular mycorrhizal fungi. Science of the Total Environment 643:238-246. https://doi.org/10.1016/j.scitotenv.2018.06.199

Sandoz F A, Bindschedler S, Dauphin B, Farinelli L, Grant J R, Hervé V (2020) Biotic and abiotic factors shape arbuscular mycorrhizal fungal communities associated with the roots of the widespread fern Botrychium lunaria (Ophioglossaceae). Environmental Microbiology Reports 12:342-354. https://doi.org/10.1111/1758-2229.12840

Schueler V, Kuemmerle T, Schröder H (2011) Impacts of surface gold mining on land use systems in Western Ghana. Ambio 40:528-539. https://doi.org/10.1007/s13280-011-0141-9

Shabani L, Sabzalian M R, Mostafavi pour S (2016) Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in Festuca arundinacea. Mycorrhiza 26:67-76. https://doi.org/10.1007/s00572-015-0647-2

Sharma J K, Kumar N, Singh N P, Santal A R (2023) Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. Frontiers in plant science 14:1076876. https://doi.org/10.3389/fpls.2023.1076876

Shi W, Zhang Y, Chen S, Polle A, Rennenberg H, Luo Z-B (2019) Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. Plant, Cell & Environment 42:1087-1103. https://doi.org/10.1111/pce.13471

Tan C, Wang H, Yang Q, Yuan L, Zhang Y, Delgado Martín J (2023) An integrated approach for quantifying source apportionment and source-oriented health risk of heavy metals in soils near an old industrial area. Environmental Pollution 323:121271. https://doi.org/10.1016/j.envpol.2023.121271

Tchounwou P B, Yedjou C G, Patlolla A K, Sutton D J (2012) Heavy metal toxicity and the environment. Experientia supplementum 101:133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

UNEP (2013) Minamata Convention on Mercury. http://www.mercuryconvention.org/

UNEP (2019) Global Mercury Assessment 2018.

Vályi K, Rillig M C, Hempel S (2015) Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. New Phytologist 205:1577-1586. https://doi.org/10.1111/nph.13236

van der Heijden M G A, Martin F M, Selosse M-A, Sanders I R (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205:1406-1423. https://doi.org/10.1111/nph.13288

Wang H-R, Zhao X-Y, Zhang J-M, Lu C, Feng F-J (2022) Arbuscular mycorrhizal fungus regulates cadmium accumulation, migration, transport, and tolerance in Medicago sativa. Journal of Hazardous Materials 435:129077. https://doi.org/10.1016/j.jhazmat.2022.129077

Wani Z A, Ahmad Z, Asgher M, Bhat J A, Sharma M, Kumar A, Sharma V, et al. (2023) Phytoremediation of Potentially Toxic Elements: Role, Status and Concerns. Plants 12:429. https://doi.org/10.3390/plants12030429

Wei Z, Gu H, Van Le Q, Peng W, Lam S S, Yang Y, Li C, Sonne C (2021) Perspectives on phytoremediation of zinc pollution in air, water and soil. Sustainable Chemistry and Pharmacy 24:100550. https://doi.org/10.1016/j.scp.2021.100550

Wężowicz K, Rozpądek P, Turnau K (2017) Interactions of arbuscular mycorrhizal and endophytic fungi improve seedling survival and growth in post-mining waste. Mycorrhiza 27:499-511. https://doi.org/10.1007/s00572-017-0768-x

Wong M H (2003) Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50:775-780. https://doi.org/10.1016/S0045-6535(02)00232-1

Wu S, Zhang X, Chen B, Wu Z, Li T, Hu Y, Sun Y, Wang Y (2016) Chromium immobilization by extraradical mycelium of arbuscular mycorrhiza contributes to plant chromium tolerance. Environmental and Experimental Botany 122:10-18. https://doi.org/10.1016/j.envexpbot.2015.08.006

Xu X, Chen C, Zhang Z, Sun Z, Chen Y, Jiang J, Shen Z (2017) The influence of environmental factors on communities of arbuscular mycorrhizal fungi associated with Chenopodium ambrosioides revealed by MiSeq sequencing investigation. Scientific reports 7:45134. https://doi.org/10.1038/srep45134

Yan A, Wang Y, Tan S N, Mohd Yusof M L, Ghosh S, Chen Z (2020) Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. Frontiers in plant science 11:359. https://doi.org/10.3389/fpls.2020.00359

Yevugah L L, Darko G, Bak J (2021) Does mercury emission from small-scale gold mining cause widespread soil pollution in Ghana? Environmental Pollution 284:116945. https://doi.org/10.1016/j.envpol.2021.116945

You Y, Ju C, Wang L, Wang X, Ma F, Wang G, Wang Y (2022) The mechanism of arbuscular mycorrhizal enhancing cadmium uptake in Phragmites australis depends on the phosphorus concentration. Journal of Hazardous Materials 440:129800. https://doi.org/10.1016/j.jhazmat.2022.129800

Zahir F, Rizwi S J, Haq S K, Khan R H (2005) Low dose mercury toxicity and human health. Environmental Toxicology and Pharmacology 20:351-360. https://doi.org/10.1016/j.etap.2005.03.007

Zhou X, Fu L, Xia Y, Zheng L, Chen C, Shen Z, Chen Y (2017) Arbuscular mycorrhizal fungi enhance the copper tolerance of Tagetes patula through the sorption and barrier mechanisms of intraradical hyphae. Metallomics 9:936-948. https://doi.org/10.1039/C7MT00072C

2 Genetic diversity and community composition of arbuscular mycorrhizal fungi associated with root and rhizosphere soil of the pioneer plant *Pueraria phaseoloides*[*]

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2.1 Abstract

There is limited knowledge about the factors that modulate abundance, diversity and selectiveness of arbuscular mycorrhizal fungi (AMF) associated with pioneer plants colonizing degraded ecosystems. To advance this information, we selected two geographically distinct, abandoned gold mining locations in Ghana (West Africa) and analyzed the genetic diversity and composition of AMF communities both in the rhizosphere and roots of the pioneer plant *Pueraria phaseoloides* (Roxb.) Benth. (tropical kudzu). Based on AMF-specific sequences obtained from the Illumina MiSeq platform, the phylogenetic analysis identified 195 amplicon sequence variants (ASV) affiliated to 8 genera of the phylum *Glomeromycota* (rhizosphere soil: 102 ASV; roots: 72 ASV; shared in both compartments: 21 ASV). The root compartment showed a lower diversity than the rhizosphere soils. Irrespective of geographical location, the community composition of AMF in rhizosphere soil differentiated from that of the root. Physico-chemical soil characteristics had no effects on community composition, but pH and Ca influenced both the richness and diversity of AMF, and Zn only affected the richness of AMF ($P < 0.05$). Co-occurrence network analysis revealed two different keystone species in the two compartments, i.e., *Acaulospora* in rhizosphere soil and *Rhizophagus* in roots. Collectively, our results indicated that plant compartmentation is the main factor shaping AMF communities associated with *P. phaseoloides*. This study suggested that the high abundance of *Rhizophagus* in the roots of *P. phaseoloides* was the result of a good match of functions between plant and AMF. Thereby, *P. phaseoloides* had a strong modulation for AMF species under the prevalent soil conditions. These fundamental insights into the complex and compartment-driven niche differentiation of plant-AMF interactions in association with *P. phaseoloides* provide an ecological basis for restoration of degraded ecosystems.

Keywords: abandoned mining sites, keystone species, plant compartment, soil conditions, geography, ecological restoration.

2.2 Highlights

- The pioneer plant *Pueraria phaseoloides* had a strong modulation effect on AMF communities.
- Irrespective of geographical location, community composition of AMF in rhizosphere soil differed from that of the root.

- Co-occurrence network analysis revealed two AMF keystone species in rhizosphere soil (*Acaulospora*) and roots (*Rhizophagus*) of *P. phaseoloides*.

2.3 Introduction

Arbuscular mycorrhizal fungi (AMF) ensure the survival of plants by facilitating access to limited resources, particularly in degraded ecosystems (Mahmoudi et al. 2019), thereby playing a crucial role in sustaining ecosystem processes and functions (Lee et al. 2013; Powell et al. 2018; Saia et al. 2022). The composition, diversity and abundance of AMF communities depend on several factors. For instance, meta-analysis revealed that AMF exhibit biogeographic patterns at global scale (Stürmer et al. 2018). At local scale, both soil and biogeographical factors were shown to determine AMF communities (Jansa et al. 2014). Numerous studies proved that soil conditions also determine AMF diversity and composition (Table S 2.1). However, AMF have an idiosyncratic response to soil conditions, and there is no consensus on their relative importance (Vályi et al. 2016). Several studies showed that soil pH is an important factor to determine AMF communities (Xu et al. 2017; Li et al. 2021). It was also reported that a higher level of phosphorus (P) limited the diversity of AMF (Cheng et al. 2013); but another study showed that soil texture, rather than pH or P, affect AMF composition in an agriculture soil (Moebius-Clune et al. 2013). This divergence may be the result of having targeted different ecosystems, host plants and sample types (Table S 2.1). More importantly, plants excert strong effects on diversity and composition of their asscociated AMF (Torrecillas et al. 2012; Deepika et al. 2021), but the specificity of the association between plant host and AMF taxa is generally low (Sepp et al. 2019). Besides, the same plant species may reveal differences in AMF communities between plant compartments, i.e. root and rhizosphere soil (Saks et al. 2014; Alguacil et al. 2016; Coleman-Derr et al. 2016; Stefani et al. 2020).

AMF have been classified into different functional groups based on biomass allocation, i.e., rhizophilic guild and edaphophilic guild (Maherali et al. 2007). The rhizophilic guild is thought to allocate more arbuscular mycorrhizal (AM) biomass to roots than soil, such as *Rhizophagus*; while the edaphophilic guild is thought to allocate more AM biomass to soil than roots, like *Acaulospora*. However, caution must be taken when classifying AMF families into guilds, due to different technologies used (Babalola et al. 2022). In addition, plants could affect AMF richness by delivering more carbon to beneficial symbionts which could facilitate the competition with others (Liu et al. 2012; Stevens et al. 2020). Especially in degraded ecosystems, ‘founder AMF’ species might benefit from plant-derived

carbon to colonize the plant roots, thus would outcompete 'AMF latecomers', thereby leading to differences between root and rhizosphere soil (Chagnon et al. 2012). Thus, understanding the extent to which various factors modulate abundance, diversity and selective root-colonization of AMF (i.e., niche differentiation) is essential not only for maintenance of ecological processes in agroecosystems (Chourasiya et al. 2021; Douglas et al. 2021), but also for facilitating the restoration of degraded ecosystems (Asmelash et al. 2016; Shuab et al. 2017; Mao et al. 2019). AMF have been verified as pioneer microorganisms in sand dunes (Corkidi et al. 1997), river floodplains (Nakatsubo et al. 1994), and volcanic areas (Fujiyoshi et al. 2005). Although the role of AMF has been studied intensively in different ecosystems with different plant species (Table S 2.1), AMF diversity and communities in association with pioneer plants in heavily degraded ecosystems remain largely unexplored. These include post-mining sites (Quoreshi 2008), which are frequently found in West Africa. This is especially true for Ghana, which is the largest producer of gold in Africa, and the sixth largest producer in the world (Council. 2020). With a rate of 2600 hectares per year, natural vegetation in Ghana has been intensively converted into gold mining sites (Barenblitt et al. 2021). After surface mining, the land is usually left abandoned and free of vegetation, offering space for colonization by pioneer plants. In Ghana, *Pueraria phaseoloides* (Roxb.) Benth. (tropical kudzu), a perennial, N_2-fixing legume, has been found as a pioneer plant species that colonizes vigorously post-mining sites (Y. Guo, personal communication). This was similarly observed in Indonesia (Singhal 2009). It could be speculated that such successful colonization and potential adaptation to various site conditions is reinforced by the symbiosis with AMF. This assumption is rationalized by an earlier study, revealing that *P. phaseoloides* failed to establish without the presence of symbiotic AMF (Waidyanatha et al. 1979).

In view of the ecological advantage of *P. phaseoloides* to colonize efficiently post-mining sites, it is imperative to disentangle the factors shaping the mycorrhizal communities associated with *P. phaseoloides*, considering benefits for degraded land restoration. Hence, we investigated the genetic diversity and composition of AMF communities in the rhizosphere and roots of *P. phaseolides* growing under the prevailing soil conditions at abandoned, highly disturbed post-mining sites in Ghana. High throughput DNA sequencing was employed to analyze the AMF communities in degraded mining soils. Different from morphological methods, which rely mainly on spore identification, DNA-based approaches capture genetic information from hyphae, mycorrhizal roots and spores (Wu et al. 2020). We hypothesized that, due to the strong adaptation potential of *P. phaseolides* in different environments, host identity is a major driving factor shaping AMF communities, assumably stronger than local factors related to geography and soil conditions.

Secondly, we hypothesized that the high adaptability of *P. phaseolides* may be reflected in the selection of AMF specific species from the rhizosphere soil. By testing this, our main ambition is to fill a gap in the understanding of the ecological status of AMF in degraded mining soils and to provide a scientific base for developing AMF-based strategies for restoring degraded lands.

2.4 Methods

2.4.1 Site description and sampling

Soil and root samples were collected at five abandoned gold mining sites distributed across two locations (45 km distance) in the Ashanti region (Ghana) in October 2019. Three sites were located in Konongo (KN, 6°37' N, 1°14' E) and two sites in Bosome-Freho (BF, 6°25' N, 1°18' E) (Figure 2.1A). At each location, all collecting sites had a distance of at least 50 m between each other. Both locations had similar climate conditions (Table S 2.2), but differed in physic-chemical soil characteristics (Table S2.3). At each site, three spatially separated plant individuals of *P. phaseoloides* with a distance of 1.5 m from each other were randomly selected to ensure independence of samples (Alimi et al. 2021). The entire plants with soil (approximately 10 cm width and 20 cm depth) were excavated and were put into poly bags and transported in cooling boxes. Intact root systems (root balls) were conserved at 4ºC (Zettler et al. 2017), permitting samples to be in a semi-natural state prior to shipping to Germany (University of Hohenheim, Stuttgart). Upon arrival, samples were conserved at 4ºC for further processing.

2.4.2 Processing of samples

The samples were processed according to the protocol (McPherson et al. 2018), with minor modifications (Figure 2.1B). Bulk soil was removed from roots by soft shaking and subjected to physic-chemical analysis (Table S 2.3). Then, 10 to 12 roots per plant with a length of 5 to 8 cm were excised. Excised roots were washed with 35 ml autoclaved, phosphate buffer mixed with 200 g L^{-1} Tween-20 to detach the rhizosphere soil from roots. The roots were transferred to a new Falcon tube (50 ml) following disinfection procedures: (1) root samples were treated with 35 ml of 50% bleach mixed with 0.01% Tween-20 for 1 min; (2) the liquid phase was replaced by 35 ml of 70% ethanol for 1 min; (3) root samples were rinsed 5 times with sterile water; (4) washed roots were dried on sterile filter paper. Then, roots were cut into small pieces using sterile forceps and pruning scissors, and conserved at -20ºC for DNA extraction. Tubes containing the rhizosphere soils were processed as follows: (1) samples were filtered (sterile 100 µm mesh cell

strainer) into a new 50 ml tube; (2) samples were centrifuged (3,000 × *g*, 5 min, room temperature (RT)) and supernatant was removed; (3) tubes were chilled on ice and 1.5 ml of sterile phosphate buffer was added, followed by vortexing; (4) the suspended phase was transferred into a clean 2 ml tube and samples were centrifuged (15,871 × *g*, 2 min, RT). Finally, the supernatant was removed and rhizosphere soil pellets were conserved at -20ºC for DNA extraction.

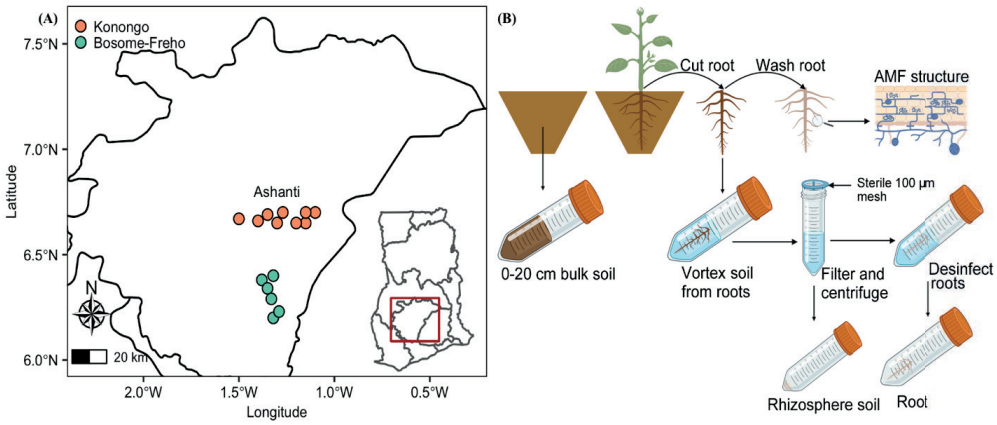

Figure 2.1 Sampling locations and sampling methods. (A) Sampling sites across two areas within the Ashanti region in Ghana. (B) Sampling methods to collect rhizosphere and root samples.

2.4.3 Amplicon sequencing

For DNA extraction, 0.1 g frozen roots of each sample homogenized in liquid N, and 0.5 g of frozen rhizosphere soil were used. DNA was extracted with the Fast DNA® spin plant kit (MP Biomedicals, Solo, Ohio, USA). To improve the quantity and quality of rhizosphere soil DNA, 30 mg polyvinylpolypyrrolidone (Thermo Fisher Scientific, Waltham, MA, USA) was added before buffer addition (Cheng et al. 2016). DNA concentration was measured by a NanoDrop spectrophotometer 2000 (Thermo Fisher Scientific). Working solutions of root (10-fold dilution) and rhizosphere soil (5 ng μl^{-1}) DNA were prepared with double-distilled water, and then stored at -20ºC for subsequent analysis. Amplicons of *Glomeromycota* were produced with nested PCR. The first PCR used the primers NS31 (Simon et al. 1992) and AMDGR (Sato et al. 2005). Each PCR (20 µl) comprised 1 µl DNA working solution, 0.2 µM of each primer, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase

(Promega GmbH, Walldorf, Germany). Reactions were run on a PeQSTAR thermal cycler (VWR International GmbH, Bruchsal, Germany) using the following conditions: 3 min initial denaturation at 95ºC, followed by 35 cycles of 95ºC for 30 s, 56º for 30 s, and 72ºC for 30 s. Reactions were completed with 72ºC for 3 min. The second nested PCR was conducted with the AMF specific primers AMV4.5NF and AMDGR (Sato et al. 2005) tagged with Illumina adapters, yielding an amplicon of approximately 300 bp. One µl of 1:10 diluted amplicon of the first PCR was used for the second PCR in 30 µl reactions, applying the same PCR run conditions as mentioned above, except that the annealing time was reduced to 20 s. Amplicons were verified by a 1.5% agarose gel electrophoresis. Then, 25 µl of amplicons with Illumina adaptors were submitted to Eurofins Genomics Europe Sequencing GmbH (Konstanz, Germany). Library construction and quality check were done by Eurofins Genomics. Illumina MiSeq was used for sequencing with a 2 × 300 sequence mode (Eurofins Genomics). The raw sequences were deposited in the Genome Sequence Archive (Chen et al. 2021) under BioProject accession number PRJ011089.

2.4.4 Bioinformatic analysis

Sequences were trimmed to exclude primer sequences and quality-filtered with quality scores > 35 in an initial step (Stefani et al. 2020). Rare amplicon sequence variants (ASV), with a frequency of less than 0.1% of the mean sequence depth, were removed. As Illumina reported to be 0.1% of reads most likely due to MiSeq bleed-through between runs (https://github.com/LangilleLab/microbiome_helper/wiki/Amplicon-SOP-v2-(qiime2-2020.2)).

Taxonomic identification of each ASV was performed according to the protocol of Stefani with minor modification (Stefani et al. 2020). First, each ASV was identified with the closest sequences against the National Center for Biotechnology Information (NCBI), using a basic local alignment search tool (BLAST). The search was set to *Glomeromycotina* (taxid:214504), whereby uncultured/environmental sample sequences were excluded and the maximum number of similar sequences retrieved (i.e., the number of top hits to record) was set to 10. BLAST results were exported as a single file XML2 and uploaded to Geneious Prime® (version 2020.2), with the aim of downloading the taxonomic information and saving only the first hit (the hit with the highest pairwise similarity and query coverage of > 95%). Then, BLAST results with taxonomic information were imported to QIIME2. Using qiime feature-classifier (classify-consensus-blast) to assign ASV, sequences belonging to ASV identified as non-*Glomeromycotina* (unclassified AMF) at the phylum level, were removed. The remaining ASV were considered as effective ASV and used for downstream analysis. Taxonomy assignment was inferred with RAxML (v8.2.12) under the GTRGAMMA

model and 1000 bootstraps via the CIPRES web-portal using "phylogenetic backbone tree". Phylogenetic backbone tree was calculated with the same producer as above, in addition to specify outgroups. The reference sequences were acquired from database (Krüger et al. 2012) and recently described AMF species in public repositories. The taxonomy of each ASV was delimitated with its position in the phylogenetic tree (Table S 2.4).

2.4.5 Statistical analysis

All statistical analyses were done in R (version 4.0.3). All sequence information is given in Table S 2.5. Low sequencing depth samples (< 1,000) were removed from the analysis to avoid any contamination with poor quality sequences (Weiss et al. 2017) (colored red in Table S 2.5). Data normality and homogeneity of variance were considered, and $\alpha = 0.05$ was defined as statistical significance. If needed, P values were adjusted for multiple comparisons using the Benjamini-Hochberg method (Benjamini et al. 1995).

Rarefaction curve was assembled individually with ASV of each compartment to confirm the sequencing depth. To eliminate errors, samples were rarefied to 1,500 sequences before calculating diversity indices. Alpha (α)-diversity indices, including observed ASV, ASV evenness (Pielou's evenness), Shannon and InvSimpson diversity, were estimated from rarefied ASV. Two-way ANOVA was used to test the significant difference in α-diversity indices within plant compartments and between locations. Post-hoc comparisons were conducted with Tukey's honest analysis.

Beta (β)-diversity of AMF communities was calculated using weighted UniFric nonmetric dimensional scaling (NMDS) ordination at the ASV level. Permutational multivariate analysis of variance (MANOVA) was carried out using Vegan's function adonis() to measure significant effects of locations and plant compartment on β-diversity. To identify distinct ASV of the two compartments, DeSeq2 was performed (Love et al. 2014). Furthermore, to verify whether this difference is related to functional variation among AMF, keystone taxa were determined in both plant compartments (i.e., rhizosphere soil, root). This analysis step was justified since keystone taxa are non-replaceable in microbiome structure and play a critical role in microbiome functioning (Banerjee et al. 2018). AMF keystone taxa were identified with co-occurrence networks which were considered as a powerful tool for inferring keystone taxa from microbial communities (Banerjee et al. 2018). Co-occurrence networks analysis was done in R, using Spearman correlation coefficient. According to strong ($R > 0.6$) and significant correlation ($P_{FDR} < 0.05$), co-occurrence models

within the rhizosphere soil and root compartment were constructed. Co-occurrence networks were visualized on Gephi platform (version 0.9.2) using the Fruchtermann-Feingold layout (Bastian et al. 2009). Those ASV with the highest betweenness centrality scores were considered as keystone taxa (Banerjee et al. 2016).

To detect the relationships between α-diversity of AMF (observed ASV richness and Shannon diversity) and soil characteristics, Pearson correlation was calculated. To further check the influence of soil characteristics on AMF composition, distance-based redundancy analysis (db-RDA) was conducted. The Variance inflation factor (VIF) was calculated to select decisive soil characteristics, whereby soil characteristics with VIF values less than 10 were selected (Hadi et al. 2006). The stepwise db-RDA was performed with Vegan's function dbrda() in R. The significance of variations in AMF composition explained by soil characteristics was tested by Monte Carlo permutation testing.

2.5 Results

2.5.1 Overall sequencing and taxonomic assignment

In total, 2,312,972 raw reads were obtained with 301 bp average read length from Illumina MiSeq® sequencing. The quality control (quality scores > 35) reduced the reads to 2,039,447 with 271 bp sequences in average (i.e., an average of 11.8% of the reads was discarded). Rare amplicon sequence variants (ASV) with a frequency of less than 0.1% of the mean sample depth were removed (see explanation in "Bioinformatic analysis" section). After removal of rare amplicon sequence variants (ASV) (16.8%), a total of 1,746,146 reads remained (Table S 2.5).

Non-*Glomeromycota* sequences were filtered according to NCBI database (see details in "Bioinformatic analysis" section), resulting in 195 ASV to the phylum *Glomeromycota* for downstream analysis. Among them, 102 ASV belonged to rhizosphere soil and 72 ASV were discovered in roots, while 21 ASV shared both compartments of rhizosphere soil and root (Figure S 2.1). Phylogenetic analysis assigned 195 ASV to 8 genera: *Acaulospora* (72), *Rhizophagus* (43), *Paraglomus* (43), *Dominikia* (16), *Claroideoglomus* (7), *Funneliformis* (7), *Septoglomus* (4), *Diversispora* (3). *Acaulospora*, *Rhizophagus, Paraglomus,* and *Septoglomus* were found both in the roots and rhizosphere soil. *Dominikia* and *Claroideoglomus* were only detected in the rhizosphere soil, while *Funneliformis* and *Diversispora* were only found in the roots (Figure 2.2A and Figure S 2.2).

2.5.2 Alpha diversity of AMF

Rarefaction curves display the number of sequences, which have reached adequate coverage (saturation) of AMF diversity, as a quality requirement for downstream analysis (Figure S 2.3). The α-diversity indices were displayed and statistical differences were annotated for both locations (Konongo, KN; Bosome-Freho, BF) (Figure 2.2C, D and Figure S 2.4). At both locations, more ASV were observed in rhizosphere soil than in roots, although this difference was not significant ($P > 0.05$) (Figure S 2.4 (a)). At both locations, ASV evenness and diversity of rhizosphere soil were, however, higher than in roots ($P < 0.05$) (Figure 2.2C, D). ASV richness of rhizosphere soil in BF was higher than in KG ($P < 0.05$), while evenness and diversity of ASV did not reveal any difference between the two locations (data not shown).

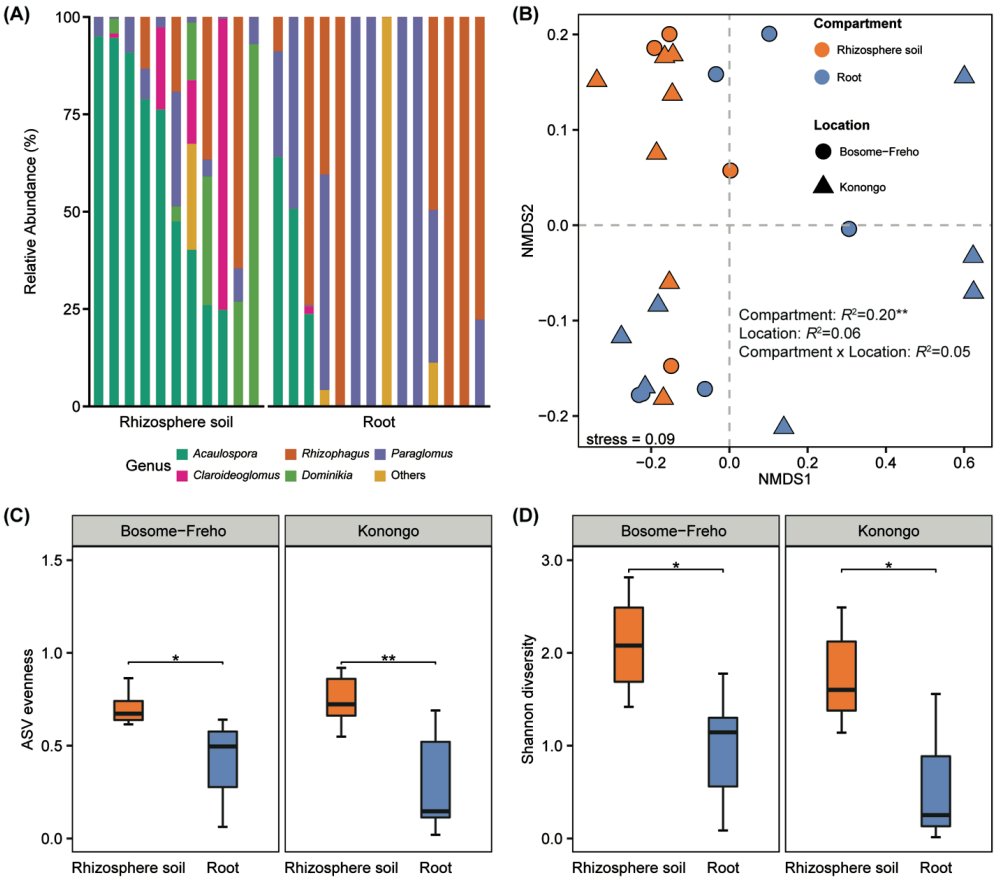

Figure 2.2 The overall structure of AMF communities. (A) Genus distribution of amplicon sequence variants (ASV) using relative abundance of AMF associated with rhizosphere soil and root. 11 rhizosphere soils and 14 root samples are displayed in seperated stacked bars. Different genera are displayed in different colors, and low abundance genera (< 10%) are grouped together (others). Relative abundance of each genus across compartments is displayed in Figure S2. (B) Nonmetric dimensional scaling (NMDS) analyses of weighted metric distance of AMF communities. (C) and (D) α-diversity of field samples. (C) Pielou's evenness. (D) Shannon diversity. The boxes represent the range between 75th and 25th quartiles. The line within the box represents the median. The whiskers represent the lowest and highest values extending 1.5 of the interquartile range. NS indicates non-significance; * denotes significance ($P < 0.05$).

2.5.3 Beta diversity (community composition) of AMF

AMF composition between two compartments was clearly separated, but not between locations (Figure 2.2B). The stress value of NMDS analysis was 0.09, reflecting the significant variation of AMF communities among factors (Clarke 1993). To verify the significance of NMDS analysis, factors (plant compartment, location) were further examined using permutational MANOVA test (Figure 2.2B). Results indicate that plant compartment was a crucial determinant to modulate AMF composition ($P < 0.05$), explaining more than 20% of variation. However, the effect of location on AMF composition was not significant, explaining only 4% of variation ($P > 0.05$).

2.5.4 Relationship of AMF with soil characteristics

Relationship between AMF richness (observed ASV) and diversity (Shannon diversity) and soil characteristics was analyzed (Table S 2.6). Contents of soil calcium (Ca) and soil pH had negative correlations with both AMF richness and diversity ($P < 0.05$). Content of soil zinc (Zn) only had a negative correlation with AMF richness ($P < 0.05$). Distance-based redundancy analysis (db-RDA) was carried out to examine the influence of soil characteristics on AMF composition. Results indicate that soil characteristics had no effect on AMF composition ($P > 0.05$) (Table S 2.7).

2.5.5 Differences in AMF communities between plant compartments

With analysis of DeSeq2, twelve ASV were found to be more abundant in the rhizosphere soil than in root samples, whereas 7 ASV were more abundant in the root compartment than in the rhizosphere soil (Figure 2.3A). In the root compartment, ASV were affiliated to two genera: *Rhizophagus* (4) and *Paraglomus* (3). In rhizosphere soils, ASV were assigned to *Acaulospora* (5), *Paraglomus* (4), *Dominikia* (2) and *Claroideoglomus* (1). Network of the rhizosphere soil had more nodes and edges (123 nodes, 948 edges) than the root compartment network (86 nodes, 468 edges). The modularity index (rhizosphere soil: 0.78; root: 0.79) was above 0.4, indicating a modular network structure (Newman 2006). The average degrees of rhizosphere soil and root were 17.6 and 10.9, respectively, and the average clustering coefficient was 0.84 for rhizosphere soil and 0.95 for root. The detailed results of co-occurrence networks are listed in Table S 2.8 and Table S 2.9. On basis of the network analysis, ASV230 (*Acaulospora*) and ASV238 (*Rhizophagus*) were determined as keystone taxa for the rhizosphere soil and root compartment, respectively (Figure 2.3B and Table S 2.8-2.9).

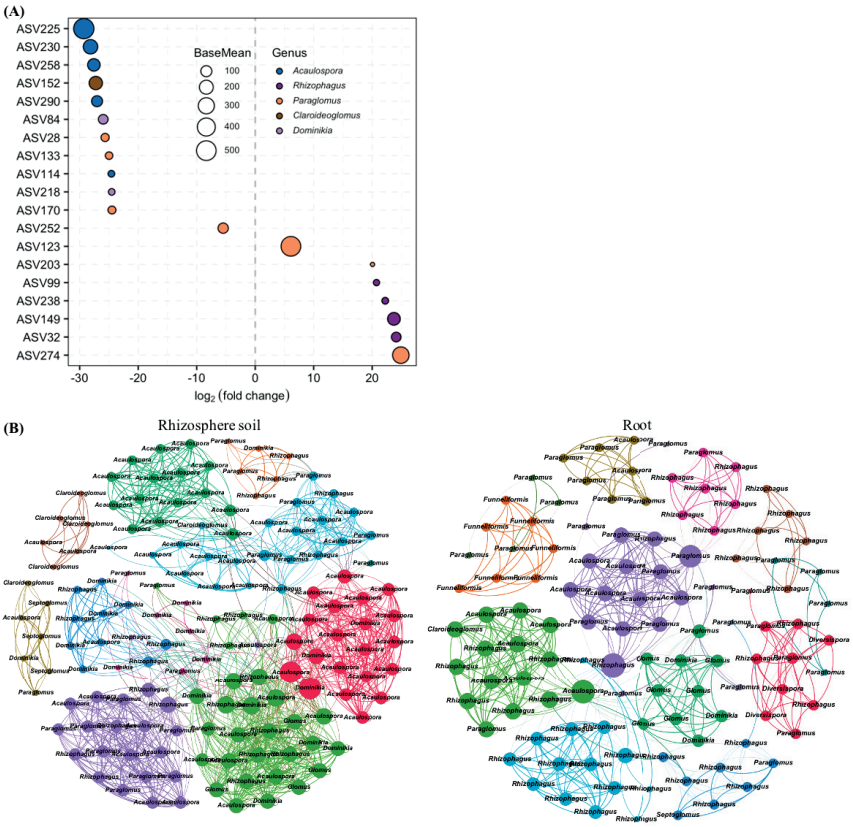

Figure 2.3 The differences of ASV and keystone taxa identification. (A) DeSeq2 plot showing the difference of amplicon sequence variants (ASV) between the two plant compartments. Different colors represent different genera, and different point sizes represent different mean values, after normalization through DeSeq2. (B) Co-occurrence network in rhizosphere soil and root compartment. Each node represents one amplicon sequence variant (ASV) labeled by genus. A node was verified by a robust (Spearman's correction coefficient $R > 0.6$) and significant ($P_{FDR} < 0.05$) correlation. The size of each node is relational to the number of connections, while nodes with the same color display the same module. The thickness of each connection between two nodes is relational to the strength of the Spearman's correlation coefficient.

2.6 Discussion

Our results showed that the root of the pioneer plant *Pueraria phaseoloides* revealed a lower amplicon sequence variants (ASV) richness and diversity of AMF than the associated rhizosphere soil, a finding in line with other plant species (Saks et al. 2014; Alguacil et al. 2016). This confirms the general view that the rhizosphere soil provides an important reservoir of AMF for plants, from which plants only recruit a proportion at a certain time (Davison et al. 2011). More importantly, the plant compartment of *P. phaseoloides* exerts, independent of geographical location or microbiome provenance, a strong effect on microbial consortia shift, indicating a selective preference for associated AMF. It was shown that geographic distance had a small effect on AMF communities, because a single plant species in agricultural land may homogenize the AMF communities over a certain distance (Gao et al. 2019). Furthermore, host compartmentalization of microbial communities facilitates the decoupling from effects of habitat fragmentation (Willing et al. 2021). In our study, *P. phaseoloides* plays an important role in shaping AMF communities, thereby overriding geographic factors. However, the symbiosis between plants and AMF is generally considered as non-specific (Smith et al. 2008), which ascribes the small number of characterized AMF species (~300) compared to that of plant species (~300,000) (Vályi et al. 2015). Nevertheless, evidence exists that a preference of AMF-plant associations was present in different ecosystems (Vályi et al. 2015; Sepp et al. 2019; Deepika et al. 2021). Apart from the release of carbonaceous root exudates triggering the most preferred symbiont (Bever et al. 2009), soil conditions have been acknowledged to modify the ability of plants to attract selected AMF (Walder et al. 2015).

Our results showed that soil conditions had no significant effect on AMF composition. This divergence seems likely due to the strong regulation of the plant host on the rhizosphere species pool via root exudates, thereby masking soil conditions in structuring the soil AMF communities. In line with other studies, it was demonstrated that the host plant exerts a much stronger selectivity for AMF colonization of roots than prevailing soil conditions (Horn et al. 2014; Sandoz et al. 2020; Deepika et al. 2021). This confirms the acknowledged community assembly concept of AMF, whereby the host filter was decisive for AMF assemblage within the plant (Vályi et al. 2016). On the other hand, the effect of soil conditions on AMF richness and diversity was detected. Firstly, AMF richness and diversity correlated negatively with soil pH, as it was observed in other situations (Alguacil et al. 2016; Xu et al. 2017; Albornoz et al. 2021). Soil pH controls AMF richness and diversity through influencing nutrient and ion availability (Neina 2019). Consequently, we found that the content of soil zinc (Zn) correlated with AMF richness but not diversity, while soil

calcium (Ca) correlated with both AMF richness and diversity. Both nutrients facilitate plant metabolic processes, and their uptake is supported by AMF (Watts-Williams et al. 2021). It is known that high Zn soil levels can negatively affect the abundance and composition of AMF in polluted soils (Zarei et al. 2008; Yang et al. 2015), while other studies showed that Zn could influence AMF diversity and richness in non-polluted ecosystems (Alguacil et al. 2016; Xu et al. 2017; Alimi et al. 2021). These inconsistent results were most likely explained by differences in ecosystem type and structure. On the other hand, only limited information is available about the influence of Ca on AMF diversity and composition. Ca is not only a nutrient, but also considered as a messenger which initializes the communication between plants and AMF (Thor 2019). Further research would be needed to explore the influence of Ca on AMF diversity and composition under the given environmental conditions of our study. Although pH, Zn and Ca had a significant effect on richness and/or diversity of AMF in our study, they did not trigger a significant difference in terms of community assembly.

Our data further supported the concept of host preference, revealing for the first time two different and highly abundant AMF keystone species in two distinct plant compartments: *Rhizophagus* in roots and *Acaulospora* in rhizosphere soils, associated with a single plant species (i.e., *P. phaseoloides*). It was reported that keystone taxa with high abundance have vital contributions for maintaining ecosystem functioning (Shetty et al. 2017). This may also apply to *Rhizophagus*, existing in high abundance in the root compartment of *P. phaseoloides. Rhizophagus* is a generally fast-growing species (r-strategy) and, based on its phenotypic traits, was classified as 'competitor' in the life history classification system (Chagnon et al. 2013). Thus, *Rhizophagus* may have a competitive advantage to occupy efficiently the root niche with immediate access to plant-derived resources. Furthermore, plants prefer to deliver more carbon to beneficial symbionts (Liu et al. 2012; Stevens et al. 2020), like *Rhizophagus*, which may help plants to be successful in the harsh environmental conditions of abandoned mining sites. With regard to the colonization of degraded ecosystems (e.g., abandoned mining sites), it was proposed that so-called 'founder AMF' species might benefit from this plant-derived carbon to colonize the plant roots in the early stage of ecological succession (Pierre-Luc et al. 2012). Accordingly, this ecological advantage would outcompete so-called 'AMF latecomers', benefiting the proliferation of *Rhizophagus* through the soil via colonization of newly formed roots. More importantly, *Rhizophagus* has been recognized as a dominant AMF species that supports plants in the early stages of growth development in agricultural system (Gao et al. 2019; Gao et al. 2022). This might also be true for degraded ecosystems. In fact, *Rhizophagus* was considered as a prominently abundant taxon, since it has been found in diverse host species

and environments (Table S2.1). However, a phylogenetic meta-analysis of most abundant AMF taxa across different ecosystems, such as *Rhizophagus,* indicated that they do not necessarily have the same phylogenetic structure (Dumbrell et al. 2010). On the other hand, *Acaulospora* is a slow growing species (k-strategy) and the trait-based framework presented *Acaulospora* as 'stress-tolerant' AMF (Chagnon et al. 2013). However, stress-tolerant AMF were believed to provide a delayed benefit to their host, which is accompanied by an excessive carbon demand from their host (Chagnon et al. 2013). Therefore, our study suggested that the abundant *Rhizophagus* in the roots of *P. phaseoloides* was the result of a good functional match between both partners in this degraded ecosystem.

2.7 Conclusions

Our results provided fundamental genetic insights of AMF communities associated with the pioneer plant *Pueraria phaseoloides* colonizing abandoned mining sites in Ghana. Our study showed that geography and prevailing soil conditions only exerted significant effects on AMF richness and diversity, but not on AMF community composition. Instead, plant compartment largely explained of the differences in AMF composition, with two different functional species in two distinct plant compartments (i.e., *Acaulospora* in rhizosphere soil; *Rhizophagus* in root). This implied that *P. phaseoloides* has a strong selectivity for AMF species, irrespective of soil conditions, emphasizing the ecological plasticity of the host in selecting AMF. The present study was based on a one-time point sampling. Hence, to fully understand the ecological effects of AMF communities in degraded ecosystems, further studies, including a broader range of abandoned mining sites with distinct environmental conditions and considering multiple AMF proxies (i.e., spore density, intraradical and extraradical hyphae) across various seasons, would provide a more profound insight into the plasticity and responsiveness of AMF compartmentation in association with *P. phaseoloides*, as a suitable ecological basis for restoration of degraded ecosystems.

2.8 References

Albornoz F E, Orchard S, Standish R J, Dickie I A, Bending G D, Hilton S, Lardner T, et al. (2021) Evidence for Niche Differentiation in the Environmental Responses of Co-occurring Mucoromycotinian Fine Root Endophytes and Glomeromycotinian Arbuscular Mycorrhizal Fungi. Microbial Ecology 81:864-873. https://doi.org/10.1007/s00248-020-01628-0

Alguacil M d M, Torres M P, Montesinos-Navarro A, Roldan A (2016) Soil Characteristics Driving Arbuscular Mycorrhizal Fungal Communities in Semiarid Mediterranean Soils. Applied and Environmental Microbiology 82:3348-3356. https://doi.org/10.1128/AEM.03982-15

Alimi A A, Ezeokoli O T, Adeleke R, Moteetee A (2021) Arbuscular mycorrhizal fungal communities colonising the roots of indigenous legumes of South Africa as revealed by high-throughput DNA metabarcoding. Rhizosphere 19:100405. https://doi.org/10.1016/j.rhisph.2021.100405

Asmelash F, Bekele T, Birhane E (2016) The Potential Role of Arbuscular Mycorrhizal Fungi in the Restoration of Degraded Lands. Frontiers in microbiology 7:1095. https://doi.org/10.3389/fmicb.2016.01095

Babalola B J, Li J, Willing C E, Zheng Y, Wang Y-L, Gan H-Y, Li X-C, et al. (2022) Nitrogen fertilisation disrupts the temporal dynamics of arbuscular mycorrhizal fungal hyphae but not spore density and community composition in a wheat field. New Phytologist 234:2057-2072. https://doi.org/10.1111/nph.18043

Banerjee S, Kirkby C A, Schmutter D, Bissett A, Kirkegaard J A, Richardson A E (2016) Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biology and Biochemistry 97:188-198. https://doi.org/10.1016/j.soilbio.2016.03.017

Banerjee S, Schlaeppi K, van der Heijden M G A (2018) Keystone taxa as drivers of microbiome structure and functioning. Nature Reviews Microbiology 16:567-576. https://doi.org/10.1038/s41579-018-0024-1

Barenblitt A, Payton A, Lagomasino D, Fatoyinbo L, Asare K, Aidoo K, Pigott H, et al. (2021) The large footprint of small-scale artisanal gold mining in Ghana. Science of the Total Environment 781:146644. https://doi.org/10.1016/j.scitotenv.2021.146644

Bastian M, Heymann S, Jacomy M (2009) Gephi: An Open Source Software for Exploring and Manipulating Networks. Proceedings of the International AAAI Conference on Web and Social Media 3:361-362. https://ojs.aaai.org/index.php/ICWSM/article/view/13937

Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological) 57:289-300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bever J D, Richardson S C, Lawrence B M, Holmes J, Watson M (2009) Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecology Letters 12:13-21. https://doi.org/10.1111/j.1461-0248.2008.01254.x

Chagnon P-L, Bradley R L, Klironomos J N (2012) Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure. New Phytologist 194:307-312. https://doi.org/10.1111/j.1469-8137.2011.04044.x

Chagnon P-L, Bradley R L, Maherali H, Klironomos J N (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends in Plant Science 18:484-491. https://doi.org/10.1016/j.tplants.2013.05.001

Chen T, Chen X, Zhang S, Zhu J, Tang B, Wang A, Dong L, et al. (2021) The Genome Sequence Archive Family: Toward Explosive Data Growth and Diverse Data Types. Genomics, Proteomics & Bioinformatics 19:578-583. https://doi.org/10.1016/j.gpb.2021.08.001

Cheng F, Hou L, Woeste K, Shang Z, Peng X, Zhao P, Zhang S (2016) Soil pretreatment and fast cell lysis for direct polymerase chain reaction from forest soils for terminal restriction fragment length polymorphism analysis of fungal communities. Brazilian Journal of Microbiology 47:817-827. https://doi.org/10.1016/j.bjm.2016.06.007

Cheng Y, Ishimoto K, Kuriyama Y, Osaki M, Ezawa T (2013) Ninety-year-, but not single, application of phosphorus fertilizer has a major impact on arbuscular mycorrhizal fungal communities. Plant and Soil 365:397-407. https://doi.org/10.1007/s11104-012-1398-x

Chourasiya D, Gupta M M, Sahni S, Oehl F, Agnihotri R, Buade R, Maheshwari H S, Prakash A, Sharma M P (2021) Unraveling the AM fungal community for understanding its ecosystem resilience to changed climate in agroecosystems. Symbiosis 84:295-310. https://doi.org/10.1007/s13199-021-00761-9

Clarke K R (1993) Non-parametric multivariate analyses of changes in community structure. Australian journal of ecology 18:117-143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez L P, Tringe S G (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytologist 209:798-811. https://doi.org/10.1111/nph.13697

Corkidi L, Rincón E (1997) Arbuscular mycorrhizae in a tropical sand dune ecosystem on the Gulf of Mexico. Mycorrhiza 7:17-23. https://doi.org/10.1007/s005720050157

Council. W G (2020) Top ten Gold producing Countries in 2020. https://www.gold.org/goldhub/data/gold-production-by-country?gclid=CjwKCAjwtIaVBhBkEiwAsr7-c2O3lJh_zO9a461Yxvqe0b_4I50cB9wOIZCjoPG_VlddRS4kJls58xoCYL8QAvD_BwE

Davison J, Öpik M, Daniell T J, Moora M, Zobel M (2011) Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiology Ecology 78:103-115. https://doi.org/10.1111/j.1574-6941.2011.01103.x

Deepika S, Kothamasi D (2021) Plant hosts may influence arbuscular mycorrhizal fungal community composition in mangrove estuaries. Mycorrhiza 31:699-711. https://doi.org/10.1007/s00572-021-01049-y

Douglas A S, Heng G, Peter E M, Jianchu X (2021) Arbuscular Mycorrhiza and Sustainable Agriculture. Circular Agricultural Systems 1:1-7. https://doi.org/10.48130/CAS-2021-0006

Dumbrell A J, Nelson M, Helgason T, Dytham C, Fitter A H (2010) Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: is there a role for stochastic processes? Journal of Ecology 98:419-428. https://doi.org/10.1111/j.1365-2745.2009.01622.x

Fujiyoshi M, Kagawa A, Nakatsubo T, Masuzawa T (2005) Successional changes in mycorrhizal type in the pioneer plant communities of a subalpine volcanic desert on Mt. Fuji, Japan. 18:60-72. http://doi.org/10.15094/00006226

Gao C, Courty P-E, Varoquaux N, Cole B, Montoya L, Xu L, Purdom E, et al. (2022) Successional adaptive strategies revealed by correlating arbuscular mycorrhizal fungal abundance with host plant gene expression. Molecular ecology 00:1-14. https://doi.org/10.1111/mec.16343

Gao C, Montoya L, Xu L, Madera M, Hollingsworth J, Purdom E, Hutmacher R B, et al. (2019) Strong succession in arbuscular mycorrhizal fungal communities. The ISME journal 13:214-226. https://doi.org/10.1038/s41396-018-0264-0

Hadi A S, Chatterjee S (2006) Regression analysis by example. Hoboken, New Jersey. https://doi.org/10.1002/0470055464.fmatter

Horn S, Caruso T, Verbruggen E, Rillig M C, Hempel S (2014) Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. The ISME journal 8:2231-2242. https://doi.org/10.1038/ismej.2014.72

Jansa J, Erb A, Oberholzer H-R, Šmilauer P, Egli S (2014) Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Molecular ecology 23:2118-2135. https://doi.org/10.1111/mec.12706

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytologist 193:970-984. https://doi.org/10.1111/j.1469-8137.2011.03962.x

Lee E-H, Eo J-K, Ka K-H, Eom A-H (2013) Diversity of Arbuscular Mycorrhizal Fungi and Their Roles in Ecosystems. Mycobiology 41:121-125. https://doi.org/10.5941/MYCO.2013.41.3.121

Li X, Qi Z, Yu X, Xu M, Liu Z, Du G, Yang Y (2021) Soil pH drives the phylogenetic clustering of the arbuscular mycorrhizal fungal community across subtropical and tropical pepper fields of China. Applied Soil Ecology 165:103978. https://doi.org/10.1016/j.apsoil.2021.103978

Liu Y, Shi G, Mao L, Cheng G, Jiang S, Ma X, An L, Du G, Collins Johnson N, Feng H (2012) Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytologist 194:523-535. https://doi.org/10.1111/j.1469-8137.2012.04050.x

Love M I, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:550. https://doi.org/10.1186/s13059-014-0550-8

Maherali H, Klironomos J N (2007) Influence of Phylogeny on Fungal Community Assembly and Ecosystem Functioning. Science 316:1746-1748. https://doi.org/10.1126/science.1143082

Mahmoudi N, Cruz C, Mahdhi M, Mars M, Caeiro M F (2019) Arbuscular mycorrhizal fungi in soil, roots and rhizosphere of Medicago truncatula: diversity and heterogeneity under semi-arid conditions. PeerJ 7:e6401. https://doi.org/10.7717/peerj.6401

Mao L, Pan J, Jiang S, Shi G, Qin M, Zhao Z, Zhang Q, An L, Feng H, Liu Y (2019) Arbuscular mycorrhizal fungal community recovers faster than plant community in historically disturbed Tibetan grasslands. Soil Biology and Biochemistry 134:131-141. https://doi.org/10.1016/j.soilbio.2019.03.026

McPherson M R, Wang P, Marsh E L, Mitchell R B, Schachtman D P (2018) Isolation and Analysis of Microbial Communities in Soil, Rhizosphere, and Roots in Perennial Grass Experiments. Journal of Visualized Experiments 137:e57932. https://doi.org/10.3791/57932

Moebius-Clune D J, Moebius-Clune B N, van Es H M, Pawlowska T E (2013) Arbuscular mycorrhizal fungi associated with a single agronomic plant host across the landscape: Community differentiation along a soil textural gradient. Soil Biology and Biochemistry 64:191-199. https://doi.org/10.1016/j.soilbio.2012.12.014

Nakatsubo T, Kaniyu M, Nakagoshi N, Horikoshi T (1994) Distribution of vesicular-arbuscular mycorrhizae in plants growing in a river floodplain. Bulletin of Japanese Society of Microbial Ecology 9:109-117. https://doi.org/10.1264/microbes1986.9.109

Neina D (2019) The Role of Soil pH in Plant Nutrition and Soil Remediation. Applied and Environmental Soil Science 2019:5794869. https://doi.org/10.1155/2019/5794869

Newman M E J (2006) Modularity and community structure in networks. Proceedings of the national academy of sciences 103:8577-8582. https://doi.org/10.1073/pnas.0601602103

Pierre-Luc C, Robert L B, John N K (2012) Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure. The New Phytologist 194:307-312. http://www.jstor.org/stable/newphytologist.194.2.307

Powell J R, Rillig M C (2018) Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. New Phytologist 220:1059-1075. https://doi.org/10.1111/nph.15119

Quoreshi A M (2008) The Use of Mycorrhizal Biotechnology in Restoration of Disturbed Ecosystem. Mycorrhizae: Sustainable Agriculture and Forestry. Springer, Dordrecht, 303-320. https://doi.org/10.1007/978-1-4020-8770-7_13

Saia S, Jansa J (2022) Editorial: Arbuscular Mycorrhizal Fungi: The Bridge Between Plants, Soils, and Humans. Frontiers in plant science 13:875958. https://doi.org/10.3389/fpls.2022.875958

Saks Ü, Davison J, Öpik M, Vasar M, Moora M, Zobel M (2014) Root-colonizing and soil-borne communities of arbuscular mycorrhizal fungi in a temperate forest understorey. Botany 92:277-285. https://doi.org/10.1139/cjb-2013-0058

Sandoz F A, Bindschedler S, Dauphin B, Farinelli L, Grant J R, Hervé V (2020) Biotic and abiotic factors shape arbuscular mycorrhizal fungal communities associated with the roots of the widespread fern Botrychium lunaria (Ophioglossaceae). Environmental Microbiology Reports 12:342-354. https://doi.org/10.1111/1758-2229.12840

Sato K, Suyama Y, Saito M, Sugawara K (2005) A new primer for discrimination of arbuscular mycorrhizal fungi with polymerase chain reaction-denature gradient gel electrophoresis. Grassland Science 51:179-181. https://doi.org/10.1111/j.1744-697X.2005.00023.x

Sepp S-K, Davison J, Jairus T, Vasar M, Moora M, Zobel M, Öpik M (2019) Non-random association patterns in a plant–mycorrhizal fungal network reveal host–symbiont specificity. Molecular ecology 28:365-378. https://doi.org/10.1111/mec.14924

Shetty S A, Hugenholtz F, Lahti L, Smidt H, de Vos W M (2017) Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies. FEMS Microbiology Reviews 41:182-199. https://doi.org/10.1093/femsre/fuw045

Shuab R, Lone R, Ahmad J, Reshi Z A (2017) RETRACTED CHAPTER: Arbuscular Mycorrhizal Fungi: A Potential Tool for Restoration of Degraded Land. Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration. Springer, Cham, 415-434. https://doi.org/10.1007/978-3-319-68867-1_22

Simon L, Lalonde M, Bruns T D (1992) Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. Applied and Environmental Microbiology 58:291-295. https://doi.org/10.1128/aem.58.1.291-295.1992

Singhal R K (2009) Mining and the Environment: From Ore to Metal. International Journal of Mining, Reclamation and Environment 23:241-241. https://doi.org/10.1080/17480930903429794

Smith S E, Read D (2008) Mycorrhizal symbiosis. Mycorrhizal Symbiosis (Third Edition). Academic Press, London, 1-9. https://doi.org/10.1016/B978-012370526-6.50002-7

Stefani F, Bencherif K, Sabourin S, Hadj-Sahraoui A L, Banchini C, Séguin S, Dalpé Y (2020) Taxonomic assignment of arbuscular mycorrhizal fungi in an 18S metagenomic dataset: a case study with saltcedar (Tamarix aphylla). Mycorrhiza 30:243-255. https://doi.org/10.1007/s00572-020-00946-y

Stevens B M, Propster J R, Öpik M, Wilson G W T, Alloway S L, Mayemba E, Johnson N C (2020) Arbuscular mycorrhizal fungi in roots and soil respond differently to biotic and abiotic factors in the Serengeti. Mycorrhiza 30:79-95. https://doi.org/10.1007/s00572-020-00931-5

Stürmer S L, Bever J D, Morton J B (2018) Biogeography of arbuscular mycorrhizal fungi (Glomeromycota): a phylogenetic perspective on species distribution patterns. Mycorrhiza 28:587-603. https://doi.org/10.1007/s00572-018-0864-6

Thor K (2019) Calcium—Nutrient and Messenger. Frontiers in plant science 10:440. https://doi.org/10.3389/fpls.2019.00440

Torrecillas E, Alguacil M, Roldán A (2012) Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. Applied and Environmental Microbiology 78:6180-6186. https://doi.org/10.1128/AEM.01287-12

Vályi K, Mardhiah U, Rillig M C, Hempel S (2016) Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. The ISME journal 10:2341-2351. https://doi.org/10.1038/ismej.2016.46

Vályi K, Rillig M C, Hempel S (2015) Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. New Phytologist 205:1577-1586. https://doi.org/10.1111/nph.13236

Waidyanatha U d S, N Yogaratnam, Ariyaratne W (1979) Mycorrhizal infection on growth and nitrogen fixation of Pueraria and Stylosanthes and uptake of phosphorus from two rock phosphates. New Phytologist 82:147-152. https://doi.org/10.1111/j.1469-8137.1979.tb07569.x

Walder F, van der Heijden M G A (2015) Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. Nature Plants 1:15159. https://doi.org/10.1038/nplants.2015.159

Watts-Williams S J, Gilbert S E (2021) Arbuscular mycorrhizal fungi affect the concentration and distribution of nutrients in the grain differently in barley compared with wheat. Plants People Planet 3:567-577. https://doi.org/10.1002/ppp3.10090

Weiss S, Xu Z Z, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, et al. (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5:27. https://doi.org/10.1186/s40168-017-0237-y

Willing C E, Pierroz G, Guzman A, Anderegg L D L, Gao C, Coleman-Derr D, Taylor J W, Bruns T D, Dawson T E (2021) Keep your friends close: Host compartmentalisation of microbial communities facilitates decoupling from effects of habitat fragmentation. Ecology Letters 24:2674-2686. https://doi.org/10.1111/ele.13886

Wu S, You F, Wu Z, Bond P, Hall M, Huang L (2020) Molecular diversity of arbuscular mycorrhizal fungal communities across the gradient of alkaline Fe ore tailings, revegetated waste rock to natural soil sites. Environmental Science and Pollution Research 27:11968-11979. https://doi.org/10.1007/s11356-020-07780-x

Xu X, Chen C, Zhang Z, Sun Z, Chen Y, Jiang J, Shen Z (2017) The influence of environmental factors on communities of arbuscular mycorrhizal fungi associated with Chenopodium ambrosioides revealed by MiSeq sequencing investigation. Scientific reports 7:45134. https://doi.org/10.1038/srep45134

Yang Y, Song Y, Scheller H V, Ghosh A, Ban Y, Chen H, Tang M (2015) Community structure of arbuscular mycorrhizal fungi associated with Robinia pseudoacacia in uncontaminated and heavy metal contaminated soils. Soil Biology and Biochemistry 86:146-158. https://doi.org/10.1016/j.soilbio.2015.03.018

Zarei M, König S, Hempel S, Nekouei M K, Savaghebi G, Buscot F (2008) Community structure of arbuscular mycorrhizal fungi associated to Veronica rechingeri at the Anguran zinc and lead mining region. Environmental Pollution 156:1277-1283. https://doi.org/10.1016/j.envpol.2008.03.006

Zettler L W, Rajaovelona L, Yokoya K, Kendon J P, Stice A L, Wood A E, Sarasan V (2017) Techniques for the collection, transportation, and isolation of orchid endophytes from afar: a case study from Madagascar. Botanical Studies 58:54. https://doi.org/10.1186/s40529-017-0209-3

2.9 Supplementary information

Table S 2.1 List of studies investigating effects of different factors on AMF diversity and communities in different ecosystems with different host plants, as well as different sample types and technologies used.

Authors	Ecosystems	Plant species	Sample types	Methods	Primers	Results (determinant factors)
Jansa et al. 2014	Swiss Agriculture soils		soil samples	qPCR	taxon-specific markers	Soil and geography are more important determinants than management practices
Xu et al. 2017	NA	*Chenopodium ambrosioides*	soil samples+root samples	Illumina MiSeq sequence	AMV4.5NF/AMDGR	Plant compartment and soil characters affected AMF communities significantly
Cheng et al. 2013	Agricultural controled system	*Lotus japonicus*+*Glycine max*	root samples	3130xl Genetic analyer	LR1/FLR2	P is major factor
Moebius-Clune et al. 2013	Agricultural soils	Maize	spore samples (trap culture)	sequencing	5'-end LSU	Soil texture is major factor
Alguacil et al. 2016	Semiarid Mediterranean soils	*Brachypodium retusum*	soil samples+root samples	cloning and sequencing	NS1/NS4+ AML1/AML2	Soil characteristics driver the AMF community
Stevens et al. 2020	Grassland soils	*Digitaria macroblephare*+ *Themeda triandra* (C4 grass)	soil samples+root samples	spore identification+fatty acid biomarker+Illumina MiSeq 300	WANDA/AML2	Regional edaphic conditions shape the site-level species pool and plant species actively select root-colonizing Fungal assemblages
Babalola et al. 2022	Agricultural soils	Wheat	soil samples+root samples	spore density+(in/ex)hyphae length+Illumina MiSeq 250	GeoA-2/AML2+ NS31/AMDGR	High nitrogen level disrupt the temporal dynamics of AM fungal hyphal growth but not sporulation and community composition
Dumbrell et al. 2011	Grassland soils		soil samples	prosequencing	NS31/AM1+ WANDA/NS31	Season is the major factor to determine the AMF community
Liu et al. 2012	Alpine Meadow and Wetland Ecosystems	*Elymus nutans* (Poaceae)	soil samples+root samples	spore density+(in/ex)hyphae length+restriction fragment length polymorphism (RFLP)	Geoa2/Geo11+ NS31/AML2	High fertilizer inputs reduced the biodiversity of AMF
Alimi et al. 2021	Agricultural soils	Eleven legumes	root samples	Illumina MiSeq sequence	AML1/AML2+ AMV4.5NF/AMDGR	Soil factors are pertinent environmental cues that shape AMF community
Deepika et al. 2021	Agricultural soils	Mangrove	root samples	RFLP	NS31/AM1	Plant identity may have a primary role in shaping AMF communities in mangroves
Johnson et al. 2016	Agricultural soils	Cowpea	soil samples+root samples	cloning and sequencing	FLR3/FLR4	AMF have no plant specifity

Sousa et al. 2022	Tropical forest	Mix of species	soil samples+root samples	Illumina MiSeq 300	AMF specific +universal fungi	Regional climate, most importantly precipitation and temperature, shape AMF biogeography
Garo et al. 2022	Agricultural soils	Enset (*Ensete ventricosum* (Welw.) Cheesman)	root samples	Illumina MiSeq sequence	AMV4.5NF/AMDGR	Intensive manure applications resulted in AMF cmoposition shift
Li et al. 2010	Hot and arid ecosystem (nature)	*Bothriochloa pertusa*+ *Heteropogon contortus*+ *Cajanus cajan*	root samples	Cloning and sequencing	LR1/FLR2+ 28G1/28G2	Habitats more influenced AMF community than plants
Mahmoudi et al. 2019	Semi-arid ecosystem (nature)	*Medicago truncatula*	root samples	prosequencing	NS1/NS4+ NS31/AM1	Abiotic facotrs has high herterogeneity of AMF community
Oehl et al. 2010	Grassland and arable land	NA	spore samples (trap culture)	spore identification		Soil type and land use intensity determine the composition of AMF community
Sandoz et al. 2020	Natural ecosystem	*Botrychium lunaria* (fern)	root samples	Illumina MiSeq 250	AMV4.5NF/AMDGR	Biotic and abiotic factors together shape AMF communitiy
Séry et al. 2018	Agricultural soils	Cassava	soil samples	Illumina MiSeq sequence	LR1/NDL22+ FLR3/FLR4	Rhizophagus is the most dominant core genus and soil type determine AMF community
Li et al. 2021	Agricultural soils	Pepper	soil samples+root samples	Illumina MiSeq 250	GeoA2/AML2+ NS31/AMDGR	Soil pH is main driver of AMF community
Valyi et al. 2015	Natural controled ecosystem	*Arrhenatherum elatius*, *Festuca pratensis*/ *Lolium perenne*/ *Lolium multiflorum* and *Poa pratensis*	soil samples+root samples	454 prosequencing	GlomWT0/Glomer1536 + NS31/AM1a+b	Land-use intensity and host plant identity shape AMF community
Varela-Cervero et al. 2015	Semi-arid ecosystem (nature)	*G. cinerea*(Vill.) DC. *L. latifolia* Medik. *Rosmarinus officinalis* L. *Thymus mastichina* L. *Thymus zygis* L	spore+mycelium+root samples	454 prosequencing	NS31/AML2	AMF composition is different among spore,mycelium and roots
Sarkodee-Addo et al. 2020	Agricultural soils	Rice	root samples	root samples	AMV4.5F/AMV4.5R+ AMV4.5NF/AMDGR	Soil properties are the key factors affecting AMF community/ Rhizophagus is the dominant geuns

Notes: NA denotes that the authors did not mention it; Blank indicates the authors did not use it.

Table S 2.2 The two study region including rainfall regime, temperature, vegetation and soil types.

	Konongo	**Bosome-Freho**
Rainfall regime	March-July September-November	April-July September-November
Temperature	25˚C	26˚C
Vegetation	Moist semi-deciduous forest	Semi-deciduous forest
Soil type	loam	Clay loam

Table S 2.3 Physical-chemical soil characteristics of each site.

	Available K mg/100g	Available P mg/100g	Ca mg/kg	K mg/kg	Mn mg/kg	P mg/kg	Zn mg/kg	SOC %	Clay %	Sand %	Silt %	Soil moisture %	N %	C %	C/N %	pH
KN-1	2.68	<2.5	851.70	615.71	319.04	162.48	41.57	0.53	23.33	32.32	44.34	80.20	0.05	0.71	13.27	6.12
KN-2	4.49	<2.5	1503.02	334.49	465.06	124.81	36.50	1.05	24.22	39.93	35.85	81.56	0.11	1.12	10.51	6.76
KN-3	4.62	<2.5	408.47	364.76	288.95	96.23	22.51	0.88	14.10	41.97	43.93	81.31	0.11	1.15	10.60	4.79
BF-1	6.53	<2.5	229.55	939.05	321.85	132.56	23.51	0.18	34.93	34.04	31.02	78.49	0.03	0.23	6.40	4.19
BF-1	15.68	<2.5	390.70	840.94	206.76	137.46	20.93	0.63	36.78	28.95	34.27	83.34	0.08	0.79	9.71	4.57

Note: KN-Konongo; BF-Bosome-Freho

Table S 2.4 The number of amplicon sequence variant (ASV) of arbuscular mycorrhizal fungi recovered from root, rhizosphere soil and shared between both root and rhizosphere soil.

ASV	Phylum	Class	Order	Family	Genus	Compartment
ASV1	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV2	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV3	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV5	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV7	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV8	Glomeromycota	Glomeromycetes	Glomerales	Claroideoglomeraceae	Claroideoglomus	Rhizosphere
ASV12	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV13	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV14	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV15	Glomeromycota	Glomeromycetes	Glomerales	Claroideoglomeraceae	Claroideoglomus	Root
ASV16	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV18	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV20	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV21	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV24	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV25	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV26	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV28	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV32	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Shared
ASV33	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV37	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV39	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV41	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV42	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV43	Glomeromycota	Glomeromycetes	Glomerales	Claroideoglomeraceae	Claroideoglomus	Rhizosphere
ASV44	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV45	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV47	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV48	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV51	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV54	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV56	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV57	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere

ASV58	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV60	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV61	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV62	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Shared
ASV63	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV64	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV65	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV66	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV67	Glomeromycota	Glomeromycetes	Diversisporales	Diversisporaceae	Diversispora	Root
ASV68	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV69	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV70	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV71	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV72	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV73	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV74	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV78	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV79	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV80	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV83	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV84	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV85	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV86	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV87	Glomeromycota	Glomeromycetes	Diversisporales	Diversisporaceae	Diversispora	Root
ASV88	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV89	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV90	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV91	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV93	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV97	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV99	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Shared
ASV100	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV101	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV103	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV105	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Shared
ASV110	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV114	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV115	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere

ASV116	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Septoglomus	Root
ASV117	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Funneliformis	Root
ASV118	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV119	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Funneliformis	Root
ASV123	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV124	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV127	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV128	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV129	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV131	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV132	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV133	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV134	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV135	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV136	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV137	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Funneliformis	Root
ASV139	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV140	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV141	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV142	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV146	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV147	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV149	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Shared
ASV150	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Funneliformis	Root
ASV151	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV152	Glomeromycota	Glomeromycetes	Glomerales	Claroideoglomeraceae	Claroideoglomus	Rhizosphere
ASV154	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV155	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV157	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV159	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV160	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV162	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV163	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV164	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV165	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV166	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV169	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV170	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared

ASV175	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV181	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Funneliformis	Root
ASV182	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV184	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV188	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV191	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV193	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV195	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV197	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV198	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Shared
ASV199	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV200	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV201	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV203	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV205	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV206	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV208	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV209	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV210	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV212	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV214	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV215	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Shared
ASV216	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV217	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV218	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV219	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV220	Glomeromycota	Glomeromycetes	Glomerales	Claroideoglomeraceae	Claroideoglomus	Rhizosphere
ASV221	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV225	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Shared
ASV227	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV228	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV229	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV230	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Shared
ASV233	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV235	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV236	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV237	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Funneliformis	Root
ASV238	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Shared

ASV240	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV243	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV244	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV246	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV247	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV248	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV249	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV252	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV253	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV255	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV257	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV258	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV259	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV260	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Septoglomus	Rhizosphere
ASV261	Glomeromycota	Glomeromycetes	Diversisporales	Diversisporaceae	Diversispora	Root
ASV262	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV263	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV264	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV265	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Funneliformis	Root
ASV267	Glomeromycota	Glomeromycetes	Glomerales	Claroideoglomeraceae	Claroideoglomus	Rhizosphere
ASV269	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV270	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV271	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Root
ASV274	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV275	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV276	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV278	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV279	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV280	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Dominikia	Rhizosphere
ASV281	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV283	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV284	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV285	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV286	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV289	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Shared
ASV290	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV291	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Rhizosphere
ASV293	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere

ASV294	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Septoglomus	Rhizosphere
ASV295	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Root
ASV296	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Shared
ASV297	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Rhizophagus	Root
ASV300	Glomeromycota	Paraglomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus	Rhizosphere
ASV301	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV302	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV303	Glomeromycota	Glomeromycetes	Glomerales	Claroideoglomeraceae	Claroideoglomus	Rhizosphere
ASV304	Glomeromycota	Glomeromycetes	Diversisporales	Acaulosporaceae	Acaulospora	Rhizosphere
ASV306	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Septoglomus	Rhizosphere

Table S 2.5 Overall information of sequences of each sample in different compartments. The number of raw reads, AMF reads and ASV are listed.

Sample ID	Location	Compartment	Raw reads	After quality filtered reads	After removing rare reads	AMF reads	ASV No.
Sample19	KN-1	Rhizosphere soil	74963	67903	52612	16690	16
Sample20	KN-1	Rhizosphere soil	77935	70801	59378	9404	16
Sample21	KN-1	Rhizosphere soil	78431	71445	60275	5879	8
Sample22	KN-2	Rhizosphere soil	86277	79050	63059	24543	8
Sample23	KN-2	Rhizosphere soil	80381	72592	57193	11454	8
Sample24	KN-2	Rhizosphere soil	80350	74121	58541	127	2
Sample25	KN-3	Rhizosphere soil	84434	77125	59676	8981	16
Sample26	KN-3	Rhizosphere soil	72164	65833	54681	1551	6
Sample27	BF-1	Rhizosphere soil	74641	66730	56823	333	3
Sample28	BF-1	Rhizosphere soil	90396	82193	62662	44103	27
Sample29	BF-1	Rhizosphere soil	87781	79665	63798	172	2
Sample33	BF-2	Rhizosphere soil	91033	83165	72999	61847	18
Sample34	BF-2	Rhizosphere soil	87934	80736	66243	60859	30
Sample35	BF-2	Rhizosphere soil	85501	78049	68231	55054	10
Sample47	KN-1	Root	84646	77581	61581	53901	11
Sample48	KN-1	Root	81592	75188	70078	41130	2
Sample49	KN-1	Root	85484	76497	66255	33976	4
Sample50	KN-2	Root	88646	7888	64671	38009	4
Sample51	KN-2	Root	90254	83893	81904	80461	8
Sample52	KN-2	Root	88244	78627	68567	53932	2
Sample53	KN-3	Root	74575	68585	47950	45289	19
Sample54	KN-3	Root	78349	71164	47271	13236	4
Sample55	BF-1	Root	87053	79340	61757	19047	10
Sample56	BF-1	Root	87173	79537	45691	42699	9
Sample57	BF-1	Root	75127	70473	69761	69080	4
Sample60	BF-2	Root	84821	77642	66756	65782	17
Sample61	BF-2	Root	67671	63220	62568	62372	6
Sample62	BF-2	Root	87071	80404	75165	74214	11
Total			**2312927**	**2039447**	**1746146**	**994125**	

Note: KN-Kononge; BF-Bosome-Freho

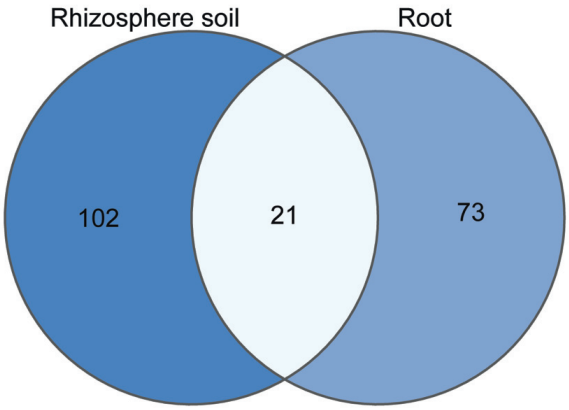

Figure S 2.1 Venn diagram showing overlap of amplicon sequence variants (ASV) between rhizosphere soil and root compartment. The numbers of ASV were annotated in the figure according to Table S 2.3.

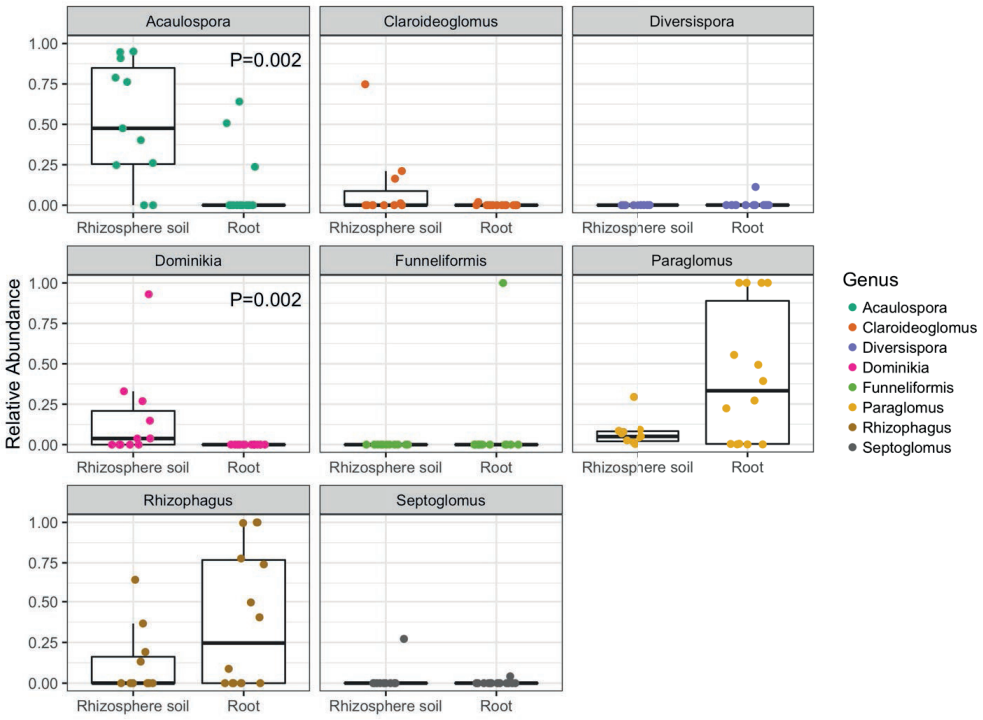

Figure S 2.2 The relative abundance of each genus in rhizosphere and root compartment with the significant differences between compartments. The relative abundance was calculated based on Figure 2.2A.

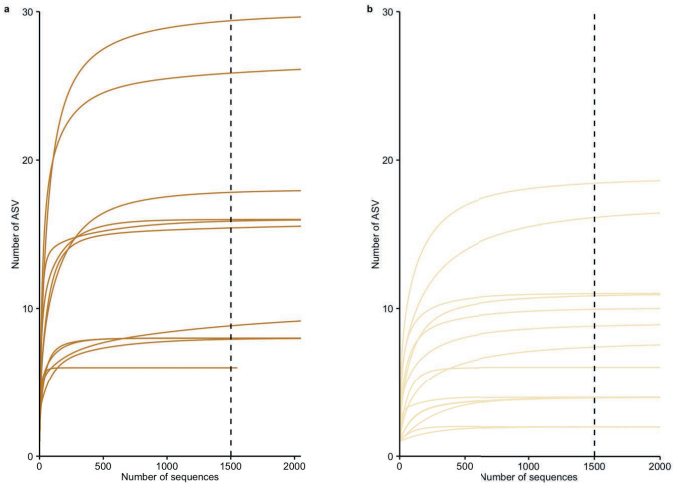

Figure S 2.3 Rarefaction curves revealing the observed amplicon sequence variants (ASV) in samples from different compartments: a) Rhizosphere soil, b) Root. The black line indicates the minimum sequence of each sample to calculate α-diversity.

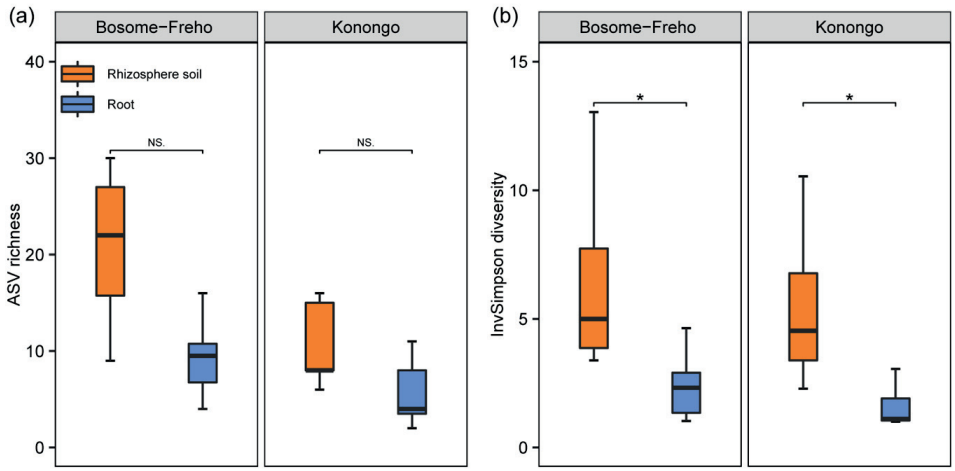

Figure S 2.4 (a) ASV richness (observed amplicon sequence variants (ASV)). (b) Inverse Simpson diversity. The boxes represent the range between 75th and 25th quartiles. The line within the box represents the median. The whiskers represent the lowest and highest values extending 1.5 of the interquartile range. NS indicates non-significance; * denotes significance ($P < 0.05$).

Table S 2.6 Pearson correlation of AMF community richness (based on observed richness) and diversity (based on Shannon diversity) with soil characteristics.

	Observed		**Shannon**	
	P	**R^2**	***P***	**R^2**
Nitrogen (%)	0.31	-0.2	0.5	-0.13
Carbon (%)	0.17	-0.27	0.32	-0.19
C/N	0.07	-0.35	0.23	-0.23
pH	0.01*	-0.51	0.01*	-0.48
Available K (mg/100g)	0.26	0.22	0.47	0.14
Ca (mg/kg)	0.01*	-0.51	0.01*	-0.51
K (mg/kg)	0.25	0.22	0.32	0.19
Mn (mg/kg)	0.12	-0.3	0.26	-0.22
P (mg/kg)	0.82	-0.05	0.67	-0.08
Zn (mg/kg)	0.02*	-0.44	0.06	-0.37
SOC (%)	0.09	-0.32	0.21	-0.25
Clay (%)	0.42	0.16	0.99	-0.01
Sand (%)	0.86	0.03	0.74	0.06
Silt (%)	0.36	-0.18	0.69	-0.08
Soil moisture (%)	0.21	0.24	0.41	0.16

Note: Significant level: $P < 0.05$

Table S 2.7 Effect of edaphic factors on AMF communities using Monte Carlo permutation test.

	Variance	*P*
Available K	0.04	0.61
Mn	0.04	0.61
P	0.03	0.81
Zn	0.05	0.61
Silt	0.04	0.63
SoilMoisture	0.04	0.61
pH	0.04	0.20
C	0.04	0.61
C/N	0.05	0.61

Table S 2.8 All statistical results of rhizosphere soil compartment based on network analysis in Gephi platform (v 0.9.2). (Due to limited space, please refer to online version).

Table S 2.9 All statistical results of root compartment based on network analysis in Gephi platform (v 0.9.2). (Due to limited space, please to online version).

3 Arbuscular mycorrhizal fungi-based bioremediation of mercury: insights from zinc and cadmium transporter studies*

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3.1 Abstract

Phytoremediation is a promising and sustainable technology in remediating mercury (Hg) contaminated soils. The efficiency of phytoremediation can be assisted by arbuscular mycorrhizal (AM) fungi, known to improve plant growth in contaminated soils and uptake of metals, including Hg. Despite having no biological function in plants, Hg can be absorbed by them due to its chemical similarity to other elements, and further transported through mechanisms that are related to other HM. Metal transporters represent the first line of metal uptake, translocation, and retention in plants. The exact transporters of Hg in plant roots remain, however, unknown. The uptake of Hg in plants may be facilitated by transporters of Zn and/or Cd, as they belong to the same group in periodic table (12/2B). The main objective of this synopsis is to provide fundamental insights into opportunities to remediate Hg-contaminated soils, with a specific focus on AM fungi. By analyzing recent studies on ecological roles of AM fungi in environments contaminated with Zn and Cd, it acknowledges that AM fungi can regulate Zn and Cd transporters, thereby affecting uptake and translocation of these HM in plants. The extent to which this regulation occurs is dependent on several factors, including the species of plant and AM fungi, and soil conditions, such as pH and elements like phosphorus. Furthermore, we compiled existing knowledge on Hg remediation, with a focus on current understanding and prospects of AM fungi-facilitated Hg uptake mechanisms. To conclude, future research directions in the discussed topic were given.

Keywords: Soil contamination, heavy metal, phytoremediation, molecular mechanisms, mercury uptake, ecosystem restoration, artisanal gold mining.

3.2 Introduction

3.2.1 Origin and consequence of mercury contamination

Environmental contamination by mercury (Hg) is a serious concern worldwide. Hg is a hazardous heavy metal (HM) that does not have any known biological function. It is toxic to both humans and other organisms, even at very low concentrations (Zahir et al. 2005). Studies have confirmed that soil contamination by Hg can lead to vegetation degradation, impact soil organisms, and ultimately result in a reduction or even loss of ecosystem functions (Gworek et al. 2020). Exposure to Hg in humans can result in gene mutations, cancer induction, and irreversible damage to the brain, lungs, and other organs in the body (Mitra et al. 2022).

Hg exists in various forms (e.g., Hg^0, Hg^{2+}, CH_3Hg^{+1}), and has low melting and boiling points (He et al. 2015). These characteristics pose significant challenges for managing this chemical element effectively. Hg is known for its capacity to transform from one form to another, which enables it to cycle through the atmosphere as well as terrestrial and aquatic ecosystem. Global Hg pollution occurs, especially in the form of Hg^0 which can be transported over long distances (Obrist et al. 2018; Pacyna 2020). The presence of Hg in the environment can be attributed to either natural deposition or anthropogenic dispersion resulting from various activities, such as manufacturing processes (e.g., production of sodium hydroxide), mining, and smelting (Driscoll et al. 2013). In quantitative terms, artisanal and small-scale gold mining (ASGM) is the primary source of Hg pollution on a global scale (UNEP 2019). This is because Hg is an essential element for amalgamating gold during the extraction process (Bugmann et al. 2022). Unfortunately, there are no practical alternatives to Hg in ASGM at present, and as a result, the use of Hg in ASGM continues to be widespread (Yevugah et al. 2021; Bugmann et al. 2022).

Soil is known to be the ultimate sink for Hg (Ahmad et al. 2021; Rashid et al. 2022). However, it is also the foundation for sustaining essential ecosystem services (Banerjee et al. 2023). Therefore, remediating soil contaminated with Hg is an urgent and critical task. Initial physical and chemical methods for remediating Hg-contaminated soil include complete removal of the contaminated soil or on-site treatments such as ion exchange, chemical precipitation, soil vapor extraction, and soil washing (He et al. 2015). Nevertheless, these techniques are frequently ineffective, costly, and may have detrimental effects on soil structure and productivity (Kuppusamy et al. 2017).

3.2.2 Principles of phytoremediation

Alternatively, phytoremediation, which involves the application of plants to remove or stabilize contaminants, is emerging as a cost-effective and environmentally friendly technology (He et al. 2015; Bhat et al. 2022). As it originates from nature, minimizes soil disturbance, and promotes ecosystem development, phytoremediation is becoming an increasingly studied approach (Fig. 3.1a). Three types of phytoremediation have been proposed for reclaiming Hg-contaminated soils: phytoextraction, phytostabilization, and phytovolatilization (Tiodar et al. 2021). In phytoextraction, Hg is taken up and accumulated in the aboveground plant parts of Hg hyperaccumulator plants, which can then be harvested for disposal or recovery. Phytostabilization involves the immobilization of Hg in plant roots, reducing its bioavailability in the soil and preventing further migration into groundwater or entry into the food chain. Phytovolatilization refers to the use of plants or genetically modified plants to convert Hg into its volatile form (Hg^0) and release it into the atmosphere. However, phytovolatilization may not be a sustainable solution, as the volatile form of Hg released into the atmosphere can be deposited back into the soil, resulting in a cycle of contamination (Vangronsveld et al. 2009; Kumar et al. 2017). Therefore, this method is not further considered in this discussion.

To date, no Hg hyperaccumulator plants have been identified, highlighting the need for complementary methods to improve the efficiency of Hg phytoremediation (Tiodar et al. 2021). In addition, the growth and survival of plants may be affected by Hg toxicity and nutrient depletion in contaminated areas, which can compromise the overall efficiency of phytoremediation. The use of plant-associated microorganisms is becoming increasingly popular to enhance plant performance under stress conditions, and thus improve the efficiency of phytoremediation. Among these microorganisms, arbuscular mycorrhizal (AM) fungi are of particular interest (Moura et al. 2022; Yang et al. 2022).

3.2.3 Role of arbuscular mycorrhizal (AM) fungi in phytoremediation

Under natural conditions, almost all vascular plants form intimate symbiosis with AM fungi, dated back to 450 million years ago (Martin et al. 2017). This co-evolution between plants and AM fungi highlights the strategic importance of their interactions for environmental adaptation (Wang et al. 2006; Strullu-Derrien et al. 2018). Numerous studies have indicated that AM fungi can facilitate the success of plant establishment in HM-polluted soils (Chen et al. 2007; Curaqueo et al. 2014; Zhan et al. 2018; Lu et al. 2020). In fact, some studies have suggested that the symbiosis between plants and AM fungi may be the most critical factor in achieving success in phytoremediation programs (Meier et al.

2011; Colombo et al. 2020; Moura et al. 2022). This is attributed to the well-known roles of AM fungi in nutrient uptake, such as phosphorus (P) and nitrogen (N), as well as water transfer beyond the root zone to plants. These functions help plants to develop resistance to various abiotic stresses, including drought, salinity, and HM toxicity, as well as biotic stresses caused by pathogens (Bücking et al. 2015). Furthermore, AM fungi are known to enhance soil structure by producing external hyphae and glomalin (Rillig et al. 2006), which can also help in the sequestration of metals from soil (Gil-Cardeza et al. 2014; Chen et al. 2018). However, the application of AM fungi in Hg-contaminated soils is not yet practical and requires a better understanding of the underlying genetic and physiological mechanisms. Nonetheless, the potential of AM fungi in phytoremediation of Hg makes it imperative to further explore and develop this approach.

Although there are several reviews available on the topic of Hg-phytoremediation assisted with AM fungi (Solís-Ramos et al. 2021; Tiodar et al. 2021; Chen et al. 2022; Sharma et al. 2022; Yu et al. 2022), there is a lack of empirical data in Hg research (Fig. 3.1c). Existing reviews provide a broad understanding of the potential of AM fungi in phytoremediation, including their ability to increase plant biomass, improve plant nutrient uptake, and enhance antioxidant activities (e.g. (Tiodar et al. 2021)). However, there is a critical lack of information on the specific molecular mechanisms involved. Understanding these mechanisms is thus crucial for the practical implementation of this technology (Yang et al. 2022).

3.2.4 Heavy metal (HM) uptake mechanisms by plants and AM fungi

The first step in HM uptake involves plant membrane transporters, followed by translocation and detoxification (Shi et al. 2019). Therefore, we emphasize exploring potential transporters and their functions in order to understand the molecular mechanisms of phytoremediation with AM fungi. Plants have evolved a vast array of genes involved in metal uptake and transport, ensuring an adequate supply of essential nutrients or metals required for vital biological processes (Tangahu et al. 2011). However, due to the structural similarity of HM with essential nutrients, they can enter the roots via similar nutrient transport pathways with the assistance of membrane transporter proteins (Manoj et al. 2020). Some examples of membrane transporters involved in metal uptake and transport include potassium (K^+) transporters, which are also involved in cesium (Cs^+) uptake (Boulois et al. 2006); silicon acid (Ma et al. 2008) and phosphate (Meharg et al. 2002) transporters facilitating arsenic (As^{3+}/As^{5+}) uptake; Zn transporters (especially *ZIP* (zinc-iron permease) (Lin et al. 2016; Zhang et al. 2017; Liu et al. 2019)), or channels of divalent cations like iron

(Fe^{2+}), copper (Cu^{2+}), calcium (Ca^{2+}) and magnesium (Mg^{2+}), facilitating cadmium (Cd^{2+}) uptake (Nakanishi et al. 2006; Song et al. 2017).

However, the specific mechanisms of Hg uptake by plants remain largely unknown, highlighting a significant knowledge gap (Fig. 3.1b, c). Along with Cd, Hg belongs to the same group of elements in the periodic table (12/2B) as Zn, and shares the same outer electronic configuration with Zn, suggesting the possibility of utilizing the same transporters for uptake. A recent study showed that root Hg concentration is negatively correlated with root Zn concentration (Guo et al. 2023). (Tiodar et al. 2021) suggested that transporters that facilitate the influx of Cd may also facilitate the uptake of Hg. Therefore, studying the mechanisms of the uptake of chemically related elements, such as Zn and Cd, which are also the most studied elements in phytoremediation (Yang et al. 2022), can provide important insights into Hg remediation mechanisms and facilitate the prospective application of Hg phytoremediation.

3.3 Synopsis structure

While it is widely accepted that Hg pollution and toxicity have significant adverse impacts on human and environmental health (Ha et al. 2017; Verma et al. 2023), research on Hg remediation remains limited (Fig. 3.1b, c). The main objective of this synopsis is to provide essential insights into the possibility of remediation of Hg-contaminated soils, with a specific focus on the role of AM fungi. We hypothesize that Hg uptake in plants may be facilitated by transporters that are involved in the uptake of chemically similar elements such as Zn and Cd, which are regulated by mycorrhization. To test this hypothesis, we conducted a comprehensive analysis of recent studies on the ecological role of AM fungi in environments contaminated with Zn and Cd, with a particular focus on the molecular mechanisms involved in metal uptake and partitioning. We also considered the effects of different P levels on metal uptake, as P plays a critical role in HM detoxification (Sharma et al. 2016; Mehmood et al. 2022). It also confers a major factor for mycorrhizal colonization, influencing the functioning of AM fungi (Bedini et al. 2018). Based on this information, we compiled existing knowledge on Hg remediation, with a particular focus on current understanding and prospects of AM fungi-facilitated Hg uptake mechanisms. We conclude this synopsis by outlining future research directions in the discussed topic.

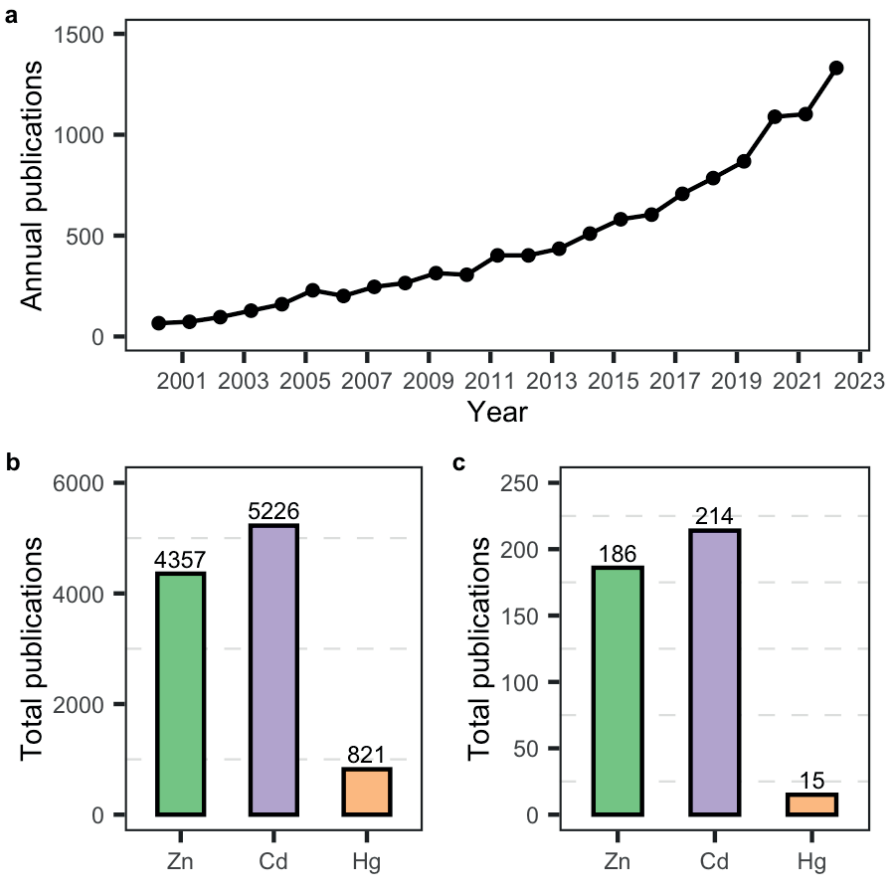

Figure 3.1 Overview of published studies. (a) The trend of the annual publications regarding phytoremediation from 2000 to 2022; (b) Total publications about phytoremediation concerning the three elements Zn (zinc), Cd (cadmium) and Hg (mercury); (c) Total publications emphasizing the three elements Zn, Cd and Hg, in which AM fungi are considered in phytoremediation. The literature search was performed using SCOPUS during January 2023, with the sequence of keywords as follows: phytoremediation OR (plant AND remediation) OR (plant AND bioremediation) AND metal AND soil for (a); phytoremediation OR (plant AND remediation) OR (plant AND bioremediation) AND Zinc (OR Zn) OR Cadmium (OR Cd) OR Mercury (OR Hg) AND soil for (b), and mycorrhiza was added to each search for (c). The titles, abstracts and keywords of the detected publications were checked. A threshold was set from 2000 to 2022.

3.4 Molecular mechanisms of AM fungi in uptake and partitioning of zinc and cadmium

3.4.1 Zinc (Zn)

Zinc (Zn) is an essential co-factor for numerous enzymes and plays an important structural role in many proteins. Although Zn is a highly reactive element, it becomes toxic at high concentrations. Several studies have reported that AM fungi can play dual roles in Zn fluxes in the soil-plant ecosystem. On the one hand, AM fungi can improve Zn uptake under Zn-limited conditions, and on the other hand, they can prevent excessive Zn accumulation in Zn-contaminated soils (Watts-Williams et al. 2013; Sarkar et al. 2017; Watts-Williams et al. 2017). This phenomenon may be attributed to the regulation of transporters (Ruytinx et al. 2020). Two transporter families, *ZIP* (zinc-iron permease or *ZRT-IRT*-like protein) and *CDF* (cation diffusion facilitator), are related to Zn homeostasis in eukaryotes. Interestingly, these two Zn transporter families work in opposite direction. *ZIP* transports Zn and/or other metal ion substrates from the extracellular space or organellar lumen into the cytoplasm, while *CDF* transports Zn and/or other metal ions from the cytoplasm into the lumen of intracellular organelles or to the outside of the cell (Ruytinx et al. 2020). *GinZnT1*, a transporter belonging to the *CDF* family, was firstly identified in *Glomus intraradices* in relation to Zn toxicity (González-Guerrero et al. 2005). This is the only gene characterized to date in AM fungi, which encodes a Zn transporter. With the advent of advanced sequencing technologies, several putative Zn transporter genes have been identified in *Rhizophagus irrgularis*, i.e., *ZRT1*, *YKE4*, *ZRT3.1* and *ZRT3.2* belonging to the *ZIP* family, and *ZnT1*, *ZnT2*, *MSC2* and *ZRG17* belonging to the *CDF* family (Tamayo et al. 2014). However, the functional characteristics of these genes have not been fully elucidated and require further research.

AM fungi are also known to regulate Zn transporters in plants. For instance, a previous study demonstrated that a mixed AM fungal inoculum can down-regulate the expression of the *MtZIP2* gene to prevent excessive Zn uptake by plants under increasing Zn fertilization (Burleigh et al. 2003). Watts-Williams et al. (2017) provided further evidence that suggests the potential involvement of *MtZIP2* genes in the detoxification of Zn stress with the help of *R. irrgularis*. In the same study, it was demonstrated that *R. irregularis* up-regulated the expression of *MtPT4*, a mycorrhizal-induced phosphate transporter, to maintain shoot P contents and biomass, resulting in reduced tissue Zn concentration and alleviated phytotoxicity under Zn stress. Another study reported that under Zn stress, *R. irrgularis* increased the biomass of *Medicago truncatula*, resulting in the dilution of Zn concentration in the plant (Nguyen et al. 2019). However, this benefit was only observed when the levels of available P in the soil were low. Hence, the hypothesis of

increased P uptake through the mycorrhizal pathway as a mechanism for plant tolerance to toxic soil Zn levels is proposed to be influenced by the availability of P in the soil.

3.4.2 Cadmium (Cd)

Cadmium (Cd) does not have any biological function in plants and is highly mobile and soluble in soil (Beesley et al. 2010). Studies have shown that Cd can be readily absorbed by plant roots through plasma membrane transporters of essential metals and transported to leaves through the xylem (Molina et al. 2020). Transporters, such as *ZIP* (*ZRT*, *IRT*-like protein), are crucial for the transport of Cd due to their structural similarity (Kaur et al. 2018). Additionally, studies have shown that natural resistance-associated macrophage proteins (*NRAMP*), which are primarily expressed in endodermal plasma membranes, are involved in loading Cd into the xylem (Takahashi et al. 2011a; Takahashi et al. 2011b). Furthermore, HM ATPase (*HMA*) plays a role in Cd translocation across the vacuolar membrane, which helps to mitigate Cd toxicity within plants (Sharma et al. 2016; Liu et al. 2017). As synergistic or antagonistic effects between Zn and Cd are possible, multiple studies have investigated the interplay between these two elements with respect to mycorrhizal colonization. Research has shown that *R. irrgularis* induces divergent patterns of transport and partitioning of Cd and Zn in *Populus trichocarpa* (De Oliveira et al. 2020). When exposed to excessive Zn, *R. irregularis* exhibited restricted transport of Cd to the shoots, resulting in higher Cd accumulation in the roots (78%) compared to the control (14%). Conversely, the translocation of Zn was promoted, leading to greater Zn accumulation in the shoots (60%) than in the roots (8%). This resulted from upregulation of *PtHAM4* genes in roots and *PtZIP1* genes in leaves induced by *R. irrgularis* (De Oliveira et al. 2020). Although recent studies confirmed that *R. irrgularis* exerted different mechanisms for Zn and Cd toxicity of *Phragmites australis*, the colonization of *R. irregularis* resulted in reduced shoot Zn concentration (10-57%) under Zn stress and increased shoot Cd concentration (17-40%) under Cd stress (You et al. 2021). These contrasting results suggest that both the plant species and the elements involved are crucial factors in determining the role of AM fungi in phytoremediation. In the same study, it was demonstrated that addition of Zn promoted the translocation of Cd to aboveground plant parts. These findings suggest that manipulating the Zn concentration in soil could enhance Cd translocation and lead to greater Cd accumulation in the shoots when colonized by AM fungi. This could be a crucial factor in the process of phytoextraction.

You et al. (2022) found that Cd uptake by *P. australis* is facilitated by different transporters, depending on the concentration of P in the substrate. Under low and medium P levels, *Funneliformis mosseae* upregulated the expression

of *ZIP* genes, which facilitated Cd uptake. However, at high P levels, *F. mosseae* upregulated the expression of *NRAMP* genes, resulting in increased Cd uptake. These results underscore the interference of soil P in Cd uptake by plants. Furthermore, the regulation of Cd transporters via AM fungi is dependent on the specific fungal species. For instance, rice inoculated with *R. intraradices* exhibited reduced expression of *NRAMP5* and *HMA3* genes in roots, which prevented Cd uptake. Conversely, the expression of these genes in roots was increased when inoculated with *F. mosseae* (Chen et al. 2019). Similarly, in *Solanum nigrum* inoculated with *R. intraradices*, there was a reduction in Cd concentration in shoots. In contrast, Cd translocation was increased when inoculated with *F. mosseae* (Li et al. 2018). (Motaharpoor et al. 2019) confirmed the downregulation of the *NRAMP1* gene in *M. sativa* when inoculated with *R. intraradices*, resulting in reduced Cd concentration in shoots.

3.5 AM fungi facilitated Hg remediation: knowledge and perspectives

Remediation of Hg-contaminated soil is an urgent and challenging task, as the molecular mechanisms involved in the transport of this element are not well understood. However, plants associated with AM fungi are being proposed as a sustainable solution to address this issue (Riaz et al. 2021; Tiodar et al. 2021). In fact, several studies have reported that mycorrhizal inoculation can enhance the resistance of plant seedlings to Hg, such as *Lactuca sativa* (Vargas Aguirre et al. 2018; Escobar-Vargas et al. 2022) and *Nauclea orientalis* (Ekamawanti et al. 2014). However, the underlying mechanisms remain unexplored. In a greenhouse pot experiment, *L. sativa* plants associated with AM fungi (a commercial inoculum containing *R. irregulris* and *F. mosseae*) exhibited better Hg tolerance compared to the control group (Cozzolino et al. 2016). The authors demonstrated that mycorrhizal inoculation improved the nutritional status of the plants, increased pigment content in plant leaves, and inhibited both the uptake and translocation of Hg from roots to shoots. Another study reported that a mixture of AM fungi (*Glomus* sp., *Acaulospora* sp., *Entrophospora* sp., *Giaspoora* sp.) conferred Hg tolerance to plants (*Lolium perenne*) by upregulating the expression of glutathione-S-transferase (*GST*) genes, which encode detoxification enzymes (Leudo et al. 2020). Yu et al. (2010) demonstrated that the AM fungus *F. mosseae* increased Hg sorption by the soil and reduced Hg bioavailability, ultimately limiting the uptake of Hg by plant roots. In contrast, *Zea mays* inoculated with *Glomus* sp. can uptake and transport Hg from the soil into the plant roots more efficiently compared to non-inoculated plants (Kodre et al. 2017). The authors further demonstrated that the increase in Hg uptake was due to a change in the ligand structure from di-thiolate (2S) to tetra-thiolate (4S) caused by AM fungal inoculation. Kodre et al. (2017) also demonstrated that native AM fungal species

from polluted environments outperformed commercial ones. Furthermore, the Hg accumulation of *Erato polymnioides*, a potential Hg hyperaccumulator plant, has been shown to be positively correlated with mycorrhizal colonization, emphasizing the importance of AM fungi in Hg remediation (Chamba et al. 2017). Recently, Li et al. (2023) investigated the co-existence of Hg and Cd, and demonstrated that the effects of Cd on Hg accumulation and transformation by *Arundo donax* followed the rule of 'low promotion and high inhibition'. However, AM fungi were not considered in this context. Another recent study demonstrated that AM fungi can enhance the accumulation of both Cd and Hg in *L. perenne* (Saldarriaga et al. 2023). These studies suggest a growing recognition of other metals such as Cd in Hg remediation.

Another mechanism that contributes to the increased uptake and accumulation of Hg is attributed to the improved nutrient status induced by AM fungi inoculation (Debeljak et al. 2018). It is widely recognized that the primary role of AM fungi on plants is to enhance mineral nutrient uptake, particularly phosphate (Karandashov et al. 2005). It has been demonstrated that phosphate is primarily transported in the form of polyphosphate via mycorrhizal hyphae (Wang et al. 2017). Coincidentally, transgenic tobacco plants engineered to express bacterial polyphosphate (polyP) exhibited higher Hg accumulation than wild-type tobacco (Nagata et al. 2006). This finding suggests that increasing the plant's polyphosphate content could be one way to enhance plant Hg accumulation, but it is unclear whether and to what extent this process is facilitated by AM fungi. Interestingly, Hg transformation and uptake can be enhanced by microorganisms under P-limiting conditions, as demonstrated in a study by (Živković et al. 2019). Given the primary role of AM fungi in facilitating phosphate transport as polyphosphates, harnessing this function could be a crucial aspect of Hg bio-augmented phytoremediation.

The bioavailability of Hg for plants is relatively low, which has led to the introduction of many chemical amendments aimed at improving the efficiency of Hg uptake (Liu et al. 2020). **T**hiol containing ligands, such as ammonium thiosulphate ($(NH_4)_2S_2O_3$) and sodium thiosulphate ($Na_2S_2O_3$), are a common choice among the many chemical amendments introduced to improve the efficiency of Hg uptake by plants (Moreno et al. 2005; Wang et al. 2011; Makarova et al. 2021). This is because Hg has a strong affinity for thiol containing ligands (Moreno et al. 2005). However, the application of such amendments in natural environments can pose a significant risk of transferring Hg to local ecosystems through enhanced leaching (Smolińska et al. 2012; Smolińska 2020). Moreover, thiosulfate has been shown to cause physiological damage to the cell membranes of roots, leading to increased plasma membrane

permeability and enhanced Hg uptake (Wang et al. 2018). Consequently, this process may induce the leakage of other ions (e.g., K), which might threaten plant performance or even plant survival. Thus, more sustainable strategies, namely the ability of AM fungi to facilitate the ligand environment for Hg uptake, should be targeted.

3.6 Conclusion and future directions of research

The observed benefits of AM fungi in Hg remediation, including enhanced plant Hg tolerance, improved Hg ligand environment, and the role of polyphosphate transfer, highlight the importance of further research into the plant-AM fungi symbiosis as a promising area of study for Hg-bioaugmented phytoremediation. As observed with Zn and Cd, AM fungi can modulate the expression of transporters, thereby enhancing or inhibiting metal uptake and translocation (Table 3.1). Consequently, understanding the underlying mechanisms of the corresponding transporters is crucial for optimizing the use of AM fungi in phytoremediation (Yang et al. 2022). Further studies should focus on identifying the relevant membrane transporters that are responsible for facilitating Hg uptake by plant roots. However, the precise transporters involved in root Hg uptake have not yet been identified. It is plausible that Zn and/or Cd transporters could play a role in Hg uptake due to their chemical similarities. Accordingly, insights from the knowledge of Zn and Cd transporters could provide a foundation for understanding Hg uptake. Additionally, other factors such as plant species, soil conditions (e.g., pH), other elements (e.g., P), and AM fungal species can also influence metal uptake and transport. Thus, future research should investigate the optimal environmental conditions under which AM fungi are effective in Hg remediation for their appropriate application.

Table 3.1 Key findings and regulations of transporters involved in the uptake of the three elements Zn (zinc), Cd (cadmium) and mercury (Hg), as influenced by mycorrhizal colonization.

Elements Concentration	AM fungal species[a]	Plant species	Key results	Mechanisms[b]	Experimental conditions	Reference
Zn						
0, 75 and 7500 µM	*Rhizophagus irregularis* (WFVAM10)	*Daucus carota*	Improved Zn stress	*GinZnT1*(CDF) ↑	*In vitro*	(González-Guerrero et al. 2005)
100 mg kg^{-1}	*Glomus versiforme* (BEG47), *Glomus intraradices* (BEG87), *Glomus caledonium* (BEG15), *Glomus claroideum* (BEG14), *Glomus mosseae*, *Scutellospora calospora*, *Gigaspora rosea* (BEG9)	*Medicago truncatula* cv. Jemalong A17	Improved Zn tolerance	*MtZIP2* ↓	Growth chamber	(Burleigh et al. 2003)
5, 20 ,40 mg kg^{-1}	*Rhizophagus irregularis* (WFVAM10)	*Medicago truncatula cv. Jemalong* A17	Improved Zn tolerance	*MtZIP2* (ZIP) ↓ *MtPT4* ↑	Glasshouse	(Watts-Williams et al. 2017)
2, 100, 300, 500, 700 mg L^{-1}	*Rhizophagus irregularis* (FR717169)	*Phragmites australis*	Reduced Zn concentration (10-57%) Alleviates Zn toxic effects	Plant biomass ↑	Greenhouse	(You et al. 2021)
0.3, 4.0, 5.8, 15.0 mg kg^{-1}	*Rhizophagus irregularis* WFVAM10	*Medicago truncatula cv. Jemalong* A17	A 'protective' role of mycorrhizal fungi at high levels of soil Zn	*MtZIP5* expression was induced both by AMF and soil Zn deficiency, *MtZIP2* was down-regulated in mycorrhizal plants, and up-regulated with increasing soil Zn concentration	Glasshouse	(Nguyen et al. 2019)

Table 3.1 *Continued*

Cd						
0.01, 1, 5, 10, 20 mg L^{-1}	*Rhizophagus irregularis* (FR717169)	*Phragmites australis*	increased Cd concentration (17-40%) Alleviates Cd toxic effects	Plant biomass ↑	Greenhouse	(You et al. 2021)
10 mg L^{-1}	*Rhizophagus irregularis* (FR717169)	*Phragmites australis*	Increased Cd uptake and retained Cd in stems	*ZnT2* (*ZIP*) ↑ *Nramp2*, *Nramp5* ↑	Greenhouse	(You et al. 2022)
0, 10 µM	*Funneliformis mosseae*	*Oryza sativa*	Higher root Cd content	*Nramp5* ↑ *HMA3* ↑	Greenhouse	(Chen et al. 2019)
	Rhizophagus intraradices		Lower root Cd content	*Nramp5* ↓ *HMA3* ↓		
14.8 mg kg^{-1}	*Funneliformis mosseae*	*Solanum nigrum*	Increased Cd translocation		Greenhouse	(Li et al. 2018)
	Rhizophagus intraradices		Reduced Cd translocation			
100 mg kg^{-1}	*Rhizophagus irregularis*	*Medicago sativa*	Increased plant biomass Reduced shoot Cd concentration	*MsIRT1* (ZIP) ↓ *MsNRAMP1* ↓	Greenhouse	(Motaharpoor et al. 2019)
3.06 (± 0.12) mg kg^{-1}	a mixture of spores of the genera *Glomus* sp., *Acaulospora* sp., *Entrophospora* sp., and *Giaspora* sp.	*Lolium perenne*	Increased accumulation of Cd		Rhizobox	(Saldarriaga et al. 2023)

Table 3.1 *Continued*

Hg						
1, 2, 4 mg kg^{-1}	*Glomus mosseae*	*Zea mays*	Increased Hg soil sorption; Reduction of bioavailability, and root uptake		Glasshouse	(Yu et al. 2010)
0, 375, 750 µM	Mycorrhizal inoculum	*Nauclea orientalis*	Improved Hg tolerance		Greenhouse	(Ekamawanti et al. 2014)
10 mg kg^{-1}	A commercial arbuscular mycorrhizal formula, containing *Rhisophagus irregularis* and *Funneliformis mosseae*	*Lactuca sativa*	Reduced Hg root uptake and translocation	plant growth ↑ P uptake ↑	Greenhouse	(Cozzolino et al. 2016)
0, 100 mg kg^{-1}	Soil mycorrhiza	*Lactuca sativa*	Improved Hg tolerance	Enhanced root elongation and seedling development	Greenhouse	(Vargas Aguirre et al. 2018)
1, 10, 100, 1000 mg kg^{-1}	Commercial mycorrhiza	*Lactuca sativa*	Improved Hg tolerance	Improved plant establishment	Greenhouse	(Escobar-Vargas et al. 2022)
50 mg kg^{-1}	*Glomus sp*.	*Zea mays*	Increased root Hg concentration and content	Change ligand from 2S to 4S complexes	Greenhouse	(Kodre et al. 2017)
50 mg kg^{-1}	*Glomus sp*.	*Zea mays*	Increased Hg uptake into the roots Increased Hg transfer to shoots	Imporve nutrient status	Greenhouse	(Debeljak et al. 2018)
3.12 (± 0.09) mg kg^{-1}	A mixture of spores of the genera *Glomus* sp., *Acaulospora* sp., *Entrophospora* sp., and *Giaspora* sp.	*Lolium perenne*	increase the accumulation of Hg	Improve rhizosphere microbiome	Rhizobox	(Saldarriaga et al. 2023)
25 mg kg^{-1}	*Rhizophagus irregularis* (QS81)	*Medicago truncatula* cv. Jemalong A17	Reduced the Hg translocation to shoots		Greenhouse	(Guo et al. 2023)
50 mg kg^{-1}			Increased the Hg translocation to shoots Reduced the Hg uptake into root		Greenhouse	

3.7 References

Ahmad W, Alharthy R D, Zubair M, Ahmed M, Hameed A, Rafique S (2021) Toxic and heavy metals contamination assessment in soil and water to evaluate human health risk. Scientific reports 11:17006. https://doi.org/10.1038/s41598-021-94616-4

Banerjee S, van der Heijden M G A (2023) Soil microbiomes and one health. Nature Reviews Microbiology 21:6-20. https://doi.org/10.1038/s41579-022-00779-w

Bedini A, Mercy L, Schneider C, Franken P, Lucic-Mercy E (2018) Unraveling the Initial Plant Hormone Signaling, Metabolic Mechanisms and Plant Defense Triggering the Endomycorrhizal Symbiosis Behavior. Frontiers in plant science 9:1800. https://doi.org/10.3389/fpls.2018.01800

Beesley L, Moreno-Jiménez E, Clemente R, Lepp N, Dickinson N (2010) Mobility of arsenic, cadmium and zinc in a multi-element contaminated soil profile assessed by in-situ soil pore water sampling, column leaching and sequential extraction. Environmental Pollution 158:155-160. https://doi.org/10.1016/j.envpol.2009.07.021

Bhat S A, Bashir O, Ul Haq S A, Amin T, Rafiq A, Ali M, Américo-Pinheiro J H P, Sher F (2022) Phytoremediation of heavy metals in soil and water: An eco-friendly, sustainable and multidisciplinary approach. Chemosphere 303:134788. https://doi.org/10.1016/j.chemosphere.2022.134788

Boulois H D d, Voets L, Delvaux B, Jakobsen I, Declerck S (2006) Transport of radiocaesium by arbuscular mycorrhizal fungi to Medicago truncatula under in vitro conditions. Environmental Microbiology 8:1926-1934. https://doi.org/10.1111/j.1462-2920.2006.01070.x

Bugmann A, Brugger F, Zongo T, van der Merwe A (2022) “Doing ASGM without mercury is like trying to make omelets without eggs”. Understanding the persistence of mercury use among artisanal gold miners in Burkina Faso. Environmental Science & Policy 133:87-97. https://doi.org/10.1016/j.envsci.2022.03.009

Burleigh S H, Kristensen B K, Bechmann I E (2003) A plasma membrane zinc transporter from Medicago truncatula is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol Biol 52:1077-1088. https://doi.org/10.1023/A:1025479701246

Chamba I, Rosado D, Kalinhoff C, Thangaswamy S, Sánchez-Rodríguez A, Gazquez M J (2017) Erato polymnioides – A novel Hg hyperaccumulator plant in ecuadorian rainforest acid soils with potential of microbe-associated phytoremediation. Chemosphere 188:633-641. https://doi.org/10.1016/j.chemosphere.2017.08.160

Chen B, Nayuki K, Kuga Y, Zhang X, Wu S, Ohtomo R (2018) Uptake and Intraradical Immobilization of Cadmium by Arbuscular Mycorrhizal Fungi as Revealed by a Stable Isotope Tracer and Synchrotron Radiation μX-Ray Fluorescence Analysis. Microbes Environ 33:257-263. https://doi.org/10.1264/jsme2.ME18010

Chen B D, Zhu Y G, Duan J, Xiao X Y, Smith S E (2007) Effects of the arbuscular mycorrhizal fungus Glomus mosseae on growth and metal uptake by four plant species in copper mine tailings. Environmental Pollution 147:374-380. https://doi.org/10.1016/j.envpol.2006.04.027

Chen L, Beiyuan J, Hu W, Zhang Z, Duan C, Cui Q, Zhu X, He H, Huang X, Fang L (2022) Phytoremediation of potentially toxic elements (PTEs) contaminated soils using alfalfa (Medicago sativa L.): A comprehensive review. Chemosphere 293:133577. https://doi.org/10.1016/j.chemosphere.2022.133577

Chen X W, Wu L, Luo N, Mo C H, Wong M H, Li H (2019) Arbuscular mycorrhizal fungi and the associated bacterial community influence the uptake of cadmium in rice. Geoderma 337:749-757. https://doi.org/10.1016/j.geoderma.2018.10.029

Colombo R P, Benavidez M E, Fernandez Bidondo L, Silvani V A, Bompadre M J, Statello M, Scorza M V, Scotti A, Godeas A M (2020) Arbuscular mycorrhizal fungi in heavy metal highly polluted soil in the Riachuelo river basin. Revista Argentina de Microbiologia 52:145-149. https://doi.org/10.1016/j.ram.2019.05.001

Cozzolino V, De Martino A, Nebbioso A, Di Meo V, Salluzzo A, Piccolo A (2016) Plant tolerance to mercury in a contaminated soil is enhanced by the combined effects of humic matter addition and inoculation with arbuscular mycorrhizal fungi. Environmental Science and Pollution Research 23:11312-11322. https://doi.org/10.1007/s11356-016-6337-6

Curaqueo G, Schoebitz M, Borie F, Caravaca F, Roldán A (2014) Inoculation with arbuscular mycorrhizal fungi and addition of composted olive-mill waste enhance plant establishment and soil properties in the regeneration of a heavy metal-polluted environment. Environmental Science and Pollution Research 21:7403-7412. https://doi.org/10.1007/s11356-014-2696-z

De Oliveira V H, Ullah I, Dunwell J M, Tibbett M (2020) Mycorrhizal symbiosis induces divergent patterns of transport and partitioning of Cd and Zn in Populus trichocarpa. Environmental and Experimental Botany 171:103925. https://doi.org/10.1016/j.envexpbot.2019.103925

Debeljak M, van Elteren J T, Špruk A, Izmer A, Vanhaecke F, Vogel-Mikuš K (2018) The role of arbuscular mycorrhiza in mercury and mineral nutrient uptake in maize. Chemosphere 212:1076-1084. https://doi.org/10.1016/j.chemosphere.2018.08.147

Driscoll C T, Mason R P, Chan H M, Jacob D J, Pirrone N (2013) Mercury as a Global Pollutant: Sources, Pathways, and Effects. Environmental Science & Technology 47:4967-4983. https://doi.org/10.1021/es305071v

Ekamawanti H A, Setiadi Y, Sopandie D, Santosa D A (2014) Mercury stress resistances in Nauclea orientalis seedlings inoculated with arbuscular mycorrhizal fungi. Agriculture Forestry and Fisheries 3:113-120. https://doi.org/10.11648/j.aff.20140302.20

Escobar-Vargas S, Vargas Aguirre C F, Rivera Páez F A (2022) Arbuscular mycorrhizal fungi prevent mercury toxicity in Lactuca sativa (L.) seed germination. Pollution 8:1014-1025. https://doi.org/10.22059/poll.2022.337840.1338

Gil-Cardeza M L, Ferri A, Cornejo P, Gomez E (2014) Distribution of chromium species in a Cr-polluted soil: presence of Cr(III) in glomalin related protein fraction. Science of the Total Environment 493:828-833. https://doi.org/10.1016/j.scitotenv.2014.06.080

González-Guerrero M, Azcón-Aguilar C, Mooney M, Valderas A, MacDiarmid C W, Eide D J, Ferrol N (2005) Characterization of a Glomus intraradices gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genetics and Biology 42:130-140. https://doi.org/10.1016/j.fgb.2004.10.007

Guo Y, Sommer N, Martin K, Rasche F (2023) Rhizophagus irregularis improves Hg tolerance of Medicago truncatula by upregulating the Zn transporter genes ZIP2 and ZIP6. Mycorrhiza https://doi.org/10.1007/s00572-022-01100-6

Gworek B, Dmuchowski W, Baczewska-Dąbrowska A H (2020) Mercury in the terrestrial environment: a review. Environmental Sciences Europe 32:128. https://doi.org/10.1186/s12302-020-00401-x

Ha E, Basu N, Bose-O'Reilly S, Dórea J G, McSorley E, Sakamoto M, Chan H M (2017) Current progress on understanding the impact of mercury on human health. Environmental Research 152:419-433. https://doi.org/10.1016/j.envres.2016.06.042

He F, Gao J, Pierce E, Strong P J, Wang H, Liang L (2015) In situ remediation technologies for mercury-contaminated soil. Environmental Science and Pollution Research 22:8124-8147. https://doi.org/10.1007/s11356-015-4316-y

Karandashov V, Bucher M (2005) Symbiotic phosphate transport in arbuscular mycorrhizas. Trends in Plant Science 10:22-29. https://doi.org/10.1016/j.tplants.2004.12.003

Kaur H, Garg N (2018) Recent Perspectives on Cross Talk Between Cadmium, Zinc, and Arbuscular Mycorrhizal Fungi in Plants. Journal of Plant Growth Regulation 37:680-693. https://doi.org/10.1007/s00344-017-9750-2

Kodre A, Arčon I, Debeljak M, Potisek M, Likar M, Vogel-Mikuš K (2017) Arbuscular mycorrhizal fungi alter Hg root uptake and ligand environment as studied by X-ray absorption fine structure. Environmental and Experimental Botany 133:12-23. https://doi.org/10.1016/j.envexpbot.2016.09.006

Kumar B, Smita K, Cumbal Flores L (2017) Plant mediated detoxification of mercury and lead. Arabian Journal of Chemistry 10:S2335-S2342. https://doi.org/10.1016/j.arabjc.2013.08.010

Kuppusamy S, Thavamani P, Venkateswarlu K, Lee Y B, Naidu R, Megharaj M (2017) Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. Chemosphere 168:944-968. https://doi.org/10.1016/j.chemosphere.2016.10.115

Li H, Li X, Xiang L, Zhao H M, Li Y W, Cai Q Y, Zhu L, Mo C H, Wong M H (2018) Phytoremediation of soil co-contaminated with Cd and BDE-209 using hyperaccumulator enhanced by AM fungi and surfactant. Science of the Total Environment 613-614:447-455. https://doi.org/10.1016/j.scitotenv.2017.09.066

Li X, Zhao L, Teng Y, Luo Y, Zhao Q (2023) Effects of cadmium on mercury accumulation and transformation by Arundo donax L. Environmental Science and Pollution Research https://doi.org/10.1007/s11356-023-26516-1

Lin Y-F, Hassan Z, Talukdar S, Schat H, Aarts M G M (2016) Expression of the ZNT1 Zinc Transporter from the Metal Hyperaccumulator Noccaea caerulescens Confers Enhanced Zinc and Cadmium Tolerance and Accumulation to Arabidopsis thaliana. PLOS ONE 11:e0149750. https://doi.org/10.1371/journal.pone.0149750

Liu H, Zhao H, Wu L, Liu A, Zhao F-J, Xu W (2017) Heavy metal ATPase 3 (HMA3) confers cadmium hypertolerance on the cadmium/zinc hyperaccumulator Sedum plumbizincicola. New Phytologist 215:687-698. https://doi.org/10.1111/nph.14622

Liu X S, Feng S J, Zhang B Q, Wang M Q, Cao H W, Rono J K, Chen X, Yang Z M (2019) OsZIP1 functions as a metal efflux transporter limiting excess zinc, copper and cadmium accumulation in rice. BMC Plant Biology 19:283. https://doi.org/10.1186/s12870-019-1899-3

Liu Z, Chen B, Wang L-a, Urbanovich O, Nagorskaya L, Li X, Tang L (2020) A review on phytoremediation of mercury contaminated soils. Journal of Hazardous Materials 400:123138. https://doi.org/10.1016/j.jhazmat.2020.123138

Lu R-R, Hu Z-H, Zhang Q-L, Li Y-Q, Lin M, Wang X-L, Wu X-N, et al. (2020) The effect of Funneliformis mosseae on the plant growth, Cd translocation and accumulation in the new Cd-hyperaccumulator Sphagneticola calendulacea. Ecotoxicology and Environmental Safety 203:110988. https://doi.org/10.1016/j.ecoenv.2020.110988

Ma J F, Yamaji N, Mitani N, Xu X Y, Su Y H, McGrath S P, Zhao F J (2008) Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Natl Acad Sci U S A 105:9931-9935. https://doi.org/10.1073/pnas.0802361105

Makarova A, Nikulina E, Tsirulnikova N, Avdeenkova T, Pishchaeva K V (2021) Potential of S-containing and P-containing complexones in improving phytoextraction of mercury by Trifolium repens L. Saudi Journal of Biological Sciences 28:3037-3048. https://doi.org/10.1016/j.sjbs.2021.02.045

Manoj S R, Karthik C, Kadirvelu K, Arulselvi P I, Shanmugasundaram T, Bruno B, Rajkumar M (2020) Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. Journal of Environmental Management 254:109779. https://doi.org/10.1016/j.jenvman.2019.109779

Martin F M, Uroz S, Barker D G (2017) Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. Science 356:eaad4501. https://doi.org/10.1126/science.aad4501

Meharg A A, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytologist 154:29-43. https://doi.org/10.1046/j.1469-8137.2002.00363.x

Mehmood T, Liu C, Bibi I, Ejaz M, Ashraf A, Haider F U, Riaz U, et al. (2022) Chapter 13 - Recent developments in phosphate-assisted phytoremediation of potentially toxic metal(loid)s-contaminated soils. Assisted Phytoremediation. Elsevier, 345-370. https://doi.org/10.1016/B978-0-12-822893-7.00014-8

Meier S, Azcón R, Cartes P, Borie F, Cornejo P (2011) Alleviation of Cu toxicity in Oenothera picensis by copper-adapted arbuscular mycorrhizal fungi and treated agrowaste residue. Applied Soil Ecology 48:117-124. https://doi.org/10.1016/j.apsoil.2011.04.005

Mitra S, Chakraborty A J, Tareq A M, Emran T B, Nainu F, Khusro A, Idris A M, et al. (2022) Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science 34:101865. https://doi.org/10.1016/j.jksus.2022.101865

Molina A S, Lugo M A, Pérez Chaca M V, Vargas-Gil S, Zirulnik F, Leporati J, Ferrol N, Azcón-Aguilar C (2020) Effect of Arbuscular Mycorrhizal Colonization on Cadmium-Mediated Oxidative Stress in Glycine max (L.) Merr. Plants 9:108. https://doi.org/10.3390/plants9010108

Moreno F N, Anderson C W N, Stewart R B, Robinson B H, Ghomshei M, Meech J A (2005) Induced plant uptake and transport of mercury in the presence of sulphur-containing ligands and humic acid. New Phytologist 166:445-454. https://doi.org/10.1111/j.1469-8137.2005.01361.x

Moreno F N, Anderson C W N, Stewart R B, Robinson B H, Nomura R, Ghomshei M, Meech J A (2005) Effect of Thioligands on Plant-Hg Accumulation and Volatilisation from Mercury-contaminated Mine Tailings. Plant and Soil 275:233. https://doi.org/10.1007/s11104-005-1755-0

Motaharpoor Z, Taheri H, Nadian H (2019) Rhizophagus irregularis modulates cadmium uptake, metal transporter, and chelator gene expression in Medicago sativa. Mycorrhiza 29:389-395. https://doi.org/10.1007/s00572-019-00900-7

Moura M A d, Oki Y, Arantes-Garcia L, Cornelissen T, Nunes Y R F, Fernandes G W (2022) Mycorrhiza fungi application as a successful tool for worldwide mine land restoration: Current state of knowledge and the way forward. Ecological Engineering 178:106580. https://doi.org/10.1016/j.ecoleng.2022.106580

Nagata T, Ishikawa C, Kiyono M, Pan-Hou H (2006) Accumulation of Mercury in Transgenic Tobacco Expressing Bacterial Polyphosphate. Biological and Pharmaceutical Bulletin 29:2350-2353. https://doi.org/10.1248/bpb.29.2350

Nakanishi H, Ogawa I, Ishimaru Y, Mori S, Nishizawa N K (2006) Iron deficiency enhances cadmium uptake and translocation mediated by the Fe2+ transporters OsIRT1 and OsIRT2 in rice. Soil Science and Plant Nutrition 52:464-469. https://doi.org/10.1111/j.1747-0765.2006.00055.x

Nguyen T D, Cavagnaro T R, Watts-Williams S J (2019) The effects of soil phosphorus and zinc availability on plant responses to mycorrhizal fungi: a physiological and molecular assessment. Scientific reports 9:14880. https://doi.org/10.1038/s41598-019-51369-5

Obrist D, Kirk J L, Zhang L, Sunderland E M, Jiskra M, Selin N E (2018) A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. Ambio 47:116-140. https://doi.org/10.1007/s13280-017-1004-9

Pacyna J M (2020) Recent advances in mercury research. Science of the Total Environment 738:139955. https://doi.org/10.1016/j.scitotenv.2020.139955

Rashid S, Shah I A, Supe Tulcan R X, Rashid W, Sillanpaa M (2022) Contamination, exposure, and health risk assessment of Hg in Pakistan: A review. Environmental Pollution 301:118995. https://doi.org/10.1016/j.envpol.2022.118995

Riaz M, Kamran M, Fang Y, Wang Q, Cao H, Yang G, Deng L, et al. (2021) Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. Journal of Hazardous Materials 402:123919. https://doi.org/10.1016/j.jhazmat.2020.123919

Rillig M C, Mummey D L (2006) Mycorrhizas and soil structure. New Phytologist 171:41-53. https://doi.org/10.1111/j.1469-8137.2006.01750.x

Ruytinx J, Kafle A, Usman M, Coninx L, Zimmermann S D, Garcia K (2020) Micronutrient transport in mycorrhizal symbiosis; zinc steals the show. Fungal Biology Reviews 34:1-9. https://doi.org/10.1016/j.fbr.2019.09.001

Saldarriaga J F, López J E, Díaz-García L, Montoya-Ruiz C (2023) Changes in Lolium perenne L. rhizosphere microbiome during phytoremediation of Cd- and Hg-contaminated soils. Environmental Science and Pollution Research https://doi.org/10.1007/s11356-023-25501-y

Sarkar A, Asaeda T, Wang Q, Kaneko Y, Rashid M H (2017) Response of Miscanthus sacchariflorus to zinc stress mediated by arbuscular mycorrhizal fungi. Flora 234:60-68. https://doi.org/10.1016/j.flora.2017.05.011

Sharma P, Parakh S K, Singh S P, Parra-Saldívar R, Kime S-H, Varjani S, Tong Y W (2022) A critical review on microbes-based treatment strategies for mitigation of toxic pollutants. Science of the Total Environment 834:155444. http://dx.doi.org/10.1016/j.scitotenv.2022.155444

Sharma S S, Dietz K-J, Mimura T (2016) Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. Plant, Cell & Environment 39:1112-1126. https://doi.org/10.1111/pce.12706

Shi W, Zhang Y, Chen S, Polle A, Rennenberg H, Luo Z-B (2019) Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. Plant, Cell & Environment 42:1087-1103. https://doi.org/10.1111/pce.13471

Smolińska B (2020) The influence of compost and nitrilotriacetic acid on mercury phytoextraction by Lepidium sativum L. Journal of Chemical Technology & Biotechnology 95:950-958. https://doi.org/10.1002/jctb.5970

Smolińska B, Król K (2012) Leaching of mercury during phytoextraction assisted by EDTA, KI and citric acid. Journal of Chemical Technology & Biotechnology 87:1360-1365. https://doi.org/10.1002/jctb.3826

Solís-Ramos L Y, Coto-López C, Andrade-Torres A (2021) Role of arbuscular mycorrhizal symbiosis in remediation of anthropogenic soil pollution. Symbiosis 84:321-336. https://doi.org/10.1007/s13199-021-00774-4

Song Y, Jin L, Wang X (2017) Cadmium absorption and transportation pathways in plants. International Journal of Phytoremediation 19:133-141. https://doi.org/10.1080/15226514.2016.1207598

Strullu-Derrien C, Selosse M-A, Kenrick P, Martin F M (2018) The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. New Phytologist 220:1012-1030. https://doi.org/10.1111/nph.15076

Takahashi R, Ishimaru Y, Nakanishi H, Nishizawa N K (2011b) Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. Plant Signaling & Behavior 6:1813-1816. https://doi.org/10.4161/psb.6.11.17587

Takahashi R, Ishimaru Y, Senoura T, Shimo H, Ishikawa S, Arao T, Nakanishi H, Nishizawa N K (2011a) The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. Journal of Experimental Botany 62:4843-4850. https://doi.org/10.1093/jxb/err136

Tamayo E, Gómez-Gallego T, Azcón-Aguilar C, Ferrol N (2014) Genome-wide analysis of copper, iron and zinc transporters in the arbuscular mycorrhizal fungus Rhizophagus irregularis. Frontiers in plant science 5:547. https://doi.org/10.3389/fpls.2014.00547

Tangahu B V, Sheikh Abdullah S R, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. International Journal of Chemical Engineering 2011:939161. https://doi.org/10.1155/2011/939161

Tiodar E D, Văcar C L, Podar D (2021) Phytoremediation and microorganisms-assisted phytoremediation of mercury-contaminated soils: Challenges and perspectives. International Journal of Environmental Research and Public Health 18:1-38. https://doi.org/10.3390/ijerph18052435

UNEP (2019) Global Mercury Assessment 2018. https://www.unep.org/resources/publication/global-mercury-assessment-2018

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, et al. (2009) Phytoremediation of contaminated soils and groundwater: Lessons from the field. Environmental Science and Pollution Research 16:765-794. https://doi.org/10.1007/s11356-009-0213-6

Vargas Aguirre C F, Rivera Páez F A, Escobar Vargas S (2018) Effect of arbuscular mycorrhizae and mercury on Lactuca sativa (Asteraceae) seedling morpho—histology. Environmental and Experimental Botany 156:197-202. https://doi.org/10.1016/j.envexpbot.2018.09.005

Verma N, Rachamalla M, Kumar P S, Dua K (2023) Chapter 6 - Assessment and impact of metal toxicity on wildlife and human health. Metals in Water. Elsevier, 93-110. https://doi.org/10.1016/B978-0-323-95919-3.00002-1

Wang B, Qiu Y L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299-363. https://doi.org/10.1007/s00572-005-0033-6

Wang J, Anderson C W N, Xing Y, Fan Y, Xia J, Shaheen S M, Rinklebe J, Feng X (2018) Thiosulphate-induced phytoextraction of mercury in Brassica juncea: Spectroscopic investigations to define a mechanism for Hg uptake. Environmental Pollution 242:986-993. https://doi.org/10.1016/j.envpol.2018.07.065

Wang J, Feng X, Anderson C W N, Qiu G, Ping L, Bao Z (2011) Ammonium thiosulphate enhanced phytoextraction from mercury contaminated soil – Results from a greenhouse study. Journal of Hazardous Materials 186:119-127. https://doi.org/10.1016/j.jhazmat.2010.10.097

Wang W, Shi J, Xie Q, Jiang Y, Yu N, Wang E (2017) Nutrient Exchange and Regulation in Arbuscular Mycorrhizal Symbiosis. Molecular Plant 10:1147-1158. https://doi.org/10.1016/j.molp.2017.07.012

Watts-Williams S J, Patti A F, Cavagnaro T R (2013) Arbuscular mycorrhizas are beneficial under both deficient and toxic soil zinc conditions. Plant and Soil 371:299-312. https://doi.org/10.1007/s11104-013-1670-8

Watts-Williams S J, Tyerman S D, Cavagnaro T R (2017) The dual benefit of arbuscular mycorrhizal fungi under soil zinc deficiency and toxicity: linking plant physiology and gene expression. Plant and Soil 420:375-388. https://doi.org/10.1007/s11104-017-3409-4

Yang L, Wang J, Yang Y, Li S, Wang T, Oleksak P, Chrienova Z, et al. (2022) Phytoremediation of heavy metal pollution: Hotspots and future prospects. Ecotoxicology and Environmental Safety 234:113403. https://doi.org/10.1016/j.ecoenv.2022.113403

Yang Z, Yang F, Liu J-L, Wu H-T, Yang H, Shi Y, Liu J, Zhang Y-F, Luo Y-R, Chen K-M (2022) Heavy metal transporters: Functional mechanisms, regulation, and application in phytoremediation. Science of the Total Environment 809:151099. https://doi.org/10.1016/j.scitotenv.2021.151099

Yevugah L L, Darko G, Bak J (2021) Does mercury emission from small-scale gold mining cause widespread soil pollution in Ghana? Environmental Pollution 284:116945. https://doi.org/10.1016/j.envpol.2021.116945

You Y, Ju C, Wang L, Wang X, Ma F, Wang G, Wang Y (2022) The mechanism of arbuscular mycorrhizal enhancing cadmium uptake in Phragmites australis depends on the phosphorus concentration. Journal of Hazardous Materials 440:129800. https://doi.org/10.1016/j.jhazmat.2022.129800

You Y, Wang L, Ju C, Wang G, Ma F, Wang Y, Yang D (2021) Effects of arbuscular mycorrhizal fungi on the growth and toxic element uptake of Phragmites australis (Cav.) Trin. ex Steud under zinc/cadmium stress. Ecotoxicology and Environmental Safety 213:112023. https://doi.org/10.1016/j.ecoenv.2021.112023

Yu Y, Li Z, Liu Y, Wang F, Liu Y, Zhao J, Li Y, Gao Y, Zhu N (2022) Roles of plant-associated microorganisms in regulating the fate of Hg in croplands: A perspective on potential pathways in maintaining sustainable agriculture. Science of the Total Environment 834:155204. https://doi.org/10.1016/j.scitotenv.2022.155204

Yu Y, Zhang S, Huang H (2010) Behavior of mercury in a soil–plant system as affected by inoculation with the arbuscular mycorrhizal fungus Glomus mosseae. Mycorrhiza 20:407-414. https://doi.org/10.1007/s00572-009-0296-4

Zahir F, Rizwi S J, Haq S K, Khan R H (2005) Low dose mercury toxicity and human health. Environmental Toxicology and Pharmacology 20:351-360. https://doi.org/10.1016/j.etap.2005.03.007

Zhan F, Li B, Jiang M, Yue X, He Y, Xia Y, Wang Y (2018) Arbuscular mycorrhizal fungi enhance antioxidant defense in the leaves and the retention of heavy metals in the roots of maize. Environmental Science and Pollution Research 25:24338-24347. https://doi.org/10.1007/s11356-018-2487-z

Zhang H, Zhao S, Li D, Xu X, Li C (2017) Genome-Wide Analysis of the ZRT, IRT-Like Protein (ZIP) Family and Their Responses to Metal Stress in Populus trichocarpa. Plant Molecular Biology Reporter 35:534-549. https://doi.org/10.1007/s11105-017-1042-2

Živković I, Fajon V, Kotnik J, Shlyapnikov Y, Obu Vazner K, Begu E, Šestanović S, et al. (2019) Relations between mercury fractions and microbial community components in seawater under the presence and absence of probable phosphorus limitation conditions. Journal of Environmental Sciences 75:145-162. https://doi.org/10.1016/j.jes.2018.03.012

4 *Rhizophagus irregularis* improves Hg tolerance of *Medicago truncatula* by upregulating the Zn transporter genes *ZIP2* and *ZIP6*[*]

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4.1 Abstract

Mercury (Hg) pollution of soils is a critical environmental problem. To rehabilitate Hg contaminated soils, arbuscular mycorrhizal (AM) fungi-based phytoremediation may be supportive, yet the functional potential of AM fungi in response to Hg exposure is unclear. In a greenhouse experiment, we assessed the response of *Medicago truncatula* (Hg tolerance index (TI), Hg partitioning) to different Hg concentrations [0 (Hg0), 25 (Hg25), 50 (Hg50) µg g^{-1}] in treatments with (AM) and without (NM) inoculation of *Rhizophagus irregularis*. Additionally, zinc (Zn) uptake and the expression of two Zn transporter genes (*ZIP2*, *ZIP6*) were examined because Zn is an essential element for plants and shares the same outer electronic configuration as Hg, implying potential competition for the same transporters. The results showed that AM plants had a higher TI than NM plants. Plant roots were identified as dominant Hg reservoirs. AM inoculation reduced the root Hg concentration under Hg50 compared to the NM treatment. There was an interaction between Hg treatment and AM inoculation on Hg stem concentration, i.e., at Hg25, AM inoculation decreased the Hg translocation from roots to stems, while Hg translocation was increased at Hg50 compared to the NM treatment. Zn acquisition was improved by *R. irregularis*. The negative relationship between Hg and Zn concentrations in the roots of AM and NM plants implied potential competition for the same transporters, although the expression of Zn transporters was upregulated by AM inoculation at all Hg levels. In conclusion, this baseline study demonstrated that *R. irregularis* may play an important role in Hg tolerance of *M. truncatula*, suggesting its potential for Hg-contaminated phytoremediation.

Keywords: Heavy metal (HM), arbuscular mycorrhizal fungi (AMF), Hg uptake, Hg accumulation, Zn transporters.

4.2 Introduction

Mercury (Hg) is a very toxic heavy metal (HM), ranked as the third most hazardous substance on earth (ATSDR 2019). Because of its high mobility and persistence in the environment, Hg has been recognized as a global pollutant and major threat to human health (Driscoll et al. 2013). Pollution with Hg occurs naturally by weathering and to a large extent through anthropogenic activities. These include artisanal gold mining, stationary combustion of coal, nonferrous metals production, and cement production. Among these, artisanal gold mining is the world's largest source of anthropogenic Hg emission (EPA 2018) and is done in many regions around the world (Wang et al. 2022). This practice often is uncontrolled and aggravates the distribution of Hg in terrestrial ecosystems, which may affect the growth of food crops, thereby compromising food safety (Gworek et al. 2020).

Decontamination of Hg polluted soils is a major concern in environmental legislation and food production. Phytoremediation, a plant-based technology, offers a green and sustainable solution to remediate Hg contaminated soils. Phytoextraction (facilitating Hg translocation to plant stems) and phytostablization (facilitating Hg storage in plant roots, while preventing Hg translocation to plant stems) are two popular approaches of phytoremediation of soil Hg contamination (Bhat et al. 2022). Plants, however, may suffer from negative effects of Hg toxicity, which limits plant growth and even threatens plant survival. To counteract such negative impacts on plant performance in the process of soil remediation, plants can be inoculated with competent soil microorganisms, specifically arbuscular mycorrhizal fungi (AMF) (Ferrol et al. 2016).

AMF are ubiquitous in terrestrial environments and form intimate relationships with the majority of vascular plants (Smith et al. 2008), reflecting the strategic importance of plant-AMF interactions for environmental adaptation (Wang et al. 2006). Several studies reported that certain arbuscular mycorrhizal (AM) fungal species facilitate HM transport to aboveground plant organs (phytoextraction) (Weissenhorn et al. 1995; Fiqri et al. 2016; Singh et al. 2019). Other studies, however, revealed that AM fungal species inhibit HM translocation by various means (Shabani et al. 2016; Chamba et al. 2017; Salazar et al. 2018). These include the binding of HM in AM fungal hyphae and glomalin, or sequestering HM in fungal structures (arbuscules, vesicles, vacuoles). With this, the transport of HM to the plant stem is prevented (phytostablization) (Garg et al. 2018; Motaharpoor et al. 2019). The extent of HM uptake and the subsequent translocation *in planta* further depends on the HM concentration in soils, even in the presence of the same AM fungal species (Huang et al. 2017).

Hg exists in three forms, i.e., elemental Hg, inorganic Hg (Hg^{1+}, Hg^{2+}) and organic Hg (Beckers et al. 2017). In soils, the predominant form is Hg^{2+} (Beckers et al. 2017; Kumari et al. 2020). Uptake of Hg^{2+} via roots is facilitated by an active process (Esteban et al. 2008; Wang et al. 2014; Ma et al. 2021), yet no precise membrane transporters involved in root Hg^{2+} have been identified. It is generally accepted that HM enter roots via nutrient transporters (Manoj et al. 2020) because of the structural similarity of HM with essential nutrients. For example, arsenate (AsO_4^{3-}) is taken up by the same transporters as phosphate (PO_4^{3-}) (Meharg et al. 2002) and arsenite shares transporters with silicic acid (Ma et al. 2008). Cadmium (Cd^{2+}) uptake by plants is facilitated via zinc (Zn) protein carriers (Kaur et al. 2018). Such pertinent information is lacking for Hg, however, despite that Hg^{2+}, like Cd^{2+}, shares the same outer electronic configuration as Zn^{2+} (Jensen 2003). Competition between Hg^{2+} and Zn^{2+} was observed in bacteria upon addition of Zn to a Hg-containing growth solution. These findings imply that Hg and Zn share affinity for the same transporters (Schaefer et al. 2014; Szczuka et al. 2015). Zn is an essential micronutrient for plants, playing vital roles in cellular and physiological functions (Fariduddin et al. 2022). Zn has a low mobility in soil solution, whereby its uptake is diffusion-limited (Lehmann et al. 2014). AMF have been recognized to play a key role in facilitating Zn tissue concentration (Ruytinx et al. 2020). These results highlight the importance of investigating the interplay between Zn and Hg uptake in relation to mycorrhizal colonization, which is fundamental to understand the mechanisms of Hg uptake and accumulation in mycorrhizal plants. Such advanced knowledge would help to optimize AMF-supported plant performance in phytoremediation.

Among the involved transporters of Zn into the cytoplasm, the Zinc-Iron-Regulated Transporter (*ZRT-IRT*) family, called *ZIP*, mostly has been studied. In *Medicago truncatula*, four *ZIP* transporters - *ZIP1*, *ZIP2*, *ZIP5* and *ZIP6* facilitating the transport of Zn^{2+} have been verified in yeast complementation assays (Burleigh et al. 2003; Stephens et al. 2011). Recent studies showed that only two of them are influenced by mycorrhizal colonization, yet under contrasting Zn conditions. Namely, *ZIP6* was up-regulated at deficient and sufficient soil Zn concentrations, while *ZIP2* was up-regulated at toxic Zn concentrations (Watts-Williams et al. 2017).

In this study, we used the model legume *Medicago truncatula* inoculated with the AM fungus *Rhizophagus irregularis* to gain fundamental insights into the underlying mechanisms of Hg uptake and accumulation by mycorrhizal plants. The aims of this study were to (1) examine the effect of *R. irregularis* on biomass and Hg accumulation of *M. truncatula* under Hg exposure; (2) determine the translocation strategies of Hg in roots, stems and leaves of *M.*

truncatula associated with *R. irregularis*; and (3) investigate the effect of *R. irregularis* on Zn nutrient uptake and Zn transporters (*ZIP2*, *ZIP6*) under Hg exposure.

4.3 Materials and methods

4.3.1 Preparation of biological materials

Medicago truncatula cv. Jemalong A17 seeds were scarified in 90% sulfuric acid for 7.5 min. Seeds were washed eight times with cold water to remove the acid, followed by 90 s surface sterilization in 3% active chlorine solution (sodium hypochlorite solution) (Garcia et al. 2006). The chlorine solution was removed by rinsing of the seeds in sterile water for 5-6 times. Sterilized seeds were soaked in sterile water overnight under darkness at room temperature (20°C). Then, the seeds were stratified in water-agar plates (0.8% (*w*/*v*)) for 24 h at 4°C and germinated at 20°C for 2 days in the dark. After germination, seeds were exposed to light for 2 days to initiate chlorophyll development. The AM fungus *Rhizophagus irregularis* (QS81) was provided by INOQ GmbH (Schnega, Germany). The inoculum of *R. irregularis* was prepared from arbuscular mycorrhizal root fragments of *Trifolium partensis* produced in sand/vermiculite 35/65 v/v in year 2019. The product was filtered through a 425 µm mesh (Grade II) and finally contained 100 million propagules kg^{-1} powder (as vesicles and spores according to the manufacturer). Prior to use, the AM fungal propagules were stored in an air-dried, well-ventilated and dark environment.

4.3.2 Experiment design and conditions

Sand quartz was twice autoclaved (121°C / 2 h) over a two day interval. For the AM fungus inoculated treatment (AM), 50 ml (80 g) sterilized sand were mixed with 25 mg (a ratio of 0.5 g L^{-1} substrate) Osmocote Exact Mini 3-4 months (NPK 15:3.9:9.1 + 1.2 Mg + trace elements, ICL Specialty Fertilizers, UK) (Senovilla et al. 2020) and 160 mg (a ratio of 3.2 g L^{-1} substrate) AM fungus inoculum (Mercy et al. 2017) was added to each plastic pot (5 cm of height, 4 cm of width and with 3 holes in the bottom). For the negative control without inoculum (NM), the same amount of sand mixed with 25 mg Osmocote Exact Mini was filled into each plastic pot. Then, one seedling (5 days) was transferred to each pot. Plants were grown in the greenhouse from 19 June to 17 August, 2021. Plants were maintained at an average temperature of 29.4ºC and an average relative humidity of 51% under natural light conditions (Phytotechnikum research station, University of Hohenheim, Stuttgart, Germany). Plants were watered daily with 5 ml tap water which did not drain from the pots. After 3 weeks, when plants showed vigorous growth, 5 ml $HgCl_2$

solution at concentrations of 25 µg g^{-1} or 50 µg g^{-1} were added to each Hg treatment pot once, respectively. The reference pots without Hg application were treated with sterile water in equivalent volume. The Hg treatment was assigned to Hg0, Hg25, and Hg50. The experiment was a 2 × 3 complete factorial design, comprising 5 replications per treatment arranged in a randomized block design. The experiment was performed for 5 weeks after Hg additions until destructive harvest.

4.3.3 Plant harvest

At the end of experiment (8 weeks), the roots were quickly washed with tap water and separated into 3 parts. One sub-sample (100 mg) of fresh root was immediately flash frozen in liquid nitrogen and stored at -80°C for RNA isolation. The second sub-sample also was stored at -80°C for AM root colonization observation. The remaining roots as well as stem and leaf biomass were dried at room temperature until weight stability to determine plant biomass weight.

The tolerance index (TI) was calculated (Eq 1) to reflect the ability of the host plant to grow in the presence of different Hg concentration (Huang et al. 2017). There, *mt* is the total dry biomass of the plant growing in each pot; *mc* denotes the average total dry biomass of the plants growing in pots without Hg under NM and AM treatment, separately.

$$TI = \frac{mt}{mc} \qquad \text{Eq 1}$$

4.3.4 Determination of Hg and Zn

Hg and Zn concentrations were determined separately for roots, stems and leaves. Each air-dried sample was milled with a centrifugal mill (Retsch GmbH, Haan, Germany) equipped with a titanium rotor and a ring sieve. Samples (0.2 g) were moistened with 1 ml of deionized H_2O and digested in 2.5 ml 69% HNO_3 in an UltraCLAVE III microwave heated digestion unit (MLS-MWS GmbH, Leutkirch, Germany). After digestion, the solutions were filled up to 10 ml with deionized H_2O. Hg concentration was analyzed via a NexION 300x inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer LAS GmbH, Rodgau, Germany), and Zn concentration was analyzed by an Agilent5100 inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent Technologies GmbH, Waldbronn, Germany) (Core Facility, University of Hohenheim, Stuttgart, Germany).

4.3.5 Mycorrhizal colonization

Frozen roots stored for AMF observation were cut into 1 cm segments and cleared using 10% NaOH in a 70°C water bath for 45 min, and then soaked in 1% HCl for 1 min at room temperature. The roots were stained in 2% PARKER QUINK blue ink (Yon et al. 2015) in a 70°C water bath for 30 min. The roots were rinsed with tap water until the water appeared transparent and then were stored in a lactoglycerol solution (v:v:v–1:1:1-latic acid:glycerol:H_2O). Thirty fragments were randomly selected, placed on a slide, and checked under a light microscope for intraradical AM structures (Trouvelot et al. 1986).

4.3.6 RNA isolation and quantitative RT-PCR

Total RNA was isolated from 100 mg root sub-samples (RNeasy® plant Mini Kit, QIAGEN GmbH, Germany). RNA integrity was checked by gel electrophoresis, following quantification of RNA by Nanodrop 2100 (Thermo Fisher Scientific). The cDNA was synthesized from 500 ng of RNA using the QIAGEN QuantiTect® Reverse Transcription Kit (QIAGEN GmbH) including a genomic DNA elimination step. Quantification of the expression of *ZIP2*, *ZIP6* and *Ri-tubulin* genes was conducted with 1 µl of 10× diluted cDNA in a 20 µl reaction using gene-specific primers and SYBR® green PCR Master Mix in the StepOnePlus™ Real-Time PCR system (Applied Biosystems, USA). The RT-PCR settings were 94°C for 5 min, then 94°C for 30 s, 60°C for 30 s and 72°C for 30 s for 40 cycles, followed by generation of a dissociation curve. *MtASPP* and *β-actin* were selected as housekeeping genes using NormFinder (Mestdagh et al. 2009). Gene expressions were normalized to the geometric mean of the two selected housekeeping genes (Vandesompele et al. 2002). Table S 3.1 displays the sequences of the forward and reverse primers used in this study.

4.4 Statistical analysis

All statistical analyses were performed in R version 4.0.3. Homogeneity and normality of data were checked with Levene's test and Shapiro-Wilk test, respectively. Data, which did not meet the criteria, were Box-Cox transformed (Box et al. 1964). Values presented in figures are non-normalized data. Response variable data were subjected to two-way analysis of variance (ANOVA), with the factors "Hg treatment" and "AM inoculation". Following two-factor ANOVA, the means of AM fungus inoculation (AM) and non-inoculated control (NM) were compared separately at each Hg level (Hg0, Hg25, Hg50) using Student's *t-test* (5%). For response variables, for which no AM fungus data

could be recorded (i.e., mycorrhizal colonization and *α-tubulin* expression of *R. irregularis* in NM treatments), one-way ANOVA along with Tukey's honestly significant difference (HSD) was used considering Hg treatment as the only factor. A correlation matrix among Hg concertation and Zn concentration in each plant part, as well as *ZIP* transporter gene expression was calculated for AM and NM treatments, respectively. Spearman's method was used and *P* value was adjusted with a Bonferroni correction.

4.5 Results

4.5.1 Plant Hg tolerance and mycorrhizal colonization

Overall, inoculation with *Rhizophagus irregularis* (AM plants) conferred higher Hg tolerance to *Medicago truncatula* compared to non-inoculated controls (NM plants) ($P < 0.001$) (Table 4.1). This effect was significant for Hg25 ($P < 0.01$) (Fig. 4.1). The results of dry biomass in each part (leaves, stems and roots) and total biomass are shown in Table S 4.2. Leaf necrosis in NM plants under Hg50 treatment was observed.

Under Hg0, Hg25 and Hg50, frequencies of mycorrhizal colonization of 42.7%, 55.8% and 34%, respectively, were determined but did not differ significantly ($P > 0.05$) (Fig. 4.2a, Table 4.1). Pictures of colonization are shown in Fig S 4.1. The expression of the *α-tubulin* gene in mycorrhizal roots of AM plants followed the same trend (Fig. 4.2b). Non-inoculated roots (NM plants) were free of mycorrhizal colonization, as confirmed by lack of *α-tubulin* gene expression in their roots.

Table 4.1 Summary of ANOVA results for all response variables. Factors in the analysis were Hg treatment and AM inoculation (*Rhizophagus irregularis*). Both the main effects and interaction term are indicated where relevant. Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant ($P > 0.05$).

	Hg treatment	AM inoculation	Hg treatment x AM inoculation
Mycorrhizal colonization (%)	ns		
Ri α-tubulin expression	ns		
TI	ns	***	ns
Leaf Hg concentration	ns	***	ns
Stem Hg concentration	***	ns	***
Root Hg concentration	***	ns	***
Leaf Hg content	ns	***	ns
Stem Hg content	ns	ns	**
Root Hg Content	***	ns	ns
Leaf Zn concentration	ns	**	*
Stem Zn concentration	ns	***	ns
Root Zn concentration	ns	**	ns
Leaf Zn content	ns	ns	ns
Stem Zn content	ns	*	ns
Root Zn content	ns	ns	ns
ZIP2 expression	ns	**	ns
ZIP6 expression	ns	***	ns

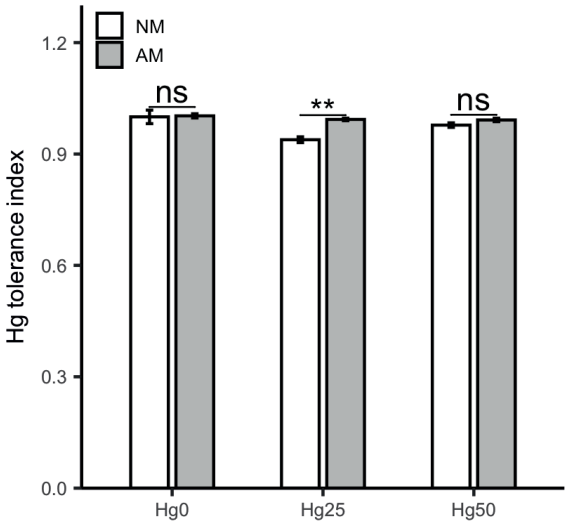

Figure 4.1 Plant mercury (Hg) tolerance index. Values present mean ± SE (n=5). Asterisks (*) denotes a significant mean difference between NM and AM treatments, as measured by the *t-test* (5%). Significance levels: **, $P < 0.01$; ns: not significant ($P > 0.05$).

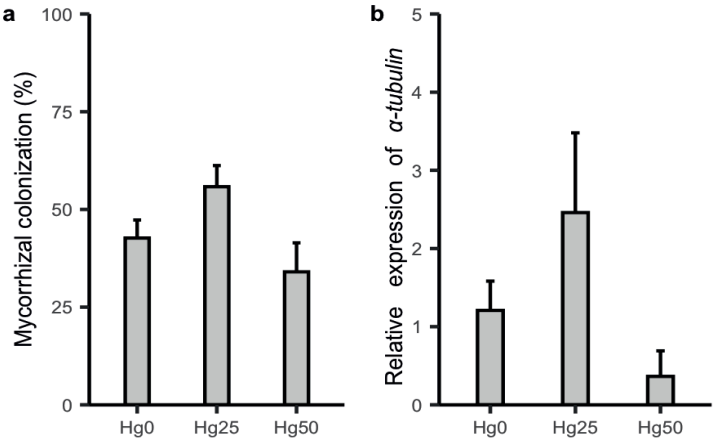

Figure 4.2 Percentage of mycorrhizal colonization (a) and relative expression of AM fungal biomass marker gene *α-tubulin* (b) in roots *of Medicago truncatula* plants inoculated with *Rhizophagus irregularis* and grown at different Hg concentrations. Values present mean ± SE (n=5). There were no significant effects of Hg treatment on both mycorrhizal colonization and relative expression of *α-tubulin* (see Table 4.1 for detailed ANOVA analysis).

Fig. 4.3 shows the Hg concentration in different plant parts (leaves, stems, roots). AM inoculation reduced the Hg concentration in leaves at all Hg concentrations compared to NM plants ($P < 0.001$) (Table 4.1, Fig. 4.3a). Hg concentrations in leaves at different Hg treatments were similar (Fig. 4.3a). There was no Hg contamination detected in the control substrate (Hg0) after the experiment (Fig. S 4.2). Therefore, Hg accumulation in leaves likely results from absorbance of atmospheric Hg, because all plants were grown in the same compartment in the greenhouse. There was a significant interaction between Hg treatment and AM fungus inoculation for Hg concentration of stems ($P < 0.001$) (Table 4.1). The Hg stem concentration under Hg25 was lower in AM plants than in NM plants ($P < 0.05$) (Fig. 4.3b). Conversely, under Hg50, the Hg stem concentration was higher in AM plants than in NM plants ($P < 0.0001$) (Fig. 4.3b). For roots, there was a significant interaction between Hg treatment and AM fungus inoculation ($P < 0.001$) (Table 3.1), which was most prominent under Hg50 ($P < 0.05$) (Fig. 4.3c). Concerning Hg contents in different plant parts, AM fungus inoculation significantly reduced the Hg content in leaves compared to NM plants ($P < 0.001$) (Table 4.1). There also was a significant interaction between Hg treatment and AM inoculation for Hg stem content ($P < 0.01$) (Table 4.1). For Hg root content, only Hg treatment had a significant main effect ($P < 0.001$) (Table 4.1). Fig. 4.4 shows the percentage of Hg content in each plant part, substantiating the root as prominent plant tissue for Hg accumulation under Hg treatment (Fig. 4.4).

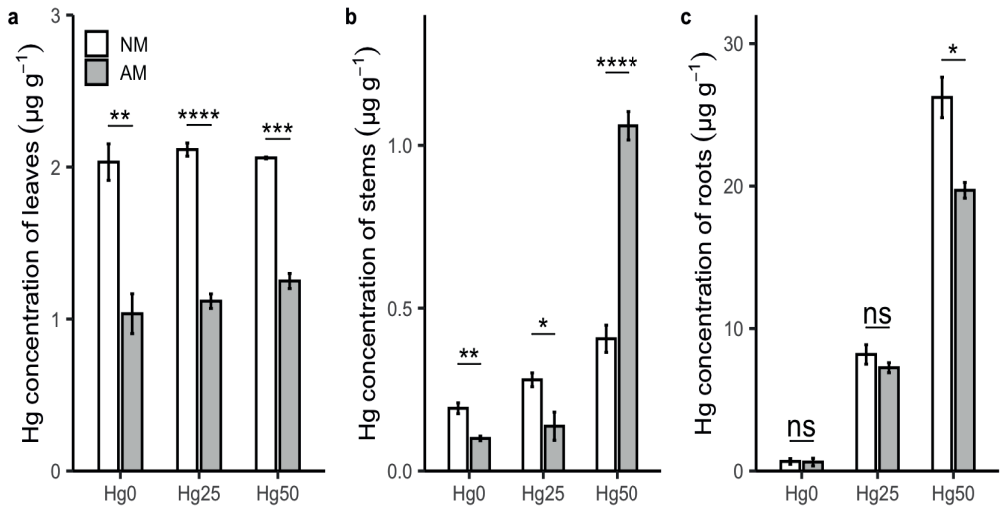

Figure 4.3 Mercury (Hg) concentration in leaves (a), stems (b) and roots (c) of non-mycorrhizal (NM) and mycorrhizal (AM) plants exposed to three different Hg levels (Hg0, Hg25, Hg50). Values present mean ± SE (n=5). Asterisk (*) denotes a significant mean difference between NM and AM treatments, as measured by the t-test (5%). Significance levels: * P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; ns, not significant (P > 0.05).

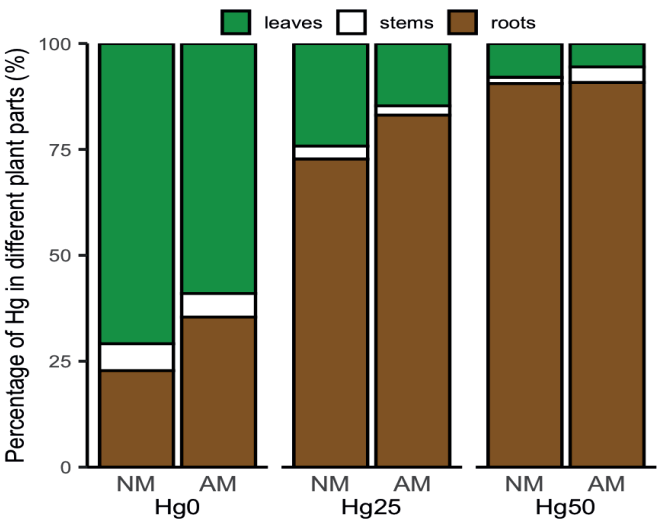

Figure 4.4 percentage mercury (Hg) content (= concentration × dry weight) in different plant parts of non-mycorrhizal (NM) and mycorrhizal (AM) plants exposed to three different Hg levels (Hg0, Hg25, Hg50).

There was a significant interaction between Hg treatment and AM fungus inoculation on Zn concentration in leaves ($P < 0.05$) (Table 4.1). Specifically, AM plants showed higher Zn concentrations than NM plants under Hg25 and Hg50 ($P < 0.05$) (Fig. 4.5a). AM fungus inoculation increased Zn concentrations in stems compared to NM plants ($P < 0.001$) (Table 4.1, Fig. 4.5b). In contrast, AM plants showed lower Zn concentrations in roots than NM plants ($P < 0.01$) (Table 4.1; Fig. 4.5c). However, considering Zn content in different plant parts, AM inoculation only had a significant effect on Zn content in stems ($P < 0.05$) (Table 4.1), by increasing the percentage of Zn content in stems when inoculated with AM fungus (Fig. 4.6). AM fungal inoculation significantly upregulated the expression of *ZIP2* ($P < 0.01$) and *ZIP6* ($P < 0.001$) genes, irrespective of Hg treatment (Table 4.1, Fig. 4.7).

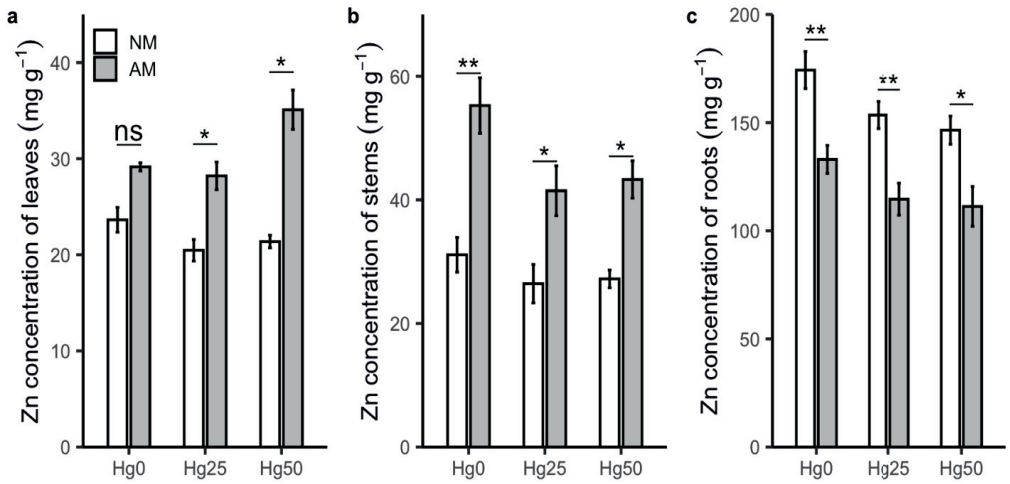

Figure 4.5 Zinc (Zn) concentration in leaves (a), stems (b) and roots (c) of non-mycorrhizal (NM) and mycorrhizal (AM) plants exposed to three different mercury (Hg) levels (Hg0, Hg25, Hg50). Values present mean ± SE (n=5). Asterisks (*) denotes a significant mean difference between NM and AM treatments, as measured by the t-test (5%). Significance levels: *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant (P > 0.05).

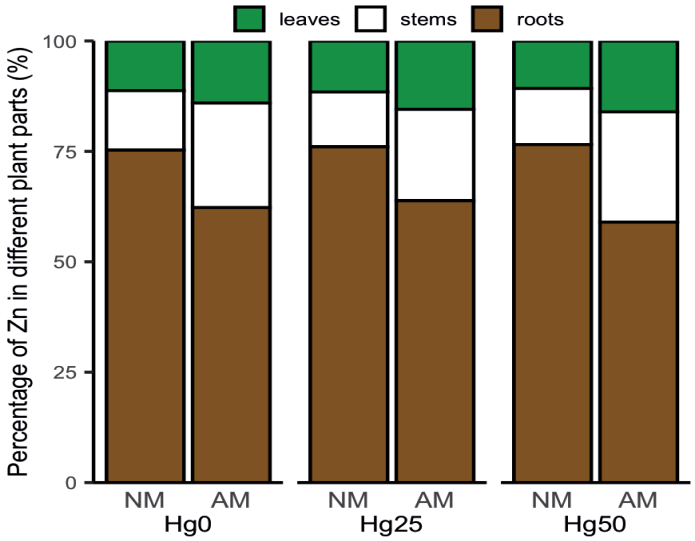

Figure 4.6 Percentage zinc (Zn) content (= concentration × dry weight) in different plant parts of non-mycorrhizal (NM) and mycorrhizal (AM) plants exposed to three different mercury (Hg) levels (Hg0, Hg25, Hg50).

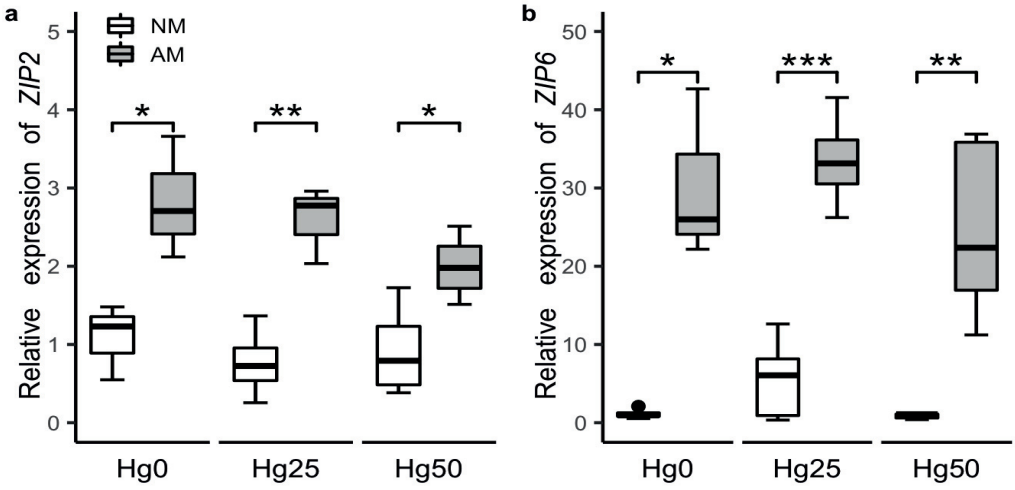

Figure 4.7 The relative gene expression of Mt*ZIP2* (a) and Mt*ZIP6* (b) in non-mycorrhizal (NM) and mycorrhizal (AM) plants exposed to three different mercury (Hg) levels (Hg0, Hg25, Hg50). Values present mean ± SE (n=5). Asterisks (*) denotes a significant mean difference between NM and AM treatment, as measure by the t-test (5%). Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

4.5.2 Correlations between Hg and Zn concentrations in roots

Correlation matrices among measured parameters were calculated for NM and AM treatments separately (Table S 4.1 and Table S 4.2). Hg concentration in stems was negatively correlated with Zn concentration in roots of the NM plants (r = -0.74; $P < 0.05$), and Hg concentration in roots was negatively correlated with Zn concentration in stems under AM treatment (r = -0.70; $P < 0.05$). Interestingly, Hg concentration in roots was negatively correlated with Zn concentration in roots for both NM and AM treatments (r = -0.71; r = -0.70, respectively; $P < 0.05$).

4.6 Discussion

4.6.1 Effects of *Rhizophagus irregularis* on Hg tolerance index and Hg partitioning

Numerous studies have indicated that AMF inoculation could alleviate HM stress of plants (Garg et al. 2018; Motaharpoor et al. 2019; Molina et al. 2020). In line with this, our results showed a positive effect of *Rhizophagus irregularis* on Hg tolerance of *Medicago truncatula*, suggesting the potential of using AM fungi in Hg phytoremediation. Furthermore, our results showed the effects of *R. irregularis* on Hg partitioning in each plant part (leaves, stems and roots). There was a significant reduction of Hg concentration and content in leaves of AM plants compared to NM plants, indicating that the strain of *R. irregularis* was able to protect leaves from Hg uptake. (Assad et al. 2016) demonstrated that Hg uptake by leaves is exclusively caused through the atmospheric pathway. This also was indicated in our study in which leaf Hg concentration was similar under different Hg concentration treatments. Thus, the reduction of Hg concentration in leaves might be attributed to the regulation of stomatal closure by *R. irregularis*. This possibility deserves further investigation. (Wang et al. 2017) showed that stomatal closure is part of the protective strategy initiated by *R. irregularis* under exposure to cadmium.

Additionally, a significant interaction between Hg treatment and AM inoculation on Hg stem concentration and content was detected in our study, indicating that the effect of *R. irregularis* on Hg translocation to stems was dependent on Hg concentration in the substrate. Prior work showed that maize associated with *Funneliformis mosseae* (formerly *Glomus mosseae*) did not influence Hg shoot concentration compared to non-inoculated plants exposed to Hg concentrations of 2 and 4 mg kg^{-1} (Yu et al. 2010). Recent studies have demonstrated, however, that *Glomus sp.* associated with maize facilitated Hg uptake and its translocation from roots to shoots at a Hg concentration of 50 mg kg^{-1} (Kodre et al. 2017; Debeljak et al. 2018). Together, these results indicate that the role of AM fungal species in

Hg translocation in plants is likely dependent on the Hg concentration in the substrate, as was corroborated by our study.

The observed increase of Hg concentrations in roots with increasing Hg levels was consistent with other reports. For example, Hg concentration in both lupin and maize roots showed a hyperbolic pattern with increasing Hg additions in the growth solution (Esteban et al. 2008; Yu et al. 2010). Furthermore, AM inoculation reduced Hg concentration at Hg50, indicating a protective role of *R. irregularis* under high Hg concentration. This reduction was generally attributed to the role of AM fungal structures, e.g., binding with hyphae and sequestration into vacuoles. However, this may not be the case in the present study because such a reduction was not observed under Hg25. There is no difference in Hg root concentrations between NM and AM treatments under Hg25, indicating the capacity of root Hg accumulation of *M. truncatula*. Within this capacity, plants can detoxify Hg by themselves (Kumar et al. 2017), but plants may need support from microbes (in this case *R. irregularis*) at high concentrations, as it was shown under Hg50. Accordingly, further work will be necessary to precisely investigate the functions of the same AM fungal species on different plant species exposed to different Hg concentrations.

4.6.2 Effects of *Rhizophagus irregularis* on Zn under Hg exposure

Generally, AM inoculation exerts a positive effect on the transport of elemental nutrients (Smith et al. 2008). In our study, AM inoculation increased Zn concentration in aboveground plant parts exposed to Hg, a finding in line with other studies (Debeljak et al. 2018; Saboor et al. 2021). Moreover, AM fungus inoculation upregulated the expression of Zn transporter genes (*ZIP2*, *ZIP6*), independent of Hg levels in the substrate. This indicates that Hg uptake by roots might not have been related to these two transporters in our study. This also was indicated by the absence of correlations between Hg root concertation and the two assayed *ZIP* transporter genes. Nevertheless, negative relationships between Hg and Zn concentrations in the roots of both AM and NM plants were found, implying potential competition between both elements for the same transporters. Our results suggest that Hg did not impair the positive regulation of Zn nutrient and its transporter genes by *R. irregularis*, which may have contributed to Hg tolerance because Zn plays a vital role in the reduction of the oxidative stress caused by Hg (Calgaroto et al. 2011). In order to fully understand the relationship between both Zn and Hg, further experiments with different Zn concentrations in the substrate and studies on other Zn-related transporter genes are needed.

4.7 Conclusions

Our results showed that the regulatory role of *R. irregularis* in Hg accumulation and translocation from roots to stems in *Medicago truncatula* is dependent on the concentration of Hg in the substrate. Additionally, a positive effect of *R. irregularis* on Hg tolerance of *M. truncatula* along with improvement of Zn nutrient status and upregulation of Zn transporter genes (*ZIP2*, *ZIP6*) was found.

4.8 References

Assad M, Parelle J, Cazaux D, Gimbert F, Chalot M, Tatin-Froux F (2016) Mercury uptake into poplar leaves. Chemosphere 146:1-7. https://doi.org/10.1016/j.chemosphere.2015.11.103

ATSDR (2019) ATSDR's Substance Priority List. Web https://www.atsdr.cdc.gov/spl/index.html#2019spl

Beckers F, Rinklebe J (2017) Cycling of mercury in the environment: Sources, fate, and human health implications: A review. Critical Reviews in Environmental Science and Technology 47:693-794. https://doi.org/10.1080/10643389.2017.1326277

Bhat S A, Bashir O, Ul Haq S A, Amin T, Rafiq A, Ali M, Américo-Pinheiro J H P, Sher F (2022) Phytoremediation of heavy metals in soil and water: An eco-friendly, sustainable and multidisciplinary approach. Chemosphere 303:134788. https://doi.org/10.1016/j.chemosphere.2022.134788

Box G E P, Cox D R (1964) An Analysis of Transformations. Journal of the Royal Statistical Society 26:211-252. https://www.jstor.org/stable/2984418

Burleigh S H, Kristensen B K, Bechmann I E (2003) A plasma membrane zinc transporter from Medicago truncatula is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Molecular Biology 52:1077-1088. https://doi.org/10.1023/A:1025479701246

Calgaroto N S, Cargnelutti D, Rossato L V, Farias J G, Nunes S T, Tabaldi L A, Antes F G, Flores E M M, Schetinger M R C, Nicoloso F T (2011) Zinc alleviates mercury-induced oxidative stress in Pfaffia glomerata (Spreng.) Pedersen. BioMetals 24:959-971. https://doi.org/10.1007/s10534-011-9457-y

Chamba I, Rosado D, Kalinhoff C, Thangaswamy S, Sánchez-Rodríguez A, Gazquez M J (2017) Erato polymnioides – A novel Hg hyperaccumulator plant in ecuadorian rainforest acid soils with potential of microbe-associated phytoremediation. Chemosphere 188:633-641. https://doi.org/10.1016/j.chemosphere.2017.08.160

Debeljak M, van Elteren J T, Špruk A, Izmer A, Vanhaecke F, Vogel-Mikuš K (2018) The role of arbuscular mycorrhiza in mercury and mineral nutrient uptake in maize. Chemosphere 212:1076-1084. https://doi.org/10.1016/j.chemosphere.2018.08.147

Driscoll C T, Mason R P, Chan H M, Jacob D J, Pirrone N (2013) Mercury as a Global Pollutant: Sources, Pathways, and Effects. Environmental Science & Technology 47:4967-4983. https://doi.org/10.1021/es305071v

EPA (2018) Global sources of mercury. https://www.epa.gov

Esteban E, Moreno E, Peñalosa J, Cabrero J I, Millán R, Zornoza P (2008) Short and long-term uptake of Hg in white lupin plants:Kinetics and stress indicators. Environmental and Experimental Botany 62:316-322. https://doi.org/10.1016/j.envexpbot.2007.10.006

Fariduddin Q, Saleem M, Khan T A, Hayat S (2022) Zinc as a Versatile Element in Plants: An Overview on Its Uptake, Translocation, Assimilatory Roles, Deficiency and Toxicity Symptoms. Microbial Biofertilizers and Micronutrient Availability: The Role of Zinc in Agriculture and Human Health. Springer International Publishing, Cham, 137-158. https://doi.org/10.1007/978-3-030-76609-2_7

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. Journal of Experimental Botany 67:6253-6265. https://doi.org/10.1093/jxb/erw403

Fiqri A, Utomo W H, Handayanto E (2016) Effect of arbuscular mycorrhizal fungi on the potential of three wild plant species for phytoextraction of mercury from small-scale gold mine tailings. JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT 3:551-558. https://doi.org/10.15243/jdmlm.2016.033.551

Garcia J, Barker D G, Journet E-P (2006) Seed storage and germination. The Medicago truncatula handbook. Samuel Roberts Noble Foundation, Ardmore, USA, 1-9.

Garg N, Singh S (2018) Arbuscular Mycorrhiza Rhizophagus irregularis and Silicon Modulate Growth, Proline Biosynthesis and Yield in Cajanus cajan L. Millsp. (pigeonpea) Genotypes Under Cadmium and Zinc Stress. Journal of Plant Growth Regulation 37:46-63. https://doi.org/10.1007/s00344-017-9708-4

Gworek B, Dmuchowski W, Baczewska-Dąbrowska A H (2020) Mercury in the terrestrial environment: a review. Environmental Sciences Europe 32:128. https://doi.org/10.1186/s12302-020-00401-x

Huang X, Ho S-H, Zhu S, Ma F, Wu J, Yang J, Wang L (2017) Adaptive response of arbuscular mycorrhizal symbiosis to accumulation of elements and translocation in Phragmites australis affected by cadmium stress. Journal of Environmental Management 197:448-455. https://doi.org/10.1016/j.jenvman.2017.04.014

Jensen W B (2003) The Place of Zinc, Cadmium, and Mercury in the Periodic Table. Journal of Chemical Education 80:952. https://doi.org/10.1021/ed080p952

Kaur H, Garg N (2018) Recent Perspectives on Cross Talk Between Cadmium, Zinc, and Arbuscular Mycorrhizal Fungi in Plants. Journal of Plant Growth Regulation 37:680-693. https://doi.org/10.1007/s00344-017-9750-2

Kodre A, Arčon I, Debeljak M, Potisek M, Likar M, Vogel-Mikuš K (2017) Arbuscular mycorrhizal fungi alter Hg root uptake and ligand environment as studied by X-ray absorption fine structure. Environmental and Experimental Botany 133:12-23. https://doi.org/10.1016/j.envexpbot.2016.09.006

Kumar B, Smita K, Cumbal Flores L (2017) Plant mediated detoxification of mercury and lead. Arabian Journal of Chemistry 10:S2335-S2342. https://doi.org/10.1016/j.arabjc.2013.08.010

Kumari S, Amit, Jamwal R, Mishra N, Singh D K (2020) Recent developments in environmental mercury bioremediation and its toxicity: A review. Environmental Nanotechnology, Monitoring & Management 13:100283. https://doi.org/10.1016/j.enmm.2020.100283

Lehmann A, Veresoglou S D, Leifheit E F, Rillig M C (2014) Arbuscular mycorrhizal influence on zinc nutrition in crop plants – A meta-analysis. Soil Biology and Biochemistry 69:123-131. https://doi.org/10.1016/j.soilbio.2013.11.001

Ma J F, Yamaji N, Mitani N, Xu X Y, Su Y H, McGrath S P, Zhao F J (2008) Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Natl Acad Sci U S A 105:9931-9935. https://doi.org/10.1073/pnas.0802361105

Ma Y, Wang G, Wang Y, Dai W, Luan Y (2021) Mercury Uptake and Transport by Plants in Aquatic Environments: A Meta-Analysis. Applied Sciences 11:https://doi.org/10.3390/app11198829

Manoj S R, Karthik C, Kadirvelu K, Arulselvi P I, Shanmugasundaram T, Bruno B, Rajkumar M (2020) Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. Journal of Environmental Management 254:109779. https://doi.org/10.1016/j.jenvman.2019.109779

Meharg A A, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytologist 154:29-43. https://doi.org/10.1046/j.1469-8137.2002.00363.x

Mercy L, Lucic-Mercy E, Nogales A, Poghosyan A, Schneider C, Arnholdt-Schmitt B (2017) A Functional Approach towards Understanding the Role of the Mitochondrial Respiratory Chain in an Endomycorrhizal Symbiosis. Frontiers in plant science 8:https://doi.org/10.3389/fpls.2017.00417

Mestdagh P, Van Vlierberghe P, De Weer A, Muth D, Westermann F, Speleman F, Vandesompele J (2009) A novel and universal method for microRNA RT-qPCR data normalization. Genome Biology 10:R64. https://doi.org/10.1186/gb-2009-10-6-r64

Molina A S, Lugo M A, Pérez Chaca M V, Vargas-Gil S, Zirulnik F, Leporati J, Ferrol N, Azcón-Aguilar C (2020) Effect of Arbuscular Mycorrhizal Colonization on Cadmium-Mediated Oxidative Stress in Glycine max (L.) Merr. Plants 9:108. https://doi.org/10.1007/s00572-021-01056-z

Motaharpoor Z, Taheri H, Nadian H (2019) Rhizophagus irregularis modulates cadmium uptake, metal transporter, and chelator gene expression in Medicago sativa. Mycorrhiza 29:389-395. https://doi.org/10.1007/s00572-019-00900-7

Ruytinx J, Kafle A, Usman M, Coninx L, Zimmermann S D, Garcia K (2020) Micronutrient transport in mycorrhizal symbiosis; zinc steals the show. Fungal Biology Reviews 34:1-9. https://doi.org/10.1016/j.fbr.2019.09.001

Saboor A, Ali M A, Danish S, Ahmed N, Fahad S, Datta R, Ansari M J, Nasif O, Rahman M H u, Glick B R (2021) Effect of arbuscular mycorrhizal fungi on the physiological functioning of maize under zinc-deficient soils. Scientific reports 11:18468. https://doi.org/10.1038/s41598-021-97742-1

Salazar M J, Menoyo E, Faggioli V, Geml J, Cabello M, Rodriguez J H, Marro N, Pardo A, Pignata M L, Becerra A G (2018) Pb accumulation in spores of arbuscular mycorrhizal fungi. Science of the Total Environment 643:238-246. https://doi.org/10.1016/j.scitotenv.2018.06.199

Schaefer J K, Szczuka A, Morel F M M (2014) Effect of Divalent Metals on Hg(II) Uptake and Methylation by Bacteria. Environmental Science & Technology 48:3007-3013. https://doi.org/10.1021/es405215v

Senovilla M, Abreu I, Escudero V, Cano C, Bago A, Imperial J, González-Guerrero M (2020) MtCOPT2 is a Cu+ transporter specifically expressed in Medicago truncatula mycorrhizal roots. Mycorrhiza 30:781-788. https://doi.org/10.1007/s00572-020-00987-3

Shabani L, Sabzalian M R, Mostafavi pour S (2016) Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in Festuca arundinacea. Mycorrhiza 26:67-76. https://doi.org/10.1007/s00572-015-0647-2

Singh G, Pankaj U, Chand S, Verma R K (2019) Arbuscular Mycorrhizal Fungi-Assisted Phytoextraction of Toxic Metals by Zea mays L. From Tannery Sludge. Soil and Sediment Contamination: An International Journal 28:729-746. https://doi.org/10.1080/15320383.2019.1657381

Smith S E, Read D (2008) Mycorrhizal symbiosis. Mycorrhizal Symbiosis (Third Edition). Academic Press, London, 1-9. https://doi.org/10.1016/B978-012370526-6.50002-7

Stephens B W, Cook D R, Grusak M A (2011) Characterization of zinc transport by divalent metal transporters of the ZIP family from the model legume Medicago truncatula. BioMetals 24:51-58. https://doi.org/10.1007/s10534-010-9373-6

Szczuka A, Morel F M M, Schaefer J K (2015) Effect of Thiols, Zinc, and Redox Conditions on Hg Uptake in Shewanella oneidensis. Environmental Science & Technology 49:7432-7438. https://doi.org/10.1021/acs.est.5b00676

Trouvelot A, Kough J L, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. V. Gianinazzi-Pearson, S. Gianinazzi (Eds.), Physiological and Genetical Aspects of Mycorrhizae INRA Press, Paris:217-221.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3:research0034.0031. https://doi.org/10.1186/gb-2002-3-7-research0034

Wang B, Qiu Y L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299-363. https://doi.org/10.1007/s00572-005-0033-6

Wang J, Ma L Q, Letcher R, Bradford S A, Feng X, Rinklebe J (2022) Biogeochemical cycle of mercury and controlling technologies: Publications in critical reviews in environmental science & technology in the period of 2017–2021. Critical Reviews in Environmental Science and Technology 1-6. https://doi.org/10.1080/10643389.2022.2071210

Wang L, Huang X, Ma F, Ho S-H, Wu J, Zhu S (2017) Role of Rhizophagus irregularis in alleviating cadmium toxicity via improving the growth, micro- and macroelements uptake in Phragmites australis. Environmental Science and Pollution Research 24:3593-3607. https://doi.org/10.1007/s11356-016-7984-3

Wang X, Tam N F-Y, Fu S, Ametkhan A, Ouyang Y, Ye Z (2014) Selenium addition alters mercury uptake, bioavailability in the rhizosphere and root anatomy of rice (Oryza sativa). Annals of Botany 114:271-278. https://doi.org/10.1093/aob/mcu117

Watts-Williams S J, Tyerman S D, Cavagnaro T R (2017) The dual benefit of arbuscular mycorrhizal fungi under soil zinc deficiency and toxicity: linking plant physiology and gene expression. Plant and Soil 420:375-388. https://doi.org/10.1007/s11104-017-3409-4

Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (Zea mays L.) in pot culture with contaminated soil. Mycorrhiza 5:245-251. https://doi.org/10.1007/BF00204957

Yon Y R, Pérez L A, Carmona A M, Pérez Y M, García L R M, Suárez K F, Echevarría A M (2015) Alternative stainning technique to determine mycorrhizal colonization. Cultivos Tropicales 36:18-21. http://dx.doi.org/10.13140/RG.2.2.10232.65287

Yu Y, Zhang S, Huang H (2010) Behavior of mercury in a soil–plant system as affected by inoculation with the arbuscular mycorrhizal fungus Glomus mosseae. Mycorrhiza 20:407-414. https://doi.org/10.1007/s00572-009-0296-4

4.9 Supplementary information

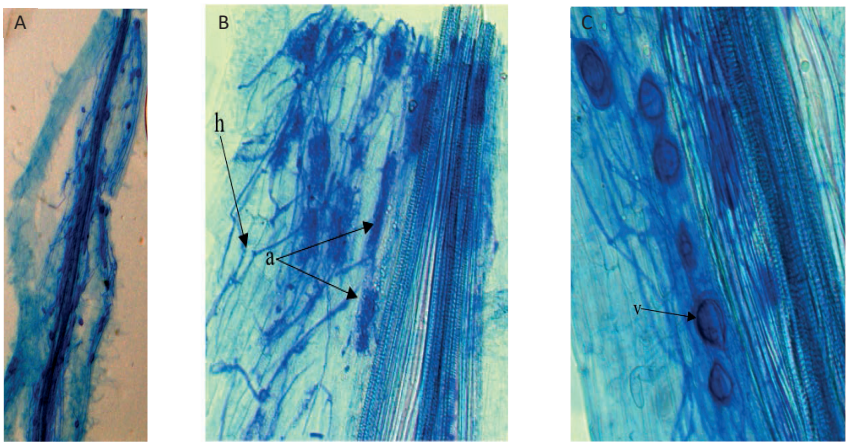

Figure S 4.1 Arbuscular mycorrhiza colonization of plant roots. (A) *Medicago truncatula* root colonized with *Rhizophagus irregularis*; (B) arbuscular (a) and hyphae (h); (C) Vesicles (v). Fungal structures were stained with ink and vinegar.

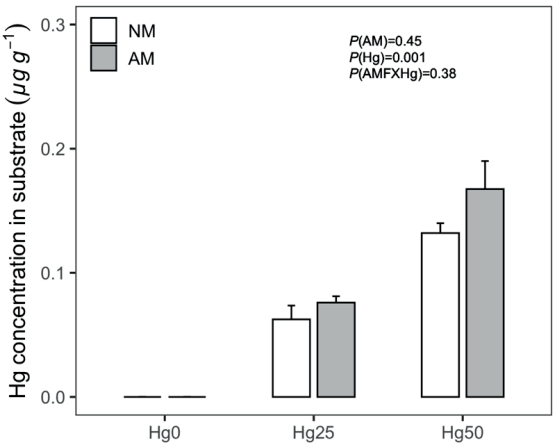

Figure S 4.2 Hg concentration in substrate in both non-mycorrhizal (NM) and arbuscular mycorrhizal (AM) treatment with three different Hg levels (0, 25 and 50 µg h-1) after experiments. The values present mean ± SE (n=5). Under Hg0, there was no detection of Hg, indicating that ther is no Hg contamination in the control substrate. Results of two-way ANOVA were annotated in the figure. There were no significant differences between NM and AM treatment means.

Table S 4.1 The list of primers used in this study.

Gene	Forward	Reverse
Beta-actin	5'-TGGGCTGCCACAGAACATTTGA-3'	5'-GCTGTGGTTGCTTTTTTGGTGTCTC-3'
MtASPP	5'-GGATCGGTCTTGGACAGTGG-3'	5'-TGGACCGCTGATTTGACTGA-3'
MtZIP2	5'-AATGGGCATTGCTTGTGGTG-3'	5'-TGTCGAAACGGCTCTTCCTC-3'
MtZIP6	5'-AGGACTTGGAATGGGAGCCT-3'	5'-CACCAAGACCCATGCCTTCA-3'
α-tubulin	5'-TGTCCAACCGGTTTTAAAGT-3'	5'-AAAGCACGTTTGGCGTACAT-3'

Table S 4.2 Leaf, stem, root and total dry biomass of non-mycorrhizal (NM) and mycorrhizal (AM) plants in the substrate exposed to three different mercury (Hg) levels (Hg0, Hg25, Hg50). Two-way ANOVA was performed to examine the effects of Hg treatment ana AM inoculation.

	Hg treatment	AM inoculation	Leaf (g/pot)	Stem (g/pot)	Root (g/pot)	Total (g/pot)
Treatment	Hg0	NM	0.67 ± 0.03a	0.63 ± 0.02a	0.65 ± 0.04a	1.95 ± 0.07a
		AM	0.62 ± 0.01a	0.60 ± 0.01b	0.61 ± 0.02ab	1.82 ± 0.02bc
	Hg25	NM	0.65 ± 0.04a	0.61 ± 0.01ab	0.61 ± 0.04ab	1.83 ± 0.23bc
		AM	0.62 ± 0.01a	0.60 ± 0.02ab	0.60 ± 0.01b	1.81 ± 0.26c
	Hg50	NM	0.65 ± 0.04a	0.62 ± 0.00ab	0.62 ± 0.01ab	1.91 ± 0.39ab
		AM	0.62 ± 0.01a	0.60 ± 0.01ab	0.58 ± 0.01b	1.80 ± 0.02c
ANOVA	Hg treatment		ns	ns	ns	ns
	AM inoculation		**	**	*	***
	Hg*AM		ns	ns	ns	*

Values presented mean ± SD, n=5
Means followed by the same letter do not differ significantly by Tukey's HSD test ($P < 0.05$)
*, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$; ns, no significant

Table S 4.3 Correlations among measured parameters in non-mycorrhizal (NM) treatment.

	Hg leaf concentration	Hg stem concentration	Hg root concentration	Zn leaf concentration	Zn stem concentration	Zn root concentration	*ZIP6*	*ZIP2*
Hg leaf concentration	1.00							
Hg stem concentration	-0.14	1.00						
Hg root concentration	0.07	**0.90**	1.00					
Zn leaf concentration	-0.11	-0.38	-0.37	1.00				
Zn stem concentration	0.17	-0.21	0.00	0.54	1.00			
Zn root concentration	0.07	**-0.74**	**-0.71**	0.41	0.46	1.00		
ZIP6	-0.30	0.05	-0.10	-0.22	-0.23	-0.39	1.00	
ZIP2	-0.05	-0.39	-0.31	0.05	0.05	0.39	0.12	1.00

Numbers presented the correlation coefficient
Bold number means significant ($P < 0.05$)

Table S 4.4 Correlations among measured parameters in mycorrhizal (AM) treatment.

	Hg leaf concentration	Hg stem concentration	Hg root concentration	Zn leaf concentration	Zn stem concentration	Zn root concentration	Colonization	*ZIP6*	*ZIP2*
Hg leaf concentration	1.00								
Hg stem concentration	0.33	1.00							
Hg root concentration	0.45	0.69	1.00						
Zn leaf concentration	0.34	0.58	0.41	1.00					
Zn stem concentration	-0.50	0.03	**-0.70**	0.22	1.00				
Zn root concentration	-0.18	-0.28	**-0.70**	-0.18	0.57	1.00			
Colonization	-0.10	-0.36	-0.15	-0.50	-0.37	-0.30	1.00		
ZIP6	0.15	0.24	0.19	0.17	0.07	-0.21	0.29	1.00	
ZIP2	-0.65	-0.69	-0.38	-0.18	01.4	0.27	0.25	-0.21	1.00

Numbers presented the correlation coefficient
Bold number means significant ($P < 0.05$)

5 General discussion

5.1 Overview

Arbuscular mycorrhizal fungi (AMF) improve plant nutrient uptake, increase plant biomass, and help plants against abiotic (drought, heat, HM) and biotic (pathogen) stress. AMF also play an important role in HM acquisition and have been considered as one of the most critical driving factors in phytoremediation (Ferrol et al. 2016; Moura et al. 2022). Although knowledge generated over past decades has advanced the understanding of mycorrhizal roles in phytoremediation, debates and knowledge gaps still remain and limit the utilization of this plant-AMF system in HM phytoremediation. Numerous studies prove that native (i.e., indigenous) AMF species performed better than commercial ones to remediate pollutants in contaminated sites (Cabello 1999; Meier et al. 2011; Kodre et al. 2017; Frew 2020), indicating the importance to select indigenous fungal species for this purpose. With this native species also circumvents ecological risks of non-indigenous species to become invasive (Hart et al. 2017). Besides, biotic and abiotic factors are known to shape the dynamics of AMF communities and thus influence the functions (Jansa et al. 2014; Alguacil et al. 2016; Coleman-Derr et al. 2016; Mahmoudi et al. 2019). However, the extent to which these factors modulate AMF communities and their relative importance to AMF functions in ecosystems remain elusive. In addition, the exact role of AMF species in phytoremediation is uncertain. That is, AMF species can either facilitate HM uptake and further translocation to aboveground parts (phytoextraction) (Weissenhorn et al. 1995; Fiqri et al. 2016; Singh et al. 2019), or inhibit HM translocation, thereby storing HM in roots (phytostabilization) (Shabani et al. 2016; Chamba et al. 2017; Salazar et al. 2018). The existence of both conflicting views regarding the roles of AMF in phytoremediation undermines the use of AMF in phytoremediation. It is suggested that a more comprehensive understanding of the underlying mechanisms of AMF in phytoremediation would help improving or refining this biotechnology to realize its potential benefits.

The main goal of this doctoral thesis was to extend the fundamental understanding of roles of AMF in phytoremediation by explicitly focusing on the contribution of AMF symbiosis in Hg-phytoremediation. In this chapter, the data and findings generated from this doctoral thesis will be discussed in a broader context and emphasize the significance of both ecological (chapter 2) and molecular (chapter 3 and chapter 4) understanding of AMF in phytoremediation. Directions of future research are suggested and implications for practical use are proposed.

5.2 Ecological understanding of AMF is important for AMF-phytoremediation

The integration of AMF into phytoremediation has gained momentum due to their crucial roles in soils, such as increasing plant nutrient uptake, influencing plant community structure and enhancing plant diversity (van der Heijden et al. 1998a; van der Heijden et al. 1998b). Furthermore, studies have demonstrated that AMF communities recover faster than plant communities in disturbed lands (Mao et al. 2019). This was exhibited by similarities of AMF community compositions in disturbed habitats compared to undisturbed habitats after three decades, while a lower vegetation coverage and plant species richness in disturbed habitats than that in undisturbed habitats was found (Mao et al. 2019). Rapid recovery of AMF communities in degraded lands potentially support vegetation development and enhance ecosystem functions in the early stages of ecosystem recovery and highlight the importance of AMF in initial plants establishment. Thus, it is desirable to introduce AMF species to accelerate process of phytoremediation, especially in mining sites where vegetation was always devoid. However, the inconsistent performance of AMF in fields has weakened the acceptance of this technology, despite its potential advantages. The source of AMF is critical since native AMF species are better adapted to the stress conditions of the target environment, whereas the distinct functional and ecological responses of AMF to different environments need to be considered (Miransari 2011; Caruso 2018; Rasmussen et al. 2018). Therefore, identifying the driving factors that influence the performance of AMF is a prerequisite for using this technology in phytoremediation. The case study represented in this doctoral thesis (chapter 2) extended the current understanding of the ecology of AMF in degraded mining sites that remained under-explored. The study revealed that AMF communities from rhizophore soil were similar irrespectively of geographic location, but a significant separation of the samples between rhizosphere soil and root compartments at both genus and ASV levels. This suggests that plant compartment exerts, independent of the microbiome source (i.e., soil), a strong recruitment effect on microbial consortia. Crucial factors underlining these phenomena are probably attributed to the nature of AMF colonization and competence (Chagnon et al. 2013), in interplay with the innate immune system of the host plant (Jones et al. 2006). Thus, the high abundance of *Rhizophagus* in the roots results from a good functional match with *P. phaseolides*. *P. phaseolides* can adapt to a range of environments, especially in harsh conditions, which might attribute to associations with AMF. However, *Rhizophagus* was also found as dominant genus in roots of rice grown in the Ashanti region of Ghana (Sarkodee-Addo et al. 2020), a location nearby the sampling sites of our study. It could be deduced that *Rhizophagus* is well-adapted to the varying environmental conditions in these areas. In this case, *Rhizophagus* could be proposed for this region, and even for regions with similar climatic conditions.

Nevertheless, this case study could not rule out the possibilities of *Acaulospora* in supporting plants since abundant *Acaulospora* in the rhizosphere soil was also detected. The ecosystem multifunctional index (measurement of multifunctional capacities of ecosystem) was positively related to AMF richness in disturbed habitats (Mao et al. 2019). It is plausible that the high abundance of *Acaulospora* in rhizosphere soil provides a favorable environment for plant development in degraded lands, particularly in nutrient-poor environments. Low soil available nutrients were found to be the key contributor to the increased AMF hyphal density and richness during grassland degradation, indicating a plant strategy to relieve nutrient deficiencies or loss as a result of degradation (Chen et al. 2023). Thus, a high abundance of *Acaulospora* in rhizosphere could attribute to low nutrients, thereby increasing hyphal density and richness outside of roots to absorb nutrients from a far distance. This may reflect the complementary functions among AMF species, as evidenced by the superiority of mixture mycorrhizal inoculum over single mycorrhizal inoculum (Crossay et al. 2019). Moreover, this suggests that available nutrients in soil are an important factor to consider when introducing AMF, as Ca and Zn were found to negatively affect AMF richness (chapter 2). Therefore, when optimizing the functions of AMF in this region, it is essential to consider at least these two nutrients. In addition, we cannot exclude other factors which were not considered in this case study may influence AMF communities, such as temporal dynamics. AMF communities have been found to shift in season and by year (Davison et al. 2011; Cotton et al. 2015). It has been demonstrated that seasonal variation coupled with low soil moisture limits the abundance of *Acaulosporaceae* (Oehl et al. 2017; Sidhoum et al. 2019). The dominance of *Acaulospora* in our study may be attributed to the sampling period in the rainy season with very high soil moisture (>78%). These provided conducive conditions for sporulation of *Acaulospora*, highlighting the need to consider seasonal temporal dynamics for further work.

Additionally, AMF achieve functions which rely on different parts of their structures. Intraradical hyphae, coils and arbuscular structures serve as interfaces for nutrient exchange with plants, while extraradical hyphae facilitate nutrient transfer to plants. Also, spores play a crucial role in propagation and development of AMF. However, it is important to note that in chapter 2 of our study, we focused solely on gaining initial insights into AMF diversity and communities using metagenomics. Although our sequencing method covered all aspects, it presented mixed information on structures, such as hyphal density and spore density, making it difficult to link specific factors to each functional part of AMF (chapter 2). Further studies incorporating additional proxies, such as AMF biomass, hyphal density inside and outside the roots, spore density and spore viable, could provide a more comprehensive understanding of AMF

communities and their functions in the field (Stevens et al. 2020).

Overall, AMF have shown potential in promoting the success of ecological restorations, yet their ecological variabilities are evident. Further research, including other factors and proxies, will provide a more thorough understandings of the ecology of AMF. Such studies would aid in providing the necessary environmental context to realize the potential roles of AMF in phytoremediation.

5.3 The necessity of understanding underlying molecular mechanisms in AMF-phytoremediation

In addition to understanding the ecological context of AMF, which includes comprehending the fundamental basis of AMF adaptation in real conditions and identifying the important factors which can facilitate their use in soil restoration and bioremediation programs (Leyval et al. 2002), it is crucial to investigate underlying molecular mechanisms by which plant-symbiosis interactions can influence the phytoremediation process. Such research not only increases the overall scientific knowledge in this realm, but also contributes to improving or refining this plant-AMF biotechnology for wide application (Hong-Bo et al. 2010; Gerhardt et al. 2017).

Uptake, translocation, detoxification, and sequestration of HM such as Hg are key processes in plants to cope with excess amounts of HM (Shi et al. 2019). Within these, transporters represent the first line to uptake metals into plants. Understanding which metal transporters are involved thus presents the initial step and would help develop strategic management to improve the efficiency of phytoremediation. For instance, via selecting different AMF species which either upregulate or down-regulate transporters will contribute to different strategies of phytoremediation such as phytostablization or phytoextraction. It is well-known that plants uptake non-essential metals via essential nutrient transporters. One prominent example is that Cd enters plants via Zn transporters, or even other divalent cations, like Fe^{2+}, or Cu^{2+}, or by Ca^{2+} and Mg^{2+} channels (Nakanishi et al. 2006; Song et al. 2017), but especially through *ZIP* transporters (Lin et al. 2016; Zhang et al. 2017; Liu et al. 2019). This is attributed to structural similarities between the two metals (Shi et al. 2019; Manoj et al. 2020), because they are in the same column in the periodic table. This offers possibilities of applying Zn fertilizer to improve Cd uptake by plants, as demonstrated with *Porphyra lasiocarpa* (Yang et al. 2022). Hg, a toxic metal, has no biological functions to plants, which indicates that plants have not evolved specific transporters for Hg uptake. In this regard, it is highly reasonable to assume that Hg may enter plants via Zn or Cd transporters. Following this, the doctoral thesis was also to investigate the Zn partitioning in plants under Hg

exposure and to examine the expression of *ZIP* transporters (*ZIP2* and *ZIP6*). Zn is an essential micronutrient with vital biological functions and is always present in plant systems. Plants have evolved specific membrane transporters for Zn (Fariduddin et al. 2022). Thus, when Hg exists in the same plant system, it may interplay with Zn, as proved in bacteria (Schaefer et al. 2014; Szczuka et al. 2015). However, the interplay between Zn and Hg within plants in association with AMF remains unknown. As our case study also reported that Zn concentrations negatively correlated with richness and diversity of AMF, suggesting manipulating Zn concentrations would enhance the functions of AMF in field. Efforts thus were first made to investigate the behavior of Zn under Hg exposure. The results showed, however, that Hg uptake did not correlate with examined Zn transporters (*ZIP2* and *ZIP6*), though AMF species significantly increased the expression of those two transporters, whereby Zn nutrient uptake was improved in plants. Thus, AMF species is enhancing Hg tolerance of plants by upregulating Zn transporter genes which might be independent of Hg uptake. Consequently, transporters that are involved in Hg uptake remain to be explored. Transcriptome analysis might be a good option to capture a series of genes which may significantly change during Hg uptake. Functional complementation of the corresponding knocked-out genes is also essential to verify the functions. This would provide solid grounds for improving Hg-phytoremediation. Nevertheless, a negative relationship between Zn concentrations and Hg concentrations in roots was detected, implying an ion antagonism. Collectively, these findings suggest that AMF species can be used to improve Zn nutrients and reduce Hg uptake when Zn concentrations are low in soil. Consequently, to what extent this positive effect holds, it requires further studies to include different Zn levels and Hg levels to find dose-dependent responses under mycorrhizal association. It is commonly believed that AMF increase Zn uptake under Zn deficiency, while AMF inhibit Zn uptake under high Zn levels (Watts-Williams et al. 2017). On the other hand, Zn concentrations affect richness and diversity of AMF (chapter 2) and were proved as an indispensable element in forming mycorrhizal symbiosis (Ruytinx et al. 2020). Hence, understanding responses of plant-AMF symbionts to Hg exposure at varying Zn concentrations is essential for the practical application of phytoremediation.

Other mechanisms which are involved in translocation, detoxification and sequestration remain also very important aspects to improve the efficiency of phytoremediation in HM polluted sites (Tonelli et al. 2020), including Hg. However, information regarding Hg is largely missing, although these have been frequently studied with other metals, such as Zn and Cd (chapter 3). For instance, metals require constant chelators once they enter the plant roots. Chelators bind with free metals and contribute to metal detoxification by reducing metabolic activities in cytosol (Clemens

2001). To date, phytochelatins (PC) and metallothioneins (MT) are the two most important chelators studied (Cobbett et al. 2002; Chaudhary et al. 2018). They have been proved to be efficient to improve plant accumulations of Zn and Cd in different plant species by overexpression (Chaudhary et al. 2018). This was attributed to thiol groups, important chemical structures within PC and MT, which can bind with HM (Chaudhary et al. 2018). Coincidentally, Hg was proved to have high affinity with thiol groups which are responsible for Hg detoxification (Wang et al. 2011; Wang et al. 2018). Some studies reported that mycorrhizal symbiosis can up-regulate their expression in plants (Cicatelli et al. 2010; Ren et al. 2019), although other factors may also influence their expression (Chaudhary et al. 2018). Altogether, these suggest that AMF can help improving Hg-phytoremediation in regulating genes which are responsible for synthesizing chelators, like PC and / or MT. However, this remains speculative and such information, in favor of expression of chelator genes under Hg exposure with mycorrhizal association, is essential for AMF to be applied as a phytoremediation enhancing technique. Chapter 3 provided perspectives to exploit AMF to improve Hg-phytoremediation, however, empirical evidence is still lacking. Understanding of underlying molecular mechanisms is the key towards better use of this biotechnology, AMF-phytoremediation.

5.4 The role of AMF in Hg foliar uptake

The finding that mycorrhizal colonization significantly reduced Hg concentrations in leaves compared to controls irrespectively of Hg concentrations in the substrate (chapter 4), is a notable and important finding. It is well-known that Hg evaporates even at room temperature (EPA 2018). Not surprisingly, Hg evaporation was recorded in our greenhouse experiment (chapter 4), which caused Hg accumulation in control plants (without Hg treatment). This is because Hg can be absorbed by leaves via stomata (Ericksen et al. 2003; Assad et al. 2016). This implies that mycorrhiza may be involved in Hg uptake by stomata which, to our best knowledge, has not been reported elsewhere. In fact, stomatal closure and decreasing CO_2 fixation are considered as a part of the protective strategy of AMF species under exposure to Cd (Pietrini et al. 2015; Wang et al. 2017). This was attributed to an avoidance mechanism for Cd uptake and translocation via immobilizing Cd in roots of AMF inoculation (Wang et al. 2017). However, it is not clear for Hg if the effects on stomata were triggered by ambient Hg or by the feedback of an avoidance mechanism of plant roots, despite roots are dominant reservoirs for Hg storage (chapter 4). In order to disentangle this mechanism of AMF under Hg exposure, a well-equipped chamber to control cross-contamination among plants would be essential. Additionally, Hg isotope analysis can be integrated to understand how AMF can affect Hg biogeochemical cycling

(Sun et al. 2019). Understanding biogeochemical cycling of Hg is very essential since, among other causes, it leads to a global distribution and harmful biotic interactions (Teixeira et al. 2018). Foliar uptake of Hg is an important route among Hg biogeochemical cycles and is considered as major deposition pathway to terrestrial surfaces (Wohlgemuth et al. 2020). It was demonstrated that foliar Hg uptake was severely affected by vapor pressure deficit (VPD) and soil water contents which both correspond to stomatal regulations (Wohlgemuth et al. 2022). The latter authors call for the implementation of environmental conditions like VPD or soil water content into a stomatal Hg deposition model to make projections about this important Hg deposition flux. Here I suggested to include soil microbes, e.g., mycorrhiza into the model to better assess future Hg cycling. Given the fact that Hg evaporation is an inherent scenario, the roles of AMF to regulate stomata under Hg exposure require further investigations. This will open a new avenue to contribute to developing effective strategies for Hg-phytoremediation. Because different AMF species can function differently (Thonar et al. 2011), this offers possibilities to screen different AMF species to serve different purposes. AMF species from the *Glomus* genus can significantly stimulate stomatal conductance (Augé et al. 2015), which is useful to cleanup atmospheric Hg pollution where Hg pollution mainly comes from air deposition. For instance, environments close to mining sites are threatened by air Hg deposition (Gworek et al. 2020); while members of *Gigasporaceae* did not increase stomatal conductance (Augé et al. 2015), which might be beneficial for food safety where Hg concentrations in leaf vegetables were highly correlated with atmospheric Hg, threatening food safety and human health (Yang et al. 2020; Addai-Arhin et al. 2022).

5.5 Future perspectives

Phytoremediation assisted with AMF has emerged as a promising and cost-effective method to remediate Hg-contaminated sites in a sustainable way. However, there is still a lack of understanding of the mechanisms involved in this approach, and field implementation is limited. To advance the practical application of AMF-assisted phytoremediation and to promote progress in remediating Hg polluted sites, future research addressing the following aspects is necessary:

1. **Plant selection**: the type of plant species used in phytoremediation plays a central role in determining the remediation process, being it phytoextraction or phytostabilization. Phytoextraction is the most popular approach since it permanently removes HM from soil. Hyperaccumulator plants, a group of plants which can actively take up exceedingly large amounts of one or more metals from the soil, are essential components in

this approach (Baker 1981; Reeves et al. 2018). For example, the threshold for consideration as a hyperaccumulator plant for zinc (Zn) and manganese (Mn) is the accumulation of 10,000 mg/kg dry weight in plant shoots. For cobalt (Co), copper (Cu), nickel (Ni), arsenic (As) and selenium (Se), it is 1000 mg/kg dry weight in plant shoots. Cadmium (Cd) has a threshold of 100 mg/kg dry weight in plant shoots. However, no Hg hyperaccumulator plants has been identified so far and consequently the threshold for Hg accumulation in hyperaccumulators is unknown. Therefore, extensive research is required to investigate and measure Hg concentrations in plant tissues to clarify the criteria of being Hg-hyperaccumulators to enhance the success of phytoremediation (Liu et al. 2020; Yin et al. 2022).

2. **Engineering microbes related to AMF**: AMF are crucial for the growth of most plants, but their functions can be enhanced by other microbes such as rhizobia and phosphorus-solubilizing bacteria (Jansa et al. 2021; Wang et al. 2022). Studies have shown that co-inoculation of AMF and rhizobia can significantly improve phytoremediation of metals like cadmium and uranium (Ren et al. 2019; Wang et al. 2021). Furthermore, both rhizobia and AMF have been shown to increase plant resistance to Hg, suggesting that co-inoculation of these microbes may also improve the efficiency of phytoremediation of Hg-contaminated sites. In addition, the use of phosphorus-solubilizing bacteria in soils can also be combined with AMF to further enhance their roles in phytoremediation, as phosphorus is often a limiting nutrient in soil. These microbial interactions can lead to improved plant growth and increased uptake of HM, ultimately resulting in a more effective remediation of contaminated sites. Further research is needed to fully understand the mechanisms involved and to optimize the co-inoculation of AMF, rhizobia, and other microbial agents for the remediation of Hg-contaminated sites.
3. **Additions of chemical or organic amendments**: amendments can be effective to make Hg more readily available for absorption by plants (Liu et al. 2020). However, it is important to note that certain amendments, such as ethylenediaminetetraacetic acid (EDTA), citric acid, and potassium iodide (KI) can cause leaching of Hg to other areas and even groundwater (Smolińska et al. 2012). AMF, with their extensive hyphal network, have the potential to prevent leaching. Combining amendments with AMF can be an effective way to remediate Hg pollutants, although research in this area is still limited. The dosage and type of amendments need to be carefully evaluated, and the remediation effect combined with AMF should be a key consideration. In addition, adding organic matter, such as compost or humic acid, can stimulate symbiosis and enhance plant

growth in conjunction with AMF (Kohler et al. 2015). The use of organic matter together with AMF could represent a sustainable and effective solution for remediating Hg-contaminated sites.

4. **Field application**: lack of (long-term) field application is not specific to Hg remediation, but a general problem with HM phytoremediation. Technical aspects certainly matter but these can be addressed through research. However, lack of interest, funding, and political and institutional constraints in phytoremediation, have been the main causes for impeding progress (Gerhardt et al. 2017). Authorities must realize that it will take a few years of experimentation to have preliminary outcomes and likely several decades to come out with definite results. During this process, other auxiliary management should be implemented to achieve the ultimate goal of phytoremediation instead of the "plant and pray" strategy which is the case currently (Gerhardt et al. 2017). This also harms the reputation of phytoremediation. Besides, authorities need to understand that there is no universal solution for remediating Hg-contaminated sites. The complex dynamics of fields such as environmental conditions including climate and soil, geographic conditions, heterogeneity of Hg pollution, the interactions between plant and microorganisms, etc. cannot be reproduced. Field investigations are certainly needed from a small to a broader scale to examine the effectiveness of phytoremediation. The development of a site-specific protocol might be necessary to take into account variabilities of all factors, achieving the optimal efficiency of phytoremediation.

Phytoremediation assisted with AMF in Hg-contaminated sites is a promising but still developing technology. This doctoral research has contributed to the understanding of ecology of AMF in post mining sites and their roles in Hg partitioning in plants, revealing valuable insights into the potential of AMF in phytoremediation. However, future research is critically needed to overcome the limitations of current phytoremediation methods and develop a systemic strategy for remediating Hg contaminants. This may involve combing suitable plant species, microbe engineering, and appropriate additions to achieve the ultimate goal of phytoremediation, such as complete removal of Hg contaminants or reduction to safe levels. Long-term field studies are also necessary to evaluate the feasibility and sustainability of this strategy in different Hg-contaminated sites.

5.6 References

Addai-Arhin S, Novirsa R, Jeong H, Phan Q D, Hirota N, Ishibashi Y, Shiratsuchi H, Arizono K (2022) Mercury waste from artisanal and small-scale gold mining facilities: a risk to farm ecosystems—a case study of Obuasi, Ghana. Environmental Science and Pollution Research https://doi.org/10.1007/s11356-022-22456-4

Alguacil M d M, Torres M P, Montesinos-Navarro A, Roldan A (2016) Soil Characteristics Driving Arbuscular Mycorrhizal Fungal Communities in Semiarid Mediterranean Soils. Applied and Environmental Microbiology 82:3348-3356. https://doi.org/10.1128/AEM.03982-15

Assad M, Parelle J, Cazaux D, Gimbert F, Chalot M, Tatin-Froux F (2016) Mercury uptake into poplar leaves. Chemosphere 146:1-7. https://doi.org/10.1016/j.chemosphere.2015.11.103

Augé R M, Toler H D, Saxton A M (2015) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25:13-24. https://doi.org/10.1007/s00572-014-0585-4

Baker A J M (1981) Accumulators and excluders -strategies in the response of plants to heavy metals. Journal of Plant Nutrition 3:643-654. https://doi.org/10.1080/01904168109362867

Cabello M N (1999) Effectiveness of indigenous arbuscular mycorrhizal fungi (AMF) isolated from hydrocarbon polluted soils. Journal of Basic Microbiology 39:89-95. https://doi.org/10.1002/(SICI)1521-4028(199905)39:2<89::AID-JOBM89>3.0.CO;2-D

Caruso T (2018) Disentangling the factors shaping arbuscular mycorrhizal fungal communities across multiple spatial scales. New Phytologist 220:954-956. https://doi.org/10.1111/nph.15212

Chagnon P-L, Bradley R L, Maherali H, Klironomos J N (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends in Plant Science 18:484-491. https://doi.org/10.1016/j.tplants.2013.05.001

Chamba I, Rosado D, Kalinhoff C, Thangaswamy S, Sánchez-Rodríguez A, Gazquez M J (2017) Erato polymnioides – A novel Hg hyperaccumulator plant in ecuadorian rainforest acid soils with potential of microbe-associated phytoremediation. Chemosphere 188:633-641. https://doi.org/10.1016/j.chemosphere.2017.08.160

Chaudhary K, Agarwal S, Khan S (2018) Role of Phytochelatins (PCs), Metallothioneins (MTs), and Heavy Metal ATPase (HMA) Genes in Heavy Metal Tolerance. Mycoremediation and Environmental Sustainability: Volume 2. Springer International Publishing, Cham, 39-60. https://doi.org/10.1007/978-3-319-77386-5_2

Chen K, Zhang J, Muneer M A, Xue K, Niu H, Ji B (2023) Plant community and soil available nutrients drive arbuscular mycorrhizal fungal community shifts during alpine meadow degradation. Fungal Ecology 62:101211. https://doi.org/10.1016/j.funeco.2022.101211

Cicatelli A, Lingua G, Todeschini V, Biondi S, Torrigiani P, Castiglione S (2010) Arbuscular mycorrhizal fungi restore normal growth in a white poplar clone grown on heavy metal-contaminated soil, and this is associated with upregulation of foliar metallothionein and polyamine biosynthetic gene expression. Annals of Botany 106:791-802. https://doi.org/10.1093/aob/mcq170

Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475-486. https://doi.org/10.1007/s004250000458

Cobbett C, Goldsbrough P (2002) PHYTOCHELATINS AND METALLOTHIONEINS: Roles in Heavy Metal Detoxification and Homeostasis. Annual Review of Plant Biology 53:159-182. https://doi.org/10.1146/annurev.arplant.53.100301.135154

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez L P, Tringe S G (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytologist 209:798-811. https://doi.org/10.1111/nph.13697

Cotton T E A, Fitter A H, Miller R M, Dumbrell A J, Helgason T (2015) Fungi in the future: interannual variation and effects of atmospheric change on arbuscular mycorrhizal fungal communities. New Phytologist 205:1598-1607. https://doi.org/10.1111/nph.13224

Crossay T, Majorel C, Redecker D, Gensous S, Medevielle V, Durrieu G, Cavaloc Y, Amir H (2019) Is a mixture of arbuscular mycorrhizal fungi better for plant growth than single-species inoculants? Mycorrhiza 29:325-339. https://doi.org/10.1007/s00572-019-00898-y

Davison J, Öpik M, Daniell T J, Moora M, Zobel M (2011) Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiology Ecology 78:103-115. https://doi.org/10.1111/j.1574-6941.2011.01103.x

EPA (2018) Global sourcs of mercury. https://www.epa.gov

Ericksen J A, Gustin M S, Schorran D E, Johnson D W, Lindberg S E, Coleman J S (2003) Accumulation of atmospheric mercury in forest foliage. Atmospheric Environment 37:1613-1622. https://doi.org/10.1016/S1352-2310(03)00008-6

Fariduddin Q, Saleem M, Khan T A, Hayat S (2022) Zinc as a Versatile Element in Plants: An Overview on Its Uptake, Translocation, Assimilatory Roles, Deficiency and Toxicity Symptoms. Microbial Biofertilizers and

Micronutrient Availability: The Role of Zinc in Agriculture and Human Health. Springer International Publishing, Cham, 137-158. https://doi.org/10.1007/978-3-030-76609-2_7

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. Journal of Experimental Botany 67:6253-6265. https://doi.org/10.1093/jxb/erw403

Fiqri A, Utomo W H, Handayanto E (2016) Effect of arbuscular mycorrhizal fungi on the potential of three wild plant species for phytoextraction of mercury from small-scale gold mine tailings. JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT 3:551-558. https://doi.org/10.15243/jdmlm.2016.033.551

Frew A (2020) Contrasting effects of commercial and native arbuscular mycorrhizal fungal inoculants on plant biomass allocation, nutrients, and phenolics. Plants People Planet 3:536-540. https://doi.org/10.1002/ppp3.10128

Gerhardt K E, Gerwing P D, Greenberg B M (2017) Opinion: Taking phytoremediation from proven technology to accepted practice. Plant Science 256:170-185. https://doi.org/10.1016/j.plantsci.2016.11.016

Gworek B, Dmuchowski W, Baczewska-Dąbrowska A H (2020) Mercury in the terrestrial environment: a review. Environmental Sciences Europe 32:128. https://doi.org/10.1186/s12302-020-00401-x

Hart M M, Antunes P M, Abbott L K (2017) Unknown risks to soil biodiversity from commercial fungal inoculants. Nature Ecology & Evolution 1:0115. https://doi.org/10.1038/s41559-017-0115

Hong-Bo S, Li-Ye C, Cheng-Jiang R, Hua L, Dong-Gang G, Wei-Xiang L (2010) Understanding molecular mechanisms for improving phytoremediation of heavy metal-contaminated soils. Critical Reviews in Biotechnology 30:23-30. https://doi.org/10.3109/07388550903208057

Jansa J, Erb A, Oberholzer H-R, Šmilauer P, Egli S (2014) Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Molecular ecology 23:2118-2135. https://doi.org/10.1111/mec.12706

Jansa J, Hodge A (2021) Swimming, gliding, or hyphal riding? On microbial migration along the arbuscular mycorrhizal hyphal highway and functional consequences thereof. New Phytologist 230:14-16. https://doi.org/10.1111/nph.17244

Jones J D G, Dangl J L (2006) The plant immune system. Nature 444:323-329. https://doi.org/10.1038/nature05286

Kodre A, Arčon I, Debeljak M, Potisek M, Likar M, Vogel-Mikuš K (2017) Arbuscular mycorrhizal fungi alter Hg root uptake and ligand environment as studied by X-ray absorption fine structure. Environmental and Experimental Botany 133:12-23. https://doi.org/10.1016/j.envexpbot.2016.09.006

Kohler J, Caravaca F, Azcón R, Díaz G, Roldán A (2015) The combination of compost addition and arbuscular mycorrhizal inoculation produced positive and synergistic effects on the phytomanagement of a semiarid mine tailing. Science of the Total Environment 514:42-48. https://doi.org/10.1016/j.scitotenv.2015.01.085

Leyval C, Joner E J, del Val C, Haselwandter K (2002) Potential of arbuscular mycorrhizal fungi for bioremediation. Mycorrhizal Technology in Agriculture: From Genes to Bioproducts. Birkhäuser Basel, Basel, 175-186. https://doi.org/10.1007/978-3-0348-8117-3_14

Lin Y-F, Hassan Z, Talukdar S, Schat H, Aarts M G M (2016) Expression of the ZNT1 Zinc Transporter from the Metal Hyperaccumulator Noccaea caerulescens Confers Enhanced Zinc and Cadmium Tolerance and Accumulation to Arabidopsis thaliana. PLOS ONE 11:e0149750. https://doi.org/10.1371/journal.pone.0149750

Liu X S, Feng S J, Zhang B Q, Wang M Q, Cao H W, Rono J K, Chen X, Yang Z M (2019) OsZIP1 functions as a metal efflux transporter limiting excess zinc, copper and cadmium accumulation in rice. BMC Plant Biology 19:283. https://doi.org/10.1186/s12870-019-1899-3

Liu Z, Chen B, Wang L-a, Urbanovich O, Nagorskaya L, Li X, Tang L (2020) A review on phytoremediation of mercury contaminated soils. Journal of Hazardous Materials 400:123138. https://doi.org/10.1016/j.jhazmat.2020.123138

Mahmoudi N, Cruz C, Mahdhi M, Mars M, Caeiro M F (2019) Arbuscular mycorrhizal fungi in soil, roots and rhizosphere of Medicago truncatula: diversity and heterogeneity under semi-arid conditions. PeerJ 7:e6401. https://doi.org/10.7717/peerj.6401

Manoj S R, Karthik C, Kadirvelu K, Arulselvi P I, Shanmugasundaram T, Bruno B, Rajkumar M (2020) Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. Journal of Environmental Management 254:109779. https://doi.org/10.1016/j.jenvman.2019.109779

Mao L, Pan J, Jiang S, Shi G, Qin M, Zhao Z, Zhang Q, An L, Feng H, Liu Y (2019) Arbuscular mycorrhizal fungal community recovers faster than plant community in historically disturbed Tibetan grasslands. Soil Biology and Biochemistry 134:131-141. https://doi.org/10.1016/j.soilbio.2019.03.026

Meier S, Azcón R, Cartes P, Borie F, Cornejo P (2011) Alleviation of Cu toxicity in Oenothera picensis by copper-adapted arbuscular mycorrhizal fungi and treated agrowaste residue. Applied Soil Ecology 48:117-124. https://doi.org/10.1016/j.apsoil.2011.04.005

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnology Advances 29:645-653. https://doi.org/10.1016/j.biotechadv.2011.04.006

Moura M A d, Oki Y, Arantes-Garcia L, Cornelissen T, Nunes Y R F, Fernandes G W (2022) Mycorrhiza fungi application as a successful tool for worldwide mine land restoration: Current state of knowledge and the way forward. Ecological Engineering 178:106580. https://doi.org/10.1016/j.ecoleng.2022.106580

Nakanishi H, Ogawa I, Ishimaru Y, Mori S, Nishizawa N K (2006) Iron deficiency enhances cadmium uptake and translocation mediated by the Fe2+ transporters OsIRT1 and OsIRT2 in rice. Soil Science and Plant Nutrition 52:464-469. https://doi.org/10.1111/j.1747-0765.2006.00055.x

Oehl F, Laczko E, Oberholzer H-R, Jansa J, Egli S (2017) Diversity and biogeography of arbuscular mycorrhizal fungi in agricultural soils. Biology and Fertility of Soils 53:777-797. https://doi.org/10.1007/s00374-017-1217-x

Pietrini F, Iori V, Bianconi D, Mughini G, Massacci A, Zacchini M (2015) Assessment of physiological and biochemical responses, metal tolerance and accumulation in two eucalypt hybrid clones for phytoremediation of cadmium-contaminated waters. Journal of Environmental Management 162:221-231. https://doi.org/10.1016/j.jenvman.2015.07.053

Rasmussen P U, Hugerth L W, Blanchet F G, Andersson A F, Lindahl B D, Tack A J M (2018) Multiscale patterns and drivers of arbuscular mycorrhizal fungal communities in the roots and root-associated soil of a wild perennial herb. New Phytologist 220:1248-1261. https://doi.org/10.1111/nph.15088

Reeves R D, Baker A J M, Jaffré T, Erskine P D, Echevarria G, van der Ent A (2018) A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytologist 218:407-411. https://doi.org/10.1111/nph.14907

Ren C G, Kong C C, Wang S X, Xie Z H (2019) Enhanced phytoremediation of uranium-contaminated soils by arbuscular mycorrhiza and rhizobium. Chemosphere 217:773-779. https://doi.org/10.1016/j.chemosphere.2018.11.085

Ruytinx J, Kafle A, Usman M, Coninx L, Zimmermann S D, Garcia K (2020) Micronutrient transport in mycorrhizal symbiosis; zinc steals the show. Fungal Biology Reviews 34:1-9. https://doi.org/10.1016/j.fbr.2019.09.001

Salazar M J, Menoyo E, Faggioli V, Geml J, Cabello M, Rodriguez J H, Marro N, Pardo A, Pignata M L, Becerra A G (2018) Pb accumulation in spores of arbuscular mycorrhizal fungi. Science of the Total Environment 643:238-246. https://doi.org/10.1016/j.scitotenv.2018.06.199

Schaefer J K, Szczuka A, Morel F M M (2014) Effect of Divalent Metals on Hg(II) Uptake and Methylation by Bacteria. Environmental Science & Technology 48:3007-3013. https://doi.org/10.1021/es405215v

Shabani L, Sabzalian M R, Mostafavi pour S (2016) Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in Festuca arundinacea. Mycorrhiza 26:67-76. https://doi.org/10.1007/s00572-015-0647-2

Shi W, Zhang Y, Chen S, Polle A, Rennenberg H, Luo Z-B (2019) Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. Plant, Cell & Environment 42:1087-1103. https://doi.org/10.1111/pce.13471

Sidhoum W, Fortas Z (2019) The beneficial role of indigenous arbuscular mycorrhizal fungi in phytoremediation of wetland plants and tolerance to metal stress. Archives of Environmental Protection vol. 45:103-114. https://doi.org/10.24425/aep.2019.125916

Singh G, Pankaj U, Chand S, Verma R K (2019) Arbuscular Mycorrhizal Fungi-Assisted Phytoextraction of Toxic Metals by Zea mays L. From Tannery Sludge. Soil and Sediment Contamination: An International Journal 28:729-746. https://doi.org/10.1080/15320383.2019.1657381

Smolińska B, Król K (2012) Leaching of mercury during phytoextraction assisted by EDTA, KI and citric acid. Journal of Chemical Technology & Biotechnology 87:1360-1365. https://doi.org/10.1002/jctb.3826

Song Y, Jin L, Wang X (2017) Cadmium absorption and transportation pathways in plants. International Journal of Phytoremediation 19:133-141. https://doi.org/10.1080/15226514.2016.1207598

Stevens B M, Propster J R, Öpik M, Wilson G W T, Alloway S L, Mayemba E, Johnson N C (2020) Arbuscular mycorrhizal fungi in roots and soil respond differently to biotic and abiotic factors in the Serengeti. Mycorrhiza 30:79-95. https://doi.org/10.1007/s00572-020-00931-5

Sun R, Jiskra M, Amos H M, Zhang Y, Sunderland E M, Sonke J E (2019) Modelling the mercury stable isotope distribution of Earth surface reservoirs: Implications for global Hg cycling. Geochimica et Cosmochimica Acta 246:156-173. https://doi.org/10.1016/j.gca.2018.11.036

Szczuka A, Morel F M M, Schaefer J K (2015) Effect of Thiols, Zinc, and Redox Conditions on Hg Uptake in Shewanella oneidensis. Environmental Science & Technology 49:7432-7438. https://doi.org/10.1021/acs.est.5b00676

Teixeira D C, Lacerda L D, Silva-Filho E V (2018) Foliar mercury content from tropical trees and its correlation with physiological parameters in situ. Environmental Pollution 242:1050-1057. https://doi.org/10.1016/j.envpol.2018.07.120

Thonar C, Schnepf A, Frossard E, Roose T, Jansa J (2011) Traits related to differences in function among three arbuscular mycorrhizal fungi. Plant and Soil 339:231-245. https://doi.org/10.1007/s11104-010-0571-3

Tonelli F M P, Tonelli F C P, de Melo Nunes N A, Lemos M S (2020) Mechanisms and Importance of Phytoremediation. Bioremediation and Biotechnology, Vol 4: Techniques for Noxious Substances Remediation. Springer International Publishing, Cham, 125-141. https://doi.org/10.1007/978-3-030-48690-7_6

van der Heijden M G A, Boller T, Wiemken A, Sanders I R (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082-2091. https://doi.org/10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2

van der Heijden M G A, Klironomos J N, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders I R (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72. https://doi.org/10.1038/23932

Wang J, Anderson C W N, Xing Y, Fan Y, Xia J, Shaheen S M, Rinklebe J, Feng X (2018) Thiosulphate-induced phytoextraction of mercury in Brassica juncea: Spectroscopic investigations to define a mechanism for Hg uptake. Environmental Pollution 242:986-993. https://doi.org/10.1016/j.envpol.2018.07.065

Wang J, Feng X, Anderson C W N, Qiu G, Ping L, Bao Z (2011) Ammonium thiosulphate enhanced phytoextraction from mercury contaminated soil – Results from a greenhouse study. Journal of Hazardous Materials 186:119-127. https://doi.org/10.1016/j.jhazmat.2010.10.097

Wang L, Huang X, Ma F, Ho S-H, Wu J, Zhu S (2017) Role of Rhizophagus irregularis in alleviating cadmium toxicity via improving the growth, micro- and macroelements uptake in Phragmites australis. Environmental Science and Pollution Research 24:3593-3607. https://doi.org/10.1007/s11356-016-7984-3

Wang L, Zhang L, George T S, Feng G (2022) A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. New Phytologist n/a:https://doi.org/10.1111/nph.18642

Wang X, Fang L, Beiyuan J, Cui Y, Peng Q, Zhu S, Wang M, Zhang X (2021) Improvement of alfalfa resistance against Cd stress through rhizobia and arbuscular mycorrhiza fungi co-inoculation in Cd-contaminated soil. Environmental Pollution 277:https://doi.org/10.1016/j.envpol.2021.116758

Watts-Williams S J, Tyerman S D, Cavagnaro T R (2017) The dual benefit of arbuscular mycorrhizal fungi under soil zinc deficiency and toxicity: linking plant physiology and gene expression. Plant and Soil 420:375-388. https://doi.org/10.1007/s11104-017-3409-4

Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (Zea mays L.) in pot culture with contaminated soil. Mycorrhiza 5:245-251. https://doi.org/10.1007/BF00204957

Wohlgemuth L, Osterwalder S, Joseph C, Kahmen A, Hoch G, Alewell C, Jiskra M (2020) A bottom-up quantification of foliar mercury uptake fluxes across Europe. Biogeosciences 17:6441-6456. https://doi.org/10.5194/bg-17-6441-2020

Wohlgemuth L, Rautio P, Ahrends B, Russ A, Vesterdal L, Waldner P, Timmermann V, et al. (2022) Physiological and climate controls on foliar mercury uptake by European tree species. Biogeosciences 19:1335-1353. https://doi.org/10.5194/bg-19-1335-2022

Yang B, Gao Y, Zhang C, Zheng X, Li B (2020) Mercury accumulation and transformation of main leaf vegetable crops in Cambosol and Ferrosol soil in China. Environmental Science and Pollution Research 27:391-398. https://doi.org/10.1007/s11356-019-06798-0

Yang Z, Yang F, Liu J-L, Wu H-T, Yang H, Shi Y, Liu J, Zhang Y-F, Luo Y-R, Chen K-M (2022) Heavy metal transporters: Functional mechanisms, regulation, and application in phytoremediation. Science of the Total Environment 809:151099. https://doi.org/10.1016/j.scitotenv.2021.151099

Yin D, Zhou X, He T, Wu P, Ran S (2022) Remediation of Mercury-Polluted Farmland Soils: A Review. Bulletin of Environmental Contamination and Toxicology 109:661-670. https://doi.org/10.1007/s00128-022-03544-0

Zhang H, Zhao S, Li D, Xu X, Li C (2017) Genome-Wide Analysis of the ZRT, IRT-Like Protein (ZIP) Family and Their Responses to Metal Stress in Populus trichocarpa. Plant Molecular Biology Reporter 35:534-549. https://doi.org/10.1007/s11105-017-1042-2